Risk factors for the development of atopic disease in infancy and early childhood

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Risk Factors for the Development of Atopic Disease in Infancy and Early Childhood

Risicofactoren voor de ontwikkeling van atopische ziekten bij het jonge kind

Proefschrift

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Overige leden:	Prof.dr. H.P. van Bever Prof.dr. H.A. Büller Prof.dr. A. Hofman

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Chapter 1

General introduction

GENERAL INTRODUCTION

The etiology of allergic diseases, including asthma, allergic rhinitis, and atopic dermatitis, is multifactorial, involving interaction of both genetic and environmental factors [1]. The prevalence of allergic diseases has doubled in the last 3 decades, especially in Western countries [2]. This sudden rise can not be explained by genetic factors and indicates that environmental factors play a crucial role in the development and clinical expression of allergic disease [3]. Various recent immunological and epidemiological studies have provided more insight into the basic patho-physiological mechanisms and genetic- and environmental risk factors for the development of allergic disease. It is clear that most children with allergic disease started to have symptoms in early life, and that early life influences are critically important in the development of allergic disease [4, 5]. A key feature in established allergic disease is the production of allergen specific IgE and the development of allergic inflammation with influx of eosinophils, basophils, mast cells and T-cells in the tissue [3]. The aims of this thesis (chapter 3) are: (1) to evaluate the role of various environmental factors on the development of symptoms of allergic disease; (2) to provide more insight in the immunological processes that result in the development of allergic disease in early childhood.

In chapter 2, the literature on the development of allergic disease is reviewed. Special emphasis is put on exposure to indoor allergens and the role of microbial stimuli during the first years of life in relation to the development of allergic disease.

One of the major difficulties in immunological and epidemiological studies on the development of allergic disease is the lack of generally accepted definitions, especially for asthma. We therefore started with evaluating the prognostic value of various respiratory symptoms in early life for the development of allergic disease in later childhood. In addition, the diagnostic criteria for asthma that were used in large prospective birth cohort studies on the development of allergic disease were reviewed (chapter 4).

In chapter 5, the study designs of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)- study and the Virus Mediated Allergy (VIGALL)-study are described.

T-cells and other inflammatory cells communicate with each other by secreting cytokines and chemokines [6]. By quantifying these proteins in children with various phenotypes of allergic disease, more insight can be obtained in the basic immunological pathways involved in the development of allergic disease. In chapter 6, we describe the association between a variety of serum markers at age 1 and the development of respiratory and skin- symptoms in the first 2 years of life.

Various risk factors for the development of allergic disease have been proposed, such as: male gender, formula feeding, exposure to environmental tobacco smoke during pregnancy, exposure to indoor allergens and a lack of microbial stimulation [7-12]. Primary prevention should be the ultimate goal for clinicians and researchers in the field of allergic disease. In chapter 7 we present the first results of a double blind placebo-controlled trial which aimed at reducing the exposure to HDM-allergen starting in the third trimester of pregnancy.

In chapter 8 the role of viral upper- and lower respiratory tract infections on the development of the infants immune system is studied. In addition, the gene (parental allergy) by environmental (daycare, having siblings) interaction in the development of upper and lower respiratory tract infections are investigated.

Finally, in chapter 9, we explore the role of ethnicity in the development of respiratory and skin symptoms.

References:

1. Evens R, Gergen PJ. Allergy, asthma, and Immunology from infancy to adulthood. Philidelphia, USA: W.B. Saunders Company; 1996.
2. von Mutius E. The rising trends in asthma and allergic disease. Clin Exp Allergy 1998;28:45-9; discussion 50-1.
3. Holgate ST. The epidemic of allergy and asthma. Nature 1999;402:B2-4.
4. Martinez FD. Recognizing early asthma. Allergy 1999;54:24-8.
5. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy 2000;55:688-97.
6. Chung KF, Barnes PJ. Cytokines in asthma. Thorax 1999;54:825-57.
7. Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993;23:504-11.
8. Oddy WH. Breastfeeding and asthma in children: findings from a West Australian study. Breastfeed Rev 2000;8:5-11.
9. Cook DG, Strachan DP. Summary of effects of parental smoking on the respiratory health of children and implications for research. Thorax 1999;54:357-366.
10. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990;323:502-7.
11. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000;343:538-43.
12. Matricardi PM, Bonini S. High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the Hygiene hypothesis? Clin Exp Allergy 2000;30:1506-10.

Chapter 2

Background of allergic disease

L.P. Koopman[1], B. Brunekreef[2], J.C. de Jongste[1], H.J. Neijens[1]

[1]Erasmus University and University Hospital/Sophia Children's Hospital, Department of Pediatrics, Rotterdam, The Netherlands. [2]University of Utrecht, Institute of Risk Assessment Sciences, The Netherlands

2.1. DEFINITIONS AND NATURAL HISTORY

The term allergic disease refers to a group of disorders and syndromes including asthma, atopic dermatitis, allergic rhinitis, food allergy, urticaria and anaphylaxis. They are linked together by a hereditary predisposition to develop specific immunoglobulin E (IgE) antibodies against various allergens: atopy or atopic sensitization [1-3]. Asthma is a heterogeneous syndrome, characterized by variable airway obstruction, bronchial hyperresponsiveness, and airway inflammation [4-6]. Atopic dermatitis or eczema is a chronically relapsing inflammatory skin disorder, characterized by itching, redness, and lichinification of the skin [7, 8]. Allergic rhinitis is characterized by atopic sensitization against indoor- or outdoor allergens, together with seasonal or perennial inflammation of the nasal mucosa, watery rhinorrhea, nasal obstruction, and itching of the nose, palate, pharynx and eyes [9]. Although most subjects start to have symptoms in early life, allergic disease in young children is often difficult to diagnose, especially asthma and allergic rhinitis. In the next paragraph, the natural history of atopic sensitization, asthma, atopic dermatitis and allergic rhinitis is discussed, with special emphasis on the first years of life.

Atopic sensitization

The natural history of atopic sensitization in the first 6 years of life was described by the German Multicenter Allergy Study (MAS) and by a prospective study by Rowntree and coworkers [10, 11]. Specific IgE antibodies against food-allergens (mainly hen's egg and cow's milk) can already be found at age 1 and their prevalence slowly diminish thereafter. In contrast, the prevalence of sensitization to outdoor inhalant allergens (birch- and grass pollen) indoor allergens (mite, cat, and dog) is very rare at age 1, and their prevalence starts to rise at age 2-3.

Asthma

The majority of asthmatic schoolchildren already had recurrent respiratory symptoms during the first 3 years of life [9, 12-15]. However, the diagnosis of asthma can only reliably be made after the age of 6, when the child is able to perform lung function tests and bronchial responsiveness can be determined. In pre-school children, the diagnosis of asthma is primarily based on the presence of respiratory symptoms, in particular wheezing. Based on findings from recent birth cohort studies, especially the Tucson Children's Respiratory Study, at least 4 wheezing phenotypes can be distinguished in early life [16-18]:

- Wheezing associated with viral infections/viral responsiveness.
- Wheezing associated with environmental tobacco smoke exposure.
- Wheezing associated with small airway size.
- Wheezing associated with atopy.

The first 3 wheezing phenotypes are usually transient and resolve before the age of 6 [18]. The 4th phenotype is associated with persistent wheezing, and the large majority of these children will eventually be diagnosed as having asthma [18]. The prognostic value of early respiratory symptoms for the development of asthma later in childhood, as well as the definitions of asthma that were used in large prospective birth cohort studies, are reviewed in chapter 4.

Atopic dermatitis
Atopic dermatitis is the most common type of allergic disease in infancy. Onset is within the first year of life in 60% of cases and within the first 5 years in 85% [9, 19]. Atopic dermatitis clears in about 40% of children but may persist in some adults as hand dermatitis [20].

Allergic rhinitis
The diagnosis of allergic rhinitis in infants is difficult because the symptoms are hard to distinguish from symptoms of common cold. On average a child experiences between 3 and 5 episodes common cold episodes in the first year of life [21-23]. In addition, sensitization to common indoor- or outdoor allergens is very rare among young children. Therefore, the diagnosis of allergic rhinitis is rarely made in young children, but it becomes more common with increasing age [12, 24, 25]. Taken these considerations into account, in this thesis the emphasis is put on wheezing and atopic dermatitis, and less on allergic rhinitis.

2.2. EPIDEMIOLOGY

Allergic diseases are common, affecting between 15-30% of all children in Western countries, depending on the definitions that are used and populations that are studied [26-29]. Because generally accepted epidemiological definitions for the diagnosis of asthma are lacking, it is difficult to obtain valid incidence- and prevalence estimates [30]. Valuable data on the worldwide prevalence of respiratory symptoms have been obtained by the International Study of Asthma and Allergies in Childhood (ISAAC) [31]. In unselected populations of Western countries, the 12-months prevalence of 'wheeze at least once' varies between 3.5% (Pamplona, Spain) and 27.2% (Christchurch, New Zealand) in 6-7 year old children and between 2.7% (Ascoli, Italy) and 33.5% (Adelaide, Australia) in 13-14 year old children. The 12–month prevalence of recurrent wheeze ($\geq$ 4 episodes) ranges from 0.7% (Pamplona, Spain) to 10.2 % (Christchurch, New Zealand) in 6-7 year old children and 0.7% (Athens, Greece) to 12.8% (Adelaide, Australia) in 13-14 year old children [32]. The prevalence of wheezing episodes in the first year of life is 25-60% [24]. The cumulative incidence of atopic dermatitis in children born after 1990 is between 2.5% and 26% (average 10%) in Western countries [12, 30]. In 13-14 year olds, the 12-month prevalence of allergic rhinitis symptoms varies between 6% (Greece) and 25% (Canada) [33]. In a study in 5-6 year old children in Germany, the prevalence of allergic rhinitis was 9.2% [30]. In the German MAS study, the cumulative incidence of allergic rhinitis between birth and age 7 years was 15% [25]. In the Tucson Children's Respiratory Study, the prevalence of doctor diagnosed allergic rhinitis at age 6 was 42% [34]. Few data are available on the prevalence of allergic disease in Dutch children. In one study, the prevalence of wheezing in the previous year was 14.0% in 2-4 year old children and 8.8% in 5-11 year old children [35]. In another Dutch study in 4 year old children, the prevalence of 'attacks of shortness of breath with wheezing in the past 12 months' and 'wheeze in the past 12 months was 11% and 20% respectively [36]. In general, the prevalence of atopic disease is much lower in developing countries than in Western countries [32]. The possible explanations for this will be discussed in paragraph 2.4.
Several studies, mainly performed in English-speaking countries, have shown that the prevalence of allergic disorders has increased substantially in the last 3 to 4 decades. In two surveys, 25 years apart in schoolchildren in Aberdeen, UK, using the same questionnaires, the

prevalence of wheeze doubled from 10.4% in 1964 to 19.8% in 1989 [37]. The prevalence of episodes of shortness of breath also rose 2-fold, the diagnosis of asthma 2.5-fold, dermatitis 2.5-fold and hay fever almost 4-fold [37]. Similar increases in the prevalence of symptoms of allergic disease have been found in Cardiff, Melbourne, Belmont & Wagga Wagga and the UK [38-41]. In a study in Rochester, USA, using data from medical records, the annual incidence of doctor diagnosed asthma in the general population rose from 183 per 100,000 in 1964 to 284 per 100,00 in 1983 [14]. The rise was entirely accounted for by increased incidence rates of asthma in children aged 1-14 year [14]. Together with the rise in prevalence and incidence of allergic disease symptoms over time, hospital admission rates and mortality rates for asthma increased substantially in the past decades [42-44]. The increased prevalence and incidence of allergic disease over time may in part be attributable to methodological factors, such as a change in diagnostic labeling by doctors or an increased public awareness of asthma and asthma-like symptoms. However, recent studies have shown that also objective measures such as airway hyper-responsiveness have increased over time [45]. Therefore, despite the aforementioned methodological problems, it is generally accepted that a large proportion of the increased prevalence and incidence of allergic disease is real [2, 46]. The possible explanations for the rising trends in allergic disease will be discussed in paragraph 2.4. It is important to note that recent studies indicate that the rising trends of allergic disease seem to have reached a plateau [47].

2.3. PATHOPHYSIOLOGY OF ALLERGIC DISEASE

IgE is thought to be a key trigger for the development of allergic inflammation, which is characterized by the influx of a variety of inflammatory cells into the tissue. Adhesion molecules play a crucial role in the recruitment of these inflammatory cells and are therefore important in the pathogenesis of allergic inflammation [48-59]. The allergic immune response is usually divided into an acute phase and a late phase [60]. The acute phase is characterized by the formation of specific IgE antibody-allergen complexes (cross-linking) which adhere to mast cells and basophils [1, 61]. Upon contact with a specific allergen, these cells release a variety of cytokines and mediators such as histamine, TNF-α, leukotrienes and chemokines, which are responsible for the acute allergic symptoms (for instance bronchoconstriction in asthma) [9, 62]. After a few hours the late phase starts, which is characterized by the influx of inflammatory cells such as eosinophils, monocytes, neutrophils, platelets and T cells [2, 9, 60]. These inflammatory cells are also sources of inflammatory mediators. Eosinophils for instance release a variety of mediators, including major basic protein, eosinophil-derived neurotoxin, -peroxidase (EPO) and- cationic protein (ECP). These mediators can damage airway epithelium and stimulate the release of mediators from mast cells and basophils [5, 9, 63-66]. In asthmatic patients, bronchial biopsies and lavage studies have shown increased numbers of inflammatory cells [67-69]. In biopsy studies of patients with allergic rhinitis or atopic dermatitis, similar inflammatory changes as in asthma have been observed [70, 71]. In the next paragraph the basic immunological mechanisms leading to allergic inflammation and the regulatory mechanisms avoiding allergic inflammation will be reviewed. In addition, the genetic background of allergic disease will be briefly discussed.

2.3.1. Basic immunology

The immune response is initiated by the binding of an allergen to antigen-presenting cells (APC) [5, 72, 73]. Various types of APC exist, such as macrophages, B-cells, dendritic cells (DC), which are the most potent APC in the lung [74, 75] and Langerhans cells, which act as APC in the skin [76]. The APC migrate to the lymphoid tissue where they present processed antigen, in association with Major Histocompatibility Class (MHC) II-molecules, to naive T helper cells which express the epitope CD4 on their membrane (Th0 cells) [72, 73]. The APC interacts with the T-cell receptor (TCR) of the Th0 cells, which results in the differentiation of the Th0 cell into either Th1 cells or Th2 cells [72]. The existence of such a Th subset system was first described in mice by Mosman et al. [77] and was later confirmed in humans by Del Prete et al. [78]. After stimulation by allergen, Th1 cells characteristically produce interferon γ (IFN-γ), interleukin 2 (IL-2), and tumor necrosis factor β (TNF-β), whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 (figure 2.1) [3, 5, 46, 68, 75, 79-82]. The cytokines produced by one subset can antagonize cytokines produced by the other subset: IFNγ is a strong inhibitor of the growth and function of Th2 cells, while IL-4, IL-10 and IL-13 inhibit the growth and function of Th1 cells (figure 2.1) [68, 82]. Th1 cytokines activate cellular cytotoxicity, production of immunglobuline (IgG_1), microbial killing and complement activation and therefore play an important role in host defense against various pathogens. Th2 cytokines stimulate allergic inflammation by promoting the differentiation and activation of eosinophils (through IL-5), the production of IgE (IL-4 and IL-13), the differentiation of B-cells into plasma cells (IL-6), as well as the growth of mast cells and basophils (IL-4, IL-9 and IL-10) [46, 75, 79, 80, 83, 84]. Many studies in children and adults provide evidence that allergic disease is a result of an imbalance between Th1 cells and Th2 cells in favor of Th2 cells. This is illustrated by the accumulation of Th2 cells in target organs and the induction of a Th2 cytokine response in peripheral blood mononuclear cells (PBMC) and isolated T-cells after allergen stimulation in allergic subjects [82, 85-87].

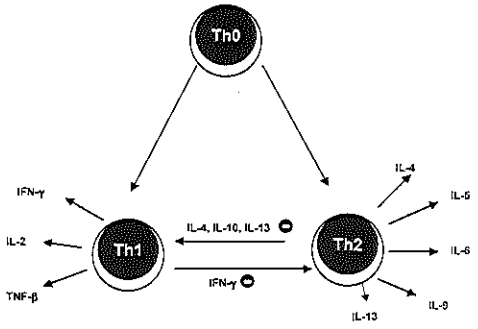

Figure 2.1. Th1/Th2 polarization. - = suppression. Abbreviations: Th = helper T-lymfocyte.

Sensitization window of opportunity in infancy

In the first years of life, the immune system of children is relatively immature compared to that of adults, which is reflected by a limited ability to develop immune responses in both quantitative and qualitative terms [88]. This period of immaturity might be essential for the development of allergic disease: the so-called 'sensitization window of opportunity in infancy' [72, 73, 89, 90]. Some studies even suggest that allergens are capable of crossing the placental barrier [91], and that in utero important events may occur for the development of allergic disease [92-95]. This is supported by studies showing that maternal atopy is stronger associated with the development of asthma and atopic dermatitis in children than paternal atopy [96-99]. However, other studies found a similar effect of maternal and paternal atopy on the development of allergic disease in their offspring [100-102]. Therefore, the issue of whether maternal atopy is stronger associated to the development of allergic disease in their children than paternal atopy is controversial. Some studies found increased T-cell proliferation responses in cord blood of infants born from mothers who were exposed to high levels of house dust mite and birch pollen allergen, suggesting the existence of some form of intrauterine sensitization [92, 93]. However, Chan-Yeung et al. and Smillie et al. found no relation between maternal exposure to HDM-allergen and CBMC's proliferation response to HDM-allergen [103, 104]. In the study of Chan-Yeung et al, no relation was found between proliferation response to HDM-allergen and the subsequent development of allergic disease in early childhood [103]. Furthermore, cord blood total IgE is poorly associated with the development of allergic disease (paragraph 2.6.) and the presence of specific IgE against common allergens has not been described in cord blood so far [105]. Therefore it might well be true that the cord blood T-cell responses against various allergens that have been found by some studies represent background (or artifact) responses rather than evidence of prenatal sensitization [106, 107].

Regulation of the immune response

Since the Th1 cytokines IFNγ, IL-2 and TNF are generally harmful for the feto-placental unit, successful pregnancy depends on a skewing of the maternal and fetal immune system towards a Th2-like phenotype [72, 93, 108-111]. In two independent prospective birth cohort studies either a weak Th2 profile [112-114] or Th0 profile [115] was found in stimulated cord blood mononuclear cells (CBMC). In the study by Prescott et al., a more pronounced Th2 cytokine profile at birth was seen in children who did not develop allergic disease in the first 2 years of life, while the cytokine profile of stimulated CBMC in the study of Laan et al. was unrelated to the development of allergic disease [113, 115]. However, in children who developed atopic disease, dominant Th2-like cytokine profiles in stimulated PBMC were found at age 6 and 12 months in both studies [113, 115]. In children who did not develop atopic disease a rapid suppression of Th2 responses were observed [113]. Together, these data suggest Th2-like responses in children who develop allergic disease in early life and Th1 or balanced Th1/Th2 responses in non-atopic children.

If we consider allergic disease being a Th2 disease, which factors are important in driving the immune response towards a Th1 or Th2 direction? Firstly, IFNγ production by stimulated CBMC's was found to be lower in children with a positive family history of allergic disease and in children who themselves develop allergic disease or atopy as compared to children without a positive family- or personal history of allergic disease [116-119]. These findings suggest that an intrinsically reduced capacity to generate IFNγ is (at least partly) responsible

for the maintenance of a Th2 phenotype that is observed in allergic children. Secondly, recent studies in murine models and adults have provided convincing evidence that APC play a central role in directing immune response [73, 120]. Due to difficulties in obtaining APC from infants and children in quantities sufficient to perform laboratory studies, no data exist on the role of APC in the immune regulation in this age group [121, 122]. Taken this important limitation into account, several factors that determine whether APC become Th1 (APC1 or DC1) or Th2 (DC2) inducers in the lung will be discussed. These factors are divided in factors reducing Th1-polarization and factors stimulating Th2-polarization.

Lack of Th1-stimulation: Bacterial wall components, such as endotoxin and various microorganisms, induce the production of IL-12 and IL-18 by APC's [120]. These cytokines, especially IL-12, seem to play a key role in determining the differentiation of Th0 cells into Th1 cells because of it's ability to stimulate the production of IFN-γ by Th1 cells [3, 5, 60, 74, 85, 90, 123-126]. When the secretion of IL-12 by APC is relatively low, for instance because of a lack of microbial stimulation, skewing towards a Th2 phenotype is more likely to occur (figure 2.2) [2].

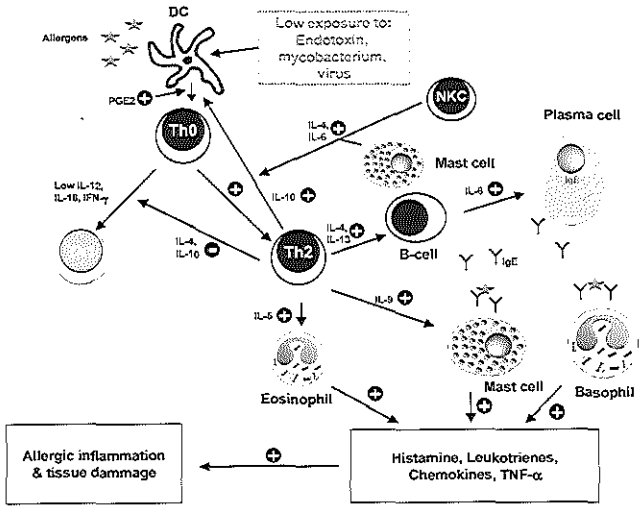

Figure 2.2. Polarization towards Th2 phenotype. + = stimulation, - = suppression, Th = helper T lymfocyte, DC = dentritic cell, NKC = natural killer cell, Th = helper T lymfocyte.

Alternatively, when the microbial load is high and relatively high levels of IL-12 are produced, skewing towards a Th1 phenotype occurs, characterized by IFN-γ, TNF-α and IL-2 production (figure 2.3) [120]. The production of IL-12 by APC is up-regulated by IFNγ (positive feed-back) and down-regulated by Th2-derived cytokines IL-4, IL-10 and IL-13 [125-130]. Another potential source of IFN-γ are natural killer cells (NK1-cells), which can directly react to microbial antigens or alternatively be stimulated by DC [120].

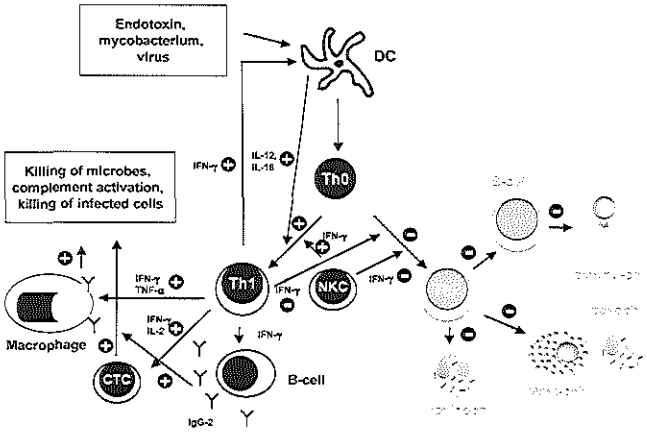

Figure 2.3. Polarization towards Th1 phenotype. + = stimulation, - = suppression. Th = helper T lymfocyte, DC = dentritic cell, NKC = natural killer cell, Th = helper T lymfocyte, CTC = cytotoxic T cell.

Stimulation of Th2-polarization: IL-4 is thought to be the most potent stimulator of Th2 differentiation, but this cytokine is not produced by DC [120]. Various allergens, including HDM-allergens, are capable of stimulating Th2-development by increasing IL-4 and IL-13 production and decreasing IFN-γ production [131, 132]. A potential source for IL-4 are natural killer cells (NK2-cells) or mast cells [120]. Another potential Th2-driving factor produced by DC is prostaglandin E_2 (PGE_2), but the factors involved in stimulating PGE_2 production are incompletely understood [74]. In paragraph 2.4, epidemiological evidence for the role of allergen exposure and microbial stimulation on the development of allergic disease will be discussed in more detail.

In conclusion, the development of allergic disease coincides with a continuation of fetal Th2-responses or a shift from fetal Th0-responses towards Th2-responses during infancy. Exposure to microorganisms is thought to deviate the infants' immune response towards a Th1-response, while exposure to allergens stimulates the continuation or deviation towards a Th2-response.

Limitations and extensions of the Th1/Th2 paradigm

Although the Th1/Th2 paradigm is clear-cut in murine models, recent studies show that in human T-cells the situation is more complex [82, 84, 133-136]. In some immune responses, T-cells do not develop distinct Th1/Th2 cytokine profiles, but may express intermediate cytokine combinations [135]. For instance, in chronic atopic dermatitis increased levels of IFNγ have been found in skin lesions [137, 138]. In addition, in a study in adults with allergic asthma and allergic rhinitis, both IL-5 and IFN-γ were increased compared to normal subjects [139]. Recent studies have provided evidence that the role of IL-10 in humans is more complex than in rodents, in which this cytokine is regarded as Th0- and Th2-derived, and acts as a strong down-regulator of Th1 cytokines [140]. Human IL-10 is produced by both Th1 and Th2 cells, T-regulatory (Tr) cells, monocytes, macrophages, B cells, mast cells and eosinophils [141]. Although IL-10 strongly inhibits the production of IL-12 by DC and is therefore

capable of reducing the production of IFN-γ [127], IL-10 has also been shown to reduce the production of IL-5 by T-cells as well as the production of various pro-inflammatory cytokines and to inhibit mast cell function (reviewed by ref. [142] and [143]). Also in clinical studies conflicting data on IL-10 exist. Increased levels of IL-10 have been found in skin biopsies and plasma of adults with atopic dermatitis and birch-pollen allergy [130, 138, 144]. In infants, the production of IL-10 by monocytes 3-4 weeks after hospitalization for RSV-bronchiolitis was found to be positively correlated with the development of recurrent wheezing after 1 year follow-up [145]. In contrast, decreased levels of IL-10 have been found after stimulation of PBMC's from asthmatic adults and from 1 year old infants with atopic disease [115, 139]. In addition, Holt and coworkers found that the magnitude of the skin response after subcutaneous injection of aeroallergens in children was inversely related to the IL-10 response of their stimulated PBMC's [146]. Therefore, in humans, IL-10 might be considered as a regulatory cytokine, damping both Th1 and Th2 polarization rather than an exclusive Th2 signature cytokine [143].
The 'Th2 hypothesis' is still a useful model in allergic disease according to most authors [82]. However, in human pathology a more complicated regulation exists, which has to be analyzed in relation to clinical features.

It is important to bare in mind that allergic immune deviation is not the only factor that leads to the development of allergic disease. In some countries about 40% of the preteen schoolchildren are sensitized to inhalant allergens, but only one quarter to one third of these children develop bronchial hyper-responsiveness and persistent wheeze [147]. Clearly, additive or synergistic factors are required for disease expression in later childhood [60]. Potential additive factors are viral infections, indoor allergens to which the atopics are sensitized, environmental tobacco smoke exposure and outdoor air pollution [60]. A key feature of chronic or persistent asthma already encountered in school-children and universally present in adults is airway remodeling: the development of airway wall thickening, subepithelial fibrosis, increased airway smooth muscle mass, myofibroblast hyperplasia and mucus metaplasia [148, 149]. Airway remodeling is thought to mediate the development of increased bronchial hyperresponsiveness, which is another hallmark of allergic airway disease [60]. Various cytokines are thought to be involved in the process of airway remodeling and the development of bronchial hyper-responsiveness, such as the Th2-cytokines IL-6 and IL-13 and the epithelial cell-derived cytokine IL-11 [148].

2.3.2. Genetics

It has long been recognized that there is a strong heritable component for asthma, atopic dermatitis and other forms of allergic disease [61, 150]. In the next paragraph, the evidence for the genetic control of allergic disease will be discussed shortly.

Family studies, twin-studies and segregation analysis

Many studies have shown that a parental history of allergic disease is the single most important risk factor for the development of allergic disease in children [18, 98, 99, 101, 151-153]. For this reason, most epidemiological studies use a family history of allergic disease to define high-risk children [154]. However, most children who will develop an allergic disease are born from non-allergic parents, because the majority of parents in the general population are non-allergic [155, 156]. In general, the risk to develop an allergic disorder is believed to be

about 20% without a positive family history of allergic disease. The risk increases to 50% if one parent is allergic and to 66% if both parents are allergic [157]. Dold en coworkers have shown that there is a strong organ-specific component in the genetics of allergic disease [100]. For instance, if one parent had atopic dermatitis, the risk of having a child with asthma was not increased (odds ratio (OR) 1.0; 95% CI 0.6-1.6, children with negative family history of allergic disease as a reference group). However, the risk of having a child with atopic dermatitis was markedly increased (OR 3.4; 95% CI 2.6-4.4). A similar pattern was seen for asthma and allergic rhinitis [100]. Studies comparing monozygotic and dizygotic twins have shown that the genetic contribution can be estimated between 36% and 79% in asthma, between 33% and 82% in hay fever, and between 71% and 74% in atopic dermatitis [158-161]. Allergic diseases are complex genetic disorders that do not conform to a simple Mendelian pattern of inheritance [162]. Segregation analysis can provide insight into the number of genes involved and the genetic model (dominant, recessive or polygenic) by studying affected families. The various segregation analyses that have been performed on asthmatic symptoms, IgE-production and bronchial hyper-responsiveness suggest that not a single gene, but many genes are involved in the etiology of allergic disease (reviewed by ref. [163]).

Linkage studies and candidate genes

Two techniques are available to identify genetic effects on a disease: candidate gene approach and positional cloning or linkage studies [61, 159]. The aim of the candidate gene approach is to find polymorphisms in a known gene and to compare the frequency of alleles in cases and controls. Positional cloning relies on linking the inheritance of specific chromosomal regions with the inheritance of the disease. In the last 10 to 15 years, many associations between candidate genes and various forms of allergic disease have been described (reviewed by ref. [61, 162-174]). Some of the genes that are possibly involved in the etiology of allergic disease will be briefly discussed.

Chromosome 5: Chromosome 5q31-q33 (q = long arm of the chromosome) contains numerous candidate genes for atopy, such as a cluster of genes encoding for CD-14 (see paragraph 2.4.), the Th2 cytokines IL-3, IL-4, IL-5, IL-9, IL-13 and the Th1 cytokine IL-12 [61, 165]. Polymorphism in the CD14 gene, IL-13 gene and IL-4 gene, have been identified that are associated with variations in IgE-levels in children and adults [164, 175, 176]. In addition, a locus or loci were found on chromosome 5q31-33 that controls for the occurence of circulating eosinophils [177].

Chromosome 6:

The genes encoding for MHC-antigen (or HLA-antigen) and TNF are localized on chromosome 6p21 (p = short arm of the chromosome) [163, 164]. Several studies found associations between polymorphisms in the MHC class II regions and the specific IgE response to mite and ragweed allergens. TNF-α is a pro-inflammatory cytokine that is abundant in asthmatic airways [61]. A polymorphism in the TNF-α gene that increases the production of TNF-α was found to be associated with asthma [163].

Chromosome 11: The β chain of the high affinity receptor for IgE (FcεRI-β, expressed on mast-cells and basophils) is localized on chromosome 11q13. A variation in the level of β chain expression may modify receptor function [164]. A polymorphism in FcεRI-β has been related to atopy, asthma, bronchial hyper-responsiveness and atopic dermatitis [163, 164].

Besides the aforementioned chromosomal regions, candidate genes for various allergic diseases have been found on chromosome 2, chromosome 7, chromosome 8, chromosome 12,

chromosome 13, chromosome 14, chromosome 16 and chromosome 17 (reviewed by ref. [61, 163, 169]).

In conclusion, it is clear that allergic diseases are polygenic, in which many genetic variants, probably in combination with environmental factors, determine small variations in immunological responses [178]. Eventually, these differences in immunological responses might result in the clinical expression of atopy and allergic disease in different individuals.

2.4. RISK FACTORS FOR ALLERGIC DISEASE

Although it is clear that allergic diseases are under genetic control, environmental factors are of comparable importance. For this reason, many epidemiological studies have been performed in the last decades focussing on identifying environmental risk factors for the development of allergic disease. The next paragraph contains a review of the literature on this topic, with special emphasis on the role of house dust mite exposure in the development of allergic disease. We also discuss the role of microbial stimulation, a concept that might, at least partly, explain the rising trends and regional differences of allergic disease as described in paragraph 2.2. Furthermore, the association between allergic disease and socioeconomic and ethnical background is debated. Finally, this paragraph provides a summary of other risk factors for the development of allergic disease.

2.4.1. House dust mite-allergen exposure

House dust mites belong to the subclass Acari of the class Arachnida [179]. House dust mites are found in almost every dwelling, but they are more abundant in humid and temperate geographical regions, because their survival is dependent on high humidity, moderate temperatures and adequate food sources [180]. The most relevant house dust mite species are Dermatophagoides pteronyssinus (Der p) and Dermatophagoides farinea (Der f) [181]. The fecal pallets of the house dust mite are the primary source of allergens. The group I allergens (e..g. Der p 1 and Der f 1), which are cysteine proteases, are considered to be the most clinically relevant allergens [182]. The role of house dust mite allergens in the development of allergic disease has been the topic of many studies. In 1987, the first International Workshop on the dust mite allergens and asthma was organized [183]. Since then, another 3 International Workshops have been organized, emphasizing the importance of house dust mite allergens in the etiology of asthma [181, 184, 185]. However, in a recent review by Pearce et al., the relative importance of house dust mite allergens for the development of allergic disease was questioned [186]. In the next paragraph, arguments pro and con the role of house dust mite allergen for the development of allergic disease, with asthma as the main focus, will be discussed.

Pro:

HDM-allergen exposure and sensitization:

Exposure to house dust mite allergen is associated with sensitization to house dust mite in pre-school children [187, 188] and schoolchildren [189, 190] and there is evidence for a dose-response relationship. At the first International Workshop it was stated that exposure to Der p 1 at a level of > 2 µg/g dust increases the risk of sensitization to HDM [183], but recent studies suggest that lower levels of exposure are also capable of inducing sensitization [191-193].

HDM-sensitization and asthma/wheeze:
In a prospective study, sensitization to HDM-allergen in wheezing infants was found to be predictive of the development of asthma in later life [194]. Many cross-sectional studies have found that sensitization to HDM-allergen is a strong risk factor for childhood asthma [195-203].
HDM-allergen exposure in established asthma:
In asthmatic children, exposure to HDM-allergen is linked to disease severity [204-206]. If HDM-allergen allergic asthmatic children are transferred to a mite-free environment, or when HDM-allergen reduction programs are applied, symptoms will improve, bronchial hyper-responsiveness decreases, and antigen-induced basophil histamine release is reduced [207-211].
HDM-allergen exposure in the light of geographical differences in asthma/wheezing:
If HDM-allergen exposure is causally related to the development of asthma, one would expect a higher prevalence of disease in areas of high mite exposure and a lower prevalence of disease in areas of low mite exposure. Indeed, as was mentioned in paragraph 2.2., the prevalence of wheezing and recurrent wheezing in children is high in the UK, New-Zealand, and some parts of Australia and the USA, were climate condition favor mite growth [32]. In contrast, in developing countries and in the Scandinavian countries, where HDM-allergen levels are low, the prevalence of respiratory symptoms in childhood is low [32].
HDM-allergen exposure and wheezing/asthma development:
In a cross-sectional study in infants aged 3-15 months, van Strien et al. found that HDM-allergen exposure was associated with an increased prevalence of parental reported respiratory symptoms (wheeze and/or prolonged cough) [212]. The strongest evidence for a direct causal relation between exposure to HDM-allergen in early life and the development of asthma in later life is provided by a prospective birth cohort study in Poole, UK [187]. In this study, children who were exposed to more than 10 μg/g Der p 1 in infancy were 4.8 times more likely to have active asthma at age 11 than children exposed to lower levels of HDM-allergen ($p = 0.05$ for difference between the 2 groups).
HDM-allergen exposure and the development of atopic dermatitis and allergic rhinitis:
No studies are available which identify exposure to HDM-allergen as a primary cause for allergic rhinitis and atopic dermatitis. However, there are many indications that HDM-allergen exposure and sensitization to HDM is associated with established allergic rhinitis and atopic dermatitis [180, 213-215].
Proposed mechanism for HDM-allergen as a cause of asthma:
Once sensitization to HDM-allergen has occurred, subsequent exposure to HDM-allergen may result in the release of mediators, the influx of inflammatory cells in the involved tissue and ultimately in the development of clinical symptoms [179]. In established asthma, exposure to HDM-allergen can result in the up-regulation of the Th2 cytokines IL-4 and IL-13 and down-regulation of the Th1 cytokine IFN-γ [131, 132], the release of histamine [189], recruitment of eosinophils and basophils and up-regulation of ICAM-1 [179]. In addition, Der p 1 is capable of cleaving the surface protein CD23, which is an important down-regulator of IgE production (see paragraph 2.3.1.)[107].

Con:
Although most studies acknowledge that HDM-allergen exposure results in sensitization to HDM-allergens, some recent studies indicate that the relationship with asthma development is less clear [186].
HDM-sensitization and asthma/wheeze:
Peat et al. studied schoolchildren in 6 different regions in Australia [202]. In these regions HDM-allergen exposure ranged from undetectable to very high levels, depending on climate conditions. Sensitization to HDM was more frequently found among children living in high HDM-allergen regions than in children living in low HDM-allergen regions. However, children in low HDM-allergen regions had a higher prevalence of sensitization to other allergens (mainly Alternaria) and the prevalence of total atopic sensitization as well as the prevalence of asthma was similar in all 6 regions. Similar findings were made in Tucson and New-Mexico, USA, which are regions with low HDM-allergen levels, but high Alternaria-levels and cat-allergen levels [216, 217]. In these studies, sensitization to HDM was rare, but sensitization to Alternaria and cat was frequently found and this was highly associated with asthma. In addition, asthma prevalence was comparable with that of studies in high HDM-allergen regions. These data suggest that genetically susceptible children will develop atopic sensitization towards the allergen that is encountered by their immune system and do not support an increased risk of asthma due to exposure to these allergens [218].
HDM-allergen exposure and wheezing/asthma development:
Although 2 studies, involving small groups of children, have found a direct link between HDM-allergen exposure and the development of wheezing in infancy and asthma at age 11 [187, 212], various studies did not find this relationship. In 3 prospective cohort studies in the USA, UK and Norway, involving relatively large groups of children, no statistically significant associations were found between early exposure to HDM-allergen and the development of wheeze in the first 2 years of life [219-221]. In a recent study in Germany, no association was found between exposure to HDM-allergen at age 6 months and the risk of current and ever wheeze or doctor diagnosed asthma at age 7 [200]. Also no association was found between exposure and various lung function parameters and bronchial hyper-responsiveness [200].

2.4.2. Exposure to micro-organisms

It is well recognized that exposure to microorganisms is a risk factor for the development of respiratory symptoms in infancy and early childhood. For instance, having siblings and attending day care are strongly positively associated with upper airway infections, lower airway infections and wheezing in the first 3 years of life [23, 222, 223]. However, in the last decade evidence has accumulated that early infections might protect against the development of allergic disease in the long run. Strachan was the first to report an inverse relationship between the number of siblings and the prevalence of hay fever [224]. The author hypothesized that allergic diseases were prevented by infections in early childhood, transmitted by contacts with older children: the so-called hygiene hypothesis. Over the past century, declining family size, improved household conditions and higher standards of personal hygiene, have reduced the opportunities for cross-infection in young families [225]. In later studies, having older siblings was found to be protective for the development of atopic sensitization, atopic dermatitis and asthma [226-228]. More recently, inverse relations have also been reported between day care attendance in early life and the development of atopic sensitization, total IgE and asthma

in later childhood [229, 230]. The hygiene hypothesis fits well in the Th1/Th2 paradigm because, as was mentioned in paragraph 2.3.1., natural immunity to bacterial and viral infections induces a Th1 cytokine release and suppresses Th2 cytokines release [231, 232]. In the last 5 years, the hygiene hypothesis is further extended by the findings that allergic disease is less common in farming communities and that the gut-flora might play an important role in directing the immune system. In the next paragraphs, factors related to hygiene and the development of allergic disease will be discussed.

2.4.2.1. Infections

Exposure to siblings and daycare attendance only indirectly reflects the infection load to which a child is exposed. More direct evidence for the inverse association between number of infections and the development of allergic disease was provided by the German MAS-study [21]. In this prospective study, Illi et al. found that the number of viral infections acquired in the first 3 years of life was inversely related to doctor's diagnosed asthma and increased bronchial hyperresponsiveness at age 7. The strongest effects on asthma and increased bronchial hyperresponsiveness were seen for episodes of runny nose and viral infections of the herpes type acquired in the first year of life. A large variety of viral and bacterial infections have been associated with the development of atopic sensitization and allergic disease. Some microorganisms have been associated with the induction of allergic disease, while others have been associated with protection.

RSV:

Respiratory Syncytial Virus (RSV) is a common cause of lower respiratory tract infection and bronchiolitis in infancy [233]. Case-control studies have shown that hospitalization for RSV-bronchiolitis in infancy is associated with recurrent wheezing in pre-school children, often extending until school age [234-236]. However, in a large prospective birth cohort study performed in Tucson, USA, the positive association between non-hospitalized lower respiratory tract illness induced by RSV and recurrent wheezing became statistically insignificant after the age of 11 [237]. The authors suggested that RSV-induced lower respiratory tract illness is associated with transient wheezing due to congenital or acquired dysfunction in the regulation of airway caliber, rather than with asthma. RSV-bronchiolitis has also been associated with various expressions of the allergic phenotype, such as eosinophilia, increased levels of total IgE and ECP, and the development of atopic sensitization. But conflicting data exists [235, 237-239]. A large number of immunological studies have provided conflicting data about the effect of RSV-infection on T-cell function. In some studies, a Th2-dominated response was found during RSV-bronchiolitis, illustrated by increased IL-4/IFN-γ and IL-10/IFN-γ ratio's in stimulated PBMC's [240, 241]. In addition, high production of IL-10 and low production of IFN-γ during RSV-bronchiolitis was predictive for the development of recurrent wheeze after 1-2 year follow-up [145, 242]. In contrast, others found increased production of IFN-γ and decreased IL-4/IFN-γ ratio's during RSV-bronchiolitis in PBMC's and nasopharyngeal secretions of infants [238, 243, 244]. Except for the Tucson Study [237], all studies on RSV-infection and the development of allergic disease have been performed in selected populations. Therefore, no definite conclusions can be made on the role of RSV infection in the development of allergic disease in the general population. Prospective population-based studies are needed to address this issue. However, as RSV infects up to 95% of all children within the first 2 years of life, a causal role for RSV in asthma etiology seems unlikely.

Measles:

In a study in Guinea-Bissau, Shaheen et al. found that children who had a measles infection in early life had approximately one-third the rate of atopy later in life (defined as a positive skin-prick test for inhalant allergens), compared with vaccinated children who did not had a measles infection [245]. However, a recent study by Paunio et al. reported that Finnish children who had a measles infection had a higher frequency of allergic disease than children who did not had a measles infection [246].

Pertussis:

Immunization with acellular and whole-cell Pertussis vaccine was reported to be associated with an increased risk of atopic disease and asthma in a prospective study and a cross-sectional study [247, 248]. However, these findings were not confirmed in a randomized, placebo-controlled trial on the effect of pertussis vaccine on atopic disease [249].

Tuberculosis:

In a study among Japanese schoolchildren, an inverse relation was found between delayed hypersensitivity to Mycobacterium tuberculosis and total IgE levels, atopy, asthma, atopic dermatitis and allergic rhinitis [250]. Interestingly, children with positive tuberculin responses at age 6 and 12 years had higher serum IFN-γ levels and lower IL-4, IL-13 and IL-10 levels at age 12 than children with negative tuberculin responses. The authors considered a positive tuberculin response to be suggestive of a past infection with Mycobacterium tuberculosis, and concluded that infection was protective for the development of allergic disease. The findings of Shirakawa et al. were supported by a study in Guinea-Bissau that reported an inverse relation between early BCG vaccination and the development of atopy in later childhood [251]. These findings might partly explain the rising trends of allergic disease in Western countries, because the prevalence of infectious diseases, including tuberculosis, has decreased substantially in the last decades [252]. However, several other studies did not find a protective effect of BCG vaccination on the development of atopy and asthma [253, 254]. A tuberculin reaction is a classic example of a cell-mediated immune response (Th1-response). Therefore, an alternative explanation for the inverse relation between tuberculin response and atopy is that atopic children have difficulties to mount an appropriate cell-mediated immune response [255].

Parasites:

Countries with a high prevalence of allergic disease are more likely to be Western, urban and industrialized, with low parasite infestation rates, whereas countries with a low prevalence of allergic disease are more likely to be rural, non-Western and with high rates of parasitic infections [256]. Therefore it is tempting to hypothesize that parasitic infestation is protective for the development of allergic disease. In a study in Gabon, van den Biggelaar et al. found that children who were infested with the helminthes Schistosoma heamatobium were less likely to be skin-test positive to HDM-allergen [257]. The protective effect of schistosomiasis seemed to be mediated through an immunosuppressive effect of parasite-induced IL-10, because IL-10 was almost undetectable in the skin-test positive children, and increased in the skin-test negative children. It is unclear how these findings on parasitic infestations fit into the Th1/Th2 hypothesis, because parasites induce a strong Th2 response, characterized by increased total IgE production [258].

2.4.2.2. Endotoxin

Several cross-sectional studies in schoolchildren have shown that children who grow up on a farm have less atopy, hay fever, wheezing and asthma [259-262]. The children who lived on farms containing livestock had the lowest prevalence of allergic disease [261, 262]. Because farming animals are a rich source of endotoxin, which is an intrinsic part of the outer membrane of Gram-negative bacteria, it has been suggested that exposure to endotoxin protects against the development of atopy and allergic disease [263]. After binding with the endotoxin-receptor CD14 on dendritic cells, endotoxin strongly stimulates the production of IL-12, resulting in upregulation of IFN-γ [255]. Gereda et al. recently reported that infants with recurrent wheeze who were exposed to high levels of house-dust endotoxin were less likely to have a positive skin test against inhalant allergens than infants who were exposed to low levels [264]. In addition, increased house-dust endotoxin concentrations correlated with an increased proportions of IFN-γ producing T-helper cells [264]. The role of endotoxin exposure in the development of recurrent wheezing in early childhood is more complex. Park et al. found that house-dust endotoxin exposure was positively associated with recurrent wheeze in infancy [265]. However, preliminary results from a prospective study of the same research group showed that endotoxin exposure in early life was associated with a decreased prevalence of recurrent wheeze after the age of 6 [266]. Hence, exposure to endotoxin potentially stimulates polarization towards a Th1 phenotype and some data are available that link exposure with protection against the development of allergic disease.

2.4.2.3. Gut flora

In a cross-sectional study in Sweden, Alm et al. found that the prevalence of atopy and allergic disease was significantly lower in children from anthroposophic families than in children from other families [267]. It is unclear which exact mechanisms underlie this protective effect of an anthroposophic lifestyle against the development of allergic disease, but potential candidates are differences in vaccination strategies and/or differences in intestinal microflora [267]. An anthroposophic lifestyle can influence the intestinal microflora in two different ways: 1) a high consumption of fermented vegetables and biodynamic food components; 2) a low consumption of antibiotics [267]. Other studies have found a positive association between the use of antibiotics in early life and the development of allergic disease in later life, especially in children who have an allergic parent [22, 248, 268]. However, the aforementioned studies all have a cross-sectional design and rely on retrospectively collected data on antibiotic use in early life. Therefore, recall bias can not be excluded. In the German MAS study, which is a prospective birth cohort study, no association was found between early antibiotic use and current wheeze, bronchial hyperreactivity and doctor diagnosed asthma at age 7 [21]. Therefore we believe that there is insufficient data to support a causal relation between early use of antibiotics and the development of allergic disease in childhood. Matricardi et al. found an inverse relation between positive serology against hepatitis A and the development of atopy in Italian military cadets aged 17-24 [269] and this finding was later extended to orofecal and foodborne microbes [270]. The authors hypothesized that hygiene and westernized, semi-sterile diet may facilitate atopy by influencing the overall pattern of commensals and pathogens that stimulate the gut associated lymphoid tissue. Various studies in Swedish and Estonian children by the group of Björksten provided more evidence for a role of the intestinal microflora on the development of allergic disease [271-273]. They found that Lactobacilli and Eubacteria were more frequently found in the feces of infants from Estonia (were the

prevalence of atopy and allergic disease is low) whereas Clostridia was more frequent in Swedish infants, where the prevalence of atopy and allergic disease is higher [273]. In addition, Estonian and Swedish infants with allergic disease were less often colonized with Lactobacilli than non-allergic infants [272]. Finally, they found differences in short chain fatty acid composition, which is an indicator of colon microflora composition, in allergic and non-allergic 13-month old children [271]. Together, these findings suggest that changes in human gut flora due to 'Western lifestyle' may be related to the increase in prevalence of allergic disease [274].

2.4.3. Ethnical and socioeconomic factors

The prevalence of atopy and allergic disease differs between ethnical groups. For instance, many studies in the USA and UK have shown that African-American and Hispanic children more often suffer from recurrent wheezing, persistent wheezing, doctor diagnosed asthma and hay-fever than Caucasian children [18, 275-279]. In addition, age of onset of asthma was lower in African-American children compared to Caucasian children [280]. The prevalence of lower respiratory illness and the incidence of RSV-bronchiolitis in early childhood were also found to be higher in Hispanic and African-American children [219, 281, 282]. The ethnical variations in the prevalence of allergic disease in continental Europe seem to show a different pattern compared to studies in the USA and UK. Cross-sectional studies in Europe found that the prevalence of atopy, atopic dermatitis, allergic rhinitis, wheezing, asthma and increased bronchial hyper-responsiveness was lower in children from Turkish immigrants than in children from German, Swedish and Dutch parents [35, 283, 284]. However, children from Chilean immigrants had a higher prevalence of asthma and allergic rhinitis than children from Swedish parents [284]. In another study in Sweden, infants from Southern European immigrants were more likely to be hospitalized for a lower respiratory tract infection [285]. The reasons for ethnical differences in the prevalence of allergic disease and respiratory tract infections are poorly understood. Genetic factors might explain ethnical differences in the prevalence of allergic disease [284]. However, some studies suggest that lifestyle factors play a more important role. Studies among Asian immigrants in Australia and Sweden have demonstrated that the low risk of atopy and allergic disease described in Asia is replaced by a high risk when Asians live in a Western environment [286, 287]. These findings seem to fit a pattern where populations with a higher standard of living and a more Western lifestyle generally have higher rates of atopy and allergic disease [288]. Studies in the USA and UK have shown that the higher prevalence of allergic disease among non-Caucasian children could be largely explained by poverty and low socioeconomic status, which is more frequently found in African-American and Hispanic families [278, 289]. These factors directly and indirectly influence factors such as diet, exposure to indoor allergens (cockroach), access to health care and lack of physical activity. Differences in socioeconomic status can not explain the lower prevalence of allergic disease that was found in Turkish children living in Western Europe, because socioeconomic status was significantly lower in the Turkish families compared to the German, Swedish and Dutch families [35, 283, 284].

2.4.4. Other risk factors for allergic disease

In table 2.1, a variety of known risk factors for the development of allergic disease are summarized. All these factors are considered to be involved in the development of allergic disease, but some of these factors might also be important in sustaining chronic inflammation.

Table 2.1. Other factors associated with the development of allergic disease

Risk factor	Effect/impact	References
Perinatal events		
Uterus related complications	Asthma ↑, recurrent wheeze↑	[290]
Fetal under-nutrition	Total IgE ↑, asthma ↑, AR ↓	[291-293]
Prematurity	Asthma ↑, recurrent wheezing ↑	[294-296]
Low birth weight	Asthma ↑, AR ↓	[293]
High gestational age	AD ↑	[297]
Birth/maternal characteristics		
Low parity	AD ↑	[297]
Low maternal age	Wheezing ↑, asthma ↑, AR ↑	[290, 293, 298]
Male gender	Wheezing ↑, asthma 0-14 yr ↑, asthma > 15 yr ↓	[9, 18, 152, 221, 299-301]
Feeding		
Breast feeding	Wheezing ↓, lower respiratory tract infection ↓, respiratory illness ↓, RSV-bronchiolitis ↓, asthma ↓ / ~, AD ↓, total IgE ↑	[234, 302-311]
Early introduction of cow's milk	Allergic disease ↑ / ~	[312-314]
Early introduction of solid food	AD ↑ / ~	[315, 316]
Processed foods, omega-6 fatty acids, salt, anti-oxidant vitamins	Allergic disease ↑	[29, 317]
Indoor environment other than HDM-exposure		
Pet allergens exposure	Atopic sensitization ↑ / ↓, asthma ↑ / ↓	[188, 198, 200, 217, 318-320]
Cockroach exposure	Atopic sensitization ↑, wheezing ↑	[219, 321, 322]
Residential dampness	Recurrent wheezing ↑	[221]
Mould spots in the home	Wheezing ↑	[323]
Evaporative cooling	Wheezing ↑	[324]
Nitrogen dioxide exposure	Respiratory illness ↑ / ~	[325, 326]
Environmental tobacco smoke exposure (ETS)	Lower respiratory illness ↑, lower respiratory tract infection ↑, wheezing ↑, cord blood IgE ↑ / ~	[327-337]

↑ = increased risk, ↓ = decreased risk, ~ = risk not altered, AD = atopic dermatitis, AR = allergic rhinitis

2.5. EARLY MARKERS OF ALLERGIC DISEASE

It is difficult to diagnose allergic disease in pre-school children. It is even more difficult to predict which pre-school child with respiratory or skin symptoms will eventually develop allergic disease. These diagnostic and predictive problems can potentially result in both over- and under-treatment of the underlying disorders. Therefore, early markers are needed which can contribute to the diagnosis and prediction of the development of allergic disease in early childhood. A major problem in the validation of early markers in relation to allergic disease in childhood is the lack of a golden diagnostic standard. In adult asthma for instance, it is feasible to use bronchial biopsies as the golden standard. Such invasive methods are unacceptable in pediatric patients [338, 339]. Therefore, in practice validation will be performed by com-

paring the values of the marker in children with and without symptoms of allergic disease. Ideally, a marker has the following properties:
It is easy and cheap to measure.
It is increased in children who have/develop allergic disease (sensitive) and not increased in children who do not have/develop allergic disease (specific).
In the next paragraph, various potential markers for the development of allergic disease that can be relatively easily measured in blood will be discussed.

Cytokines and cytokine receptors in serum/plasma
Various cytokines can be measured in serum or (preferential) in plasma [144]. However, surprisingly few studies have related serum- or plasma cytokine levels in early childhood to respiratory and skin symptoms and the development of allergic disease. Serum levels of IL-4 were found to be increased in children with atopic dermatitis and asthma as compared to non-allergic controls [340]. There are some studies available on soluble IL-2 receptor (sIL-2R), which is considered to be a marker of T-cell activation [341]. Levels of sIL-2R have been found to be increased in children aged 1-5 with atopic dermatitis [341] and in children aged 3-12 year with asthma or asthma-exacerbation's [340-342], as compared to normal controls. SIL-2R was found to be a rather good predictor of the persistence of wheeze in children who started wheezing between 3 months and 3 years of age [343]. However, this finding was not confirmed in a large unselected cohort study [344]. Serum levels of IL-4 were found to be increased in children with atopic dermatitis and asthma as compared to non-allergic controls [340].

Cord blood IgE
Some early reports suggested that cord blood total IgE is a good predictor for the development of allergic disease [345-349]. However, various more recent studies did not find any correlation between cord blood IgE levels and allergic disease, or found a rather low positive predictive value [350-358]. It is now generally accepted that cord blood IgE is a poor predictor for the development of allergic disease in childhood [359].

Total- and specific IgE and skin-tests in pre-school children
Infants with atopic dermatitis have higher levels of total IgE and are more often sensitized to cow's milk and egg at age 6 months to 1 year than children without atopic dermatitis [151, 360, 361]. One study suggested that specific IgE against food-allergens precedes the clinical expression atopic dermatitis in infancy [362], although another study could not confirm this [360]. Both studies however concluded that the clinical usefulness of the test was rather low because of the low sensitivity [360, 362]. Most studies did not find an association between total and specific IgE and the prevalence of wheezing in the first 2 years of life [151, 361, 363, 364]. An exception is the Isle of Wight study, in which a positive skin prick test at age 1 was associated with the development of asthma-like symptoms in infancy [300]. Various prospective studies have shown that the positive association between specific IgE against common allergens and wheezing or asthma-like symptoms becomes apparent starting from age 3-4 years [200, 361]. So it seems that total and specific IgE in pre-school children is moderately related to atopic dermatitis and weakly or unrelated to wheezing.

Eosinophil counts and serum ECP
Eosinophil counts in peripheral blood are usually not elevated in infants with atopic dermatitis or wheezing [360, 365]. However, one study found that infants who developed allergic disease in the first 18 months of life (mainly atopic dermatitis) had higher eosinophil counts at 3 months of age compared to children who did not develop allergic disease [366]. Eosinophil Cationic Protein (ECP), an eosinophil degranulation product that can be measured in serum as sECP, is thought to reflect eosinophilic activation [64, 66]. Serum ECP was found to be elevated in children aged 0-2 with recurrent wheezing and in school children with asthma [66, 367]. In addition, in one prospective study, sECP in wheezing infants was found to be predictive for the development of asthma after 1 year follow-up [368]. However, this finding was not confirmed by another study on the predictive value of sECP for the development of asthma [365]. In conclusion, the predictive value of sECP in early childhood for the development of allergic disease is still controversial [338].

Adhesion molecules
Limited data are available on adhesion molecules as a marker of allergic disease in childhood. Some studies indicate that levels of sE-selectin, sICAM and sVCAM are elevated in infants with atopic dermatitis and schoolchildren with asthma [52, 369]. However, in another study, similar levels of sE-selectin, sICAM and sVCAM were found in pre-school children with stable asthma, atopic dermatitis and normal controls [58]. In the same study it was found that sE-selectin in early infancy did not predict the development of allergic disease after 2 year follow-up [360].

In conclusion, no currently available blood markers have been shown to be sensitive and specific enough to either diagnose or predict the development of allergic disease in early childhood. Further studies are needed to evaluate the diagnostic and predictive value of a variety of markers, such as serum/plasma cytokines, chemokines and adhesion molecules. Non-invasive methods have recently been developed to investigate airway inflammation, including measurement of Nitric Oxide in exhaled air (eNO) [370]. This marker promises to be valuable in the diagnosis and monitoring of asthma in childhood [338]. Studies in pre-school children about the predictive value of eNO for the development of asthma in later childhood will be available in the near future.

2.6. PREVENTION OF ALLERGIC DISEASE

In view of the increasing prevalence of allergic disease and accumulating health care and community costs, an important goal of governments, physicians and patients is primary prevention of allergic diseases [371]. In addition, a prevention study is considered to be the most conclusive study design to proof causality between exposure and disease [105]. Therefore, a variety of allergen avoidance studies have been performed aiming at reducing the development of allergic disease. These studies on food-allergen avoidance, combined food- and indoor allergen avoidance, indoor allergen avoidance and probiotics will be discussed in the next paragraph.

Table 2.2. Food-allergen avoidance studies

Reference	Subjects	Maternal diet	Maternal lactation diet	Infant diet	Follow up	Effect
Fälth-Magnusson [372, 373]	212, high risk	Active: no CM and egg. Control: no restrictions	No restrictions	No restrictions	5 year	No effect on allergic disease and sensitization
Zeiger [374-376]	288, high risk	Active: no CM, egg, peanut, soy. Control: no restrictions	Active: no CM, egg, peanut, soy. Control: no restrictions	Active: hypo-allergen milk if no BF, SF > 6 mo. Control: CM if no BF, SF > 4 mo.	7 year	Age 2: active group less any allergic disorder, less skin + food-allergy. Age 4 and age 7: no effect.
Hattevig [377]	115, high risk	No restrictions	Active: no CM, eggs and fish. Control: no restrictions	No restrictions	18 mo.	18 mo.: active group less AD
Halken [378, 379]	105, high risk	No restrictions	No restrictions	Active: hypo-allergen milk if no BF. Control: CM if no BF.	18 mo.	18 mo.: active group less any allergic disorder, recurrent wheeze, AD, vomiting, food-allergy
Miskelly [380]	487, high risk	No restrictions	No restrictions	Active: soy milk if no BF. Control: CM if no BF	1 year	No effect on allergic disease
Kjellman [27]	51, high risk	No restrictions	No restrictions	Active: soy milk if no BF. Control: CM if no BF	4 year	No effect on allergic disease
Oldaeus [28]	155, high risk	No restrictions	No restrictions	Group 1: CM, group 2: low-allergen milk, group 3: very low allergen milk, group 4: BF	18 mo.	18 mo.: in group 3 less any allergic disorder, AD and sensitization to egg than in group 1, 2 and 4.
Chandra [26, 381]	263, high risk	No restrictions	No restrictions	Group 1: CM, group 2: soy milk, group 3: low-allergen milk, group 4: BF	18 mo.	18 mo.: in group 3 and 4 less AD and atopic sensitization than in group 1 and 2
Marini [382]	359, high risk	No restrictions	Group 1 and 2: reduction of CM consumption, no egg. Group 3: no restrictions	Group 1: BF or hypo-allergen milk, group 2: BF or CM, group 3: no intervention	3 years	0-3 years: in group 1 and 2 less any allergic disorder and AD than in group 3. In group 1 less AD than in group 2.
Lucas [312]	777, pre-term infants	No restrictions	No restrictions	Group 1: human milk, group 2: pre term formula, group 3: term formula	18 mo.	No effect on allergic disease
Kajosaari [383]	135, high risk	No restrictions	No restrictions	Active: SF > 6 mo, Control: SF > 3 mo	5 year	Age 1: active group less AD and food-allergy. Age 5: active group less hay fever. No other differences

CM = cow's milk, BF = breast feeding, SF = solid food, AD = atopic dermatitis

Food-allergen avoidance

In the last decades, various studies aiming at the prevention of allergic disease have been performed. The majority of these studies focussed on prenatal or postnatal avoidance of food allergens in children with at least one allergic parent (high risk). These studies are summarized in table 2.2. Many methodological problems exist, including difficulties in study design (double blind, placebo-controlled, the impossibility to exclude breastfeeding), parent's compliance and problems with defining the endpoints. However, it is reasonable to conclude that a maternal hypo-allergenic diet during pregnancy and infant feeding with soy-milk does not prevent the development of allergic disease in early childhood [384, 385]. In addition, prolonged avoidance of food-allergens by the lactating mother or by feeding the infant with hypo-allergenic milk has resulted in a delay in the manifestation of atopic dermatitis and food-allergy, but has little or no effect on asthma development [386].

Combined food- and indoor allergen avoidance

Two studies with a rather similar combined food and indoor allergen avoidance program have been published: the Isle of Wight study and a study performed in Winnipeg and Vancouver, Canada [387-390]. In table 2.3, the results of these studies are summarized. The Canadian study has only published data about the effect of avoidance measures on symptoms in the first year of life, showing small effects on recurrent wheeze/cough and rhinitis without a cold [390]. In the Isle of Wight study, the initial effect of the intervention on respiratory symptoms disappeared after age 1 [388, 389]. The effect of the intervention on atopic dermatitis and allergic sensitization was still seen at age 4, although the differences between the active group and the control group became smaller with increasing age [389].

Table 2.3. Combined food- and indoor allergen avoidance studies

Study	Subjects	Intervention	Effect on allergens and clinical symptoms
Isle of Wight [387-389]	120, high risk	Active (n = 58): maternal diet pregnancy/lactation: no eggs, fish and nuts. Infant diet: BF or hypo-allergenic milk, no CM before 9 mo. Allergen avoidance: polyvinyl covered mattresses, Acarosan anti-HDM treatment of the home Control (n = 62): usual care, no information on avoidance measures	Significantly lower level of HDM-allergen in active group than control group at 9 mo. after birth. Age 1: less any allergic disease, recurrent wheeze and cough (asthma) and AD in active group. Age 2: less any allergic disease, allergic rhinitis, and atopy in active group, no difference in asthma Age 4: less atopy, any allergic disease and AD in active group, no effect on allergic rhinitis and asthma
Winnipeg/ Vancouver [390]	545, high risk	Active (n = 279): maternal diet pregnancy/lactation: no nuts, fish. Infant diet: BF or hypo-allergenic milk, SF > 6 mo., no CM, fish. Allergen avoidance: mattress encasings, Acarosan-treatment, pet avoidance. Other interventions: smoking cessation counseling, avoid day care Control (n = 266): usual care, no information on avoidance measures	Significantly lower level of HDM-allergen in active group than in control group at age 1. No effect on cat-allergens. Age 1: less wheeze or cough and rhinitis without colds in active group. Effect on AD unknown.

BF = breast feeding, CM = cow's milk, SF = solid food

Indoor allergen avoidance

Several primary prevention studies, specifically aiming on reducing exposure to HDM-allergen, have been started in Southampton, Manchester, Canada and Australia [200]. Recently published data from the Manchester Asthma and Allergy Study (MAAS) show that rigorous avoidance measures including mattress encasings, smooth floor covering, high filtration vacuum cleaning, and application of benzyl benzoate (Acarosan) can achieve and maintain very low HDM-allergen exposition in infancy [391]. In the same study, the intervention did not result in a lower prevalence of wheezing, night cough, apart from colds, eczema and sensitization against HDM-allergen in the first year of life compared to the control group [392]. However, a significantly lower prevalence of attacks of severe wheeze with shortness of breath (6% versus 14%) and prescription of medication for wheezy attacks (27% versus 16%) was seen in the active group compared to the control group [392]. There is one published report on the effect of HDM-allergen avoidance in infants who already developed atopic dermatitis but were not sensitized to HDM-allergen (secondary prevention) [393]. In this study in Japan, infants who received mattress encasings impermeable for HDM-allergen (active group) were compared with infants receiving placebo encasings. In the active group, the 1-year incidence of sensitization against HDM-allergen was 31% compared to 63% in the placebo group. Recurrent wheezing was observed in 11% of the infants in the active group and in 37% of the placebo group. Many studies have shown a positive effect of HDM-allergen avoidance on clinical symptoms in HDM allergic patients with established allergic disease (tertiary prevention), but this goes beyond the topic of this thesis.

Probiotics

Probiotics are cultures of bacteria of healthy gut flora, which can potentially stimulate Th1 development and antagonize Th2 development [394, 395]. The only published study on probiotics as a primary prevention strategy for the development of allergic disease showed promising results [394]. In a randomized placebo-controlled trial in Finland, the probiotic Lactobacillus GG was given to women in the last 2-4 weeks of pregnancy, and after delivery to the lactating mother or orally to the child if it was not breastfed. The cumulative 2-years incidence of atopic dermatitis was 23% in the group receiving Lactobacillus, and 46% in the placebo group. However, no differences in total IgE, specific IgE and skin test reactions were seen between the 2 groups and no data were reported on the prevalence of wheezing [394].

In conclusion, a variety of intervention trials have been designed to investigate the effect of a reduction of exposure to food-allergens, aeroallergens and a combination of these allergens on the development of allergic disease in childhood. Preliminary results indicate that avoidance of allergenic food, such as cow's milk and hen's egg, might delay the clinical expression or protect against the development of atopic dermatitis, but it is ineffective in preventing asthma. Combined food- and indoor allergen avoidance studies indicate that this strategy might be effective in preventing the development of atopic dermatitis and sensitization against house dust mite allergens, but the effect on asthma development is less clear. The various studies on reducing early exposure to house dust mite allergen have not published data on clinical symptoms yet, so this issue remains unresolved.

2.7. CONCLUDING REMARKS

In the previous chapter, an overview was given of the currently available literature on some important factors involved in the development of allergic disease in early childhood, with special emphasis on allergen exposure and exposure to micro-organisms. It is clear that the etiology of allergic disease is multi-factorial, with both genetic and environmental factors playing an important role. The observations of various repetitive cross-sectional studies that the prevalence of allergic disease has increased dramatically in the last few decades can not be explained by genetic factors and therefore points towards the importance of environmental factors for the development of allergic disease. The basic immunological mechanisms underlying allergic disease are complex, but studies in the last decade have provided much insight. From a pathophysiological perspective, allergic diseases might be considered as a dysregulation in the immune response, resulting in the production of allergen specific IgE, recruitment of inflammatory cells such as eosinophils, basophils and mast cells and the production and release of inflammatory mediators in the affected tissue. The regulation of this inflammatory process is only partly understood, but the development of T-cell polarization and the production of cytokines and chemokines by a variety of inflammatory cells, including T-cells (Th0, Th1, Th2, Tr, cytotoxic T cells), NK-cells, mast cells, eosinophils, basophils, macrophages and dendritic cells are of critical importance. A central question is which environmental factors are involved in the dysregulation of the immune response towards allergy. In the past decades, much emphasis was put on the role of exposure to indoor allergens, especially HDM-allergens, in the development of allergic disease. This has resulted in designing a variety of intervention studies aiming at reducing HDM-allergen exposure. More recently, attention has shifted from the role of early allergen exposure towards the role of decreased microbial stimulation of the immune system in explaining the development of allergic disease. Together with the increased awareness that allergic disease is more prevalent in societies adapted to a Western lifestyle, this has resulted in the proposition of the so called 'hygiene hypothesis'. This hypothesis has now been accepted as an important etiological framework to explain the development of allergic disease. However, some very important questions concerning the development of allergic disease are still unresolved. In this thesis, we have studied basic immunological factors that are relevant for the development of allergic disease in early childhood. In addition, we investigated risk factors for the development of allergic disease.

References:

1. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. J Allergy Clin Immunol 2001:107(3):429-40.
2. Holgate ST. The epidemic of allergy and asthma. Nature 1999:402(6760 Suppl):B2-4.
3. Corry DB, Kheradmand F. Induction and regulation of the IgE response. Nature 1999:402(6760 Suppl):B18-23.
4. Sheffer AL. The National Asthma Education Program attacks asthma. J Allergy Clin Immunol 1991:87(2):468-9.
5. Busse WW, Lemanske RF, Jr. Asthma. N Engl J Med 2001:344(5):350-62.
6. Sears MR. Descriptive epidemiology of asthma. Lancet 1997:350(Suppl 2):SII1-4.
7. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Dermatovener 1980:Suppl. 92:44-47.
8. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993:186(1):23-31.

9. Allergy, Asthma, and Immunology from Infancy to Adulthood. Philadelphia, USA: W.B. Saunders Company; 1996.
10. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. J Allergy Clin Immunol 1999:103(6):1173-9.
11. Rowntree S, Cogswell JJ, Platts-Mills TA, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. Arch Dis Child 1985:60(8):727-35.
12. Kjellman NI. Natural course of asthma and allergy in childhood. Pediatr Allergy Immunol 1994:5(6 Suppl):13-8.
13. Gergen PJ, Turkeltaub PC, Kramer RA. Age of onset in childhood asthma: data from a national cohort. Ann Allergy 1992:68(6):507-14.
14. Yunginger JW, Reed CE, O'Connell EJ, Melton LJd, O'Fallon WM, Silverstein MD. A community-based study of the epidemiology of asthma. Incidence rates, 1964-1983. Am Rev Respir Dis 1992:146(4):888-94.
15. Martinez FD. Recognizing early asthma. Allergy 1999:54(Suppl 49):24-8.
16. Silverman M, Wilson N. Wheezing phenotypes in childhood. Thorax 1997:52(11):936-7.
17. Martinez FD, Helms PJ. Types of asthma and wheezing. Eur Respir J Suppl 1998:27:3s-8s.
18. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995:332:133-8.
19. Bonifazi E, Meneghini CL. Atopic dermatitis in the first six months of life. Acta Derm Venereol Suppl (Stockh) 1989:144:20-2.
20. Rudikoff D, Lebwohl M. Atopic dermatitis. Lancet 1998:351(9117):1715-21.
21. Illi S, von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. Bmj 2001:322(7283):390-5.
22. von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. Eur Respir J 1999:14(1):4-11.
23. Louhiala PJ, Jaakkola N, Ruotsalainen R, Jaakkola JJ. Form of day care and respiratory infections among Finnish children. Am J Public Health 1995:85(8 Pt 1):1109-12.
24. Silverman M. Clinical diagnosis and assesment in infants. Philidelphia: Lipincott-Raven; 1997.
25. Kulig M, Klettke U, Wahn V, Forster J, Bauer CP, Wahn U. Development of seasonal allergic rhinitis during the first 7 years of life. J Allergy Clin Immunol 2000:106(5):832-9.
26. Chandra RK, Singh G, Shridhara B. Effect of feeding whey hydrolysate, soy and conventional cow milk formulas on incidence of atopic disease in high risk infants. Ann Allergy 1989:63(2):102-6.
27. Kjellman NI, Johansson SG. Soy versus cow's milk in infants with a biparental history of atopic disease: development of atopic disease and immunoglobulins from birth to 4 years of age. Clin Allergy 1979:9(4):347-58.
28. Oldaeus G, Anjou K, Bjorksten B, Moran JR, Kjellman NI. Extensively and partially hydrolysed infant formulas for allergy prophylaxis. Arch Dis Child 1997:77(1):4-10.
29. Peat JK. Prevention of asthma. Eur Respir J 1996:9(7):1545-55.
30. Schafer T, Ring J. Epidemiology of allergic diseases. Allergy 1997:52(38 Suppl):14-22; discussion 35-6.
31. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995:8(3):483-91.
32. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998:12(2):315-35.
33. Anonymous. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998:351(9111):1225-32.
34. Wright AL, Holberg CJ, Martinez FD, Halonen M, Morgan W, Taussig LM. Epidemiology of physician-diagnosed allergic rhinitis in childhood. Pediatrics 1994:94(6 Pt 1):895-901.
35. van der Wal MF RB. Asthmatic symptoms among Dutch and non-Dutch 2-11 year old children in Amsterdam. Tijdschr Soc Gezondheidsz 1995:73:42-50.
36. Janssen NAH, Zock JP, Brunekreef B, de Groot BJA, Rijcken B. Prevalence of respiratory symptoms in Dutch schoolchildren. Tijdsch Soc Gezondheidsz 1994:72:3-8.
37. Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart [published erratum appears in BMJ 1992 May 2;304(6835):1157]. Bmj 1992:304(6831):873-5.

38. Burr ML, Butland BK, King S, Vaughan-Williams E. Changes in asthma prevalence: two surveys 15 years apart. Arch Dis Child 1989:64(10):1452-6.
39. Robertson CF, Heycock E, Bishop J, Nolan T, Olinsky A, Phelan PD. Prevalence of asthma in Melbourne schoolchildren: changes over 26 years. Bmj 1991:302(6785):1116-8.
40. Peat JK, van den Berg RH, Green WF, Mellis CM, Leeder SR, Woolcock AJ. Changing prevalence of asthma in Australian children. Bmj 1994:308(6944):1591-6.
41. Burney PG, Chinn S, Rona RJ. Has the prevalence of asthma increased in children? Evidence from the national study of health and growth 1973-86. Bmj 1990:300(6735):1306-10.
42. Wever-Hess J, Wever AM, Yntema JL. Mortality and morbidity from respiratory diseases in childhood in The Netherlands, 1980-1987. Eur Respir J 1991:4(4):429-33.
43. Mitchell EA. International trends in hospital admission rates for asthma. Arch Dis Child 1985:60(4):376-8.
44. Evens R, Gergen PJ. Allergy, asthma, and Immunology from infancy to adulthood. Philidelphia, USA: W.B. Saunders Company; 1996.
45. von Mutius E. The rising trends in asthma and allergic disease. Clin Exp Allergy 1998:28(Suppl 5):45-9; discussion 50-1.
46. Kay AB. Allergy and allergic diseases. First of two parts. N Engl J Med 2001:344(1):30-7.
47. Ronchetti R, Villa MP, Barreto M, Rota R, Pagani J, Martella S, et al. Is the increase in childhood asthma coming to an end? Findings from three surveys of schoolchildren in Rome, Italy. Eur Respir J 2001:17(5):881-6.
48. Bochner BS. Cellular adhesion and its antagonism. J Allergy Clin Immunol 1997:100(5):581-5.
49. Chung HL, Hwang JB, Kwon YD, Park MH, Shin WJ, Park JB. Deposition of eosinophil-granule major basic protein and expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in the mucosa of the small intestine in infants with cow's milk- sensitive enteropathy. J Allergy Clin Immunol 1999:103(6):1195-201.
50. Yamashita N, Kaneko S, Kouro O, Furue M, Yamamoto S, Sakane T. Soluble E-selectin as a marker of disease activity in atopic dermatitis. J Allergy Clin Immunol 1997:99(3):410-6.
51. Kobayashi T, Hashimoto S, Imai K, Amemiya E, Yamaguchi M, Yachi A, et al. Elevation of serum soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin levels in bronchial asthma. Clin Exp Immunol 1994:96(1):110-5.
52. El-Sawy IH, Badr-El-Din OM, El-Azzouni OE, Motawae HA. Soluble intercellular adhesion molecule-1 in sera of children with bronchial asthma exacerbation. Int Arch Allergy Immunol 1999:119(2):126-32.
53. Oh JW, Shin JC, Jang SJ, Lee HB. Expression of ICAM-1 on conjunctival epithelium and ECP in tears and serum from children with allergic conjunctivitis. Ann Allergy Asthma Immunol 1999:82(6):579-85.
54. Huang JL, Lee WY, Chen LC, Kuo ML, Hsieh KH. Changes of serum levels of interleukin-2, intercellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1 and Th1 and Th2 cell in severe atopic dermatitis after intravenous immunoglobulin therapy. Ann Allergy Asthma Immunol 2000:84(3):345-52.
55. Koizumi A, Hashimoto S, Kobayashi T, Imai K, Yachi A, Horie T. Elevation of serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels in bronchial asthma. Clin Exp Immunol 1995:101(3):468-73.
56. Stanciu LA, Djukanovic R. The role of ICAM-1 on T-cells in the pathogenesis of asthma. Eur Respir J 1998:11(4):949-57.
57. Riise GC, Larsson S, Lowhagen O, Andersson BA. Circulating leukocyte adhesion molecules in stable asthma and nonobstructive chronic bronchitis. Allergy 1995:50(8):693-8.
58. Laan MP, Koning H, Baert MR, Oranje AP, Buurman WA, Savelkoul HF, et al. Levels of soluble intercellular adhesion molecule-1, soluble E- selectin, tumor necrosis factor-alpha, and soluble tumor necrosis factor receptor p55 and p75 in atopic children. Allergy 1998:53(1):51-8.
59. Wolkerstorfer A, Laan MP, Savelkoul HF, Neijens HJ, Mulder PG, Oudesluys-Murphy AM, et al. Soluble E-selectin, other markers of inflammation and disease severity in children with atopic dermatitis. Br J Dermatol 1998:138(3):431-5.
60. Holt PG, Macaubas C, Stumbles PA, Sly PD. The role of allergy in the development of asthma. Nature 1999:402(6760 Suppl):B12-7.
61. Cookson W. The alliance of genes and environment in asthma and allergy. Nature 1999:402(6760 Suppl):B5-11.
62. Parronchi P, Brugnolo F, Sampognaro S, Maggi E. Genetic and environmental factors contributing to the onset of allergic disorders. Int Arch Allergy Immunol 2000:121(1):2-9.

63. Biedermann T, Rocken M. Th1/Th2 balance in atopy. Springer Semin Immunopathol 1999:21(3):295-316.
64. Venge P, Bystrom J, Carlson M, Hakansson L, Karawacjzyk M, Peterson C, et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. Clin Exp Allergy 1999:29(9):1172-86.
65. Arshad SH, Nanabhay Y. Early biomarkers of allergic disease in children. Clin Exp Allergy 1999:29(5):576-8.
66. Carlsen KH, Halvorsen R, Pettersen M, Carlsen KC. Inflammation markers and symptom activity in children with bronchial asthma. Influence of atopy and eczema. Pediatr Allergy Immunol 1997:8(3):112-20.
67. Vignola AM, Chanez P, Campbell AM, Souques F, Lebel B, Enander I, et al. Airway inflammation in mild intermittent and in persistent asthma. Am J Respir Crit Care Med 1998:157(2):403-9.
68. Riffo-Vasquez Y, Pitchford S, Spina D. Cytokines in airway inflammation. Int J Biochem Cell Biol 2000:32(8):833-53.
69. McFadden ER, Jr., Gilbert IA. Asthma. N Engl J Med 1992:327(27):1928-37.
70. Bousquet J, Vignola AM, Campbell AM, Michel FB. Pathophysiology of allergic rhinitis. Int Arch Allergy Immunol 1996:110:207-18.
71. Leung DY. Pathogenesis of atopic dermatitis. J Allergy Clin Immunol 1999:104(3 Pt 2):S99-108.
72. Holt PG. Programming for responsiveness to environmental antigens that trigger allergic respiratory disease in adulthood is initiated during the perinatal period. Environ Health Perspect 1998:106(Suppl 3):795-800.
73. Martinez FD. Maturation of immune responses at the beginning of asthma. J Allergy Clin Immunol 1999:103(3 Pt 1):355-61.
74. Kapsenberg ML, Hilkens CM, Wierenga EA, Kalinski P. The paradigm of type 1 and type 2 antigen-presenting cells. Implications for atopic allergy. Clin Exp Allergy 1999:29(Suppl 2):33-6.
75. Sinigaglia F, D'Ambrosio D. Regulation of helper T cell differentiation and recruitment in airway inflammation. Am J Respir Crit Care Med 2000:162(4 Pt 2):S157-60.
76. Charlesworth EN. Atopic eczema and the allergist. Allergy Asthma Proc 1999:20(5):305-10.
77. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986:136(7):2348-57.
78. Del Prete GF, De Carli M, Mastromauro C, Biagiotti R, Macchia D, Falagiani P, et al. Purified protein derivative of Mycobacterium tuberculosis and excretory-secretory antigen(s) of Toxocara canis expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991:88(1):346-50.
79. Fearon DT, Locksley RM. The instructive role of innate immunity in the acquired immune response. Science 1996:272(5258):50-3.
80. Chung KF, Barnes PJ. Cytokines in asthma. Thorax 1999:54(9):825-57.
81. Jansen HM. [Immunology in medical practice. XXIX.Pathogenesis of allergic and immunologic pulmonary diseases]. Ned Tijdschr Geneeskd 2000:144(28):1341-6.
82. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol 2000:105(3):399-408.
83. Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T-cell priming and Th1/Th2- mediated responses. Immunol Today 1998:19(12):568-74.
84. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994:12:227-57.
85. Savelkoul HF. Immune parameters in high-risk atopic individuals during early childhood. Am J Respir Crit Care Med 2000:162(3 Pt 2):S100-4.
86. Koning H, Neijens HJ, Baert MR, Oranje AP, Savelkoul HF. T cell subsets and cytokines in allergic and non-allergic children. I. Analysis of IL-4, IFN-gamma and IL-13 mRNA expression and protein production. Cytokine 1997:9(6):416-26.
87. Laan M. Analysis of T cell differentiation during the development of atopy in children . Rotterdam, The Netherlands: Erasmus University/Academic Hospital Rotterdam; 1999.
88. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy 2000:55(8):688-97.
89. Holt PG, C. M, Nelson D. Primary sensitisation to inhalant allergens during infancy. Pediatr Allergy Immunol 1990:1:3-13.

90. Savelkoul HF, Neijens HJ. Immune responses during allergic sensitization and the development of atopy. Allergy 2000:55(11):989-97.
91. Szepfalusi Z, Loibichler C, Pichler J, Reisenberger K, Ebner C, Urbanek R. Direct evidence for transplacental allergen transfer. Pediatr Res 2000:48(3):404-7.
92. Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. Pediatr Allergy Immunol 1996:7(3):109-16.
93. Warner JA, Jones AC, Miles EA, Warner JO. Prenatal sensitisation. Pediatr Allergy Immunol 1996:7(9 Suppl):98-101.
94. Warner JA, Jones AC, Miles EA, Colwell BM, Warner JO. Prenatal origins of asthma and allergy. Ciba Found Symp 1997:206:220-8; discussion 228-32.
95. Szepfalusi Z, Pichler J, Elsasser S, van Duren K, Ebner C, Bernaschek G, et al. Transplacental priming of the human immune system with environmental allergens can occur early in gestation. J Allergy Clin Immunol 2000:106(3):530-6.
96. Halonen M, Stern DA, Lohman C, Wright AL, Brown MA, Martinez FD. Two subphenotypes of childhood asthma that differ in maternal and paternal influences on asthma risk. Am J Respir Crit Care Med 1999:160(2):564-70.
97. Ruiz RG, Kemeny DM, Price JF. Higher risk of infantile atopic dermatitis from maternal atopy than from paternal atopy. Clin Exp Allergy 1992:22(8):762-6.
98. Rusconi F, Galassi C, Corbo GM, Forastiere F, Biggeri A, Ciccone G, et al. Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group. Am J Respir Crit Care Med 1999:160(5 Pt 1):1617-22.
99. Sears MR, Holdaway MD, Flannery EM, Herbison GP, Silva PA. Parental and neonatal risk factors for atopy, airway hyper-responsiveness, and asthma. Arch Dis Child 1996:75(5):392-8.
100. Dold S, Wjst M, von Mutius E, Reitmeir P, Stiepel E. Genetic risk for asthma, allergic rhinitis, and atopic dermatitis. Arch Dis Child 1992:67(8):1018-22.
101. Tariq SM, Matthews SM, Hakim EA, Stevens M, Arshad SH, Hide DW. The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. J Allergy Clin Immunol 1998:101(5):587-93.
102. Ehrlich RI, Du Toit D, Jordaan E, Zwarenstein M, Potter P, Volmink JA, et al. Risk factors for childhood asthma and wheezing. Importance of maternal and household smoking. Am J Respir Crit Care Med 1996:154(3 Pt 1):681-8.
103. Chan-Yeung M, Ferguson A, Chan H, Dimich-Ward H, Watson W, Manfreda J, et al. Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma. J Allergy Clin Immunol 1999:104(2 Pt 1):317-21.
104. Smillie FI, Elderfield AJ, Patel F, Cain G, Tavenier G, Brutsche M, et al. Lymphoproliferative responses in cord blood and at one year: no evidence for the effect of in utero exposure to dust mite allergens. Clin Exp Allergy 2001:31(8):1194-204.
105. Wahn U, von Mutius E. Childhood risk factors for atopy and the importance of early intervention. J Allergy Clin Immunol 2001:107(4):567-74.
106. Bjorksten B. Risk factors in early childhood for the development of atopic diseases. Allergy 1994:49(6):400-7.
107. Platts-Mills TA, Wheatley LM, Aalberse RC. Indoor versus outdoor allergens in allergic respiratory disease. Curr Opin Immunol 1998:10(6):634-9.
108. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon?. Immunol Today 1993:14(7):353-6.
109. Jones CA, Kilburn SA, Warner JA, Warner JO. Intrauterine environment and fetal allergic sensitization. Clin Exp Allergy 1998:28(6):655-9.
110. Warner JA, Jones AC, Miles EA, Colwell BM, Warner JO. Maternofetal interaction and allergy. Allergy 1996:51(7):447-51.
111. Holt PG, Macaubas C, Prescott SL, Sly PD. Primary sensitization to inhalant allergens. Am J Respir Crit Care Med 2000:162(3 Pt 2):S91-4.
112. Prescott SL, Sly PD, Holt PG. Raised serum IgE associated with reduced responsiveness to DPT vaccination during infancy. Lancet 1998:351(9114):1489.
113. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. Lancet 1999:353(9148):196-200.
114. Holt PG, Macaubas C. Development of long-term tolerance versus sensitisation to environmental allergens during the perinatal period. Curr Opin Immunol 1997:9(6):782-7.

115. Van Der Velden VH, Laan MP, Baert MR, De Waal Malefyt R, Neijens HJ, Savelkoul HF. Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. Clin Exp Allergy 2001:31(7):997-1006.
116. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema?. Clin Exp Allergy 1994:24(5):423-30.
117. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. J Immunol 1998:160(10):4730-7.
118. Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet 1994:344(8928):983-5.
119. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. J Allergy Clin Immunol 1995:96(5 Pt 1):652-60.
120. Lambrecht BN. The dendritic cell in allergic airway diseases: a new player to the game. Clin Exp Allergy 2001:31(2):206-18.
121. Holt PG. Dendritic cell ontogeny as an aetiological factor in respiratory tract diseases in early life. Thorax 2001:56(6):419-20.
122. Tschernig T, Debertin AS, Paulsen F, Kleemann WJ, Pabst R. Dendritic cells in the mucosa of the human trachea are not regularly found in the first year of life. Thorax 2001:56(6):427-31.
123. Kapsenberg ML, Hilkens CM, van Der Pouw Kraan TC, Wierenga EA, Kalinski P. Atopic allergy: a failure of antigen-presenting cells to properly polarize helper T cells? Am J Respir Crit Care Med 2000:162(3 Pt 2):S76-80.
124. Agarwal SK, Marshall GD, Jr. Beta-adrenergic modulation of human type-1/type-2 cytokine balance. J Allergy Clin Immunol 2000:105(1 Pt 1):91-8.
125. Wills-Karp M. Interleukin-12 as a target for modulation of the inflammatory response in asthma. Allergy 1998:53(2):113-9.
126. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. J Allergy Clin Immunol 2001:107(1):9-18.
127. Meyaard L, Hovenkamp E, Otto SA, Miedema F. IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J Immunol 1996:156(8):2776-82.
128. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993:178(3):1041-8.
129. Kapsenberg ML, Hilkens CM, Jansen HM, Bos JD, Snijders A, Wierenga EA. Production and modulation of T-cell cytokines in atopic allergy. Int Arch Allergy Immunol 1996:110(2):107-13.
130. Moverare R, Elfman L, Stalenheim G, Bjornsson E. Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. Allergy 2000:55(2):171-5.
131. Comoy EE, Pestel J, Duez C, Stewart GA, Vendeville C, Fournier C, et al. The house dust mite allergen, Dermatophagoides pteronyssinus, promotes type 2 responses by modulating the balance between IL-4 and IFN-gamma. J Immunol 1998:160(5):2456-62.
132. Shimizu Y, Shichijo M, Hiramatsu K, Takeuchi M, Nagai H, Takagi K. Mite antigen-induced IL-4 and IL-13 production by basophils derived from atopic asthma patients. Clin Exp Allergy 1998:28(4):497-503.
133. Colavita AM, Reinach AJ, Peters SP. Contributing factors to the pathobiology of asthma. The Th1/Th2 paradigm. Clin Chest Med 2000:21(2):263-77, viii.
134. Allen JE, Maizels RM. Th1-Th2: reliable paradigm or dangerous dogma? Immunol Today 1997:18(8):387-92.
135. Borish L, Rosenwasser L. TH1/TH2 lymphocytes: doubt some more. J Allergy Clin Immunol 1997:99(2):161-4.
136. Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 1998:19(8):359-61.
137. Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon-gamma in atopic eczema. Lancet 1994:343(8888):25-6.
138. Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzykowski R, Kim J, et al. Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. J Immunol 1995:154(4):1956-63.

139. Kenyon NJ, Kelly EA, Jarjour NN. Enhanced cytokine generation by peripheral blood mononuclear cells in allergic and asthma subjects. Ann Allergy Asthma Immunol 2000:85(2):115-20.
140. Mosmann TR, Moore KW. The role of IL-10 in crossregulation of TH1 and TH2 responses. Immunol Today 1991:12(3):A49-53.
141. Koulis A, Robinson DS. The anti-inflammatory effects of interleukin-10 in allergic disease. Clin Exp Allergy 2000:30(6):747-50.
142. Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. Clin Exp Allergy 1999:29(9):1164-71.
143. Borish L. IL-10: evolving concepts. J Allergy Clin Immunol 1998:101(3):293-7.
144. Niwa Y, Akamatsu H, Sumi H, Ozaki Y, Abe A. Evidence for degradation of cytokines in the serum of patients with atopic dermatitis by calcium-dependent protease. Arch Dermatol Res 2000:292(8):391-6.
145. Bont L, Heijnen CJ, Kavelaars A, van Aalderen WM, Brus F, Draaisma JT, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. Am J Respir Crit Care Med 2000:161(5):1518-23.
146. Macaubas C, Sly PD, Burton P, Tiller K, Yabuhara A, Holt BJ, et al. Regulation of T-helper cell responses to inhalant allergen during early childhood. Clin Exp Allergy 1999:29(9):1223-31.
147. Holt PG. Potential role of environmental factors in the etiology and pathogenesis of atopy: a working model. Environ Health Perspect 1999:107(Suppl 3):485-7.
148. Elias JA. Airway remodeling in asthma. Unanswered questions. Am J Respir Crit Care Med 2000:161(3 Pt 2):S168-71.
149. Djukanovic R. Asthma: A disease of inflammation and repair. J Allergy Clin Immunol 2000:105(2 Pt 2):S522-6.
150. Cooke RA, Van der Veer A. Human sensitization. J Immunol 1916:1:201-305.
151. Bergmann RL, Bergmann KE, Lau-Schadensdorf S, Luck W, Dannemann A, Bauer CP, et al. Atopic diseases in infancy. The German multicenter atopy study (MAS-90). Pediatr Allergy Immunol 1994:5(6 Suppl):19-25.
152. Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993:23(6):504-11.
153. Sherman CB, Tosteson TD, Tager IB, Speizer FE, Weiss ST. Early childhood predictors of asthma. Am J Epidemiol 1990:132(1):83-95.
154. Hansen LG. How do we define the high-risk groups for development of allergic disease? Pediatr Allergy Immunol 1996:7(9 Suppl):95-7.
155. Wahn U. What drives the allergic march? Allergy 2000:55(7):591-9.
156. Bergmann R, Woodcock A. Whole population or high-risk group? Childhood asthma. Eur Respir J Suppl 1998:27:9s-12s.
157. Ownby DR. Environmental factors versus genetic determinants of childhood inhalant allergies. J Allergy Clin Immunol 1990:86(3 Pt 1):279-87.
158. Koppelman GH, Los H, Postma DS. Genetic and environment in asthma: the answer of twin studies. Eur Respir J 1999:13(1):2-4.
159. Koppelman GH, Meijer GG, Bleecker ER. Genetics of Asthma. In: Clark TJH, Godfrey S, Lee TH, Thomson NC, editors. Asthma. London: Arnold; 2000. p. 146-74.
160. Lichtenstein P, Svartengren M. Genes, environments, and sex: factors of importance in atopic diseases in 7-9-year-old Swedish twins. Allergy 1997:52(11):1079-86.
161. Rasanen M, Laitinen T, Kaprio J, Koskenvuo M, Laitinen LA. Hay fever--a Finnish nationwide study of adolescent twins and their parents. Allergy 1998:53(9):885-90.
162. Sandford AJ, Pare PD. The genetics of asthma. The important questions. Am J Respir Crit Care Med 2000:161(3 Pt 2):S202-6.
163. Los H, Koppelman GH, Postma DS. The importance of genetic influences in asthma. Eur Respir J 1999:14(5):1210-27.
164. Cookson WO, Moffatt MF. Genetics of asthma and allergic disease. Hum Mol Genet 2000:9(16):2359-64.
165. Postma DS, Koppelman GH, Meyers DA. The genetics of atopy and airway hyperresponsiveness. Am J Respir Crit Care Med 2000:162(3 Pt 2):S118-23.
166. Borish L. Genetics of allergy and asthma. Ann Allergy Asthma Immunol 1999:82(5):413-24; quiz 424-6.
167. Koppelman GH, Postma DS. De genetica van atopie. Nederlands Tijdschrift voor Allergie, in press .
168. Barnes KC. Atopy and asthma genes--where do we stand? Allergy 2000:55(9):803-17.

169. Holloway JW, Beghe B, Holgate ST. The genetic basis of atopic asthma. Clin Exp Allergy 1999:29(8):1023-32.
170. Barnes KC. Gene-environment and gene-gene interaction studies in the molecular genetic analysis of asthma and atopy. Clin Exp Allergy 1999:29(Suppl 4):47-51.
171. Wiesch DG, Meyers DA, Bleecker ER. Genetics of asthma. J Allergy Clin Immunol 1999:104(5):895-901.
172. Holgate ST. Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol 1999:104(6):1139-46.
173. Rosenwasser LJ, Borish L. Genetics of atopy and asthma: the rationale behind promoter-based candidate gene studies (IL-4 and IL-10). Am J Respir Crit Care Med 1997:156(4 Pt 2):S152-5.
174. Wierenga EA, Messer G. Regulation of interleukin 4 gene transcription: alterations in atopic disease? Am J Respir Crit Care Med 2000:162(3 Pt 2):S81-5.
175. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. Am J Respir Cell Mol Biol 1999:20(5):976-83.
176. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritzsch C, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. J Allergy Clin Immunol 2000:105(3):506-13.
177. Martinez FD, Solomon S, Holberg CJ, Graves PE, Baldini M, Erickson RP. Linkage of circulating eosinophils to markers on chromosome 5q. Am J Respir Crit Care Med 1998:158(6):1739-44.
178. Patino CM, Martinez FD. Interactions between genes and environment in the development of asthma. Allergy 2001:56(4):279-86.
179. Sporik R, Chapman MD, Platts-Mills TA. House dust mite exposure as a cause of asthma. Clin Exp Allergy 1992:22(10):897-906.
180. Colloff MJ, Ayres J, Carswell F, Howarth PH, Merrett TG, Mitchell EB, et al. The control of allergens of dust mites and domestic pets: a position paper. Clin Exp Allergy 1992:22(Suppl 2):1-28.
181. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol 1997:100(6 Pt 1):S2-24.
182. Stewart GA, Thompson PJ. The biochemistry of common aeroallergens. Clin Exp Allergy 1996:26(9):1020-44.
183. Anonymous. Dust mite allergens and asthma: a worldwide problem. International Workshop report. Bull World Health Organ 1988:66(6):769-80.
184. Platts-Mills TA, Thomas WR, Aalberse RC, Vervloet D, Champman MD. Dust mite allergens and asthma: report of a second international workshop. J Allergy Clin Immunol 1992:89(5):1046-60.
185. The house-dust mite: its biology and role in allergy. Proceedings of an international scientific workshop. Oslo, Norway, 4-7 September 1997. Allergy 1998:53(48 Suppl):1-135.
186. Pearce N, Douwes J, Beasley R. Is allergen exposure the major primary cause of asthma? Thorax 2000:55(5):424-31.
187. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990:323(8):502-7.
188. Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. J Allergy Clin Immunol 1997:99(6 Pt 1):763-9.
189. Lau S, Falkenhorst G, Weber A, Werthmann I, Lind P, Buettner-Goetz P, et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol 1989:84(5 Pt 1):718-25.
190. Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. J Allergy Clin Immunol 1994:94(1):44-52.
191. Warner JA, Little SA, Pollock I, Longbottom JL, Warner JO. The influence of exposure to house dust mite, cat, pollen and fungal allergens on primary sensitization in asthma. Pediatr Allergy Immunol 1990:1:79-86.
192. Marks GB. House dust mite exposure as a risk factor for asthma: benefits of avoidance. Allergy 1998:53(48 Suppl):108-14.
193. Wickman M, Nordvall SL, Pershagen G, Sundell J, Schwartz B. House dust mite sensitization in children and residential characteristics in a temperate region. J Allergy Clin Immunol 1991:88(1):89-95.
194. Delacourt C, Labbe D, Vassault A, Brunet-Langot D, de Blic J, Scheinmann P. Sensitization to inhalant allergens in wheezing infants is predictive of the development of infantile asthma. Allergy 1994:49(10):843-7.

195. Ulrik CS, Backer V, Hesse B, Dirksen A. Risk factors for development of asthma in children and adolescents: findings from a longitudinal population study. Respir Med 1996:90(10):623-30.
196. Henderson FW, Henry MM, Ivins SS, Morris R, Neebe EC, Leu SY, et al. Correlates of recurrent wheezing in school-age children. The Physicians of Raleigh Pediatric Associates. Am J Respir Crit Care Med 1995:151(6):1786-93.
197. Peat JK, Woolcock AJ. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. Clin Exp Allergy 1991:21(5):573-81.
198. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. Clin Exp Allergy 1989:19(4):419-24.
199. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. Clin Exp Allergy 1993:23(11):941-8.
200. Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. Lancet 2000:356(9239):1392-7.
201. Squillace SP, Sporik RB, Rakes G, Couture N, Lawrence A, Merriam S, et al. Sensitization to dust mites as a dominant risk factor for asthma among adolescents living in central Virginia. Multiple regression analysis of a population-based study. Am J Respir Crit Care Med 1997:156(6):1760-4.
202. Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. Am J Respir Crit Care Med 1996:153(1):141-6.
203. Kuehr J, Frischer T, Meinert R, Barth R, Schraub S, Urbanek R, et al. Sensitization to mite allergens is a risk factor for early and late onset of asthma and for persistence of asthmatic signs in children. J Allergy Clin Immunol 1995:95(3):655-62.
204. Chan-Yeung M, Manfreda J, Dimich-Ward H, Lam J, Ferguson A, Warren P, et al. Mite and cat allergen levels in homes and severity of asthma. Am J Respir Crit Care Med 1995:152(6 Pt 1):1805-11.
205. Peat JK, Tovey E, Gray EJ, Mellis CM, Woolcock AJ. Asthma severity and morbidity in a population sample of Sydney schoolchildren: Part II--Importance of house dust mite allergens. Aust N Z J Med 1994:24(3):270-6.
206. de Blay F, Pauli G, Velten M, Bessot JC. Influence of mite exposure on symptoms of mite-sensitive patients with asthma. J Allergy Clin Immunol 1994:93(1 Pt 1):136-8.
207. Custovic A, Simpson A, Chapman MD, Woodcock A. Allergen avoidance in the treatment of asthma and atopic disorders. Thorax 1998:53(1):63-72.
208. Carswell F, Birmingham K, Oliver J, Crewes A, Weeks J. The respiratory effects of reduction of mite allergen in the bedrooms of asthmatic children--a double-blind controlled trial. Clin Exp Allergy 1996:26(4):386-96.
209. Marks GB, Tovey ER, Green W, Shearer M, Salome CM, Woolcock AJ. House dust mite allergen avoidance: a randomized controlled trial of surface chemical treatment and encasement of bedding. Clin Exp Allergy 1994:24(11):1078-83.
210. Boner AL, Peroni D, Sette L, Valletta EA, Piacentini G. Effects of allergen exposure-avoidance on inflammation in asthmatic children. Allergy 1993:48(17 Suppl):119-23; discussion 124.
211. Piacentini GL, Martinati L, Fornari A, Comis A, Carcereri L, Boccagni P, et al. Antigen avoidance in a mountain environment: influence on basophil releasability in children with allergic asthma. J Allergy Clin Immunol 1993:92(5):644-50.
212. van Strien RT, Verhoeff AP, van Wijnen JH, Doekes G, de Meer G, Brunekreef B. Infant respiratory symptoms in relation to mite allergen exposure. Eur Respir J 1996:9(5):926-31.
213. Friedmann PS. The role of dust mite antigen sensitization and atopic dermatitis. Clin Exp Allergy 1999:29(7):869-72.
214. Tupker RA, De Monchy JG, Coenraads PJ, Homan A, van der Meer JB. Induction of atopic dermatitis by inhalation of house dust mite. J Allergy Clin Immunol 1996:97(5):1064-70.
215. Norris PG, Schofield O, Camp RD. A study of the role of house dust mite in atopic dermatitis. Br J Dermatol 1988:118(3):435-40.
216. Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. Alternaria as a major allergen for asthma in children raised in a desert environment. Am J Respir Crit Care Med 1997:155(4):1356-61.
217. Sporik R, Ingram JM, Price W, Sussman JH, Honsinger RW, Platts-Mills TA. Association of asthma with serum IgE and skin test reactivity to allergens among children living at high altitude. Tickling the dragon's breath. Am J Respir Crit Care Med 1995:151(5):1388-92.

218. Martinez FD. Gene by environment interactions in the development of asthma. Clin Exp Allergy 1998:28(Suppl 5):21-5; discussion 26-8.
219. Gold DR, Burge HA, Carey V, Milton DK, Platts MT, Weiss ST. Predictors of repeated wheeze in the first year of life. The relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. Am J Respir Crit Care Med 1999:160(1):227-36.
220. Burr ML, Miskelly FG, Butland BK, Merrett TG, Vaughan-Williams E. Environmental factors and symptoms in infants at high risk of allergy. J Epidemiol Community Health 1989:43(2):125-32.
221. Nafstad P, Oie L, Mehl R, Gaarder PI, Lodrup-Carlsen KC, Botten G, et al. Residential dampness problems and symptoms and signs of bronchial obstruction in young Norwegian children. Am J Respir Crit Care Med 1998:157(2):410-4.
222. Celedon JC, Litonjua AA, Weiss ST, Gold DR. Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. Pediatrics 1999:104(3 Pt 1):495-500.
223. Holberg CJ, Wright AL, Martinez FD, Morgan WJ, Taussig LM. Child day care, smoking by caregivers, and lower respiratory tract illness in the first 3 years of life. Group Health Medical Associates. Pediatrics 1993:91(5):885-92.
224. Strachan DP. Hay fever, hygiene, and household size. Bmj 1989:299(6710):1259-60.
225. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis&quot. Thorax 2000:55(Suppl 1):S2-10.
226. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH. Skin test reactivity and number of siblings. Bmj 1994:308(6930):692-5.
227. Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. The Aberdeen WHEASE Group. Thorax 1998:53(1):28-32.
228. Mattes J, Karmaus W, Moseler M, Frischer T, Kuehr J. Accumulation of atopic disorders within families: a sibling effect only in the offspring of atopic fathers. Clin Exp Allergy 1998:28(12):1480-6.
229. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999:353(9151):450-4.
230. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000:343(8):538-43.
231. Romagnani S. The Th1/Th2 paradigm and allergic disorders. Allergy 1998:53(46 Suppl):12-5.
232. Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. Lancet 1994:344(8920):456-8.
233. Openshaw PJM, Pala P, Sparer T, Matthews S, Pennycook A, Hussell T. T-cell subsets and lung inflammation: lessons from respiratory syncytial virus. Eur Respir Rev 2000:10(70):108-111.
234. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995:95(4):500-5.
235. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000:161(5):1501-7.
236. Noble V, Murray M, Webb MS, Alexander J, Swarbrick AS, Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. Arch Dis Child 1997:76(4):315-9.
237. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999:354(9178):541-5.
238. Welliver RC. Immunology of respiratory syncytial virus infection: eosinophils, cytokines, chemokines and asthma. Pediatr Infect Dis J 2000:19(8):780-3; discussion 784-5; 811-3.
239. Forster J, Tacke U, Krebs H, Streckert HJ, Werchau H, Bergmann RL, et al. Respiratory syncytial virus infection: its role in aeroallergen sensitization during the first two years of life. Pediatr Allergy Immunol 1996:7(2):55-60.
240. Roman M, Calhoun WJ, Hinton KL, Avendano LF, Simon V, Escobar AM, et al. Respiratory syncytial virus infection in infants is associated with predominant Th-2-like response. Am J Respir Crit Care Med 1997:156(1):190-5.
241. Diaz PV, Calhoun WJ, Hinton KL, Avendano LF, Gaggero A, Simon V, et al. Differential effects of respiratory syncytial virus and adenovirus on mononuclear cell cytokine responses. Am J Respir Crit Care Med 1999:160(4):1157-64.
242. Renzi PM, Turgeon JP, Marcotte JE, Drblik SP, Berube D, Gagnon MF, et al. Reduced interferon-gamma production in infants with bronchiolitis and asthma. Am J Respir Crit Care Med 1999:159(5 Pt 1):1417-22.

243. Brandenburg AH, Kleinjan A, van Het Land B, Moll HA, Timmerman HH, de Swart RL, et al. Type 1-like immune response is found in children with respiratory syncytial virus infection regardless of clinical severity. J Med Virol 2000:62(2):267-77.
244. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC, 2nd, Welliver RC. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. J Allergy Clin Immunol 1999:103(4):630-6.
245. Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, et al. Measles and atopy in Guinea-Bissau. Lancet 1996:347(9018):1792-6.
246. Paunio M, Heinonen OP, Virtanen M, Leinikki P, Patja A, Peltola H. Measles history and atopic diseases: a population-based cross-sectional study. Jama 2000:283(3):343-6.
247. Odent MR, Culpin EE, Kimmel T. Pertussis vaccination and asthma: is there a link?. Jama 1994:272(8):592-3.
248. Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. Thorax 1998:53(11):927-32.
249. Nilsson L, Kjellman NI, Bjorksten B. A randomized controlled trial of the effect of pertussis vaccines on atopic disease. Arch Pediatr Adolesc Med 1998:152(8):734-8.
250. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science 1997:275(5296):77-9.
251. Aaby P, Shaheen SO, Heyes CB, Goudiaby A, Hall AJ, Shiell AW, et al. Early BCG vaccination and reduction in atopy in guinea-bissau. *Clin Exp Allergy* 2000:30:644-50.
252. Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. Lancet 1999:354 Suppl 2:SII12-5.
253. Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. Lancet 1997:350(9075):400-3.
254. Strannegard IL, Larsson LO, Wennergren G, Strannegard O. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. Allergy 1998:53(3):249-54.
255. Herz U, Lacy P, Renz H, Erb K. The influence of infections on the development and severity of allergic disorders. Curr Opin Immunol 2000:12(6):632-40.
256. Weiss ST. Parasites and asthma/allergy: what is the relationship? J Allergy Clin Immunol 2000:105(2 Pt 1):205-10.
257. van den Biggelaar AH, van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG, et al. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. Lancet 2000:356(9243):1723-7.
258. Lynch NR, Goldblatt J, Le Souef PN. Parasite infections and the risk of asthma and atopy. Thorax 1999:54(8):659-60.
259. Downs SH, Marks GB, Mitakakis TZ, Leuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. Clin Exp Allergy 2001:31(4):570-5.
260. Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. Clin Exp Allergy 2000:30(2):201-8.
261. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 2000:30(2):194-200.
262. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 2000:30(2):187-93.
263. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 2000:30(9):1230-4.
264. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000:355(9216):1680-3.
265. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 2001:163(2):322-8.
266. Litonjua A, Milton D, Celedon JC, Ryan L, Sredl D, Weiss ST, et al. Longitudinal association house dust endotoxin levels and wheeze in young children. Am J Respir Crit Care Med 2001:163(5):A845.
267. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. Lancet 1999:353(9163):1485-8.
268. Droste JH, Wieringa MH, Weyler JJ, Nelen VJ, Vermeire PA, Van Bever HP. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? Clin Exp Allergy 2000:30(11):1547-53.

269. Matricardi PM, Rosmini F, Ferrigno L, Nisini R, Rapicetta M, Chionne P, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. Bmj 1997:314(7086):999-1003.
270. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. Bmj 2000:320(7232):412-7.
271. Bottcher MF, Nordin EK, Sandin A, Midtvedt T, Bjorksten B. Microflora-associated characteristics in faeces from allergic and nonallergic infants. Clin Exp Allergy 2000:30(11):1590-6.
272. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy 1999:29(3):342-6.
273. Sepp E, Julge K, Vasar M, Naaber P, Bjorksten B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. Acta Paediatr 1997:86(9):956-61.
274. Matricardi PM, Bonini S. High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the Hygiene hypothesis? Clin Exp Allergy 2000:30(11):1506-10.
275. Cunningham J, Dockery DW, Gold DR, Speizer FE. Racial differences in the association between maternal smoking during pregnancy and lung function in children. Am J Respir Crit Care Med 1995:152(2):565-9.
276. Wissow LS, Gittelsohn AM, Szklo M, Starfield B, Mussman M. Poverty, race, and hospitalization for childhood asthma. Am J Public Health 1988:78(7):777-82.
277. Halfon N, Newacheck PW. Childhood asthma and poverty: differential impacts and utilization of health services. Pediatrics 1993:91(1):56-61.
278. Duran-Tauleria E, Rona RJ. Geographical and socioeconomic variation in the prevalence of asthma symptoms in English and Scottish children. Thorax 1999:54(6):476-81.
279. Gold DR, Rotnitzky A, Damokosh AI, Ware JH, Speizer FE, Ferris BG, et al. Race and gender differences in respiratory illness prevalence and their relationship to environmental exposures in children 7 to 14 years of age. Am Rev Respir Dis 1993:148(1):10-8.
280. Weitzman M, Gortmaker S, Sobol A. Racial, social, and environmental risks for childhood asthma. Am J Dis Child 1990:144(11):1189-94.
281. Margolis PA, Greenberg RA, Keyes LL, LaVange LM, Chapman RS, Denny FW, et al. Lower respiratory illness in infants and low socioeconomic status. Am J Public Health 1992:82(8):1119-26.
282. Glezen WP, Paredes A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 1981:98(5):708-15.
283. Kabesch M, Schaal W, Nicolai T, von Mutius E. Lower prevalence of asthma and atopy in Turkish children living in Germany. Eur Respir J 1999:13(3):577-82.
284. Hjern A, Haglund B, Hedlin G. Ethnicity, childhood environment and atopic disorder. Clin Exp Allergy 2000:30(4):521-8.
285. Hjern A, Haglund B, Bremberg S. Lower respiratory tract infections in an ethnic and social context. Paediatr Perinat Epidemiol 2000:14:53-60.
286. Leung RC, Carlin JB, Burdon JG, Czarny D. Asthma, allergy and atopy in Asian immigrants in Melbourne. Med J Aust 1994:161(7):418-25.
287. Hjern A, Rasmussen F, Johansson M, Aberg N. Migration and atopic disorder in Swedish conscripts. Pediatr Allergy Immunol 1999:10(3):209-15.
288. von Mutius E, Weiland SK, Fritzsch C, Duhme H, Keil U. Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. Lancet 1998:351(9106):862-6.
289. Malveaux FJ, Fletcher-Vincent SA. Environmental risk factors of childhood asthma in urban centers. Environ Health Perspect 1995:103(Suppl 6):59-62.
290. Nafstad P, Magnus P, Jaakkola JJ. Risk of childhood asthma and allergic rhinitis in relation to pregnancy complications. J Allergy Clin Immunol 2000:106(5):867-73.
291. Godfrey KM, Barker DJ, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994:24(7):641-8.
292. Fergusson DM, Crane J, Beasley R, Horwood LJ. Perinatal factors and atopic disease in childhood. Clin Exp Allergy 1997:27(12):1394-401.
293. Braback L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. Clin Exp Allergy 1998:28(8):936-42.
294. Kelly YJ, Brabin BJ, Milligan P, Heaf DP, Reid J, Pearson MG. Maternal asthma, premature birth, and the risk of respiratory morbidity in schoolchildren in Merseyside. Thorax 1995:50(5):525-30.

295. Greenough A, Giffin FJ, Yuksel B. Respiratory morbidity in preschool children born prematurely. Relationship to adverse neonatal events. Acta Paediatr 1996:85(7):772-7.
296. Lucas A, Brooke OG, Cole TJ, Morley R, Bamford MF. Food and drug reactions, wheezing, and eczema in preterm infants. Arch Dis Child 1990:65(4):411-5.
297. Olesen AB, Ellingsen AR, Olesen H, Juul S, Thestrup-Pedersen K. Atopic dermatitis and birth factors: historical follow up by record linkage. Bmj 1997:314(7086):1003-8.
298. Martinez FD, Wright AL, Holberg CJ, Morgan WJ, Taussig LM. Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. Am J Epidemiol 1992:136(10):1258-68.
299. Bisgaard H, Klug B. Lung function measurement in awake young children. Eur Respir J 1995:8(12):2067-75.
300. Arshad SH, Hide DW. Effect of environmental factors on the development of allergic disorders in infancy. J Allergy Clin Immunol 1992:90(2):235-41.
301. Lewis S, Richards D, Bynner J, Butler N, Britton J. Prospective study of risk factors for early and persistent wheezing in childhood. Eur Respir J 1995:8(3):349-56.
302. Burr ML, Limb ES, Maguire MJ, Amarah L, Eldridge BA, Layzell JC, et al. Infant feeding, wheezing, and allergy: a prospective study. Arch Dis Child 1993:68(6):724-8.
303. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. Lancet 1995:346(8982):1065-9.
304. Watkins CJ, Leeder SR, Corkhill RT. The relationship between breast and bottle feeding and respiratory illness in the first year of life. J Epidemiol Community Health 1979:33(3):180-2.
305. Nafstad P, Jaakkola JJ, Hagen JA, Botten G, Kongerud J. Breastfeeding, maternal smoking and lower respiratory tract infections. Eur Respir J 1996:9(12):2623-9.
306. Cushing AH, Samet JM, Lambert WE, Skipper BJ, Hunt WC, Young SA, et al. Breastfeeding reduces risk of respiratory illness in infants. Am J Epidemiol 1998:147(9):863-70.
307. Rylander E, Eriksson M, Pershagen G, Nordvall L, Ehrnst A, Ziegler T. Wheezing bronchitis in children. Incidence, viral infections, and other risk factors in a defined population. Pediatr Allergy Immunol 1996:7(1):6-11.
308. Oddy WH. Breastfeeding and asthma in children: findings from a West Australian study. Breastfeed Rev 2000:8(1):5-11.
309. Chandra RK. Prospective studies of the effect of breast feeding on incidence of infection and allergy. Acta Paediatr Scand 1979:68(5):691-4.
310. Kramer MS. Does breast feeding help protect against atopic disease? Biology, methodology, and a golden jubilee of controversy. J Pediatr 1988:112(2):181-90.
311. Wright AL, Sherrill D, Holberg CJ, Halonen M, Martinez FD. Breast-feeding, maternal IgE, and total serum IgE in childhood. J Allergy Clin Immunol 1999:104(3 Pt 1):589-94.
312. Lucas A, Brooke OG, Morley R, Cole TJ, Bamford MF. Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. Bmj 1990:300(6728):837-40.
313. Gustafsson D, Lowhagen T, Andersson K. Risk of developing atopic disease after early feeding with cows' milk based formula. Arch Dis Child 1992:67(8):1008-10.
314. de Jong MH, Scharp-van der Linden VT, Aalberse RC, Oosting J, Tijssen JG, de Groot CJ. Randomised controlled trial of brief neonatal exposure to cows' milk on the development of atopy. Arch Dis Child 1998:79(2):126-30.
315. Fergusson DM, Horwood LJ. Early solid food diet and eczema in childhood: a 10-year longitudinal study. Pediatr Allergy Immunol 1994:5(6 Suppl):44-7.
316. Forsyth JS, Ogston SA, Clark A, Florey CD, Howie PW. Relation between early introduction of solid food to infants and their weight and illnesses during the first two years of life. Bmj 1993:306(6892):1572-6.
317. Peat J, Bjorksten B. Primary and secondary prevention of allergic asthma. Eur Respir J Suppl 1998:27:28s-34s.
318. Hesselmar B, Aberg N, Aberg B, Eriksson B, Bjorksten B. Does early exposure to cat or dog protect against later allergy development?. Clin Exp Allergy 1999:29(5):611-7.
319. Nafstad P, Magnus P, Gaarder PI, Jaakkola JJ. Exposure to pets and atopy-related diseases in the first 4 years of life. Allergy 2001:56(4):307-12.
320. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001:357(9258):752-6.

321. Eggleston PA, Rosenstreich D, Lynn H, Gergen P, Baker D, Kattan M, et al. Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. J Allergy Clin Immunol 1998:102(4 Pt 1):563-70.
322. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997:336(19):1356-63.
323. Peat JK, Dickerson J, Li J. Effects of damp and mould in the home on respiratory health: a review of the literature. Allergy 1998:53(2):120-8.
324. Aldous MB, Holberg CJ, Wright AL, Martinez FD, Taussig LM. Evaporative cooling and other home factors and lower respiratory tract illness during the first year of life. Group Health Medical Associates. Am J Epidemiol 1996:143(5):423-30.
325. Ogston SA, Florey CD, Walker CH. The Tayside infant morbidity and mortality study: effect on health of using gas for cooking. Br Med J (Clin Res Ed) 1985:290(6473):957-60.
326. Samet JM, Lambert WE, Skipper BJ, Cushing AH, Hunt WC, Young SA, et al. Nitrogen dioxide and respiratory illnesses in infants. Am Rev Respir Dis 1993:148(5):1258-65.
327. Cook DG, Strachan DP. Summary of effects of parental smoking on the respiratory health of children and implications for research. Thorax 1999:54(4):357-366.
328. Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma. Am J Respir Crit Care Med 1999:159(2):403-10.
329. Young S, Arnott J, O'Keeffe PT, Le Souef PN, Landau LI. The association between early life lung function and wheezing during the first 2 yrs of life. Eur Respir J 2000:15(1):151-7.
330. Morgan WJ. Maternal smoking and infant lung function. Further evidence for an in utero effect. Am J Respir Crit Care Med 1998:158(3):689-90.
331. Ey JL, Holberg CJ, Aldous MB, Wright AL, Martinez FD, Taussig LM. Passive smoke exposure and otitis media in the first year of life. Group Health Medical Associates. Pediatrics 1995:95(5):670-7.
332. Magnusson CG. Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. J Allergy Clin Immunol 1986:78(5 Pt 1):898-904.
333. Bergmann RL, Schulz J, Gunther S, Dudenhausen JW, Bergmann KE, Bauer CP, et al. Determinants of cord-blood IgE concentrations in 6401 German neonates. Allergy 1995:50(1):65-71.
334. Bjerke T, Hedegaard M, Henriksen TB, Nielsen BW, Schiotz PO. Several genetic and environmental factors influence cord blood IgE concentration. Pediatr Allergy Immunol 1994:5(2):88-94.
335. Kaan A, Dimich-Ward H, Manfreda J, Becker A, Watson W, Ferguson A, et al. Cord blood IgE: its determinants and prediction of development of asthma and other allergic disorders at 12 months. Ann Allergy Asthma Immunol 2000:84(1):37-42.
336. Ownby DR, Johnson CC, Peterson EL. Maternal smoking does not influence cord serum IgE or IgD concentrations. J Allergy Clin Immunol 1991:88(4):555-60.
337. Oryszczyn MP, Godin J, Annesi I, Hellier G, Kauffmann F. In utero exposure to parental smoking, cotinine measurements, and cord blood IgE. J Allergy Clin Immunol 1991:87(6):1169-74.
338. de Jongste JC. Surrogate markers of airway inflammation:inflammometry in paediatric respiratory medicine. Paediatric Respiratory Reviews 2000:I:354-360.
339. Branthwaite MA. Ethical problems in respiratory care: the role of the law. Thorax 2001:56(1):78-81.
340. Matsumoto T, Miike T, Yamaguchi K, Murakami M, Kawabe T, Yodoi J. Serum levels of soluble IL-2 receptor, IL-4 and IgE-binding factors in childhood allergic diseases. Clin Exp Immunol 1991:85(2):288-92.
341. Kim JT, Kim CK, Koh YY. Serum levels of soluble interleukin-2 receptor at acute asthma exacerbation: relationship with severity of exacerbation and bronchodilator response. Int Arch Allergy Immunol 1998:117(4):263-9.
342. Shi HZ, Sun JJ, Pan HL, Lu JQ, Zhang JL, Jiang JD. Alterations of T-lymphocyte subsets, soluble IL-2 receptor, and IgE in peripheral blood of children with acute asthma attacks. J Allergy Clin Immunol 1999:103(3 Pt 1):388-94.
343. Clough JB, Keeping KA, Edwards LC, Freeman WM, Warner JA, Warner JO. Can we predict which wheezy infants will continue to wheeze? Am J Respir Crit Care Med 1999:160(5 Pt 1):1473-80.
344. Miller AL, Stern DA, Martinez FD, Wright AL, Taussig LM, Halonen M. Serum levels of the soluble low affinity receptor for IgE and soluble interleukin-2 receptor in childhood, and their relation to age, gender, atopy and allergic disease. Pediatr Allergy Immunol 1996:7(2):68-74.
345. Businco L, Marchetti F, Pellegrini G, Perlini R. Predictive value of cord blood IgE levels in 'at-risk' newborn babies and influence of type of feeding. Clin Allergy 1983:13(6):503-8.

346. Magnusson CG. Cord serum IgE in relation to family history and as predictor of atopic disease in early infancy. Allergy 1988:43(4):241-51.
347. Michel FB, Bousquet J, Greillier P, Robinet-Levy M, Coulomb Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. J Allergy Clin Immunol 1980:65(6):422-30.
348. Chandra RK, Puri S, Cheema PS. Predictive value of cord blood IgE in the development of atopic disease and role of breast-feeding in its prevention. Clin Allergy 1985:15(6):517-22.
349. Croner S, Kjellman NI, Eriksson B, Roth A. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. Arch Dis Child 1982:57(5):364-8.
350. Halonen M, Stern D, Taussig LM, Wright A, Ray CG, Martinez FD. The predictive relationship between serum IgE levels at birth and subsequent incidences of lower respiratory illnesses and eczema in infants. Am Rev Respir Dis 1992:146(4):866-70.
351. Varonier HS, Lacourt GC, Assimacopoulos A. Cord serum IgE and early detection of the atopic phenotype: suitable for routine screening? Eur J Pediatr 1991:150(12):844-6.
352. Bergmann RL, Edenharter G, Bergmann KE, Guggenmoos-Holzmann I, Forster J, Bauer CP, et al. Predictability of early atopy by cord blood-IgE and parental history. Clin Exp Allergy 1997:27(7):752-60.
353. Hansen LG, Host A, Halken S, Holmskov A, Husby S, Lassen LB, et al. Cord blood IgE. II. Prediction of atopic disease. A follow-up at the age of 18 months. Allergy 1992:47(4 Pt 2):397-403.
354. Edenharter G, Bergmann RL, Bergmann KE, Wahn V, Forster J, Zepp F, et al. Cord blood-IgE as risk factor and predictor for atopic diseases. Clin Exp Allergy 1998:28(6):671-8.
355. Hide DW, Arshad SH, Twiselton R, Stevens M. Cord serum IgE: an insensitive method for prediction of atopy. Clin Exp Allergy 1991:21(6):739-43.
356. Ruiz RG, Richards D, Kemeny DM, Price JF. Neonatal IgE: a poor screen for atopic disease. Clin Exp Allergy 1991:21(4):467-72.
357. Eiriksson TH, Sigurgeirsson B, Ardal B, Sigfusson A, Valdimarsson H. Cord blood IgE levels are influenced by gestational age but do not predict allergic manifestations in infants. Pediatr Allergy Immunol 1994:5(1):5-10.
358. Kobayashi Y, Kondo N, Shinoda S, Agata H, Fukutomi O, Orii T. Predictive values of cord blood IgE and cord blood lymphocyte responses to food antigens in allergic disorders during infancy. J Allergy Clin Immunol 1994:94(5):907-16.
359. Kjellman NI. IgE in neonates is not suitable for general allergy risk screening. Pediatr Allergy Immunol 1994:5(1):1-4.
360. Laan MP, Baert MR, Bijl AM, Vredendaal AE, De Waard-van der Spek FB, Oranje AP, et al. Markers for early sensitization and inflammation in relation to clinical manifestations of atopic disease up to 2 years of age in 133 high-risk children. Clin Exp Allergy 2000:30(7):944-53.
361. Burr ML, Merrett TG, Dunstan FD, Maguire MJ. The development of allergy in high-risk children. Clin Exp Allergy 1997:27(11):1247-53.
362. Lilja G, Oman H. Prediction of atopic disease in infancy by determination of immunological parameters: IgE, IgE- and IgG-antibodies to food allergens, skin prick tests and T lymphocyte subsets. Pediatr Allergy Immunol 1991:2:6-13.
363. Sporik R, Holgate ST, Cogswell JJ. Natural history of asthma in childhood--a birth cohort study. Arch Dis Child 1991:66(9):1050-3.
364. Wilson NM, Dore CJ, Silverman M. Factors relating to the severity of symptoms at 5 yrs in children with severe wheeze in the first 2 yrs of life. Eur Respir J 1997:10(2):346-53.
365. Oymar K, Bjerknes R. Is serum eosinophil cationic protein in bronchiolitis a predictor of asthma? Pediatr Allergy Immunol 1998:9(4):204-7.
366. Borres MP, Odelram H, Irander K, Kjellman NI, Bjorksten B. Peripheral blood eosinophilia in infants at 3 months of age is associated with subsequent development of atopic disease in early childhood. J Allergy Clin Immunol 1995:95(3):694-8.
367. Lodrup Carlsen KC, Halvorsen R, Carlsen KH. Serum inflammatory markers and effects of age and tobacco smoke exposure in young non-asthmatic children. Acta Paediatr 1998:87(5):559-64.
368. Koller DY, Wojnarowski C, Herkner KR, Weinlander G, Raderer M, Eichler I, et al. High levels of eosinophil cationic protein in wheezing infants predict the development of asthma. J Allergy Clin Immunol 1997:99(6 Pt 1):752-6.
369. Kimata H. Increased serum levels of soluble adhesion molecules in young children with atopic dermatitis. Eur J Pediatr 1999:158(6):529-30.

370. Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001:163(7):1693-722.
371. von Mutius E. Towards prevention. Lancet 1997:350(Suppl 2):SII14-7.
372. Falth-Magnusson K, Kjellman NI. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy--a randomized study. J Allergy Clin Immunol 1987:80(6):868-75.
373. Falth-Magnusson K, Kjellman NI. Allergy prevention by maternal elimination diet during late pregnancy--a 5-year follow-up of a randomized study. J Allergy Clin Immunol 1992:89(3):709-13.
374. Zeiger RS, Heller S, Mellon MH, Forsythe AB, RD OC, Hamburger RN, et al. Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: a randomized study [published erratum appears in J Allergy Clin Immunol 1989 Nov;84(5 Pt 1):677]. J Allergy Clin Immunol 1989:84(1):72-89.
375. Zeiger RS, Heller S, Mellon MH, Halsey JF, Hamburger RN, Sampson HA. Genetic and environmental factors affecting the development of atopy through age 4 in children of atopic parents: a prospective randomized study of food allergen avoidance. Pediatr Allergy Immunol 1992:3:110-127.
376. Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. J Allergy Clin Immunol 1995:95(6):1179-90.
377. Hattevig G, Kjellman B, Sigurs N, Bjorksten B, Kjellman NI. Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. Clin Exp Allergy 1989:19(1):27-32.
378. Halken S, Host A, Hansen LG, Osterballe O. Effect of an allergy prevention programme on incidence of atopic symptoms in infancy. A prospective study of 159 "high-risk" infants. Allergy 1992:47(5):545-53.
379. Halken S, Host A, Hansen LG, Osterballe O. Preventive effect of feeding high-risk infants a casein hydrolysate formula or an ultrafiltrated whey hydrolysate formula. A prospective, randomized, comparative clinical study. Pediatr Allergy Immunol 1993:4(4):173-81.
380. Miskelly FG, Burr ML, Vaughan-Williams E, Fehily AM, Butland BK, Merrett TG. Infant feeding and allergy. Arch Dis Child 1988:63(4):388-93.
381. Chandra RK, Hamed A. Cumulative incidence of atopic disorders in high risk infants fed whey hydrolysate, soy, and conventional cow milk formulas. Ann Allergy 1991:67(2 Pt 1):129-32.
382. Marini A, Agosti M, Motta G, Mosca F. Effects of a dietary and environmental prevention programme on the incidence of allergic symptoms in high atopic risk infants: three years' follow-up. Acta Paediatr Suppl 1996:414:1-21.
383. Kajosaari M. Atopy prevention in childhood: the role of diet. Prospective 5-year follow-up of high-risk infants with six months exclusive breastfeeding and solid food elimination. Pediatr Allergy Immunol 1994:5(6 Suppl):26-8.
384. Falth-Magnusson K. Is maternal diet worthwhile? Pediatr Allergy Immunol 1994:5(6 Suppl):29-32.
385. Halken S, Host A. The lessons of noninterventional and interventional prospective studies on the development of atopic disease during childhood. Allergy 2000:55(9):793-802.
386. Zeiger RS. Dietary manipulations in infants and their mothers and the natural course of atopic disease. Pediatr Allergy Immunol 1994:5(6 Suppl):33-43.
387. Arshad SH, Matthews S, Gant C, Hide DW. Effect of allergen avoidance on development of allergic disorders in infancy. Lancet 1992:339(8808):1493-7.
388. Hide DW, Matthews S, Matthews L, Stevens M, Ridout S, Twiselton R, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. J Allergy Clin Immunol 1994:93(5):842-6.
389. Hide DW, Matthews S, Tariq S, Arshad SH. Allergen avoidance in infancy and allergy at 4 years of age. Allergy 1996:51(2):89-93.
390. Chan-Yeung M, Manfreda J, Dimich-Ward H, Ferguson A, Watson W, Becker A. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. Arch Pediatr Adolesc Med 2000:154(7):657-63.
391. Custovic A, Simpson BM, Simpson A, Hallam C, Craven M, Brutsche M, et al. Manchester Asthma and Allergy Study: low-allergen environment can be achieved and maintained during pregnancy and in early life. J Allergy Clin Immunol 2000:105(2 Pt 1):252-8.
392. Custovic A, Simpson BM, Simpson A, Kissen P, Woodcock A. Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial. Lancet 2001:358(9277):188-93.
393. Nishioka K, Yasueda H, Saito H. Preventive effect of bedding encasement with microfine fibers on mite sensitization. J Allergy Clin Immunol 1998:101(1 Pt 1):28-32.

394. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 2001:357(9262):1076-9.
395. Holt PG, Macaubas C, Sly PD. Strategic targets for primary prevention of allergic disease in childhood. Allergy 1998:53(45 Suppl):72-6.

Chapter 3

Study aims

STUDY AIMS

- A major difficulty in studying the pathophysiology and epidemiology of allergic disease is the lack of clear-cut and generally accepted definitions of allergic disease, especially in early childhood. This issue is dealt with in more detail in **chapter 4**. The aims of this chapter are: 1) To review the prognostic value of early respiratory symptoms for the development of asthma later in life, and 2) To evaluate the outcome variables used to describe respiratory symptoms and disease in large prospective cohort studies on the development of allergic disease.
- Although the first symptoms of asthma and atopic eczema start in early life, indications for IgE-mediated disease are present in only a minority of pre-school children. Several possible explanations for this paradox can be proposed. Firstly, various prospective birth cohort studies have shown that a large proportion of pre-school children with wheezing do not suffer from asthma. Therefore, it is comprehensible that in the majority of wheezing infants no signs of allergic inflammation are present. Alternatively, it might be possible that traditional allergy markers, such as specific IgE and eosinophils in peripheral blood are too insensitive for early identification of allergic disease. Both explanations warrant the need for easy obtainable blood markers that either support the diagnosis or predict the development of allergic disease in early childhood. If the first explanation is correct, markers are needed to distinguish the children with viral-induced wheeze from children with early-onset asthma. If the second explanation is true, other than traditional markers should be sought that better reflect the development of allergic disease in pre-school children. In **chapter 6**, a variety of serum markers are evaluated in relation to respiratory-and skin symptoms in early life.
- In paragraph 2.4. it was mentioned that indoor allergens might play an important role in the development of allergic disease. If house dust mite is important in the etiology of allergic disease, measures aimed at reducing HDM-allergen exposure can prevent the development of allergic disease. An important goal of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)- study is investigating the effect of mattress encasings impermeable to HDM-allergen on the development of sensitization to HDM and allergic disease in childhood. In **chapter 7**, the effect of the mattress encasings on HDM-allergen levels as well as the development of sensitization and airway- and skin symptoms in the first 2 years of life will be reported.
- Recent studies have shown that exposure to microorganisms in early life, either indirectly estimated by having siblings and early daycare visits or directly assessed by the number of viral upper airway infections, protects against the development of allergic disease in later childhood. However, it is unclear in which way the exposure to microorganisms influences to the development of the infant immune system. The aim of the Virus Mediated Allergy (VIGALL)-study is to investigate the effect of viral upper airway infections in early life on the development of the infant immune system and the development of allergic disease. Special emphasis is put on Th1 and Th2-like cytokine responses in peripheral blood and nasal brush samples. In this thesis (**chapter 8**), the first results with respect to the nasal mucosal immune response in RSV-bronchiolitis versus RSV-upper airway infection will be described. In addition, the interaction between a family history of allergic disease and conditions of increased risk of microbial exposure (by child care or having siblings) for the development of upper- and lower airway infections will be described.
- From studies in the USA and UK it has become clear that ethnicity and socioeconomic status are associated with allergic disease in childhood. In continental Europe, very few studies have been done on this topic, and no prospective studies are available. Therefore the relationship between ethnicity and socioeconomic status on the one hand and the de-

velopment of respiratory and- skin symptoms in the first 2 years of life on the other hand will be examined in the context of the PIAMA-study (**chapter 9**).

Chapter 4

Definition of allergic disease

4.1. Definition of respiratory symptoms and disease in early childhood in large prospective birth cohort studies that predict the development of asthma

L.P. Koopman[1], B. Brunekreef[2], J.C. de Jongste[1], H.J. Neijens[1]

[1]Erasmus University and University Hospital/Sophia Children's Hospital, Department of Pediatrics, Rotterdam, The Netherlands. [2]University of Utrecht, Institute of Risk Assessment Sciences, The Netherlands

ABSTRACT

We have reviewed the prospective value of early respiratory symptoms for determining the risk of development of asthma later in life by using data from studies based on the general population, hospital population and general practices. Although 'wheezing' in infancy generally has a good prognosis, it is an important risk factor for the development of asthma later in life. The prognostic value of 'coughing' and 'shortness of breath' in infancy for the later development of asthma is less clear. Despite the fact that no internationally accepted criteria for the definition of asthma in early childhood are available, many studies have been performed on this topic. We also have investigated the outcome variables that were used to describe respiratory symptoms and disease in early childhood in the publications of nine large prospective birth cohort studies on the development of asthma. From seven of these studies, we reviewed the original questionnaires. We found that various studies used different outcome variables, but the data that were actually collected were similar. This is an important observation because it implies that comparisons between studies can be markedly improved by data sharing among investigators.

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INTRODUCTION

Systematic international comparison of the prevalence of asthma have been made possible for school-age children by the International Study of Asthma and Allergy in Childhood (ISAAC) [1, 2]. Unfortunately, no valid criteria for asthma are available for infants and pre-school children. Comparison of the prevalence of early respiratory symptoms in different parts of the world is clearly important for learning about the etiology of asthma in greater detail [3]. The most important symptom of asthma is wheeze; other symptoms include shortness of breath and recurrent cough [4]. Wheezing and cough are especially common in pre-school children, usually in whom they usually occur in association with respiratory infections, and asthma will develop in only a minority of the children who ever wheezed. This raises the question of which respiratory symptoms in early life are relevant for the subsequent risk of developing asthma. The first aim of this work was to assess the prognostic value of respiratory symptoms in pre-school children with respect to subsequent development of asthma. The second aim was to compare the respiratory outcome variables that have been used for children of 0-3 years of age in publications of large birth cohort studies in regard to risk factors for the development of asthma in childhood. In addition, we collected the original questionnaires of most of these studies in order to compare the data that were actually gathered.

PROGNOSTIC VALUE OF RESPIRATORY SYMPTOMS IN PRE-SCHOOL CHILDREN

Wheezing:

Wheezing is the most frequently studied respiratory symptom in pre-school children with regard to its prognostic value for the later development of asthma. However, wheeze is a problematic symptom. Members of non-wheezing families do not necessarily recognize a wheeze properly [5] and upper respiratory noises, such as sniffles, snoring and stridor are difficult to differentiate from wheeze. Furthermore, when questionnaires are used for international comparison, many languages do not have an equivalent for wheezing.

Wheezing is a relatively common symptom in infancy and early childhood and affects ≈ 25% to 60% of children in the first few years of life, depending on the definition that is used and the population that is studied [6]. Wheezing in early life is highly associated with viral infections [7]; however, until the end of the 1980s no distinction was made between descriptive terms such as virus associated wheezy bronchitis, recurrent bronchiolitis and asthma. These syndromes were considered as having a common underlying disorder [8, 9]. To date no diagnostic tools are available to distinguish between transient and persistent wheezing at an early stage [10, 11]. Nevertheless, even though the majority of children who wheeze in infancy and early childhood do not develop asthma later in life, follow-up studies indicate that wheezing in infancy remains an important risk factor for wheezing later in life. In the Tucson cohort, 40% of the children who wheezed at least once in the first 3 years of life also wheezed at age 6, compared to only 22% of the children who did not wheeze in the first three years of life [10]. Infants with wheezing lower respiratory disease in infancy are four to five times more likely to have recurrent wheezing at age 11 [11]. These findings are in agreement with various other population-based studies [12-18]. In highly selected hospital- or general practice-based populations, some studies showed remission of wheezing in more than 50% of the patients

[19-21], while other studies showed persistence of symptoms in later life in the majority of patients [8, 22, 23]. Most studies indicate that early onset wheezing has a better outcome than late onset wheezing [15, 17, 20, 24], but some controversy exists [8, 16, 23].
One retrospective population-based study showed that the number of wheezing episodes in the first year of life was positively associated with the persistence of wheezing episodes at 10 years of age [16]. The result of this study can also be explained by recall-bias. However, this finding is in agreement with the conclusions of three other studies in symptomatic children [8, 22, 25]. We are not aware of any population-based prospective birth cohort study that investigated the prognostic value of the frequency of wheezing attacks on the persistence of symptoms. Moreover, it is unclear whether the length of wheezing periods in pre-school children has a prognostic value for the development of asthma later in life.

Chronic coughing and shortness of breath:
In asthmatic schoolchildren, chronic coughing has been described as the 'cough variant asthma' [26]. In addition, in cases of chronic coughing in infants and pre-school children a diagnosis of 'cough variant asthma' is frequently made [27]. Minimal data exists on the relevance of chronic coughing in relation to the subsequent development of asthma later in life. The prevalence of chronic coughing in the absence of cold in pre-school children, recruited from the general population, was estimated to be 10% in the United States and 20 % in the UK [14, 28]. In one British study in pre-school children, recurrent coughing, defined as a positive answer to the question 'does your child usually cough without a cold?', was not associated with the development of wheezing, bronchial responsiveness, pulmonary function or peak flow variability after 3 years of follow-up [13, 29]. Although in this study nearly 60% was lost to follow up, these findings are in agreement with another prospective study in the USA, were no associations were found between frequent or chronic coughing in the first 2 years of life and the diagnosis of asthma at 10 years of age [14]. In the same study, a statistically significant association was found between reported shortness of breath at 1 to 2 years of age and the development of asthma at 10 years of age. This association was not significant for the 0 to 1 age-group despite an odds ratio of 4.8.
Little data is available concerning the prognostic value of a combination of respiratory symptoms in early life on the subsequent development of asthma. Dodge et al. found that the risk of developing asthma was significantly higher among infants with any one of three respiratory tract symptoms (frequent cough, wheezing without colds, or attacks of shortness of breath with wheeze) when they were compared with infants without any of these symptoms [14].

RESPIRATORY OUTCOME VARIABLES USED IN COHORT STUDIES

Many prospective birth cohort studies have been initiated to identify risk factors for the development of asthma. These studies can also be used to determine the prognostic values of respiratory symptoms in early childhood for the development of asthma later in life. From this perspective, it is interesting to evaluate which symptoms are used in these studies to define respiratory disease in the first 3 years of life. We performed a medline search using the following inclusion criteria: articles written in English between 1980 and 1999, prospective birth cohort study, respiratory symptoms (wheezing, cough, shortness of breath, dyspnea) and

Table 1: Outcome variables used to describe respiratory symptoms and disease in 9 prospective birth cohort studies designed to study the development of allergic disease.

Author	Year	Sub-jects	Follow-up (months)	Definition used for the respiratory illness	Type of information	Personnel who completed /obtained the information
Croner [38], Sweden	1982	1701	18	Obvious bronchial asthma = recurrent wheezing, observed by study doctor. Possible bronchial asthma = recurrent wheezing during infections or other Occasions.	Questionnaire Physical examination (only children with symptoms)	Parents Doctor
De Jong [30], The Nether-lands	1998	1482	6,12,24	Obvious asthma = $\geq$ 3 episodes of asthma or wheezing a year. Possible asthma = respiratory symptoms suggesting asthma 1 or 2 times a year (asthma not defined by authors)	Weekly symptom cart Questionnaire Physical examination	Parents Parents Doctor
Bergmann [31], Germany	1994	1239	1,3,6,12, 18,24	Obvious asthma = $\geq$ 2 episodes of wheezing with shortness of breath in first and second year. Possible asthma = not defined by authors.	Diary for symptoms Questionnaire Structured interview Physical examination	Parents Parents From parents, by doctor Doctor
Fergusson [34], New Zealand	1981	1180	12,24	Lower respiratory symptoms = medically treated bronchitis or pneumonia or > 1 episode of parental reported wheezing or chesty cold	Diary for symptoms Structured interview Hospital records	Parents From parents, by doctor Unclear
Arshad [36], UK	1992	1167	12,24	Asthma = $\geq$ 3 episodes of cough and wheezing in first year.	Observation Questionnaire Physical examination (only children with symptoms)	Study nurse Parents Doctor
Cushing [35], USA, Albequerque	1993	1051	0-18	Lower respiratory illness = $\geq$ 2 consecutive days of any upper respiratory symptoms and either wet cough or wheezing or both for at least 1 day	Diary for symptoms Telephone interview	Parents From parents, by nurse
Martinez [10], USA, Tucson	1992	826	0-36	Parents were instructed to contact SD in case of signs of respiratory illness (deep or wet chest cough, wheezing, hoarseness, stridor or shortness of breath). Lower respiratory tract illness with wheezing: if wheezing was found at physical examination	Questionnaire Structured interview and Physical examination	Parents Pediatricians of health maintenance organization
Lucas [32], UK	1990	777	9,18	Asthma or wheezing = diagnosis of asthma or $\geq$ 1 episode of wheezing in first 18 month. Asthma not defined by authors.	Structured interview and physical examination	Clinical scientists and doctor
Hansen [33], Denmark	1991	762	6,12,18	Definite asthma = Bronchial asthma (not further defined by author) in the first 18 months. Possible asthma = $\geq$ 2 episodes of wheezy bronchitis	Questionnaire Telephone interview Physical examination	Parents Unclear Pediatricians

SD = study doctor

Table 2: Information available in seven prospective cohort studies based on reviewing the original questionnaires

	Croner [38], Sweden	De Jong [30], The Netherlands	Bergmann [31], Germany	Fergusson [34], New Zealand	Arshad [36], UK	Martinez [10], Tucson, USA	Hansen [33], Denmark
Exact question on wheezing in questionnaire/interview	'Did your child have wheezy or noisy breathing or serious irritating cough or shortness of breath"	" Did your child have a wheezing respiration?"	"Did your child have a wheezing or whistling respiration?'	'Has your child visited a doctor/ hospital for chesty cold or wheezy chest?'	'Did the child have any wheezing episodes?'	'Did your child have wheezing?'	Unknown†
Information on number of wheezing episodes*	2 categories: less than 3 episodes or at least 3 episodes	Exact number of episodes recorded	Exact number of episodes recorded	7 categories, ranging from 0 to more than 10 episodes	3 categories: ranging from 0 to more than 3 episodes	Exact number of episodes recorded	3 categories: 0, 1-2 and 3 or more episodes
Information on duration of Wheezing episodes*	No	Yes	Yes	No	No	Yes	No
Information on symptoms of cold during wheezing episode*	Yes	Yes	Yes	No	No	Yes	Yes
Information on cough*	Yes	Yes	Yes	No	Yes	Yes	No
Information on shortness of breath*	Yes	Yes	Yes	No	No	Yes	No
Information on other respiratory symptoms or disease*	Rhinitis, sneezing, nasal obstruction, whooping cough, laryngitis	Noisy breathing, nasal discharge, fever	Noisy breathing, nasal discharge, fever, respiratory infections, bronchitis	Colds/flu, hay fever, upper airway infections, whooping cough	Rhinitis, chest infections	Colds/flu, fever, upper airway symptoms, bronchitis, bronchiolitis, pneumonia	Rhinitis
Information on doctors visit for any disease	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Information on respiratory Medication	No	Yes	Yes	Yes	Yes	Yes	Yes

* Information collected by a standard question in a questionnaire or structured interview, † Exact question unknown, diagnose based on interview: asthma = at least three episodes of wheezing requiring bronchodilator treatment and diagnosed by a physician. Recurrent wheezy bronchitis: at least 2 episodes of respiratory infections associated with wheezing, requiring bronchodilator treatment and diagnosed by a physician.

disease (asthma, obstructive disease, wheezing disorders, recurrent wheezing). Only studies containing published data on respiratory outcome after pre-school age or which stated that subjects will be followed-up for the development of asthma later in life, were included. We decided to review only studies in which the results are based on at least 750 subjects. We found nine studies that fulfilled these criteria and the characteristics of these studies are summarized in table 1. For each study only 1 article is mentioned as a reference, although most groups published more than one article. We have focussed on the outcome variables that were used to describe respiratory symptoms and disease in the first three years of life, the method and time interval of data collection, and relevant supplementary investigations. In addition, we asked the 9 corresponding authors to send us the original questionnaire or structured interview used in their study. In response, seven sent us their questionnaires.

Published outcome variables used to describe respiratory symptoms and disease:
All nine studies used wheezing as the most important symptom of respiratory disease and six used the term 'asthma' to describe these symptoms. Four studies provided an incomplete definition in the method section [30-33]. Three studies considered a single wheezing episode a year as a case [10, 32, 34], while in other studies this required more than 2 episodes [31, 33, 35] or more than 3 episodes [30, 36]. In one study, the frequency of wheezing episodes was not mentioned [37]. In the same study, obvious asthma was only diagnosed when symptoms were confirmed by a physical examination. Only one study defined the minimal duration of a wheezing episode [35]. Although most studies used wheezing as the only relevant symptom, one also included shortness of breath [31] and another study used coughing [36] as a criterion to be considered as a case. In a study from New Zealand, medically documented bronchitis or pneumonia was incorporated in the definition of lower respiratory illness [34]. In three studies, the presence or absence of signs of rhinitis or infection in relation to wheezing were considered as criteria [10, 35, 37].

Method of data collection:
Follow-up intervals varied from four times a year [31] to once in 18 months [37]. In one study, the follow-up period was a continuum, and subjects were seen during episodes of respiratory disease [10]. Eight out of nine studies used questionnaires completed by the parents and four studies used weekly or monthly symptom cards [30, 31, 34, 35]. In seven out of nine studies, the subjects were seen and interviewed by a study doctor or nurse [10, 30-33, 36, 38], the other three studies relied on questionnaires or medical records only. In two of the seven studies in which a physical examination was performed, the children were examined only when they had signs and symptoms of atopic disease [36, 37], while in the remaining five studies all were examined.

Relevant supplementary investigations:
Lung function measurements were performed in only 1 of the 9 studies [10]. Viral cultures were taken in 2 studies [10, 35].

Information collected on respiratory symptoms and disease, based on review of the original questionnaires or structured interviews:
Table 2 summarizes the data on respiratory symptoms or disease in early childhood that were actually collected by questionnaire or structured interview in seven prospective cohort studies

on the development of asthma. All studies collected data on the number of wheezing episodes, and the categories are generally comparable. Data on coughing were available in five out of seven studies. Information on shortness of breath and symptoms of cold during wheezing episodes was less extensively collected. All studies collected data on symptoms of cold or rhinitis independent of wheezing episodes and most studies also gathered information about lower respiratory tract infections. Six out of seven studies collected data on respiratory medication. Finally, in all seven studies the study doctor or nurse recorded additional information about the respiratory health of the child in a non-structured fashion.

CONCLUSIONS

Recent birth cohort studies have contributed greatly to our understanding of the natural history and prognosis of respiratory symptoms and disease in early childhood, but many issues are still unresolved. It is now well established that early wheezing is associated with asthma later in life, but the majority of wheezing infants will not develop asthma. The relative prognostic value of the frequency or duration of wheezing episodes, and the significance of the absence or presence of signs of common cold during wheezing episodes in pre-school children for the development of asthma later in life needs further study. Chronic or persistent cough in pre-school children seems to be a poor predictor of the development of asthma later in life. The definitions and terminology that have been used in publications to describe lower respiratory symptoms and disease in early childhood differed widely between large prospective birth cohort studies. In addition, the methods used to collect data differed between studies. This may contribute, to a large extent, to the considerable variation reported in the prevalence and incidence rates for respiratory symptoms and disease in infancy and early childhood. Only a limited part of the large data sets of recent studies has been published. However, based on review of the original questionnaires, the various studies are considerably more comparable than can be concluded from the published literature. Pooling data from different cohort studies could therefore be attempted to investigate the prospective value of different respiratory symptoms in early life that might determine the risk of later asthma. This would be useful for defining the criteria for respiratory symptoms in early life that can be used for future birth cohort studies.

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References

1. Anonymous. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee . Lancet 1998;351:1225-32.
2. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
3. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med 1994;149:358-64.

4. Weis KB GP. The epidemiology of asthma. Philidelphia: Lipincott-Raven; 1997.
5. Burr ML. Diagnosing asthma by questionnaire in epidemiological surveys. Clin Exp Allergy 1992;22:509-10.
6. Silverman M. Clinical diagnosis and assesment in infants. Philidelphia: Lipincott-Raven; 1997.
7. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am J Epidemiol 1989;129:1232-46.
8. Williams H, McNicol KN. Prevalence, natural history, and relationship of wheezy bronchitis and asthma in children. An epidemiological study. Br Med J 1969;4:321-5.
9. Anderson HR, Pottier AC, Strachan DP. Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. Thorax 1992;47:537-42.
10. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates . N Engl J Med 1995;332:133-8.
11. Martinez FD. Recognizing early asthma. Allergy 1999;54:24-8.
12. Tariq SM, Matthews SM, Hakim EA, Stevens M, Arshad SH, Hide DW. The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. J Allergy Clin Immunol 1998;101:587-93.
13. Brooke AM, Lambert PC, Burton PR, Clarke C, Luyt DK, Simpson H. The natural history of respiratory symptoms in preschool children. Am J Respir Crit Care Med 1995;152:1872-8.
14. Dodge R, Martinez FD, Cline MG, Lebowitz MD, Burrows B. Early childhood respiratory symptoms and the subsequent diagnosis of asthma. J Allergy Clin Immunol 1996;98:48-54.
15. Sporik R, Holgate ST, Cogswell JJ. Natural history of asthma in childhood--a birth cohort study. Arch Dis Child 1991;66:1050-3.
16. Park ES, Golding J, Carswell F, Stewart-Brown S. Preschool wheezing and prognosis at 10. Arch Dis Child 1986;61:642-6.
17. Aberg N, Engstrom I. Natural history of allergic diseases in children. Acta Paediatr Scand 1990;79:206-11.
18. Anderson HR, Bland JM, Patel S, Peckham C. The natural history of asthma in childhood. J Epidemiol Community Health 1986;40:121-9.
19. Blair H. Natural history of wheezing in childhood. J R Soc Med 1979;72:42-8.
20. Foucard T, Sjoberg O. A prospective 12-year follow-up study of children with wheezy bronchitis. Acta Paediatr Scand 1984;73:577-83.
21. Wennergren G, Hansson S, Engstrom I, et al. Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. Acta Paediatr 1992;81:40-5.
22. Johnstone DE. A study of the natural history of bronchial asthma in children. Am J Dis Child 1968;115:213-6.
23. Buffum WP, Settipane GA. Prognosis of asthma in childhood. Am J Dis Child 1966;112:214-7.
24. Gergen PJ, Turkeltaub PC, Kramer RA. Age of onset in childhood asthma: data from a national cohort. Ann Allergy 1992;68:507-14.
25. Blair H. Natural history of childhood asthma. 20-year follow-up. Arch Dis Child 1977;52:613-9.
26. Corrao WM, Braman SS, Irwin RS. Chronic cough as the sole presenting manifestation of bronchial asthma. N Engl J Med 1979;300:633-7.
27. Holinger LD, Sanders AD. Chronic cough in infants and children: an update. Laryngoscope 1991;101:596-605.
28. Luyt DK, Burton PR, Simpson H. Epidemiological study of wheeze, doctor diagnosed asthma, and cough in preschool children in Leicestershire. Bmj 1993;306:1386-90.
29. Brooke AM, Lambert PC, Burton PR, Clarke C, Luyt DK, Simpson H. Recurrent cough: natural history and significance in infancy and early childhood. Pediatr Pulmonol 1998;26:256-61.
30. de Jong MH, Scharp-van der Linden VT, Aalberse RC, Oosting J, Tijssen JG, de Groot CJ. Randomised controlled trial of brief neonatal exposure to cows' milk on the development of atopy . Arch Dis Child 1998;79:126-30.
31. Bergmann RL, Bergmann KE, Lau-Schadensdorf S, et al. Atopic diseases in infancy. The German multicenter atopy study (MAS-90). Pediatr Allergy Immunol 1994;5:19-25.
32. Lucas A, Brooke OG, Morley R, Cole TJ, Bamford MF. Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. Bmj 1990;300:837-40.
33. Hansen LG, Host A, Halken S, et al. Cord blood IgE. II. Prediction of atopic disease. A follow-up at the age of 18 months. Allergy 1992;47:397-403.
34. Fergusson DM, Horwood LJ, Shannon FT, Taylor B. Parental smoking and lower respiratory illness in the first three years of life. J Epidemiol Community Health 1981;35:180-4.

35. Cushing AH, Samet JM, Lambert WE, et al. Breastfeeding reduces risk of respiratory illness in infants. Am J Epidemiol 1998;147:863-70.
36. Arshad SH, Hide DW. Effect of environmental factors on the development of allergic disorders in infancy. J Allergy Clin Immunol 1992;90:235-41.
37. Croner S, Kjellman NI, Eriksson B, Roth A. IgE screening in 1701 newborn infants and the development of atopic disease during infancy. Arch Dis Child 1982;57:364-8.
38. Croner S, Kjellman NI. Natural history of bronchial asthma in childhood. A prospective study from birth up to 12-14 years of age. Allergy 1992;47:150-7.

Chapter 5

Patients and methods

PATIENTS AND METHODS

The backbone of this thesis is formed by data from the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)- study (section 6.1., 7.1., 7.2., 8.1. and 9.1.). The study design of the PIAMA-study will be discussed in detail in paragraph 5.1. Data from the Virus Mediated Allergy (acronym in Dutch, VIGALL)-study is used in section 6.1. and 8.2, and the study-design of this study will be briefly discussed in paragraph 5.2.

5.1. PIAMA STUDY DESIGN

In 1992, the Public Health Council of the Netherlands published a report entitled 'Indoor allergens, allergy, and the development of chronic respiratory diseases'[1]. A central question in this report was whether early reduction of indoor allergen exposure could prevent or delay the subsequent development of inhalant allergies and asthma in children. In order to answer this question, a prospective, randomized, and placebo-controlled trial was designed: the Intervention part of the PIAMA-study. Parallel to the Intervention part of the PIAMA-study, the National Institute of Public Health and the Environment (RIVM) initiated the Natural History part of the PIAMA-study. The primary aim of this part of the study was to investigate the incidence of allergic disease in childhood in The Netherlands and to evaluate the role of environmental and dietary risk factors for the development of allergic disease.
The PIAMA-study is a multi-center study, conducted in the north (Groningen and surroundings), center and south-west (greater Rotterdam area) of the Netherlands. The recruitment of the PIAMA-study participants is illustrated by figure 5.1.

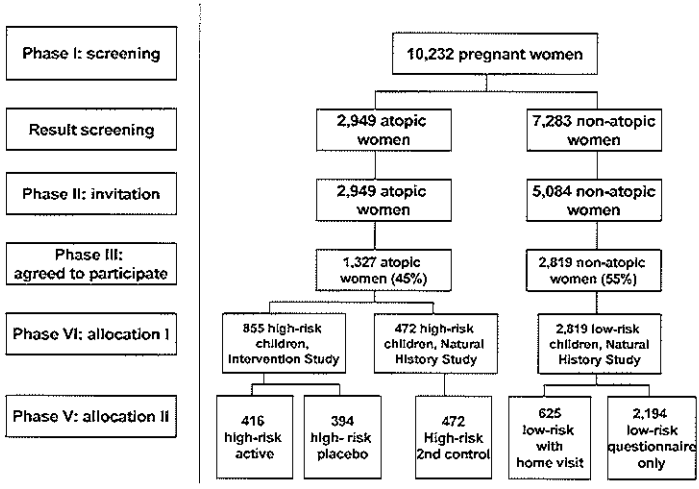

Figure 5.1: Recruitment-scheme PIAMA-study

Recruitment took place between March 1996 and May 1997, and the children were born between May 1996 and December 1997. In the second trimester of pregnancy, expecting mothers were asked to complete a validated screening questionnaire on asthma and allergy when they visited the prenatal health clinic [2]. Women with self-reported asthma, hay-fever, house dust mite allergy, or pet allergy were considered to be allergic, and their children were defined as high-risk children. Women who did not report these symptoms were considered to be non-allergic, and their children were defined as low-risk children. All allergic pregnant women, and a random sample of the non-allergic pregnant women were invited to participate in the PIAMA-study, and approximately 50% agreed and gave informed consent (n = 4,146). Approximately two-third of the high risk children were randomly allocated into an intervention part of the PIAMA-study, and one-third was allocated into the natural history part of the PIAMA-study. All low-risk children were allocated to the natural history part of the study. In figure 5.2, the observation scheme of both the intervention study (IS) and the natural history study (NHS) is summarized.

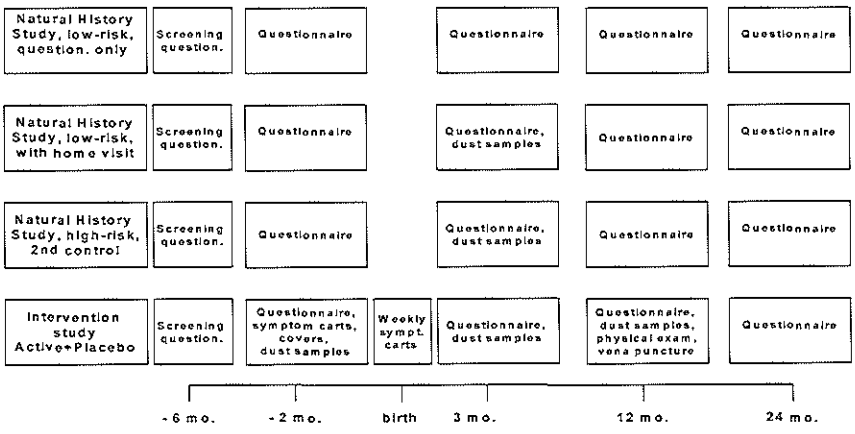

Figure 5.2: PIAMA observation scheme

Intervention and dust sampling:

In 45 of the 855 families who had signed informed consent no intervention took place due to loss to follow-up in the period between completing the screening questionnaire and conducting the intervention. The intervention consisted of the application of mattress covers for the infant and parents' bed and covers for the parents' pillows. The active group received HDM-allergen impermeable mattress covers (Acb™, allergy control products®, USA). The placebo group received cotton placebo covers, made by a social work place. Both the investigator and the parents were unaware of group assignment. Dust samples of the infant- and parents' mattress were collected at the time the mattress covers were provided, and 3 months after birth. The planned sample size of the IS was 800 (400 intervention group, 400 placebo group). This was based on a power calculation, assuming a drop out of 50% during the study period of 8 years, a cumulative incidence of allergic disease of 50% in the placebo group, and assuming a

1.5-fold reduction in incidence in the active group (33% cumulative incidence in the active group).
No intervention was performed in families participating in the NHS. Dust samples were taken at 3 months of age in all the high-risk children participating in the NHS and a random sample of the low-risk children.

Physical examination and blood collection:
At age 1, all children participating in the IS underwent an examination of the skin for signs of visible dermatitis and parents were interviewed on skin symptoms of their babies. A vena puncture was performed in order to collect serum for allergy tests. The definition of atopic dermatitis was made in accordance with the UK Working Party's Criteria for Atopic Dermatitis [3]. In short, children were considered having atopic dermatitis when they have had an itchy skin condition in the previous year (obligatory), together with at least 3 of the 4 following criteria:

- History of atopic disease in at least 1 parent.
- History of dermatitis of the skin creases (e.g. elbow folds, behind the knees, front of ankles or neck).
- History of general dry skin
- Visible dermatitis in the elbow folds, behind the knees, front of the ankles, lower part of the leg, forearm, around the eyes, on the cheeks, or front of the neck.

Questionnaires: outcome variables and covariables:
The parents completed questionnaires at the following time points: second trimester of pregnancy (screening questionnaire), third trimester of pregnancy (pregnancy questionnaire), at 3 months, 12 months and 24 months of age.
Pregnancy questionnaire: data was collected on duration of pregnancy, parental age, environmental tobacco smoke exposure, paternal allergy [2], maternal diet, maternal medication, and pet ownership.
Weekly symptom cards: families participating in the IS completed a diary to record weekly occurrence of fever-, respiratory-, skin- and gastrointestinal symptoms in the first 12 months of life.
3-months questionnaire: birth characteristics, respiratory- and skin symptoms in the first 3 months of life, infants feeding, pet ownership, housing characteristics, indoor environmental factors, environmental tobacco smoke exposure (ETS), number of siblings, and allergic-, respiratory- and skin symptoms of the parents and siblings. The assessment of ETS by questionnaire correlated well with nicotine concentrations measured in living room air [4].
12-months questionnaire: anthropometric data, data on vaccinations, respiratory- and skin symptoms, doctor diagnosed eczema, -asthma, -upper and lower respiratory tract infections in the first 12 months of life, food allergy, infant feeding, day care attendance, ETS, parental employment, parental level of education.
24-months questionnaire: anthropometric data, respiratory- and skin symptoms, doctor diagnosed eczema, -asthma, -upper- and lower respiratory tract infections in the previous 12 months, food allergy, infant feeding, day care attendance, ETS, ethnical background of the parents

The data of all questionnaires have been entered by a professional data entry company in duplicate. A difference between two data entries of 0.5% was accepted. Extensive data cleaning procedures were conducted in order to get the most reliable and complete data set possible.

Follow-up:
In this thesis, data of the first 2 years of life have been used. Of the 4,146 included families, 4,034 (97%) were still participating with the study at age 1, and 3,879 (93%) at age 2. At the end of 2001, dust samples will have been collected, and a physical examination at age 4 will have been performed in all children of the IS, all high-risk children of the NHS and all low-risk children of the NHS were dust samples were taken at age 3 months. The physical examination includes lung function tests such as measurement of exhaled nitric oxide (eNO), airway resistance (Rint) and peak flow. In addition, blood will be collected and the presence dermatitis will be examined. The next evaluation of the total cohort is planned in 2003-2004, when all children are 8 years. Between 3 and 8 years questionnaires on the development of allergic symptoms will be send to the parents on a yearly basis.

5.2. VIGALL STUDY-DESIGN

The aim of the VIGALL –study is to investigate the effect of viral upper airway infections in early life on the development of the infants' immune system, and the development of allergic disease. The VIGALL study is a prospective birth cohort study, and the design is summarized in figure 5.3. Recruitment of children around the time of their birth took place between August 1996 and November 1998.

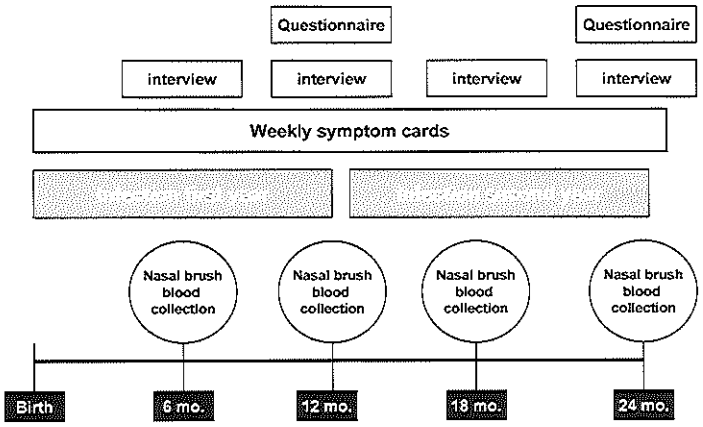

Figure 5.3: VIGALL study design

In total, 129 children were included in the study, of which 52 (40%) also participated in the PIAMA-study. Children with at least 1 allergic parent (high risk children) were over-sampled:

89 high risk children (69%) versus 40 low risk children. Starting from 6 months of life, the children were examined routinely every 6 months until age 2. During these visits, a physical examination took place, with special focus on upper airway symptoms, symptoms of airway obstruction, and skin symptoms suggestive for atopic dermatitis (using the same protocol as was used in the PIAMA-study [3]). In addition, blood was taken and mucosal cells from the naso-pharynx were harvested with the nasal brush technique as previously described by Godthelp et al. [5]. The nasal brush cells were also used for viral isolations. Besides the half yearly routine visits, parents were asked to contact the investigators when their child had signs of an acute upper airway infection (runny nose with at least one of the following accompanying symptoms: fever, malaise, sleeping difficulties or loss of appetite). During this acute infection and 2 weeks after the infection (convalescent phase), the same procedures were performed as during the routine visits.

Parents completed weekly symptom cards and underwent a structured interview each half year in order to obtain detailed information on the number and duration of upper airway infections in the first 2 years of life. Questionnaires at age 1 and 2 (similar as the PIAMA-questionnaires) and half-yearly structured interviews provided information on the development of symptoms of allergic disease and the distribution of various cofactors.

From the 129 children included around birth, 129 (100%) were still participating at 6 month, 110 (85%) at 12 months, 87 (67%) at 18 months, and 83 (64%) at 24 months of age. Similar as the children participating in the PIAMA-study, the children in the VIGALL-study will be followed-up at the age of 4 years and 8 years in order to assess the development of allergic disease.

References:

1. Indoor allergens, allergy, and the development of chronic respiratory diseases. The Hague, The Netherlands: Health Council of The Netherlands; 1992.
2. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
3. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PG. A protocol for recording the sign of flexural dermatitis in children [see comments]. Br J Dermatol 1995;133:941-9.
4. Brunekreef B, Leaderer BP, van Strien R, et al. Using nicotine measurements and parental reports to assess indoor air: the PIAMA birth cohort study. Prevention and Incidence of Asthma and Mite Allergy. Epidemiology 2000;11:350-2.
5. Godthelp T, Holm AF, Fokkens WJ, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97:800-11.

Chapter 6

Markers of allergic disease

6.1. Increased serum IL-10/IL-12 ratio in wheezing infants

L.P. Koopman[1], H. Savelkoul[2], I.J. van Benten[3], J. Gerritsen[4], B. Brunekreef[5] and H.J. Neijens[1]

[1]Erasmus University Medical Center Rotterdam, Sophia Children's Hospital, Department of Pediatrics, [2]Wageningen University, Department of Cell Biology, [3]Erasmus University Medical Center Rotterdam, Department of Otorhinolaryngology, [4]Groningen University, Beatrix Children's Hospital, Department of Pediatrics, [5]University of Utrecht, Institute for Risk Assessment Sciences, Department of Environmental and Occupational Health, The Netherlands

ABSTRACT

Objective: To investigate the association between various serum markers and atopic symptoms in the first year of life, and to evaluate the prognostic value of these markers for the development of wheezing and skin rash in the second year of life.
Methods: Data of 86 children on the development of wheezing and skin rash in the first two years of life was collected prospectively, making use of parental completed questionnaires, weekly symptom cards, structured interview and physical examination. Serum markers (IL-10, IL-12, IL-13, eotaxin, sE-selectin, sICAM-1, sIL-2R) and total and- specific IgE were determined at age 1.
Results: Children who developed wheezing in the first year of life had lower serum levels of IL-12 than children without symptoms (median 40.3 pg/ml versus 49.0 pg/ml, $p = 0.01$) and a higher serum IL-10/IL-12 ratio (0.41 versus 0.31, $p = 0.001$) at age 1. The IL-10/IL-12 ratio increased with an increasing number of wheezing episodes. Levels of sE-selectin in children with wheezing and in children with itchy skin rash in the first year of life were higher than in symptom free children (6.1 ng/ml and 5.9 ng/ml versus 4.9 ng/ml, $p = 0.01$ and $p = 0.03$ respectively). Children who developed wheezing in the second year of life already had increased sICAM-1 levels at age 1.
Conclusion: children who developed wheezing in the first year of life showed a serum cytokine response that is skewed towards a T-helper 2 profile, with lower IL-12 levels and an increased IL-10/IL-12 ratio. Children who developed wheezing in the second year of life had elevated sICAM-1 levels at age 1. Follow-up of the children is needed to evaluate the prognostic value of various serum markers for the development of allergic disease in later childhood.

Submitted

INTRODUCTION

The diagnosis of atopic eczema and asthma in early childhood is difficult [1, 2]. This is partly due to the incomplete understanding of the involved patho-physiological mechanisms [3, 4]. In addition, in young children, asthmatic symptoms are impossible to distinguish from respiratory symptoms caused by airway infection or symptoms associated with small airway caliber [2]. Traditional immunological markers, such as eosinophils counts and total and-specific IgE reflect allergic disease in early childhood insufficiently [5]. Therefore, the identification of other markers might be useful for supporting the diagnosis, prediction and clinical evaluation of allergic disease in early childhood. To be of clinical use, the markers should be easily measurable by routine laboratories in small quantities of blood or serum. Various potential markers for allergic disease have been identified, but most studies have been performed on school-age children and adults. An imbalance between CD4+ helper T cell subsets (Th1 cells and Th2 cells) in favor of the Th2 subtype, is thought to contribute to allergic disease [6]. Interleukin (IL)-4 is an important cytokine steering the outgrowth of Th2 cells, while IL-10 and IL-13 are considered to be signature Th2 cytokines. IL-12, on the other hand, plays an important role in driving the immune response towards a Th-1 direction, which by the release of interferon (IFN)-γ counteracts Th2 activity in vivo [7]. Eotaxin is an important mediator of allergic inflammation by recruiting eosinophils, basophils and Th-2 cells [7]. Adhesion molecules such as E-selectin and intra-cellular adhesion molecule I (ICAM-1) are preferentially expressed on endothelial cells, and their production is up-regulated during local inflammation in response to various cytokines [8]. These adhesion molecules are shed into the circulation in a soluble form: sICAM-1 and sE-selectin. Another potential marker is soluble IL-2 receptor (sIL-2R), which is a marker of T-cell activation [9]. We investigated the association between serum levels of IL-10, IL-12, IL-13, eotaxin, sE-selectin, sICAM-1, and sIL-2R assessed at age 1 on the one hand and the development of wheezing and itchy skin rash in the first year of life on the other hand. We also investigated the prospective value of the aforementioned markers on the development of wheezing and skin rash in the second year of life.

PATIENTS & METHODS

Study population:
Eighty-six children were randomly selected from 2 ongoing prospective birth cohort studies: the PIAMA-study and the VIGALL-study. The PIAMA (Prevention and Incidence of Asthma and Mite Allergy)- study (n ~ 4000) focuses on identifying risk factors for the development of atopic disease, with special interest on the role in house dust mite exposure. The VIGALL (Virus Mediated Allergy)-study was designed to investigate the role of virus infections on the maturation of the immune system in children (n = 129). Details of the study design of both studies have been published elsewhere [10, 11]. In both studies, children with allergic parents were over sampled. Children were born between August 1996 and October 1998 in greater Rotterdam area, The Netherlands. Both studies were approved by the medical ethical committee of the University Hospital Rotterdam.

Data collection:
The development of wheezing and skin rash was assessed by using questionnaires at age 1 and 2 [12], a structured interview at age 1 and parental completed weekly symptom cards. Wheezing was defined as at least 1 reported episode of wheezing in the previous year, recurrent wheezing was defined as at least 4 episodes of wheezing. Skin rash was defined as parental reported itchy skin rash on localization typical for eczema (e.g. around the ears or eyes, on the neck, front of the ankle or in the knee or- elbow folds). In order to assess visible dermatitis, a physical examination was performed by the study doctor (LK) at age 1. Together with a structured interview, the diagnosis of atopic dermatitis was made, according to the UK working Party's Diagnostic Criteria for Atopic Dermatitis [1]. Information on duration of pregnancy, birth weight, gender, type of feeding, environmental tobacco smoke exposure, having siblings and day care was collected by using questionnaires. Allergy in the parents was assessed by using a validated questionnaire [13].

Laboratory procedures:
Blood was collected by vena puncture at age 1. After centrifugation, the serum was stored at – 20 °C until analysis. IL-10, IL-12, IL-13, eotaxin, sE-selectin, sICAM-1, and sIL-2R were measured with commercially available ELISA-kits (Biosource, Nivelle, Belgium). All measurements were performed in duplicate dilutions according to the manufacturer instructions. Total IgE at age 1 was measured as described previously by Stallman and Aalberse [14] and specific IgE against HDM, cat, dog, and food-allergens was determined with a radioallergosorbent fluorescent immunoassay [15].

Data analysis:
Statistical analyses were performed by SPSS (Version 10.0, Chicago, USA). Pearson χ^2 tests were used to compare categorical data, and Mann-Whitney U tests and Kruskal-Wallis tests were used to assess differences in the concentration of various markers between the groups. The Spearman rank correlation was used to assess the interdependent relation of the various markers, including total IgE. Statistical significance was defined at $p < 0.05$. For each individual child, the ratio of IL-10 and IL-12, as well as the ratio of IL-13 and IL-12 was calculated.

RESULTS

Study population:
From the 86 children, 38 (44%) developed neither wheezing nor skin rash, 22 (25%) only wheezing, 18 (21%) only skin rash, and 8 (9%) both wheezing and skin rash in the first year of life. Of the 30 children with wheezing in the first year of life, 15 (17% of the total group) experienced 4 or more episodes of wheezing. No child wheezed at the time of vena puncture. From the 26 children with an itchy skin rash during the first year of life, 8 (9% of the total group), had visible dermatitis at the time of vena puncture, and thereby fulfilled the strict criteria of atopic dermatitis [1].

General characteristics:
The general characteristics of the study population, stratified for wheezing and itchy rash in the first year of life, are summarized in table 1. Children with both wheezing and itchy skin rash more often had an allergic parent, had a lower birth weight, were less often breastfed, and were more often exposed to environmental tobacco smoke, than children without symptoms, although the differences between the four symptom groups did not reach statistical significance (Pearson χ^2 -test). No differences were seen in total IgE and specific IgE (mainly against cow's milk) between the symptom groups.

Table 1: general characteristics of the study population, stratified for wheezing and atopic eczema at age 1:

Characteristic	Wheeze -/ skin rash - (n=38)	Wheeze +/ skin rash - (n=22)	Wheeze -/ Skin rash + (n=18)	Wheeze +/ skin rash + (n=8)	Total (n=86)
Allergic parent (%)	76	95	83	100	85
Duration of pregnancy (mean weeks, SD)	39.7 (1.4)	39.6 (1.7)	39.1 (2.5)	38.2 (3.4)	39.4 (2.0)
Birth weight (g, SD)	3433 (386)	3494 (526)	3258 (667)	3196 (754)	3391 (530)
Male gender (%)	43	27	41	37	38
Exclusive breastfeeding at 3 months (%)	21	14	13	12	17
Smoke exposure at age 1 (%)	18	23	22	37	22
Having siblings (%)	58	41	40	62	50
No day care (%)	23	25	53	37	31
Small day care* (%)	42	40	27	50	39
Large day care* (%)	35	35	20	13	30
Total IgE age 1 (IU/ml, geometric mean, geometric SD)	9.3 (3.8)	5.9 (2.9)	6.3 (2.6)	9.2 (2.6)	7.6 (3.2)
Child RAST $\geq$ 1 at age 1	21	9	28	12	19

* small day care was defined as attending day care settings with less than 5 other children for at least 4 hours a week, large day care was defined as attending day care settings with at least 10 other children for at least 4 hours a week.

Serum markers at age 1 in relation to wheezing and skin rash in the first year of life:
Children with wheezing in the first year of life showed lower levels of IL-12 ($p = 0.01$) and a higher IL-10/IL-12 ratio ($p = 0.001$) than children without wheezing and skin rash (table 2). Figure 1 shows the relation between the number of wheezing episodes in the first year of life and the corresponding IL-10/IL-12 ratio (analysis were performed irrespective of having skin rash). The IL-10/IL-12 ratio increased with an increasing number of wheezing episodes, and a highly significant difference in IL-10/IL-12 ratio was observed between children with recurrent wheezing and no wheezing ($p = 0.009$). Children with only wheezing and children with only an itchy skin rash in the first year of life had higher levels of sE-selectin at age 1 than children without these symptoms (table 2). When the children with an itchy skin rash without visible dermatitis ($n = 22$) were compared to children with visible dermatitis ($n = 8$), no statistically significant difference in sE-selectin was found (median 6.5 ng/ml versus 5.9 ng/ml). No differences in IL-10, IL-13, the IL-13/IL-12 ratio, eotaxin, sICAM-1, and sIL-2R between the four symptom groups were observed. The same pattern was seen when analyzing the children with visible dermatitis separately (data not shown). No association was found between elevated total IgE levels and specific IgE against food or inhalant allergens at age 1 and the various markers that were studied (data not shown).

Table 2: Serum markers at age 1 in relation to wheezing and skin rash in the first year of life. Results are expressed as median (range).

	Wheeze -/ skin rash - (n=38)	Wheeze +/ skin rash - (n=22)	Wheeze -/ skin rash + (n=18)	Wheeze +/ skin rash + (n=8)	p-value for trend[¶]
IL-10 (pg/ml)	15.6 (4-23)	15.8 (13-25)	14.7 (11-25)	16.4 (13-21)	0.44
IL-12 (pg/ml)	49.0 (22-90)	40.3 (25-107)*	49.7 (24-116)	54.8 (30-78)	0.02
IL-13 (pg/ml)	2.3 (0.5-31)	2.8 (0.5-15.1)	2.6 (0.5-34)	2.2 (2-6)	0.97
IL-10/IL-12 ratio	0.31 (0.1-0.6)	0.41 (0.2-0.7)[‡]	0.31 (0.1-0.7)	0.28 (0.2-0.5)	0.003
IL-13/IL-12 ratio	0.045 (0.01-1.1)	0.056 (0.01-0.6)	0.046 (0.01-1.41)	0.040 (0.01-0.2)	0.92
Eotaxin (pg/ml)	97.9 (47-204)	97.9 (57-255)	86.4 (55-187)	95.1 (76-175)	0.59
sE-selectin (ng/ml)	4.9 (0.1- 11)	6.1 (4-10)*	5.9 (0.8-11)[†]	4.4 (3-11)	0.01
SICAM (ng/ml)	69.9 (21-124)	70.3 (52-109)	65.7 (11-105)	64.9 (40-134)	0.76
sIL-2R (ng/ml)	73.4 (26-290)	91.4 (46-206)	70.4 (49-204)	90.4 (43-179)	0.52

[¶] Kruskal-Wallis test, * p = 0.01 for difference between wheeze -/skin rash - and wheeze +/skin rash -, Mann-Whitney U test, [†] p = 0.03 for difference between wheeze -/skin rash and wheeze -/skin rash +, Mann-Whitney U test, [‡] p = 0.001 for difference between wheeze -/skin rash - and wheeze +/skin rash -, Mann-Whitney U test

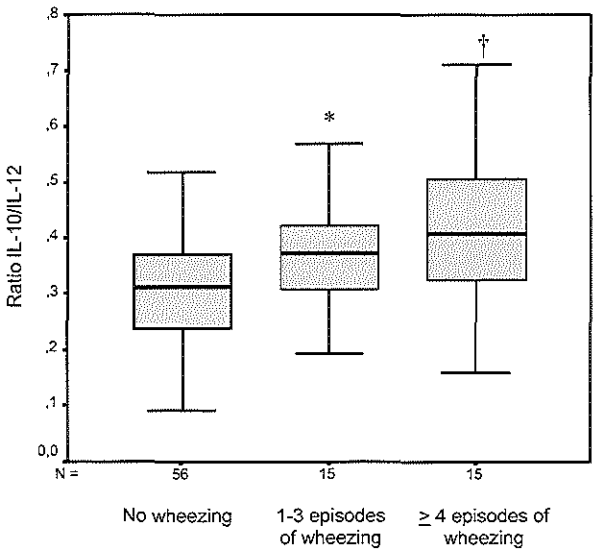

Figure 1: Box-plot of IL-10/IL-12 ratio in association with frequency of wheezing first year of life (p = 0.01 for trend, Kruskal-Wallis test). Solid lines in box represent median, boxes represent the 25th and 75th percentile, and lines represent 5th and 95th percentile. * p = 0.06 for difference between no wheezing and 1-3 episodes of wheezing, Mann-Whitney U test. [†] p = 0.009 for difference between no wheezing and ≥ 4 episodes of wheezing, Mann-Whitney U test

The prognostic value of serum markers at age 1 for the development of symptoms in the second year:

Information on the prevalence of wheezing and skin rash in the second year of life was available for 79 (92%) children. A higher IL-10/IL-12 ratio was found in children with wheezing only in the first year of life compared to children who never wheezed (table 3). Children who

developed wheezing in the second year of life (and did not wheeze in the first year of life) had significantly higher levels of sICAM at age 1 compared to children who never wheezed. Children with persistent wheezing (both in the first and second year of life), had higher levels of sIL-2R. No statistically significant differences in levels of serum markers were observed between children without itchy skin rash, skin rash only in the first year, skin rash only in the second year and skin rash both in the first and second year (data not shown).

Table 3: Serum markers at age 1 in relation to wheezing in the first 2 years of life. Results are expressed as median (range).

	Never wheeze (n = 44)	Wheeze only 1st year (n = 18)	Wheeze only 2nd year (n = 7)	Wheeze 1st & 2nd year (n = 10)	p-value for trend
IL-10 (pg/ml)	15.4 (9-25)	15.8 (13-25)	14.6 (4-19)	15.7 (13-19)	0.35
IL-12 (pg/ml)	48.7 (22-116)	40.35 (25-107)	49.3 (36-67)	45.1 (28-61)	0.16
IL-13 (pg/ml)	2.8 (0.5-34)	2.8 (0.5-15)	1.6 (0.6-31)	1.8 (1-9)	0.48
IL-10/IL-12 ratio	0.31 (0.1-0.7)	0.41 (0.2-0.7)‡	0.26 (0.1-0.5)	0.37 (0.3-0.6)	0.02
IL-13/IL-12 ratio	0.048 (0.01-1.4)	0.050 (0.01-0.6)	0.042 (0.01-0.47)	0.051 (0.03-0.33)	0.82
Eotaxin (pg/ml)	93.0 (47-204)	112 (57-255)	80.3 (52-130)	86.2 (75-164)	0.32
sE-selectin (ng/ml)	5.3 (0.1-11)	5.2 (5-11)	5.8 (5-11)	6.3 (4-10)	0.36
SICAM (ng/ml)	66.5 (11-124)	69.7 (47-134)	86.3 (66-103)*	61.0 (40-95)	0.19
sIL-2R (ng/ml)	71.7 (26-290)	85.0 (46-179)	86.2 (51-204)	143.7 (67-206)†	0.03

* p = 0.03 for difference between never wheeze and wheeze only 2nd year, † p = 0.004 for difference between never wheeze and wheeze in 1st and & second year, ‡ p = 0.02 for difference between never wheeze and wheeze 1st year, Mann-Whitney U test

Interdependent relationship of various serum markers:

For the whole group, a strong correlation was found between sE-selectin and sICAM-1 (figure 2a) and between sE-selectin and sIL-2R (figure 2b).

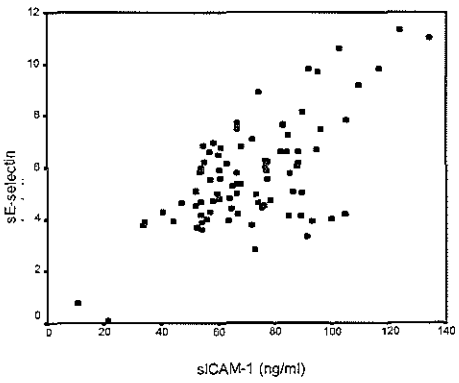

Figure 2a: Correlation between sE-selectin and sICAM-1, Spearman's rho= 0.47, p < 0.001

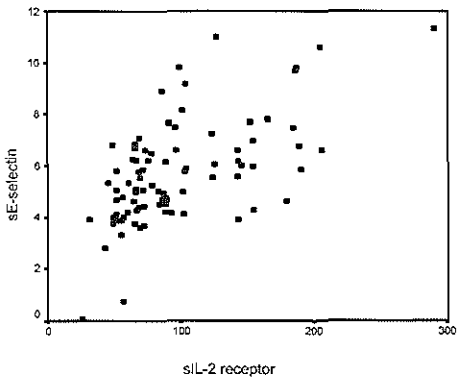

Figure 2b: Correlation between sE-selectin and sIL-2 receptor, Spearman's rho= 0.58, p < 0.001

Also a significant correlation was found between sE-selectine and IL-12 (rho = - 0.31, p = 0.004), sIL-2R and IL-13 (rho = - 0.26, p = 0.01), sIL-2R and sICAM-1 (rho = 0.23, p = 0.03) as well as IL-10 and IL-12 (rho = 0.27, p = 0.01). A correlation of borderline statistical

significance was found for IL-13 and total IgE (rho = 0.22, p = 0.05). In children with wheezing in the first year of life (n = 30), associations tended to be stronger than for the whole group: sE-selectin and sICAM-1; rho = 0.55, p = 0.002, sE-selectine and IL-12; rho = - 0.48, p = 0.008. The same pattern was seen in children with itchy skin rash (n = 24): sE-selectin and sICAM-1; rho = 0.64, p = 0.001, sE-selectin and IL-12; rho = - 0.52, p = 0.009, sIL-2R and IL-10; rho = - 0.53, p = 0.008, sIL-2R and IL-12; rho = 0.48, p = 0.01. The strongest correlation between sE-selectin and sICAM-1 was seen in the children with itchy skin rash with visible dermatitis (n= 8): rho = 0.95, p < 0.001.

Relation between serum markers and various potential confounders:
Girls had higher levels of sIL-2R than boys (median 87.5 ng/ml versus 69.2 ng/ml, p = 0.05). Children with allergic parents had higher sE-selectin levels (median 5.8 ng/ml versus 4.8 ng/ml, p = 0.03) and higher eotaxin levels (median 95.9 ng/ml versus 81.2 ng/ml, p = 0.05) than children with non-allergic parents. Children with siblings had lower levels of IL-10 (median 14.7 pg/ml versus 16.2 pg/ml, p = 0.03) than children without siblings. Children who attended small day care settings (contact with 1-5 children) had lower levels of IL-10 than children who did not attend day care or children who attended large day-care (contact with > 10 children): median 15.1 pg/ml versus 16.6 pg/ml versus 15.9 pg/ml respectively, p = 0.02 for difference between small day-care and no day-care). No association was found between duration of pregnancy, birth weight, having siblings, exposure to environmental tobacco smoke, type of feeding, and serum markers.

DISCUSSION

Using data from 2 prospective birth cohort studies, we evaluated the association between various serum markers and the development of wheezing and itchy skin rash in the first 2 years of life. We found that children who developed wheezing in the first year of life had lower serum levels of IL-12 and a higher serum IL-10/IL-12 ratio at age 1 than children without symptoms. The increased IL-10/IL-12 ratio was positively related to the number of wheezing episodes. We also found a higher level of sE-selectin in children with wheezing and itchy skin rash in the first year of life. In addition, we found that children who newly develop wheezing in the second year of life already had a higher sICAM-1 level at age 1. Finally, children with wheezing in both the first and second year of life had higher levels of sIL-2R than children who never wheezed.

Serum cytokines and symptoms in infants:
In humans, IL-12 is mainly produced by antigen-presenting cells after stimulation by pathogens such as bacteria and bacterial products [16]. IL-12 is a powerful inducer of IFN-γ production and a suppresser of IL-4, and therefore capable of directing the differentiation of T-cells into Th-1 cytokine producing cells [17]. IL-12 production is up-regulated by IFNγ [17, 18]. Naseer et al found lower numbers of IL-12-expressing cells in bronchial biopsies of asthmatic adults compared with normal controls [19]. Chou et al. found that the level of IL-12 produced by peripheral blood mononuclear cells (PBMC's) was lower in asthmatic children than in healthy controls [20]. IL-10 has been shown to block the production of IL-12 in human PBMC's in vitro, resulting in inhibition of the production of IFNγ [21]. Increased levels

of IL-10 have been found in skin biopsies and plasma of adult patients with atopic dermatitis [22, 23]. In infants, the production of IL-10 by monocytes 3-4 weeks after RSV-bronchiolitis was found to be associated with an increased risk to develop recurrent wheezing after 1 year follow-up [24]. Therefore, the balance between micro-environmental IL-10 and IL-12 might be critical for determining the fate of T cells, with increased ratio's representing a Th-2 response [18, 20]. Our findings of decreased IL-12 levels and an increased IL-10/IL-12 ratio in wheezing children are in line with the above mentioned studies.
However, some important immunological and methodological consideration should be made. In rodents, IL-10 is mainly produced by Th2 cells and naive T-helper cells (Th0 cells), and it strongly inhibits the synthesis of cytokines by Th1 cells [25]. In contrast to rodents, human IL-10 is produced by both Th1 and Th2 cells, monocytes, macrophages, B cells, mast cells and eosinophils [26]. IL-10 has also been shown to prevent IL-5 production by human T cells and the production of various pro-inflammatory cytokines and chemokines as well as to inhibit mast cell function (reviewed by ref. [27]). Because of the inhibition of both Th1 and Th2 cytokines in human, IL-10 might therefore be considered as a inhibitory cytokine, resulting in tolerance or immunological unresponsiveness [28].
Wheezing in infancy only partly reflects the presentation of an asthmatic phenotype, and that wheezing in this age group is strongly associated with viral infections and small airway caliber [29]. A recent study found increased, rather than decreased serum IL-12 levels in children with acute Respiratory Syncytial Virus (RSV)- bronchiolitis [30]. Together with our finding that especially children with recurrent wheezing had decreased levels of IL-12 and an increased IL-10/IL-12 ratio, we speculate that at least a considerable part of the children with wheezing in our cohort might reflect early asthma. Follow-up of the cohort will give more insight on this topic. No association was found between IL-10 or IL-12 and skin rash or atopic dermatitis, so these markers have limited value for the evaluation of skin problems in early childhood.
In contrast to children with only wheezing at age 1, the children with both wheezing and skin rash had similar IL-12 and IL-10 levels and a similar IL-10/IL-12 ratio compared to children without symptoms. This difference is difficult to explain, but might be a result of the small sample size of the group with both wheezing and skin rash. Therefore results must be interpreted with caution.
Interestingly, we found a significant trend of lower levels of IL-10 in children exposed to other children (siblings or day care), an observation which fits in the observation that exposure to microorganisms in early life protects against the development of asthma and allergy in later life: the so called hygiene hypothesis [31].
IL-13, which is mainly produced by Th-2 cells, is a strong stimulant of IgE production by B-cells [7]. Although we could find a weak correlation between serum IL-13 and total IgE, no association was found with wheezing or skin rash. Our data indicates that serum IL-13 is not a suitable early marker of allergic disease, at least not in this age group.

Eotaxin and symptoms:
No association was found between the chemokine eotaxin and the development of wheezing and skin rash in the first 2 years of life. Therefore, we believe that in this age group eotaxin plays a limited role in the pathophysiology of allergic disease, or that measurement of serum levels is not sensitive enough to evaluate it's role in the disease process.

Adhesion molecules and symptoms:
Several studies have found elevated serum levels of sE-selectin and sICAM-1 in infants and school-children with atopic eczema and school-children with stable asthma and asthma exacerbation's [32, 33] although others could not confirm this [34, 35]. Our group recently described a strong correlation between levels of sE-selectin and disease severity in atopic dermatitis [34, 36]. Furthermore, in children with atopic dermatitis, a fast and significant reduction of sE-selectin, sICAM-1 and sVCAM-1 was found after anti-inflammatory treatment [32]. We found slightly increased levels of sE-selectin in children with wheezing or with skin rash in the first year of life (especially children with visible dermatitis). Considerable overlap between the groups was observed, and unexpectedly, children with both wheezing and skin rash in the first year of life did not have increased levels of sE-selectin. sICAM-1 was found to be elevated in children who newly develop wheezing in the second year of life. Also in this case considerable overlap with the sICAM-1 levels of children without any wheezing existed, but we believe this is an interesting finding which warrants further investigations. A strong correlation was found between sE-selectine and sICAM, which is consistent with findings of another study performed by our group [34].

Soluble IL-2 receptor and symptoms:
Several studies have found increased levels of sIL-2R in serum and BAL-fluid in children and adults with atopic eczema and asthma [9, 37], although other studies could not confirm this [38, 39]. We were also unable to show an association between sIL-2R and wheezing and skin rash in the first or second year of life. We found markedly increased levels of sIL-2R in children who wheezed in both the first and second year of life. It would be interesting to study whether this increased sIL-2R level will remain present after longer follow-up. If this is the case, sIL-2R is a potential marker for disease persistence.

Validation issues:
In this study we tested 7 different markers in relation to a variety of symptoms. It should be realized that based on chance, significant relations are likely to be found. Nevertheless, various p-values were rather small, so we are confident that the majority of associations we found were real. In addition, the clinical outcome variables we chose were largely based on parental reported symptoms (except for visible dermatitis), which can potentially lead to reporting bias. However, we related the clinical symptoms to objective parameters (e.g. serum markers), and it is very unlikely that selective parental report is related to a skewed distribution of these markers. Finally, most children participating in the studies had at least one allergic parent, and results might not be translated to children without a positive family history of allergic disease.

In conclusion, we found that several serum markers of allergic inflammation were related to the development of wheezing and skin rash in the first two years of life. The strongest and most consistent relation was found between wheezing and recurrent wheezing on the one hand and decreased IL-12 levels and an increased IL-10/IL-12 ratio on the other hand. Increased levels of sE-selectin were found in children with wheezing and skin rash in the first year of life. Finally, increased levels of sICAM-1 at age 1 were found in children who developed wheezing in the second year of life, and increased levels of sIL-2R were found in children with persistent wheezing. Follow-up of the cohort is needed to determine the prognostic

value of the various serum markers of allergic inflammation for the development of allergic disease in later childhood.

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References:

1. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PG. A protocol for recording the sign of flexural dermatitis in children. Br J Dermatol 1995;133:941-9.
2. Martinez FD. Recognizing early asthma. Allergy 1999;54:24-8.
3. Leung DY. Pathogenesis of atopic dermatitis. J Allergy Clin Immunol 1999;104:S99-108.
4. Kay AB. Allergy and allergic diseases. First of two parts. N Engl J Med 2001;344:30-7.
5. Laan MP, Baert MR, Bijl AM, et al. Markers for early sensitization and inflammation in relation to clinical manifestations of atopic disease up to 2 years of age in 133 high-risk children. Clin Exp Allergy 2000;30:944-53.
6. Sinigaglia F, D'Ambrosio D. Regulation of helper T cell differentiation and recruitment in airway inflammation. Am J Respir Crit Care Med 2000;162:S157-60.
7. Busse WW, Lemanske RF, Jr. Asthma. N Engl J Med 2001;344:350-62.
8. Bochner BS. Cellular adhesion and its antagonism. J Allergy Clin Immunol 1997;100:581-5.
9. Matsumoto T, Miike T, Yamaguchi K, Murakami M, Kawabe T, Yodoi J. Serum levels of soluble IL-2 receptor, IL-4 and IgE-binding factors in childhood allergic diseases. Clin Exp Immunol 1991;85:288-92.
10. Wijga A, Smit HA, Brunekreef B, et al. Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. Clin Exp Allergy 2001;31:576-81.
11. Koopman LP, van Benten IJ, de Waal L, Fokkens WJ, Osterhaus ADME, Neijens HJ. Virus mediated allergy (VIGALL)-study: study design and clinical results 1 year follow-up. Am J Respir Crit Care Med 2000;161:A347.
12. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
13. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
14. Stallman PJ, Wagenaar s, Swierenga J, van der Wal RJ, Feltkamp-Vroom TM. Cell-bound IgE on human mast cells and basophilic granulocytes in atopic and nonatopic subjects. Int Arch Allergy Appl Immunol 1977;54:443-50.
15. Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. J Allergy Clin Immunol 1997;99:545-50.

16. Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. Blood 1994;84:4008-27.
17. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. J Allergy Clin Immunol 2001;107:9-18.
18. Meyaard L, Hovenkamp E, Otto SA, Miedema F. IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J Immunol 1996;156:2776-82.
19. Naseer T, Minshall EM, Leung DY, et al. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. Am J Respir Crit Care Med 1997;155:845-51.
20. Chou CC, Huang MS, Hsieh KH, Chiang BL. Reduced IL-12 level correlates with decreased IFN-gamma secreting T cells but not natural killer cell activity in asthmatic children. Ann Allergy Asthma Immunol 1999;82:479-84.
21. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041-8.
22. Ohmen JD, Hanifin JM, Nickoloff BJ, et al. Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. J Immunol 1995;154:1956-63.
23. Niwa Y, Akamatsu H, Sumi H, Ozaki Y, Abe A. Evidence for degradation of cytokines in the serum of patients with atopic dermatitis by calcium-dependent protease. Arch Dermatol Res 2000;292:391-6.
24. Bont L, Heijnen CJ, Kavelaars A, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. Am J Respir Crit Care Med 2000;161:1518-23.
25. Mosmann TR, Moore KW. The role of IL-10 in crossregulation of TH1 and TH2 responses. Immunol Today 1991;12:A49-53.
26. Koulis A, Robinson DS. The anti-inflammatory effects of interleukin-10 in allergic disease. Clin Exp Allergy 2000;30:747-50.
27. Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. Clin Exp Allergy 1999;29:1164-71.
28. Barnes PJ, Lim S. Inhibitory cytokines in asthma. Mol Med Today 1998;4:452-8.
29. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
30. Blanco-Quiros A, Gonzalez H, Arranz E, Lapena S. Decreased interleukin-12 levels in umbilical cord blood in children who developed acute bronchiolitis. Pediatr Pulmonol 1999;28:175-80.
31. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000;343:538-43.
32. Kimata H. Increased serum levels of soluble adhesion molecules in young children with atopic dermatitis. Eur J Pediatr 1999;158:529-30.
33. Yamashita N, Kaneko S, Kouro O, Furue M, Yamamoto S, Sakane T. Soluble E-selectin as a marker of disease activity in atopic dermatitis. J Allergy Clin Immunol 1997;99:410-6.
34. Laan MP, Koning H, Baert MR, et al. Levels of soluble intercellular adhesion molecule-1, soluble E-selectin, tumor necrosis factor-alpha, and soluble tumor necrosis factor receptor p55 and p75 in atopic children. Allergy 1998;53:51-8.
35. Riise GC, Larsson S, Lowhagen O, Andersson BA. Circulating leukocyte adhesion molecules in stable asthma and nonobstructive chronic bronchitis. Allergy 1995;50:693-8.
36. Wolkerstorfer A, Laan MP, Savelkoul HF, et al. Soluble E-selectin, other markers of inflammation and disease severity in children with atopic dermatitis. Br J Dermatol 1998;138:431-5.
37. Shi HZ, Sun JJ, Pan HL, Lu JQ, Zhang JL, Jiang JD. Alterations of T-lymphocyte subsets, soluble IL-2 receptor, and IgE in peripheral blood of children with acute asthma attacks. J Allergy Clin Immunol 1999;103:388-94.
38. Miller AL, Stern DA, Martinez FD, Wright AL, Taussig LM, Halonen M. Serum levels of the soluble low affinity receptor for IgE and soluble interleukin-2 receptor in childhood, and their relation to age, gender, atopy and allergic disease. Pediatr Allergy Immunol 1996;7:68-74.
39. Lanz MJ, Leung DY, McCormick DR, Harbeck R, Szefler SJ, White CW. Comparison of exhaled nitric oxide, serum eosinophilic cationic protein, and soluble interleukin-2 receptor in exacerbations of pediatric asthma. Pediatr Pulmonol 1997;24:305-11.

Chapter 7

The indoor environment

7.1. Mattress encasings and mite allergen levels in the PIAMA-study

RT van Strien[1], LP Koopman[2], M Kerkhof[3], M Oldenwening[1], JC de Jongste[2], J Gerritsen[4], HJ Neijens[2], RC Aalberse[5], HA Smit[6], B Brunekreef[1]

[1] Department of Environmental and Occupational Health, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, [2] Department of Pediatrics, Sophia Children's Hospital, Rotterdam, [3] Department of Epidemiology and Statistics, University Groningen, Groningen, [4] Department of Pediatric Respiratory Medicine, University Hospital Groningen, Groningen, [5] Central Laboratory for the Blood Transfusion Service, Department of Allergy, Amsterdam, [6] National Institute of Public Health and the Environment, Bilthoven

ABSTRACT

Background: Reduction of allergen exposure from birth may reduce sensitization and subsequent allergic disease
Objective: To measure the influence of mite allergen impermeable mattress covers and cotton placebo covers on the amount of dust and mite allergen in beds.
Methods: 810 children take part in the PIAMA (=Prevention and Incidence of Asthma and Mite Allergy) study. They were selected for having an allergic mother. Allergen impermeable and placebo mattress covers were applied to the child's and the parental bed before birth. Dust samples were taken from the beds of children and their parents before birth and three and twelve months after birth. Extracts of dust samples were analyzed for mite allergens (Der p 1 and Der f 1).
Results: Active mattress covers were significantly more effective in reducing dust and mite allergen levels than placebo covers. Mite allergen levels were low in general and the treatment effect was modest. Twelve months after birth mattresses with active mattress covers had about half the amount of Der 1 (=Der p 1 + Der f 1)/m^2, compared to mattresses with placebo covers, for the child's and the parental mattress.
Conclusion: This study shows that mite impermeable mattress covers have a significant but modest effect on dust and mite allergen levels on mattresses with low initial mite allergen levels.

Submitted

INTRODUCTION

Allergies to house dust mites are common in asthmatics in the Netherlands and in many other countries where climatic conditions favor the growth of mites [1-3]. Mite allergic asthmatics benefit from the use of mite-impermeable mattress encasings, as these lower mite allergen exposure [4-8]. A number of studies have suggested that allergen exposure during early childhood is associated with a higher risk of sensitization and possibly with asthma later in life [9-12]. Some of these studies addressed the issue of primary prevention of mite allergy by influencing the home environment of the newborn child. Avoidance measures should preferably be as simple and feasible as possible in such studies, because the measures are intended for symptom free children who are not allergic yet, which may influence compliance with more demanding interventions.
In the PIAMA-study (Prevention and Incidence of Asthma and Mite Allergy) 'high risk' subjects were included in the third trimester of pregnancy. The subjects were randomly allocated to an active and a placebo group. The active group was supplied with mite impermeable mattress- and pillow encasings for the parental bed and the child's bed (Allergy Control™) . The placebo group was supplied with placebo cotton encasings. In the present study we examined the influence of this relatively simple avoidance measure on mite allergen levels in dust collected from beds. Effects on health status of the infants are reported elsewhere.

METHODS

Study population:
The PIAMA study is a prospective birth cohort study, which is partly natural history and partly an intervention study. Ten thousand two hundred and thirty two pregnant women in the Netherlands completed a validated screening questionnaire on allergies and asthma in 1996 and 1997 [13]. For the Intervention study, 2,062 allergic mothers were invited to participate and 855 (=41.5%) agreed. In 810 of these 855, the intervention measures were actually applied. This paper deals with these 810 subjects. The Medical Ethical Committees of all participating institutes approved the study protocol. Children were born between May 1996 and December 1997. The subjects were randomly allocated to two groups. The active group was supplied with mite impermeable encasings (Acb™) and the placebo group was supplied with placebo cotton encasings. Before the intervention was applied, a home visit was performed in the third trimester of pregnancy. Dust samples were taken from the parental mattress and, if already present, the child's mattress. Mattress- and pillow encasings for the parental bed and mattress encasings for the child's bed were supplied, including instructions on how to use the encasings. The parents were instructed to take all bedding from the mattress and to put the encasing around the mattress. After this, all bedding should be re-installed as it was previously. All parents were advised to wash the bedding regularly at temperatures high enough to kill mites. Furthermore, 'allergen avoidance' brochures from the Netherlands Asthma Fund were supplied to all participants. About three and twelve months after the birth of the child, a second and a third home visit were conducted and new dust samples were taken.

Dust sampling and analysis:
Dust was sampled, by trained fieldworkers, from the parental mattress and the child's mattress after the blankets (but not the undersheets) were removed. In the Netherlands it is common to have a fleecy cotton underblanket underneath the sheets, to protect the mattress. Furthermore, the child's mattress was often equipped with a plastic sheet against bedwetting. All these items, if present, were left in place during sampling. So dust was sampled from the sheets, on which the subjects actually slept. Before birth, the child's bed was usually not equipped with any bedding.
Dust was sampled, using a Rowenta Dymbo vacuum cleaner at 1200 Watt, with an ALK-filter holder. Paper filters (Schleicher & Schuell 589) were put in plastic petri dishes and weighed before sampling after at least 24 hours equilibration at 20°C and 50% relative humidity. At the sampling site, the filter was placed in the filter holder using a forceps. Sampling was done from each bed for a period of two minutes, regardless of the surface area of the mattress. After sampling the dust and the filter were put back into the petri dish and frozen at the end of the day (-20°C). After thawing and adjustment to the controlled temperature and relative humidity for 24 hours, the petri dish was weighed again and the total amount of dust was recorded. The limit of detection for this procedure was 11 mg of dust, based on repeated registration of the weight of blank samples. The dust and the filter were extracted for 2 hours by shaking in PBS with 0.05% Na-azide (8 g NaCl, 0.2g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4/liter (pH 7.2). For <300 mg dust 3 ml was used, for 300-500 mg 5 ml was used, for 500-1000 mg 10 ml was used and for more than 1000 mg 20 ml was used. After this aliquots of the extracts were stored at –20°C until analysis.

Dust extracts were analyzed using reagents for a sandwich EIA, supplied by Indoor Biotechnologies (Cardiff, UK). As catching antibody the Der p 1 and Der f 1 assays use mouse IgG anti mite monoclonal antibodies 5H8 and 6A8 respectively. Both assays use biotinylated Mouse IgG1 anti mite monoclonal 4C1 for marker antibody. Avidine peroxidase is used as conjugate (DAKO, Glostrup, Denmark). OPD was used as substrate and the reaction is stopped with 2M HCl. After this absorbance is read at 492 nm. All extracts were diluted five-, ten- and twenty fold, and more if required. The median Coefficient of Variation (CV) was 7.9% and 8.1% for Der p 1 and Der f 1. The lower limit of detection was 8 ng/ml for Der p 1 and 6 ng/ml for Der f 1 for five fold diluted samples. About 20% of the samples were analyzed in duplicate from a second aliquot on a different day. In about half of these samples, allergen levels were undetectable both times. For samples detectable on one or both occasions the CV was calculated. For Der p 1 and Der f 1 the median CV was 15.2% and 19.0% respectively.
All dust samples taken during the first two home visits were analyzed for Der p 1 and Der f 1. Of the samples taken during the third visit (at twelve months), a randomly selected subset of 200 was analyzed for both allergens, 100 from homes with active mattress covers and 100 from homes with placebo covers.

Statistical analysis:
Statistical analysis was done using SAS statistical software for PC v6.12. As distributions were found to be extremely right-skewed, due to a large number of undetectable samples, medians and interquartile ranges were used to describe the distributions of dust, Der p 1, Der f 1 and total Der 1 (=sum of Der p 1 and Der f 1) per m^2 and the amount of Der p 1, Der f 1 and

total Der 1 per gram of dust. Spearman correlation coefficients were calculated to investigate the correlation between Der p 1 and Der f 1 in samples taken from the parental and the child's beds. Differences in median dust and allergen levels between active and placebo covers were tested for statistical significance using Wilcoxon two sample tests. P-values smaller than 0.05 were considered statistically significant.

RESULTS

Table 1 and 2 show dust and allergen levels in the child's and the parental mattress before birth and three and twelve months after birth. Table 1 shows results for the Child's mattress and table 2 for the parental mattress. The intervention was implemented 1-2 months before birth so that samples taken after birth were taken 4-5 months after the intervention had been implemented. 77% of the samples taken from children's mattresses before birth, and 94% of the samples taken 3 months after birth contained a detectable quantity of dust. A detectable amount of dust was almost always obtained from the parental mattress. Of all samples taken from the child's mattress, 41-46% did not contain detectable amounts of Der p 1 or Der f 1 (tables 1 and 2). Of all samples taken from the parental mattress, 12-24% did not contain detectable quantities of Der p 1 or Der f 1. For the child's bed it is important to realize that many of the beds were new, and therefore never used when the baseline samples were taken.

Table 1: Levels of dust (mg/m^2) and Der 1 (=Der p 1 + Der f 1) (ng/m^2 en ng/gram dust) before the intervention and after the intervention in the child's mattress (differences between active and placebo assessed using Wilcoxon two sample test: # p<0.10, * p<0.05, ** p<0.01).

	2 months before birth			3 months after birth			12 months after birth		
Placebo	n/nd[a]	Median	IQR[b]	n/nd	Median	IQR	n/nd	Median	IQR
Mg dust/m^2	322/ 81	94	26-221	333/ 15	159	86-268	94/ 1	271	142-572
Ng Der 1/m^2	282/132	114	65-575	294/122	154	65-455	95/26	398	130-1676
Ng Der 1/g	207/ 79	1346	726-4782	282/117	974	629-2310	93/24	1468	634-7313
Active	n/nd	Median	IQR	n/nd	Median	IQR	n/nd	Median	IQR
Mg dust/m^2	340/ 74	87	30-223	352/ 29	129**	68-240	97/ 1	201	123-572
Ng Der 1/m^2	314/140	136	65-668	327/146	130**	65-298	97/32	190*	83-691
Ng Der 1/g	247/ 92	1525	753-6030	300/126	1028	657-1928	96/31	913#	569-2443

[a] n/nd=number of samples/number of non detectable samples
[b] IQR=Inter Quartile Range

Table 2: Levels of dust (mg/m^2) and Der 1 (=Der p 1 + Der f 1) (ng/m^2 en ng/gram dust) before the intervention and after the intervention in the parental mattress (differences between active and placebo assessed using Wilcoxon two sample test: # p<0.10, * p<0.05, ** p<0.01).

	2 months before birth			3 months after birth			12 months after birth		
Placebo	n/nd[a]	Median	IQR[b]	n/nd	Median	IQR	n/nd	Median	IQR
Mg dust/m^2	350/ 0	202	133-341	335/ 0	216	146-323	95/ 0	176	104-265
Ng Der 1/m^2	295/38	414	85-1361	299/46	281	88-809	95/13	309	87-1268
Ng Der 1/g	293/38	1851	607-5957	297/45	1507	470-3202	95/13	2097	568-6002
Active	n/nd	Median	IQR	n/nd	Median	IQR	n/nd	Median	IQR
Mg dust/m^2	382/ 3	202	128-313	351/ 0	167**	96-247	98/ 0	154	89-234
Ng Der 1/m^2	338/40	298	73-1140	321/79	113**	47-430	98/21	158*	59-496
Ng Der 1/g	336/40	1676	419-5602	321/79	807**	357-2424	98/21	1018	465-3179

[a] n/nd=number of samples/number of non detectable samples
[b] IQR=Inter Quartile Range

Figures 1 and 2 show the association between the amounts of Der p 1 and Der f 1 expressed per gram dust and per m^2 for the child's bed and the parental bed. Only when the levels of Der p 1 and Der f 1 were both undetectable samples were not entered into the figures. The figures show that Der p 1 and Der f 1 are hardly related to each other. Correlations are low, and for different mattresses the amount of Der p 1can be high while the amount of Der f 1 is low and vice versa.

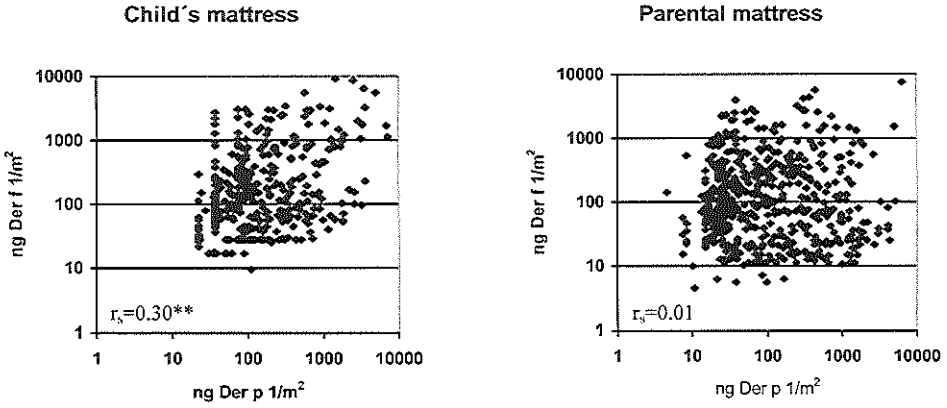

Figure 1: The amount of Der p 1 vs. Der f 1 per m^2 for the child's and the parental mattress (samples for which Der p 1 and Der f 1 were both undetectable are not shown) (r_s=Spearman correlation coefficient: ** p<0.01).

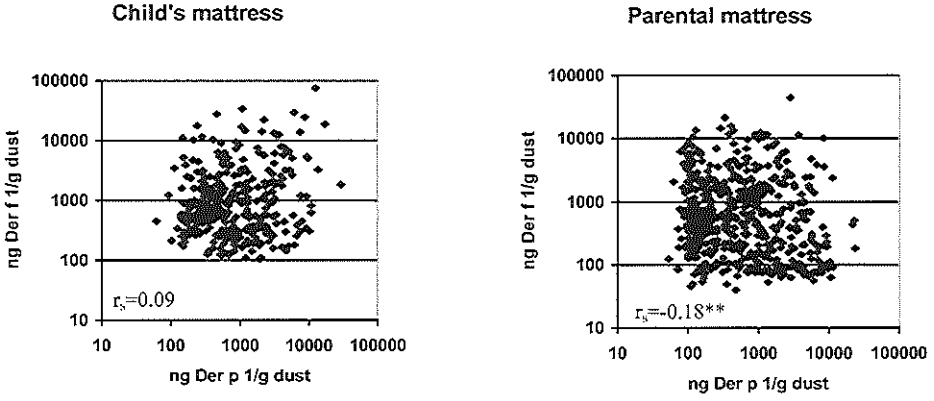

Figure 2: The amount of Der p 1 vs. Der f 1 per gram dust for the child's and the parental mattress (samples for which Der p 1 and Der f 1 were both undetectable are not shown)(r_s=Spearman correlation coefficient: ** p<0.01).

Figures 3 and 4 show the concentrations of Der $1/m^2$, Der 1/g, Der p 1/g and Der f 1/g for the active and the placebo group at the three different moments dust samples were taken (2 months before birth, 3 months after birth and 12 months after birth), for the child's and the parental bed respectively. To make sure concentrations are comparable over time, in this

figure data are shown only for the subset of mattresses for which samples were analyzed at twelve months after birth. The figure shows that the total amount of mite allergen/m^2 is reduced in the parental beds with active covers, compared to placebo, after 3 months and then remained stable, whereas allergen levels increased in the placebo as well as in the active group in baby mattresses at 3 and 12 months. However, in the child's as well as in the parental bed, the differences between active and placebo covers were larger at 12 months than at 3 months. At twelve months after birth total mite allergen levels, expressed per m^2 as well as per gram dust were about two-fold lower in the active group, compared to placebo.

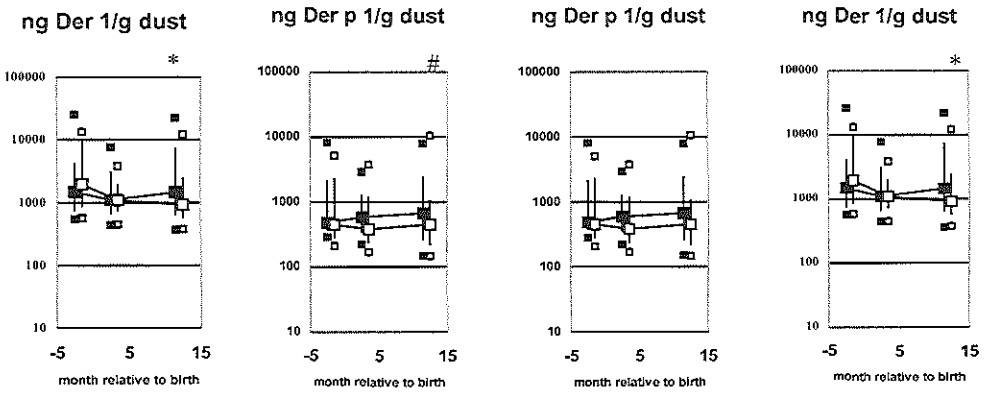

Figure 3: Total mite allergen content and Der p 1 and Der f 1 concentrations in dust from the child's mattress, shown for the placebo (filled squares) and the active covers (open squares) separately. The figures show median, interquartile range (error bars) and 10 and 90 percentiles (small squares), for the samples taken before birth, at 3 months after birth and at 12 months after birth (differences between active and placebo assessed using Wilcoxon two sample test: # $p<0.10$, * $p<0.05$).

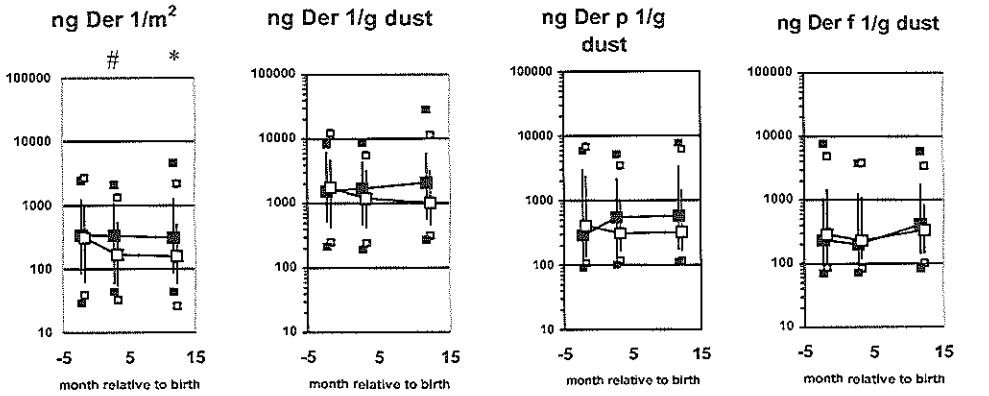

Figure 4: Total mite allergen content and Der p 1 and Der f 1 concentrations in dust from the parental mattress, shown for the placebo (filled squares) and the active covers (open squares) separately. The figures show median, interquartile range (error bars) and 10 and 90 percentiles (small squares), for the samples taken before birth, at 3 months after birth and at 12 months after birth (differences between active and placebo assessed using Wilcoxon two sample test: # $p<0.10$, * $p<0.05$).

DISCUSSION

This study showed that at relatively low mite allergen levels, the application of mite impermeable mattress covers resulted in modest, but statistically significant changes in dust and mite allergen exposure, compared to placebo. The effect of the application of mite impermeable mattress covers, compared to placebo, was bigger after one year than it was after three months.
The dust and mite allergen content of the mattresses studied here was in low, not only in the children's mattresses, which was expected after other studies [12,14], but also in the mattresses of the parents. These low allergen levels are in contrast with our previous findings in population based studies, where allergen levels in mattress dust were up to ten times higher [15,16]. Possible explanations include a strong downward shift of allergen exposure in recent years and self-selection of subjects joining this study who might be actively practicing mite allergen avoidance measures already. In a previous study, allergic parents were shown to have lower mite allergen levels in their homes than non-allergic parents [16]. This makes the low mite allergen levels found in the PIAMA Intervention study plausible, because participants were chosen on the basis of self-reported allergies and asthma. Another possible explanation is that in previous studies all dust samples were taken in October and November, this is the season in which mites are most abundant. Due to the nature of the PIAMA study dust samples were taken year round, and this could have been another reason for the low allergen levels found in this study. When more samples, including samples from homes of non-allergic parents will be available in the PIAMA study, these possible explanations will be examined further.
This finding of low mite allergen levels in the study population participating in the intervention part of the study has some possibly interesting consequences. If families with an allergic mother are already reducing mite allergen exposure fairly effectively, the relative small gains related to the use of mite impermeable mattress covers cannot be expected to have the same clinical effect as when baseline levels of allergen exposure would have been higher, and thus more amenable to change.

Low correlations were found between Der p 1 and Der f 1 levels. This was in accordance with results found by Gross and coworkers [17]. Der p 1 is important in some mattresses with no Der f 1 and vice versa. This finding makes it important to not only determine Der p 1, but also Der f 1.

The main goal of the PIAMA study is to find out whether mite allergen impermeable mattress covers are able to reduce the allergen exposure of children and thereby, possibly, the development of allergic diseases. We found a statistically significant difference between the amount of Der 1/m^2 for the parental as well as for the child's mattress at age 3 months and at age 12 months between the active and the placebo groups. This difference was on average never larger than a factor two. The amount of Der p 1 as well as the amount of Der f 1 decreased in the active group compared to placebo, for both mattresses, but in none of the analyses significantly so.
The levels and changes of the Der p 1 concentration in the child's bed are comparable to the results reported by Custovic and coworkers [14] from Manchester. The main difference lies in the fact that we have a placebo controlled design whereas no covers were used at all in the

study reported from Manchester. Mite allergen concentrations were reduced in the active group as well as in the placebo group, but in the active group the effect seems to persist for a longer period of time compared to the placebo group. Relative to a control group using no mattress covers at all, the effect of the active intervention may have been underestimated. Nevertheless, the active intervention reduced the mite allergen concentration in dust from the parental and the child's bed more than placebo, especially after 12 months.

At this moment a number of other studies is going on in which the development of sensitization and allergic disease is being studied in relation to different types of intervention [11,12,14]. In a few years time, we will be able to reach more definitive conclusions about the effect of allergen avoidance measures for preventing the development of sensitization and allergic disease in early childhood.

References

1. Colloff MJ, Ayres J, Carswell F, Howarth PH, Merret TG, Mitchell EB, Walshaw MJ, Warner JO, Warner JA, Woodcock AA. The control of allergens of dust mites and domestic pets: a position paper. Clin Exp Allergy 1992;22 (Suppl2):1-28.
2. Arlian LG, Platts-Mills TAE. The biology of dust mites and the remediation of mite allergens in allergic disease. J Allergy Clin Immunol 2001;107:S406-13.
3. Platts-Mills TAE, de Weck, AL. Dust mite allergens and asthma: a worldwide problem. J Allergy Clin Immunol 1992;89:1046-60.
4. Nishioka K, Yasueda H, Saito H. Preventive effect of bedding encasement with microfine fibers on mite sensitization. J Allergy Clin Immunol 1998;101:28-32.
5. Vaughan JW, Mc Laughlin TE, Perzanowski MS, Platts-Mills, TAE. Evaluation of materials used for bedding encasement: Effect of pore size in blocking cat and dust mite allergen. J Allergy and Clin Immunol 1999;103:227-31.
6. Tovey E, Marks G. Methods of effectiveness of environmental control. J Allergy Clin Immunol 1999;103:179-91.
7. Frederick JM, Warner JO, Jessop WJ, Enander I, Warner JA. Effect of a bed covering system in children with asthma and house dust mite hypersensitivity. Eur Respir J 1997;10:361-66.
8. Van der Heide S, Kauffman HF, Dubois AEJ, de Monchy, JGR. Allergen-avoidance measures in the homes of house-dust-mite allergic asthmatic patients: effects of acaricides and mattress encasings. Allergy 1997;52:921-7.
9. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. N Engl J Med 1990;323:502-7.
10. Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993;23:504-11.
11. Hide DW, Matthews S, Tariq S, Arshad SH. Allergen avoidance in infancy and allergy at 4 years of age. Allergy 1996;51:89-93.
12. Wahn U, Lau S, Bergmann R, Kulig M, Forster J, Bergmann K, Bauer C, Guggenmoos-Holzmann I. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. J Allergy Clin Immunol 1997;99:763-9.
13. Lakwijk N, van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allerg 1998;28:454-8.
14. Custovic A, Simpson BM, Simpson A, Hallam C, Craven M, Brutsche M, Woodcock A. Manchester Asthma and Allergy Study: Low-allergen environment can be achieved and maintained during pregnancy and in early life. J Allergy Clin Immunol 2000;105:252-8.
15. Van Strien RT, Verhoeff AP, Brunekreef B, van Wijnen JH. Mite antigen in house dust: relationship with different housing characteristics in the Netherlands. Clin Exp Allergy 1994;843-53.
16. Van Strien RT, Verhoeff AP, van Wijnen JH, Doekes G, de Meer G, Brunekreef B. Der p I concentrations in mattress surface and floor dust collected from infant's bedrooms. Clin Exp Allergy 1995;1184-9.
17. Gross I, Heinrich J, Fahlbusch B, Jäger L, Bischof W, Wichmann HE. Indoor determinants of Der p 1 and Der f 1 concentrations in house dust are different. Clin Exp Allergy 2000;30:376-82.

7.2. Placebo-controlled trial of house dust mite impermeable mattress covers: effect on symptoms in early childhood

L.P. Koopman[1], R.T. van Strien[2], M. Kerkhof[3], H.A. Smit[4], Alet Wijga4, J.C. de Jongste[1], J. Gerritsen[5], R.C. Aalberse[6], B. Brunekreef[2] and H.J. Neijens[1] on behalf of the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study

[1]Erasmus University Medical Center / Sophia Children's Hospital Rotterdam, Department of Pediatrics. [2]University of Utrecht, Institute for Risk Assessment Sciences, Department of Environmental and Occupational Health. [3]Groningen University, department of Epidemiology and Statistics. [4]National Institute of Public Health and the Environment, Department of Chronic Disease Epidemiology. [5]Beatrix Children's University Hospital Groningen, department of Pediatric Pulmonology. [6]Central Laboratory for the Blood Transfusion Services, Amsterdam, The Netherlands

ABSTRACT

We investigated the effect of house dust mite-allergen avoidance on the development of respiratory symptoms, atopic dermatitis and atopic sensitization by performing a double blind, placebo controlled trial. In total, 1282 allergic pregnant women were selected, of which 810 were randomly allocated into the intervention study, and 472 into the natural history study. 416 participants of the intervention study received house dust mite- allergen impermeable mattress covers (active), and 394 received cotton covers (placebo), for the parents and child's mattress in the third trimester of pregnancy. In the natural history study, no intervention took place. Data on allergen exposure, clinical symptoms, and IgE were collected prospectively. Allergen levels were reduced more by the active than by the placebo covers, especially in the parents' bed. The prevalence of night cough without a cold in the second year of life was lower in the group with active covers compared to the placebo covers (adjusted odds ratio 0.65; 95% CI 0.4-1.0). However, the prevalence of night cough without a cold in the second year of life in the second control group (natural history study) was significantly lower compared to the placebo group (adjusted odds ratio 0.69; 95% CI 0.4-1.0). No effect of house dust mite- allergen impermeable mattress covers was seen on other respiratory symptoms, atopic dermatitis, and total and- specific IgE. Additionally, we analyzed the association between exposure to house dust mite- allergen (independent of group allocation). We found that allergen exposure was positively associated with wheezing at least once in the first year of life (no detectable allergens versus exposure to 200-500 ng/m^2 Der 1, adjusted odds ratio 1.75, 95% CI 1.0-3.1). Exposure was negatively associated with atopic dermatitis at age 1 (no detectable allergens versus exposure to > 500 ng/m^2 Der 1, adjusted odds ratio 0.39; 95% CI 0.2-0.9). Conclusion: application of house dust mite impermeable mattress covers on the child and parents bed reduced night cough, but not other respiratory symptoms, atopic dermatitis and atopic sensitization in the first 2 years of life. Follow-up will determine the long- term effect of the intervention on the development of atopic disease.

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INTRODUCTION

Several studies have shown that exposure to house dust mite (HDM)-allergens is associated with mite sensitization in early childhood [1, 2] and that sensitization to HDM-allergen is a strong risk factor for the development of atopic disease [3, 4]. Whether there is a causal relation between exposure to HDM-allergen and the development of atopic disease is controversial [5]. In a prospective study, exposure to HDM-allergen at 1 year of age was related to sensitization to HDM and asthmatic symptoms at the age of 11 [1]. Furthermore, a cross-sectional study in infants found a positive association between levels of HDM-allergen in the home and respiratory symptoms at age 1 [6], although other studies could not confirm these findings [7, 8]. In two combined food- and indoor allergen avoidance studies, allergen avoidance decreased the prevalence of atopic disease at the age of 1 and sensitization to food and-inhalant allergens at age 2 [9-11]. Because various avoidance measures were taken, the specific effect of avoidance of HDM-allergens could not be separated from avoidance of food-allergens. In a Japanese study in infants with atopic dermatitis and food allergy but no sensitization to HDM, reduction of HDM-allergen exposure significantly reduced the subsequent development of sensitization to HDM and wheezing after 1 year of follow-up [12]. In a recently published primary prevention study from Manchester, UK, stringent environmental manipulations aiming on reducing HDM-allergen exposure were not associated with a reduction in the prevalence of wheezing, night cough apart from colds and eczema in the first year of life [13]. However, in the same study, a significantly lower prevalence of attacks of severe wheeze with shortness of breath, prescription of medication for wheezy attacks and wheeze after playing was observed in the active group compared to the control group [13].
We performed a randomized, placebo controlled study to investigate the effect of a simple HDM-allergen avoidance strategy (application of HDM-allergen impermeable mattress covers), starting in the third trimester of pregnancy, on the development of respiratory symptoms, atopic eczema and mite sensitization in children born to allergic mothers. We now report data from the first 2 years of life.

METHODS

Approximately 10,000 pregnant women completed a validated screening questionnaire on allergic disease [14]. Of 2,825 allergic women, 1,327 (47%) agreed to participate in the PIAMA study and gave informed consent. Women were randomly allocated into an Intervention Study (IS, n=855) or a Natural History Study (NHS, n=472). In 810 of the 855 families participating in the IS, the intervention was actually applied. The remaining 45 families could not be reached or decided to withdraw from the study when application of the intervention was planned. Children were born between May 1996 and December 1997. The study was approved by the local Medical Ethical Committees.

The 810 remaining participants of the IS were almost equally distributed among an active group (IS-active, n=416) or a placebo group (IS-placebo, n=394). Mattress covers for the beds of the infant and parents and pillow covers for the parents were applied by the investigator in the third trimester of pregnancy. The IS-active group received polyester-cotton mattress covers (ACb™, Allergy Control Products®, USA), the IS-placebo group received cotton placebo covers. The investigators and parents were blinded to group assignment. In the NHS (n =

472), no intervention took place, and these children were considered as a second control group.
Parents completed questionnaires during pregnancy, and when the children were 3 months, 1 year, and 2 years old (IS and NHS). Uniform criteria for the definition of atopic disease in early childhood are lacking [10]. Therefore we chose a symptom-based approach, using questionnaires about respiratory symptoms in the previous year at age 1 and 2 (adapted from the ISAAC-protocol [15]). Data were collected on wheezing, recurrent wheezing (at least 4 episodes/year), night cough without a cold, runny nose without a cold, use of asthma medication, and sleep disturbance due to wheezing. In addition, data were collected on birth characteristics, and indoor environmental-, socioeconomic-, lifestyle- and demographic factors. Allergic disease in the father and siblings was assessed by questionnaires [14, 15]. Atopic dermatitis at age 1 was determined by physical examination and questionnaire, using the UK Working Party's Diagnostic Criteria for Atopic Dermatitis (IS only) [16].
Total IgE at age 1 was measured as described previously by Stallman and Aalberse [17]. Specific IgE against HDM, cat, dog, and food-allergens was determined with a radioallergosorbent fluorescent immunoassay (IS only) [18].
Dust samples were taken by trained fieldworkers from the parents' and infant's mattresses in the third trimester of pregnancy and 3 months after birth. The sampling, storing and extraction procedures are described in detail elsewhere [19]. By adding the amount of Dermatophagoides (Der) p $1/m^2$ to the amount of Der f $1/m^2$, the total exposure to group 1 HDM-allergens was calculated (Der $1/m^2$). Data on HDM-allergen exposure were only available for children participating in the IS.
Statistical analyses were performed by SPSS (version 10.0). We used Pearson's χ^2 tests to compare categorical data, independent samples T-tests for parametric continuous data and Mann-Whitney U tests for non-parametric continuous data. An association was considered statistically significant when the p-value was ≤ 0.05. Multivariate regression analyses were used to estimate the independent effect of the intervention on various outcome variables. In order to investigate the effect of HDM-allergen exposure (independent of group assignment) on the development of symptoms, the children were arbitrarily divided into four groups: I) children without detectable HDM-allergens on their mattress at 3 months of age (= reference); II) Der 1 < 200 ng/m^2; III) Der 1 = 200-500 ng/m^2; Der 1 > 500 ng/m^2.

RESULTS

Response:
The flow of the participants through each stage of the study until the age of 2 years is summarized in figure 1 [20]. In the children participating in the NHS, no dust samples before the birth of the child were taken, no blood samples at age 1 were collected, and no physical examinations at age 1 were performed. Loss to follow-up and missing data were more common in the IS than in the NHS. Within the IS, loss to follow-up and missing data was somewhat more common in the IS-placebo group compared to the IS-active group. In order to assess selective loss to follow-up, the children with incomplete data for the 1 or 2 years questionnaire (17% of the total study population, IS and NHS) were compared with children with complete data. Children with incomplete data were not different from the other children with respect to atopic disease in the father and siblings, parental age, gender, season of birth, dura-

tion of pregnancy, number of siblings, day care attendance, pet keeping, and indoor environmental factors. However, children with incomplete data were more likely to have an asthmatic mother (33% versus 24%, p = 0.01), a mother who smoked during pregnancy (27% versus 16%, p = 0.002), to be exposed to environmental tobacco smoke at age 1 (37% versus 24%, p = 0.03), to be exclusively formula fed at age 3 months (61% versus 51%, p = 0.009), to have a non-working mother (47% versus 33%, p = 0.01), to have a non-working father (13% versus 4%, p = 0.02) and to have two parents with low level of education (14% versus 7%, p = 0.04). In addition, children with incomplete data had a lower birth weight (3.364 kg versus 3.508 kg, p = 0.02). Before birth, the geometric mean quantity of HDM-allergen (Der p 1 + Der f 1) on the parents' mattresses of families with incomplete data was 427 ng/m^2 as compared to 295 ng/m^2 for families with complete data (p = 0.02). No statistically significant differences in HDM-allergen exposure between families with incomplete data and complete data were observed on the child's mattresses before birth (267 ng/m^2 versus 206 ng/m^2) and after birth (201 ng/m^2 versus 188 ng/m^2) and on the parents' mattress after birth (231 ng/m^2 versus 198 ng/m^2).

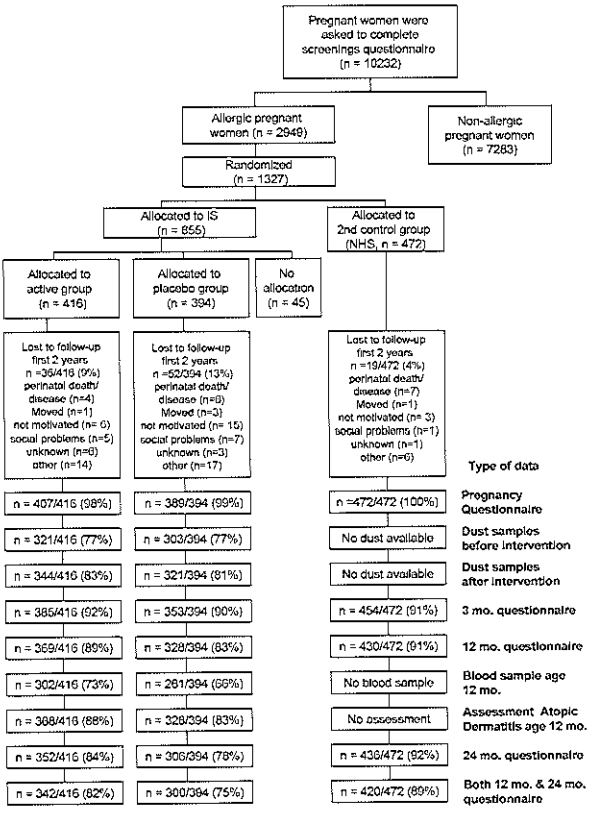

Figure 1: PIAMA flow chart.

General Characteristics:

In table 1 the general characteristics of the study population are summarized. Children participating in the IS more often had a mother who was allergic to HDM-allergen, and were less often born in the winter compared to children in the NHS. The IS-active group contained fewer boys compared to the IS-placebo group and the NHS group. Pet ownership and exclusive formula feeding at age 3 months were more common in the IS-active group and NHS group as compared to placebo group. All other characteristics were similar for the three groups.

Table 1: general characteristics of the study population, stratified for group allocation

	IS-active (n=416)	IS-placebo (n=394)	NHS (n=472)
Mother allergy for HDM (questionnaire)	58%	61%	51%
Mother ever asthma	26%	28%	23%
Allergic father (questionnaire)	37%	34%	30%
Father allergy for HDM (questionnaire)	15%	13%	16%
Father ever asthma	7%	8%	6%
Allergic sibling	32%	29%	31%
Sibling ever asthma	7%	8%	7%
Gender boy	46%	55%	54%
Smoking during pregnancy	16%	17%	20%
Smoke exposure in the home at age 1	24%	26%	25%
Born in winter	16%	18%	25%
Premature birth (< 37 weeks of gestation)	4%	7%	5%
Mean birth weight (kg, standard deviation)	3.487 (0.561)	3.482 (0.567)	3.502 (0.564)
Mean weight at age 1 (kg, standard deviation)	9.7 (1.1)	9.8 (1.2)	9.8 (1.1)
Exclusive formula feeding at 3 months	55%	48%	54%
At least 1 sibling	51%	47%	48%
Day care at age 1	67%	66%	63%
Exposure to pets	55%	46%	54%
Living in apartment-flat	8%	10%	9%
Damp- or mould spots in the home	10%	11%	13%
Carpet floor bedroom child	47%	45%	54%
Carpet floor bedroom parents	59%	64%	65%
Median age mattress child (years, 5th, 95th percentile)	1 (0-8)	1 (0-9)	1 (0-9)
Mean age mother of the child (years, SD)	30.5 (3.9)	29.9 (3.9)	29.8 (3.8)
Both parents low level of education	8%	7%	7%
Mother with paid job	65%	66%	68%
Father with paid job	96%	96%	96%

Effect of the intervention

Effect of the intervention on house dust mite-allergen exposure:

HDM-allergens were detectable on 58% of the children's mattresses at 3 months after birth. In the period between the first (1-2 months before birth) and second (3 months after birth) sampling time, HDM-allergen levels were reduced more by the allergen impermeable covers (active group) than by the placebo covers (placebo group). This effect was more pronounced for the parental beds (which had all been in use prior to the study) than for the child's mattresses (many of which had not been used before the child was born). Detailed information about the effect of the intervention on HDM-allergen levels will be published separately.

Effect of the intervention on the prevalence of clinical symptoms:
Figures 2a-d show the unadjusted prevalences of respiratory symptoms in the first and second year of life and the percentage of children with persistent respiratory symptoms, depending on group allocation.

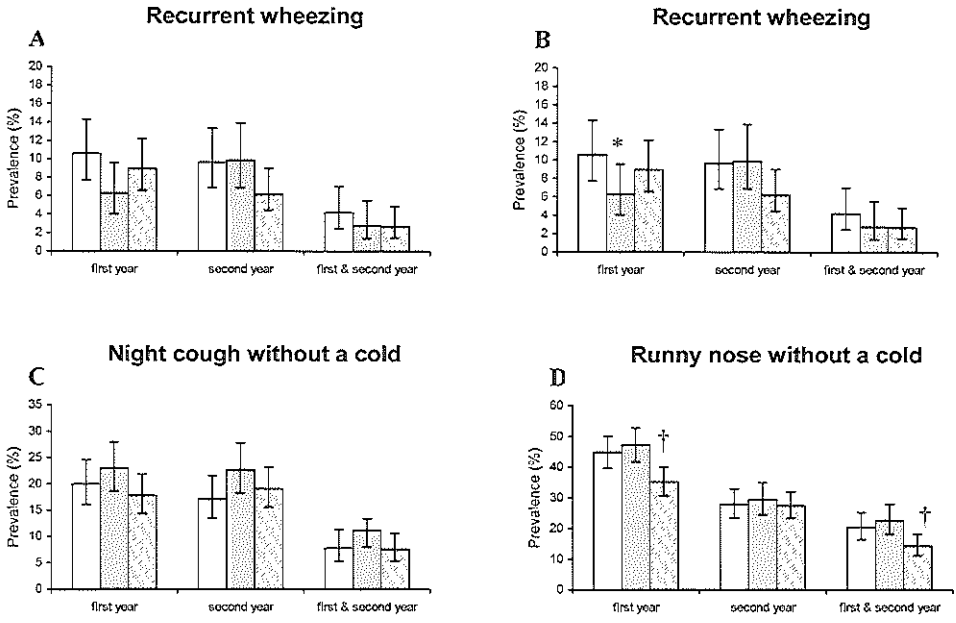

Figure 2a-d: Prevalence of parental reported wheeze at least once, recurrent wheeze, night cough without a cold and runny nose without a cold in the first and second year of life, and the percentage of children with persistent wheeze. White bars = IS-active, gray bars = IS-placebo, striped bars = NHS. Error bars represent the 95% confidence interval of the given prevalence [29]. * $p = 0.05$ for difference between IS-active and IS-placebo, † $p < 0.01$ for difference between IS-placebo and NHS.

IS-active versus IS-placebo: No statistically significant differences in the prevalence of wheezing at least once (Figure 2a), night cough without a cold (figure 2c) or runny nose without a cold (figure 2d) were observed between children in the active- and the placebo group. Children in the IS-active group had a higher prevalence of recurrent wheeze in the first year of life than those in the IS-placebo group (figure 2b, 10.6% versus 6.3%, $p = 0.05$). The prevalence of atopic dermatitis in the first year of life was similar in both groups (13.3% in the IS-active group versus 14.9% in the IS-placebo group, $p = 0.6$).
IS-placebo versus NHS: The prevalence of runny nose without a cold in the first year of life and in the first & second year of life was significantly higher in the IS-placebo group than in the NHS group (47.2% versus 35.2%, $p = 0.001$ and 22.7% versus 14.3%, $p = 0.004$ respectively).
Logistic regression analysis: after adjustment for potential confounders (table 2), the children in the IS-active group were less likely to develop night cough without a cold in the second year of life compared to children in the IS-placebo group (adjusted odds ratio (AOR) 0.65;

95% confidence interval (CI) 0.4-1.0). However, children participating in the NHS were also less likely to develop night cough without a cold in the second year of life compared to the IS-placebo group (AOR 0.69; 95% CI 0.4-1.0). No significant differences between the IS-active group, IS-placebo group and NHS group were observed for wheezing, recurrent wheezing, runny nose without a cold, any respiratory symptoms, and atopic dermatitis (table 2).

Table 2: Adjusted odds ratios (AOR) and 95% confidence interval (95% CI) for group allocation in relation to the development of wheezing, night cough without a cold and runny nose without a cold in the first 2 years of life and atopic dermatitis in the first year of life, using the placebo group as the index group.

		First year		Second year		First & second year	
	Study	AOR*	95% CI	AOR*	95% CI	AOR*	95% CI
Wheezing at least once	IS-placebo	1		1		1	
	IS-active	0.97	0.7-1.4	0.98	0.6-1.4	0.96	0.6-1.6
	NHS	1.20	0.8-1.7	0.80	0.5-1.2	1.04	0.6-1.7
Recurrent wheezing	IS-placebo	1		1		1	
	IS-active	1.57	0.8-3.0	0.96	0.5-1.7	1.83	0.6-5.3
	NHS	1.77	1.0-3.2	0.61	0.3-1.1	1.28	0.4-3.7
Night cough without a cold	IS-placebo	1		1		1	
	IS-active	0.87	0.6-1.3	0.65	0.4-1.0	0.65	0.3-1.2
	NHS	0.77	0.5-1.1	0.69	0.4-1.0	0.63	0.3-1.3
Runny nose without a cold	IS-placebo	1		1		1	
	IS-active	0.90	0.6-1.2	0.82	0.5-1.2	0.80	0.5-1.2
	NHS	0.61	0.4-0.8	0.80	0.5-1.1	0.52	0.3-0.8
Any respiratory symptom†	IS-placebo	1		1		1	
	IS-active	0.81	0.6-1.1	0.76	0.5-1.1	0.78	0.5-1.1
	NHS	0.78	0.6-1.1	0.74	0.5-1.0	0.74	0.5-1.0
Atopic dermatitis	IS-placebo	1					
	IS-active	1.05	0.6-1.7		No data		
	NHS	no data	no data				

*adjusted for maternal asthma, atopic disease father and sibling, gender, maternal smoking during pregnancy, type of feeding at 3 months, birth weight, having siblings, day care, maternal age, parental education and having pets. † any respiratory symptom = 1 or more of the following symptoms: wheeze, night cough without cold, or runny nose without a cold

Effect of the intervention on symptom severity:

In the first year of life, asthma medication was prescribed in 6.6% of the children in the IS-active group, in 8.3% of the children in the IS-placebo group, and in 7.3% of the children in the NHS ($p = 0.59$ for trend, chi-square). Of the children who were wheezing in the first year of life, sleep disturbance for at least one night/week was reported in 23.0% of the cases in the IS-active group, 22.8% of the cases in the IS-placebo group, and in 18.0% of the cases in the NHS ($p = 0.40$ for trend, chi-square). Also in the second year of life, no statistically significant differences were seen between the three groups in the prescription of asthma medication and prevalence of sleep disturbance due to wheezing (data not shown).

Effect of the intervention on total and specific IgE:

Serum levels of total IgE at age 1 were similar for the IS-active group and the IS-placebo group (table 3). Specific IgE against HDM (RAST-class ≥ 1) was found in only 4 children (2 in the IS-active group and 2 in the IS-placebo group). Ten children had specific IgE against cat dander and 8 children had specific IgE against dog dander. Fourteen % of the children were IgE positive for at least 1 allergen (mainly cow's milk or hen's egg) if a cut-of value of

RAST class 2 was chosen. No differences in specific IgE at age 1 were found between the IS-active group and the IS-placebo group.

Table 3: Total- and specific IgE at age 1

	IS-active (n=296)	IS-placebo (n=256)	p-value
Total IgE (geometric mean IU/ml, geometric SD)	6.83 (4.34)	6.57 (4.42)	0.78*
Specific IgE against HDM (% RAST-class ≥ 1)	0.7	0.8	0.57†
Specific IgE against any allergen, % RAST-class ≥ 2	14.4	14.0	0.90†

* Difference between IS-active group and IS-placebo group calculated by means of t-test, † Difference between active group and placebo group calculated by means of Chi-square

Relation between HDM-allergen exposure and the development of symptoms:
Table 4 shows the associations between various levels of exposure to HDM-allergen (Der 1) at 3 months of age, and the development of respiratory symptoms and atopic dermatitis in the first 2 years of life, using children without detectable levels of HDM-allergen on their mattress as the reference group (42% of the total IS cohort).

Table 4: Adjusted odds ratio's (AOR) and 95% confidence interval (95% CI) for association between exposure to HDM allergen at 3 months after birth (only IS, independent of group allocation) and the development of respiratory symptoms and atopic dermatitis in first 2 years of life. Group I: Der 1 (Der p1 + Der f1) undetectable, n = 280 (= reference); group II: Der 1 < 200ng/m2, n = 134; group III: Der 1 = 200-500 ng/m2, n = 114; group IV: Der 1 > 500 ng/m2, n = 135.

		First year		Second year		First & second year	
	Exposure HDM	AOR*	95% CI	AOR*	95% CI	AOR*	95% CI
Wheezing at least once	Group I	1		1		1	
	Group II	1.46	0.8-2.5	1.64	0.9-2.9	1.66	0.8-3.6
	Group III	1.75	1.0-3.1	1.69	0.9-3.1	1.98	0.9-4.6
	Group IV	1.53	0.9-2.6	1.30	0.7-2.4	1.65	0.7-3.7
Recurrent wheezing	Group I	1		1		1	
	Group II	0.78	0.3-1.9	1.54	0.7-3.5	1.32	0.3-5.3
	Group III	0.89	0.3-2.3	1.36	0.5-3.4	1.34	0.3-6.0
	Group IV	0.70	0.3-1.8	1.44	0.6-3.5	0.97	0.2-4.5
Night cough without a cold	Group I	1		1		1	
	Group II	1.42	0.8-2.5	1.14	0.6-2.1	1.15	0.5-2.8
	Group III	1.10	0.6-2.1	1.36	0.7-2.6	1.40	0.6-3.5
	Group IV	0.96	0.5-1.8	1.21	0.6-2.3	1.04	0.4-2.6
Runny nose without a cold	Group I	1		1		1	
	Group II	0.76	0.5-1.2	1.49	0.8-2.6	1.07	0.6-2.0
	Group III	1.22	0.7-2.0	1.67	0.9-3.0	1.81	0.9-3.5
	Group IV	0.68	0.4-1.1	1.49	0.8-2.7	0.86	0.4-1.8
Any respiratory symptom†	Group I	1		1		1	
	Group II	1.05	0.6-1.7	1.49	0.9-2.5	1.43	0.7-2.8
	Group III	1.41	0.8-2.4	1.59	0.9-2.7	1.65	0.8-3.3
	Group IV	0.88	0.5-1.5	1.36	0.8-2.3	1.08	0.5-2.1
Atopic dermatitis	Group I	1					
	Group II	0.43	0.2-1.0		No data available		
	Group III	0.49	0.2-1.2				
	Group IV	0.39	0.2-0.9				

* adjusted for maternal asthma, atopic disease father and sibling, gender, maternal smoking during pregnancy, type of feeding at 3 months, birth weight, having siblings, day care, maternal age, parental education and having pets. † any respiratory symptom = 1 or more of the following symptoms: wheeze, night cough without cold, or runny nose without a cold

Results given are for the total group (active and placebo), similar results were seen when we stratified for group allocation (data not shown). Atopic dermatitis in the first year of life was less prevalent in children who were exposed to HDM-allergens on the mattress as compared to children who were not exposed to HDM-allergens. Exposure to HDM-allergen was associated with wheezing at least once in the first year of life, and a trend was seen for wheezing in the second year of life and persistent wheezing. All other associations between HDM-allergen exposure and the development of respiratory symptoms were not statistically significant. No dose-response relation was observed between exposure to HDM-allergen and the development of atopic dermatitis and wheezing, since the odds ratio's for exposure groups II, III, and IV were similar.

DISCUSSION

In this large randomized and placebo controlled birth cohort study we found that a simple HDM-allergen avoidance strategy can reduce exposure to HDM-allergens in early childhood. In the children who received HDM-allergen impermeable mattress covers (IS-active group), a slightly, but significantly lower prevalence of night cough without a cold in the second year of life was observed. No significant effect of the mattress covers was seen on the development of wheezing, runny nose without a cold, atopic dermatitis, specific IgE against HDM, and respiratory symptom severity in the first 2 years of life. Exposure to HDM-allergens at 3 months of age (independent of group allocation) was associated with an increased risk to wheeze at least once in the first year of life. Surprisingly, we found a decreased prevalence of atopic dermatitis at age 1 in children who were exposed to HDM-allergen at 3 months of age. No evidence for a dose-response relation between HDM-allergen exposure and wheezing and atopic dermatitis was found.

Choice of outcome parameters:
No clear definition of atopic disease in early childhood exists, therefore most studies rely on the description of symptoms [21]. The diagnosis of asthma is particularly difficult, because no objective parameters are available for this age group. Wheezing and recurrent wheezing are usually chosen as a surrogate markers of asthma in epidemiological studies, although it is clearly established that wheezing during the first years of life is strongly associated with viral infections and the majority of wheezy children will not develop asthma later in life [22]. Also other respiratory symptoms in early childhood, such as night cough and runny nose without a cold, only partly reflect atopic disease. For this reason, this early evaluation of the effect of the intervention does not exclude possible benefits in a later stage, and the study will therefore be continued after 2 years of age.

Effect of the intervention on exposure and symptoms:
We chose a simple strategy to reduce HDM-allergen exposure by applying covers for the parents and child's mattress. Studies, such as the Manchester Allergy and Asthma Study (MAAS), that rely on more strenuous reduction programs affecting factors such as ventilation, furniture and floor covers might be more successful in reducing HDM-allergen exposure, but it is very difficult to conduct such studies in a double-blind, placebo-controlled fashion [23].

Also, simple measures are more likely to be implemented widely if they can be shown to have a reasonable effectiveness.
One could argue that the difference in allergen exposure between the active group and the placebo group in the IS was too small to expect a clinical effect of the intervention. Overall HDM-allergen levels were low in both the IS-active and IS-placebo group, and levels in the placebo group were 2 to 10-fold lower than previously found in other studies in the Netherlands [19, 24]. This can be explained because most infants were sleeping on new mattresses at the time of dust sampling, so mite infestation could only have taken place for a limited time period. HDM-allergen quantities were higher on the parents' mattresses, which were older, than on the infant mattresses, and the effect of the intervention was larger. It is likely that, in time, the allergen load on the child's mattress will further increase in the placebo arm of the study. Another possible explanation for the low allergen levels is an increased public awareness of the potential adverse effect of allergen exposure, in particular among atopic families [24]. In addition, participation in an intervention trial might have resulted in allergen avoidance and consequently low allergen levels in the IS-placebo group. This is supported by results of the MAAS, in which even lower HDM-allergen levels were found in the control arm of the study compared to the placebo arm of the PIAMA-study [23]. Therefore, our results can not be extrapolated to countries such as the United Kingdom and Australia, were allergen levels were shown to be much higher [1, 25].
Similar to the PIAMA-study, in the MAAS no reduction of the prevalence of wheezing, night cough apart from colds, eczema and sensitization to HDM-allergen was observed in the active group as compared to the control group [13]. In contrast to the PIAMA-study, the prevalence of severe respiratory manifestations, such as recurrent wheeze with shortness of breath and medication prescription for wheezing was found to be lower in the active group compared to the control group in the MAAS. Various possible explanations for this discrepancy can be postulated. The definitions for disease severity that were used in the PIAMA-study were slightly different from the definitions used in the MAAS. Furthermore, as was mentioned earlier, the method of intervention and the study design differed between the studies. It should be mentioned that although a significant difference in respiratory symptom severity was found between the active group and the control group of the MAAS, the absolute numbers of affected children was small, resulting in wide confidence intervals. In addition, no attempt was made to control for potential confounders.

According to a review by Pearce at al., the causal relationship between exposure to HDM-allergens and the development of asthma can be questioned [26]. The key study that links early allergen exposure to the development of asthma later in life in fact found a non significant trend towards an association [1]. In a recent publication from the German MAS study, no consistent association was found between HDM-allergen exposure at 6 months of age and current wheeze, ever wheeze and doctors' diagnosed asthma at age 7 [27]. In the same study, a strong association was found between early allergen exposure and mite-sensitization after the age of 5 on the one hand, and an association between mite-sensitization and wheezing after the age of 3 on the other hand. Based on these findings, the authors concluded that the strong association between sensitization to HDM and asthma reflects the susceptibility of an asthmatic individual to become sensitized to perennial allergens that are most prevalent in the environment rather than an increased risk of asthma when exposed to allergens. Despite overall low levels of HDM-allergen exposure, the prevalence of respiratory symptoms and atopic

dermatitis in our study was not lower than in other prospective birth cohort studies [11, 22, 28]. This might also indicate that HDM-allergen exposure is not an important factor for the development of respiratory and –skin symptoms, at least in the first years of life.

Atopic dermatitis and respiratory symptoms in relation to allergen exposure:
We found an inverse relation between exposure to HDM-allergen at 3 months of age and the development of atopic dermatitis in the first year of life. This association remained highly statistically significant after adjusting for potential confounders, and was independent of group allocation. To our knowledge, this kind of inverse association has not been published previously and we can only speculate about the potential mechanism. A possible explanation might be that parents of children who develop skin symptoms in the first weeks will increase the frequency of cleaning, resulting in lower HDM-allergen levels in the dust samples. However, parents completed weekly symptom cards in the first years of life, and we could not find an association between reported skin symptoms in the first weeks of life and the amount of HDM-allergen found on the child's mattress at 3 months of life (data not shown). We found a positive association between HDM-allergen exposure and wheezing at least once in the first year of life, and an association of borderline statistical significance for wheezing at least once in the second year of life and persistent wheezing in both the first and second year of life. Because no such association was found for recurrent wheezing, this relationship may not be due to allergic inflammation. An alternative explanation might be that proteolytic digestive enzymes present in animal feces increase the risk of non-allergic airway inflammation [8].

Validation issues:
In general, the prevalence of respiratory symptoms seemed to be somewhat lower in children participating in the NHS compared to children in the IS- placebo group. This is also the case for night cough without a cold in the second year of life, which is the only symptom that differs significantly between the IS-active group and the IS-placebo group, after adjustment for confounders. This observation might be the effect of enrollment into an intervention study resulting in over-reporting of respiratory symptoms. Responses to questionnaires at age 1 and 2 were higher in the NHS compared to the IS, which probably reflects the less intense study procedures used in the NHS. Within the IS, responses to questionnaires were slightly higher in the IS-active group as compared to the IS-placebo group, and non-responders had somewhat higher initial HDM-allergen quantities on the parents' mattress at the beginning of the study. The differences in response between the two groups might be a result of parents' awareness into which group they were allocated, because the appearance of the active cover and the placebo cover were slightly different. An alternative explanation is that the difference is a result of chance. Absolute follow-up differences were small, and overall follow-up rates were highly acceptable in both groups. So, although there are some indications for participation bias, we do not believe that this can explain the limited effect of the intervention so far. Because non-smoking parents of high socioeconomic status were more likely to provide complete data, the generalisability of the study for the total population might be somewhat limited.

In conclusion, we were unable to demonstrate a protective effect of house dust mite impermeable mattress covers on the prevalence and severity of respiratory symptoms, atopic dermatitis

and atopic sensitization in the first 2 years of life. Follow-up of the cohort will determine whether this intervention can reduce the prevalence of asthma and atopy later in childhood.

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References:

1. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990;323:502-7.
2. Wahn U, Lau S, Bergmann R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. J Allergy Clin Immunol 1997;99:763-9.
3. Peat JK, Woolcock AJ. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. Clin Exp Allergy 1991;21:573-81.
4. Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. Clin Exp Allergy 1989;19:419-24.
5. Platts-Mills TA, Sporik RB, Wheatley LM, Heymann PW. Is there a dose-response relationship between exposure to indoor allergens and symptoms of asthma? J Allergy Clin Immunol 1995;96:435-40.
6. van Strien RT, Verhoeff AP, van Wijnen JH, Doekes G, de Meer G, Brunekreef B. Infant respiratory symptoms in relation to mite allergen exposure. Eur Respir J 1996;9:926-31.
7. Burr ML, Miskelly FG, Butland BK, Merrett TG, Vaughan-Williams E. Environmental factors and symptoms in infants at high risk of allergy. J Epidemiol Community Health 1989;43:125-32.
8. Gold DR, Burge HA, Carey V, Milton DK, Platts MT, Weiss ST. Predictors of repeated wheeze in the first year of life. The relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. Am J Respir Crit Care Med 1999;160:227-36.
9. Arshad SH, Matthews S, Gant C, Hide DW. Effect of allergen avoidance on development of allergic disorders in infancy. Lancet 1992;339:1493-7.
10. Chan-Yeung M, Manfreda J, Dimich-Ward H, Ferguson A, Watson W, Becker A. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. Arch Pediatr Adolesc Med 2000;154:657-63.
11. Hide DW, Matthews S, Matthews L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. J Allergy Clin Immunol 1994;93:842-6.
12. Nishioka K, Yasueda H, Saito H. Preventive effect of bedding encasement with microfine fibers on mite sensitization. J Allergy Clin Immunol 1998;101:28-32.
13. Custovic A, Simpson BM, Simpson A, Kissen P, Woodcock A. Effect of environmental manipulation in pregnancy and early life on respiratory symptoms and atopy during first year of life: a randomised trial. Lancet 2001;358:188-93.
14. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
15. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
16. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PG. A protocol for recording the sign of flexural dermatitis in children. Br J Dermatol 1995;133:941-9.

17. Stallman PJ, Wagenaar s, Swierenga J, van der Wal RJ, Feltkamp-Vroom TM. Cell-bound IgE on human mast cells and basophilic granulocytes in atopic and nonatopic subjects. Int Arch Allergy Appl Immunol 1977;54:443-50.
18. Schuurman J, Perdok GJ, Lourens TE, Parren PW, Chapman MD, Aalberse RC. Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE. J Allergy Clin Immunol 1997;99:545-50.
19. Van Strien RT, Verhoeff AP, Brunekreef B, Van Wijnen JH. Mite antigen in house dust: relationship with different housing characteristics in The Netherlands. Clin Exp Allergy 1994;24:843-53.
20. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. BMC Med Res Methodol 2001;1:2.
21. Koopman LP, Brunekreef B, de Jongste JC, Neijens HJ. Definition of respiratory symptoms and disease in early childhood in large prospective birth cohort studies that predict the development of asthma. Pediatr Allergy Immunol 2001;12:118-24.
22. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133-8.
23. Custovic A, Simpson BM, Simpson A, et al. Manchester Asthma and Allergy Study: low-allergen environment can be achieved and maintained during pregnancy and in early life. J Allergy Clin Immunol 2000;105:252-8.
24. van Strien RT, Verhoeff AP, van Wijnen JH, Doekes G, de Meer GE, Brunekreef B. Der p I concentrations in mattress surface and floor dust collected from infants' bedrooms. Clin Exp Allergy 1995;25:1184-9.
25. Peat JK, Tovey E, Gray EJ, Mellis CM, Woolcock AJ. Asthma severity and morbidity in a population sample of Sydney schoolchildren: Part II--Importance of house dust mite allergens. Aust N Z J Med 1994;24:270-6.
26. Pearce N, Douwes J, Beasley R. Is allergen exposure the major primary cause of asthma? Thorax 2000;55:424-31.
27. Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. Lancet 2000;356:1392-7.
28. Bergmann RL, Bergmann KE, Lau-Schadensdorf S, et al. Atopic diseases in infancy. The German multicenter atopy study (MAS-90). Pediatr Allergy Immunol 1994;5:19-25.
29. Fleis J. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley & sons; 1981.

Chapter 8

Infection

8.1. Respiratory infections in infants: interaction of parental allergy, child care and siblings: the PIAMA study

L.P. Koopman[1], H.A. Smit[2], M.A. Heijnen[2], A. Wijga[2], R.T. van Strien[3], M. Kerkhof[4], J. Gerritsen[4], B. Brunekreef[3], J.C. de Jongste[1], and H.J. Neijens[1]

[1]Sophia Children's Hospital, Erasmus University Rotterdam, Department of Pediatrics, [2]National Institute of Public Health and the Environment, Department of Chronic Disease Epidemiology and Department of Infectious Diseases Epidemiology, [3]University of Utrecht, Institute for Risk Assessment Sciences, Department of Environmental and Occupational Health, [4]Beatrix Children's Hospital, Groningen University

ABSTRACT

Objective: To investigate the association between contacts with other children and the development of respiratory infections in the first year of life in children with or without genetic predisposition for allergy.
Methods: Children (n=4146) who participate in a prospective birth cohort study (Prevention and Incidence of Asthma and Mite Allergy study) were investigated. Questionnaires were used to obtain information on doctor-diagnosed upper respiratory tract infection (URTI) and lower respiratory tract infection (LRTI), child care attendance, having siblings, family history of allergic disease and various potential confounders.
Results: Childcare attendance in the first year of life was associated with doctor-diagnosed URTI (adjusted odds ratio [AOR] 2.7; 95% confidence interval [CI]: 2.1-3.4 for large child care facility versus no child care) and doctor-diagnosed LRTI (AOR 5.6; 95% CI: 3.9-7.9). Having siblings was associated with doctor-diagnosed LRTI (AOR 2.6; 95% CI 2.0-3.4). In addition, children who have allergic parents and attend child care or have older siblings have a higher risk of developing doctor-diagnosed LRTI than do children who have non-allergic parents.
Conclusions: Child care attendance or having siblings increases the risk of developing doctor-diagnosed LRTI in the first year of life to a greater extent in allergy-prone children than in children who are not allergy-prone.

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INTRODUCTION

Respiratory infections in infancy are common and account for considerable morbidity, doctor visits, hospital admissions, and health care costs [1, 2]. Various risk factors for upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI) in children have been identified, such as child care attendance, having older siblings, exposure to environmental tobacco smoke, young motherhood, low socioeconomic status, male gender, premature birth, low birth weight, born in the winter and formula feeding [2-15]. Although a parental history of allergic disease is thought to be associated with an increased risk of upper and lower respiratory symptoms [16, 17], few studies have addressed this issue in relation to upper and lower respiratory tract infections in early life [3].

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study is a combined effort of the University of Utrecht, the Sophia Children's Hospital Rotterdam, the Beatrix Children's Hospital of University Hospital Groningen, the Central Laboratory of the Red Cross Blood Transfusion Service, and the National Institute of Public Health and the Environment. This prospective birth cohort study includes children from both allergic and non-allergic parents. The genetic background of our cohort gave us the opportunity to study whether the parental history of allergic disease interacts with child care attendance and having siblings in the development of respiratory tract infections.

METHODS

Study population:

Recruitment of the cohort took place during the first trimester of pregnancy and was carried out by 52 midwife practices in three different regions in the Netherlands: north (Groningen and surroundings), central (Bilthoven and Wageningen and surroundings) and southwest (Rotterdam and surroundings). In total, 7862 pregnant women were asked to participate in the study, and 4146 (53%) agreed. The 4146 children have been followed from birth. In this cohort, 1327 have an allergic mother and are considered as high-risk children. The remaining 2819 children have a non-allergic mother and are considered low-risk children. The study includes an intervention part and a natural history part.

Some of the high-risk children (855) were allocated to the intervention part of the study. Half of those children received house dust mite impermeable mattress covers for the parental and infant bed (active group), and the other half received cotton mattress covers (placebo group). In the natural history part of the study (no intervention), 472 high-risk children (second control group to the intervention study) and 2819 low-risk children were included.

The participating children were born between May 1996 and December 1997. The definition of allergy in the mother was based on a validated screening questionnaire [18]. Women with any of the following self-reported symptoms were defined as allergic, and their children were defined as high-risk: asthma, hay fever, house dust allergy, house dust mite allergy, or pet allergy. Women who reported not having any of these symptoms were defined as non allergic (and their children were defined as low-risk). The PIAMA study was approved by the local medical ethical committees.

Data collection:
Questionnaires were sent to the participating parents during the third trimester of pregnancy, when the child was 3 months of age, and when the child was 1 year of age. Information was collected on birth characteristics (duration of pregnancy, gender, birth weight, length and head circumference, season of birth), indoor environmental factors (smoking by the parents, having pets, housing characteristics), socioeconomic characteristics (parental education and employment status), lifestyle factors (method of feeding, child care) and demographic factors (siblings, parental age).

Outcome variables:
When the children were 1 year of age, parents were asked whether their child had a doctor-diagnosed flu/serious cold, throat infection, middle ear infection, bronchitis, pneumonia, pertussis, or any other serious respiratory tract infections. On the basis of the information mentioned above, three outcome groups were created:

Doctor-diagnosed upper respiratory tract infection (URTI): children who had a doctor-diagnosed flu/serious cold, throat infection, or middle ear infection; but without doctor-diagnosed bronchitis, pneumonia, pertussis, or any other serious respiratory infection.
Doctor-diagnosed lower respiratory tract infection (LRTI): children with a doctor's diagnosed bronchitis or pneumonia, irrespective of any doctor-diagnosed flu/serious cold, throat infection, middle ear infection, pertussis, or any other serious respiratory infection.
Healthy control group: children without any report of doctor-diagnosed flu/serious cold, throat infection, middle ear infection, bronchitis, pneumonia, pertussis, or any other serious respiratory infection.

We anticipated that many children would develop both a doctor-diagnosed URTI and LRTI in the first year of life, which might interfere with the calculation of the associations between these two outcomes and various risk factors. For this reason we defined doctor-diagnosed URTI as the development of a doctor-diagnosed flu/serious cold, middle-ear infection, or throat infection without any other respiratory infections. Ideally, doctor-diagnosed LRTI should have been defined as doctor-diagnosed bronchitis or pneumonia without any other respiratory disease. Because a large majority of children with LRTI also experienced an URTI or other serious infections, we decided to include all the children with reported doctor-diagnosed bronchitis or pneumonia in the group with doctor-diagnosed LRTI.

Exposures of interest:
Child care attendance was divided in 3 groups: 1) children who did not attend any form of child care in the first year of life and did not have regular contact with children other than their siblings (no child care), 2) children who were cared for regularly by relatives or foster parents and have contact with small numbers of children other than their siblings (small child care groups with <5 children), and children who attend large child care facilities (large child care, groups usually >10). The allergic disease status of the father was assessed by using the same validated questionnaire as was used for the mother. The allergic status of the siblings was assessed by using the ISAAC questionnaire [19].

Statistical analysis

Crude odds ratios, adjusted odds ratios (AOR) and 95% confidence intervals (CI) were estimated for the association between various risk factors and respiratory tract infections by using cross-tabulation and logistic regression analysis (SPSS, version 8.0, SPSS Inc., Chicago, IL). The healthy control group was used as the reference group in all analyses. The following 9 potential confounders were included in the model: type of study (intervention or natural history), maternal age, gender, season of birth, parental history of allergic disease, type of feeding, maternal smoking during pregnancy, maternal employment status and level of education of the parents. On the basis of the parental history of allergic disease in combination with known risk factors for the development of respiratory infections (e.g. child care attendance and having siblings), the infants were stratified into various sub-groups. Interaction variables were constructed in order to test the joint impact of the parental history of allergic disease and exposure to other known risk factors on the development of respiratory infections. For instance, the 2 dummy variables of child care attendance were multiplied by the 3 dummy variables of parental history of allergic disease, resulting in an interaction term, which was entered into the logistic regression model.

RESULTS

Response:

From the 4146 participants, 3745 (90%) completed a questionnaire when the child was 1 year of age. From the remaining 401 families, 112 dropped out before the child was 1 year of age for various reasons (perinatal death, moved, language barrier, not interested); 289 parents did not complete a 1-year questionnaire but are still participating in the study. Another group of 327 parents completed a 1-year questionnaire, but data were incomplete for one or more confounders. A complete data set with the outcome variables and confounders was available for 3418 participants (82%). Children with incomplete data were more likely (in comparison with children with complete data): to have an allergic mother (44% vs 29%), to have an asthmatic mother (12% vs 7%), to have a mother who smoked during the whole pregnancy (21% versus 14%), to be exposed to environmental tobacco smoke at 3 months (39% vs 27%), to live in an apartment (11% vs 6%) and to be bottle fed exclusively at 3 months of age (62% vs 52%). All other characteristics were similar in both groups. In this paper, data used are from 3418 children with complete data at 1 year of age.

General characteristics:

The general characteristics of the study population, stratified for allergic disease status of the parents, are shown in Table 1. Children from allergic parents were slightly more likely to have been breastfed at 3 months of age and less likely to have been exposed to maternal smoking during the whole pregnancy as compared with children from non-allergic parents.

Incidence of respiratory infections

The cumulative incidences of URTI and LRTI in the first year of life are summarized in Table 2. Medication was prescribed in 66% of the children with a flu/serious cold, in 77% of the children with a throat infection, and in 84% of the children with a middle-ear infection. Ninety-two percent of the children with bronchitis received medication, and hospitalization

was needed in 4% of the children. All children with reported pneumonia received medication and 43% were hospitalized. The majority of children (78%) who experienced doctor-diagnosed LRTI also had a doctor-diagnosed URTI in the first year of life.

Table 1: General characteristics of the study population, stratified for parental history of allergic disease

Characteristic	Both parents non-allergic (n=1700)	Mother allergic (n=677)	Father allergic (n=710)	Both parents allergic (n=331)	Total (n=3418)
Male gender (%)	50.8	52.7	52.3	47.4	51.1
Maternal age (mean years ± standard deviation)	30.4 (3.9)	30.1 (3.7)	30.5 (3.8)	30.0 (4.1)	30.3 (3.9)
Duration of pregnancy:					
37-40 weeks (%)	44.4	43.0	47.7	48.5	45.2
> 40 weeks (%)	51.0	52.2	48.4	45.7	50.2
< 37 weeks (%)	4.6	4.8	3.8	5.9	4.6
Birth weight (mean g ± standard deviation)	3512 (539)	3502 (570)	3527 (518)	3452 (398)	3507 (547)
Season of birth:					
Winter (%)	17.1	20.8	19.4	19.0	18.5
Spring (%)	25.1	24.4	22.5	20.8	24.0
Summer (%)	31.4	23.6	31.1	27.8	29.5
Fall (%)	26.4	31.2	26.9	32.3	28.0
Method of feeding:					
Only breast feeding at 3 mo of age (%)	30.2	31.6	32.7	36.0	31.5
Breastfeeding and formula at 3 mo (%)	16.8	17.4	17.2	15.4	16.9
Only formula feeding at 3 mo (%)	53.0	51.0	50.1	48.6	51.6
% with at least 1 older sibling	52.1	48.2	51.7	47.4	50.8
Siblings with allergic disease (% of total)*	22.3	30.2	28.7	30.6	26.0
Form of child care†					
No child care (%)	35.1	36.3	31.8	34.4	34.6
Child care small group (%)	41.8	40.8	41.0	41.4	41.4
Child care large group (%)	23.1	22.9	27.2	24.2	24.0
Smoking mother during pregnancy:					
No smoking (%)	79.6	83.3	82.8	83.4	81.4
Only first trimester (%)	4.4	3.8	4.5	4.8	4.3
Whole pregnancy (%)	16.0	12.9	12.7	11.8	14.3
Both parents low level of education‡ (%)	6.5	6.6	5.6	6.9	6.4

*Allergic disease in siblings defined as having a history of asthma, eczema, hay fever or other inhalant allergies. † No child care = no form of child care at all, small child care = regularly cared for by relatives or foster parents, and contact with small numbers of children other than their siblings and large child care = child care centers, usually 10 or more children. ‡ Both parents have a maximum of 4 years high school education

Risk factors for doctor-diagnosed URTI and LRTI

AOR for the association between contacts with other children, parental history of allergic disease and URTI and LRTI are shown in Table 3. Child care attendance increased the risk for doctor-diagnosed LRTI and, to a lesser extent, for doctor-diagnosed URTI. Furthermore, the size of the child care group was positive related directly to the incidence of respiratory tract infections. Having one or more siblings (all siblings are older than the study subject) was a strong risk factor for doctor-diagnosed LRTI, and was only weakly related to doctor-diagnosed URTI. Having an allergic father and having 2 allergic parents was weakly associated with the development of doctor-diagnosed LRTI, but was not associated with doctor-diagnosed URTI.

In a multivariate analysis, maternal age, season of birth, type of feeding, and low level of education were associated with doctor-diagnosed URTI. Male gender, maternal age and type of feeding were all risk factors for doctor-diagnosed LRTI (table 3).

Table 2: Cumulative 12 months incidence (%) of URTI and LRTI, stratified for parental history of allergic disease

Type of respiratory tract infection	Both parents non-allergic (n=1700)	Mother allergic (n=677)	Father allergic (n=710)	Both parents allergic (n=331)	Total (n=3418)
Doctor- diagnosed flu/serious cold	42.2	48.7	45.8	53.5	45.3
Doctor-diagnosed throat infection	5.8	7.4	8.2	9.1	6.9
Doctor-diagnosed middle-ear infection	17.2	16.8	18.3	19.6	17.6
Doctor-diagnosed bronchitis	13.8	15.1	15.6	16.9	14.7
Doctor-diagnosed pneumonia	2.1	2.5	3.2	2.7	2.5
Pertussis	0.9	1.5	1.4	1.2	1.2
Other respiratory tract infections*	11.7	15.7	13.4	13.1	13.0
Doctor-diagnosed URTI †	31.8	33.7	30.0	34.4	32.1
Doctor-diagnosed LRTI ‡	15.0	15.8	17.6	19.0	16.1

* Parents were also asked "did your child have any other respiratory tract infections than flu/serious cold, throat infection, middle ear infection, bronchitis or pneumonia?" † Cumulative 12 months incidence of doctors diagnosed URTI: flu/serious cold, throat infection or middle ear infection. Children with bronchitis, pneumonia, pertussis or other respiratory infections excluded, ‡ Cumulative 12 months incidence of doctors diagnosed LRTI: bronchitis or pneumonia, irrespective of other respiratory disease.

Relation between contacts with other children and respiratory tract infections, stratified for parental history of allergic disease

Figures 1 and 2 show the AOR for the association between contact with other children (child care attendance and having siblings) and doctor-diagnosed LRTI, stratified for parental history of allergic disease. The OR for doctor-diagnosed LRTI gradually increase when the children attend small or large child care centers (Fig 1). When the child does not attend any form of child care, parental allergy does not influence the risk for doctor-diagnosed LRTI; but when the child attends small or large child care facilities, the risk of developing a LRTI is markedly influenced by the parental allergic status. For instance, a child who attends small child care and has two allergic parents has a 3.2 fold increased risk to develop a LRTI as compared to the reference group (no child care, no allergic parents), whereas a child who attends small child care and has no allergic parents only has a 1.4 fold increased risk to develop a LRTI as compared to the reference group. A similar pattern is seen for increasing number of allergic parents and having siblings in relation to doctor-diagnosed LRTI (Fig 2). When interaction variables were entered in the logistic regression model for doctor-diagnosed LRTI, statistically significant interaction was found for "father allergic × small child care" (P = .02) and "father allergic × large child care" (P = .04) and "both parents allergic × small child care" (P = .05). The interaction term "both parents allergic × having siblings" was borderline statistically significant (P = .06). When the interaction variables were entered in the logistic regression model for doctor-diagnosed URTI, no significant associations were found (data not shown).

Table 3: Contacts with other children and parental history of allergic disease and other factors in relation to respiratory tract infections in the first 12 months of life

	URTI		LRTI	
	AOR*	95% CI	AOR*	95% CI
No child care	1		1	
Child care, small group	1.4	1.2-1.7	2.0	1.5-2.6
Child care, large group	2.7	2.1-3.4	4.8	3.5-6.9
Siblings	1.2	1.0-1.5	2.5	1.9-3.1
No parent allergic	1		1	
Only mother allergic	1.1	0.8-1.5	1.2	0.8-1.8
Only father allergic	1.0	0.8-1.2	1.2	0.9-1.6
Both parents allergic	1.2	0.8-1.7	1.6	1.0-2.5
Maternal smoking pregnancy				
No smoking	1		1	
Only smoking first trimester	1.3	0.9-1.9	1.5	0.9-2.8
Smoking whole pregnancy	1.0	0.7-1.2	1.3	0.9-1.7
Male gender	1.0	0.8-1.1	1.4	1.1-1.7
Maternal age (for each additional year)	0.97	0.95-1.00	0.93	0.90-0.96
Season of birth				
Winter	1		1	
Spring	0.9	0.7-1.2	0.9	0.7-1.3
Summer	0.9	0.7-1.2	0.9	0.7-1.3
Fall	0.8	0.6-1.0	0.8	0.6-1.2
Type of feeding at 3 mo†				
Exclusive breastfeeding	1		1	
Breastfeeding and formula	1.4	1.1-1.8	1.0	0.7-1.5
Exclusive formula	1.6	1.3-1.9	1.5	1.1-1.9
Low level of parental education	1.4	1.0-2.0	0.8	0.5-1.3

* Adjusted for type of study, employment of the mother and the other risk factors, eg, results for sibs are adjusted for child care attendance, parental history of allergic disease, maternal smoking during pregnancy, gender, maternal age, season of birth, type of feeding, level of parental education, † OR adjusted like above*, and also adjusted for birth weight and duration of pregnancy

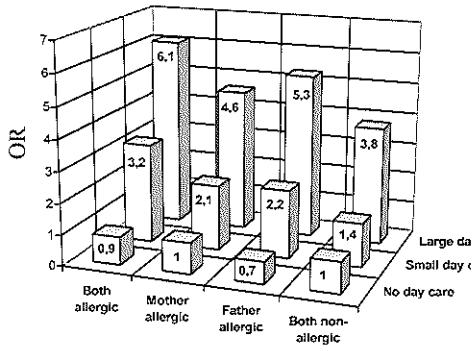

Figure 1: AOR for doctor-diagnosed LRTI in relation to type of child care, stratified for parental history of allergic disease.

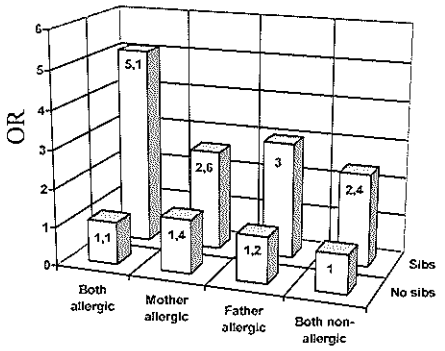

Figure 2: AOR for doctor-diagnosed LRTI in relation to having siblings, stratified for parental history of allergic disease.

DISCUSSION

In this prospective birth cohort study, we found that in allergy-prone children, child care attendance and having siblings increase the risk of developing a doctor-diagnosed LRTI in infancy as compared to children without predisposition for allergy. This increased risk is higher than can be explained by the independent effect of the individual risk factors, which is suggestive for interaction.

Validation issues:
The parental reporting of respiratory tract infections could be related to child care, having siblings, or the allergic status of the parents, resulting in reporting bias. In our study, parents also reported on cough, runny nose, earache, fever and doctors visits for these symptoms, in the month before completing the 12-month questionnaire. We found that for these respiratory symptoms in the month before completion of the questionnaire, type of child care, having siblings, and allergic status of the parents were not related to doctor visits for these symptoms (data not shown). Therefore, it is unlikely that our results can be explained by reporting bias, although we have no data on potential reporting bias for lower respiratory symptoms. Symptoms of asthma or asthma-like disease could have been labeled inappropriately as LRTI, because in this age group a distinction between these diseases is impossible. However, for reporting bias to explain the interaction that we found, it would have to operate selectively in allergic parents whose child goes to daycare, and/or has an older sibling, as in children without older siblings or daycare, parental allergy had little influence on LRTI reporting. This seems unlikely.

Cumulative incidence of respiratory tract infections:
We found a similar cumulative incidence of doctor-diagnosed LRTI as in a Norwegian Study that used the same criteria to define LRTI [14], but a lower incidence as described in studies using the term lower respiratory illness [1, 7]. The latter definition also includes various forms of wheezing illness and probably reflects a less severe outcome [14]. The cumulative 1-year incidence of otitis media in our study (18%) is comparable with some studies [20, 21], but low compared with other studies who found a cumulative incidence of more than 60% [9, 15]. In the latter studies, the diagnosis was made by regular inspection of the tympanum, probably incorporating a-symptomatic otitis media in the definition and therefore reflecting a less severe outcome.

Child care attendance, having siblings and parental history of allergic disease in relation to respiratory tract infections:
There is little doubt that child care attendance is associated with an increased incidence of URTI and LRTI [3-5, 9, 22-24] and probably reflects an increased exposure to microorganisms. For instance, Celedon et al. [3] reported in a high risk population (at least 1 parent was allergic) an OR of 1.6 (95% CI: 1.0-2.4) for lower respiratory tract infection at age 1 in children who attended some form of child care. In the same study, child care in large groups was associated with a much higher risk of developing a lower respiratory tract infection as compared with child care in small groups. In a study by Marbury et al. [4], child care attendance was associated with the development of lower respiratory tract illness in the first year of life [OR of 2.0 (95% CI 1.7-2.3]. Also in their study, the risk of developing a lower respiratory

tract illness increased with increasing group size. Our finding that having older siblings is related to doctor-diagnosed LRTI is in agreement with various other studies [3, 6, 13]. Few data are available about the relationship between parental history of allergic disease and respiratory infections in their children. Our finding that having two allergic parents was associated with an increased risk of developing doctor-diagnosed LRTI (Table 3) is in agreement with the findings of two other studies. Leeder et al. [6] reported a higher incidence of bronchitis and pneumonia in children from asthmatic or wheezing parents than in children from symptom-free parents. Ponsonby et al. [13] found that having a family member with asthma was an independent predictor of developing a lower respiratory tract infection in the first year of life.

Interaction between parental history of allergic disease and contacts with other children:
To our knowledge, this is the first publication in which interaction between the parental history of allergic disease and contacts with other children (child care attendance and having siblings) for the development of respiratory infections in infancy is reported. Our results suggest that the combination of having allergic parents and high exposure to microorganisms increases the risk of developing a doctor-diagnosed LRTI to a greater extent than can be expected from the independent effect of the individual risk factors. A possible explanation is that children from allergic parents have airways that are, for an unknown reason, more vulnerable to infections than airways of children from non-allergic parents. If children are not exposed to a high load of microorganisms (eg, in the case of a single child, when the child is cared for at home), then this increased vulnerability may not lead to serious infections. In contrast, if the infection load is higher (having siblings or, to a higher extent, attending child care), then the increased vulnerability as a result of genetic predisposition leads to an excessive increase in doctor-diagnosed LRTI. It is interesting that the interaction effect was greatest for children with an allergic father compared to children with an allergic mothers or children with 2 allergic parents.

What are the implications of our findings for the prevention of LRTI in infancy? Obviously, decreasing contacts with siblings is mostly not desirable, but high exposure to microorganisms in child care facilities potentially can be avoided. It remains to be seen whether avoiding contact with other children in early life is beneficial in the long run. Two recent studies found an inverse relationship between child care attendance or having siblings in early life and the development of asthma and allergy in later childhood [25, 26]. The authors speculated that infections in early life stimulate the infant's immune response to shift from a predominantly type 2 helper T cell- (which is associated with the allergic phenotype) toward a type 1 helper T cell-dominated response. So if in allergy-prone children child care attendance or having siblings increased the risk of LRTI (as was documented in the PIAMA study), and child care and having siblings in early life protects against asthma and allergy in later childhood [25], then an interesting question will be, "do children from allergic parents benefit more from child care attendance and having older siblings than children from non allergic parents, with respect to the later development of asthma?" Follow-up of our cohort might give more insight on this topic.

CONCLUSION

We found that child care attendance and having siblings increases the risk of developing doctor-diagnosed LRTI in the first year of life to a greater extent in allergy-prone children than in children who are not allergy-prone. The relevance of this finding for future risk of allergic disease requires a long-term follow-up.

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References:

1. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am J Epidemiol 1989;129:1232-46.
2. Ey JL, Holberg CJ, Aldous MB, Wright AL, Martinez FD, Taussig LM. Passive smoke exposure and otitis media in the first year of life. Group Health Medical Associates. Pediatrics 1995;95:670-7.
3. Celedon JC, Litonjua AA, Weiss ST, Gold DR. Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. Pediatrics 1999;104:495-500.
4. Marbury MC, Maldonado G, Waller L. Lower respiratory illness, recurrent wheezing, and day care attendance. Am J Respir Crit Care Med 1997;155:156-61.
5. Holberg CJ, Wright AL, Martinez FD, Morgan WJ, Taussig LM. Child day care, smoking by caregivers, and lower respiratory tract illness in the first 3 years of life. Group Health Medical Associates. Pediatrics 1993;91:885-92.
6. Leeder SR, Corkhill R, Irwig LM, Holland WW, Colley JR. Influence of family factors on the incidence of lower respiratory illness during the first year of life. Br J Prev Soc Med 1976;30:203-12.
7. Fergusson DM, Horwood LJ, Shannon FT, Taylor B. Parental smoking and lower respiratory illness in the first three years of life. J Epidemiol Community Health 1981;35:180-4.
8. Martinez FD, Wright AL, Holberg CJ, Morgan WJ, Taussig LM. Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. Am J Epidemiol 1992;136:1258-68.
9. Paradise JL, Rockette HE, Colborn DK, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. Pediatrics 1997;99:318-33.
10. Margolis PA, Greenberg RA, Keyes LL, et al. Lower respiratory illness in infants and low socioeconomic status. Am J Public Health 1992;82:1119-26.
11. Greenough A, Giffin FJ, Yuksel B. Respiratory morbidity in preschool children born prematurely. Relationship to adverse neonatal events. Acta Paediatr 1996;85:772-7.
12. Rylander E, Eriksson M, Pershagen G, Nordvall L, Ehrnst A, Ziegler T. Wheezing bronchitis in children. Incidence, viral infections, and other risk factors in a defined population. Pediatr Allergy Immunol 1996;7:6-11.
13. Ponsonby AL, Couper D, Dwyer T, Carmichael A, Kemp A. Relationship between early life respiratory illness, family size over time, and the development of asthma and hay fever: a seven year follow up study. Thorax 1999;54:664-9.
14. Nafstad P, Jaakkola JJ, Hagen JA, Botten G, Kongerud J. Breastfeeding, maternal smoking and lower respiratory tract infections. Eur Respir J 1996;9:2623-9.
15. Duncan B, Ey J, Holberg CJ, Wright AL, Martinez FD, Taussig LM. Exclusive breast-feeding for at least 4 months protects against otitis media. Pediatrics 1993;91:867-72.
16. Bergmann RL, Bergmann KE, Lau-Schadensdorf S, et al. Atopic diseases in infancy. The German multicenter atopy study (MAS-90). Pediatr Allergy Immunol 1994;5:19-25.
17. Camilli AE, Holberg CJ, Wright AL, Taussig LM. Parental childhood respiratory illness and respiratory illness in their infants. Group Health Medical Associates. Pediatr Pulmonol 1993;16:275-80.

18. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
19. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
20. Benediktsdottir B. Upper airway infections in preschool children--frequency and risk factors. Scand J Prim Health Care 1993;11:197-201.
21. Aniansson G, Alm B, Andersson B, et al. A prospective cohort study on breast-feeding and otitis media in Swedish infants. Pediatr Infect Dis J 1994;13:183-8.
22. Louhiala PJ, Jaakkola N, Ruotsalainen R, Jaakkola JJ. Form of day care and respiratory infections among Finnish children. Am J Public Health 1995;85:1109-12.
23. Alho OP, Laara E, Oja H. Public health impact of various risk factors for acute otitis media in northern Finland. Am J Epidemiol 1996;143:1149-56.
24. Uhari M, Mantysaari K, Niemela M. A meta-analytic review of the risk factors for acute otitis media. Clin Infect Dis 1996;22:1079-83.
25. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000;343:538-43.
26. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999;353:450-4.

8.2. Enhanced nasal pro-inflammatory cytokine production rather than a Th2-like response in RSV bronchiolitis

I.J. van Benten[1], L.P. Koopman[2], A. KleinJan[1], B.C. van Middelkoop[1], L.de Waal[3], A. D.M.E. Osterhaus[3], H.J. Neijens[2], and W.J. Fokkens[1]

[1]Departments of Otorhinolaryngology and [2]Pediatrics and [3]Institute for Virology, Erasmus University Medical Center Rotterdam, The Netherlands

ABSTRACT

Background: Respiratory syncytial virus (RSV) can induce both severe infection of the lower respiratory tract (bronchiolitis) and mild infection of the upper respiratory tract in infants. *Objective:* To investigate differences in numbers of nasal mucosal inflammatory cells and cytokine positive cells in infants with RSV bronchiolitis and RSV upper respiratory tract infections (URTI).
Methods: Nasal brush samples were obtained from 14 infants with RSV bronchiolitis and 8 infants with RSV URTI during the acute phase and convalescent phase (2 to 4 weeks later) of infection. Cytospin preparations were stained immunohistochemically for CD3 (T cells), CD68 (macrophages), major basic protein (eosinophils), IgE, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, IFNγ and ICAM-1.
Results: During the acute phase of RSV infection, bronchiolitis and URTI, marked influxes of macrophages and T lymphocytes were observed. During the acute phase of bronchiolitis, but not URTI, higher numbers of IL-6, IL-12, IL-18 and ICAM-1 positive cells were observed compared to convalescence. Infants with bronchiolitis had, during the acute phase of infection, higher numbers of IL-18 positive cells than infants with URTI. In addition, infants with bronchiolitis had higher numbers of eosinophils during the convalescent phase than infants with URTI. For both bronchiolitis and URTI, no differences in IgE, IL-4, IL-8, IL-10 and IFNγ were observed.
Conclusion: Increased numbers of IL-6, IL-12 and IL-18 positive cells during RSV bronchiolitis may indicate enhanced pro-inflammatory responses rather than T helper 2-like responses in the nasal mucosa. These may well lead to excessive mucus secretion, airway narrowing and prolonged wheezing symptoms following bronchiolitis.

Submitted

INTRODUCTION

Respiratory syncytial virus (RSV) infection is a major cause of upper and lower respiratory tract disease. Most infants infected with RSV only develop upper respiratory tract infection (URTI). However, a small percentage is hospitalized due to severe lower respiratory tract symptoms (bronchiolitis or pneumonia) [1,2]. Risk factors for developing bronchiolitis are prematurity, lung and heart disease and immune and metabolic disorders [3-5]. However, RSV bronchiolitis may occur in healthy non-risk infants.
Several prospective studies found an association between severe RSV bronchiolitis during early life and the development of wheezing episodes and asthma during later childhood [6-8]. However, others could not confirm these findings and found no increased risk of wheezing or allergic sensitization in 13 year-old children following bronchiolitis during infancy [9]. Increased levels of eosinophil cationic protein (ECP), RSV specific and total IgE have been found in nasal lavage samples during bronchiolitis [6, 10-13]. High peripheral blood eosinophil counts at the time of bronchiolitis are correlated to wheezing symptoms following infection [14,15]. In addition, in RSV infected mice and T cell culture systems infected with RSV, enhanced T helper 2 (Th2) responses have been observed [16-17]. These findings may explain the development of asthma after RSV bronchiolitis. On the other hand, pro-inflammatory and Th1 responses have also been found in bronchiolitis. Neutrophils, their activation products [18-20] and IL-1, IL-6 and IL-8 have been observed in nasal lavage or blood samples during infection [18, 20-23].
We compared nasal mucosal immune responses in 14 infants with RSV bronchiolitis and 8 infants with URTI without bronchiolitis to determine whether differences in numbers of inflammatory cells and cytokine positive cells can be found. Differences in cell and cytokine responses between the two patient groups could explain why in bronchiolitis patients wheezing and asthma-like symptoms are found.

METHODS

Patients and study design:
During the winters of 1998 and 1999, 14 infants (mean age 9 weeks) seen at the Sophia's Children Hospital in Rotterdam (The Netherlands) with RSV induced lower respiratory tract infection (bronchiolitis) accompanied by upper respiratory tract symptoms were selected for inclusion in the study. Clinical criteria of bronchiolitis were the need for hospital care due to the severity of RSV infection. Bronchiolitis was treated with either antibiotics or bronchodilators. Eight infants (mean age 29 weeks) who participated in a prospective birth cohort study and presented with mild URTI symptoms without bronchiolitis caused by RSV were selected as controls. None of the children with URTI presented to the hospital. Physical examination was performed and nasal brush samples were taken within the first few days after the onset of infection for the URTI group (median 3 days) and within 24 hours after arrival in the hospital for the bronchiolitis group (median 5 days of URTI symptoms). During the convalescent phase (2 to 4 weeks later) of RSV infection, nasal brush samples were taken from all infants with URTI and 11 of 14 infants with bronchiolitis. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre Rotterdam. Written informed consent was given by all parents for participation of their child in this study.

Nasal brushes and viral diagnostics:
Cells were harvested from the nasopharynx with a cytobrush (Medscand Medical, Sweden) and processed as previously described by Godthelp et al. [24]. Cells were washed in RPMI 1640 medium (Life Technologies) and cytospin preparations made on 10% poly-L-lysine (Sigma) coated microscope slides were stored at -80°C. RSV infection was confirmed by direct immunofluorescent staining on nasal brush cells with antiviral antibodies and/or by virus isolation from nasal brush supernatant.

Immunohistochemical staining of CD3, CD68, MBP, IgE, ICAM-1 and IL-18:
Slides were fixed in acetone and placed in a semi-automatic stainer (Sequenza, Shandon, Amsterdam, The Netherlands). Immunohistochemical staining was performed as previously described by Godthelp et al. [24]. In brief, slides were pre-incubated with 10% normal goat serum (CLB, The Netherlands) (10 minutes) and subsequently for 60 minutes with mouse anti-human monoclonal antibodies directed against CD3, CD68, major basic protein (MBP), IgE, ICAM-1 and IL-18 diluted in PBS supplemented with 1% blocking reagent (Boehringer Mannheim, Germany) (PBS/block) (Table 1). After incubation for 30 minutes with biotinylated goat anti-mouse Ig serum, slides were incubated either with streptavidin alkaline phosphatase for CD3, CD68, MBP staining or with polyclonal goat anti-biotin antibody for IgE, ICAM-1 and IL-18 staining for 30 minutes. After incubation with New Fuchsin substrate (Chroma, Kongen, Germany), sections were counterstained with Gill's haematoxilin and mounted in glycerin-gelatin. Control staining was performed by the substitution of primary monoclonal antibody with isotypic control antibody.

Table 1: Monoclonal antibodies

Antibody	Specificity	Cell type/cytokine	Conc (μg/ml)	Source
T3-4B5	CD3	T lymphocytes	4.2	DAKO, Denmark
EBM11	CD68	Macrophages	4.6	DAKO, Denmark
BMK-13	MBP	Eosinophils	0.2	Sanbio, The Netherlands
MH25M	IgE	IgE	1	CLB, The Netherlands
MEM-112	CD54	ICAM-1	144	Sanbio, The Netherlands
1-41-1	IL-4	IL-4	12	Novartis, Switserland
IC25-471	IL-10	IL-10	10	Instruchemie, The Netherlands
24945.11	IL-12p70	IL-12	5	R&D systems, United Kingdom
500-M87	IL-18	IL-18	20	Peprotech, United Kingdom
MD-1	IFNγ	IFNγ	2.5	Innogenetics, Belgium
B-E8	IL-6	IL-6	10	Bender Medsystems, Austria
NAP II	IL-8	IL-8	1	Bender Medsystems, Austria

Tyramide signal amplification (TSA) staining for IL-4, IL-8, IL-10, IL-12 and IFNγ:
A modified amplified protocol based on the alkalic phosphatase method described in the previous section was made. Slides were incubated with mouse anti-human monoclonal antibodies directed against IL-4, IL-8, IL-10, IL-12, IFNγ or an isotypic control antibody for 60 minutes (table I). After incubation with biotinylated goat anti-mouse Ig serum, endogenous peroxidase was blocked using azide (0.2 %), peroxidase (0.02 %) and methanol (50 %) in PBS. Slides were then subsequently incubated with streptavidin conjugated peroxidase (30 minutes) (NEN Inc., Boston, MA, USA), biotinyl tyramide in Tris/HCL buffer (10 minutes) for amplification

of the staining signal, with alkaline-phophatase conjugated goat-anti-biotin (30 minutes) and new fuchsin substrate.

Immunohistochemical staining of IL-6:
Sections were stained for IL-6 using the *poly*MICA immunohistochemical staining system of The Binding Site Ltd (Birmingham, UK). In brief, sections were fixed in acetone and endogenous peroxidase was blocked using azide (0.01 %) and peroxidase (0.1 %) in PBS. After incubation with mouse monoclonal antibody directed against IL-6 (table I) for 60 minutes, slides were incubated with reagents in accordance with the manufacturer's instructions. Finally, slides were incubated with DAB substrate and nucleus staining was performed with Gill's haematoxilin.

Light-microscopic evaluation:
1000 cells stained with a purple-blue nucleus were counted in every nasal brush sample. All slides were blinded and counted by two independent investigators in order to guarantee an objective analysis. The number of positively stained cells was calculated as a percentage of 1000 nasal brush cells. CD3 and ICAM-1 positively stained cells had a red cell membrane. Red cytoplasmic staining was found for CD68, IgE, MBP, IL-4, IL-8, IL-10, IL-12, IL-18 and IFNγ. IL-6 positive cells had dark brown cytoplasmic staining. On basis of morphology both inflammatory and ciliated epithelial cells were found to stain positive for cytokines. Cells were counted at a magnification of 400x.

Statistical analysis:
The statistical analysis of cell numbers was performed with SPSS. Percentages of positive cells were log-transformed to obtain a normal distribution among all data. Differences between the two sampling moments were analyzed with the paired sample T test. Differences in cytokine positive cells between patients with bronchiolitis and between different patient characteristics were analyzed with the independent sample T test. Differences between patient groups and sampling moments were considered statistically significant when the p value ≤ 0.05.

RESULTS

Patient characteristics:
All but one of 14 patients with RSV bronchiolitis needed hospital admission. Six infants were admitted to the medium care unit and 7 to the intensive care unit (ICU). Of the infants admitted to intensive care, 3 needed mechanical ventilation. All bronchiolitis infants suffered from a runny nose and cough and 3 infants showed wheezing symptoms. None of the eight control infants with mild RSV URTI symptoms were admitted to the hospital and only suffered from mild common cold symptoms. Patient characteristics are summarized in table 2.

Macrophages, T cells and ICAM-1 positive cells:
Numbers of macrophages found in nasal brush samples varied between 0.1 and 17.9% and T lymphocytes between 0 and 7.4% (figure 1A and 1B). During the acute phase of bronchiolitis as well as URTI, there was a marked increase of macrophages in nasal brush samples com-

pared to convalescent samples (p=0.001 and p=0.009 respectively). Statistically significant increases in numbers of T lymphocytes were observed for URTI (p=0.02), as well as a trend towards increased numbers of T lymphocytes (p=0.06) in bronchiolitis during the acute phase of infection compared to convalescence. Many ICAM-1 positive cells were found (range 0.8-64.2%) during the acute and convalescent phase of RSV infection (figure 1C). Significantly elevated numbers of ICAM-1 positive cells were found during the acute phase of bronchiolitis (p=0.04) compared to convalescence. No differences in T lymphocytes, macrophages and ICAM-1 positive cells were found between bronchiolitis and URTI at each sampling time point.

Table 2: Patient characteristics

	Bronchiolitis	URTI
Number of patients	14	8
Age (weeks) *	9	29
Gender (male)	36%	63%
Smoking parent	35%	13%
ICU	50%	0%
Birth weight (grams)	3343	3830
Duration pregnancy (weeks)	38.7	40.2

* p=0.005 Fisher's Exact test; ICU, Intensive care unit

Eosinophils and IgE positive cells:
Small numbers of eosinophils and IgE positive cells were found in nasal brush samples. Maximum percentages of positive cells were 0.4% for eosinophils (figure 1D) and 0.1% for IgE positive cells (data not shown). No differences were observed in eosinophils and IgE positive cells between acute and convalescent samples nor between bronchiolitis and URTI in the acute phase. During the convalescent phase in bronchiolitis, significantly more eosinophils were detected than in patients with URTI (p=0.05).

Th1 and Th2 type cytokines: IL-4, IL-10, IL-12 and IFNγ:
Nasal brush samples were stained for Th1 cytokines IL-12 and IFNγ and for Th2 cytokines IL-4 and IL-10 (figure 2). Numbers of IL-12 positive cells ranged between 0.3 and 36.1% and IFNγ positive cells between 0 and 21.5%. Numbers of IL-4 positive cells ranged between 3.7 and 47.7% and IL-10 positive cells between 2.4 and 58.1%. Median percentages of Th1 positive cells were less than 10%, whereas median percentages of Th2 positive cells were about 20%. In bronchiolitis, numbers of IL-12 positive cells increased during the acute phase as compared to the convalescent phase (p=0.04). No differences were found for IL-4, IL-10 and IFNγ positive cells between the two sampling moments. During both the acute and convalescent phase, no differences were found in IL-4, IL-10, IL-12 and IFNγ positive cells between bronchiolitis and URTI. When Th2/Th1 balances were expressed as IL-4/IFNγ and IL-10/IL-12 ratios, no differences were demonstrated between patients with RSV bronchiolitis and URTI at both sampling time points. Increased IL-10/IL-12 ratios were measured during the convalescent phase of bronchiolitis as compared to the acute phase (p=0.04).

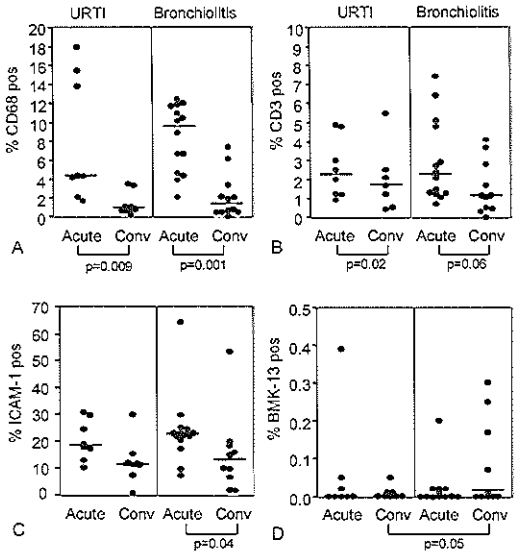

Figure 1: Numbers of (A) macrophages (CD68 positive), (B) T lymphocytes (CD3 positive) and (C) ICAM-1 positive cells, and (D) eosinophils (MBP positive) during the acute and convalescent phase (conv) of RSV induced URTI and bronchiolitis. Bars represent median percentages.

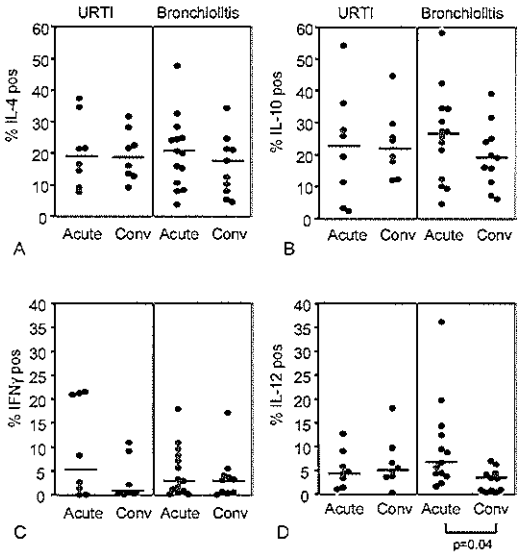

Figure 2: Numbers of IL-4 (A), IL-10 (B), IFNγ (C) and IL-12 (D) positive cells during the acute and convalescent phase (conv) of RSV induced URTI and bronchiolitis. Bars represent median percentages.

Pro-inflammatory cytokines: IL-6, IL-8 and IL-18:
High numbers of IL-18 positive cells were found in nasal brush samples (range 3.0-99.0% of nasal brush cells) (figure 3A). Statistically significant increases in numbers of IL-18 positive cells were found during the acute phase of bronchiolitis compared to the convalescent phase (p=0.01). During the acute phase of RSV bronchiolitis, higher numbers of IL-18 positive cells were found as compared to RSV URTI (p=0.001). Numbers of IL-6 and IL-8 positive cells ranged from 3.1 and 79.1% for IL-6 (figure 3B) and between 0.5 and 45.1% for IL-8 (data not shown). In bronchiolitis, higher numbers of IL-6 positive cells were observed during the acute phase compared to convalescence (p=0.03). No differences between acute and convalescent sampling were found for IL-8 positive cells and no differences were observed between bronchiolitis and URTI for IL-6 and IL-8 positive cells.

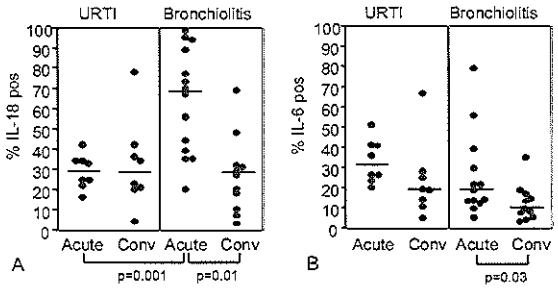

Figure 3: Numbers of IL-18 (A) and IL-6 (B) positive cells during the acute phase and convalescent phase (conv) of RSV induced URTI and bronchiolitis. Bars represent median percentages.

Correlation with patient characteristics:
No statistically significant associations were found between cell and cytokine responses on the one hand and gender, birth weight and the age of the child on the other when all patients were analyzed. Infants with smoking parents had increased numbers of macrophages (p<0.001) and IL-10 positive cells (p=0.03) during infection compared to infants with non-smoking parents. Infants with RSV bronchiolitis admitted to the ICU had more IL-10 positive cells during acute infection compared to infants admitted to the medium care unit (p=0.05) (data not shown).

DISCUSSION

Differences in numbers of inflammatory cells and cytokine positive cells were studied in nasal brush samples of infants with RSV bronchiolitis and infants with URTI. Differences in cell numbers may explain why infants with bronchiolitis often present with wheezing symptoms and an asthma-like phenotype during and after infection. T cell culture studies and studies performed in mouse models has led to the hypothesis that a shift towards a Th2 response during bronchiolitis explains asthma/wheezing in these infants [16, 17, 25]. This study inves-

tigated whether these findings could be extrapolated to human immunological responses during bronchiolitis.
We could not find marked differences in the balance between Th1 and Th2 type cytokine positive cells in infants with RSV bronchiolitis and URTI. The present study supports pro-inflammatory and Th1-like responses, which may differ according to disease severity [23, 26, 27].
In bronchiolitis, increased numbers of IL-6, IL-12 and IL-18 positive cells were observed. Interleukin 18 is involved in anti-viral mechanisms and is, in combination with IL-12, a powerful inducer of IFNγ production from T lymphocytes and natural killer cells. IL-18 can therefore stimulate Th1 responses. In addition, IL-18 is – like IL-6 – often regarded as a pro-inflammatory cytokine [28]. The effect of IL-18 is closely related to that of IL-1 and induces expression of TNFα, IL-1, IL-6, IL-8, GM-CSF, ICAM-1, Fas ligand and several chemokines and inhibits IL-10 and IgE production [29, 30]. Enhanced production of IL-6, IL-8, IL-12, RANTES and MIP-1α during RSV bronchiolitis and virus-induced asthma exacerbations has also been shown by others [18, 20-23, 26, 31]. Although it has recently been shown that in mice IL-18 may induce Th2 responses [32, 33], and IL-4 and histamine release from cultured bone marrow cells [34], these responses depend to a major extent on the genetic background of the mouse strain and cytokine microenvironment of cultured cells. Since we predominantly observed Th1-type cytokine positive cells (IL-12), we believe that IL-18 is more likely to support a pro-inflammatory response. No increase in numbers of IFNγ positive cells was found. This may indicate a defective IFNγ production independent of IL-12 and IL-18. Actual quantities of IFNγ have to be measured in nasal samples to examine whether infants are incapable of IFNγ production.
Can this type of immune response explain lower airway pathology? Increased pro-inflammatory cytokine production during infection may induce excessive mucus secretion, leading to airway narrowing and wheezing symptoms. Infiltration of inflammatory cells may damage airway epithelium and subsequent remodeling mechanisms may prolong lower airway pathology following bronchiolitis. This may explain prolonged wheezing after RSV bronchiolitis which probably is not related to a Th2-mediated asthmatic response.
A secondary Th2-like response following bronchiolitis cannot be totally ruled out. During convalescence, there was a trend towards higher numbers of eosinophils in infants with bronchiolitis, but no increase in numbers of ICAM-1 positive cells. In adult allergic patients, prolonged eosinophilia was also documented following viral infection [35]. This may prime them for subsequent allergic symptoms. Whether prolonged eosinophilia indicates allergy-like immune responses remains questionable because numbers of eosinophils detected in this study were low and this issue requires further study.
Nasal brushes are easy to perform in infants and can adequately document cellular immune responses in the nose. These nasal responses are closely linked to pulmonary immune responses [36]. Until now, most investigators studied immune responses in infants with severe RSV bronchiolitis, as compared to healthy non-infected infants or infants with mild bronchiolitis [20, 37, 38]. We found clear differences between infants with bronchiolitis compared to infants with only URTI caused by RSV. The mean age of infants with bronchiolitis was significantly lower than those with URTI and could have skewed the results. An effect of age is however implausible, because the number of cytokine and cell-marker positive cells is not correlated to the age of the child. Moreover, if there is an age-effect, higher numbers of Th2 cytokine positive cells (IL-4 and IL-10) and lower numbers of Th1 cytokine positive cells (IL-

12, IL-18 and IFNγ) would have been expected in the younger age group (bronchiolitis-group) than the URTI group which is in contrast to our findings [39].
In conclusion, no increase in Th2 cytokine positive cells was observed in infants during the acute phase of RSV bronchiolitis. What we found was an increase in Th1 or pro-inflammatory cytokine positive cells. Additional follow-up studies will have to be performed to investigate in detail secondary local immune responses following RSV bronchiolitis.

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We would like to thank all children and parents who participated in this study, Afke Brandenburg for collecting nasal brush and blood samples of infants with bronchiolitis and Novartis for kindly providing the IL-4 antibody. This study was supported by the Netherlands Asthma Foundation and the Netherlands Organisation for Health Science.

References:

1. Green M, Brayer AF, Schenkman KA, Wald ER. Duration of hospitalization in previously well infants with respiratory syncytial virus infection. Pediatr Infect Dis J 1989;8(9):601-5.
2. Everard ML, Milner AD. The respiratory syncytial virus and its role in acute bronchiolitis. Eur J Pediatr 1992;151(9):638-51.
3. Groothuis JR, Gutierrez KM, Lauer BA. Respiratory syncytial virus infection in children with bronchopulmonary dysplasia. Pediatrics 1988;82(2):199-203.
4. MacDonald NE, Hall CB, Suffin SC, Alexson C, Harris PJ, Manning JA. Respiratory syncytial viral infection in infants with congenital heart disease. N Engl J Med 1982;307(7):397-400.
5. Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial viral infection in children with compromised immune function. N Engl J Med 1986;315(2):77-81.
6. Welliver RC, Sun M, Rinaldo D, Ogra PL. Predictive value of respiratory syncytial virus-specific IgE responses for recurrent wheezing following bronchiolitis. J Pediatr 1986;109(5):776-80.
7. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics 1995;95(4):500-5.
8. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000;161(5):1501-7.
9. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999;354(9178):541-5.
10. Garofalo R, Kimpen JL, Welliver RC, Ogra PL. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. J Pediatr 1992;120(1):28-32.
11. Garofalo R, Dorris A, Ahlstedt S, Welliver RC. Peripheral blood eosinophil counts and eosinophil cationic protein content of respiratory secretions in bronchiolitis: relationship to severity of disease. Pediatr Allergy Immunol 1994;5(2):111-7.
12. Koller DY, Wojnarowski C, Herkner KR, et al. High levels of eosinophil cationic protein in wheezing infants predict the development of asthma. J Allergy Clin Immunol 1997;99(6 Pt 1):752-6.
13. Welliver RC, Duffy L. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at age 7-8 years. Pediatr Pulmonol 1993;15(1):19-27.
14. Ehlenfield DR, Cameron K, Welliver RC. Eosinophilia at the time of respiratory syncytial virus bronchiolitis predicts childhood reactive airway disease. Pediatrics 2000;105(1 Pt 1):79-83.
15. Martinez FD, Stern DA, Wright AL, Taussig LM, Halonen M. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. J Allergy Clin Immunol 1998;102(6 Pt 1):915-20.
16. Roman M, Calhoun WJ, Hinton KL, et al. Respiratory syncytial virus infection in infants is associated with predominant Th-2-like response. Am J Respir Crit Care Med 1997;156(1):190-5.
17. van Schaik SM, Welliver RC, Kimpen JL. Novel pathways in the pathogenesis of respiratory syncytial virus disease. Pediatr Pulmonol 2000;30(2):131-8.

18. Teran LM, Johnston SL, Schroder JM, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. Am J Respir Crit Care Med 1997;155(4):1362-6.
19. Everard ML, Swarbrick A, Wrightham M, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. Arch Dis Child 1994;71(5):428-32.
20. Abu-Harb M, Bell F, Finn A, et al. IL-8 and neutrophil elastase levels in the respiratory tract of infants with RSV bronchiolitis. Eur Respir J 1999;14(1):139-43.
21. Roseler S, Holtappels G, Wagenmann M, Bachert C. Elevated levels of interleukins IL-1 beta, IL-6 and IL-8 in naturally acquired viral rhinitis. Eur Arch Otorhinolaryngol Suppl 1995;1:S61-3.
22. Bont L, Heijnen CJ, Kavelaars A, et al. Peripheral blood cytokine responses and disease severity in respiratory syncytial virus bronchiolitis. Eur Respir J 1999;14(1):144-9.
23. Brandenburg AH, Kleinjan A, van Het Land B, et al. Type 1-like immune response is found in children with respiratory syncytial virus infection regardless of clinical severity. J Med Virol 2000;62(2):267-77.
24. Godthelp T, Holm AF, Fokkens WJ, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.
25. Bendelja K, Gagro A, Bace A, et al. Predominant type-2 response in infants with respiratory syncytial virus (RSV) infection demonstrated by cytokine flow cytometry. Clin Exp Immunol 2000;121(2):332-8.
26. Bont L, Kavelaars A, Heijnen CJ, van Vught AJ, Kimpen JL. Monocyte interleukin-12 production is inversely related to duration of respiratory failure in respiratory syncytial virus bronchiolitis. J Infect Dis 2000;181(5):1772-5.
27. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC, 2nd, Welliver RC. Increased production of IFN-gamma and cysteinyl leukotrienes in virus- induced wheezing. J Allergy Clin Immunol 1999;103(4):630-6.
28. Dinarello CA. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999;103(1 Pt 1):11-24.
29. Wang W, Tanaka T, Okamura H, et al. Interleukin-18 enhances the production of interleukin-8 by eosinophils. Eur J Immunol 2001;31(4):1010-6.
30. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 2001;12(1):53-72.
31. Teran LM, Seminario MC, Shute JK, et al. RANTES, macrophage-inhibitory protein 1alpha, and the eosinophil product major basic protein are released into upper respiratory secretions during virus-induced asthma exacerbations in children. J Infect Dis 1999;179(3):677-81.
32. Wild JS, Sigounas A, Sur N, et al. IFN-gamma-inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol 2000;164(5):2701-10.
33. Leite-De-Moraes MC, Hameg A, Pacilio M, et al. IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: a pro-Th2 effect of IL-18 exerted through NKT cells. J Immunol 2001;166(2):945-51.
34. Yoshimoto T, Tsutsui H, Tominaga K, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci U S A 1999;96(24):13962-6.
35. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995;151(3 Pt 1):879-86.
36. Braunstahl GJ, Kleinjan A, Overbeek SE, Prins JB, Hoogsteden HC, Fokkens WJ. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. Am J Respir Crit Care Med 2000;161(6):2051-7.
37. Everard ML, Fox G, Walls AF, et al. Tryptase and IgE concentrations in the respiratory tract of infants with acute bronchiolitis. Arch Dis Child 1995;72(1):64-9.
38. Renzi PM, Turgeon JP, Yang JP, et al. Cellular immunity is activated and a TH-2 response is associated with early wheezing in infants after bronchiolitis. J Pediatr 1997;130(4):584-93.
39. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. Lancet 1999;353(9148):196-200.

Chapter 9

Ethnicity, socioeconomic status and allergic disease

9.1. Early respiratory and- skin symptoms in relation to ethnical background: the importance of socioeconomic status; the PIAMA-Study

L.P. Koopman[1], A. Wijga[2], H.A. Smit[2], J.C. de Jongste[1], M. Kerkhof[3], J. Gerritsen, A.P.H. Vos[4], R.T. van Strien[4], B. Brunekreef[4] and H.J. Neijens, MD[1]

[1]Erasmus University Medical Center / Sophia Children's Hospital Rotterdam, Department of Pediatrics. [2]National Institute of Public Health and the Environment, Department of Chronic Disease Epidemiology. [3]Groningen University/Beatrix Children's Hospital Groningen, department of Epidemiology/Pediatric Pulmonology.[4]University of Utrecht, Institute for Risk Assessment Sciences, Department of Environmental and Occupational Health.

ABSTRACT

Objective: evaluate ethnical differences in the prevalence of respiratory and- skin symptoms in the first 2 years of life.
Design: prospective birth cohort study.
Participants and setting: 4146 children participating in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)- study.
Main outcome measures and covariables: Parents completed questionnaires on respiratory and- skin symptoms, ethnical background and other potential confounders during pregnancy, and at 3 months, 1 year, and 2 years of age.
Results: in the first year, 'Non-Dutch' children (compared with 'Dutch' children) had a higher prevalence of runny nose with itchy/watery eyes (11.0% versus 5.0%). In the second year, a higher prevalence of wheeze at least once (26.7% versus 18.5%), night cough without a cold (24.6% versus 15.5%), runny nose without a cold (34.1% versus 21.3%) and runny nose with itchy/watery eyes (13.7% versus 4.6%) was found. Adjustment for various confounders, especially adjustment for socioeconomic factors reduced most associations between ethnicity and respiratory symptoms. Only runny nose with itchy/watery eyes in the second year of life was independently associated with 'Non-Dutch' ethnicity (adjusted odds ratio 2.89, 95% confidence interval; 1.3-6.4).
Conclusions: 'Non-Dutch' children more often had respiratory symptoms in the first 2 years of life than 'Dutch' children. This could largely be explained by differences in socioeconomic status. Follow-up of the cohort will determine whether this higher prevalence of respiratory symptoms in children with 'Non-Dutch' ethnicity represents an increased risk to develop allergic disease rather than non-specific or infection-related respiratory symptoms.

Submitted

INTRODUCTION

The prevalence of allergic disease varies considerably throughout the world, with in general a high prevalence in Western countries and a low prevalence in developing countries [1]. Within countries, the prevalence of allergic disease varies among different ethnical groups. In the USA and the UK, a consistently higher prevalence of asthma and recurrent wheezing is found among African-American, Hispanic, and Afro-Caribbean children as compared to white children [2-4]. Although immigration in the last decades has lead to marked demographic changes in continental Western Europe, few data are available about ethnical differences in the prevalence of allergic disease in this part of the world. Cross-sectional studies in Germany, Sweden and the Netherlands indicate that children born from Turkish immigrants have a lower prevalence of asthma, recurrent wheezing and atopic dermatitis than children born from German, Swedish or Dutch parents [5-7]. In Sweden, a higher prevalence of asthma and allergic rhinitis was found among children from Chilean immigrants compared with children from Swedish born parents [5]. Studies from the USA and UK indicate that ethnical differences in allergic disease can largely be explained by differences in socioeconomic status (SES) rather than genetic factors [8-10]. However, in the studies from Germany, Sweden and the Netherlands, the association between ethnicity and allergic disease was independent of SES [5-7].
Previous studies in Western Europe have investigated ethnical differences in allergic disease and respiratory symptoms in schoolchildren [5-7] and toddlers [7]. Few data are available for children aged 0 to 2 year. Using data from a large prospective birth cohort study (PIAMA), we have investigated ethnical differences in the prevalence of respiratory and- skin symptoms in the first 2 years of life. Because the diagnosis of allergic disease in this age group is difficult, we used a symptom based approach instead of a disease diagnosis.

METHODS

Study population:
Participants were recruited during the first trimester of pregnancy from 52 midwife practices in 3 different regions in the Netherlands: the North (greater Groningen), central (Bilthoven, Wageningen, and surroundings) and South-West (greater Rotterdam). In total, 7862 pregnant women were invited to participate in the study, and 4146 (53 %) gave informed consent. Their children were all born in The Netherlands between May 1996 and December 1997.

Study design:
Details of the study design have been published previously [11, 12]. Pregnant women completed a validated allergy screening questionnaire in the second trimester of pregnancy [13]. Mothers with self-reported symptoms of asthma and/or allergy were defined as allergic. Of the cohort, 1327 children have an allergic mother (high risk children) and 2819 children have a non-allergic mother (low risk children). The study includes an intervention part and a natural history part. Eight-hundred fifty-five high-risk children were allocated to the intervention part of the study. Half of those children received house dust mite impermeable mattress covers for the parental and infant bed (active group), and the other half received cotton mattress covers (placebo group). In the natural history part of the study (no intervention), 472 high-risk

children and 2819 low-risk children were included. The study was approved by the local medical ethical committees.

Data collection:
Questionnaires, in Dutch, were sent to the participating families during pregnancy, and when the child was 3 months, 1 year and 2 years of age.
Measures of outcome: In the 1 and- 2 years questionnaire, parents were asked whether their child had wheezing episodes, cough without a cold, runny nose without a cold (and if yes, if this was accompanied by watery, irritated eyes), and itchy skin rash in the previous year. Children with at least 4 episodes of wheeze were defined as children with recurrent wheeze. Only children with skin rash at localization typical for eczema (around the eyes/ears, in the neck, in the knee or-elbow folds and at the front of the ankles) were defined as having an itchy skin rash. Parents were also asked whether their child had a cough, a runny nose, earache, or fever in the month prior to completing the 1 and- 2 years questionnaire and information was collected on doctor visits for these symptoms.
Definition of ethnicity: When the children were 2 years, we asked in which country the parents were born and to which ethnical group they consider themselves belonging [14]. Based on the answers we defined two different ethnical groups: 'Dutch': children from mothers born in the Netherlands which consider themselves Dutch, and 'non-Dutch': children from mothers born in Non-Western countries (mainly Suriname, Netherlands Antilles, Indonesia, Turkey, and Morocco) or children from mothers who consider themselves belonging to a non-Western ethnical group. Children from mothers born in other Western countries (e.g. all the countries belonging to the European Union, Switzerland, USA, Canada, Australia, New Zealand, and South Africa) were excluded from this analysis.
Potential confounders: In the pregnancy questionnaire, data were collected on parental age, expected birth date, smoking by the mother and allergy in the father (by using the same validated questionnaire as was used for the mother [13]). In the 3 months questionnaire, data were collected on birth characteristics, number of siblings, type of feeding, housing characteristics, pet keeping and atopic disease in the siblings [15]. In the 1- year questionnaire, data were collected on weight and height, day care attendance, environmental tobacco smoke exposure and SES (parental employment, parental education). The level of education of the parents was divided into 7 categories, ranging from only elementary school to college education. Parents in the lowest 2 categories (only elementary school or technical/vocational training until age 16) were defined as having 'a low level of education'.

Statistical analysis:
Statistical analysis were performed using SPSS (version 10.0, Chicago, USA). The maximum number of children with data on ethnicity were used in all analysis (for instance, the prevalence of respiratory symptoms at age 2 was estimated by using all children with data on this topic at age 2 and not only for children who also had complete data during pregnancy, at 3 months and 1 year). Categorical data were compared by using the Pearson's χ^2 test, and continuous data were compared by using the independent sample T-test. A p-value < 0.05 was considered statistically significant. Ninety-five % confidence intervals (95% CI) for proportions were calculated according to Fleiss et al. [16]. Multivariate logistic regression analyses were used to estimate the independent effect of ethnicity on the development of respiratory symptoms in the first 2 years of life. Various models were made to evaluate the effect of so-

cioeconomic factors, housing characteristics and other potential confounders on the relation between ethnicity and respiratory and skin symptoms.

RESULTS

Response:
Of the 4146 children, 267 (6.4%) were lost to follow-up in the first 2 years of life. Reasons for loss to follow-up were: perinatal death, premature birth, illness of the child, moved abroad, untraceable, social problems in the family and lack of time. Data on ethnicity and symptoms at 2 and 1 year of age were available for respectively 89% and 86% of the total cohort. Children with incomplete data for either a 1 or 2 years questionnaire were similar to children with complete data with respect to gender, paternal allergy and asthma, duration of pregnancy, season of birth, number of siblings, day care, having pets, damp or- mould spots in the home and paternal age. Children with incomplete data for a 1 or 2 years questionnaire were more likely (compared to children with complete data) to have an asthmatic mother (13.7% versus 7.2%, $p < 0.001$), to have an allergic mother (45.5% versus 29.7%, $p < 0.001$), to have an asthmatic sibling (8.0% versus 4.2%, $p = 0.001$), to have a mother who smoked during pregnancy (30.6% versus 18.3%, $p < 0.001$), to be exclusively formula fed at 3 months of age (63.9% versus 51.7%, $p < 0.001$), to live in an apartment flat 12.4% versus 6.2%, $p < 0.001$), to live in South-West Netherlands (42.6% versus 27.3%, $p < 0.001$), to have a mother without a paid job (45.9% versus 34.2%, $p < 0.001$), to have a mother with low level of education (20.8% versus 12.7%, $p = 0.002$), to have a father without a paid job (8.2% versus 2.8%, $p < 0.001$) and to have a father with low level of education (25.7% versus 19.0%, $p = 0.03$). In addition, children with incomplete data had a lower birth weight (3.448 kg versus 3.514 kg, $p = 0.03$) and had younger mothers (mean 29.3 year versus 30.4 year, $p < 0.001$).

General characteristics:
Seventy-three mothers were born in Western countries other than the Netherlands (mainly Germany, UK, and Belgium) and their children were excluded from further analysis. From the remaining children, 3490 were defined as 'Dutch', and 131 as 'non-Dutch'. From the latter group, the mothers were born in the following countries/regions: The Netherlands, but with a self-reported non-Western ethnicity (14), Indonesia (8), Dutch Antilles (16), Suriname (27), Turkey (20), Northern-Africa (14), Central & Eastern Europe (10), other (22). The general characteristics of the study population are summarized in table 1. Children with 'non-Dutch' ethnicity (as compared to children with 'Dutch' ethnicity) had a lower birth weight, were less often exposed to pets and more often exposed to environmental tobacco smoke at age 1. In addition, there was more dampness and crowding in their homes, they more often lived in apartment-flats, they more often lived in Rotterdam and the SES of their parents (measured by level of education and employment status) was lower.

Symptoms in relation to ethnicity:
In the first year of life, parents from children with 'non-Dutch' ethnicity more often reported a runny nose with itchy/watery eyes as compared to parents of children with 'Dutch' ethnicity (figure 1a). In the second year of life, children with 'non-Dutch' ethnicity had a higher prevalence of wheeze at least once, night cough without a cold, runny nose without a cold, and

Table 1: General characteristics of the study population

	'Dutch' (n=3490)	'non-Dutch' (n=131)	p-value
Mother ever asthma (%)	7.2	6.2	0.4
Atopic mother (%)	29.4	35.1	0.2
Father ever asthma (%)	7.8	6.3	0.7
Atopic father (%)	30.3	29.2	0.8
Sibling ever asthma (%)	4.2	3.2	0.8
Allergic sibling (%)	25.7	28.1	0.6
Gender boy (%)	51.8	50.4	0.8
Smoking during pregnancy (%)	18.6	22.4	0.5
Smoke exposure in the home at age 1 (%)	26.9	38.7	0.02
Premature birth (< 37 weeks of gestation) (%)	4.8	7.3	0.4
Mean birth weight (kg, SD)	3.519 (0.542)	3.271 (0.567)	< 0.001
Mean weight at age 1 (kg, SD)	9.8 (1.1)	9.7 (1.3)	0.2
Exclusive formula feeding at 3 months (%)	52.7	50.4	0.7
At least 1 sibling (%)	51.7	46.4	0.3
Day care at age 1 (%)	65.5	61.7	0.7
Crowding [< 1.25 room/person] (%)	41.0	53.2	0.007
Exposure to pets (%)	52.7	25.4	< 0.001
Living in apartment-flat (%)	5.6	27.8	< 0.001
Damp- or mould spots in the home (%)	13.6	23.3	0.009
Carpet floor bedroom child (%)	56.4	51.6	0.3
Carpet floor bedroom parents (%)	70.1	64.7	0.2
Mechanical ventilation in the home (%)	54.2	67.5	0.003
Mean age mother of the child (years, SD)	30.5 (3.7)	28.8 (4.9)	< 0.001
Mean age father of the child (years, SD)	32.8 (4.5)	32.0 (5.3)	0.1
Groningen (%)	32.8	11.5	< 0.001
Utrecht (%)	40.5	32.8	
Rotterdam (%)	26.6	55.7	
Mother low level of education (%)	12.6	21.4	0.01
Father low level of education (%)	19.3	22.0	0.5
Mother unemployed (%)	33.9	41.2	0.1
Father unemployed (%)	2.4	15.5	< 0.001
Natural History Study (%)	82.5	80.9	0.5
Intervention Study Active (%)	9.5	8.4	
Intervention Study Placebo (%)	8.4	10.7	

runny nose with itchy/watery eyes (figure 1b). No association was found between ethnicity and recurrent wheezing as well as itchy skin rash in the first two years of life. Table 2 shows the prevalence of respiratory symptoms in the various ethnical subgroups. Although the number of children included in the subgroups is too small to draw definite conclusions, it is obvious that the group of 'Non-Dutch' ethnicity is heterogeneous. Parents of 'Turkish', 'Surinamese', and 'North-African' children reported more respiratory symptoms than parents of 'Dutch' children and 'Non-Dutch' children with Indonesian, Antillean, Eastern-European, and other ethnicity.

Symptoms in relation to exposure to pets, housing characteristics and SES:
For the total study population, the prevalence of respiratory symptoms in the first 2 years of life was similar in homes with and without pets at age 3 months (data not shown). Having pets in the home was inversely related to itchy skin rash in the first year of life (crude odds ratio (cOR) 0.74, 95% confidence interval (CI); 0.6-0.9) and second year of life (cOR 0.79, 95% CI; 0.7-0.9). Living in an apartment flat was unrelated to the development of respiratory and-

skin symptoms in the first 2 years of life (data not shown). Having damp or- mould spots in the home was associated with wheezing at least once in the first- (cOR 1.28, 95% CI; 1.0-1.6), and second year of life (cOR 1.36, 95% CI; 1.1-1.7). Crowding was associated with runny nose with itchy/watery eyes in the first year of life (cOR 1.42, 95% CI; 1.0-1.9) and second year of life (cOR 1.53, 95% CI; 1.1-2.1). Table 3 shows the association between respiratory symptoms and several indicators of SES (independent of ethnical background). Having a mother with a low level of education and especially having a father without a paid job was associated with various respiratory symptoms in the first two years of life. No association was found between socioeconomic indices and the development of skin rash in the first 2 years of life (data not shown).

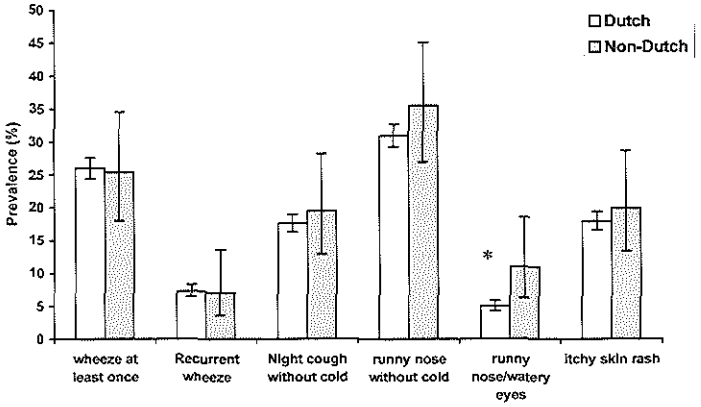

Figure 1a: prevalence of respiratory and- skin symptoms (%) in the first year of life, stratified for ethnic background. Error bars represent 95% Confidence Intervals. * p = 0.01, chi-square

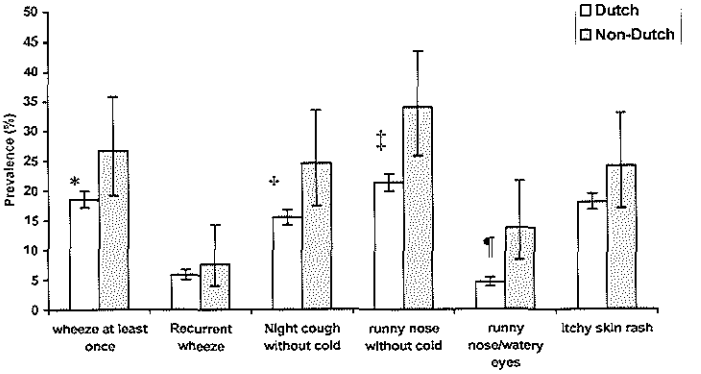

Figure 1b: prevalence of respiratory symptoms (%) in the second year of life, stratified for ethnic background. Error bars represent 95% Confidence Intervals.* p = 0.02, † p = 0.01, ‡ p = 0.001, ¶ p < 0.001, all chi-square

Table 2: Prevalence (%) of respiratory symptoms, stratified for ethnicity of the child

	Dutch	Indonesian	Antillean	Surinamese	Turkish	North-African	Eastern European	Other
Symptoms 0-1 year:	N = 3365	N = 10	N = 16	N = 28	N = 21	N = 13	N = 9	N = 21
Wheeze at least once	26	10	25	29	38	38	22	10
Recurrent wheezing	7	0	6	11	9	18	0	0
Night cough without a cold	18	9	0	25	48	17	0	14
Runny nose without a cold	31	18	27	50	57	31	11	24
Runny nose with itchy/watery eyes	5	0	0	14	29	15	0	5
Symptoms 1-2 year:	N = 3467	N = 11	N = 17	N = 30	N = 23	N = 14	N = 10	N = 26
Wheeze at least once	18	27	12	27	48	21	10	27
Recurrent wheezing	6	0	0	10	4	7	0	19
Night cough without a cold	15	18	18	27	39	36	0	20
Runny nose without a cold	21	0	23	43	48	42	0	42
Runny nose with itchy/watery eyes	4	0	18	7	30	29	0	8

Table 3: Crude odd ratios and 95% confidence interval for association between socioeconomic factors and the development of respiratory symptoms in the first 2 years of life. Differences with $p < 0.05$ are printed in bold.

	Wheeze at least once	Recurrent wheezing	Night cough without a cold	Runny nose without cold	Runny nose with itchy/ watery eyes
First year of life					
Mother paid job	1	1	1	1	1
Mother no paid job	0.96 (0.8-1.1)	1.05 (0.8-1.4)	0.98 (0.8-1.2)	1.00 (0.9-1.2)	0.85 (0.6-1.2)
Father paid job	1	1	1	1	1
Father no paid job	**1.71 (1.1-2.6)**	**2.00 (1.1-3.7)**	1.48 (0.9-2.4)	**2.07 (1.4-3.1)**	**2.05 (1.0-4.1)**
Mother education high	1	1	1	1	1
Mother education low	1.14 (0.9-1.4)	1.25 (0.9-1.8)	1.00 (0.8-1.3)	**1.36 (1.1-1.7)**	1.40 (0.9-2.1)
Father education high	1	1	1	1	1
Father education low	1.00 (0.8-1.2)	1.21 (0.9-1.7)	**1.19 (1.0-1.5)**	1.12 (0.9-1.3)	0.77 (0.5-1.2)
Second year of life					
Mother paid job	1	1	1	1	1
Mother no paid job	1.16 (1.0-1.4)	1.10 (0.8-1.5)	1.14 (0.9-1.4)	**1.28 (1.1-1.5)**	**1.56 (1.1-2.1)**
Father paid job	1	1	1	1	1
Father no paid job	**1.58 (1.0-2.5)**	**2.94 (1.6-5.3)**	**1.68 (1.0-2.7)**	1.51 (0.9-2.4)	1.68 (0.8-3.7)
Mother education high	1	1	1	1	1
Mother education low	1.29 (1.0-1.6)	1.29 (0.9-1.9)	1.12 (0.8-1.5)	**1.67 (1.3-2.1)**	**1.75 (1.2-2.6)**
Father education high	1	1	1	1	1
Father education low	**1.26 (1.0-1.6)**	**1.38 (1.0-1.9)**	1.09 (0.9-1.4)	0.95 (0.8-1.2)	1.02 (0.7-1.5)

Multivariate regression analysis:

To evaluate the independent effect of 'Non-Dutch' ethnicity on the development of symptoms in the first 2 years of life, various logistic regression models were used (figure 2). Because no associations were found between ethnicity and skin rash in the unadjusted and adjusted analyses (data not shown), figure 2 focuses on respiratory symptoms. Controlling for a standard set of confounders (gender, allergic family history, day care, birth weight, smoke exposure in the

home at age 1, type of feeding at 3 months of age and study type) had little effect on the association between ethnicity and symptoms (model 2). This is illustrated by similar crude (model 1) and adjusted odd ratios (model 2). The association between 'non-Dutch' ethnicity and the development of various respiratory symptoms disappeared after controlling for SES (model 3), except for runny nose with itchy/watery eyes in the first and second year of life (adjusted odds ratio (aOR) 2.18, 95% CI; 1.0-4.5 and aOR 2.87, 95%; CI 1.4-5.8 respectively). After additional controlling for housing characteristics (living in an apartment and having damp or mould spots in the home, not in figure 2), the association between 'non-Dutch' ethnicity and runny nose with itchy/watery eyes in the second year remained statistically significant (aOR 2.89, 95% CI; 1.3-6.4), but this was not the case for runny nose with itchy/watery eyes in the first year of life (aOR 1.62, 95% CI: 0.6-4.0). Adding region of birth and having pets in the home to the model did not alter the adjusted odd ratios given in figure 2.

Doctor visits for upper airway symptoms in relation to ethnicity:
Parental report of symptoms might be influenced by the parents' readiness to consult a doctor. We therefore investigated ethnical differences in upper airway symptoms in the month prior to completing the 1 and- 2 years questionnaire, and doctor visits for these symptoms. Children with 'non-Dutch' ethnicity had a similar risk of developing upper airway symptoms as children with 'Dutch' ethnicity (0-1 year: aOR 0.70, 95% CI; 0.4-1.1. 1-2 year: aOR 0.83, 95% CI; 0.5-1.3, 'Dutch' children = reference). If a child had upper airway symptoms, parents of 'Non-Dutch' children visited a doctor (for these symptoms) more frequently than parents of 'Dutch' children (0-1 year: aOR 1.32, 95% CI 0.8-2.2. 1-2 year: aOR 2.34, 95% CI 1.3-4.0).

DISCUSSION

In this large prospective birth cohort study, we found that the prevalence of runny nose with itchy watery eyes in the first year of life was higher in children with a 'Non-Dutch' ethnicity than in children with a 'Dutch' ethnicity. The prevalence of wheeze at least once, night cough without a cold, runny nose without a cold, and runny nose with itchy watery eyes in the second year of life was higher in children with a 'Non-Dutch' ethnicity than in children with a 'Dutch' ethnicity. No association was found between 'Non-Dutch' ethnicity, and recurrent wheeze or itchy skin rash. Unemployment of the father and low level of education of the mother was associated with an increased risk of developing respiratory symptoms in the first 2 years of life. After adjustment for these socioeconomic indices, the association between 'Non-Dutch' ethnicity and respiratory symptoms disappeared, except for runny nose with itchy/watery eyes in the second year of life. In addition, parents from children with a 'Non-Dutch' ethnicity were more likely to visit a doctor when their child had respiratory symptoms in the first 2 years of life.

Ethnical differences in symptoms:
Only few studies have investigated the role of ethnicity in the development of respiratory and skin morbidity in young children. In a study in Boston, USA, black children were two times more likely to develop repeated wheeze (2 or more episodes) in the first year of life as compared to white children [17]. Also in the USA, Hispanic children were found to have an

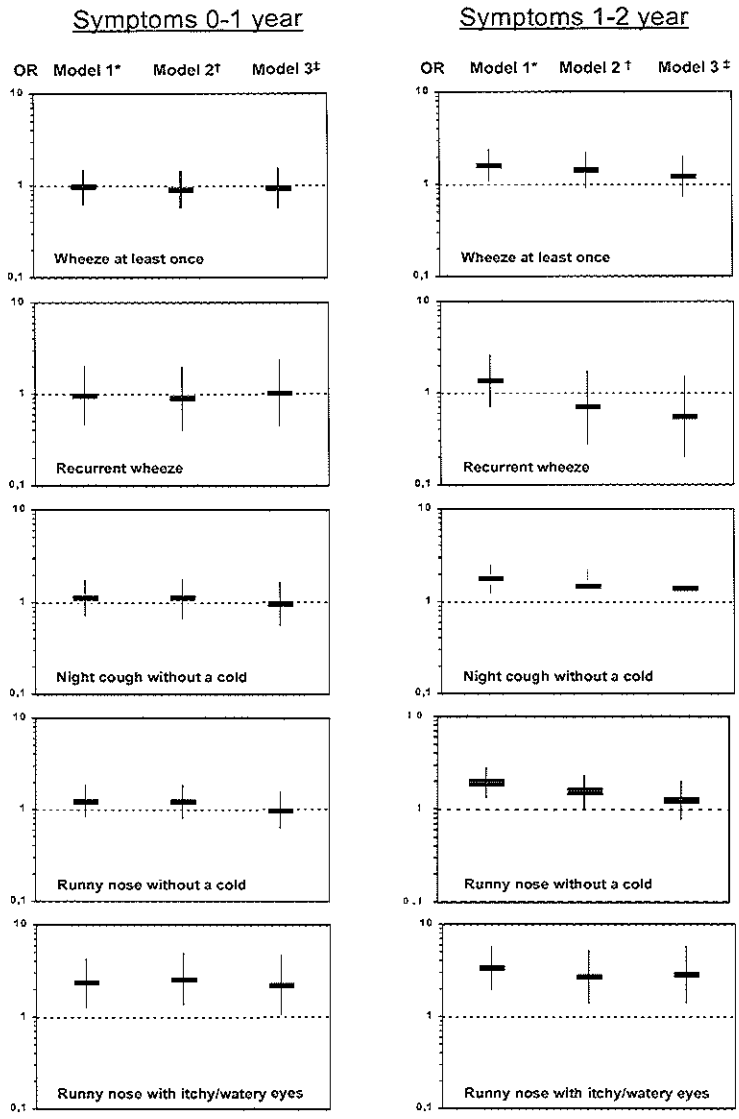

Figure 2: cOR and aOR (horizontal bars) and 95% CI (vertical lines) for the association between 'Non-Dutch' ethnicity and the development of respiratory symptoms in the first two years of life, using 'Dutch' children as the reference group. * model 1, crude odds ratios, † model 2, adjusted for gender, allergic family history, day care, birth weight, smoke exposure in the home at age 1, type of feeding at 3 months of age, and study type (natural history, intervention active, intervention placebo) ‡ model 3, as in model 1, but also adjusted for maternal age, parental employment and maternal education

increased risk to develop wheeze and RSV bronchiolitis [18, 19]. In another American study, black infants were more likely to develop persistent respiratory symptoms (cough or wheeze) as compared to white children [20]. In Sweden, hospital admissions for lower respiratory tract infections in the first year of life were found to be more common in children of immigrants from Southern Europe than in children from Swedish parents [21]. To our knowledge, no European studies focussing on respiratory symptoms in this age group are available, so our findings are difficult to compare with other studies. However, we found only a weak association between 'Non-Dutch' ethnicity and wheezing in the second year of life and no association with recurrent wheeze, so it seems that ethnical differences in respiratory morbidity found in the USA can not be translated to the European situation, at least not in the Netherlands. We found some differences between 'Dutch' and 'Non-Dutch' children in the prevalence of night cough and runny nose (with and without itchy/watery eyes). The differences in respiratory symptoms were more prominent in the second year of life as compared to the first year of life. It is unknown whether these symptoms reflect an increased prevalence of respiratory tract infections or early allergic disease in the children from 'Non-Dutch' ethnicity. An interesting observation is that we did not find differences in the prevalence of itchy skin rash between 'Dutch' and 'Non-Dutch' children. Together with the absence of a difference in recurrent wheezing, which is used in most prospective cohort studies as a marker for early respiratory allergic disease [22], our study points towards a difference in non-specific or infectious symptoms, rather than allergic symptoms. Follow-up of the PIAMA cohort might give more insight on this matter.

Relevance of higher prevalence of respiratory symptoms in a subgroup of 'Non-Dutch' ethnicity:

The prevalence of 'wheezing at least once' was much higher in children with 'Turkish' ethnicity as compared to children with 'Dutch' ethnicity. The number of children with 'Turkish' ethnicity included in the study was small (n = 23), but taking this limitation into account, this finding is in contrast with other studies in Turkish immigrants in Sweden, Germany and the Netherlands [5-7]. In these studies, children with 'Turkish' ethnicity were found to have a decreased risk rather than an increased risk to develop wheezing episodes. A possible explanation might be that we have studied symptoms in children age 0-2, while the other studies investigated children age 2-11 [5-7]. Further studies that include larger ethnical subgroups are needed to confirm our findings in these young children.

Role of socioeconomic factors:

Adjustment for socioeconomic factors largely eliminated the differences in odds ratio's between 'Non-Dutch' and 'Dutch' children. This indicates that a socioeconomic disadvantaged position, which is more frequently found in 'Non-Dutch' families as compared to 'Dutch' families, rather than the genetic or- ethnical background is responsible for the increased prevalence of respiratory morbidity. This finding is in agreement with other studies on respiratory morbidity in children in relation to ethnical background. In a study in schoolchildren in the UK, the association between ethnical background and persistent wheeze disappeared after controlling for Townsend score, which is a parameter of SES [4]. In a study performed in the USA, Wissow et al. conclude that asthma is more prevalent among black children than in white children, but this difference could be largely explained by differences in poverty [10].

In Sweden, the association between ethnicity and admissions for lower respiratory tract infection in infancy disappeared after adjusting for socioeconomic factors [21]. In contrast, adjustment for socioeconomic factors did not alter the decreased risk for allergic disease in children of Turkish immigrants and the increased risk in children from Chilean immigrants in studies performed in Germany, The Netherlands and Sweden [5-7].

Validation issues:
The strength of the present study is the large sample size, minimal loss of follow-up, and the prospective design. However, this study has some methodological limitations. The group of 'Non-Dutch' children was relatively small and heterogeneous. Because participants had to be able to read and understand the Dutch language, we probably over-sampled children with 'Non-Dutch' parents who are relatively well adjusted to the Dutch society, resulting in some selection bias. Another potential source of bias is cultural and linguistic differences in reporting symptoms. Because no objective measures of disease activity are available in this age group, it is difficult to address this issue. There were some indications in this study that parents from 'Non-Dutch' children with upper airway symptoms consulted a doctor more easily as compared to parents from 'Dutch' children, even after correction for SES. It seems plausible that this can have some interaction with the parental report of the outcome measures we studied, leading to a higher prevalence of our outcome measures. We also encountered some selective loss of follow up related to low SES, which is frequently found in prospective cohort studies. Loss of follow up and missing data were more frequent in the city of Rotterdam. Because asthmatic and allergic mothers were over-sampled in this area, the children with missing data were more likely to have an asthmatic or allergic mother. The definition of ethnical background was made at the end of the follow up period (at age 2). Hence, we were unable to determine whether the frequency of loss to follow up and missing data in the first two years of life was unequally distributed among the different ethnical groups. Because the group of children with 'Non-Dutch' ethnicity had a lower SES, it is likely that loss to follow up was more frequent in the children with 'Non-Dutch' ethnicity. However, complete data on ethnicity and symptoms were available for over 86% of the study population, so we believe that selective loss to follow up can not explain our findings.

In conclusion, we found that the prevalence of some respiratory symptoms (night cough, runny nose without a cold) was increased in children from 'Non-Dutch' ethnicity, but that the prevalence of recurrent wheeze was similar in both ethnical groups during the first 2 years of life. The differences in respiratory symptoms could largely be explained by differences in SES. Follow up of our cohort can provide more insight in the impact of ethnical differences on childhood allergic disease.

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References:

1. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998;12:315-35.
2. Cunningham J, Dockery DW, Gold DR, Speizer FE. Racial differences in the association between maternal smoking during pregnancy and lung function in children. Am J Respir Crit Care Med 1995;152:565-9.
3. Stein RT, Holberg CJ, Sherrill D, et al. Influence of parental smoking on respiratory symptoms during the first decade of life: the Tucson Children's Respiratory Study. Am J Epidemiol 1999;149:1030-7.
4. Duran-Tauleria E, Rona RJ. Geographical and socioeconomic variation in the prevalence of asthma symptoms in English and Scottish children. Thorax 1999;54:476-81.
5. Hjern A, Haglund B, Hedlin G. Ethnicity, childhood environment and atopic disorder. Clin Exp Allergy 2000;30:521-8.
6. Kabesch M, Schaal W, Nicolai T, von Mutius E. Lower prevalence of asthma and atopy in Turkish children living in Germany. Eur Respir J 1999;13:577-82.
7. van der Wal MF. Asthmatic symptoms among Dutch and non-Dutch 2-11 year old children in Amsterdam. Tijdschr Soc Gezondheidsz 1995;73:42-50.
8. Halfon N, Newacheck PW. Childhood asthma and poverty: differential impacts and utilization of health services. Pediatrics 1993;91:56-61.
9. Malveaux FJ, Fletcher-Vincent SA. Environmental risk factors of childhood asthma in urban centers. Environ Health Perspect 1995;103:59-62.
10. Wissow LS, Gittelsohn AM, Szklo M, Starfield B, Mussman M. Poverty, race, and hospitalization for childhood asthma. Am J Public Health 1988;78:777-82.
11. Wijga A, Smit HA, Brunekreef B, et al. Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. Clin Exp Allergy 2001;31:576-81.
12. Koopman LP, Smit HA, Heijnen MA, et al. Respiratory infections in infancy: interaction of parental allergy, day care and siblings: the PIAMA-study. Pediatrics, in press.
13. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
14. Bruijnzeels M. Measuring ethnical background. The Hague, The Netherlands: ZorgOnderzoek Nederland; 1999.
15. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
16. Fleis J. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley & sons; 1981.
17. Gold DR, Burge HA, Carey V, Milton DK, Platts MT, Weiss ST. Predictors of repeated wheeze in the first year of life. The relative roles of cockroach, birth weight, acute lower respiratory illness, and maternal smoking. Am J Respir Crit Care Med 1999;160:227-36.
18. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson Children's Respiratory Study. II. Lower respiratory tract illness in the first year of life. Am J Epidemiol 1989;129:1232-46.
19. Glezen WP, Paredes A, Allison JE, Taber LH, Frank AL. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. J Pediatr 1981;98:708-15.
20. Margolis PA, Greenberg RA, Keyes LL, et al. Lower respiratory illness in infants and low socioeconomic status. Am J Public Health 1992;82:1119-26.
21. Hjern A, Haglund B, Bremberg S. Lower respiratory tract infections in an ethnic and social context. Paediatr Perinat Epidemiol 2000;14:53-60.
22. Koopman LP, Brunekreef B, de Jongste JC, Neijens HJ. Definition of respiratory symptoms and disease in early childhood in large prospective birth cohort studies that predict the development of asthma. Pediatr Allergy Immunol 2001;12:118-24.

Chapter 10

Summary, general discussion, future research

10.1. SUMMARY

The etiology of allergic disease is multi-factorial, involving both genetic and environmental factors. The exact role of exposure to indoor allergens such as HDM-allergen and pet-allergen, exposure to microorganisms and the role of ethnicity on the development of allergic disease in early childhood are only partly established. In addition, our knowledge about the immunological mechanisms underlying the development of allergic disease has increased in the past decades, especially concerning the role of Th1 and Th2 cell development. However, the precise regulatory mechanisms that are involved in the development of allergic disease are only partly understood. The aims of this thesis are:
Evaluate the role of various environmental factors on the development of symptoms of allergic disease.
Provide insight in the immunological processes associated with the development of allergic disease in early childhood.

Chapter 1 contains a general introduction of the thesis.
Chapter 2 reviews the literature regarding the development of allergic disease in early childhood, with special emphasis on HDM-allergen exposure and microbial stimulation.
In **chapter 3**, the study aims are discussed in detail.
In **chapter 4**, we review the predictive value of early respiratory symptoms for the development of asthma later in life. We also evaluated the outcome variables that were used to describe respiratory disease in early childhood in large prospective cohort studies on the development of allergic disease. We concluded that from all respiratory symptoms during infancy, wheezing is most closely associated with the development of asthma in later childhood, despite the observation that only about 40% of the wheezing infants continue to wheeze later in life. In the prospective cohort studies we evaluated, wheezing was always incorporated in the case definition, although all studies used different criteria in their publications. However, the data that were actually collected by questionnaire and structured interview were comparable for all studies. This makes it essentially possible to pool data from various cohort studies in order to investigate the prospective value of various respiratory symptoms for the development of asthma in more detail.
In this thesis, we analyzed data from two prospective birth cohort studies: the PIAMA and VIGALL-study. **In chapter 5**, the study designs of these studies are described.
In **chapter 6**, we describe the association between the serum markers interleukin (IL)-10, IL-12, IL-13, eotaxin, soluble E-selectin (sE-selectin), soluble intra-cellular adhesion molecule (ICAM)-1 and soluble IL-2 receptor (sIL-2R) on the one hand and the development of atopic symptoms in the first 2 years of life on the other hand. We found that children who developed wheezing in the first year of life had lower serum levels of IL-12 and a higher IL-10/IL-12 ratio compared to healthy infants. The IL-10/IL-12 ratio increased with an increasing number of wheezing episodes during the first 2 years of life. Infants with wheezing in the first year of life and children with itchy skin rash in the first year of life had higher serum sE-selectin levels than healthy infants. Finally, we found that children who developed wheezing in the second year of life had increased serum sICAM levels at age 1. Our data suggest that serum cytokine responses at age 1 in wheezing infants were skewed towards a T-helper 2 direction. Between the various symptom groups, considerable overlap existed in the concentrations of

all markers that were investigated. Therefore the predictive value of the serum markers, within the time span of our observations, is limited.

In **chapter 7**, we describe the first results of a randomized, placebo controlled trial on avoidance of house dust mite (HDM)-allergen exposure in early life. We evaluated the effect of application of HDM impermeable encasings, provided for the parents' and infants mattresses and the parents' pillows, on HDM-allergen exposure as well as on the development of respiratory and skin symptoms in a birth cohort of children with an allergic mother. The covers were applied in the last trimester of pregnancy. We found that at age 1 year, 13-14 months after the application of mattress encasings, on both the children's and parents' mattress the amount of HDM-allergens was 2 times lower in the active group than in the placebo group. However, HDM-allergen levels were unexpectedly low in the placebo group as well. We found a moderately protective effect of the mattress encasings on night cough without a cold in the second year of life (adjusted odds ratio 0.65, 95% confidence interval 0.4-1.0, placebo group was used as reference group). No effect was seen on other respiratory symptoms (including wheezing), atopic dermatitis and total- and specific IgE in the first 2 years of life. Therefore, we concluded that application of mattress encasings during pregnancy is not associated with a clinically relevant reduction of symptoms suggestive for allergic disease in early childhood. However, since a reliable diagnosis of allergic disease is difficult in this age group, follow-up of the cohort is needed to determine any long-term effect.

In **chapter 8**, we studied the development of allergic disease in the context of respiratory infections. We investigated the association between contact with other children (attending child care or having siblings), a family history of allergic disease, and the development of respiratory tract infections in the first years of life. We found that attending child care facilities and having siblings increases the risk of developing a doctor diagnosed lower respiratory tract infection (LRTI) in infancy to a greater extent in children with allergic parents than in children without allergic parents. These data suggest the existence of gene (allergic parents) by environment (child care, siblings) interaction.

In another study we investigated differences in the nasal mucosal immune responses in children suffering from Respiratory Syncytial Virus induced bronchiolitis (RSV-bronchiolitis) and RSV-induced upper respiratory tract infection (RSV-URTI). Nasal brushes were obtained and responses of inflammatory cells, cytokines, and ICAM-1 were measured during the acute phase and 2-4 weeks thereafter (convalescent phase). The number of IL-18 positive cells that we found during acute RSV-bronchiolitis was two-fold higher compared to RSV-URTI. In addition, higher numbers of macrophages and T-cells were found during both acute RSV-bronchiolitis and acute RSV-URTI compared to convalescence, indicating an influx of inflammatory cells into the nose during acute infection, which was independent of disease severity. During the acute phase of RSV-bronchiolitis, a higher numbers of cells stained positive for IL-6, IL-12, IL-18 and ICAM-1 compared to convalescence. These increased responses during infection were not found in RSV-URTI. Together, these data indicate that in the nose, pro-inflammatory and/or Th1 responses develop during both RSV-bronchiolitis and RSV-URTI, with higher responses during RSV-bronchiolitis than in RSV-URTI. No convincing evidence for a Th2 response was found in both RSV-bronchiolitis and RSV-URTI.

In **chapter 9**, we have studied ethnical differences in the prevalence of respiratory and skin symptoms in the first 2 years of life. Children with 'Non-Dutch' ethnicity had a higher prevalence of runny nose with itchy/watery eyes in the first year of life compared to 'Dutch' children. In addition, 'Non-Dutch' children also had a higher prevalence of wheeze at least once,

night cough without a cold, runny nose without a cold and runny nose with itchy/watery eyes in the second year of life. Adjustment for parental employment and parental education strongly reduced the strength of the association between ethnicity and respiratory symptoms. Therefore, we propose that the differences in the prevalence of respiratory symptoms between 'Non-Dutch' and 'Dutch' children can largely be explained by differences in socioeconomic status.

10.2. GENERAL DISCUSSION AND FUTURE RESEARCH

In the next paragraphs, we will discuss the most relevant findings of this thesis, and, if possible, the practical implications. In addition, we provide suggestions for future research. We start to discuss the findings of the various chapters separately. Making use of the insight obtained from the PIAMA and VIGALL-study, as well as information from other studies, we propose a model about the factors involved in the development of allergic disease and viral wheezing.

The work presented in this thesis was based on a large prospective (partly placebo controlled) cohort study, the Prevention and Incidence of Asthma and Mite Allergy (PIAMA)-study, and on a smaller cohort study entitled Virus Mediated Allergy (VIGALL)-study. In epidemiology, a prospective study design is considered to be more valid compared to a cross-sectional or retrospective study design, mainly because the risk of various types of bias is smaller [1, 2]. The large size of the cohort ($n \approx 4000$), as well as the relative low proportion of loss to follow-up (< 10%) strengthens the validity of the PIAMA-study. In case of the VIGALL-study ($n = 129$), loss to follow-up was small in the first year of life (15%), but somewhat larger in the second year of life (36% in total), probably due to the more invasive methods used in this study compared to the PIAMA-study. Another strength of the study design is the validation of various co-factors. We have published validation studies on the definition of allergic disease in the parents and on environmental tobacco smoke exposure [3, 4].
The thesis deals with the development of allergic disease in the first 2 years of life. The definition of asthma in early childhood is controversial. No objective measurements for the diagnosis of asthma in this age group exist and no uniform criteria for epidemiological studies are available. We therefore reviewed the prognostic value of various respiratory symptoms in early life on the development of asthma in later childhood. We also examined the criteria that were used in large prospective cohort studies to describe respiratory symptoms and disease (**chapter 4**). In this thesis, we chose for a symptom-based approach and thus avoided using the diagnosis of asthma as an outcome variable. We used yearly questionnaires on respiratory symptoms that were internationally validated in 6-7 year and 13-14 year old children [5]. The advantage of this approach is that we collect similar data at various time points during the prospective follow-up, which makes the data comparable over time and with studies in other parts of the world [6].
An attempt should be made to pool data from various existing birth-cohort studies, including the PIAMA-study, in order to investigate which symptoms or combinations of symptoms in early life predict the development of asthma in later life most accurately. Based on this data-sharing project, uniform criteria for respiratory symptoms can be selected, which should be used for future birth cohort studies.

10.2.1. Allergen exposure

The application of mattress encasings during pregnancy in the PIAMA-study was not associated with a clinically relevant reduction of symptoms suggestive for allergic disease in early childhood. The following explanations for this important finding can be considered:

- The magnitude of allergen reduction that was established in the PIAMA-study may have been insufficient to have an effect on the development of clinical symptoms. The initial HDM-allergen levels and the effect of the intervention program on HDM-allergen levels in the PIAMA-study are comparable with another primary prevention study performed in Canada in high-risk children [7]. However, in 2 primary prevention studies in high-risk children performed in the UK (the Isle of Wight Study and the Manchester Asthma and Allergy Study [MAAS]), the magnitude of reduction of allergen exposure was higher compared to the PIAMA-study [8, 9]. The greater success of the Isle of Wight Study, compared to the PIAMA-study and the Canadian study, in reducing allergen exposure could entirely be explained by the higher initial HDM-allergen levels [7, 8]. In the MAAS, more rigorous intervention measures were taken, and therefore the difference between the active and the control group was somewhat larger, with almost undetectable HDM-allergen levels in the active group [9]. In the PIAMA-study and, to a greater extent, in the MAAS, surprisingly low levels of HDM-allergen levels were found in the infants' beds of the control group, with geometric mean levels of Der p1 far below the 2 μg/ g dust [9]. In earlier studies from The Netherlands and the UK much higher allergen levels were found in high-risk children [10, 11]. We initially speculated that parents in the control group have taken allergen control measures on their own initiative, probably because of an increased awareness of the adverse effects of allergen exposure in high-risk families [12]. However, preliminary analysis of dust samples of children participating in the natural history part of the PIAMA-study indicate that a family history of asthma and/or allergy is not associated with the allergen levels in our study (Rob van Strien, personal communication). An alternative explanation for the low overall HDM-allergen levels might be that the majority of the infants slept on new mattresses, on which mite infestation had not taken place yet.
- The follow-up period was still relatively short. Although the majority of children with asthma already have symptoms in early life, the definite diagnosis can only be made at an older age. Therefore it is possible that in wheezing children allocated to the placebo group, a larger proportion will continue to wheeze in later life compared to those allocated to the active group. This makes further follow-up until the age of 8 years crucial for evaluating the impact of our intervention. The difference in HDM-allergen exposure between the intervention group and the placebo group increased considerably between age 3 months and age 1 year of the children, in particular in the children's beds. This observation is agreement with the observation of the MAAS [9], and further stresses the importance of longer follow-up of the PIAMA-cohort.
- HDM-allergen exposure is causally associated with the development of atopic sensitization but not with asthma. If this hypothesis is true, no differences between the active group and placebo group will be found after longer follow-up. Even if it turns out that HDM-allergens are not associated with the development of asthma and allergic disease, their proteolytic activity might be capable of inducing non-allergic inflammation of the airway mucosa and skin [13, 14]. We analyzed the association between exposure to HDM-allergen at 3 months of age and the development of respiratory symptoms in the

first 2 years of life. We indeed found indications that children who were exposed to any HDM-allergen, independent of group assignment, were more likely to develop wheezing, recurrent wheezing and any respiratory symptoms in the first 2 years of life than children who were not exposed to detectable quantities of HDM-allergen. Together with the convincing evidence that HDM-allergen exposure is an important trigger of respiratory symptoms in established asthma and atopic dermatitis and that patients with allergic disease benefit from allergen reduction [15, 16], it is tempting to speculate that the intervention we performed might result in secondary prevention of symptoms in established allergic disease.

10.2.2. Child care, siblings, parental allergy and risk for LRTI

Contact with other children by attending child care or having siblings results in an increased exposure to microorganisms [17]. We analyzed the association between contact with other children and the development of respiratory infection in the context of parental allergy. We showed that child care attendance and having older siblings are strong risk factors for both doctor-diagnosed upper and lower respiratory tract infections in infancy. These findings are in agreement with other studies about the association between contact with other children and the development of respiratory tract infections [18-21]. A new finding of our study was the interaction between allergy in the parents and contacts with other children resulting in an increased risk of doctor-diagnosed LRTI. In other words, children with two allergic parents who attend small child care facilities were 2 times more likely to develop a doctor diagnosed LRTI in the first year of life than children with no allergic parents who attend the same form of child care. An important question is how this interaction between allergic predisposition and contact with other children for the development of LRTI can be explained. In 8-week old healthy infants with a first degree relative with asthma, Dezateux et al. found a smaller airway caliber and a higher airway resistance, compared to infants without a positive family history of asthma [22]. The Tucson Children's Respiratory Health Study has shown that small airway caliber and decreased lung function in early life predispose for the development of LRTI in infancy [23]. We therefore hypothesize that the interaction between allergic predisposition and contact with other children for the development of LRTI could be explained by an intrinsic abnormality of the airways in children with allergic parents, resulting in an increased vulnerability to respiratory infections.

- Although contacts with other children is clearly associated with an increased risk to develop respiratory infections in early life, recent studies indicate that these contacts might actually be beneficial in the long run by preventing asthma in later childhood [17, 24]. Whether this also holds true for children with allergic parents is unclear, but follow-up of the PIAMA-cohort might answer this question.

10.2.3. Nasal immune response during RSV-bronchiolitis and RSV-URTI

An imbalance between CD4+ helper T cell subsets (Th1 cells and Th2 cells) in favor of the Th2 subtype, is thought to contribute to allergic disease. Immunological studies on the role of RSV infection in the development of asthma and allergy show conflicting results; some studies indicate a Th2 response, some studies indicate a Th1 response, and other studies show a balanced

Th1/Th2 response (reviewed by ref. [25]). A similar degree of controversy as in immunological studies can be found in epidemiological studies. In several studies, hospitalization for RSV-bronchiolitis in infancy was found to be associated with the development of asthma later in childhood [26, 27]. However, in a population based study in Tucson, USA, no association was found between mild RSV-bronchiolitis in the first 3 years of life and asthma and atopy at age 11-13, although the risk of wheezing was clearly increased until age 8 [28]. In the VIGALL-study, measuring the nasal mucosal immune response during RSV-infection, we found no evidence for Th2 polarization. Instead, we found increased numbers of IL-6, IL-12, IL-18 and ICAM-1 positive cells, especially in children with RSV-bronchiolitis. How can these findings be explained in view of the well-known association between RSV-bronchiolitis and prolonged episodes of recurrent wheezing [29]? IL-18 and IL-12 are considered Th1 cytokines, whereas IL-6 is a Th2 cytokine [30, 31]. However, these 3 cytokines also have pro-inflammatory properties, which might result in tissue injury, epithelial damage and mucus hypersecretion [29, 32]. We therefore speculate that this pro-inflammatory response is capable of inducing prolonged wheezing episodes, without leading to a Th2 related asthmatic response. RSV-infection has also been associated with increased production of IL-11 by airway epithelial cells, with increased IL-11 levels in RSV-bronchiolitis, but not in RSV-URTI [33]. This particular cytokine is associated with the accumulation of fibroblasts, myofibroblast, myocytes, increased bronchial hyperresponsiveness, and airway remodeling [31] and increased mucus secretion [25], which might also contribute to persistent wheezing episodes.

- In humans, only indirect evidence exists that exposure to microorganisms might protect against the development of allergic disease [17]. In the VIGALL-study extensive data is collected on child care, having siblings and the number of airway infections. In combination with detailed prospectively collected information on the local and peripheral immune responses, this study can potentially provide direct evidence about the relation between exposure to microorganisms and the development of the immune system and allergic disease.
- Besides RSV, various other viral pathogens, such as para-influenza virus, rhinovirus and coronavirus, can cause upper and lower airway infections. In the VIGALL-study, various different viruses are being studied in relation to the development of the nasal and peripheral immune response.

10.2.4. Markers of allergic inflammation

Various serum markers measured at age 1, including IL-12, the ratio between IL-10 and IL-12, sE-selectine, sICAM-1 and sIL-2R, correlated with the development of wheezing and, to a lesser extent, skin rash during the first 2 years of life (**chapter 6**). In particular the finding of decreased serum IL-12 levels in wheezing children provides new insight in the immune responses in the first year of life. IL-12 is mainly produced by APC and is thought to be an important regulatory cytokine, involved in the up-regulation of IFN-γ production, resulting in the differentiation of Th0 cells into Th1 cells [34]. Three possible mechanisms underlying these decreased levels of IL-12 can be postulated:

1. An intrinsic or genetic defect in the production of IL-12.
2. A lack of environmental factors that stimulate the production of IL-12.
3. Children with virus-associated wheezing are incapable of mounting proper Th1-like responses, including an inability to produce adequate quantities of IL-12.

If the first or the second mechanism is true, low levels of IL-12 might result in a disturbed balance of the Th1/Th2 cytokines in favor of Th2 cytokines, leading to the development of allergic airway inflammation and wheezing. A defect in the production of IFN-γ by stimulated cord blood mononuclear cells in children from allergic parents has been described by various groups and the authors suggested that this defect might partly explain the increased risk for the development of allergic disease in these children [35, 36]. To our knowledge, no data from prospective birth cohort studies on the development of IL-12 responses in the first year of life are available. Therefore, it is unknown whether children who develop allergic disease are born with an intrinsic defect of IL-12 production. We did not find any relation between parental allergy and serum IL-12 levels in their children at age 1, therefore we believe that an intrinsic defect in IL-12 production is less likely. No data on exposure to environmental factors that stimulate IL-12 production, such as endotoxin, were available in this study, so we cannot rule out this explanation. However, no association was found between contact with other children (siblings or child care) and serum IL-12 levels. If the third explanation is true, the low levels of IL-12 can be interpreted as a marker of viral infection, rather than a marker of allergic disease. Based on the results of the study described in **chapter 8.2**, we believe that this explanation is unlikely. In this study about the nasal immune response in children with RSV-infection, we found increased, instead of decreased, numbers of cells staining positive for IL-12 during RSV-bronchiolitis.

We found a considerable overlap of the levels of serum markers between healthy children and children with allergic symptoms, as has been reported by other groups [37-40]. In view of the heterogeneous nature of the disease processes, this overlap is not an unexpected finding. Therefore we believe that it is unlikely that a single serum marker will be found that can perfectly predict the development of allergic disease in early childhood. Instead, a combination of serum markers, reflecting different aspects of the pathophysiological mechanism involved in the development of allergic disease, may be more useful.

- The small sample size of our study limits the possibility to investigate the combinations of serum markers using multiple regression models. Increasing the sample size could overcome this problem.
- Longer follow-up is needed to evaluate the prognostic value of the measured serum markers on the development of allergic disease.
- It would be interesting to study the development of infant IL-12 responses in relation to exposure to environmental factors that may stimulate IL-12 production, such as endotoxin and other components of microorganisms . Ideally, such a study should have a prospective design. Endotoxin exposure should be measured at birth and at various time points during the first years of life. Simultaneously, IL-12 responses of stimulated peripheral blood cells and serum IL-12 levels should be documented.

10.2.5. Ethnical differences in atopic symptoms

We compared the prevalence of respiratory and skin symptoms between 'Dutch' children and 'Non-Dutch' children. We found a higher prevalence of respiratory symptoms in the first 2 years of life in 'Non-Dutch' children compared to 'Dutch' children, including wheeze, night cough and runny nose without a cold. Ethnical differences in the prevalence of a disease can be either caused by genetic differences or by environmental differences between the ethnical groups. Our data indicate that environmental factors are more important than genetic factors

in determining the association between ethnicity and respiratory symptoms, because adjustment for socioeconomic status had a strong impact on the associations we found. Although the prevalence of respiratory symptoms was higher in 'Non-Dutch' children, the prevalence of skin rash suggestive for allergic dermatitis was similar between the ethnical groups, and a trend was seen for a lower prevalence of recurrent wheezing in the second year of life in the 'Non-Dutch' group. These data suggest that the differences that were seen in respiratory symptoms were caused by differences in the prevalence of respiratory infections rather than differences in the prevalence of allergic disease. Theoretically, this increased prevalence of early respiratory tract infections might confer protection against the development of atopy and allergic disease in later childhood [41]. This might also partly explain the findings of studies in Germany and Sweden which found a decreased prevalence of asthma, recurrent wheezing and atopic dermatitis in schoolchildren born from Turkish immigrants [42, 43].

10.2.6. Concluding remarks:

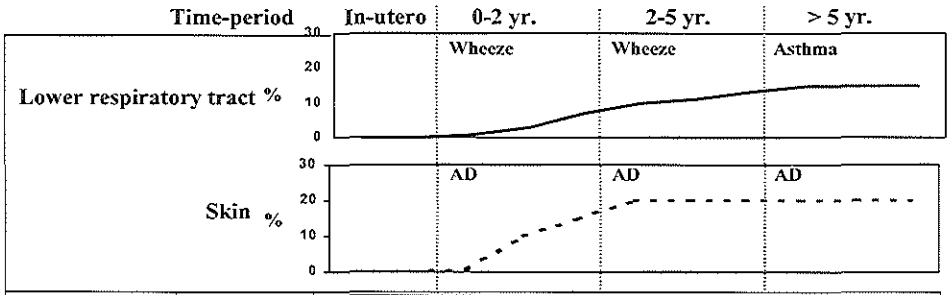
We present a simplified model about factors influencing the development of allergic disease or Th2-disease (figure 10.1) and viral-associated wheezing (figure 10.2), based on results of this thesis and the results of other studies. We distinguish the following time-periods: in-utero, infancy/early childhood, pre-school, and childhood, based on the chronological sequence of the involved immunological processes. We provide an estimation of the cumulative incidence of asthma, atopic dermatitis and viral-associated wheeze over time. Note that for all three categories the majority of children already express symptoms before the age of 2 years, although the curves are steeper during the first years of life for atopic dermatitis and viral-associated wheezing, compared to asthma. In addition, the pathophysiological and immunological development associated with Th2-disease and viral-associated wheeze are summarized.

In the models we distinguish the following types of associations:

1. Modulating factor; causal related, positive associations (risk factor)
2. Modulating factor; causal related, negative association (protective factor)
3. Triggering factor in established disease

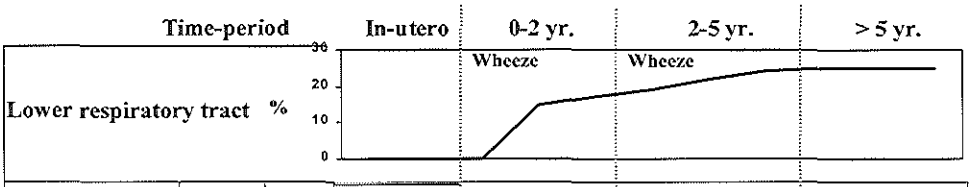
From the models it becomes clear that a given factor can be a risk factor, a protective factor and an triggering factor, depending on the time of impact, the type of disease and the type of exposure. For instance, early microbial stimulation in the form of endotoxin might protect against the development of Th2-polarization and subsequent development of atopic disease and wheezing later in life (figure 10.1.). In contrast, endotoxin exposure can be a triggering factor in established asthma. In addition, endotoxin exposure has also been associated with wheezing in early life, possibly by inducing non-allergic airway inflammation and facilitating the development of viral infections (figure 10.2). Similar complex associations can be postulated for RSV-infection, allergen exposure and exposure to irritants such as environmental tobacco smoke and outdoor air pollution. The complexity of the disease process, characterized by the combined operation of variety of factors, including genetic factors, environmental factors and time, makes allergic disease a challenging research topic. Various prospective birth cohort studies, including the PIAMA- and VIGALL-study are currently preformed. These studies are contributing to our knowledge about the patho-physiology of, and risk factors for allergic diseases, and will probably reveal important new information. Together with ongoing developments in the field of human genetics and basic immunology it can be expected that in

the next few decades a better prediction at an early stage can be made on which children will develop allergic disease. This will hopefully contribute to the development of effective prevention strategies against the development of allergic disease, and improve the treatment of established disease.



Pathofysiology	Th2				
	IgE				
	Inflammation				
	Remodeling				
	BHR ↑				
Genetics	Modulating	+	+	+	+
	Triggering	n.a.	n.a	n.a.	n.a.
HDM-allergens	Modulating	~	~	+/~	+/~
	Triggering	n.a.	+/~	+	+
RSV-infection	Modulating	n.d.	~	~	~
	Triggering	n.a.	+	+	+
Microbial Stimulation[1]	Modulating	n.d	-	-	-
	Triggering	n.a.	+	+	+
ETS/irritants	Modulating	+/~	+/~	+/~	+/~
	Triggering	n.a.	+	+	+

Figure 10.1. Schematical presentation of factors involved in the development of allergic disease (Th2-disease) during childhood. Time course is divided in 4 periods: in utero, infancy/early childhood (0-2 year), pre-school (2-4 year) and childhood (> 5 year). The top 2 figures represent an estimation of the cumulative incidence of asthma and atopic dermatitis respectively. The bars represent the time course of the immunological and pathophysiological responses. The lowest part of the scheme describes the association between Th2-disease and various genetic and environmental factors. Abbreviation and symbols: n.d. = not enough data, n.a. = not applicable, + = positive association, - = negative association, ~ = no association, +/~ = positive association or no association, -/~ negative association or no association, AD = atopic dermatitis, Th = helper T-lymfocyte, IgE = immunoglobulin E , eo's = eosinophils, BHR ↑ = increased bronchial hyperreactivity, HDM = house dust mite, RSV = Respiratory Syncytial Virus, ETS = environmental tobacco smoke exposure. [1] Microbial stimulation = exposure to (parts) of microorganisms other than RSV



Pathofysiology	Growth ↓				
	Small airways				
	IL-18 ↑				
Genetics	Causal	+	+	+	n.a.
	Exacerbating	n.a.	n.a.	n.a.	n.a.
HDM-allergens	Causal	n.d.	~	~	n.a.
	Exacerbating	n.a.	~/+	~/+	n.a.
RSV-infection	Causal	n.d.	+	+	n.a.
	Exacerbating	n.a.	+	+	n.a.
Microbial Stimulation[1]	Causal	n.d	+	+	n.a.
	Exacerbating	n.a.	+	+	n.a.
ETS/irritants	Causal	+	+	+	n.a.
	Exacerbating	n.a.	+	+	n.a.

Figure 10.2. Schematical presentation of factors involved in the development of transient wheezing during childhood. Time course is divided in 4 periods: in utero, infancy/early childhood (0-2 year), pre-school (2-4 year) and childhood (> 5 year). The top figure represent an estimation of the cumulative incidence transient wheezing. The bars represent the time course of the immunological and pathophysiological responses. The lowest part of the scheme describes the association between transient wheezing and various genetic and environmental factors. Abbreviation and symbols: n.d. = not enough data, n.a. = not applicable, + = positive association, - = negative association, ~ = no association, +/ ~ = positive association or no association, -/ ~ negative association or no association, IL = interleukin, HDM = house dust mite, RSV = Respiratory Syncytial Virus, ETS = environmental tobacco smoke exposure. [1] Microbial stimulation = exposure to (parts) of microorganisms other than RSV

References:

1. Vandenbroucke JP, Hofman A, van Stiphout WAHJ. Grondslagen der epidemiologie. Utrecht, The Netherlands: Bunge; 1991.
2. Wahn U, von Mutius E. Childhood risk factors for atopy and the importance of early intervention. J Allergy Clin Immunol 2001;107:567-74.
3. Brunekreef B, Leaderer BP, van Strien R, et al. Using nicotine measurements and parental reports to assess indoor air: the PIAMA birth cohort study. Prevention and Incidence of Asthma and Mite Allergy. Epidemiology 2000;11:350-2.
4. Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. Clin Exp Allergy 1998;28:454-8.
5. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
6. Pearce N, Sunyer J, Cheng S, et al. Comparison of asthma prevalence in the ISAAC and the ECRHS. ISAAC Steering Committee and the European Community Respiratory Health Survey. International Study of Asthma and Allergies in Childhood. Eur Respir J 2000;16:420-6.
7. Chan-Yeung M, Manfreda J, Dimich-Ward H, Ferguson A, Watson W, Becker A. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. Arch Pediatr Adolesc Med 2000;154:657-63.

8. Hide DW, Matthews S, Matthews L, et al. Effect of allergen avoidance in infancy on allergic manifestations at age two years. J Allergy Clin Immunol 1994;93:842-6.
9. Custovic A, Simpson BM, Simpson A, et al. Manchester Asthma and Allergy Study: low-allergen environment can be achieved and maintained during pregnancy and in early life. J Allergy Clin Immunol 2000;105:252-8.
10. van Strien RT, Verhoeff AP, van Wijnen JH, Doekes G, de Meer GE, Brunekreef B. Der p I concentrations in mattress surface and floor dust collected from infants' bedrooms. Clin Exp Allergy 1995;25:1184-9.
11. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990;323:502-7.
12. Wijga A, Smit HA, Brunekreef B, et al. Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. Clin Exp Allergy 2001;31:576-81.
13. Platts-Mills TA, Wheatley LM, Aalberse RC. Indoor versus outdoor allergens in allergic respiratory disease. Curr Opin Immunol 1998;10:634-9.
14. Friedmann PS. The role of dust mite antigen sensitization and atopic dermatitis. Clin Exp Allergy 1999;29:869-72.
15. Custovic A, Simpson A, Chapman MD, Woodcock A. Allergen avoidance in the treatment of asthma and atopic disorders. Thorax 1998;53:63-72.
16. Tan BB, Weald D, Strickland I, Friedmann PS. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. Lancet 1996;347:15-8.
17. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N Engl J Med 2000;343:538-43.
18. Paradise JL, Rockette HE, Colborn DK, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. Pediatrics 1997;99:318-33.
19. Celedon JC, Litonjua AA, Weiss ST, Gold DR. Day care attendance in the first year of life and illnesses of the upper and lower respiratory tract in children with a familial history of atopy. Pediatrics 1999;104:495-500.
20. Holberg CJ, Wright AL, Martinez FD, Morgan WJ, Taussig LM. Child day care, smoking by caregivers, and lower respiratory tract illness in the first 3 years of life. Group Health Medical Associates. Pediatrics 1993;91:885-92.
21. Marbury MC, Maldonado G, Waller L. Lower respiratory illness, recurrent wheezing, and day care attendance. Am J Respir Crit Care Med 1997;155:156-61.
22. Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired airway function and wheezing in infancy: the influence of maternal smoking and a genetic predisposition to asthma. Am J Respir Crit Care Med 1999;159:403-10.
23. Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988;319:1112-7.
24. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999;353:450-4.
25. van Schaik SM, Welliver RC, Kimpen JL. Novel pathways in the pathogenesis of respiratory syncytial virus disease. Pediatr Pulmonol 2000;30:131-8.
26. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000;161:1501-7.
27. Noble V, Murray M, Webb MS, Alexander J, Swarbrick AS, Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. Arch Dis Child 1997;76:315-9.
28. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999;354:541-5.
29. Gern JE, Busse WW. The role of viral infections in the natural history of asthma. J Allergy Clin Immunol 2000;106:201-12.
30. Dinarello CA. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999;103:11-24.
31. Elias JA. Airway remodeling in asthma. Unanswered questions. Am J Respir Crit Care Med 2000;161:S168-71.
32. Okamura H, Kashiwamura S, Tsutsui H, Yoshimoto T, Nakanishi K. Regulation of interferon-gamma production by IL-12 and IL-18. Curr Opin Immunol 1998;10:259-64.

33. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. J Clin Invest 1996;97:915-24.
34. Corry DB, Kheradmand F. Induction and regulation of the IgE response. Nature 1999;402:B18-23.
35. Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. Lancet 1994;344:983-5.
36. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? [see comments]. Clin Exp Allergy 1994;24:423-30.
37. Blanco-Quiros A, Gonzalez H, Arranz E, Lapena S. Decreased interleukin-12 levels in umbilical cord blood in children who developed acute bronchiolitis. Pediatr Pulmonol 1999;28:175-80.
38. Kimata H. Increased serum levels of soluble adhesion molecules in young children with atopic dermatitis. Eur J Pediatr 1999;158:529-30.
39. Yamashita N, Kaneko S, Kouro O, Furue M, Yamamoto S, Sakane T. Soluble E-selectin as a marker of disease activity in atopic dermatitis. J Allergy Clin Immunol 1997;99:410-6.
40. Laan MP, Koning H, Baert MR, et al. Levels of soluble intercellular adhesion molecule-1, soluble E-selectin, tumor necrosis factor-alpha, and soluble tumor necrosis factor receptor p55 and p75 in atopic children. Allergy 1998;53:51-8.
41. Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. Bmj 2001;322:390-5.
42. Kabesch M, Schaal W, Nicolai T, von Mutius E. Lower prevalence of asthma and atopy in Turkish children living in Germany. Eur Respir J 1999;13:577-82.
43. Hjern A, Haglund B, Hedlin G. Ethnicity, childhood environment and atopic disorder. Clin Exp Allergy 2000;30:521-8.

Nederlandse samenvatting

Bij het ontstaan van allergische ziekten zijn zowel erfelijke factoren als omgevingsfactoren betrokken. De precieze rol van blootstelling aan huisstofmijt allergenen en allergenen afkomstig van huisdieren bij de ontwikkeling van allergische ziekten is onduidelijk. Ook het effect van blootstelling aan bacteriën, virussen en parasieten en de rol van etniciteit in de ontwikkeling van astma en allergie is onvoldoende onderzocht. De laatste jaren is veel vooruitgang geboekt in het achterhalen van de onderliggend immunologische mechanismen die leiden tot de ontwikkeling van allergische ziekten. Met name de rol van zogenaamde T-helper 1 en T-helper 2 lymfocyten is uitgebreid bestudeerd. Er is echter weinig bekend over de diverse sturingsmechanismen die de ontwikkeling van allergische ziekten stimuleren dan wel voorkomen.
Het doel van dit proefschrift was:
Het bestuderen van de rol van diverse omgevingsfactoren bij de ontwikkeling van allergische ziekten.
Immunologische processen bestuderen die betrokken zijn bij de ontwikkeling van allergische ziekten in het jonge kind.

Hoofdstuk 1 bevat een algemene inleiding van het proefschrift.
Hoofdstuk 2 geeft een samenvatting van de literatuur met betrekking tot de ontwikkeling van allergische ziekten bij het jonge kind, met de nadruk op de rol van blootstelling aan huisstofmijt allergenen en micro organismen.
Hoofdstuk 3 behandelt de diverse studiedoelen van het proefschrift.
In **hoofdstuk 4** geven we een overzicht van de voorspellende waarde van vroege luchtweg symptomen voor de ontwikkeling van astma op latere leeftijd. Daarnaast worden de uitkomst variabelen geëvalueerd die zijn gebruikt in diverse geboortecohortstudies op het gebied van de ontwikkeling van astma en allergie. Wij concluderen dat van alle luchtwegsymptomen, piepende ademhaling op de vroege kinderleeftijd het sterkst is geassocieerd met de ontwikkeling van astma op latere leeftijd. In de tien cohortstudies die we hebben onderzocht werden steeds verschillende criteria gebruikt voor het beschrijven van een ziektegeval, hoewel in alle studies het aanwezig zijn van piepende ademhaling een belangrijke factor was. Echter, de gegevens die in de verschillende studies zijn verzameld met betrekking tot de aan- of afwezigheid van luchtweg symptomen zijn grotendeels vergelijkbaar. Hierdoor is het in principe mogelijk om de data van de verschillende cohortstudies bij elkaar te voegen en zodoende meer inzicht te krijgen in de voorspellende waarden van verschillende luchtweg symptomen voor de ontwikkeling van allergische ziekten.
In dit proefschrift wordt gebruik gemaakt van gegevens van twee prospectieve geboorte cohort studies: de Preventie en Incidentie van Astma en Mijt Allergie (PIAMA) studie en de Virus Gemedieerde Allergie (VIGALL) studie. In **hoofdstuk 5** wordt de opzet van beide studies besproken.
In **hoofdstuk 6** beschrijven we diverse bloedmarkers (IL-10, IL-12, IL-13, eotaxine, sE-selectine, sICAM-1, sIL-2R) op de leeftijd van 1 jaar in relatie tot de ontwikkeling van allergische ziekten in de eerste 2 levensjaren. Deze bloedmarkers spelen een belangrijke rol in de regulatie van het immuunsysteem. We vonden dat kinderen met een piepende ademhaling in het eerste levensjaar een lagere IL-12 waarde en een hogere IL-10/IL-12 ratio in het bloed hadden dan gezonde kinderen. Kinderen met een piepende ademhaling of kinderen met een

jeukende huidaandoening in het eerste levensjaar hadden hogere bloed sE-selectine waarden vergeleken met gezonde kinderen. Tenslotte vonden we dat kinderen die in het tweede levensjaar een piepende ademhaling ontwikkelden al op de leeftijd van 1 jaar een verhoogde bloed sICAM-1 waarde hadden. Bovenstaande gegevens suggereren dat bij kinderen met een piepende ademhaling in het eerste levensjaar een immuun respons wordt gevonden die past bij een T-helper 2 ontwikkeling.

In **hoofdstuk 7** beschrijven we de eerste resultaten van het interventie gedeelte van het PIAMA onderzoek. In deze studie heeft de helft van de deelnemers matrashoezen voor het bed van de ouders en het kind gekregen die de blootstelling aan huisstofmijt allergeen verminderen (actieve groep), terwijl de andere helft zogenaamde placebohoezen heeft gekregen (placebo groep). De interventie vond plaats in de laatste 3 maanden van de zwangerschap. Op de leeftijd van 1 jaar was in de actieve groep de blootstelling aan huisstofmijt allergenen ongeveer de helft lager dan in de placebo groep. De toepassing van huisstofmijt werende matrashoezen had een beschermend effect op de ontwikkeling van nachtelijk hoesten in het tweede levensjaar. Er werd geen verschil gevonden tussen de actieve groep en de placebo groep met betrekking tot de prevalentie van andere luchtweg symptomen zoals piepende ademhaling en de prevalentie van eczeem en allergie tegen huisstofmijt-allergenen. We concluderen dat het toepassen van huisstofmijt-allergeen werende matrashoezen niet leidt tot een klinisch relevante vermindering van de ontwikkeling van symptomen suggestief voor allergische ziekten in de eerste 2 levensjaren. Het is echter van belang dat de deelnemers aan het PIAMA onderzoek tenminste tot het 8^{e} levensjaar worden gevolgd, omdat de definitieve diagnose van astma en allergie pas op latere leeftijd is te stellen.

In **hoofdstuk 8** bestuderen we de ontwikkeling van allergische ziekten in relatie tot luchtweg infecties. Wij onderzochten de ontwikkeling van luchtweg infecties in het eerste levensjaar in samenhang met het contact met andere kinderen (bijvoorbeeld contact met broertjes en zusjes en het verblijf op de crèche) en het hebben van één of twee allergische ouders. Wij vonden dat kinderen die én naar de crèche gingen én een allergische ouder hadden een veel grotere kans hadden om een lagere luchtweginfectie te krijgen dan op grond van de afzonderlijke risico factoren kan worden voorspeld.

In een ander onderzoek hebben we bij kinderen de immunologische respons in de neus onderzocht tijdens Respiratoir Syncytieel Virus (RSV)-longontsteking en RSV-verkoudheid. In de acute fase van een RSV infectie en 2-4 weken na de infectie werd door middel van neusborstels de hoeveelheid ontstekingscellen, de cytokine productie en ICAM-1 in de neus gemeten. Wij vonden dat de productie van het cytokine IL-18 twee keer zo hoog was in de neus-borstel cellen van kinderen met een acute RSV-longontsteking dan in kinderen met een acute RSV-verkoudheid. Daarnaast werd in de acute fase van zowel de RSV-longontsteking als RSV-verkoudheid een toename gezien van ontstekingscellen zoals macrofagen en T-cellen. Tijdens de acute fase van RSV-longontsteking werd een groter aantal cellen gevonden die positief kleurden voor IL-6, IL-12, IL-18 en ICAM-1 vergeleken met 2-4 weken na de RSV-longontsteking. Wij concluderen dat tijdens RSV-verkoudheid, en in sterkere mate tijdens RSV-longontsteking, een pro-inflammatoire en/of T helper 1 respons in gang wordt gezet.

In **hoofdstuk 9** hebben we etnische verschillen in de prevalentie van luchtweg en- huidsymptomen in de eerste 2 levensjaren bestudeerd. Kinderen met een 'Niet-Nederlandse' etniciteit hadden vaker symptomen van loopneus + tranende ogen in het eerste levensjaar dan 'Nederlandse' kinderen. 'Niet-Nederlandse' kinderen hadden ook vaker last van piepende ademhaling, nachtelijk hoesten, loopneus en loopneus + tranende ogen in het 2^{e} levensjaar vergeleken

met Nederlandse kinderen. Echter, na correctie voor sociaal-economische status verdween de associatie tussen etniciteit en luchtwegsymptomen grotendeels. Deze bevindingen suggereren dat verschillen in de prevalentie van luchtwegsymptomen tussen 'Niet-Nederlandse' en 'Nederlandse' kinderen voor een belangrijk deel kunnen worden verklaard door verschillen in sociaal-economische status tussen beide groepen.

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Curriculum vitae

Laurens Pieter Koopman was born in Delft, on October 1, 1969. He passed his secondary school exam (VWO) at the 'Haagse Montessori Lyceum' in 1987. In 1987 he started his medical training at the Medical Faculty of the Erasmus University Rotterdam, The Netherlands. During his study he did a clinical training in Durban, South-Africa, and participated in a research project at the department of Pediatric Pulmonology of the Sophia Children's Hospital Rotterdam (supervisor dr. H.A.W.M. Tiddens, head: Prof.dr. J.C. de Jongste). After obtaining his Medical Degree in 1995, he performed research at the department of Pediatric Pulmonology of the Sophia Children's Hospital Rotterdam (head: Prof.dr. J.C. de Jongste), worked as a Senior House Officer Internal Medicine in various hospitals in the UK and worked as a medical examiner for an insurance company (Zorgvoorzieningen Leeuwarden). During 1996-1997 he was a Senior House Officer (AGNIO) in Pediatrics at the Beatrix Children's Hospital Groningen (head: dr. J.L. Kimpen). From November 1997 until September 2001 he worked as a research fellow at the department of Pediatric Pulmonology (head: Prof.dr. J.C. de Jongste) and department of Allergy&Immunology (head: Prof.dr. H.J. Neijens) of the Sophia Children's Hospital Rotterdam. The research performed in this period is presented in this thesis. In October 2001 he started his specialist training in Pediatrics at the Sophia Children's Hospital Rotterdam (head: Prof.dr. H.A. Büller).

List of publications

Koopman LP, Plotz FB, Meuzelaar JJ, Knoester H. Thymic cyst haemorrhages and transient cholestasis in a 4-week-old infant. *Eur J Pediatr. 1998 Mar;157(3):236-8.*

Tiddens HA, **Koopman LP**, Lambert RK, Elliott WM, Hop WC, van der Mark TW, de Boer WJ, de Jongste JC. Cartilaginous airway wall dimensions and airway resistance in cystic fibrosis lungs. *Eur Respir J. 2000 Apr;15(4):735-42.*

Brunekreef B, Leaderer BP, van Strien R, Oldenwening M, Smit HA, **Koopman LP**, Kerkhof M. Using nicotine measurements and parental reports to assess indoor air: the PIAMA birth cohort study. Prevention and Incidence of Asthma and Mite Allergy. *Epidemiology 2000 May;11(3):350-2.*

Wijga A, Smit HA, Brunekreef B, Gerritsen J, Kerkhof M, **Koopman LP**, Neijens HJ. Are children at high familial risk of developing allergy born into a low risk environment? The PIAMA Birth Cohort Study. Prevention and Incidence of Asthma and Mite Allergy. *Clin Exp Allergy 2001 Apr;31(4):576-81.*

Koopman LP, Brunekreef B, de Jongste JC, Neijens HJ. Definition of respiratory symptoms and disease in early childhood in large prospective birth cohort studies that predict the development of asthma. *Pediatr Allergy Immunol 2001 Jun;12(3):118-24.*

Koopman LP, Smit HA, Heijnen ML, Wijga A, van Strien RT, Kerkhof M, Gerritsen J, Brunekreef B, de Jongste JC, Neijens HJ. Respiratory infections in infants: interaction of parental allergy, child care, and siblings-the PIAMA study. *Pediatrics 2001 Oct;108(4):943-8*

Koopman LP, van Strien RT, Kerkhof M, Smit HA, Wijga A, de Jongste JC, Gerritsen J, Aalberse RC, Brunekreef B, Neijens HJ. Placebo-controlled trial of house dust mite impermeable mattress covers: effect on symptoms in early childhood. *Provisionally accepted for publication by Am. J. Respir. Crit. Care Med.*

Koopman LP, Savelkoul H, van Benten IJ, Gerritsen J, Brunekreef B, Neijens HJ. Increased serum IL-10/IL-12 ratio in wheezing infants. *Submitted.*

Van Strien RT, **Koopman LP**, Kerkhof M, Oldenwening M, de Jongste JC, Gerritsen J, Neijens HJ, Aalberse RC, Smit HA, Brunekreef B. Mattress encasings and mite allergen levels in the PIAMA-study. *Submitted.*

Van Benten IJ, **Koopman LP**, KleinJan A, van Middelkoop BC, de Waal L, Osterhaus ADME, Neijens HJ, Fokkens WJ. Enhanced nasal pro-inflammatory cytokine production rather than a Th2-like response in RSV bronchiolitis. *Submitted.*

Koopman LP, Wijga A, Smit HA, de Jongste JC, Kerkhof M, Gerritsen J, Vos APH, van Strien RT, Brunekreef B, Neijens HJ. Early respiratory and- skin symptoms in relation to ethnical background: the importance of socioeconomic status; the PIAMA-Study. *Submitted.*

List of abbreviations

Acb™	Allergy control products®
AD	Atopic dermatitis
AR	Allergic rhinitis
APC	Antigen-presenting cell
BF	Breast feeding
CBMC	Cord blood mononucleasr cells
CD	Cluster of differentiation
CI	Confidence interval
CM	Cow's milk
CTC	Cytotoxic T-cell
DC	Dentritic cells
Der f	Dermatophagoides farinea
Der p	Dermatophagoides pteronyssinus
ECP	Eosinophil-derived cationic protein
eNO	Nitric oxide in exhailed air
EPO	Eosinophil-derived peroxidase
ETS	Environmental tobacco smoke exposure
HDM	House dust mite
HLA	Human leukocyte antigen
ICAM	Intra-cellular adhesion molecule
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IQR	Inter quartile range
IS	Intervention study
ISAAC	International Study of Asthma and Allergies in Childhood
IU	International unit
MAAS	Manchester Asthma and Allergy Study
MAS	Multicenter Allergy Study
MHC	Major Histocompatibility Class
NHS	Natural history study
NK-cells	Natural killer cells
OR	Odds ratio
PBMC	Peripheral blood mononuclear cells
PG	Prostoglandin
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
RAST	Radioallergosorbent fluorescent immunoassay
Rint	Airway resistance by the interruptor technique
RSV	Respiratory Syncytial Virus
SD	Study doctor
SF	Solid food
sIL-2R	Soluble interleukin 2 receptor
TCR	T-cell receptor
Th-cells	T-helper cells
TNF	Tumor necrosis factor
Tr-cells	T-regulatory cells
VCAM	Vascular cell adhesion molecule
VIGALL	Virus Gemedieerde Allergie