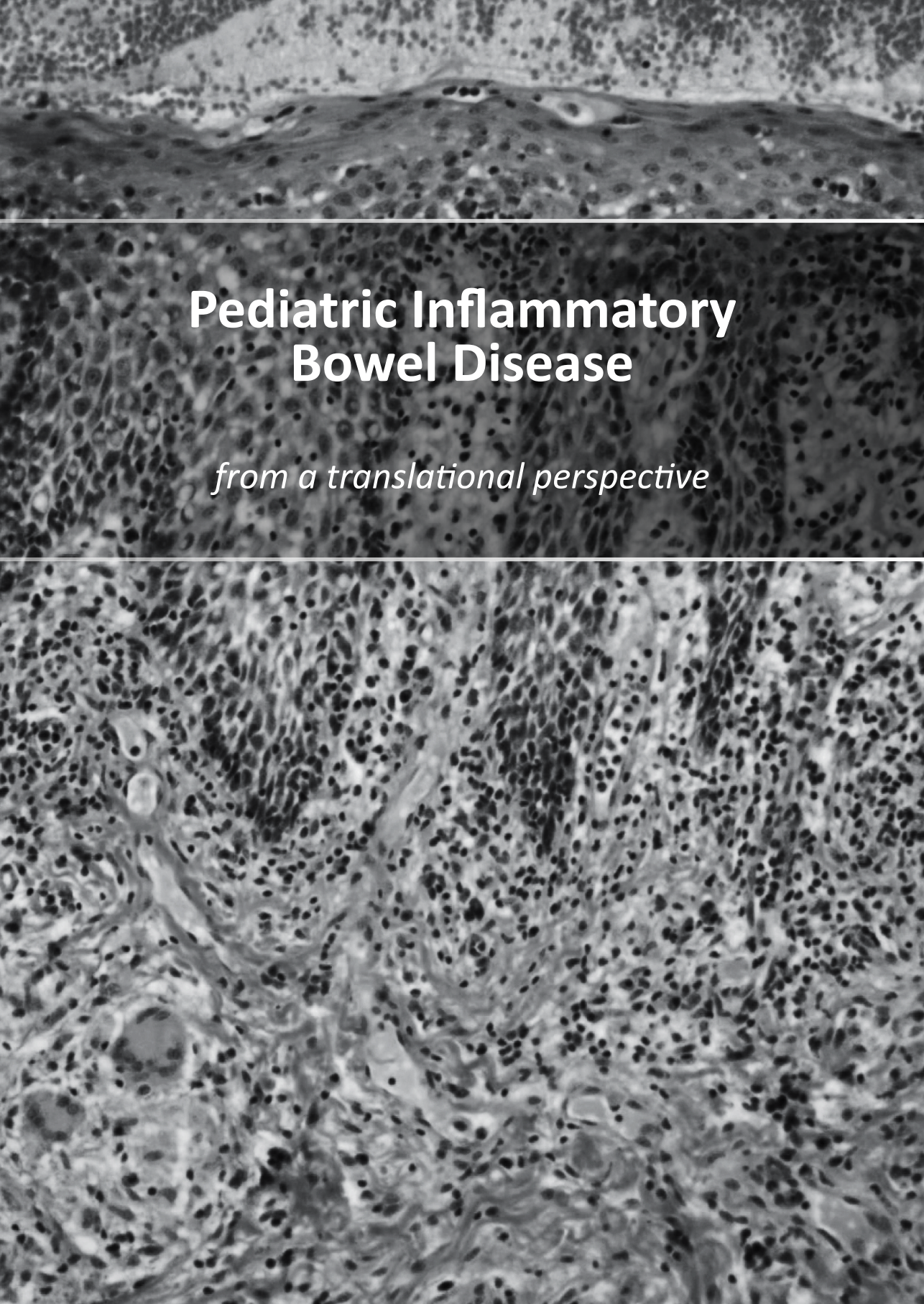


Pediatric Inflammatory Bowel Disease

from a translational perspective

Part of the research in this thesis is financially supported by Mrace (Medische Research Advies Commissie van het Erasmus MC)

The publication of this thesis is supported by Nestlé Nutrition, Mead Johnson Nutrition, Ferring, Schering-Plough and Solvay Pharma, for which I am grateful.

Design and print: NPN drukkers B.V., Breda

ISBN/EAN: 978-90-78343-04-2

The cover illustrates granulomas in the upper lip of an 8 year old boy with a swollen upper lip and elevated angiotensin converting enzyme, who developed gastrointestinal Crohn's disease 4 months later.

Pediatric Inflammatory Bowel Disease,

from a translational perspective

proefschrift

ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof. dr. H.G. Schmidt

en volgens besluit van het College voor Promoties

de openbare verdediging zal plaatsvinden op 14 april 2010 om 13.30 uur

door

Gerardus Maria Damen

geboren te Oosterhout (N-B)

Promotiecommissie

Promotoren:	Prof. Dr. A.J. van der Heijden
	Prof. Dr. E.E.S. Nieuwenhuis
Co-promotoren:	Dr. J.C. Escher
	Dr. J.N. Samsom
Overige leden:	Prof. Dr. D. Tibboel
	Prof. Dr. J.B. van Goudoever
	Dr. C.J. van der Woude
	Prof. Dr. J.D. Laman
	Prof. Dr. J.H.J.M. van Krieken
	Dr. D.J. de Jong

CONTENTS

Chapter 1	Introduction and outline of the thesis	7
Chapter 2	Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn's disease	21
Chapter 3	Defective acute inflammation in Crohn's disease	37
Chapter 4	NOD-2 polymorphisms are not associated with altered buccal epithelial responsiveness in pediatric Crohn's disease patients	41
Chapter 5	Production of IL-12p70 and IL-23 by monocyte-derived dendritic cells in children with inflammatory bowel disease	49
Chapter 6	Niet-invasieve markers bij inflammatoire darmziekten op de kinderleeftijd	57
Chapter 7	Overlap, common features and essential differences in granulomatous inflammatory bowel disease	65
Chapter 8	Differences in histology of biopsies of the small bowel and the colon between pediatric-onset and adult-onset inflammatory bowel disease	85
Chapter 9	Catch-up growth is often lacking in patients with childhood-onset Inflammatory Bowel Disease	99
Chapter 10	Summary and discussion	117
	Samenvatting en discussie	122
	Curriculum Vitae	129
	List of publications	130
	Dankwoord	132

CHAPTER 1

Introduction

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), the two main subtypes of inflammatory bowel disease (IBD), are chronic relapsing inflammatory disorders of the gastrointestinal tract that have a peak age of onset in the second decade of life in children. There is strong evidence to support that dysregulation of the normally controlled immune response to commensal bacteria in a genetically susceptible individual drives IBD.[1,2] Patients typically suffer from frequent and chronically relapsing flares, resulting in abdominal pain, diarrhea, rectal bleeding and weight loss. In CD, inflammation is transmural and often discontinuous. In UC, inflammatory changes typically involve the superficial mucosal and submucosal layers of the intestinal wall. CD most commonly involves the ileum and colon, but can affect any region of the gut. UC classically involves the rectum and inflammation may extend as far as the caecum in a typical continuous pattern. Patients with IBD may have various extra-intestinal symptoms such as oral ulcers, uveitis, arthalgias or arthritis and sclerosing cholangitis.[3-5]

IBD is heritable, 5 to 20% of the patients have a family history of the disease.[6,7] This positive family history of IBD is more frequently observed in patients with CD than in UC.[7] In IBD, there is a significantly higher rate of disease concordance in monozygotic twins compared with dizygotic twins.[8-11]

Genetics

Genetic epidemiology data provide compelling evidence that CD and UC are related polygenic diseases.[12] In 2001 *Nod2/CARD15* was identified as the first CD susceptibility gene by hypothesis-free linkage analyses and positional cloning.[13,14] The technology of genome-wide association (GWA) scanning is now yielding multiple susceptibility loci both in CD and UC. In CD, GWA studies identified additional variants in the genes *ATG16L1, IRGM, IL23R, IL12B, JAK2, STAT3, NKX2-3, TNFSF15, PTPN22, ICOSLG, CDKAL1, ITLN1, CCR6* and *ECM1*.[1,12,15] Within UC, there is a known contribution of the major histocompatibility complex[16], and GWA studies further identified associations between UC and variants in *MST1, ECM1, NELL1, MDR1, STAT3, IL12B, IL18RAP, JAK2, LYRM4, CDKAL1, BSN, NKX2-3, HERC2, CCNY* and a borderline association at *PTPN2*.[1,12,15-21]

Many of the polymorphisms and associations mentioned above are related to two emerging major pathogenic themes.[1,12] Firstly, there is a critical contribution of defects in innate immunity as has been confirmed with the polymorphisms in the *Nod2* genes and the autophagy genes *ATG16L1* and *IRGM* in CD. Polymorphisms in these genes suggest that alterations in the recognition and intracellular processing of bacterial components will have a role in the immunopathogenesis of disease. Mutant *Nod2* will not sense muramyl dipeptide − a bacterial component of peptidoglycan − properly and might be associated with unopposed Toll-like receptor(TLR)2 signaling, leading to enhanced IL-12 production.[22] Autophagy has an important role in restricting growth of certain microorganisms.[1] Mutations in the autophagy-related genes *ATG16L1* and *IRGM* may result in an impaired capacity

to eliminate intracellular pathogens such as *S. typhimurium, Toxoplasma gondii, L. monocytogenes* and mycobacteriae.[23-25] Secondly, in IBD, defects in the adaptive immune system may result in alternative signalling within the interleukin (IL)-12/IL-23 axis and the induction of IL-17 expression by T helper 17 (T_H17) cells. The regulated expression of IL-23R and IL-23Rβ2 has a key role in T_H-cell subset differentiation. Following engagement of IL-23R by IL-23, Janus kinase 2 (JAK2) is activated, resulting in JAK2 autophosphorylation and tyrosine phosphorylation of IL-23R. This in turn results in the recruitment, phosphorylation, homodimerization and nuclear translocation of STAT3 as well as other STAT's.[26] STAT3 and STAT4 have central roles in the differentiation of T_H17 cells and T_H1 cells (through IL-12 activation).[27]

Immunopathogenesis

The single cell layer that forms the epithelial barrier includes absorptive cells, secretory cells, microfold cells (M cells), goblet cells and Paneth cells. Goblet cells contribute to the formation of a protective mucus layer, whereas Paneth cells are located near the base of small intestinal crypts and secrete potent antimicrobial peptides known as defensins. Patients with IBD are found to have a diminished expression of alpha-defensins that is most pronounced in patients with Nod2-mutations.[28,29] M cells and dendritic cells sample intestinal mucosal contents.[30,31] Pathogenic bacteria or disruptions in epithelial-cell barrier function activate dendritic cells, triggering their transport to the mesenteric lymph nodes, where they promote the differentiation of naive T cells into effector and regulatory T cells. The cytokine milieu secreted in part by the dendritic cells skews the differentiation of naive CD4+ T cells into T_H1, T_H2, T_H17- or regulatory T-cell subsets.[1] Analysis of the mucosal T cell phenotype largely supported the concept of CD as a T_H1 disease, driven by IL-12 and characterized by the production of the signature cytokine IFN-gamma, and UC as an atypical T_H2 disease characterized by production of cytokines such as IL-13.[32,33] More recently, the discovery of the T_H17 lineage, driven by IL-23 (as well as IL-6 and transforming growth factor beta) and characterized by IL-17 production has led to a re-evaluation and realization that a number of inflammatory conditions in which T_H1 was previously considered central, may actually be T_H17 dependent.[33]

Epidemiology

In Western Europe, the incidence rate of IBD in children has increased to 5.2 new cases per 100.000 children per year.[34-41] In pediatric IBD there is a predominance of CD over UC, with a clearly increasing incidence of CD the past decade.[41] In agreement with the hygiene hypothesis, the incidence of (auto)immune diseases, including IBD, increased while the incidence of infectious diseases decreased.[1,41,42] In Western society, there is an increase in living standards and hygiene, while family crowding and contact with animals and cattle decreases.

Clinical presentation

The clinical manifestations are well reported in a large cohort of children with IBD by Sawczenko et al.[43] In UC, children had diarrhoea (74%), bleeding (84%) and abdominal pain (62%). Nevertheless, 26% of the patients lacked diarrhoea and 16% of the patients lacked bleeding. In CD, children had abdominal pain (72%), weight loss (58%), diarrhoea (56%) and/or bleeding (22%). In CD, 44% of the children lacked diarrhoea and almost 80% lacked bleeding. In pediatric IBD, especially in CD, the clinical presentation may be quite diverse. Patients may present with lethargy, anorexia, nausea, vomiting, constipation, soiling, anemia, oral ulcers, uveitis, arthralgias and arthritis, perianal abscesses, ulcers or fistulas, growth failure, delayed puberty and skin manifestations such as erythema nodosum. Growth failure is one of unique features of childhood-onset IBD, and is present in 10-40% of the affected children at diagnosis.[44-51] Growth failure is ascribed to a complex interaction between nutritional status, inflammation, disease activity and severity, and genotype, causing resistance to the effects of growth hormone.[52-54]

Diagnostics

In IBD patients, blood tests may present anemia, thrombocytosis, increased inflammation parameters such as ESR and CRP, and hypoalbuminemia.[55,56] In children suspected of having IBD, anemia and thrombocytosis are the strongest indicators of IBD.[55] The ESR is increased (>25 mm/hr) in 85% of the children with CD and in 23% of the children with UC.[56] Antibodies against *Saccharomyces cerevisiae* are associated with CD.[57-59] These antibodies are positive in 46-60% of the children with CD, in 4-12% of the children with UC and in 3-5% of healthy controls. Perinucleair antineutrophilic cytoplasmatic antibodies are positive in 48-82% of the children with UC and in 5-19% of the children with CD. In order to examine bowel inflammation several indicators of inflammation can be determined in the stools, such as alpha-1-antitrypsin, calprotectin and lactoferrin. In children, the diagnosis of IBD has to be verified by gastroduodenoscopy and ileocolonoscopy including histology on multiple biopsies, as well as radiological examination of the small bowel.[60] Examination of the small bowel may include small bowel follow through, MRI or video endocapsule investigations.

Histology

Architectural changes, epithelial cell abnormalities and inflammatory features are microscopic features of IBD.[61-65] Architectural changes include crypt architectural irregularity, crypt branching, crypt distortion, crypt atrophy and surface irregularity; epithelial cell abnormalities are erosions, ulcerations, mucin depletion and Paneth cell metaplasia; and inflammatory features include poly-

morph exudates, crypt epithelial polymorphs, cryptitis, crypt abscesses, increased lamina propria cellularity (polymorphs and/or eosinophils), basal plasmacytosis and granulomas. In UC, inflammatory changes typically involve the superficial mucosal and submucosal layers of the intestinal wall. UC classically involves the rectum and inflammation may extend as far as the caecum in a typical continuous (diffuse) pattern. A gradient of inflammation along the colon, from proximal to distal, will support a diagnosis UC. In adults, a diagnosis of UC is based upon the combination of basal plasmacytosis, heavy, diffuse transmucosal lamina propria cell increase and widespread mucosal or crypt architectural distortion.[65] In CD, inflammation is transmural and often discontinuous (patchy or focal); the ileum and colon are most commonly involved, but any region of the intestinal tract may be affected. The generally accepted microscopic features of CD are discontinuous and/or patchy chronic inflammation (with lymphocytes and plasma cells), focal crypt irregularity (with discontinuous crypt distortion) and granulomas (not related to crypt injury).[63] In resected specimens of the bowel of patients with CD, transmural inflammation, transmural presence of lymphoid aggregates, skipped lesions, and the presence of fissures, sinuses and fistulae may be seen.

In children with CD, non-caseating granulomas are found in about 50% of the mucosal biopsies.[66,67] Granulomas are sarcoid-like, non-necrotic, and can be well-formed.[68-70] They are largely composed of epitheloid cells and multinucleated giant cells. Necrosis is usually absent, and if necrosis occurs it will be limited to a small central area. Granulomas may be found anywhere in the wall of the bowel (including serosa), in the regional lymph nodes and at any other site of involvement; they can be seen in isolation away from areas of active disease.

Early versus late onset IBD

In early-onset CD, patients less often have isolated ileal involvement and more often have large bowel involvement compared with adults.[71-73] In early-onset CD, there is rather frequently upper gastrointestinal (GI) involvement[66,67], however, in adult-onset CD there is no routine in upper GI-endoscopy with biopsies at diagnosis. In early-onset UC, at diagnosis, disease localization is often more extensive compared with adults. In pediatric UC, pancolitis is present in 70-80% of the patients and proctitis is present only in a minority of patients (4-13%).[43,74,75] Next to the more extensive localization of disease, there is an occurrence of rectal sparing in up to 30% of pediatric UC patients.[76] Due to the more extensive localization of disease the clinical presentation is often more severe in children compared with adults, and the occurrence of colorectal carcinoma at an adult age is clearly increased in patients younger than 15 years of age at diagnosis.[77] Finally, a number of genetic mutations are more frequently present in early-onset IBD compared with adults.[78,79] It is speculated that the contribution of innate immune defects to the pathogenesis of IBD are inversely related to the age of disease onset.[40]

Outline of this Thesis

Inflammatory Bowel Diseases (IBD) represent an aberrant immune response by the mucosal immune system to luminal bacteria. Since the oral mucosa harbors the first epithelial cells that interact with microorganisms, we assessed the immunological activity of buccal epithelium in children with IBD and adults with Crohn's disease (CD). In **Chapter 2**, buccal epithelial cells were obtained from children and adults with CD, children with ulcerative colitis (UC) and healthy controls. In vitro, the chemokine production by buccal epithelial cells was assessed, with and without microbial stimulation. We aimed to obtain a new, rapid and non-invasive test for screening and classification of IBD in children. We hypothesized that only buccal epithelium in patients with (early-onset) CD would be immunological active. We studied whether buccal epithelial cells from patients with (early-onset) CD might demonstrate an inducible production of chemokines upon microbial stimulation. Finally, we studied whether an enhanced chemokine production was restricted to cells derived from the epithelial barrier.

It has been suggested that CD patients have a general defect in the innate mucosal immune response, including a diminished CXCL-8 production. In a letter, in **Chapter 3**, we come to a different conclusion. A diminished CXCL-8 production does not necessarily represent a general mucosal paradigm for CD, as we have demonstrated. We argue that results from one study cannot be extrapolated to other involved immune cells or to specific patients such as children with CD.

In **Chapter 4**, the inducible production of chemokines by buccal epithelial cells is studied into more detail. Furthermore, we address whether an aberrant epithelial response pattern is associated with Nod2 polymorphisms, an intracellular pattern-recognition molecule that is implicated in the detection of microbial peptidoglycan.

In **Chapter 5**, we study Toll-like receptor (TLR)-driven cytokine responses from monocyte-derived dendritic cells (moDCs) in children with IBD. We study whether the intrinsic ability of these dendritic cells to secrete interleukin (IL)-12 and IL-23 is related to the type of IBD, duration of disease (early versus late) or treatment.

In **Chapter 6**, the value of non-invasive markers of inflammation in the blood and stool are discussed in children suspected of having IBD. A correct interpretation of the results of blood tests may help in the decision for more invasive investigations. Markers may be of value in determining the type of IBD and disease activity. Presence of specific antibodies might be related to age of presentation, localization of disease, results of treatment and outcome. Different fecal markers can be determined, however, the question is whether these markers are specific and whether the levels of these markers correlate with disease activity.

In **Chapter 7** overlap, common features and essential differences in pediatric granulomatous inflammatory bowel disease are discussed. This chapter is based on clinical problems that we encountered in patients with overlap in CD and sarcoidosis, as well as overlap in CD and the Hermansky-Pudlak syndrome. In this review, granulomatous inflammatory bowel diseases in children are addressed including chronic granulomatous disease (CGD), sarcoidosis, CD, Hermansky-Pudlak syndrome and abdominal tuberculosis. Substantial overlap may exist in the clinical presentation of these diseases, with common symptoms such as anorexia, vomiting, abdominal pain, abdominal distension, weight loss, (bloody) diarrhea and growth failure, and less common clinical manifestations such as peri-anal abscesses or fistulas, hepatic abscesses, intestinal obstruction, pulmonary manifestations and rheumatologic manifestations like arthritis and uveitis. By addressing common features as well as essential differences in clinical presentation, physical examination, laboratory results, radiology, endoscopy and histology, instructions for specific diagnosis and respective treatments are provided. An accurate interpretation of histology is discussed into detail.

To establish a complete and correct diagnosis of IBD is of great importance as treatment options, course of the disease and prognosis differ between CD and UC. Recent guidelines have focused on the definition of histological criteria for IBD, but were mainly derived from adult patients. In **Chapter 8** we verify whether these criteria for biopsy diagnosis are applicable in children with IBD. Predetermined quantitative and qualitative criteria are formulated from the existing guidelines. In patients (children and adults) with a first presentation of IBD histology of mucosal biopsies of the upper gastrointestinal tract, the terminal ileum and the colon are reviewed by a pathologist and a pediatric gastroenterologist. Clinical and endoscopic data are collected. We study the differences in histology between pediatric (early-onset) and adult (late-onset) patients with IBD. Furthermore, we determine which histological criteria discriminate best between CD and UC in children.

In children with IBD growth failure is a unique feature. Treatment aimed to reverse growth failure is not always successful. This outcome seems to be influenced by the degree of growth failure at diagnosis, use of corticosteroids and jejunal disease. On the other hand, delayed maturation and delayed puberty seem to compensate for the period of poor growth earlier in life. In **Chapter 9** we assess growth failure at the time of diagnosis, the progression of linear growth in the years thereafter and adult height in patients with childhood-onset IBD. Retrospectively, we analyze growth data in 318 patients with childhood-onset IBD (178 patients with CD, 131 patients with UC and 9 patients with unclassified colitis). A total of 162 patients already reached adulthood. We correlate height at diagnosis, linear growth and adult height with variables such as age at diagnosis, diagnostic delay, type of IBD, localization of disease, disease activity and therapy.

References

1. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nature Reviews Immunology 2008;8:458-66.
2. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002;347:417–29.
3. Jose FA, Heyman MB. Extraintestinal manifestations of Inflammatory Bowel Disease. J Ped Gastroenterol Nutr 2008;46:124-33.
4. Ardizzone S, Sarzi Puttini P, Cassinotti A, Bianchi Porro G. Extraintestinal manifestations of inflammatory bowel disease. Dig Liver Dis 2008;40S:S253-9.
5. Jose FA, Garnett EA, Vittinghoff E, et al. Development of extraintestinal manifestations in pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis 2009;15:63-8.
6. Binder V. Epidemiology of IBD during the twentieth century: an integrated view. Best Pract Res Clin Gastroenterol 2004;18:463-79.
7. Yang H, Rotter JI. In: Inflammatory Bowel Disease, pages 301-331, eds Kirsner JB, Shorter RG, (Williams & Wilkins, Baltimore, 1995).
8. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut 1988;29:990–6.
9. Halfvarson J, Bodin L, Tysk C, et al. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology 2003;124:1767–73.
10. Thompson NP, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: results of a British twin study. BMJ 1996;312:95–6.
11. Orholm M, Binder V, Sorensen TI, et al. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. Scand J Gastroenterol 2000;35:1075-81.
12. Anderson CA, Massey DC, Barrett JC, et al. Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. Gastroenterology 2009;136:523-9.
13. Hugot J-P, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599–603.
14. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603–6.
15. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008;40:955-62.
16. Fisher SA, Tremelling M, Anderson CA, et al. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet 2008;40:710–712.
17. Schwab M, Schaeffeler E, Marx C, et al. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. Gastroenterology 2003;124:26–33.
18. Brant SR, Panhuysen CI, Nicolae D, et al. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. Am J Hum Genet 2003;73:1282–1292.
19. Franke A, Hampe J, Rosenstiel P, et al. Systematic association mapping identifies NELL1 as a novel IBD disease gene. PLoS ONE 2007;2:e691.

20. Franke A, Balschun T, Karlsen TH, et al. Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. Nat Genet 2008;40:713–715.
21. Zhernakova A, Festen EM, Franke L, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. Am J Hum Genet 2008;82:1202–1210.
22. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004;5:800-8.
23. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nature Genet 2007;39:596-604.
24. Collazo CM, Yap GS, Sempowski GD, et al. Inactivation of LRG-47 and IRG-47 reveals a family of interferon γ-inducible genes with essential, pathogen-specific roles in resistance to infection. J Exp Med 2001;194:181–8.
25. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science 2006;313:1438-41.
26. Parham C, Chirica M, Timans J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rβ1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002;168:5699-5708.
27. Watford WT, Jissong BD, Bream JH, et al. Signaling by IL-12 and IL-23 and the immune-regulatory roles of STAT4. Immunol Rev 2004;202:139-56.
28. Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 2004;53:1658–64.
29. Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc Natl Acad Sci U S A 2005;102:18129–34.
30. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361–7.
31. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 2005;307:254–8.
32. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003;3:521-33.
33. Shale M, Ghosh S. Beyond TNF, Th1 and Th2 in inflammatory bowel disease. Gut 2008;57:1349-51.
34. Lindberg E, Lindquist B, Holmquist L, Hildebrand H. Inflammatory bowel disease in children and adolescents in Sweden, 1984-1995. J Ped Gastroenterol Nutr 2000;30:259-64.
35. Armitage E, Drummond HE, Wilson DC, Ghosh S. Increasing incidence of both juvenile-onset Crohn's disease and ulcerative colitis in Scotland. Eur J Gastroenterol Hepatol 2001;13:1439-47.
36. Stordal K, Jahnsen J, Bentsen BS, Moum B. Pediatric inflammatory bowel disease in southeastern Norway: a five-year follow-up study. Digestion 2004;70:226-30.
37. van der Zaag-Loonen HJ, Casparie M, Taminiau JA, et al. The incidence of pediatric inflammatory bowel disease in the Netherlands : 1999-2001. J Ped Gastroenterol Nutr 2004;38:302-7.

38. Auvin S, Molinie F, Gower-Rousseau C, et al. Incidence, clinical presentation and location at diagnosis of pediatric inflammatory bowel disease: a prospective population-based study in northern France (1088-1999). J Ped Gastroenterol Nutr 2005;41:49-55.
39. Turunen P, Kolho K, Auvinen A, et al. Incidence of inflammatory bowel disease in Finnish children, 1987-2003. Inflamm Bowel Dis 2006;12:677-83.
40. Nieuwenhuis EES, Escher JC. Early onset IBD : What's the difference ? Dig Liver Dis 2008;40:12-5.
41. de Mesquita MB, Civitelli F, Levine A. Epidemiology, genes and inflammatory bowel diseases in childhood. Dig Liver Dis 2008;40:3-11.
42. Gent AE, Hellier MD, Grace RH, et al. Inflammatory bowel disease and domestic hygiene in infancy. Lancet 1994;343:766-7.
43. Sawczenko A, Sandhu BK. Presenting features of inflammatory bowel disease. Arch Dis Child 2003;88:995-1000.
44. Kanof ME, Lake AM, Bayless TM. Decreased height velocity in children and adolescents before the diagnosis of Crohn's disease. Gastroenterology 1988;95:1523-7.
45. Motil KJ, Grand RJ, Davis-Kraft L, et al. Growth failure in children with inflammatory bowel disease: a prospective study. Gastroenterology 1993;105:681-691.
46. Griffiths AM, Nguyen P, Smith C, et al. Growth an clinical course of children with Crohn's disease. Gut 1993;34:939-43.
47. Markowitz J, Grancher K, Rosa J, et al. Growth failure in pediatric inflammatory bowel disease. J Ped Gastroenterol nutr 1993;16:373-380.
48. Hildebrand AM, Karlberg J, Kristiansson B. Longitudinal growth in children and adolescents with inflammatory bowel disease. J Ped Gastroenterol Nutr 1994;18:165-173.
49. Wine E, Reif SS, Leshinsky-Silver E, et al. Pediatric Crohn's Disease and Growth Retardation: The Role of Genotype, Phenotype, and Disease Severity. Pediatrics 2006;114:1281-1286.
50. Sawczenko A, Ballinger AB, Savage MO, Sanderson IR. Clinical Features Affecting Final Adult Height in Patients With Pediatric-Onset Crohn's Disease. Pediatrics 2006;118:124-9.
51. Pfefferkorn M, Burke G, Griffiths A, et al. Growth abnormalities persist in newly diagnosed children with crohn disease despite current treatment paradigms. J Ped Gastroenterol Nutr 2009;48:168-74.
52. Ballinger AB, Camach-Hübner C, Croft NM. Growth failure and intestinal inflammation. QJM 2001;94:121-5.
53. Heuschkel R, Salvestrini C, Beattie RM, et al. Guidelines for the management of growth failure in childhood inflammatory bowel disease. Inflamm Bowel Dis 2008;14:839-49.
54. Shamir R, Phillip M, Levine A. Growth retardation in pediatric Crohn's disease: pathogenesis and interventions. Inflamm Bowel Dis 2007;13:620-8.
55. Cabrera-Abreu JC, Davies P, Matek Z, Murphy MS. Performance of blood tests in diagnosis of inflammatory bowel disease in a specialist clinic. Arch Dis Child 2004;89:69-71.
56. Beattie RM, Walker-Smith JA, Murch SH. Indications for investigation of chronic gastrointestinal symptoms. Arch Dis Child 1995;73:345-5.

57. Khan K, Schwarzenberg SJ, Sharp H, et al. Role of serology and routine laboratory tests in childhood inflammatory bowel disease. Inflamm Bowel Dis 2002;8:325-9.
58. Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. J Pediatr 1999;134:447-52.
59. Ruemmelle FM, Targan SR, Levy G, et al. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1998;115:822-9.
60. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis – the Porto criteria. J Ped Gastroenterol Nutr 2005;41:1-7.
61. D'Haens G, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenteroloy 1998;114:262-7.
62. Bentley E, Jenkins D, Campbell F, Warren B. How could pathologists improve the initial diagnosis of colitis? Evidence from an international workshop. J Clin Pathol 2002;55:955-60.
63. Stange EF, Travis SPL, Vermeire S, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut 2006;55(suppl I):i1-i15.
64. Differentiating Ulcerative Colitis from Crohn Disease in Children and Young Adults: Report of a Working Group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and Crohn's and Colitis Foundation of America. J Ped Gastroenterol Nutr 2007;44:653-74.
65. Stange EF, Travis SP, Vermeire S, et al. European evidence-based Consensus on the diagnosis and management of Ulcerative colitis: Definitions and diagnosis. J Crohn's Colitis 2008;2:1-23.
66. Abdullah BA, Gupta SK, Croffie JM, et al. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. J Ped Gastroenterol Nutr 2002;35:636-40.
67. Tobin JM, Sinha B, Ramani P, et al. Upper gastrointestinal mucosal disease in pediatric Crohn disease and ulcerative colitis: a blinded, controlled study. J Ped Gastroenterol Nutr 2001;32:443-8.
68. Rosai J, Ackerman LV. Surgical Pathology. 9th ed. St Louis: Mosby, 2004. Chapter 11: gastrointestinal tract, p721-3.
69. Russo P, Brown K, Baldassano RN. Chapter 5: Colitis in childhood, p108-17. In: Russo P, Ruchelli E, Piccoli DA, eds. Pathology of Pediatric Gastrointestinal and Liver Disease. New York: Spinger, 2004.
70. Yantiss RK, Odze RD. Diagnostic difficulties in inflammatory bowel disease pathology. Review. Histopathology 2006;48:116-32.
71. Mamula P, Telega GW, Markowitz JE, et al. Inflammatory bowel disease in children 5 years of age and younger. Am J Gastroenterol 2002;97:2005-10.
72. Sawczenko A, Sandhu BK, Logan RF, et al. Prospective surgery of childhood inflammatory bowel disease in the British Isles. Lancet 2001;357:1093-4.
73. Heyman MB, Kirschner BS, Gold BD, et al. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. J Pediatr 2005;146:35-40.

74. Kugathasan S, Judd RH, Hoffmann RG, et al. Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. J Pediatr 2003;143:525-31.
75. Griffiths AM. Specificities of inflammatory bowel disease in childhood. Best Pract Res Clin Gastroenterol 2004 ;18 :509-23.
76. Glickman JN, Bousvaros A, Farraye FA, et al. Pediatric patients with untreated ulcerative colitis may present initially with unusual morphologic findings. Am J Surg Pathol 2004;28:190-7.
77. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis : a meta-analysis. Gut 2001;48:526-35.
78. de Ridder L, Weersma RK, Dijkstra G, et al. Genetic susceptibility has a more important role in pediatric-onset Crohn's disease than in adult-onset Crohn's disease. Inflamm Bowel Dis 2007;13:1083-92.
79. Sun L, Roesler J, Rösen-Wolff A, et al. CARD15 genotype and phenotype analysis in 55 pediatric patients with Crohn disease from Saxony, Germany. J Ped Gastroenterol Nutr 2003;37:492-7.

CHAPTER 2

Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn's disease

Damen GM [1]
Hol J [1]
de Ruiter L [1]
Bouquet J [1]
Sinaasappel M [1]
van der Woude J [2]
Laman JD [3]
Hop WCJ [4]
Büller HA [1]
Escher JC [1]
Nieuwenhuis EES [1]

[1] Dept. of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC – Sophia Children's Hospital, University Medical Center, Rotterdam
[2] Dept. of Gastroenterology, Erasmus University Medical Center
[3] Dept. of Immunology, Erasmus University Medical Center
[4] Dept. of Epidemiology and Biostatistics, Erasmus University Medical Center

J Ped Gastroenterol Nutr 2006;42:142-9

Abstract

Objectives

Inflammatory Bowel Diseases (IBD) represent an aberrant immune response by the mucosal immune system to luminal bacteria. Since the oral mucosa harbors the first epithelial cells that interact with microorganisms, we assessed the immunological activity of buccal epithelium in children with IBD and adults with Crohn's disease.

Methods

Buccal epithelial cells were obtained from 17 children and 14 adults with Crohn's disease, 18 children with ulcerative colitis, and 40 controls. Cells were cultured with and without microbial stimulation. Chemokine levels were determined in culture-supernatants by cytometric bead array and ELISA. CXCL-8 production was studied by immunohistochemical analysis of these cells. CXCL-8 production by lipopolysaccharide stimulated monocyte-derived dendritic cells from these patients was determined.

Results

Compared to controls, pediatric ulcerative colitis patients, and adult Crohn's disease patients, only in children with Crohn's disease buccal epithelial cells exhibited enhanced production of CXCL-8, CXCL-9, and CXCL-10. *In vitro* stimulation with lipopolysaccharide or zymosan resulted in a further increase of chemokine-levels only in cells from pediatric Crohn's disease patients. CXCL-8 production by stimulated monocyte-derived dendritic cells from children with Crohn's disease was equal to that of children with ulcerative colitis.

Conclusions

Buccal epithelium of children with Crohn's disease is immunologically active, even in the absence of oral lesions. The enhanced chemokine production is associated with pediatric Crohn's disease and seems restricted to cells derived from the epithelial barrier. Assessment of chemokine production by buccal epithelial cells may become a new, rapid, non-invasive test for screening and classification of IBD in children.

Introduction

Crohn's disease (CD), ulcerative colitis (UC) and indeterminate colitis represent diseases of chronic intestinal inflammation, also called inflammatory bowel diseases (IBD). Genetic susceptibility, environmental triggers and immune dysregulation have been described as the main factors involved in the establishment and development of IBD.[1] The aberrant response of the mucosal immune system associated with IBD is thought to be directed towards microorganisms that are present within the intestinal lumen.[2-3] This hypothesis is supported by the evidence of a mutation of the bacterial sensing gene NOD2 being strongly associated with susceptibility to CD.[4,5]

Intestinal epithelial cells can play a role in initiating and regulating mucosal innate and acquired immune responses through the secretion of cytokines and chemokines. In IBD patients, epithelial cells derived from colonic specimens are able to produce significant amounts of chemokines including CXCL-1 (Groα), -2 (Groβ), -5 (ENA-78), -8 (IL-8), and CCL-2 (MCP-1), -3 (MIP-1-α), -4 (MIP-1-β), -7 (MCP-3), -8 (MCP-2).[6-15] These studies indicate that intestinal epithelial cells are an important source of chemokines that play a role in the recruitment of neutrophils and T lymphocytes to the epithelial layer, which may initiate and/or promote intestinal inflammation in IBD.

CD can be localized throughout the entire digestive tract. In up to 40% of children with CD, biopsies from the upper gastrointestinal tract may reveal granulomas, even in mucosa that appears normal on endoscopy.[16-17] Accordingly, we speculate that even in the absence of oral lesions buccal epithelial cells from children with CD may display pro-inflammatory immune responses.

We studied the chemokine production by buccal epithelial cells in pediatric IBD patients, in adult CD, as well as in healthy controls. CXCL-8 (i.e. interleukin-8) production was assessed as it represents the most commonly produced chemokine by the epithelial cells. The production of related chemokines, such as CXCL-9 (i.e. monokine induced by interferon-gamma [Mig]) and CXCL-10 (interferon-inducible protein-10 [IP-10]) was also determined. In addition CCL-2 (monocyte chemoattractant protein-1 [MCP-1]) and CCL-5 (regulated upon activation, normal T cell expressed, and secreted [Rantes]) were determined as several studies have indicated enhanced production of these molecules in colonic biopsies from IBD patients. Next to the evaluation of spontaneous production of these chemokines we also established an *in vitro* assay to determine whether these molecules could be induced by microbial stimuli. Finally, we determined the response of monocyte-derived dendritic cells (moDCs) to lipopolysaccharide (LPS). MoDCs were included as they represent a non-epithelial cell-type that is involved in mucosal microbial-host interactions.[3, 18, 19]

Materials and methods

Patient characteristics

All children with (suspected) IBD that were admitted to the clinic or outpatient clinic of the department of Pediatric Gastroenterology at the Sophia Childrens Hospital from September 2003 to September 2004 were included. The included adult CD patients visited the clinic or outpatient clinic of the department of Gastroenterology at the ErasmusMC in September 2003. The study was approved by the Medical Ethical Committee of the Erasmus MC, and the Central Committee on Research Involving Human Subjects.

In children with UC, disease activity was assessed by the modified Truelove and Witts score.[20] This score is based on clinical symptoms (number of stools a day and amount of bloodloss in the stools), laboratory parameters (hemoglobin levels and erythrocyte sedimentation rate [ESR]), and physical examination (axillary temperature and pulse rate). On a scale of 6-18 points, a score of 6 indicates inactive disease, 7-10 mild disease, 11-14 moderate disease, and 15-18 severe disease. In children with CD, disease activity was expressed by means of the Paediatric Crohn's Disease Activity Index (PCDAI).[21] The PCDAI is a disease activity index based on symptoms, laboratory parameters (haematocrit, ESR, and albumin), and physical examination, including changes in linear growth. On a scale of 0-100 points, a score <15 indicates inactive disease, 15-30 mild disease, and >30 moderate to severe disease.

Buccal epithelium

Buccal epithelial cells were collected by gently rubbing a Cytobrush® Plus (Medscand Medical AB, Sweden) over the inside of the cheeks. Cells were washed twice in RPMI 1640 (Invitrogen, Merelbeke, Belgium). In a 96-wells flat bottom plate 3,5 x 10^4 cells per well were incubated in 200 µl medium RPMI 1640 supplemented with 10% fetal calf serum (FCS; Integro, Leuvenheim, the Netherlands), hepes 15 mM, L-glutamine 2 mM, penicilline 100 U/ml, streptomycin 100 µg/ml, amphotericin B 500 µg/ml and mercaptopurine 50 µM. When more than 10,5 x 10^4 cells were obtained lipopolysaccharide (from *E. Coli* serotype 005:B5, Sigma-Aldrich, Zwijndrecht, the Netherlands) and/or zymosan A (from *Saccharomyces cerevisiae*, Sigma-Aldrich) were added at different concentrations (as indicated), starting from February 2004. Buccal epithelial cells do not proliferate *in vitro* and have limited viability. In a pilot experiment CXCL-8 was produced already within the first hours of incubation, and reached a plateau at 8 to 12 hours. This is in agreement with another publication concerning the production of CXCL-8 by buccal epithelial cells upon microbial stimulation[10]. Therefore, we decided to collect (and store at minus 80°C) the supernatants of the cell-cultures at 24 h of incubation. The remaining cells were washed thrice with phosphate-buffered saline (PBS) and suspended in 50 µl PBS and 50 µl 10% human serum albumin (HSA). Cytospins were prepared by centrifugation (50g, 7 min) of the cell suspension. Cytospins were air-dried on silicagel overnight and either used directly or stored at minus 20°C.

Monocyte-derived dendritic cells (MoDCs)

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation of anti-coagulated human blood from IBD patients using Lymphoprep™ (Nycomed, Oslo, Norway). $CD14^+$ cells were isolated by applying MACS CD14 MicroBeads and the Magnetic cell separator MidiMACS (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified cells were typically >95% $CD14^+$ as determined by flow cytometry. To obtain MoDCs [22], $CD14^+$ cells (5×10^5 cells/well) were cultured in RPMI 1640 with L-glutamine (Invitrogen), 10% FCS (Integro), gentamycin 56 mg/ml, recombinant human GM-CSF (molgramostim) at 500 U/ml (Novartis, Arnhem, the Netherlands) and recombinant human IL-4 at 250 U/ml (Pepro Tech, Rocky Hill, USA). At day 6 LPS was added to a subset of wells, at indicated concentrations. Supernatants were collected at day 7.

Measurement of chemokines in the cell culture supernatants

CXCL-8 levels in the cell culture supernatants were determined by ELISA according the manufacturers protocol (BD Biosciences, San Diego, USA). CXCL-9 and -10, and CCL-2 and -5 were detected by application of the standard protocol of the Cytometric Bead Array (CBA) using the human chemokine-I kit specific for these chemokines (BD Biosciences).

Immunohistochemical staining of buccal epithelial cells for CXCL-8

For immunohistochemical detection of CXCL-8 the peroxidase-labeled avidin-biotin method was used. Buccal epithelial cells on the cytospins were fixed by immersion into fresh acetone containing 0.02% (vol/vol) H_2O_2. Slides were then air-dried for 10 min. Histochemical revelation of endogenous peroxidase activity was performed with 4-chloride-1-naphtol (4-Cl-1-napthol). A solution of 80 mg 4-Cl-1-napthol in 1 ml of ethanol 100% was added to 200 ml Tris-HCL buffer together with 6.2 µl 30% H_2O_2. This solution was filtered. Slides were immersed into this solution for 15 min at RT. Slides were washed with phosphate-buffered saline (PBS) 1x1 min and with PBS/Tween20 0.05% 1x10 min. Subsequently cells were incubated with the primary mouse antihuman CXCL-8 antibody (BD Biosciences) overnight at 4°C in a humidified atmosphere, or with the irrelevant isotype-match antibody (IgG2b; Dako, Glostrup, Denmark). The next morning, cells were incubated with the secondary biotinylated rabbit anti-mouse antibody (Dako) for 30 min at RT. Between incubation steps slides were washed twice with PBS/Tween20 0.05%. Subsequently cells were incubated with avidin-biotin compex labeled horseradish peroxidase-conjugated (ABC complex HRP; Dako) for 1 h at RT. Slides were washed twice in PBS. The peroxidase activity was revealed by incubation with 3-amino-9-ethyl-carbazole (AEC; Sigma-Aldrich) for 10 min at RT, leading to a bright red precipitate. Again slides were washed twice with PBS. Finally cells were counterstained using Mayer's haematoxylin (Merck) and embedded in glycerol gelatin. The same method was used for detection of CD45 (using a primary mouse anti-human CD45 antibody; BD Biosciences).

Statistical analysis

In order to obtain approximate normal distributions patient groups were compared using the t-test after logarithmic transformation. Outcomes less than the lower limit of detection were set at this limit, and the resulting value was analyzed as a left-censored observation using STATA software (proc CNREG). Paired data (LPS 0,01 versus LPS 0,1 mcg/ml) were compared using the Wilcoxon signed-rank test. Correlation coefficients given are Spearman's (r_s). P = 0,05 (two-sided) was considered the limit of significance.

Results

Patient characteristics and yield of buccal epithelial cells

September 2004 thirty-five children with IBD were included, as well as 14 adult CD patients and 40 controls (children and adults). Patient demographics and disease characteristics are shown in table 1. In IBD patients as well as in adult CD patients disease activity ranged from mild to severe.

Table 1: Patient characteristics and patient demographics

	Pediatric UC	Pediatric CD	Adult CD	Controls
Number	18	17	14	40
Male / female	10 / 8	10 / 7	4 / 10	18 / 22
Age at diagnosis (in years)	1.5–15 (median 11)	1.2–16 (median 10)	> 18 years	n.a.
Age at study entry (in years)	4–17 (median 13)	1.7–17 (median 12)	29-63 (median 42)	1-50 (median 17)
Newly diagnosed at study entry	6 / 18	6 / 17	0 / 14	n.a.
PCDAI for children	n.a.	7,5–55 (median 24)	n.a.	n.a.
CDAI for adults	n.a.	n.a.	47-325 (median 164)	n.a.
Modified Truelove-Witts score	7–16 (median 10)	n.a.	n.a.	n.a.

n.a. = not applicable

Six children with CD and 6 children with UC were newly diagnosed with IBD and had not received anti-inflammatory medication yet. None of the adult CD patients was newly diagnosed (they were all tertiary admitted). Treatment strategies of the IBD patients, children as well as adults, were very different. Five adolescents stopped their medication without consulting the pediatric gastro-enterologist. Other patients used 5-ASA, prednisone, azathioprine, methotrexate, anti-tumor necrosis factor, or a combination of these. Most of the controls (children and adults) were healthy. Others were admitted under the suspicion of IBD, but turned out not to have IBD.

The median yield of buccal epithelial cells for the pediatric CD patient was 30 x 10^4 cells per patient (ranging from 3.5 to 81 x 10^4 cells per patient). The median yield of buccal epithelial cells for the pediatric UC patient was comparable (24 x 10^4 cells per patient, ranging from 3.5 to 85.6 x

10^4 cells per patient). The yield of buccal epithelial cells for the adult CD patients and the controls was also within the same range.

CXCL-8 production by buccal epithelial cells is exclusively enhanced in children with Crohn's disease

Figure 1: CXCL-8 is exclusively enhanced in pediatric CD patients. CXCL-8 levels in the culture-supernatants of buccal epithelial cells of controls (adults and children), children with ulcerative colitis (ped UC), children with Crohn's disease (ped CD), and adults with Crohn's disease (adult CD). Samples were taken at 24 h of incubation. In children with CD, CXCL-8 production was significantly enhanced compared to children with UC ($p<0.001$) and controls ($p<0.001$). Adults with CD did not show enhanced CXCL-8 production.

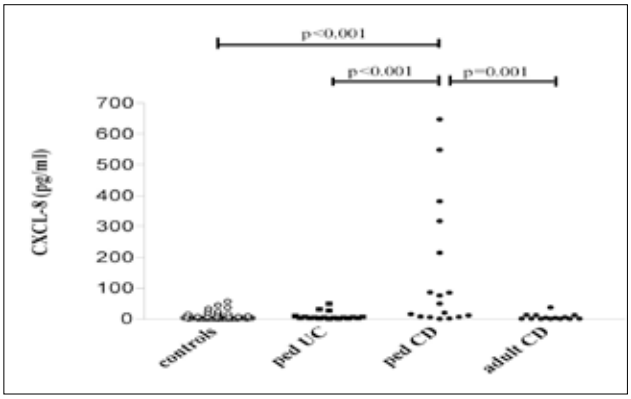


Figure 1 shows the levels of CXCL-8 in the culture supernatants of buccal epithelial cells. In children with CD, CXCL-8 production was significantly higher than in children with UC ($p=0.001$), or controls ($p<0.001$). Four out of 6 children with CD were newly diagnosed, had a moderate to severe disease activity and were not receiving medication yet. These four patients presented with a high CXCL-8 production (of more than 300 pg/ml). CXCL-8 production by buccal epithelial cells derived from adult CD patients was comparable to that of controls. In children with CD the CXCL-8 production by buccal epithelial cells was correlated with the erythrocyte sedimentation rate ($r_s=0.61$; $p=0.016$) as is shown in figure 2.

In these children, CXCL-8 production did not correlate with the C-reactive protein ($r_s=-0.13$; ns), the hemoglobin ($r_s=-0.37$; ns), the thrombocytes ($r_s=-0.06$; ns), the leukocytes ($r_s=-0.10$; ns), or the albumin ($r_s=-0.42$; ns) in the blood (data not shown). Finally, the CXCL-8 production was not related to clinical disease activity expressed as PCDAI ($r_s=0.33$; ns)(data not shown).

In order to conclusively identify the epithelial cell as the source for the CXCL-8, we performed immunohistochemical analysis of these cells. Figure 3 shows representative examples of CXCL-8 production by buccal epithelial cells. CXCL-8 was particularly detected at the periphery of the cells, and was located in granules. As shown, buccal epithelial cells were not contaminated with cells positive for the haematopoietic marker CD45 (i.e. monocytic cells or macrophages).

Figure 2: In children with CD, CXCL-8 production by buccal epithelium correlates with ESR. CXCL-8 levels in the culture-supernatant of buccal epithelial cells derived from children with CD correlate with the erythrocyte sedimentation rate in the blood of these patients (rs=0.61; p=0.016). The line represents least-squares regression line after logarithmic transformation of both axes.

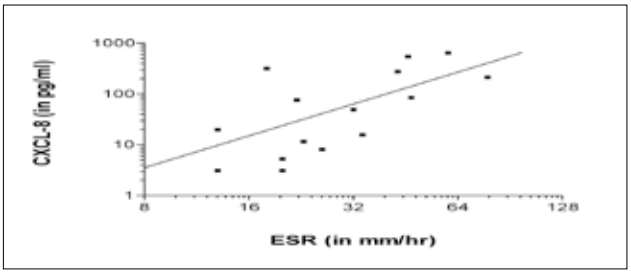
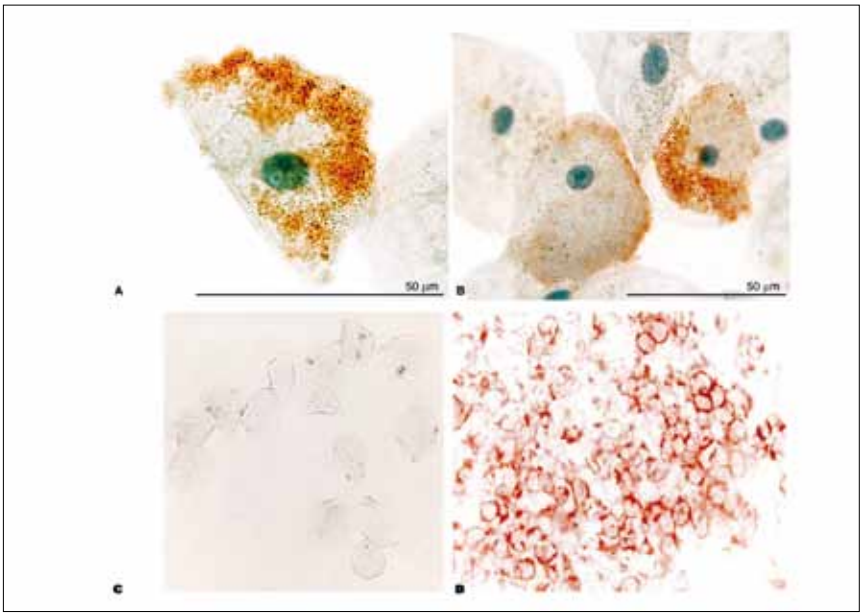


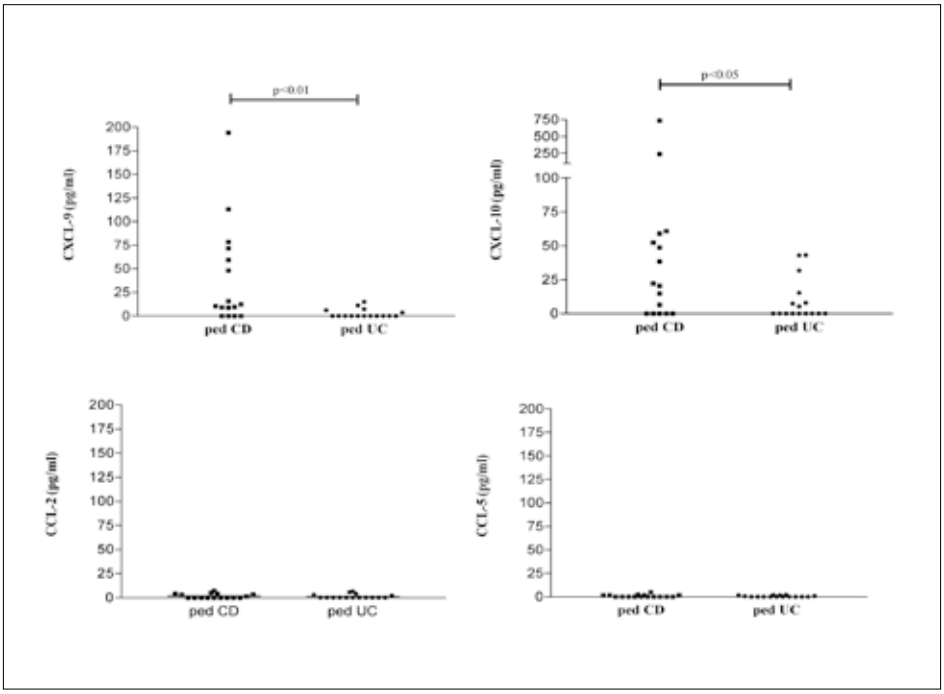
Figure 3: Representative examples of CXCL-8 production by buccal epithelial cells are shown. Immunohistochemical analysis for CXCL-8 was performed on cytospins of buccal epithelial cells. In the examples above (figure 3A and 3B) buccal epithelial cells of a child with CD were identified as the source for CXCL-8. The orange-red pigment indicates positive CXCL-8 staining. In contrast to bone marrow cells, positive for CD45 (i.e. the red staining, figure 3D), buccal epithelial cells were all negative for the haematopoietic marker CD45 (figure 3C) which excludes contamination with monocytic cells or macrophages.



Enhanced production of other chemokines by buccal epithelial cells from pediatric CD patients. Next to CXCL-8, the levels of CXCL-9, CXCL-10, CCL-2 and CCL-5 were determined in the culture-supernatant of the buccal epithelial cells at 24 hours of incubation by using cytometric bead array.

Figure 4 shows that in children with CD, CXCL-9 production was significantly higher compared to children with UC (p=0,008). In children with CD, CXCL-10 production was also higher compared to children with UC (p=0,024). The production of CCL-2 and CCL-5 were equally low in both patient groups.

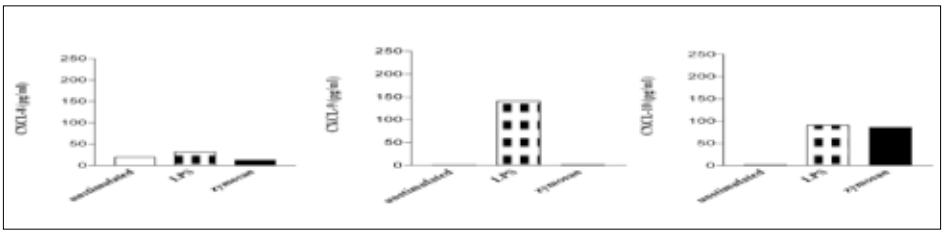
Figure 4: Levels of CXCL-9, CXCL-10, CCL-2 and CCL-5 in the culture-supernatants of buccal epithelial cells of children with Crohn's disease (ped CD) and ulcerative colitis (ped UC). Samples were taken at 24 h of incubation. CXCL-9 and CXCL-10 production was significantly enhanced in CD patients compared to UC patients ($p<0.01$ and <0.05 respectively). CCL-2 and CCL-5 levels were very low in both CD and UC patients.



Stimulation of buccal epithelial cells with LPS or zymosan

In order to determine whether the buccal epithelium of pediatric CD patients produced more CXCL-8 as a result of a lower threshold for microbial stimulation we next performed an *in vitro* stimulation assay. In this assay, buccal epithelial cells were cultured in the presence of LPS or zymosan at different concentrations. In 4 out of 10 children with CD the production of CXCL-8, CXCL-9 and/or CXCL-10 increased more than 50 pg/ml. Figure 5 shows a representative example of enhanced chemokine production upon microbial stimulation. No induction (either by LPS or zymosan) of chemokine production (CXCL-8, CXCL-9, CXCL-10, CCL-2 and/or CCL-5) was found in any of the buccal epithelial cells that were derived either from children with UC (n=10), or in cells from healthy controls (n=20).

Figure 5: Representavive example of inducible chemokine production by buccal epithelial cells from pediatric CD patients. Cells were stimulated with lipopolysaccharide (LPS) at 0.1 mcg/ml or zymosan at 100 mcg/ml. At 24h of incubation chemokine production was determined in the culture-supernatants.



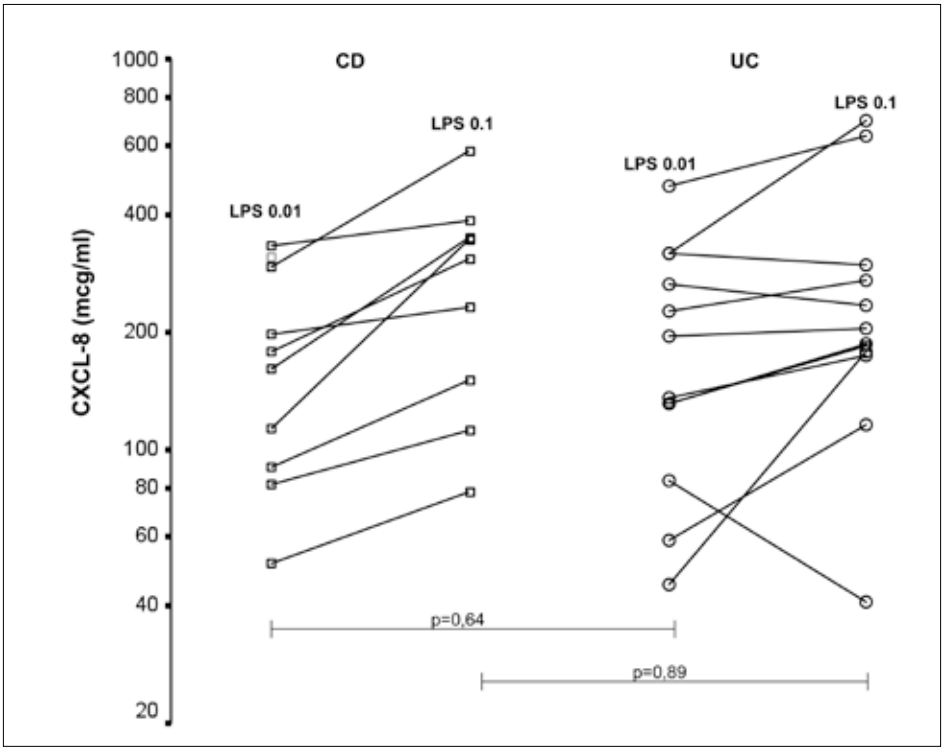
The response of monocyte-derived dendritic cells to lipopolysaccharide

Next we determined whether the enhanced chemokine production by buccal epithelium demonstrated in pediatric CD could be extended to other immunocompetent non-epithelial cells. As such, CXCL-8 production by moDCs in response to LPS was measured in pediatric IBD patients. MoDCs were derived from children with CD and UC and stimulated with different concentrations of LPS as described. As is illustrated in figure 6, CXCL-8 production in response to LPS 0.1 mcg/ml generally was higher than in response to LPS 0.01 mcg/ml (p=0.004 for CD; p=0.04 for UC). In response to LPS 0.01 mcg/ml moDCs of children with CD produced the same amounts of CXCL-8 as those of children with UC (p=0.64; ns). In response to LPS 0.1mcg/ml moDCs of children with CD also produced comparable amounts of CXCL-8 compared to that of children with UC (p=0.89; ns). In conclusion, in response to LPS, moDCs of children with CD did produce comparable amounts of CXCL-8 as did moDCs of children with UC.

Discussion

One hypothesis on the etiology of IBD is that these diseases represent an aberrant immune response by the mucosal immune system, to either pathogenic or resident luminal bacteria. According to this hypothesis, resident bacteria can persistently stimulate the mucosal and systemic immune system, thereby perpetuating the inflammatory response. Intestinal epithelial cells (IECs) and dendritic cells are among the first cells that are capable of sensing microbial signals through the expression of pattern recognition molecules such as the Toll-like receptors. Through the presentation of antigens and the production of chemokines and cytokines, IECs and DCs are involved in the initiation and regulation of the acquired immune response. Several studies have identified the IECs as a major source of chemokines that play an important role in chemotaxis, adhesion, activation and degranulation of migratory immune cells. In response to microorganisms or pro-inflammatory cytokines, IECs are capable of producing CXCL-1, -3 (GROγ), -5, -8, -9, -10, and -11 (I-TAC) as well as CCL2, -3, -4, and –5.[10, 23-28] In freshly obtained specimens

Figure 6: CXCL-8 production by stimulated monocyte-derived dendritic cells in children with CD is comparable to that of children with UC. Levels of CXCL-8 in the culture-supernatant of monocyte-derived DCs (moDCs) of children with CD (n=10) and UC (n=13) are compared. CXCL-8 production in response to LPS 0.1 mcg/ml generally was higher than in response to LPS 0.01 mcg/ml (p=0.004 for CD; p=0.04 for UC). In response to LPS 0.01 mcg/ml moDCs of children with CD produced the same amounts of CXCL-8 as those of children with UC (p=0.64; ns). In response to LPS 0.1mcg/ml moDCs of children with CD also produced comparable amounts of CXCL-8 compared to that of children with UC (p=0.89; ns).



from IBD patients, epithelial cells of the lower GI tract showed an increased expression of CXCL-1, -5, and –8, as well as CCL-2, -3, -4, -7 and -8.[6-15] These studies used in situ hybridisation with radiolabelled probes of the chemokine-genes, immunohistochemical analyses, and/or specific protocols for the isolation of intestinal epithelial cells. Importantly, other studies that made use of the same techniques failed to demonstrate such chemokine production by colonic epithelial cells in vivo. [29-32] Although buccal epithelial cells seem to be an obvious subject for experimentation as these cells are so readily available without the need for endoscopy or biopsies, to our knowledge this is the first functional study on these cells in IBD patients. In fact, chemokine production or expression by buccal epithelial cells in these patients has never been studied before.

Here we demonstrate that in children with CD, buccal epithelial cells produce significantly higher levels of CXCL-8, CXCL-9 and CXCL-10, in comparison to children with UC, to controls or to adults

with CD. Interestingly, not all newly diagnosed children with CD presented with an enhanced chemokine production by buccal epithelial cells. Also, no relation was found between the disease activity (pCDAI) or a specific drug that was used and chemokine production. These findings may suggest that the enhanced chemokine production is specifically associated with a subset of patients with a specific (genetic) ethiopathogenesis.

In children with CD, the production of CXCL-8 by buccal epithelial cells was correlated with the ESR in the blood. No such correlation was found in children with UC despite the fact that the range of ESR in these children was comparable to that of the children with CD. The correlation between ESR and CXCL-8 production in CD patients may represent a phenomenon that is associated only with pediatric CD, and not with pediatric UC. As an enhanced ESR is found in active pediatric CD as well as in active pediatric UC, CXCL-8 production by buccal epithelial cells seems the better test to discriminate between the two diseases.

Upon stimulation with LPS or zymosan, only buccal epithelial cells derived from pediatric CD patients show an inducible production of chemokines. Based on our preliminary data (11 out of 17 children with CD had an enhanced release of CXCL-8, CXCL-9 and/or CXCL–10 spontaneously, and 4 out of 10 children with CD had an increased release upon microbial stimulation), we estimate that over 72% of all children with CD will exhibit an enhanced chemokine production, spontaneous or upon microbial stimulation. These striking differences in response patterns by buccal epithelial cells can be explained by various mechanisms. Alterations in the local (oral) flora of pediatric CD patients may be associated with an enhanced chemokine production. The fact that chemokine production by microbial stimuli could only be induced in pediatric CD patients points to an enhanced ability of these cells to become stimulated. As we could not show the same differences in moDCs, this mechanism seems to be limited to epithelial cells of pediatric CD patients. The enhanced chemokine production by buccal epithelial cells from pediatric CD patients may be associated with mutations in the NOD2 molecule, such as described.[33] A recent paper by Watanabe et al[34] elucidated how signaling through mutated NOD2/CARD15 molecules may lead to disease by causing an excessive T_H1 response. The authors present a model whereby NOD2 senses muramyl dipeptide (a breakdown product of peptidoglycan) within the cell which leads to a blockade of TLR2 signaling upon activation by peptidoglycan at the cell surface. This may in fact represent a physiological mechanism through which the inflammatory response to gut flora is limited. Mutant NOD2 (in Crohn's disease) will not sense MDP and will be associated with unopposed TLR2 signaling, which leads to enhanced IL-12 production, one of the essential mediators of intestinal inflammation in IBD. Another approach may be that the expression of molecules such as TLR2 and 4 is specifically enhanced in the epithelial cells of pediatric CD patients as is suggested by various authors.[35-37] Finally, the results may also be explained by alterations in the expression of molecules such a TOLLIP. Recently it was reported that these types of molecules might contribute to a state of hypo-responsiveness of epithelial cells to microbial stimuli.[38-40]

Finally, we found a striking difference in epithelial chemokine-response patterns by comparing pediatric to adult IBD patients. None of the adult CD patients in this study were diagnosed in

childhood. The question whether pediatric CD patients will also exhibit this enhanced chemokine production into adulthood remains to be elucidated. Interestingly, recent reports suggest that, in comparison to adults, pediatric CD may represent a distinct disease that may be associated with an enhanced incidence of NOD2/CARD 15 mutations.[41,42]

A high production of CXCL-8 or CXCL-9 by the buccal epithelium, either spontaneously or upon microbial stimulation, increases the suspicion of CD in non-diagnosed children. In children with indeterminate colitis an enhanced production of these chemokines may contribute to further discrimination. Determining the chemokine production by these cells can be of great value in making the correct diagnosis, and in deciding on a specific treatment modality. Enhanced chemokine production by buccal epithelial cells may well provide us with the first soluble marker that is exclusively linked to pediatric CD, and may therefore become a new, rapid, and non-invasive test in children with (suspected) IBD.

References

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002;347: 417-29.
2. Sartor RB. Innate immunity in the pathogenesis and therapy of IBD. J Gastroenterol 2003;38 Suppl 15:43-7.
3. Nieuwenhuis EE, Blumberg RS. The role of the epithelial barrier in Inflammatory Bowel Disease. In: Blumberg RS, Neurath M, eds. Immune mechanisms in Inflammatory Bowel Disease. Georgetown USA, Landes 2004.
4. Hugot J-P, Chamaillard M, Zouali H, Lesage S, Cézard J-P, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel J-F, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599-603.
5. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr H, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuňez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603-6.
6. Mazzucchelli L, Hauser C, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. Am J Pathol 1994;144:997-1007.
7. Izutani R, Loh EY, Reinecker HC, et al. Increased expression of interleukin 8 mRNA in ulcerative colitis and Crohn's disease mucosa and epithelial cells. Inflammatory Bowel Disease 1995;1:37-47.
8. Reinecker HC, Loh EY, Ringler DJ, Mehta A, Rombeau JL, MacDermott RP. Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa. Gastroenterology 1995;108:40-50.
9. Z'Graggen K, Walz A, Mazzucchelli L, Strieter RM, Mueller C. The C-X-C chemokine ENA-78 is preferentially expressed in intestinal epithelium in inflammatory bowel disease. Gastroenterology 1997;113:808-16.

10. Keates S, Keates AC, Mizoguchi E, Bhan A, Kelly CP. Enterocytes are the primary source of the chemokine ENA-78 in normal colon and ulcerative colitis. Am J Physiol 1997;273:G75-82.
11. Wedemeyer J, Lorentz A, Goke M, Meier PN, Flemming P, Dahinden CA, Manns MP, Bischoff SC. Enhanced production of monocyte chemotactic protein 3 in inflammatory bowel disease mucosa. Gut 1999;44:629-35.
12. MacDermott RP. Chemokines in the inflammatory bowel diseases. J Clin Immunol 1999;19:266-72.
13. Imada A, Ina K, Shimada M, Yokoyama T, Yokoyama Y, Nishio Y, Yamaguchi T, Ando T, Kusugami K. Coordinate upregulation of interleukin-8 and growth-related gene product-alpha is present in the colonic mucosa of inflammatory bowel. Scand J Gastroenterol 2001;36:854-64.
14. Autschbach F, Giese T, Gassler N, Sido B, Heuschen G, Heuschen U, Zuna I, Schulz P, Weckauf H, Berger I, Otto HF, Meuer SC. Cytokine/chemokine messenger-RNA expression profiles in ulcerative colitis and Crohn's disease. Virchows Arch 2002;441:500-13.
15. Banks C, Bateman A, Payne R, Johnson P, Sheron N. Chemokine expression in IBD. Mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease. J Pathol 2003;199:28-35.
16. Abdullah BA, Gupta SK, Croffie JM, Pfefferkorn MD, Molleston JP, Corkins MR, Fitzgerald JF. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. J Ped Gastroenterol Nutr 2002;35:636-40.
17. Tobin JM, Sinha B, Ramani P, Saleh ARH, Murphy MS. Upper gastrointestinal mucosal disease in pediatric Crohn disease and ulcerative colitis: a blinded, controlled study. J Ped Gastroenterol Nutr 2001;32:443-8.
18. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361-7.
19. Blumberg RS, Strober W. Prospects for research in inflammatory bowel disease. Jama 2001;285:643-7.
20. Truelove SC, Witts LJ. Cortisone in ulcerative colitis. Final report on a therapeutic trial. BMJ 1955;2:1041–8.
21. Hyams JS, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, Griffiths AM, Katz AJ, Grand RJ, Boyle JT, et al. Development and validation of a pediatric Crohn's disease activity index. J Ped Gastroenterol Nutr 1991;12:439-47.
22. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179:1109-18.
23. Eckmann L, Jung HC, Schürer-Maly C, Panja A, Morzycka-Wroblewska E, Kagnoff MF. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. Gastroenterology 1993;105:1689-97.
24. Jung HC, Eckmann L, Yang S-K, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest 1995;95:55-65.

25. Yang S-K, Eckmann L, Panja A, Kagnoff MF. Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. Gastroenterology 1997;113:1214-23.
26. Kagnoff MF, Eckmann L. Epithelial cells as sensors for microbial infection. J Clin Invest 1997;100:6-10.
27. Dwinell MB, Lügering N, Eckmann L, Kagnoff MF. Regulated production of interferon-inducible T-cell chemoattractants by human intestinal epithelial cells. Gastroenterology 2001;120:49-59.
28. Marion R, Coëffier M, Gargala G, Ducrotté P, Déchelotte P. Glutamine and CXC chemokines IL-8, Mig, IP-10 and I-TAC in human intestinal epithelial cells. Clinical Nutrition 2004;23:579-85.
29. Grimm MC, Elsbury SK, Pavli P, Doe WF. Interleukin 8: cells of origin in inflammatory bowel disease. Gut 1996;38:90-8.
30. Daig R, Andus T, Aschenbrenner E, Falk W, Scholmerich J, Gross V. Increased interleukin 8 expression in the colon mucosa of patients with inflammatory bowel disease. Gut 1996;38:216-22.
30. Uguccioni M, Gionchetti P, Robbiani DF, Rizzello F, Peruzzo S, Campieri M, Baggiolini M. Increased expression of IP-10, IL-8, MCP-1, and MCP-3 in ulcerative colitis. Am J Pathol 1999;155:331-6.
32. Daig R, Rogler G, Aschenbrenner E, Vogl D, Falk W, Gross V, Scholmerich J, Andus T. Human intestinal epithelial cells secrete interleukin-1 receptor antagonist and interleukin-8 but not interleukin-1 or interleukin-6. Gut 2000;46:350-8.
33. Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 2003;3:371-82.
34. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004;5:800-8.
35. Abreu MT, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. J Immunol 2001;167:1609-17.
36. Abreu MT, Arnold ET, Thomas LS, Gonsky R, Zhou Y, Hu B, Arditi M. TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. J Biol Chem 2002;277:20431-7.
37. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000;68:7010-7.
38. Zhang G, Ghosh S. Negative regulation of toll-like receptor-mediated signaling by Tollip. J Biol Chem 2002;277:7059-65.
39. Melmed G, Thomas LS, Lee N, Tesfay SY, Lukasek K, Michelsen KS, Zhou Y, Hu B, Arditi M, Abreu MT. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. J Immunol 2003;170:1406-15.
40. Otte J-M, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004;126:1054-70.
41. Sun L, Roesler J, Rösen-Wolff A, Winkler U, Koch R, Thürigen A, Henker J. CARD15 genotype and phenotype analysis in 55 pediatric patients with Crohn disease from Saxony, Germany. J Ped Gastroenterol Nutr 2003;37:492-7.
42. Heyman MB, Kirschner Heyman, Gold BD, Ferry G, Baldassano R, Cohen SA, Winter HS, Fain P, King C, Smith T, El-Serag HB. Children with early-onset inflammatory bowel disease (IBD): Analysis of a pediatric IBD consortium registry. J Pediatr 2005;146:35-40.

CHAPTER 3

Defective acute inflammation in Crohn's disease

Van Lierop PPE
Damen GM
Escher JC
Samsom JN
Nieuwenhuis EES

Dept. of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC - Sophia Children's Hospital, University Medical Center, Rotterdam

Lancet 2006;368:578

Daniel Marks and colleagues suggest that patients with Crohn's disease have a general defect in the innate mucosal immune response, including diminished interleukin 8 production.[1] We are not convinced. Various studies show an increased number of neutrophils-probably associated with the respective induction of specific chemokines, such as interleukin 8-rather than a diminished neutrophilic influx.[2] Moreover, intestinal epithelial cells are an important source of interleukin 8, in ulcerative colitis as well as in Crohn's disease.[3] Taken together, these data indicate a limitation to the general constitutional abnormality in Crohn's disease as postulated by Marks and colleagues.

By contrast with Marks and colleagues' findings, we have shown increased release of interleukin 8 (on stimulation and at basal levels) by buccal epithelial cells derived from children with Crohn's disease when compared with healthy controls and children with ulcerative colitis.[4] Furthermore, monocyte-derived dendritic cells from our children with inflammatory bowel disease did not show this striking difference in chemokine production. Whether epithelial cells represent a cell type with a particular responsiveness that might not be seen in myeloid cells such as macrophages remains to be clarified. Finally, childhood Crohn's disease might represent a specific disease entity. Indeed, reports suggest that, in comparison with adults, children with the disease have a higher number of associated mutations, different kinetics, and differences in location of inflammation and responsiveness to immunosuppression.[5]

Therefore, we suggest that the differences in immune responses found by Marks and colleagues do not necessarily represent a general mucosal paradigm for Crohn's disease. We argue that their results cannot be extrapolated to other involved immune cells or to specific patients such as children with Crohn's disease.

We declare that we have no conflict of interest.

P.P.E. van Lierop, G.M. Damen, J.C. Escher, J.N. Samsom,*E.E.S. Nieuwenhuis

e.nieuwenhuis@erasmusmc.nl

Department of Paediatric Gastroenterology and Laboratory of Paediatrics, Erasmus MC, Sophia Children's Hospital, University Medical Center, 3015 GE Rotterdam, Netherlands

1. Marks DJ, Harbord MW, MacAllister R, et al. Defective acute inflammation in Crohn's disease: a clinical investigation. Lancet 2006;367: 668–78.
2. Foell D, Kucharzik T, Kraft M, et al. Neutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease. Gut 2003; 52: 847–53.
3. Nieuwenhuis EES, Blumberg RS. The role of the epithelial barrier in inflammatory bowel disease. Adv Exp Med Biol 2006; 579: 108–16.
4. Damen GM, Hol J, de Ruiter L, et al. Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn disease. J Ped Gastroenterol Nutr 2006; 42: 142–49.
5. Heyman MB, Kirschner BS, Gold BD, et al. Children with early-onset inflammatory bowel disease (IBD): analysis of a pediatric IBD consortium registry. J Pediatr 2005; 146: 35–40.

CHAPTER 4

NOD-2 polymorphisms are not associated with altered buccal epithelial responsiveness in pediatric Crohn's disease patients

Damen GM [1,2]
Van Lierop PPE [1]
De Ruiter L [1]
Borm ME [3]
Samsom JN [1]
Escher JC [1]
Nieuwenhuis EES [1]

[1] Dept. of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC - Sophia Children's Hospital, University Medical Center, Rotterdam
[2] Dept. of Pediatric Gastroenterology, Radboud University Nijmegen Medical Center
[3] Dept. of Molecular Cell Biology and Immunology, Free University Medical Centre, Amsterdam

Submitted

Abstract

Objectives

Previously we demonstrated that buccal epithelium displays an enhanced chemokine production exclusively in children with Crohn's disease (CD). In this study, responsiveness of buccal epithelial cells (BECs) upon microbial stimulation is studied in children with CD and ulcerative colitis (UC) into more detail. Furthermore, we address whether an aberrant epithelial response pattern is associated with Nod2 polymorphisms.

Material and Methods

BECs and blood samples were taken in children with CD (n=27) and UC (n=24). BECs were incubated in medium and stimulated with the TLR ligands lipopolysaccharide, zymosan and pam3cys. After overnight incubation, supernatants of the cell cultures were collected, and in the supernatants CXCL-8 and CXCL-9 were determined by Cytometric Bead Array. Nod2 polymorphisms Arg702Trp, Gly908Arg and Leu1007fsinsC were determined in the blood samples.

Results

In children with CD, BECs have a significant increase in CXCL-8 production in response to LPS (p=0.003) and in response to zymosan (p=0.012). In response to pam3cys, there is no significant increase in production of CXCL-8. In children with UC, the production of CXCL-8 is not significantly enhanced in response to either of the TLR ligands. The same stands for the production of CXCL-9. Two CD patients are homozygote for the Arg702Trp mutation and several CD and UC patients are heterozygote for one of the three mutations. There is no correlation between the enhanced chemokine production of BECs (unstimulated or upon microbial stimulation) and specific Nod2 mutations. Of the two patients homozygote for the Arg702Trp mutation, BECs demonstrate a moderate CXCL-8 production, while the levels of CXCL-9 and CXCL-10 are below the lower limit of detection. Upon microbial stimulation, BECs do not demonstrate an enhancement chemokine production in these two patients.

Conclusions

In response to LPS and zymosan, the production of the chemokines CXCL-8 and -9 by BECs increased significantly only in children with CD. However, this aberrant epithelial response pattern was not associated with Nod2 polymorphisms.

Introduction

Nucleotide-binding oligomerization domain 2 (Nod2) is an intracellular pattern-recognition molecule that is implicated in the detection of bacterial peptidoglycan.[1,2] Microbial recognition induces proinflammatory signaling pathways such as nuclear factor kB (NfkB) and mitogen-activated protein kinases (MAPKs). Nod-proteins participate in a complex system that involves various other classes of microbial pattern recognition molecules e.g. Toll-like receptors (TLRs). To date, the mechanisms involved in the cross-talk between Nod and TLR pathways are not completely elucidated but may include synergy as well as counterregulation.[3-5]

Previously we demonstrated that buccal epithelium displays an enhanced chemokine production exclusively in children with Crohn's disease.[6] In this study, responsiveness of buccal epithelial cells upon microbial stimulation is studied in children with inflammatory bowel disease into more detail. Furthermore, we address whether an aberrant epithelial response pattern is associated with Nod2 polymorphisms.

Patients and methods

In 27 children with Crohn's disease (CD) and 24 children with ulcerative colitis (UC) buccal epithelial cells (BECs) were obtained and blood samples were taken. The patients visited the outpatient clinic or clinic of our hospital. Patient characteristics are depicted in table 1.

Table 1: Patient characteristics and patient demographics

	CD	UC
Number (male)	27 (15)	24 (12)
Age at diagnosis, median (range)	12.9 yrs (2.2 – 16.9)	11.0 yrs (3.1 – 15.7)
Age at study entry, median (range)	12.9 yrs (4.3 – 16.9)	13.5 yrs (3.1 – 18.1)
Newly diagnosed at study entry	6	4
PCDAI (CD; median and range) Leigtiger score (UC; median, range)	20 (range 0 – 60)	 3 (range 1 – 15)
Disease localization (CD / UC)	only upper GI n=1 (4%) only ileum n=3 (11%) only colon n=7 (26%) ileocolon n=16 (59%)	extensive n=20 (83%) left-sided n=4 (17%)
Medication	5-ASA n=2 (7%) Steroids n=6 (22%) Azathioprine n=11 (41%) Anti-TNF n=0	5-ASA n=16 (67%) Steroids n=4 (17%) Azathioprine n=6 (25%) Anti-TNF n=1 (4%)

Patient data were collected including gender, age at diagnosis, disease localization, disease activity, disease severity, type of IBD, use of medication and clinical characteristics such as fistulas. Several blood parameters were determined such as hemoglobulin, ESR, CRP and serum albumin. BECs were collected as we have described previously.[6] In a 96-well flat bottom plate, 35.000 cells per well were incubated in 200 µl medium. These cells were stimulated with different concentrations of lipopolysaccharide (*Escherichia coli,* serotype 005:B5, Sigma-Aldrich, Zwijndrecht, Netherlands), zymosan (*Saccharomyces cerevisiae*, Sigma-Aldrich) and/or synthetic pam3cys (EMC Microcollections, Tübingen, Germany). After overnight incubation the supernatants of the cell cultures were collected and stored at -80^{0}C. In the cell culture supernatants, the levels of CXCL-8 and CXCL-9 were determined by Cytometric Bead Array using the human chemokine-I kit specific for these chemokines (BD Biosciences). Blood samples were collected in order to determine three well-known Nod-2 mutations, i.e. Arg702Trp, Gly908Arg and Leu1007fsinsC. Responsiveness of the buccal epithelium upon microbial stimulation was studied. Enhanced responsiveness of the buccal epithelium was correlated with disease characteristics (such as type of IBD and early versus late onset IBD) and Nod2 polymorphisms.

Results

In children with CD, BECs have a significant increase in CXCL-8 production in response to LPS (p=0.003) and in response to zymosan (p=0.012). In response to pam3cys, there is no significant increase in the production of CXCL-8. In children with UC, the production of CXCL-8 is not significantly enhanced in response to either LPS, zymosan or pam3cys. The same stands for the production of CXCL-9.

Table 2 demonstrates the frequency of the Nod-2 mutations Arg702Trp, Gly908Arg and Leu1007fsinsC within the study population. Only 2 patients are homozygote for the Arg702Trp mutation, both patients are diagnosed with CD. Several CD and UC patients are heterozygote for one of the three mutations Arg702Trp, Gly908Arg and Leu1007fsinsC. The enhanced production of chemokines by BECs is studied in relation to these Nod2-polymorphisms. However, the enhanced chemokine production by BECs (unstimulated and upon microbial stimulation) does not

Table 2: Results of genetic investigations in children with CD (n=27) and UC (n=24)

	Arg702Trp		Gly908Arg		Leu1007frsinsC	
	Het.	Hom.	Het.	Hom.	Het.	Hom.
CD	7%	7%	7%	0	15%	0
UC	4%	0	13%	0	4%	0

Het. = heterozygote, Hom. = homozygote

correlate with any of the Nod-2 mutations. Of the two patients homozygote for the Arg702Trp mutation, BECs demonstrate a moderate production of the chemokine CXCL-8 (75 and 208 pg/ml respectively), while the level of CXCL-9 is below the lower limit of detection. Upon microbial stimulation, these BECs don't demonstrate an enhancement in chemokine production.

Discussion

In this study we demonstrate that, in response to LPS and zymosan, the production of the chemokines CXCL-8 and -9 by BECs increased significantly in children with CD. This increase was not observed in children with UC. However, there was no correlation between this aberrant epithelial response pattern and Nod2 polymorphisms.

In a previous study we hypothesized that there could be a correlation between the enhanced chemokine production by BECs in children with CD and Nod2 polymorphisms.[6] Mutant Nod2 will not sense MDP properly and might be associated with unopposed TLR2 signaling, leading to enhanced IL-12 production.[7] However, we still do not understand how specific Nod2-mediated functions contribute to optimal intestinal immune homeostasis and how deregulation contribute to the increased propensity to develop CD.[8] Loss-of-function mutations in Nod2 may result in altered host–microbial interactions through various mechanisms, including altered colonization of mucosal surfaces (resulting from decreased production of α-defensins by epithelial cells), impaired clearance of oral pathogens and through altered tolerance to chronic bacterial stimulation.[9] As the intestine is constantly exposed to microbes, it is important that chronic activation of the Nod2 signaling pathway leads to hyporesponsiveness of cells to subsequent stimulation with Nod and TLR ligands.[10,11] In individuals with disease-associated Nod2 mutations, however, the tolerizing effects of chronic stimulation by commensal bacteria were shown to be impaired.[10,11]

In our study, in patients with CD, BECs were enhanced responsive to TLR2 agonist zymosan and TLR4 agonist LPS, but not to TLR2 agonist pam3cys. Pam3Cys-Ser-(Lys)4 hydrochloride is a cell-permeable, water-soluble, synthetic, cationic lipohexapeptide analog of the immunologically active N-terminal portion of bacterial lipoprotein that potently activates monocytes and macrophages, promoting the translocation of NF-κB, enhancing tyrosine protein phosphorylation and activating ERK1/2 and MEK1/2. It is unclear why BECs were responsive to zymosan and not to pam3cys. Perhaps this was due to technical reasons; *in vitro*, pam3cys was soluble only at a high temperature with a surplus of medium. Another explanation could be the necessary presence of cofactors, microbes or other TLR agonists, in order to have an *in vitro* response of BECs to pam3cys.

When the enhanced chemokine production by BECs in children with CD is not associated with Nod2 polymorphisms, we have to look for other explanations. These explanations might include an enhanced expression of molecules such as TLR2 and 4 in epithelial cells of children with CD[12-14] or an alteration in the expression of regulatory molecules such as TOLLIP.[15-17] Also

other explanations could be possible such as alteration in the local oral flora in patients with CD compared with UC and the presence of primary defects in the NF-κB signaling in buccal epithelial cells disrupting immune homeostasis.[18]

In conclusion, we demonstrate that, in response to LPS and zymosan, production of the chemokines CXCL-8 and -9 by BECs increased significantly only in children with CD. This aberrant epithelial response pattern, however, was not associated with Nod2 polymorphisms.

References

1. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nuñez G. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003;278:5509-12.
2. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003;278:8869-72.
3. Abraham C, Cho JH. Functional Consequences of *NOD2 (CARD15)* Mutations. Inflamm Bowel Dis 2006;12:641-50.
4. Van Lierop PPE, Samsom JN, Escher JC, Nieuwenhuis EES. Role of the Innate Immune System in the Pathogenesis of Inflammatory Bowel Disease. J Ped Gastroenterol Nutr 2009;48:142-51.
5. Rescigno M, Nieuwenhuis EES. The role of altered microbial signaling via mutant NODs in intestinal inflammation. Curr Opin Gastroenterol 2007;23:21-6.
6. Damen GM, Hol J, de Ruiter L, Bouquet J, Sinaasappel M, van der Woude J, Laman JD, Hop WC, Buller HA, Escher JC, Nieuwenhuis EE. Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn disease. J Ped Gastroenterol Nutr 2006;42:142-9.
7. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004;5:800-8.
8. Cho JH, Abraham C. Inflammatory Bowel Disease Genetics: Nod2. Annu Rev Med 2007;58:401-16.
9. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nature Reviews Immunology 2008;8:458-66.
10. Hedl M, Li J, Cho JH, Abraham C. Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc Natl Acad Sci USA 2007;104:19440–5.
11. Watanabe T, Asano N, Murray PJ, Ozato K, Tailor P, Fuss IJ, Kitani A, Strober W. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J Clin Invest 2008;118:545–9.
12. Abreu MT, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. J Immunol 2001;167:1609-17.
13. Abreu MT, Arnold ET, Thomas LS, Gonsky R, Zhou Y, Hu B, Arditi M. TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. J Biol Chem 2002;277:20431-7.

14. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000;68:7010-7.
15. Zhang G, Ghosh S. Negative regulation of toll-like receptor-mediated signaling by Tollip. J Biol Chem 2002;277:7059-65.
16. Melmed G, Thomas LS, Lee N, Tesfay SY, Lukasek K, Michelsen KS, Zhou Y, Hu B, Arditi M, Abreu MT. Human intestinal epithelial cells are broadly unresponsive to Toll-like receptor 2-dependent bacterial ligands: implications for host-microbial interactions in the gut. J Immunol 2003;170:1406-15.
17. Otte J-M, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004;126:1054-70.
18. Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, Huth M, Nikolaev A, Neufert C, Madison B, Gumucio D, Neurath MF, Pasparakis M. Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature 2007;446:557-61.

CHAPTER 5

Production of IL-12p70 and IL-23 by monocyte-derived dendritic cells in children with inflammatory bowel disease

Damen GM [1,2]
van Lierop PPE [1]
de Ruiter L [1]
Escher JC [1]
Donders R [3]
Samsom JN [1]
Nieuwenhuis EES [1]

[1] Dept. of Pediatric Gastroenterology and Laboratory of Pediatrics, Erasmus MC-Sophia Children's Hospital, University Medical Center, Rotterdam
[2] Dept. of Pediatric Gastroenterology, Radboud University Nijmegen Medical Center
[3] Dept. of Epidemiology, Biostatistics, and HTPA, EPIB 133, Radboud University Nijmegen Medical Center

Gut 2008;57:1480

Quoted in two editorials:

1. Shale M, Ghosh S. Beyond TNF, Th1 and Th2 in inflammatory bowel disease. Gut 2008;57:1349-51.
2. Kugathasan S, Cohen S. Searching for New Clues in Inflammatory Bowel Disease: Tell Tales From Pediatric IBD Natural History Studies. Gastroenterology 2008;135:1038-41

Introduction

In the past, analysis of the mucosal T cell phenotype largely supported the concept of Crohn's disease (CD) as a T helper 1 (T_H1) disease, driven by interleukin-12 (IL-12) and characterized by the production of the signature cytokine interferon (IFN)-gamma, and ulcerative colitis (UC) as an atypical T_H2 disease characterized by production of cytokines such as IL-13.[1] Nowadays, we know that the cytokine milieu secreted in part by the dendritic cells skews the differentiation of naïve $CD4^+$ T cells not only in T_H1 and T_H2, but also in T_H17- or regulatory T-cell subsets.[2,3] The discovery of the T_H17 lineage, driven by IL-23 (as well as IL-6 and transforming growth factor beta) and characterized by IL-17 production has led to re-evaluation, and the realization that a number of inflammatory conditions in which T_H1 was previously considered central, may actually be T_H17 dependent.[2,3] Amongst these are several experimental models of colitis, and there is emerging evidence of CD as a T_H17 disease. To make it more complex, in humans a significant proportion of T_H17 cells also secrete IFN-gamma, thus displaying a mixed T_H17/T_H1 cytokine phenotype, particularly in the gut.[4] A number of observations regarding T_H17 cells challenge the concepts of terminal T cell lineage commitment, with the demonstration that regulatory T cells can differentiate into inflammatory T_H17 cells.[5] A number of ubiquitous molecules such as retinoic acid regulate the reciprocal relationship between T_H17 and regulatory T lymphocytes. IL-17 also regulates intestinal epithelial barrier function thorough the extracelluar signal-regulated (ERK)–mitogen-activated protein kinase (MAPK) pathway, and therefore antagonizing IL-17 may have potentially conflicting effects on intestinal inflammation.[6] While re-examining the concepts of the various T cell phenotypes in IBD, it has become apparent that our understanding of the complex cytokine networks regulating these cells is far from complete.

Overall, we are unable to determine the relative contribution of each cytokine to the initiation and perpetuation of inflammation in IBD.[2] We know that IL-12 (containing a p35 and a p40 subunit) and IL-23 (containing a p19 and a p40 subunit) are both produced in large quantities, along with other cytokines, by activated antigen-presenting cells (APCs), such as dendritic cells (DCs). *In vitro*, their secretion can be studied using artificial innate ligands which activate receptors such as Toll-like receptors (TLRs) and *Nod2*-receptors. It is evident that there are significant differences in the regulation of transcription of the component subunits (p19, p35 and p40), and indeed the kinetics and time-course of secretion differ, raising the possibility that they act differentially in acute and chronic inflammation.[7] In addition, the promiscuity of p40 is far from unique in the IL-12 family of cytokines; the recent demonstration of the combination of p35 with EBI-3 (itself a component of IL-27) to form the regulatory cytokine IL-35 illustrates how much more we have to learn in the field of cytokine regulation.[8] The activation status of the immune cells and the relative synergy between multiple TLR signals determine how different pathogen-associated molecular patterns (PAMPs) elicit different cytokine-driven T lymphocyte phenotypes. The ability of an APC to secrete a cytokine does not confirm a relevant biological role, as postsecretory effects are modified by the relative expression of the appropriate receptor on the target cell, and by the local presence

of other cytokines and regulatory T cells, which are capable of modifying T cell responses to cytokines.[2] Kugathasan et al. recently compared T cell clones derived from the colonic mucosa of pediatric patients with newly diagnosed CD with those from patients with established disease or infective colitis.[9] They were able to examine the relative secretion of T cell cytokines, and the susceptibility to modulation by APC-derived cytokines. Overall, they found significant differences between T cells from early and late disease. Early disease was characterized by a strongly polarized T_H1 response indistinguishable from that evoked in infective colitis, expressing high levels of the specific IL-12 receptor β2 subunit and IFN-gamma, and sensitivity to cytokine modulation. In contrast, clones from established disease produced more IL-4 and IL-10, and demonstrated relative insensitivity to the effects of modulation when cultured with cytokines. Of note, clones derived from early disease had the ability to upregulate markedly the secretion of the immunoregulatory cytokines IL-10 and IL-4 in response to the appropriate stimulus. This study raises a number of important questions regarding the role of cytokines in shaping the mucosal infiltrate in IBD, and the potential for disease modifying therapies. In particular, given the apparent insensitivity of T cells in established disease (at least to the cytokines used), it will be important to establish the additional mechanisms active in controlling the mucosal adaptive response, such as co-stimulatory molecules, microbial antigens and the effects of regulatory T cells.[10] Perhaps the most intriguing question is whether targeting the disease early (in immunological terms), when cytokines are still dominant players in shaping the immune response and establishing chronicity, might offer greater potential to modify the course of disease than intervening late with anticytokine therapy.[2] This concept is supported by clinical observations of greater efficacy with biological therapies when used in early IBD. Further studies looking at the earliest immunological events in IBD are needed to guide the rational selection of the most appropriate cytokine target, as are longitudinal studies of the balance of cytokines in the evolution of chronic IBD. One of the remaining questions is, what will determine the development of these typical effector T cells: the expression of specific T cell receptors or the production of differentiating cytokines by antigen presenting cells? In the Gut paper, we directly addressed whether the capacity of dendritic cells to produce IL-12 and/or IL-23 could be linked to a specific subset of pediatric IBD patients.

References

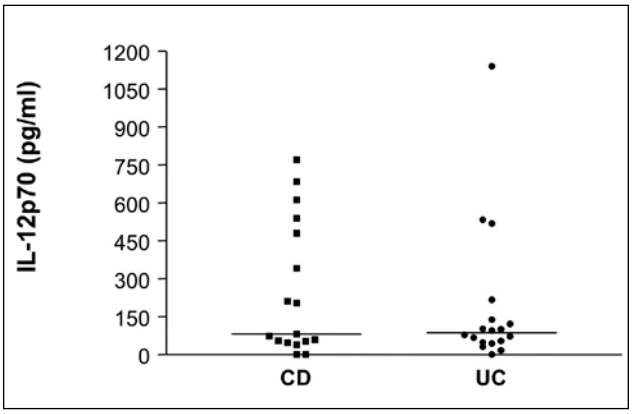
1. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003;3:521-33.
2. Shale M, Ghosh S. Beyond TNF, Th1 and Th2 in inflammatory bowel disease. Gut 2008;57:1349-51.
3. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nature Reviews Immunology 2008;8:458-66.
4. Annunziato F, Cosmi L, Santarlasci V, et al. Phenotypic and functional features of human Th17 cells. J Exp Med 2007;204:1849–61.
5. Xu LL, Kitani A, Fuss I, et al. Cutting edge: regulatory T cells induce CD4+CD25+Foxp3+ T cells or are selfinduced to become Th17 cells in the absence of exogenous TGF-b. J Immunol 2007;178:6725–9.
6. Kinugasa T, Sakaguchi T, Gu X, et al. Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology 2000;118:1001–11.
7. Goriely S, Neurath MF, Goldman M. How microorganisms tip the balance between interleukin-12 family members. Nat Rev Immunol 2008;8:81–6.
8. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007;450:566-9.
9. Kugathasan S, Saubermann LJ, Smith L, et al. Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. Gut 2007;56:1696-1705.
10. Huibregtse IL, van Lent AU, van Deventer SJ. Immunopathogenesis of IBD: insufficient suppressor function in the gut? Gut 2007;56:584–92.

Gut paper

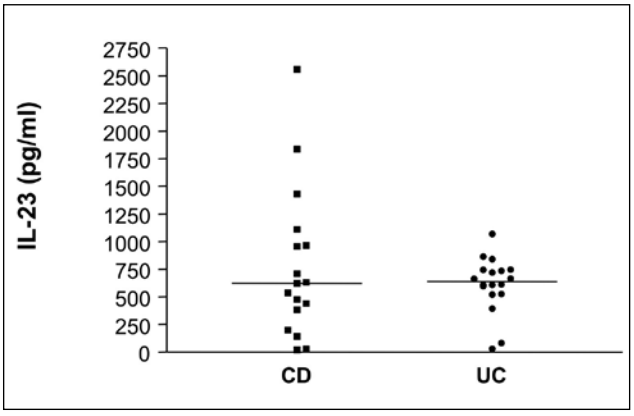
Inflammatory bowel disease (IBD) patients represent a heterogeneous group of patients that may need novel classification beyond just Crohns' disease (CD) and ulcerative colitis (UC). Notably, based on patient specifics such as genetics, disease location, immune responses and drug responsiveness, it seems likely that early-onset IBDs represents a specific disease entity.[1] Consequently, various disease-associated effector T cells have been identified, probably generated under the control of cytokines that are produced by antigen-presenting cells. In their recent publication, Kugathsan et al demonstrated that the level of IL12β2 (interleukin 12 receptor) expression by mucosal T cells may be a major determinant during the initial manifestations of CD for the development of the typically associated mucosal T helper 1 (T_H1) cytokine profile.[2] In this case, the presence of a specific T cell receptor correlated with the development of early CD. Similar mechanisms have been proposed for another recently discovered effector T cell subset. As such, T cell differentiation into IL-17-producing cells (T_H17) may, in part, depend on T cell expression of the IL-23 receptor (IL-23R).[3] Specifically, germline variations of IL-23R have been implicated in conferring protection to ileal CD.[4] The question now is, what will determine the development of these typical effector T cells; the expression of specific T cell receptors or the production of differentiating cytokines by antigen-presenting cells? In our studies we directly addressed whether the capacity of dendritic cells to produce IL-12 and/or IL-23 could be linked to a specific subset of paediatric IBD patients.

To this aim, we cultured monocyte-derived dendritic cells (moDCs) obtained from 17 children with CD (age 6.9–18 years, median 14.1 years) and 18 children with UC (age 3.2–17.2 years, median 13.3 years).[5] After harvesting, the moDCs ($2x10^5$ cells per well, 200 µl) were stimulated overnight with various microbial ligands at different concentrations: lipopolysaccharide (*Escherichia coli*, serotype 055:B5 L-4005, Sigma-Aldrich, Schnelldorf, Germany), zymosan (Sigma-Aldrich) and pam3cys (EMC Microcollections, Tubingen, Germany). Cell supernatants were collected, and the production of IL-12p70 and IL-23 was determined by means of Cytometric Bead Array (BD Biosciences, San Jose, California, USA) and ELISA (coating, affinity purified anti-human IL-23p19 (clone eBio473P19); detection, antihuman IL-12p40/70 (clone C8.6; eBioscience)), respectively. For statistical analyses, Pearson correlation coefficients and Spearman rank correlation coefficients were calculated both for the original values and for the logarithmic transformed values. Furthermore, analysis of variance (ANOVA) and linear mixed models with a unstructured correlation matrix for the residuals to account for dependencies (due to repeated measurements) were used.

Within the two patient groups, there was a high variability in IL-12p70 and/or IL-23 production upon microbial stimulation (fig 1A,B). The levels of either IL-12 or IL-23 could not be related to disease activity (expressed by the Paediatric Crohn's Disease Activity Index (PCDAI) or Lichtiger

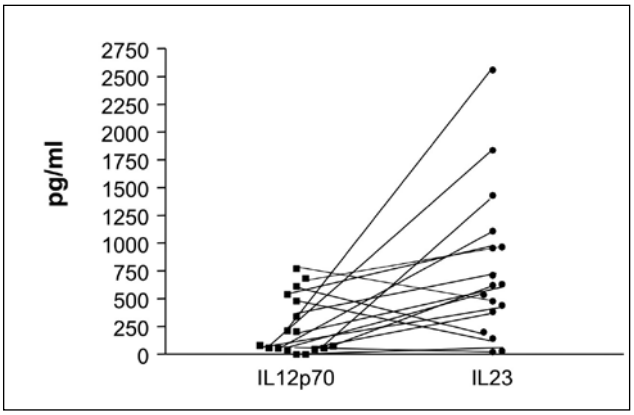


Legend to figure 1a:
High variability in the production of interleukin 12p70 (IL-12p70) by monocyte-derived dendritic cells (moDCs) from children with Crohn's disease (CD) vs ulcerative colitis (UC). moDCs were stimulated overnight with lipopolysaccharide (LPS) at 0.1 µg/ml.



Legend to figure 1b:
High variability in the production of IL-23 by moDCs obtained from children with CD vs UC. moDCs were stimulated with LPS 0.1 at µg/ml.

Colitis Activity Index (LCAI), respectively), disease location or medication. There was no difference in cytokine production by moDCs when we compared early with late IBD (for CD as well as for UC). There was a clear dose dependent relationship between the production of IL-12 or IL-23 and all the used microbial ligands. As such, we could subcategorize the patient moDCs into high, intermediate or low responders, irrespective of the used microbial ligand (data not shown). These data indicate that neither of the specific IBD subsets (CD or UC) may be associated with a ''hardwired'' predisposition for IL-12 or IL-23 production. We did not find any relationship between IL-12p70 and IL-23 levels (figure 2), confirming recent reports that the production of these two cytokine family members is differentially regulated. We conclude that the intrinsic capacity of moDCs to produce IL-12p70 and/or IL-23 is not associated with a specific subtype or stage of IBD. We therefore hypothesize that the presence of specific mucosal T effector cells that have been identified in CD or UC results from the local amount of particular microbial ligands and T cell expression of specific receptors such as IL-12β2 and IL-23R.



Legend to figure 2:

No relationship between the production of interleukin 12p70 (IL-12p70) and IL-23 by monocyte-derived dendritic cells (moDCs). moDCs were derived from children with Crohn's disease and stimulated with lipopolysaccharide at 0.1 µg/ml.

References

1. Nieuwenhuis EE, Escher JC. Early onset IBD: what's the difference? Dig Liver Dis 2008;40:12-5.
2. Kugathasan S, Saubermann LJ, Smith L, Kou D, Itoh J, Binion DG, Levine AD, Blumberg RS, Fiocchi C. Mucosal T-cell immunoregulation varies in early and late inflammatory bowel disease. Gut 2007;56:1696-1705.
3. McGovern D, Powrie F. The IL23 axis plays a key role in the pathogenesis of IBD. Gut 2007;56:1333-6.
4. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006;314:1461-3.
5. Damen GM, Hol J, de Ruiter L, Bouquet J, Sinaasappel M, van der Woude J, Laman JD, Hop WCJ, Büller HA, Escher JC, Nieuwenhuis EES. Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn's disease. J Ped Gastroenterol Nutr 2006;42:142-9.

CHAPTER 6

Niet-invasieve markers bij inflammatoire darmziekten op de kinderleeftijd

Damen GM
Nieuwenhuis EES

Afdeling Kindergeneeskunde, subafdeling Kinder MDL, Erasmus MC - Sophia Kinderziekenhuis, Rotterdam

Tijdschrift voor Kindergeneeskunde 2005;73:17-21

Samenvatting

Niet-invasief aanvullend onderzoek kan worden gebruikt indien het klinisch beeld niet geheel duidelijk is en wanneer men zich afvraagt of invasief onderzoek wel of niet is geïndiceerd. Het niet-invasieve onderzoek kan worden gebruikt om meer duidelijkheid te krijgen in het type inflammatoire darmziekte, en om de mate van ziekteactiviteit vast te stellen. Anemie en trombocytose zijn algemene indicatoren voor IBD. Een verhoogde bezinking wordt vooral gezien bij de ziekte van Crohn. ASCA- en p-ANCA-bepalingen kunnen uitkomst bieden voor het maken van onderscheid tussen de ziekte van Crohn en colitis ulcerosa. Het wel of niet aanwezig zijn van deze antistoffen lijkt in enige mate samen te hangen met de leeftijd van presentatie, de lokalisatie van de ziekte, het beloop en de effectiviteit van de ingestelde behandeling. Fecaal alfa-1-antitrypsine is vooral verhoogd bij de ziekte van Crohn, maar is niet duidelijk gerelateerd aan de ziekteactiviteit. Bepaling van fecaal calprotectine bij IBD lijkt meer betrouwbaar, maar bij onderzoeken met volwassenen bleek het fecaal calprotectine ook verhoogd te zijn bij een colorectaal carcinoom en bij een NSAID-geïnduceerde enteropathie. Het fecaal lactoferrine helpt niet voor het onderscheid tussen CD en UC, maar lijkt wel gerelateerd aan de ziekteactiviteit.

Summary

Non-invasive markers of inflammation in blood or stool samples may be of help in children suspected of inflammatory bowel disease (IBD). Positive results will support the need for more invasive investigations, while some markers are of value in determining the type of IBD, as well as disease activity. Anemia and thrombocytosis are general indicators of IBD. Elevated erythrocyte sedimentation rate is often found in active Crohn's disease. In IBD colitis, ASCA and p-ANCA can be used in order to categorize the disease as either Crohn's disease or ulcerative colitis. The presence of these antibodies seems to be related to age of presentation, localization of disease, results of treatment and outcome. Fecal alpha-1-antitrypsin is mainly increased in Crohn's disease, but is not clearly related to disease activity. Determination of fecal calprotectin in IBD seems to be more reliable, though in adults fecal calprotectin levels may be increased in colorectal carcinoma and NSAID-induced enteropathy. Fecal lactoferrin does not discriminate between Crohn's disease and ulcerative colitis, but is related to disease activity.

Inleiding

Indien bij een kind anamnese en bevindingen bij lichamelijk onderzoek sterk wijzen op een chronische inflammatoire darmziekte (IBD) zal vrij snel worden besloten tot het verrichten van invasief onderzoek, bestaande uit endoscopie van de tractus digestivus en een passagefoto van de dunne darm. Indien het klinisch beeld minder duidelijk is, kan men twijfelen of invasief aanvullend onderzoek is geïndiceerd. In dit geval kunnen de uitslagen van niet-invasief aanvullend onderzoek ondersteuning bieden. Ook wanneer de diagnose ziekte van Crohn of colitis ulcerosa al is gesteld, bestaat de behoefte met behulp van niet-invasief aanvullend onderzoek de ziekteactiviteit te bepalen. Als op basis van endoscopie met histologie en radiologisch onderzoek het type IBD niet is te bepalen, is er sprake van indeterminate colitis. Dit is bij 10-20% van de kinderen het geval en in deze groep kan niet-invasief aanvullend onderzoek een rol spelen bij het stellen van de juiste diagnose. Dit kan belangrijke gevolgen hebben voor de behandeling van de ziekte.

Bloedbeeld, bezinking, CRP en albumine

De belangrijkste bepalingen in het bloed bij een chronische inflammatoire darmziekte zijn het hemoglobine, de celindices, het aantal trombocyten, het CRP (C-reactieve proteïne), de bezinking en het albumine. Bloedarmoede kan optreden als gevolg van malabsorptie, bloedverlies en/of een verminderde aanmaak ten gevolge van de chronische ontsteking. Malabsorptie zal vooral optreden bij aantasting van de dunne darm, zoals bij de ziekte van Crohn (CD) kan voorkomen. Bij colitis ulcerosa (UC) of een Crohnse colitis zal de bloedarmoede vooral het gevolg zijn van intestinaal bloedverlies. De aanmaak kan onderdrukt zijn door de chronische inflammatie en/of door een ijzergebrek (bij veel bloedverlies). Wanneer sprake is van een ernstig eiwitverlies via de darm kan er sprake zijn van hypoalbuminemie.
In een recent onderzoek werden de uitkomsten onderzocht van bloedonderzoek bij 153 kinderen die onder de verdenking van IBD waren ingestuurd naar een secundair/tertiair ziekenhuis.[1] Bij 103 van de 153 verwezen kinderen werd de diagnose IBD gesteld. Analyse van de bloedparameters van de kinderen met IBD versus de kinderen zonder IBD toonde dat anemie en trombocytose de belangrijkste indicatoren waren voor IBD (oddsratio's respectievelijk 10,6 en 6,4). Voor de diagnose IBD hadden anemie of trombocytose een sensitiviteit van 91%, een specificiteit van 80%, een positief voorspellende waarde van 90% en een negatief voorspellende waarde van 81%. Op basis van de laboratoriumuitslagen was evenwel geen onderscheid te maken tussen de ziekte van Crohn en colitis ulcerosa. Minder betrouwbaar voor de diagnose 'IBD of geen IBD' waren het serumalbumine en de bezinking (oddsratio's respectievelijk 2,3 en 1,6).
Beattie et al. verrichtten een onderzoek bij 91 kinderen die werden verwezen voor endoscopisch onderzoek.[2] Bij respectievelijk 26 en 13 kinderen werd de diagnose CD dan wel UC gesteld. Als afwijkende bevinding bij bloedanalyse werden in de meeste gevallen trombocytose (>400 x 10^9/l)

en een verhoogd CRP vastgesteld. Bij kinderen met CD was de bezinking vaak verhoogd (85% van de kinderen had een BSE van >25 mm/uur), bij colitis ulcerosa was dit slechts bij 23% van de kinderen het geval. Omgekeerd kan worden gesteld dat de klinische verdenking op IBD laag is wanneer bij geen van bovenstaande parameters abnormale waarden worden aangetoond.

ASCA en P-ANCA

Serologische tests die indicatief kunnen zijn voor de diagnose IBD zijn de bepaling van het perinucleair antineutrofiel cytoplasmatisch antilichaam (p-ANCA) en het anti-*Saccharomyces cerevisiae* antilichaam (ASCA). De p-ANCA-antistoffen zijn gericht tegen perinucleaire componenten in het cytoplasma van de neutrofiel. Deze antistoffen kunnen specifiek gericht zijn tegen cytoplasmatisch myeloperoxidase, elastase, lactoferrine en/of cathepsine.[3] ASCA-antistoffen herkennen mannosesequenties in de celwand van de gist *Saccharomyces cerevisiae*.
Bij 46-60% van de kinderen met CD kunnen deze antistoffen in het serum worden aangetoond.[4-6] Bij kinderen met UC is dit slechts het geval bij 4-12%; bij gezonde kinderen is de ASCA positief bij 3-5%. De sensitiviteit varieert dus van 46-60%, de specificiteit bedraagt 82-95%. Bij 48-82% van de kinderen met UC is de p-ANCA in het serum positief terwijl dit slechts bij 5-19% van de kinderen met CD het geval is.[4,6-9] In het onderzoek van Hoffenberg et al. bedroeg dit percentage zelfs 35, maar het patiëntenaantal in dit onderzoek was laag.[5] De sensitiviteit varieert dus van 48 tot 82%, de specificiteit van 65 tot 97%. Aan de hand van bovenstaande onderzoeken kan worden gesteld dat p-ANCA-antistoffen geassocieerd kunnen worden met colitis ulcerosa, en ASCA-antistoffen met de ziekte van Crohn. Een positieve uitslag kan wel richting geven aan de uiteindelijke diagnose, maar geeft beslist geen zekerheid. Vanwege de lage sensitiviteit van de serologische parameters mag een negatieve uitslag echter nooit leiden tot afstel van endoscopie bij een klinische verdenking op IBD.
Als er ondanks volledige endoscopische, histologische en radiologische diagnostiek sprake is van indeterminate colitis, kan serologie soms uitkomst bieden. In een onderzoek bij volwassen IBD-patiënten werden 'likelihood ratios' berekend. Patiënten die p-ANCA-positief en ASCA-negatief waren, hadden een negentien maal zo grote kans om colitis ulcerosa te hebben. Patiënten die p-ANCA-negatief en ASCA-positief waren, hadden een zestien maal zo grote kans om de ziekte van Crohn te hebben.[10] In het geval van indeterminate colitis kon op basis van deze serologische onderzoeken circa 60% geclassificeerd worden als CD of UC.
De antistoffen p-ANCA en ASCA zijn niet te gebruiken bij het vaststellen van de ziekteactiviteit. Het is daarom ook niet zinvol deze parameters te volgen in de tijd.
Uit recente onderzoeken bij volwassenen is gebleken dat de aanwezigheid van ASCA bij patiënten met CD geassocieerd is met presentatie op jongere leeftijd, ziekte van de dunne darm, vorming van fibrose en stenose, en met meervoudig operatief ingrijpen.[11]
Bij patiënten met in het colon gelokaliseerde ziekte van Crohn blijkt p-ANCA vaker positief dan

bij patiënten met de ziekte in de dunne darm.[12] ASCA is vaker positief bij inflammatie van de dunne darm en minder vaak bij de ziekte van Crohn gelokaliseerd in het colon. Bij onderzoeken onder volwassenen lijkt de aanwezigheid van ANCA-antistoffen bij IBD geassocieerd met meer therapieresistente ziekte en de noodzaak tot een vroegtijdig operatief ingrijpen.[13] Bij therapieresistente linkszijdige colitis waren de ANCA-antistoffen positief bij 90% van de patiënten, bij een linkszijdige colitis die goed reageerde op behandeling waren de ANCA-antistoffen bij 62% positief. Bij patiënten met de ziekte van Crohn met een $ANCA^{+}/ASCA^{-}$ serologisch profiel is een verminderde gevoeligheid voor infliximab (anti-tumor necrose factor) gesuggereerd.[12,14]

Feces algemeen

Onderzoek van de ontlasting kan worden uitgevoerd om de mate van darmontsteking te beoordelen. Alfa-1-antitrypsine, calprotectine, lactoferrine, lysozym, elastase, leukocyte-esterase en tumornecrosefactor-α (TNF-α) zijn ontstekingsparameters die in de feces kunnen worden bepaald. Drie ontstekingsmediatoren die het beste zijn bestudeerd worden besproken:

Fecaal alfa-1-antitrypsine

Alfa-1-antitrypsine (α1AT) is een in humaan serum aanwezige proteïnaseremmer die wordt geproduceerd door levercellen, macrofagen en intestinaal epitheel. Bij eiwitverlies in de darm is de fecaal α1AT verhoogd. Protein-losing enteropathy is een verzamelnaam van ziektebeelden die aanleiding geven tot eiwitverlies in de darm. Een verhoogd α1AT in de ontlasting duidt op eiwitverlies in het algemeen en is niet specifiek voor IBD. Bij bloederige ontlasting is er altijd een verhoogd α1AT. De α1AT in de ontlasting kan eenvoudig en goedkoop worden gemeten. De uitslag van deze test is meer betrouwbaar in 24-uurs feces dan in een portie. Het aantal onderzoeken bij kinderen met IBD en de bepaling van α1AT in de ontlasting is beperkt.[15-17]

In het onderzoek van Griffiths et al. is de α1AT-klaring (ml per dag) verhoogd bij 84% van de kinderen met CD.[15] De klaring was niet gerelateerd aan de ziekteactiviteit. Zo werd gerapporteerd dat ondanks klinische remissie de klaring bij een aantal kinderen nog steeds verhoogd was. Bij kinderen met UC was de α1AT-klaring ook bij verscheidene patiënten verhoogd, maar een percentage werd niet weergegeven. In het onderzoek van Thomas et al. bestond bij 96% van de kinderen met CD een verhoogde α1AT-uitscheiding.[16] Kinderen met UC werden niet bestudeerd. Wanneer sprake was van slechts matig bloedverlies was dit niet gecorreleerd met de klaring van α1AT. In het onderzoek van Grill et al. met veertien kinderen werd een niet-significante correlatie gevonden tussen α1AT-klaring en de klinische ziekteactiviteit.[17] Klaring van α1AT bleek voornamelijk verhoogd bij inflammatie in de dunne darm.

Fecaal calprotectine

Calprotectine is een eiwit dat in het cytosol van de neutrofiel voorkomt. Het bindt calcium en het

behoort tot de zogenoemde S100-eiwitten. Het eiwit heeft bacteriostatische en fungostatische activiteit en is resistent tegen bacteriële afbraak in het colon. Mogelijk remt het microbiële groei door competitie met zink. Calprotectine kan worden bepaald in een kleine hoeveelheid (5 gram) feces die tot zeven dagen op kamertemperatuur kan worden bewaard. De bepaling is mogelijk met behulp van een commercieel verkrijgbare ELISA-kit. Onderzoeken bij volwassenen toonden aan dat de uitscheiding van calprotectine in de ontlasting is verhoogd bij inflammatoire darmziekte, maar ook bij een colorectaal carcinoom en bij NSAID-geïnduceerde enteropathie. Bij patiënten met IBD werd een sterke correlatie gevonden tussen fecaal calprotectine en endoscopische en histologische bevindingen van inflammatie bij IBD. Bij succesvolle behandeling van IBD werd een daling van de concentratie van fecaal calprotectine gezien.[18]

Het aantal onderzoeken bij kinderen is beperkt en de patiëntenaantallen zijn klein.[19-22] In het onderzoek van Bunn et al. werden de resultaten beoordeeld bij 21 kinderen met CD, 16 kinderen met UC en bij 31 controles.[19] Het fecaal calprotectine bij controles was maximaal 6,3 mg/l. Bij 17 van de 21 kinderen met CD was het calprotectine verhoogd (gemiddeld 14 mg/l; range 1-60 mg/l). Bij 13 van de 16 kinderen met UC was het eiwit verhoogd (gemiddeld 11,5 mg/l; range 1-273 mg/l). Het fecaal calprotectine bleek te correleren met de klinische ziekteactiviteit. In een vervolgonderzoek van dezelfde auteurs werd aangetoond dat het fecaal calprotectine correleerde met ziekteactiviteit op endoscopisch en histologische niveau alsook met de mate van inflammatie van het colon, vastgesteld met behulp van technetium-gelabelde leukocytenonderzoeken.[20] In het onderzoek van Olafsdottir et al. was fecaal calprotectine laag bij gezonde kinderen van 1 tot 13 jaar en bij kinderen met *recurrent abdominal pain* (RAP).[21] Bij kinderen met IBD werden vergelijkbare resultaten gezien als met de eerdere onderzoeken bij kinderen en volwassenen. Wel werd een fors verhoogde calprotectine-uitscheiding gerapporteerd bij zuigelingen met kolieken, bij zuigelingen met een (transiënte) lactose-intolerantie en bij gezonde zuigelingen jonger dan 10 weken.

Ten slotte kan het fecaal calprotectine zijn verhoogd in het geval van *protein-losing enteropathy*. Om de definitieve diagnostische waarde van deze bepaling bij kinderen met IBD te kunnen bepalen, is aanvullend onderzoek vereist.

Fecaal lactoferrine

Lactoferrine is een glycoproteïne dat ijzer bindt en antibacteriële eigenschappen heeft. Lactoferrine wordt gesecreteerd door verscheidene slijmvliezen. Het is aanwezig in granulae in neutrofielen en niet in monocyten of lymfocyten. Wanneer neutrofielen de darmmucosa penetreren, zoals bij een infectieuze colitis, resulteert dit in een toegenomen concentratie lactoferrine in de ontlasting. Het eiwit is stabiel en bestand tegen proteolyse en vriezen.

Kane et al. verrichtten een onderzoek met 104 patiënten met CD, 80 patiënten met UC, 31 patiënten met een prikkelbaredarmsyndroom (IBS) en gezonde controles.[23] De lactoferrine in de feces was bij deze groepen 440 ± 128 µg/g voor patiënten met CD, 1125 ± 498 µg/g voor de patiënten met UC en kleiner dan 2 µg/g voor IBS en gezonde controles. In dit onderzoek bestond

de groep van patiënten met IBD voor 15% uit kinderen (10-18 jaar). Er was geen correlatie tussen de uitslag van het fecaal lactoferrine en de lokalisatie van de IBD, de leeftijd of het geslacht. Wel was de concentratie van het fecaal lactoferrine gerelateerd aan de ziekteactiviteit. Andere onderzoeken bij volwassenen toonden vergelijkbare resultaten. Onderzoeken naar fecaal lactoferrine bij kinderen zijn nog nauwelijks beschikbaar. Eén onderzoek in een zeer kleine groep kinderen (*n*=8) met IBD toonde aan dat de concentratie van fecaal lactoferrine was gerelateerd aan de ziekteactiviteit en dat deze parameter bruikbaar was bij het vervolgen van de ziekteactiviteit.[24]

Samenvatting

Samenvattend kan worden gesteld dat er wel degelijk een indicatie is voor het verrichten van niet-invasieve diagnostiek bij kinderen met een (verdenking op) inflammatoire darmziekte. Anemie en trombocytose zijn algemene indicatoren voor IBD. ASCA- en p-ANCA-bepalingen kunnen uitkomst bieden voor het onderscheid tussen CD en UC. Fecaal alfa-1-antitrypsine lijkt vooral verhoogd bij CD, maar is niet duidelijk gerelateerd aan de ziekteactiviteit. Bepaling van fecaal calprotectine lijkt veelbelovend, maar de uitscheiding kan behalve bij IBD bij een groot aantal andere ziektebeelden verhoogd zijn. Fecaal lactoferrine helpt niet voor het onderscheid tussen CD en UC, maar lijkt wel gerelateerd aan de ziekteactiviteit.

References

1. Cabrera-Abreu JC, Davies P, Matek Z, Murphy MS. Performance of blood tests in diagnosis of inflammatory bowel disease in a specialist clinic. Arch Dis Child 2004;89:69-71.
2. Beattie RM, Walker-Smith JA, Murch SH. Indications for investigation of chronic gastrointestinal symptoms. Arch Dis Child 1995;73:354-5.
3. Colombel JF, Reumaux D, Duthilleul P, et al. Antineutrophil cytoplasmic autoantibodies in inflammatory bowel diseases. Gastroenterol Clin Biol 1992;16:656-60.
4. Khan K, Schwarzenberg SJ, Sharp H, et al. Role of serology and routine laboratory tests in childhood inflammatory bowel disease. Inflamm Bowel Dis 2002;8(5):325-9.
5. Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. J Pediatr 1999;134(4):447-52.
6. Ruemmele FM, Targan SR, Levy G, et al. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1998;115(4):822-9.
7. Olives JP, Breton A, Hugot J-P, et al. Antineutrophil cytoplasmic antibodies in children with inflammatory bowel disease: prevalence and diagnostic value. J Ped Gastroenterol Nutr 1997;25(2):142-8.
8. Winter HS, Landers CJ, Winkelstein A, et al. Anti-neutrophil cytoplasmic antibodies in children with ulcerative colitis. J Pediatr 1994;125(5 Pt 1):707-11.

9. Proujansky R, Fawcett PT, Gibney KM, et al. Examination of anti-neutrophil cytoplasmic antibodies in childhood inflammatory bowel disease. J Ped Gastroenterol Nutr 1993;17(2): 193-7.
10. Quinton JF, Sendid B, Reumaux D, et al. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998;42:788-91.
11. Reumaux D, Sendid B, Duthilleul P, et al. Serological markers in inflammatory bowel diseases. Best Pract Res Clin Gastroenterol 2003;17:19-35.
12. Griffiths AM, Zachos M. Antineutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibody: clinical tools or clues for research? J Ped Gastroenterol Nutr 2000;30:1-2.
13. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. Mayo Clin Proc 1996;71:431-6.
14. Rutgeerts P, Vermeire S. Clinical value of the detection of antibodies in the serum for diagnosis and treatment of inflammatory bowel disease. Gastroenterology 1998;115:1006-9.
15. Griffiths AM, Drobnies A, Soldin SJ, Hamilton JR. Enteric protein loss measured by fecal alpha 1-antitrypsin clearance in the assessment of Crohn's disease activity: a study of children and adolescents. J Ped Gastroenterol Nutr 1986;5(6):907-11.
16. Thomas DW, Sinatra FR, Merritt RJ. Fecal alpha 1-antitrypsin excretion in young people with Crohn's disease. J Ped Gastroenterol Nutr 1983;2(3):491-6.
17. Grill BB, Tinghitella T, Hillemeier C, Gryboski J. Fecal alpha 1-antitrypsin clearance in patients with inflammatory bowel disease. J Ped Gastroenterol Nutr 1984;3(1):56-61.
18. Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. World J Gastroenterol 2001;7:460-5.
19. Bunn SK, Bisset WM, Main MJ, Golden BE. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. J Ped Gastroenterol Nutr 2001;32:171-7.
20. Bunn SK, Bisset WM, Main MJ, et al. Fecal calprotectin: validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. J Ped Gastroenterol Nutr 2001;33:14-22.
21. Olafsdottir E, Aksnes L, Fluge G, Berstad A. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. Acta Paediatr 2002;91:45-50.
22. Bjarnason I, Sherwood R. Fecal calprotectin: a significant step in the noninvasive assessment of intestinal inflammation. J Ped Gastroenterol Nutr 2001;33:11-3.
23. Kane SV, Sandborn WJ, Rufo PA, et al. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. Am J Gastroenterol 2003;98:1309-14.
24. Buderus S, Boone J, Lyerly D, Lentze MJ. Fecal lactoferrin: sensitive marker for monitoring inflammatory bowel disease in pediatric patients [abstract]. J Ped Gastroenterol Nutr 2002;34(4):466.

CHAPTER 7

Overlap, common features and essential differences in pediatric granulomatous inflammatory bowel disease

Damen GM [1]
van Krieken JH [2]
Hoppenreijs E [1]
van Os E [1]
Tolboom JJM [1]
Warris A [1]
Yntema J [1]
Nieuwenhuis EES [3]
Escher JC [3]

[1] Dept. of Pediatrics, Radboud University Nijmegen Medical Center
[2] Dept. of Pathology, Radboud University Nijmegen Medical Center
[3] Dept. of Pediatric Gastroenterology, Erasmus MC- Sophia Children's Hospital, University Medical Center, Rotterdam

Accepted by J Ped Gastroenterol Nutr

Abstract

Overlap in the clinical presentation of pediatric granulomatous inflammatory bowel disease may be substantial, depending on the mode of presentation. Chronic granulomatous disease (CGD) may present with granulomatous colitis, perianal abscesses, hepatic abscesses or granulomas, failure to thrive and obstruction of the gastrointestinal tract (including esophageal strictures and dysmotility, delayed gastric emptying and small bowel obstruction). Anemia, thrombocytosis, elevated CRP and ESR and hypoalbuminemia are nonspecific and may occur in any of the granulomatous inflammatory bowel diseases. In histology macrophages with cytoplasmic inclusions will be rather specific for CGD. Sarcoidosis may present with abdominal pain or discomfort, diarrhoea, weight loss, growth failure, delayed puberty, erythema nodosum, arthritis, uveitis and hepatic granulomata. Only in 55% of the patients angiotensin-converting enzyme will be elevated. The non-caseating epitheloid granulomata will be unspecific. Bronchoalveolar lymphocytosis and abnormalities in pulmonary function are reported in sarcoidosis and in Crohn's disease (CD) and CGD. Importantly, CD patients may present with granulomatous lung disease, fibrosing alveolitis and drug-induced pneumonitis. Sarcoidosis and concomitant gastrointestinal CD have been reported in patients, as well as coexistence of CD and sarcoidosis in siblings. Common susceptibility loci have been indentified in CD and sarcoidosis. CD and CGD share defects in the defense mechanisms against different microbes. In this review, common features and essential differences are discussed in clinical presentation and diagnostics - including histology - in CGD, sarcoidosis and CD, together with two other granulomatous inflammatory bowel diseases, namely abdominal tuberculosis and Hermansky-Pudlak syndrome. Instructions for specific diagnosis and respective treatments are provided.

Depending on the mode of presentation, overlap in the clinical presentation of pediatric granulomatous inflammatory bowel disease may be substantial, and the physician will be challenged to establish a definite and accurate diagnosis. Overlap may also exist in the results from laboratory tests, radiology, endoscopy and histology. In this review we will address overlap, common features and essential differences in the granulomatous inflammatory bowel diseases chronic granulomatous disease (CGD), sarcoidosis, Crohn's disease (CD), abdominal tuberculosis and Hermansky-Pudlak syndrome. Results from histology are provided for each of them.

1. Chronic Granulomatous Disease (CGD)

In CGD, a immunodeficiency, phagocytes are unable to kill certain bacteria and fungi. Phagocyte function tests demonstrate a reduced capacity to produce superoxide and hydrogen peroxide. The incidence of CGD is 1 in 250.000 live births in the USA and 1 in 450.000 in Sweden.[1-3] Mutations occur in the *CYBB* gene, encoding gp91^{phox} and located on the X-chromosome, or in the genes *NCF1* (encoding p47^{phox}), *NCF2* (p67^{phox}) and *CTBA* (p22^{phox}).[1,2,4] Patients suffer from life-threatening infections, but may also present with granulomatous inflammation.

Clinical presentation

Due to the type of inheritance, the majority of patients are males. Seventy-five percent of the patients are diagnosed before 5 years of age.[1,2,5] Patients may present with (recurrent) pneumonia, subcutaneous abscesses, suppurative adenitis, cellulitis, osteomyelitis, sepsis and meningitis, and patients may suffer from abscesses in the liver, the lung and brain. Colitis, peri-anal abscesses and intestinal obstruction may be the first symptoms of disease.[1-23] In CGD colitis, an infection may initiate bowel inflammation, subsequently deregulation of the immune response may perpetuate and exacerbate bowel inflammation.[24] Further gastointestinal (GI) manifestations include oral ulcers, stomatitis, vomiting, abdominal pain, abdominal distension, (bloody) diarrhea, tenesmus, incontinence, weight loss, growth failure, hepatosplenomegaly and ascites. Fever, abdominal pain, weight loss and night sweats may point to the presence of a hepatic abscess. Progressive dysphagia, oesophageal dysmotility and delayed gastric emptying have been described, as well as obstruction of the GI tract ranging from esophageal strictures, to gastric outlet syndrome and small bowel obstruction.[1,2,5-10,16,17,19,20] In general, GI manifestations precede the diagnosis of CGD in up to 17% of the patients.[1] In CGD, obstructive lesions of the urinary tract are reported, as well as discoid and systemic lupus erythematosus, chorioretinitis, idiopathic or immune thrombocytopenia and Behçet syndrome.[1,2]

Diagnostics

Frequent findings in CGD include anemia, thrombocytosis, elevated ESR, hypergammaglobulinemia and hypoalbuminemia. There will be quite a variability in the results of additional investigations, due to the variability in clinical presentation. In case of GI involvement radiological imaging of the intestines may reveal mucosal thickening, narrowing of the lumen, prestenotic dilatation, cobblestone pattern and fistulation; endoscopy may show (diffuse) colitis, patchy friability, ulcers and pseudopolyps. Phagocyte function test (like the nitrogen blue test) and mutational analysis are specific tests in CGD.

Histology

In case of CGD colitis, histology may show diffuse or focal colitis with different degrees of severity, characterized by cryptitis, crypt abscesses, predominance of eosinophils, paucity of neutrophils and, rather specific, presence of large macrophages with either brown granular cytoplasm or pink eosinophilic crystalline cytoplasmic inclusions (illustrated in figure 1a).[4,6,10,13,15,17,19,22,23,25]

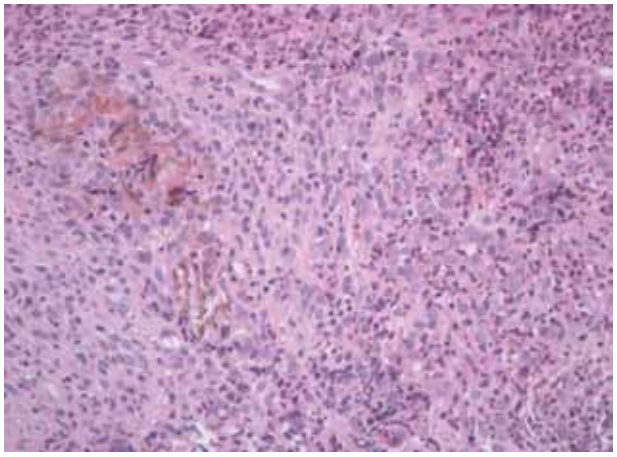


Figure 1a:
Severe inflammation of the mucosa with many macrophages and (eosinophilic) granulocytes in a child with CGD colitis; characteristic brown intra- and extracellular material is present (HE staining 600x).

Nuclear debris may be present within the epithelial cells and lamina propria, as well as microscopic ulcers and epitheloid granulomas (sporadic or disseminated). In CGD colitis, granulomata are sharply defined aggregates of epitheloid histiocytes, surrounded by a cuff of dense lymphocytic inflammation (figure 1b). Characteristics of chronic colitis may be present, such as crypt shortening, thickening of the muscularis mucosa and Paneth cell metaplasia.

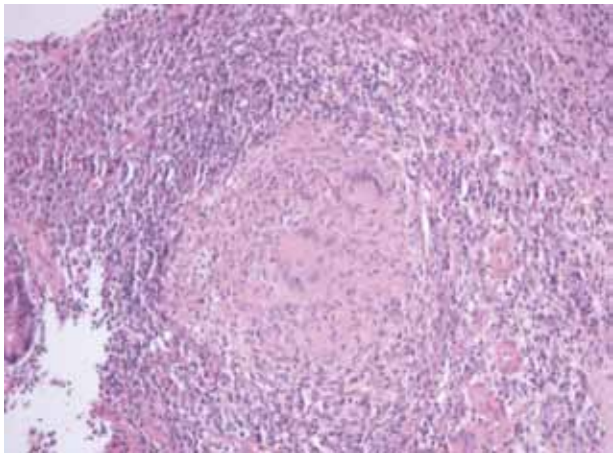


Figure 1b:
A granuloma consisting of epitheloid histiocytes and Langhans giant cells is surrounded by a cuff of lymphocytes in a child with CGD colitis; few eosinophilic granulocytes are present (HE staining 250x).

Treatment

In case of infections, aggressive and appropriate antimicrobial and/or anti-fungal therapy is required. Most CGD patients with hepatic abscesses will also require corticosteroids. In CGD colitis, the majority of patients will respond to mesalazine and corticosteroids.[4,6,8,11,14,17,22,24,26,27] Corticosteroids reduce the activation, proliferation, differentiation and survival of inflammatory cells such as macrophages and lymphocytes, key cells in granuloma formation.[27] One study reports the use of cyclosporine, in a patient with CGD colitis, blocking T-cell activation.[24] In this report, a rapid initial response was followed by a nearly fatal pneumonia. Treatment with granulocyte colony stimulating factor has been reported to be effective in two adults with CGD enteritis.[12] In one patient with CGD colitis, infliximab was temporarily successful, allowing for tapering of prednisone.[6] Notably, immunomodulation may be accompanied by an increased risk of infective complications.

2. Sarcoidosis

Sarcoidosis is a systemic immune disorder characterized by formation of non-necrotizing epitheloid granulomas.[28-32] In Northern Europe, the incidence of sarcoidosis is 0.3 per 100.000 person-years in children younger than 16 years.[32] Sarcoidosis is polygenic disease and is associated with certain HLA loci. Genetic associations include cytokine genes (e.g. promoter region tumor necrosis alpha), receptor genes (such as the *BTNL2* gene) and candidate genes (e.g. Heat shock proteins).[33] Recently, a susceptibility locus on chromosome 10p12.2 and a strong association signal mapping to the annexin A11 gene on chromosome 10q22.3 were demonstrated.[34,35]

Clinical presentation

In pediatric sarcoidosis, common clinical manifestations include cough, chest pain, fever, (hilar) lymphadenopathy and exertional dyspnoea. Other manifestations are sarcoid arthritis, sarcoid skin lesions or erythema nodosum, uveitis or iridocyclitis and central nervous symptoms.[41,45,57,58] Gastrointestinal manifestations include anorexia, nausea, vomiting, abdominal pain or discomfort and weight loss. Sporadic cases have been reported with hepatic granulomata, diarrhoea, growth failure and delayed puberty.[36,37]

Diagnostics

In 55% of the children, serum angiotensin-converting enzyme is elevated; hypercalciemia and leukopenia are present in 30% of the children.[32] Less frequently are reported: increased plasma creatinin, leukocytosis, thrombocytosis, increased plasma alkaline phosphatase, albuminuria and hematuria. In the diagnostic work-up, histology from different organs such as the lungs, lymph nodes, skin, nasal or labial mucosa, parotid gland, liver and appendix may need to be obtained. In patients with overt pulmonary symptoms, imaging of the lungs (X-ray or CT-scan), in association with clinical features and specific laboratory findings will substantiate the diagnosis of sarcoidosis.

Histology

Independent of the affected organ, histology may show non-caseating epitheloid cell granulomas. These granulomas may contain Langhans' giant cells and lymphocytes.[38] Hyalinization of granulomas and diffuse interstitial fibrosis may render the diagnosis difficult. In the vast majority of granulomas necrosis is absent, but small foci of necrosis may be found in the center. Sarcoid granulomas may exhibit Schaumann bodies (lamellated, concentric inclusions in the cytoplasm of multinucleated giant cells, containing calcium and proteins), which are unspecific.[39] Figure 2 shows a single granuloma in a biopsy from the stomach in a 16 year old boy with sarcoidosis.

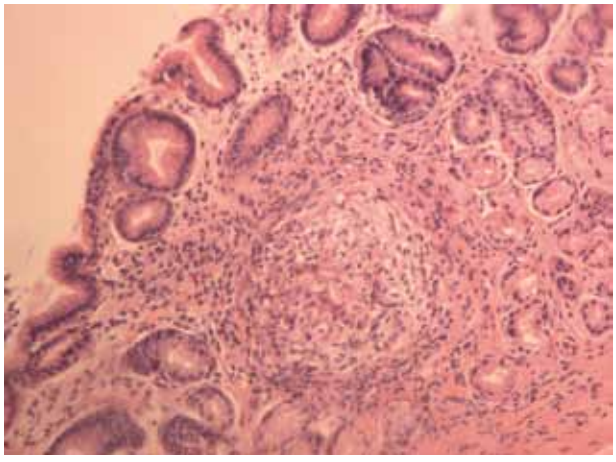


Figure 2:
A granuloma in a biopsy from the stomach of a male adolescent with sarcoidosis (HE staining 180X).

Treatment

Treatment of sarcoidosis is mostly limited to a short course of glucocorticosteroids, without the need for maintenance therapy.[37] In steroid-resistant sarcoidosis treatment with infliximab was successful.[40-42]

3. Common features, overlap and essential differences in CGD, sarcoidosis and Crohn's disease

Clinical manifestations

Table 1 illustrates the overlap in clinical manifestations in CGD, sarcoidosis and CD. Abdominal pain or discomfort and weight loss are the most frequent overlapping symptoms. Diarrhea, perianal abscesses and failure to thrive are common in CGD and CD, as well as vomiting, stomatitis, oral

Table 1: Clinical manifestations in children with CGD, sarcoidosis, and CD in larger patient groups with varying presentations. The presence of the specific clinical manifestations is expressed as: rare (-/+), less frequent than 25% (+), 25 to 50% (++), 50 to 75% (+++), and >75% (++++).

Clinical manifestations in	CGD	Sarcoidosis	CD
Anorexia / lethargy	++	+	+/++
Nausea / vomiting	++	+	+
Oral ulcers / stomatitis	+	?	+
Abdominal pain or discomfort	++/+++	+/++	+++
Weight loss	++	++/+++	+++
Growth failure / delayed puberty	++/+++	-/+	++/+++
Diarrhea	+/++	-/+	++/+++
Rectal bleeding	+	-	+/++
Constipation / soiling	+	-/+	+
Peri-rectal abscess / ulcer / fistula	+/++	-	+
Hepatic granuloma / abscess	+/++	+	-/+
Intestinal obstruction	+	-/+	+
Skin manifestations	-/+	++	+
Arthritis or arthropathy	+	+	+
Uveitis / iridocyclitis / chorioretinitis	-/+	+/++	+
Fever	+++	++	+
Cough	++/+++	+++	-/+
Chest pain	+	+	-/+
Exertional dyspnoea	+/++	++	?
Lymphadenopathy (hilar)	++/+++	+++	+
Neurological symptoms	+	+/++	-/+

ulcers, and strictures or obstruction of the GI-tract. In CGD and sarcoidosis weight loss, fever, cough, exertional dyspnoea and (hilar) lymphadenopathy are frequent symptoms. In all three disease there is a variety of skin manifestations as is illustrated in Table 2.[2,43-47] Depending on the mode of presentation, overlap in clinical manifestations can be quite substantial, resulting in a diagnostic challenge.

Tabel 2: Skin manifestations in CGD, sarcoidosis and CD.

CGD:	Positive skin rashed
	Dermatitis
	Acne
	Furunculosis
	Abscess
	BCGitis
	Aseptic granulomas
Sarcoidosis:	Erythema nodosum
	Maculopapules or maculopapular eruptions[1]
	Erythematous/violaceous infiltrated plaques
	Annular plaques with central hypopigmentation, or Annular plaques with an infiltrative active border, atrophy and teleangiectasias, or Sarcoid plaques
	Acneiform erythematous papules on the face
	Skin-colored subcutaneous (sarcoid) nodules
	Infiltrative scars
	Lupus pernio
	Alopecia
	Ulcerative lesions
	Ichthyosiform sarcoidosis
	Hypopigmentation
	Nail dystrophy
CD:	Erythema nodosum
	Pyoderma gangrenosum
	Sweet's syndrome[2]
	Pyostomatitis vegetans
	Psoriasis
	Erythema multiforme
	Epidermolysis bullosa acquista
	Metastatic CD

[1] red-brown to purple; often affecting eyelids, periorbital area, nasolabial folds

[2] Sweet's syndrome is characterized by tender, red inflammatory nodules or papulae, usually affecting the upper limbs, face or neck.

Pulmonary manifestations

Pulmonary manifestations are frequent in CGD and sarcoidosis. In sarcoidosis, patients may have a decreased diffusion capacity, FEV1 and vital capacity, and bronchoalveolar lymphocytosis.[32,48] In CD, pulmonary manifestations may include granulomatous lung disease, fibrosing alveolitis and drug-induced (sulphazalazine) pneumonitis.[32,48-55] In CD, the pulmonary function may be restricted, the lung transfer factor for carbon monoxide may be reduced, bronchoalveolar lymphocytosis may be present and an increased superoxide anion production by alveolar macrophages is reported.[56-65]

Coexistence of diseases in patients and in siblings

Patients with sarcoidosis and concomitant gastrointestinal CD have been reported, even with an interval up to several years.[48-50,66] Al-Binali *et al.* reported an 11-year-old child with recurrent cough and exertional dyspnea during 8 months, preceding the emergence of gastrointestinal symptoms.[50] This child had granuloma formation in the lungs, and developed erosions and aphtous lesions in the duodenum and colon. There seems to be a disease spectrum with overlap between sarcoidosis and CD. Within our patient cohort, we treat a patient that presented with granulomas in the upper lip (Figure 3) and an elevated ACE, who developed gastrointestinal CD 4 months later [data unpublished]. Gastrointestinal CD may coincide with granulomatous pulmonary disease, but may also precede granulomatous pulmonary disease.[37,48-55,66,67] Interestingly, coexistence of CD and sarcoidosis occur in siblings.[68-70] Within our patient cohort, a patient was diagnosed with pulmonary sarcoidosis and a granuloma in the stomach, while his sister had CD with an ileocoecal localization [data unpublished]. In summary, coexistence of sarcoidosis and CD occurs in patients as well as in siblings, simultaneous or in time.

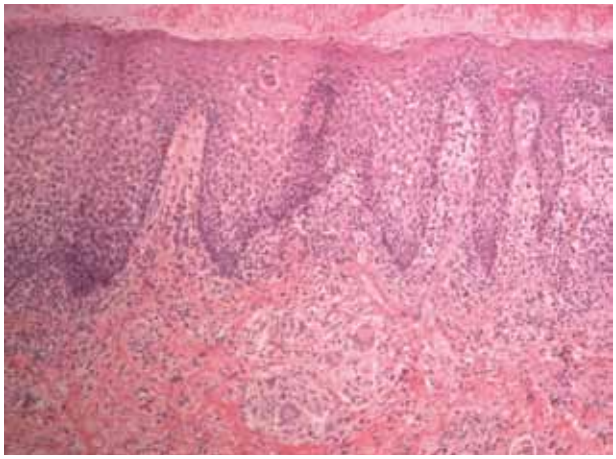


Figure 3:
Multiple granulomas in a biopsy from the upper lip (HE staining 125x)

Common genes

In CGD, gene mutations are well defined. In CD, genetic analyses have identified several polymorphisms, including the best characterized polymorphisms *Nod2/CARD15*, *ATG16L1* and *IRGM*.[71-76] Polymorphisms in *Nod2/CARD15* result in impaired recognition of bacterial peptidoglycan and might result in unopposed TLR2 signaling and enhanced IL-12 production.[77] Polymorphisms in *ATG16L1* and *IRGM* impede autophagy, an intracellular process that contributes to the degradation of intracellular of pathogens, antigen processing, regulation of cell signaling and regulation of T-cell homeostasis.[78] Both CD and CGD enteritis might relate to a primary abnormality in the phagocytic response, resulting in inadequate intracellular processing and digestion of bacteria.[4] In sarcoidosis, both positive and negative association studies of *Nod2/CARD15* polymorphisms have been reported.[79] The *BTNL2* association may be common between sarcoidosis and inflammatory bowel disease, however, this relation was reported in ulcerative colitis and sarcoidosis, not in CD.[80] In patients with sarcoidosis and CD, a genome-wide association study identified common susceptibility loci on chromosome 10p12.2.[34]

Diagnostics

In CGD, phagocyte function tests are specific and discriminative blood tests, as well as DNA-testing. Proteinuria, hematuria, increase in plasma creatinin and elevated serum ACE point to sarcoidosis, but unfortunately, these findings quite often are normal. Antibodies against *Saccharomyces Cerevisiae* are present in 40 to 70% of CD patients, but can also exist in healthy controls.[81-83] Blood tests that are not discriminative between CGD, sarcoidosis and CD are diminished serum albumin, leukocytopenia of leukocytosis, thrombocytosis and elevation in ESR and CRP.

Granulomas in the GI-tract

In CGD colitis, pigment-laden macrophages with brown granular cytoplasm or pink eosinophilic crystalline cytoplasmic inclusions are characteristic and discriminative, however, these characteristics quite often are absent. In CGD, granulomas may also be present in the small bowel and/or liver. In sarcoidosis, upper GI endoscopy will not be performed routinely, but granulomas may be present in the stomach.[37,50] Both in sarcoidosis and CD, granulomas may be present outside the GI-tract, e.g. in the upper lip. Involvement of the small bowel has been reported in sarcoidosis.[84] This occurrence however is very exceptional and one has to be aware that patients with sarcoidosis may develop CD in time. In CD, non-caseating granulomas are found in about 50% of the mucosal biopsies of the GI-tract.[85,86] These sarcoid-like granulomas are composed of epitheloid cells and multinucleated giant cells, and can be well-formed.[87-89] Necrosis is usually absent (or limited to a small central area). The granulomas may be found anywhere in the bowel wall (including serosa), in regional lymph nodes and at any other site of involvement; they can also been seen in isolation away from areas of active disease. Other histological characteristics of CD

are focal inflammation, focal crypt abscesses and relative preservation of goblet cells. In resected bowel specimens, transmural inflammation, transmural presence of lymphoid aggregates, skipped lesions and presence of fissures, sinuses and fistulae may be seen.

Common treatment

In CGD, sarcoidosis and CD, patients respond well to steroids. Antimicrobial therapy may have some effect in CD, and is the mainstay of therapy in CGD. Tumor necrosis factor alpha blockade is successful in CD and has been shown to be effective in steroid-resistant sarcoidosis; in CGD, tumor necrosis factor alpha blockade was temporarily successful, allowing for tapering of prednisone.

4. Abdominal tuberculosis

In the Western world, abdominal tuberculosis (TB) is a rare form of extrapulmonary TB. However, the presenting symptoms of abdominal TB, physical examination and the results from diagnostics often are nonspecific and indistinguishable from CD.[90-100]

Clinical presentation

In 19 to 64% of the patients with abdominal TB, clinical features of pulmonary TB are present, including fever, night sweats, cough, haemoptysis, chest pain, pneumonia, pleural effusion, lymphadenopathy and weight loss. [91,93,96,97,100-104] In abdominal TB clinical features may include anorexia, nausea, vomiting, abdominal pain, abdominal distension, abdominal mass, weight loss, diarrhea, hepatomegaly, splenomegaly and ascites.[91,93,96,100-102] In colonic TB, rectal bleeding can be present and, more rarely, fistulas have been demonstrated.[97,105-107] Patients may also present with jaundice or an acute abdomen. Thickening of the bowel wall and ulceroconstrictive lesions may cause stricturing and bowel obstruction, especially in the small intestine. Perforation, haemorrhage, fistulae and malabsorption syndrome have been reported.[91,93,96,97,100-107]

Diagnostics

Laboratory results may show anemia, lymphopenia or lymphocytosis, diminished serum albumin and elevation in ESR and CRP. Radiological analysis may show abdominal lymphadenopathy (with calcification), thickening of the bowel wall and mesentery, mass lesions, ascites, diffuse regular thickening of the peritoneum, calcifications and granulomas in the liver, spleen and pancreas. Tuberculous involvement of the intestines commonly involves the ileocoecal region, but is variable. Endoscopy may reveal inflammatory polyps or nodules, well-defined ulcers (with surrounding erythema), cobblestones and strictures. Colonic TB can appear as segmental ulcers

with rectal sparing, proctocolitis or generalized colitis. It has been postulated that anorectal lesions, longitudinal or aphthous ulcers and cobblestone appearance are more common in patients with CD, while involvement of a limited number of segments (<4), a patulous ileocecal valve, transverse ulcers and scars or pseudopolyps were more frequently observed in intestinal TB.[108] Tuberculin skin testing (TST) can be false-negative in 12-50% of patients with abdominal TB.[93,96,109] The diagnosis of abdominal TB is confirmed by detection of acid-fast bacilli, by PCR and by culture of the tubercle bacillus on tissue biopsy specimens obtained through endoscopy, needle aspiration, laparoscopy and/or surgery. Gastric fluid, pleural fluid and ascites can also be studied for this reason. One of the more recent techniques in the investigation for tuberculosis include T cell interferon gamma release assay (QuantiFERON).[110-112]

Histology

In abdominal TB, mucosal biopsies obtained may demonstrate caseating granulomas, which usually are well-defined, large, florid, coalescent, located in the submucosa.[95,97,113,114] Biopsies should be obtained from ulcer margins.[97,115,116] Importantly, in more than one third of patients with abdominal TB granulomas may be noncaseating.[92,95,99]

5. Hermansky-Pudlak syndrome

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by oculocutaneous albinism, platelet dysfunction and systemic complications, including pulmonary fibrosis, renal disease and granulomatous colitis.[117-118] Ceroid accumulate in the reticuloendothelial system in HPS. Pulmonary disease begins with a restrictive component, progressing to death usually in the 4th or 5th decade. Earlier in the course of disease, patient may have a granulomatous colitis, skin tags, fistulas and anorectal strictures, and less frequently, inflammation of the small bowel.[119-125,127] Platelet dense bodies are absent, triggering the secondary aggregation of platelets. Patients have easy bruisability of soft tissues and prolonged bleeding after dental extraction and surgical procedures.

Diagnostics may show hypoalbuminemia, anemia, thrombocytosis, and elevation of ESR and CRP. Endoscopy may reveal diffuse colitis, but also pseudopolyps, scattered ulcers and segmental colitis have been reported.[121] In HPS, histology may show accumulation of ceroid in the macrophages, brown granular pigmentation, chronic inflammation with crypt architectural abnormalities and non-necrotizing epithelioid granulomas (Figure 4).[119,121,123,125,127]

In quite some children with HPS, treatment of granulomatous colitis has been unsuccessful, including therapy with mercaptopurine and corticosteroids. The number of patients needing colectomy is relatively high.[118] Recently, it has been demonstrated that infliximab may be effective in patients with HPS and granulomatous colitis.[120-122,124] During interventions prophylaxis with desmopressin can be effective, in order to prevent bleeding, and avoidance of aspirin and NSAID's is essential.

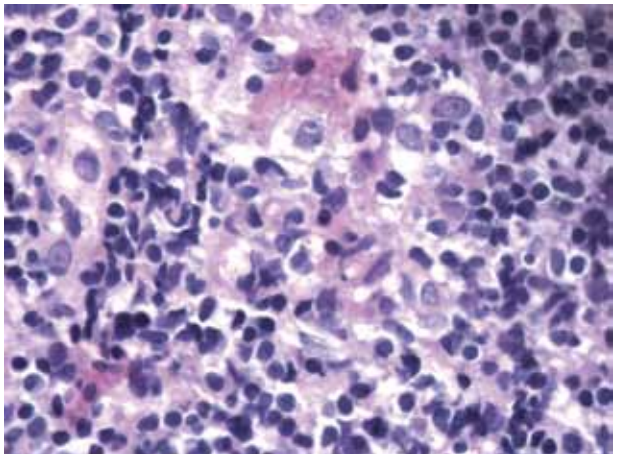


Figure 4a:
Brown granular pigmentation (ceroid deposition) in a child with granulomatous colitis and the Hermansky-Pudlak syndrome (HE staining 800x).

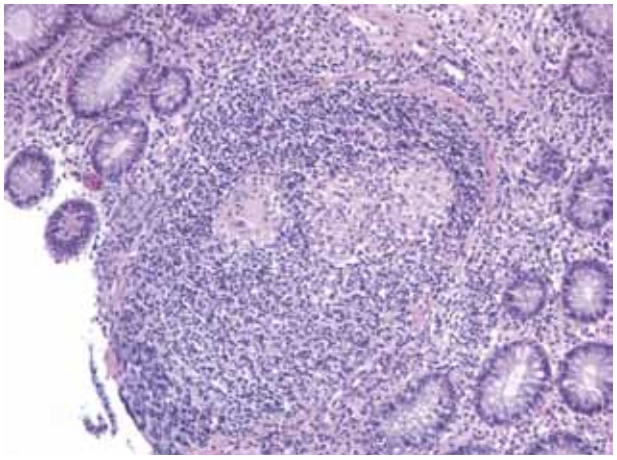


Figure 4b:
Small, non-necrotizing, epitheloid granulomas are surrounded by abundant lymphocytes, in a child with colitis and the Hermansky-Pudlak syndrome (HE staining 150x).

References

1. Winkelstein JA, Marino MC, Johnston RB Jr, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 2000;79:155–169.
2. Van den Berg JM, van Koppen E, Ahlin A, et al. Chronic Granulomatous Disease: The European Experience. PLoS One 2009;4:e5234.
3. Ahlin A, de Boer M, Roos D, et al. Prevalence, genetics and clinical presentation of chronic granulomatous disease in Sweden. Acta Paediatr 1995;84:1386-94.
4. Marks DJ, Miyagi K, Rahman FZ, et al. Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am J Gastroenterol 2009;104:117-24.
5. Segal BH, Leto TL, Gallin JI, et al. Genetic, biochemical, and clinical features of chronic granulomatous disease. Medicine (Baltimore) 2000;79:170–200.
6. Marciano BE, Rosenzweig SD, David E, et al. Gastrointestinal involvement in Chronic Granulomatous Disease. Pediatrics 2004;114:462-8.
7. Soler-Palacin P, Margareto C, Llobet P, et al. Chronic granulomatous disease in pediatric patients: 25 years of experience. Allergol Immunopathol 2007;35:83-9.
8. Huang A, Abbasakoor F, Vaizey CJ. Gastrointestinal manifestations of chronic granulomatous disease. Colorectal Dis 2006;8:637-44.
9. Carnide EG, Jacob CA, Castro AM, Pastorino AC. Clinical and laboratory aspects of chronic granulomatous disease in description of eighteen patients. Pediatr Allergy Immunol 2005;16:5-9.
10. Schäppi MG, Klein NJ, Lindley KJ, et al. The nature of colitis in Chronic Granulomatous Disease. J Ped Gastroenterol Nutr 2003;36:623-31.
11. Schäppi MG, Smith VV, Goldblatt D, et al. Colitis in chronic granulomatous disease. Arch Dis Child 2001;84:147–151.
12. Myrup B, Valerius NH, Mortensen PB. Treatment of enteritis in chronic granulomatous disease with granulocyte colony stimulating factor. Gut 1997;42:127–30.
13. Sloan JM, Cameron CHS, Maxwell RJ, et al. Colitis complicating chronic granulomatous disease: A clinicopathologic case report. Gut 1996;38:619–22.
14. Fisher A, Segal AW, Seger R, Weening RS. The management of chronic granulomatous disease. Eur J Ped 1993;152:896–9.
15. Isaacs D, Wright VM, Shaw DG, et al. Chronic granulomatous disease mimicking Crohn's disease. J Ped Gastroenterol Nutr 1985;4:498–501.
16. Lindahl JA, Williams FH, Newman SL. Small bowel obstruction in chronic granulomatous disease. J Ped Gastroenterol Nutr 1984;3:637–640.
17. Werlin SL, Chusid MJ, Caya J, Oechler HW. Colitis in chronic granulomatous disease. Gastroenterology 1982;82:328–331.
18. Markowitz JF, Aronow E, Rausen AR, Aet al. Progressive esophageal dysfunction in chronic granulomatous disease. J Ped Gastroenterol Nutr 1982;1:145–149.
19. Yogman MW, Touloukian RJ, Gallagher R. Intestinal granulomatosis in chronic granulomatous disease and in Crohn's disease. N Engl J Med 1974;290:228.

20. Griscom NT, Kirkpatrick JA Jr, Girdany BR, et al. Gastric antral narrowing in chronic granulomatous disease of childhood. Pediatrics 1974;54:456-60.
21. Harris BH, Boles ET. Intestinal lesions in chronic granulomatous disease of childhood. J Ped Surg 1973;8,955.
22. Ament ME. Intestinal granulomatosis in chronic granulomatous disease and in Crohn's disease. N Engl J Med 1974;290:228.
23. Ament ME, Ochs HD. Gastrointestinal manifestations of chronic granulomatous disease. N Engl J Med 1973;288:382–7.
24. Rosh JR, Tang HB, Mayer L. Treatment of intractable gastrointestinal manifestations of chronic granulomatous disease with cyclosporine. J Pediatr 1995;126:143–4.
25. Levine S, Smith VV, Malone M, Sebire NJ. Histopathological features of chronic granulomatous disease (CGD) in childhood. Histopathology 2005;47:508-16.
26. Quie PG, Belani K. Corticosteroids for chronic granulomatous disease. J Pediatr 1987;111:393–4.
27. Chin TW, Stiehm ER, Falloon J, Gallin JI. Corticosteroids in treatment of obstructive lesions of chronic granulomatous disease. J Pediatr 1987;11:349–352.
28. Fauraux B, Clement A. Paediatric sarcoidosis. Paediatr Respir Rev 2005;6:128-33.
29. Thomas KW, Hunninghake GW. Sarcoidosis. JAMA 2003;289:3300-3.
30. Cimaz R, Ansell BM. Sarcoidosis in the pediatric age. Clin Exp Rheumatol 2002;20:231-7.
31. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. Review article. N Eng J Med 2007;357:2153-65.
32. Hoffmann AL, Milman N, Byg KE. Childhood sarcoidosis in Denmark 1979-1994: incidence, clinical features and laboratory results at presentation in 48 children. Acta Paediatr 2004;93:30-6.
33. Smith G, Brownell I, Sanchez M, Prystowsky S. Advances in the genetics of sarcoidosis. Clin Genet 2008;73:401-12.
34. Franke A, Fischer A, Nothnagel M, et al. Genome-wide association analysis in sarcoidosis and Crohn's disease unravels a common susceptibility locus on 10p12.2. Gastroenterology 2008;135:1207-15.
35. Hofmann S, Franke A, Fischer A, et al. Genome-wide association study identifies ANXA11 as a new susceptibility locus for sarcoidosis. Nat Genet 2008;40:1103-6.
36. Hunninghake GW, Costabel U, Ando M, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis 1999;16:149-73.
37. Baculard A, Blanc N, Boule M, et al. Pulmonary sarcoidosis in children: a follow-up study. Eur Respir J 2001; 17:628-35.
38. Rosai J, Ackerman LV. Surgical Pathology. 9th ed. St Louis: Mosby, 2004. Chapter 11: gastrointestinal tract, p725.
39. Hsu RM, Connors AF Jr, Tomashefski JF Jr. Histologic, microbiologic, and clinical correlates of the diagnosis of sarcoidosis by transbronchial biopsy. Arch Pathol Lab Med 1996;120:364-8.
40. Denys BG, Bogaerts Y, Coenegrachts KL, de Vriese AS. Steroid-resistant sarcoidosis: is antagonism of TNF-alpha the answer? Clin Sci (Lond) 2007;112:281-9.

41. Ulbricht KU, Stoll M, Bierwirth J, et al. Successful tumor necrosis factor alpha blockade treatment in therapy-resistant sarcoidosis. Arthritis Rheum 2003;48:3542-3.
42. Alrashid AI, Brown RD, Mihalov ML, et al. Crohn's disease involving the lung: resolution with infliximab. Dig Dis Sci 2001;46:1736-9.
43. Cale CM, Morton L, Goldblatt D. Cutaneous and other lupus-like symptoms in carriers of X-linked chronic granulomatous disease: incidence and autoimmune serology. Clin Exp Immunol 2007;148:79-84.
44. Fernandez-Faith E, McDonnell J. Cutaneous sarcoidosis: differential diagnosis. Clinics Dermatol 2007;25:276-87.
45. Tchernev G, Patterson JW, Nenoff P, Horn LC. Sarcoidosis of the skin - A dermatological puzzle: important differential diagnostic aspects and guidelines for clinical and histopathological recognition. J Eur Acad Dermatol Venereol 2009 jul 17 (Epub ahead of print).
46. Jose FA, Heyman MB. Extraintestinal manifestations of inflammatory bowel disease. J Ped Gastroenterol Nutr 2008;46:124-33.
47. Ardizzone S, Sarzi Puttini P, Cassinotti A, Bianchi Porro G. Extraintestinal manifestations of inflammatory bowel disease. Digest Liver Dis 2008;40S:S253-9.
48. Omori H, Asahi H, Inoue Y, et al. Pulmonary Involvement in Crohn's Disease, report of a case and review of the Literature. Inflamm Bowel Dis 2004;10:129-134.
49. Munck A, Murciana D, Pariente R, et al. Latent pulmonary function abnormalities in children with Crohn's disease. Eur Respir J 1995;8:377–380.
50. Al-Binali AM, Scott B, Al-Garni A, et al. Granulomatous pulmonary disease in a child: an unusual presentation of Crohn's disease. Pediatr Pulmonol 2003;36:76-80.
51. Bentur L, Lachter J, Koren I, et al. Severe pulmonary disease in association with Crohn's disease in a 13-year-old girl. Pediatr Pulmonol 2000;29:151–154.
52. Minic P, Perisic VN, Minic A. Case report of metastatic Crohn's disease. J Ped Gastroenterol Nutr 1998;27:338–341.
53. Calder CJ, Lacy D, Raafat F, et al. Crohn's disease with pulmonary involvement in a 3 year old child. Gut 1993;34:1636–1638.
54. Puntis JWL, Tarlow MJ, Raafat F, Booth IW. Crohn's disease of the lung. Arch Dis Childh 1990;65:1270–1271.
55. Lemann M, Messing B, D'Agay F, Modigliani R. Crohn's disease with respiratory tract involvement. Gut 1987;28:1669–1672.
56. Storch I, Sachar D, Katz S. Pulmonary manifestations of inflammatory bowel disease. Inflamm Bowel Dis 2003;9:104-15.
57. Tzanakis N, Bouros D, Samiou M, et al. Lung function in patients with inflammatory bowel disease. Respir Med 1998;92:516–522.
58. Neilly JB, Main ANH, McSharry C, et al. Pulmonary abnormalities in Crohn's disease. Respir Med 1989;83:487–491.

59. Pasquis P, Colin R, Denis P, et al. Transient pulmonary impairment during attacks of Crohn's disease. Respiration 1981;41:56–59.
60. Bewig B, Manske I, Bottcher H, et al. Crohn's disease mimicking sarcoidosis in bronchoalveolar lavage. Respiration 1999;66:467–9.
61. Winterbauer R, Lammert J, Selland M, et al. Broncheolar lavage cell populations in the diagnosis of sarcoidosis. Chest 1993;104:352–61.
62. Bonniere P, Wallaert B, Cortot A, et al. Latent pulmonary involvement in Crohn's disease: biological, functional, bronchoalveolar lavage and scintigraphic studies. Gut 1986;27:919–925.
63. Smiejan J-M, Cosnes J, Chollet-Martin S, et al. Sarcoid like lymphocytosis of the lower respiratory tract in patients with active Crohn's disease. Ann Intern Med 1986;104:17–21.
64. Wallaert B, Colombel JF, Tonnel AB, et al. Evidence of lymphocyte alveolitis in Crohn's disease. Chest 1985;87:363-7.
65. Valetta E, Bertini M, Sette L, et al. Early broncho-pulmonary involvement in Crohn disease: a case report. MNC Gastroenterology 2001;1:13-6.
66. Fries W, Grassi SA, Leone L, et al. Association between inflammatory bowel disease and sarcoidosis. Scand J Gastroenterol 1995;30:1221–1223.
67. Wirnsberger RM, de Vries J, Wouters EF, Drent M. Clinical presentation of sarcoidosis in The Netherlands an epidemiological study. Neth J Med 1998;53:53-60.
68. Bambery P, Kaur U, Bhusnurmath SR, Dilawari JB. Familial idiopathic granulomatosis: sarcoidosis and Crohn's disease in two Indian families. Thorax 1991;46:919–921.
69. Gronhagen-Riska C, Fyhrquist F, Hortling L, Koskimies S. Familial sarcoidosis and Crohn's disease. Lancet 1983;1:1287–1288.
70. Willoughby JMT, Mitchell DN, Wilson JD. Sarcoidosis and Crohn's disease in siblings. Am Rev Respir Dis 1971;104:249–253.
71. Hugot J-P, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599–603.
72. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603–6.
73. Hampe J, Franke A, Rosenstiel P, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nature Genet 2007;39:207–211.
74. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nature Genet 2007;39:596–604.
75. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–8.
76. Parkes M, Barret JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nature Genet 2007;39:830–2.
77. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004;5:800-8.

78. Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. Nature Rev Immunol 2007;7:767–77.
79. Cho JH, Abraham C. Inflammatory Bowel Disease Genetics: Nod2. Annu Rev Med 2007;58:401-16.
80. Fischer SA, Tremelling M, Anderson CA, et al. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet 2008;40:710–712.
81. Khan K, Schwarzenberg SJ, Sharp H, et al. Role of serology and routine laboratory tests in childhood inflammatory bowel disease. Inflamm Bowel Dis 2002;8:325-9.
82. Hoffenberg EJ, Fidanza S, Sauaia A. Serologic testing for inflammatory bowel disease. J Pediatr 1999;134:447-52.
83. Ruemmele FM, Targan SR, Levy G, et al. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. Gastroenterology 1998;115:822-9.
84. Sprague R, Harper P, McClain S, et al. Disseminated Gastro-intestinal sarcoidosis. Case report and review of the literature. Gastroenterology 1984;87:421-5
85. Abdullan BA, Gupta SK, Croffie JM et al. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. J Ped Gastroenterol Nutr 2002;35:636-40.
86. Tobin HM, Sinha B, Ramani P, et al. Upper gastrointestinal mucosal disease in pediatric Crohn disease and ulcerative colitis: a blinded, controlled study. J Ped Gastroenterol Nutr 2001;32:443-8.
87. Rosai J, Ackerman LV. Surgical Pathology. 9th ed. St Louis: Mosby, 2004. Chapter 11: gastrointestinal tract, p721-3.
88. Russo P, Brown K, Baldassano RN. Chapter 5: Colitis in childhood, p108-17. In: Russo P, Ruchelli E, Piccoli DA, eds. Pathology of Pediatric Gastrointestinal and Liver Disease. New York: Spinger, 2004.
89. Yantiss RK, Odze RD. Diagnostic difficulties in inflammatory bowel disease pathology. Review. Histopathology 2006;48:116-32.
90. Myers AL, Colombo J, Jackson MA, et al. Tuberculous colitis mimicking Crohn disease. J Ped Gastroenterol Nutr 2007;45:607-10.
91. Zhou ZY, Luo HS. Differential diagnosis between Crohn's disease and intestinal tuberculosis in China. Int J Clin Pract 2006;60:212-4.
92. Gan HT, Chen YQ, Ouyang Q, et al. Differentiation between intestinal tuberculosis and Crohn's disease in endoscopic biopsy specimens by polymerase chain reaction. Am J Gastroenterol 2002;97:1446-51.
93. Saczek KB, Schaaf HS, Voss M, et al. Diagnostic dilemmas in abdominal tuberculosis in children. Pediatr Surg Int 2001;17:111–5.
94. Bernhard JS, Bhatia G, Knauer CM. Gastrointestinal tuberculosis: an eighteen-patient experience and review. J Clin Gastroenterol 2000;30:397–402.
95. Pulimood AB, Ramakrishna BS, Kurian G, et al. Endoscopic mucosal biopsies are useful in distinguishing granulomatous colitis due to Crohn's disease from tuberculosis. Gut 1999;45:537–41.
96. Veeragandham RS, Lynch FP, Canty TG, et al. Abdominal tuberculosis in children: review of 26 cases. J Pediatr Surg 1996;31:170–6.

97. Marshall JB. Tuberculosis of the gastrointestinal tract and peritoneum. Am J Gastroenterol 1993;88:989–99.
98. Shah S, Thomas V, Mathan M, et al. Colonoscopic study of 50 patients with colonic tuberculosis. Gut 1992;33:347–51.
99. Bhansali SK. Abdominal tuberculosis. Experiences with 300 cases. Am J Gastroenterol 1977;67:324–37.
100. Basu S, Ganguly S, Chandra PK, Basu S. Clinical profile and outcome of abdominal tuberculosis in Indian children. Singapore Med J 2007;48:900-5.
101. Tanrikulu AC, Aldemir M, Gurkan F, et al. Clinical review of tuberculous peritonitis in 39 patients in Diyarbakir, Turkey. J Gastroenterol Hepatol 2005; 20:906-9.
102. Demir K, Okten A, Kaymakoglu S, et al. Tuberculous peritonitis--reports of 26 cases, detailing diagnostic and therapeutic problems. Eur J Gastroenterol Hepatol 2001; 13:581-5.
103. Lin YS, Huang YC, Chang LY, et al. Clinical characteristics of tuberculosis in children in the north of Taiwan. J Microbiol Immunol Infect 2005; 38:41-6.
104. Ibrahim M, Osoba AO. Abdominal tuberculosis. On-going challenge to gastroenterologists. Saudi Med J 2005; 26:274-80.
105. Sultan S, Azria F, Bauer P, at al. Anoperineal tuberculosis: diagnostic and management considerations in seven cases. Dis Colon Rectum 2002;45: 407–10.
106. Koniaris LG, Seibel JL. Tuberculosis presenting as a perirectal mass: report of a case. Dis Colon Rectum 2000; 43: 1604–5.
107. Candela F, Serrano P, Arriero JM, et al. Perianal disease of tuberculous origin: report of a case and review of the literature. Dis Colon Rectum 1999;42: 110–2.
108. Lee YJ, Yang SK, Byeon JS, et al. Analysis of colonoscopic findings in the differential diagnosis between intestinal tuberculosis and Crohn's disease. Endoscopy 2006;38:592-7.
109. Andronikou S,Welman CJ, Kader E. The CT features of abdominal tuberculosis in children. Pediatr Radiol 2002;32:75–81.
110. Lighter J, Rigaud M, Eduardo R, et al. Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold In-Tube test. Pediatrics 2009;123:30-7.
111. Bergamini BM, Losi M, Vaienti F, et al. Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. Pediatrics 2009;123:e419-24.
112. Bianchi L, Galli L, Moriondo M, et al. Interferon-gamma release assay improves the diagnosis of tuberculosis in children. Pediatr Infect Dis J 2009;28:510-4.
113. Anand BS, Schneider FE, Elzaatari FAK, et al. Diagnosis of intestinal tuberculosis by polymerase chain reaction on endoscopic biopsy specimens. Am J Gastroenterol 1994;89:2248–9.
114. Bhargava DK, Kushwaha AK, Dasarathy S, et al. Endoscopic diagnosis of segmental colonic tuberculosis. Gastrointest Endosc 1992;38:571-4.
115. Chatzicostas C, Koutroubakis IE, Tzardi M, et al. Colonic tuberculosis mimicking Crohn's disease: case report. BMC Gastroenterol 2002;2:10.
116. Arnold C, Moradpour D, Blum HE. Tuberculous colitis mimicking Crohn's disease. Am J Gastroenterol 1998;93:2294–6.

117. Hermansky F, Pudlak P. Albinism associated with hemorrhagic diathesis and unusual pigmented cells in the bone marrow; report of two cases with histochemical studies. Blood 1959;14:162–9.
118. Gahl WA, Brantly M, Kaiser-Kupfer MI et al. Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky–Pudlak syndrome). N Engl J Med 1998; 338: 1258–64.
119. Schinella RA, Greco MA, Cobert BL, et al. Hermansky-Pudlak syndrome with granulomatous colitis. Ann Intern Med 1980;92:20–3.
120. Kouklakis G, Efremidou EI, Papageorgiou MS, et al. Complicated Crohn's-like colitis, associated with Hermansky-Pudlak syndrome, treated with Infliximab: a case report and brief review of the literature. J Med Case Reports 2007;8;176.
121. Grucela AL, Patel P, Goldstein E, et al. Granulomatous enterocolitis associated with Hermansky-Pudlak syndrome. Am J Gastroenterol 2006;101:2090-5.
122. Erzin Y, Cosgun S, Dobrucali A, et al. Complicated granulomatous colitis in patient with Hermansky-Pudlak syndrome, succesfully treated with infliximab. Acta Gastroenterol Belg 2006;69:213-6.
123. Hazzan D, Seward S, Stock H, et al. Cohn's-like colitis, enterocolitis and perianal disease in Hermansky-Pudlak syndrome. Colorectal Dis 2006;8:539-43.
124. de Leusse A, Dupuy E, Huizing M, et al. Ileal Crohn's disease in a woman with Hermansky-Pudlak syndrome. Gastroenterol Clin Biol 2006;30:621-4.
125. GK, Sadler MA. Small bowel stricture in a woman with oculocutaneous albinism (Hermansky–Pudlak syndrome). Am J Rad 2000; 174: 1163–4.
126. Mahadeo R, Markowitz J, Fisher F, et al. Hermansky-Pudlak syndrome with granulomatous colitis in children. J Pediatr 1991;118;904–6.
127. Sherman A, Genuth L, Hazzi CG, et al. Perirectal abscess in the Hermansky-Pudlak syndrome. Am J Gastroenterol 1989;84:552–6.

CHAPTER 8

Differences in histology of biopsies of the gastrointestinal-tract in pediatric- and adult-onset inflammatory bowel disease

Damen GM [1]
Van Krieken JH [2]
De Jong D [3]
Van Rheenen R [1]
Nieuwenhuis EES [4]
Escher JC [4]

[1] Dept. of Pediatric Gastroenterology, Radboud University Nijmegen Medical Center
[2] Dept. of Pathology, Radboud University Nijmegen Medical Center
[3] Dept. of Gastroenterology, Radboud University Nijmegen Medical Center
[4] Dept. of Pediatric Gastroenterology, Erasmus MC- Sophia Children's Hospital, University Medical Center, Rotterdam

Submitted

Abstract

It is important to establish a correct diagnosis of Crohn's disease (CD) and ulcerative colitis (UC) as treatment options, course of disease and prognosis differ. Recent guidelines focusing on histological criteria of inflammatory bowel diseases (IBD) are mainly derived from adult patients. In this study, we studied histological criteria discriminating best between pediatric CD and UC and differences in histology between children and adults. In patients with a debut of IBD, histology of mucosal biopsies of the colon, terminal ileum and upper gastrointestinal-tract were reviewed blindly, using predetermined criteria. In children, histology of the colon showed that crypt distortion, basal plasmacytosis and transmucosal distribution of inflammation were not discriminative. Strong arguments for pediatric UC were diffuse crypt architectural irregularity, diffuse chronic inflammation, diffuse crypt epithelial polymorphs and villous transformation. In pediatric CD, crypt abscesses were present in 38% of the patients. Strong arguments for pediatric CD were discontinuous, focal or patchy chronic inflammation, focal crypt epithelial polymorphs and focal crypt irregularity; epitheloid granulomas were present in 46% of patients. In UC, crypt branching and distortion were more frequently present in children and mucin depletion in adults. In CD, epitheloid granulomas and polymorph exudates were more frequently present in children, basal plasmacytosis in adults. In CD chronic inflammation was mostly focal in adults and focal, patchy or diffuse in children. Also histology of the ileum and upper GI-tract demonstrated differences in pediatric- and adult-onset IBD. This study confirms differences in histology of biopsies of the gastrointestinal-tract in pediatric- versus adult-onset IBD.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD) characterized by chronic (intermittent) intestinal inflammation. The diagnosis of CD and UC is based on clinical signs and symptoms, radiology and endoscopy with histology. In pediatric IBD patients, the diagnostic work-up includes upper gastrointestinal (GI) endoscopy, colonoscopy with intubation of the terminal ileum and radiologic imaging of the small bowel (MRI or small bowel follow through).[1] Multiple biopsies from all segments of the GI-tract are needed for a complete histological evaluation.[1-4] It is essential to diagnose IBD early in the course of disease and to distinguish CD from UC, since choices in the clinical management, as well as the course and prognosis of the disease, depend on the type, location and extent of the disease at first presentation. In pediatric IBD any delay in diagnosis may result in delay of treatment, more pronounced growth retardation and suboptimal quality of life. In case of an incorrect diagnosis choices of treatment may be inadequate. In addition, the life-time risk of intestinal dysplasia and cancer, and the concomitant need for chemical prevention and/or endoscopic surveillance, is not the same in CD and UC, again illustrating the importance of a correct diagnosis. Once therapy is started it may become more and more difficult to distinguish UC from CD.[5,6] Histology changes and, in ongoing disease, crypt architecture may be restored, while inflammation may diminish. Eventually, this may result in segmental and discontinuous inflammation in patients with UC, seemingly more suggestive of a diagnosis of CD.

In adults with IBD, a large number of microscopic features has been evaluated including architectural changes, epithelial cell abnormalities and inflammatory features.[7-11] Architectural changes include crypt architectural irregularity, crypt branching, crypt distortion, crypt atrophy and surface irregularity.[9] Epithelial cell abnormalities are erosions, ulcerations, mucin depletion and Paneth cell metaplasia. Inflammatory features include polymorph exudates, crypt epithelial polymorphs, cryptitis, crypt abscesses, increased lamina propria cellularity (polymorphs and/or eosinophils), basal plasmacytosis and granulomas. The inflammation may be diffuse, patchy, focal, superficial and/or transmucosal. A gradient of inflammation along the colon, from proximal to distal, will support a diagnosis UC. In adults, a diagnosis of UC is based upon the combination of basal plasmacytosis, heavy, diffuse transmucosal lamina propria cell increase and widespread mucosal or crypt architectural distortion.[11] In adults the generally accepted microscopic features of CD are discontinuous and/or patchy chronic inflammation (with lymphocytes and plasma cells), focal crypt irregularity (with discontinuous crypt distortion) and granulomas (not related to crypt injury).[9]

In this study, criteria for biopsy diagnosis (including the recent ECCO criteria) are applied in children and adults with IBD.[7-11] In children, histological criteria that discriminate best between CD and UC were studied. Furthermore, we assessed differences in histology in pediatric- and adult-onset IBD.

Patients and methods

Patients that were included were referred to the (outpatient) clinic of Gastroenterology and Pediatric Gastroenterology at the Radboud University Nijmegen Medical Centre in 2008 or 2009. Fourteen children and 14 adults with CD as well as 14 children and 14 adults with UC were included; all with a first presentation of disease. Their patient characteristics are described in Table 1.

Table 1A: In patients with ulcerative colitis (UC), gender, age at diagnosis and disease localization are shown.

	Children with UC n=14	**Adults with UC n=14**
Age at diagnosis, median *(range)*	13 yrs *(7-16 yrs)*	36 yrs *(20-58 yrs)*
Female	6	7
Proctitis	3	6
Left-sided colitis	0	3
Extensive colitis	11	5

Tabel 1B: In patients with Crohn's disease (CD) , gender, age at diagnosis and disease localization are shown.

	Children with CD n=14	**Adults with CD n=14**
Age at diagnosis, median *(range)*	12 yrs *(5-16 yrs)*	27 yrs *(19-76 yrs)*
Female	6	7
Upper GI	3	2
Ileum only	1	2
Colon only	3	2
Ileum+colon	10	10

Clinical data of the patients were studied in detail, including the results of upper GI-endoscopy, ileocolonoscopy and small bowel follow through (SBFT). Biopsies obtained from the upper and lower GI-tract (2 or more from each segment) were formalin fixed, paraffin embedded and stained with haematotoxylin and eosinophil. Histology of these patients was assessed by a pathologist (HvK) and a pediatric gastroenterologist (GD), who were both blind to the endoscopic outcome and the diagnosis. Histology of the colon was reviewed and the most affected biopsy specimen of the colon was scored according to a scoring system based on the study of Bentley et al and again on recent ECCO criteria (Appendix A).[8,9,11] Histology of the terminal ileum was scored according to a scoring system based on the study of D'Haens et al. as well as on recent ECCO criteria.[7,8] Histology of the upper GI-tract was studied without a predefined scoring system.

Colonoscopy

Table 1 shows the macroscopic localization of the disease in the colon in pediatric- and adult-onset IBD. In pediatric-onset CD, colonoscopy demonstrated a normal mucosa (n=2), segmental colitis (n=4), apthoid lesions or circumscript ulcera in each segment of the colon (n=3), proximal colitis or vulnerability of the cecum (n=3) or extensive colitis (n=2). In adult-onset CD, colonoscopy demonstrated a normal mucosa (n=1), segmental colitis (n=4), apthoid ulcerations in each segment of the colon (n=2), active (apthoid) ulcerative inflammation of the cecum and/or proximal colon with and without the transverse colon (n=5), necrotizing colitis (n=1) or extensive colitis (n=1).

Appendix A: scoring system for histological investigations of biopsies of the colon (refs 8,9 and 11)

Architecture	
• crypt architectural irregularity	absent / focal / diffuse
• crypt distortion	absent / present
• crypt branching	absent / present
• reduced crypt numbers / atrophy	no / yes
• irregular surface (villous transf.)	no / yes
Polymorph inflammation	
• crypt abscess	no / yes
• cryptitis	no / yes
• lamina propria polymorphs	absent / present
• lamina propria eosinophils	absent / present
• crypt epithelial polymorphs	absent / focally increased / diffusely increased
• polymorph exudates	absent / present
Chronic inflammation	
• increase in intensity	no / focal / patchy / diffuse
• distribution	superficial / transmucosal
• (increase in) basal plasma cells	no / yes
• granulomas	absent / epitheloid / mucin
Epithelial changes	
• erosion / ulceration	absent / present
• mucin	preservation / depletion
• Paneth cells distal to hepatic flex	absent / present
Epithelial associated changes	
• increased intraepith. lymphocytes > 15	absent / present
• Increased subepithelial collagen	absent / present
Comparison between different segments	
• Distribution of inflammation along the colon: gradient from proximal to distal	yes / no
• Nr of biopsies focal cell infiltration : nr biopsies mononuclear cell infiltration	… / …

Ileoscopy

In pediatric-onset CD, the mucosa of the ileum was normal (n=3) or there was ileitis (n=6). In 5 patients, the ileum could not be intubated (due to obstruction or technical reasons); SBFT demonstrated ileitis terminalis in 4 of them. In pediatric-onset UC, intubation of the ileum was successful in 13 patients, only 1 patient had slight redness of the mucosa of the ileum (in the presence of pancolitis). In adult-onset CD, the mucosa of the ileum was normal (n=2) or there was ileitis (n=4). In 8 patients the ileum was not intubated (due to obstruction, pain or technical reasons); in 7 of them SBFT showed ileitis terminalis and/or a stenosis. In adult-onset UC, the diagnostic work-up was limited to endoscopy of the transverse and descending colon (none of the patients had undergone ileoscopy).

Upper gastrointestinal endoscopy

In pediatric-onset CD, upper GI-endoscopy had been performed in 10 out of 14 patients. Mucosal abnormalities were demonstrated in 4 patients, including apthoid lesions, erosions and/or ulcerations in the duodenum (n=3), the stomach (n=3) and redness in the esophagus (n=2). In pediatric-onset UC, upper GI-endoscopy had been performed in 9 out of 14 patients. Mucosal abnormalities were demonstrated in 3 patients, including edema, redness and vulnerability in the stomach (n=2) and the duodenal bulb (n=1).

In adult-onset CD, only in 2 out of the 14 patients upper GI-endoscopy had been performed. In both patients there was inflammation of the stomach; one of them also had erosions and ulcerations in the duodenal bulb. Upper GI-endoscopy had not been performed in adult-onset UC patients.

Results

Histology of the most affected biopsies of the colon

Table 2 shows the results from the histological scoring of the most affected biopsies of the colon in children with CD and UC. In children, chronic inflammation was diffuse in 93% of the UC patients and patchy or focal in 77% of the CD patients. In children with UC, crypt epithelial polymorphs and crypt architectural irregularity was diffusely present in 43-75% of the patients; both items were absent or focally present in CD. In 46% of the children with CD epitheloid granulomas were present. Crypt distortion and transmucosal distribution of chronic inflammation were frequently present both in CD and UC, whereas basal plasmacytosis was more frequently present in children with CD than in UC. Crypt branching and crypt abscesses were more frequently seen in children with UC than in CD, whereas an irregular surface or villous transformation was seen only in UC. In children with IBD, epithelial associated changes like increased intra-epithelial lymphocytes and increased subepithelial collagen were absent.

Table 2: Histological score of the most affected biopsies of the colon (% of patients)	**CD adults (n=14)**	**ped CD (n=14)**	**ped UC (n=14)**	**UC adults (n=14)**
Crypt architectural irregularity diffusely	0	0	75 %	86 %
Crypt architectural irregularity focally	71 %	77 %	18 %	7 %
Crypt architectural irregularity absent	29 %	23 %	7 %	7 %
Crypt distortion	57 %	62 %	86 %	64 %
Crypt branching	7 %	23 %	68 %	36 %
Reduced crypt numbers or atrophy	14 %	15 %	32 %	21 %
Irregular surface or villous transformation	0	0	50 %	64 %
Crypt abscess	29 %	38 %	71 %	71 %
Cryptitis	86 %	85 %	93 %	93 %
Lamina propria polymorphs	100 %	85 %	100 %	100 %
Lamina propria eosinophils	86 %	85 %	86 %	100 %
Crypt epithelial polymorphs diffusely	0	0	43 %	79 %
Crypt epithelial polymorphs focally	100 %	92 %	43 %	21 %
Crypt epithelial polymorphs absent	0	8 %	14 %	0
Polymorph exudates	0	35 %	25 %	29 %
Chronic inflammation diffusely	7 %	15 %	93 %	100 %
Chronic inflammation patchy	0	35 %	7 %	0
Chronic inflammation focally	93 %	42 %	0	0
Chronic inflammation no increase in intensity	0	9 %	0	0
Distribution transmucosal	100 %	100 %	93 %	100 %
Distribution superficial	0	0	7 %	0
(Increase in) basal plasma cells	64 %	27 %	54 %	57 %
Granulomas epitheloid	17 %	46 %	0	0
Granulomas mucin	0	9 %	7 %	7 %
Erosions and/or ulcerations	21 %	38 %	39 %	50 %
Mucin depletion	17 %	38 %	36 %	71 %
Paneth cells distal hepatic flexure	0	9 %	7 %	0
Epithelial associated changes [#]	0	0	0	0
Distribution along the axis (distal most affected)	0	43 %	55 %	? [*]
All biopies showing focal cell infiltration	93%	divers	0	0
All biopsies mononuclear cell infiltration	0	divers	93 %	100 %

This scoring system is based on three publications, namely on the study of Bentley et al and on recent ECCO criteria.[8,9,11]

[#] None of the patients had epithelial associated changes, such as an increased number of intra-epithelial or subepithelial lymphocytes.

[*] In a number of patients the distribution of the biopsies along the axis could not be determined, since biopsies from different segments had been put together in one vial.

Table 2 also shows the results from the histological scoring of the most affected biopsies of the colon in pediatric-onset compared to adult-onset IBD. In UC, children more often demonstrated crypt branching and slightly more often demonstrated crypt distortion in comparison to adults, whereas mucin depletion was more common in adults. In adult-onset CD chronic inflammation was mostly focal (93%), while in pediatric-onset CD chronic inflammation was focal (42%), patchy (35%) or diffuse (15%). Crypt architectural irregularity was focal in about 75% of both children and adults with CD. Epitheloid granulomas were less frequently present in adult-onset CD (17%) than in pediatric-onset CD (46%), while basal plasmacytosis was more frequently present in adults.

Histology of the ileum

In pediatric-onset UC, histology of the biopsies of the terminal ileum was completely normal in 12 of the 13 patients, only in 1 patient histology demonstrated focal epithelial damage with a moderate increase in polymorphonuclear cells in the epithelium, cryptitis and crypt abscesses.
In pediatric- and adult-onset CD, results of histology of biopsies of the terminal ileum are demonstrated in Table 3.

Table 3: Histology of the terminal ileum [7,8]	pediatric CD (n=9)	CD adults (n=6)
Architectural changes < 50% disturbed	33 %	17 %
Architectural changes > 50% disturbed	33 %	17 %
Villus irregularity	56 %	33 %
Crypt architectural irregularity - focal	56 %	33 %
Crypt architectural irregularity - diffuse	0	0
Epithelial damage or change - focal	56 %	50 %
Epithelial damage or change - extensive	11 %	0
Polymorphonuclear cells in surface epithelium	33 %	33 %
Cryptitis	11 %	50 %
Crypt abscess	0	0
Mononuclear cells lamina propria - moderate	61 %	50 %
Mononuclear cells lamina propria - severe	11 %	0
Polymorphonuclear cells epithelium - moderate	61 %	83 %
Polymorphonuclear cells epithelium - severe	11 %	0
Epitheloid granulomas	44 %	0

This scoring system is based on two studies, namely on the study of D'Haens et al. and on recent ECCO criteria.[7,8]
Granulomas were present in 4 (of 9) children with CD, and were absent in adult-onset CD. There were no crypt abscesses and cryptitis was more frequently present in adult-onset CD than in children.

Histology of the upper GI-tract

In pediatric-onset CD, 10 patients underwent upper GI-endoscopy. Histology of the biopsies of the duodenum demonstrated patchy or more diffuse chronic inflammation (n=2). Histology of the biopsies of the stomach demonstrated epitheloid granulomas (n=2), focally enhanced gastritis (n=5) and a more diffuse gastritis in one patient (all *Helicobater Pylori* negative). Biopsies of the esophagus demonstrated active inflammation in 1 patient. In pediatric onset UC, 9 patients underwent upper GI-endoscopy. Histology of the biopsies of the duodenal bulb demonstrated inflammation (n=2). Histology of the biopsies of the stomach demonstrated focal chronic active inflammation (n=2) and focally enhanced gastritis (n=3), in 1 patient there was a crypt-associated granuloma in the mucosa of the stomach as is depicted in Figure 1 (all *Helicobacter Pylori* negative). In adult-onset IBD, only 2 patients with CD underwent upper GI-endoscopy. Histology of biopsies of the stomach demonstrated granulomas (n=1), HP-associated gastritis (n=1) and focally enhanced gastritis (n=2).

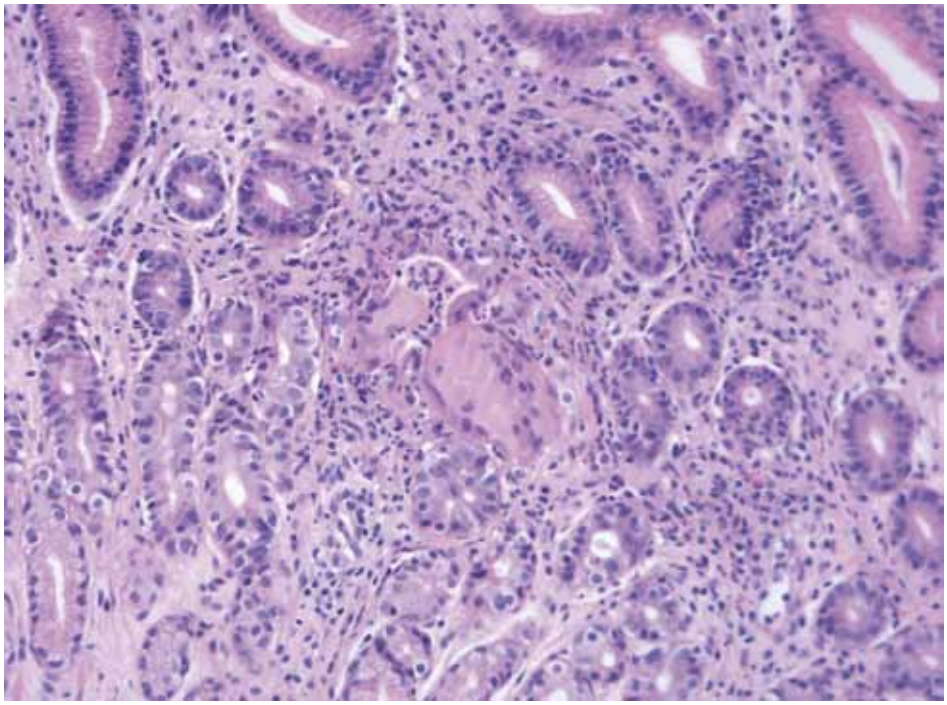


Figure 1 demonstrates a crypt-associated granuloma in the mucosa of the stomach in a child with UC (14 yr, with an extensive colitis and normal ileum; x250). The picture shows a foveolus that is damaged by an acute, granulocytic inflammation. A crypt abcess is still present and a large multinucleated histiocyte is located attached to the damaged epithelium. Since this is presumed to be a respons to mucin, this lesion is often referred to as mucin granuloma.

Discussion

In pediatric-onset IBD, histology of the most affected biopsies of the colon demonstrated that diffuse crypt architectural irregularity, diffuse chronic inflammation and diffuse crypt epithelial polymorphs were strong arguments for UC, while discontinuous, focal or patchy chronic inflammation, focal crypt epithelial polymorphs and focal crypt irregularity were strong arguments for CD. In pediatric-onset UC, crypt branching and crypt abscesses were more frequently present than in CD. In children, irregular surface or villous transformation was only seen in UC. In 46% of the children with CD, epitheloid granulomas were present in the most affected biopsies of the colon. Abnormalities such as crypt distortion, basal plasmacytosis and a transmucosal distribution of chronic inflammation were frequently present both in pediatric-onset CD and UC, and were not discriminative.

In comparison to adult-onset IBD, histology of the most affected biopsies of the colon was clearly different in pediatric-onset IBD. In UC, crypt distortion and especially crypt branching were more frequently present in children, while mucin depletion was more frequently present in adults. In pediatric-onset UC, crypt epithelial polymorphs were present both focally and diffusely, while in adult-onset UC crypt epithelial polymorphs were more diffusely present. In CD, polymorph exudates and epitheloid granulomas were more frequently present in children, while basal plasmacytosis was more frequently present in adults. In pediatric-onset CD, chronic inflammation was focal, patchy or diffuse, while chronic inflammation was mostly focal in adult-onset CD. Differences in histology in pediatric and adult onset IBD might be explained by several causes.[12] In the pathogenesis of IBD genetic mutations are thought to play a larger role in pediatric-onset IBD than in adult-onset IBD. Contributions of the innate immune defects to the pathogenesis of IBD seem to be inversely related to the age of disease onset. Early onset IBD may have a more severe clinical presentation as compared to adults. And finally, disease location is different in pediatric onset IBD compared to adult onset IBD. In pediatric-onset CD, there is more upper GI involvement, less isolated ileum involvement and more colonic involvement compared to adult-onset CD. In UC, extensive colitis is more frequently present in children than in adults.[14-16] In our study, 79% of the children with UC had an extensive colitis compared to 36% of the adult patients. Remarkably, one child with UC demonstrated a proctitis and a patchy lesion in the cecum. This occurrence is quite exceptional, but has been published in adults with UC.[17-19] Furthermore, it is important to notice that in UC, crypt-associated granulomas may be present, but not epitheloid granulomas.[10,20]

In this study, not every patient with adult-onset UC underwent complete colonoscopy. One could argue that every patient with a first presentation of IBD should undergo complete colonoscopy. Bentley et al. demonstrated that histological judgment of full colonoscopic series resulted in a far more accurate diagnosis of the type of IBD compared with the judgment of rectal biopsies alone.[8] On the rectal biopsies only 18% of reference CD cases were correctly diagnosed, whereas 62% were correct with the full series. The percentage of UC cases correctly diagnosed improved only from 64% using the rectal biopsies to 74% with the full series. The same is true in pediatric-onset

IBD, where sigmoidoscopy will be insufficient.[13]

In children and adults with CD, SBFT demonstrated ileitis terminalis in respectively 71 and 79% of the patients. When endoscopy of the ileum was successful, aphtoid lesions, erosions and/or ulcerations were demonstrated in 44% of the children with CD and in 67% of the adults with CD. Histology, on the other hand, demonstrated remarkable differences. Histology of the ileum demonstrated granulomas in 44% of the children with CD and none in the adults. Next to the endoscopic findings, histology of the ileum demonstrated erosions and/or ulcerations in 44% of the children with CD, however, in adults, hardly any erosions or ulcerations were seen in histology; in these patients cryptitis was more frequently present than in children. We were not able to find a difference in the way biopsies were taken in children and adults.

Upper GI-endoscopy is included in the diagnostic work-up of pediatric-onset IBD. In our study, in pediatric-onset IBD, apthoid lesions, erosions and/or ulcerations in the upper GI-tract predisposed to CD. Literature however demonstrated that these lesions may also occur in pediatric-onset UC.[21-23] Granulomas in the upper GI tract have been demonstrated in 24 to 40% of the children with CD.[2,21-27] Even without mucosal abnormalities, it is important to take biopsies from each segment of the upper GI-tract. In 20% of the children with CD in our study, epitheloid granulomas were identified in the upper GI-tract with normal endoscopic findings. In pediatric-onset CD, other studies showed that epitheloid granulomas were found solely in the upper GI-tract in a rather high percentage (even up to 39%).[22,23] Another frequent finding in pediatric-onset IBD is focally enhanced gastritis, which is more often present in CD than in UC. Focally enhanced gastritis however does not discriminate between both disease entities and can also be found in other diseases.[23,26,28-31]

In conclusion, our study reveals that there are important histological differences in pediatric-onset and adult-onset IBD. When the pathologist is aware of these, he or she will be able to establish a correct diagnosis of IBD more accurately.

References

1. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis – the Porto criteria. J Ped Gastroenterol Nutr 2005;41:1-7
2. Mashako MN, Cezard JP, Navarro J, Mougenot JF, Sonsino E, Gargouri A, Maherzi A. Crohn's disease lesions in the upper gastrointestinal tract: correlation between clinical, radiological, endoscopic, and histological features in adolescents and children. J Ped Gastroenterol Nutr 1989;8:442-6.
3. Sanderson IR, Boyle S, Williams CB, Walker-Smith JA. Histological abnormalities in biopsies from macroscopically normal colonoscopies. Arch Dis Child 1986;61:274-7.
4. Dejaco C, Oesterreicher C, Angelberger S, Püspök A, Birner P, Poetzi R, et al. Diagnosing colitis: a prospective study on essential parameters for reaching a diagnosis. Endoscopy 2003;35:1004-8.

5. Bernstein CN, Shanahan F, Anton PA, Weinstein WM. Patchiness of mucosal inflammation in treated ulcerative colitis: a prospective study. Gastrointest Endosc 1995; 42: 232-237.
6. Geboes K, Dalle I. Influence of treatment on morphological features of mucosal inflammation. Gut 2002;50(Suppl III): iii37-iii42.
7. D'Haens G, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenteroloy 1998;114:262-7
8. Bentley E, Jenkins D, Campbell F, Warren B. How could pathologists improve the initial diagnosis of colitis? Evidence from an international workshop. J Clin Pathol 2002;55:955-60
9. Stange EF, Travis SPL, Vermeire S, Bedlinger C, Kupcinskas L, Geboes K, Barakauskiene et al. for the European Crohn's and Colitis Organisation (ECCO). European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut 2006;55(suppl I):i1-i15
10. Differentiating Ulcerative Colitis from Crohn Disease in Children and Young Adults: Report of a Working Group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. J Ped Gastroenterol Nutr 2007;44:653-74
11. Stange EF, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, et al. for the European Crohn's and Colitis Organisation (ECCO). European evidence-based Consensus on the diagnosis and management of Ulcerative colitis: Definitions and diagnosis. J Crohn's Colitis 2008;2:1-23
12. Nieuwenhuis EES, Escher JC. Early onset IBD: What's the difference? Dig Liver Dis 2008;40:12-15
13. Escher JC, ten Kate F, Lichtenbelt K, Schornagel I, Büller H, Derkx B, Taminiau J. Value of rectosigmoidoscopy with biopsies for diagnosis of inflammatory bowel disease in children. Inflamm Bowel Dis 2002;8:16-22.
14. Kugathasan S, Judd RH, Hoffmann RG, Heikenen J, Telega G, Khan F, et al.; Wisconsin Pediatric Inflammatory Bowel Disease Alliance. Epdidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. J Pediatr 2003;143:525-31.
15. Sawczenko A, Sandhu BK. Presenting features of inflammatory bowel disease in Great Britain and Ireland. Arch Dis Child 2003;88:995-1000.
16. Griffiths AM. Specificities of inflammatory bowel disease in childhood. Best Pract Res Clin Gastroenterol 2004;18:509-23.
17. D'Haens G, Geboes K, Peeters M, Baert F, Ectors N, Rutgeerts P. Patchy cecal inflammation associated with distal ulcerative colitis: a prospective endoscopic study. Am J of Gastroenterology 1997;92:1275-9.
18. Mutinga ML, Odze RD, Wang HH, Hornick JL, Farraye FA. The clinical significance of right-sided colonic inflammation in patients with left-sided chronic ulcerative colitis. Inflamm Bowel Dis 2004;10:215-9.
19. Dendrinos K, Cerda S, Farraye FA. The "cecal patch" in patients with ulcerative colitis. Gastrointest Endosc 2008;68:1006-7.
20. Mahadeva U, Martin JP, Patel NK, Price AB. Granulomatous ulcerative colitis: a re-appraisal of the mucosal granuloma in the distinction of Crohn's disease from ulcerative colitis. Histopathology 2002;41:50-5.
21. Ruuska T, Vaajalahti P, Arajarvi P, Maki M. Prospective evaluation of upper gastrointestinal mucosal lesions in children with ulcerative colitis and Crohn's disease. J Ped Gastroenterol Nutr 1994;19:181-6.

22. Tobin JM, Sinha B, Ramani P, Saleh AR, Murphy MS. Upper gastrointestinal mucosal disease in pediatric Crohn disease and ulcerative colitis: a blinded, controlled study. J Ped Gastroenterol Nutr 2001;32:443-448.
23. Abdullah BA, Gupta SK, Croffie JM, Pfefferkorn MD, Molleston JP, Corkins MR, Fitzgerald JF. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. J Ped Gastroenterol Nutr 2002; 35: 636-640.
24. Lenaerts C, Roy CC, Vaillancourt M, Weber AM, Morin CL, Seidman E. High incidence of upper gastrointestinal tract involvement in children with Crohn disease. Pediatrics 1989;83:777–81.
25. Schmidt-Sommerfeld E, Kirschner BS, Stephens JK. Endoscopic and histologic findings in the upper gastrointestinal tract of children with Crohn's disease. J Ped Gastroenterol Nutr 1990;11:448–54.
26. Castellaneta SP, Afzal NA, Greenberg M, Deere H, Davies S, Murch SH, et al. Diagnostic role of upper gastrointestinal endoscopy in pediatric inflammatory bowel disease. J Ped Gastroenterol Nutr 2004;39:257-61.
27. Lemberg DA, Clarkson CM, Bohane TD, Day AS. Role of esophagogastroduodenoscopy in the initial assessment of children with inflammatory bowel disease. J Gastroenterol Hepatol 2005;20:1696-700.
28. Parente F, Cucino C, Bollani S, Imbesi V, Maconi G, Bonetto S, et al. Focal gastric inflammatory infiltrates in inflammatory bowel diseases: prevalence, immunohistochemical characteristics, and diagnostic role. Am J Gastroenterol 2000;95:705-711.
29. Kundhal PS, Stormon MO, Zachos M, Critch JN, Cutz E, Griffiths AM. Gastral antral biopsy in the differentiation of pediatric colitides. Am J Gastroenterol 2003; 98: 557-561.
30. Oberhuber G, Puspok A, Oesterreicher C, Novacek G, Zauner C, Burghuber M, et al. Focally enhanced gastritis: a frequent type of gastritis in patients with Crohn's disease. Gastroenterology 1997;112:698-706.
31. Sharif F, McDermott M, Dillon M, Drumm B, Rowland M, Imrie C, et al. Focally enhanced gastritis in children with Crohn's disease and ulcerative colitis. Am J Gastroenterol 2002;97:1415-1420.

CHAPTER 9

Catch-up growth is often lacking in patients with childhood-onset Inflammatory Bowel Disease

Damen GM *[1]
van Rheenen R *[1]
Tolboom JJM [1]
Otten BJ [1]
de Jong D [2]
van der Woude CJ [3]
Nieuwenhuis EES [4]
Escher JC [4]

* the first two authors contributed equally

[1] Dept. of Pediatrics, Radboud University Nijmegen Medical Center
[2] Dept. of Gastroenterology, Radboud University Nijmegen Medical Center
[3] Dept. of Gastroenterology, Erasmus University Medical Center, Rotterdam
[4] Dept. of Pediatric Gastroenterology, Erasmus MC- Sophia Children's Hospital, University Medical Center, Rotterdam

Submitted

Abstract

Background

Growth failure is a unique feature of inflammatory bowel disease (IBD) in children. Treatment aimed to reverse growth failure are not always successful. Jejunal disease, the degree of growth failure at diagnosis and corticosteroids have been attributed to influence this outcome.

Objectives

We assess growth failure at the time of diagnosis, progression of growth in the years thereafter and adult height in patients with childhood-onset IBD.

Methods

Growth was analyzed in 318 patients with childhood-onset IBD (Crohn's disease [CD] n=178; ulcerative colitis [UC] n=131; unclassified colitis n=9). A total of 162 patients already reached adulthood.

Results

At diagnosis, patients had a mean height SDS of -0.8 in CD and -0.4 in UC, which was significantly different ($p<0.01$). Height at diagnosis significantly correlated to the diagnostic delay ($p<0,001$). The first 5 years of treatment catch-up growth was often disappointing. Especially children with UC and severe growth retardation were prone for persistent growth failure. At an adult age, the mean height SDS was -0.7 in CD and -0.4 in UC. Adult height was significantly correlated with height at diagnosis ($p<0,001$). There was no correlation between adult height and localization of disease, disease activity or therapy. Height expressed in SDS did not clearly improve in the group of patients treated with infliximab (n=39). Several CD patients still had important growth (up to 13 cm) after the age of 18 years, until their early twenties.

Conclusion

Height at diagnosis (expressed in height SDS) is the most important predictor of adult height.

Introduction

Crohn's disease (CD), ulcerative colitis (UC) and unclassified colitis are chronic inflammatory diseases of the gastrointestinal tract. The incidence of inflammatory bowel disease (IBD) in children is 3.1 to 5.7 per 100.000 in Northern Europe.[1-6] Growth failure is one of unique features of childhood-onset IBD, and is present in 10% to 40% of the affected children at diagnosis.[7-14] Variation in the reported prevalence is dependent on the definition of growth failure, and may be dependent on both the age of the children included and the study population (population based or tertiary referral centre).[15] In IBD, growth failure is ascribed to a complex interaction between nutritional status, inflammation, disease activity and severity, and genotype.[16-17] This complex interaction causes resistance to the effects of growth hormone.[18] In animal models, inflammatory cytokines such as TNF-alpha and IL-6 have been shown to suppress IGF-1 concentrations.[19-21]
Treatments aimed at reversing growth failure need to address nutrition and also target the underlying inflammatory process.[15,17] The use of corticosteroids has been one of the mainstays in the treatment of children with IBD, however, one of the adverse effects of corticosteroids is inhibition of growth. In one study of children with CD, the use of corticosteroids *in puberty* did have a negative effect on adult height.[22] Other studies were not able to demonstrate a relationship between the use of corticosteroids and adult height.[13,23] In general, growth suppression due to suboptimal control of disease will have more negative impact on growth than the adverse affects of corticosteroids.[8] Control of disease will be better by using immunosuppressives such as 6-mercaptopurine (6-MP).[24] However, there was no significant difference in linear growth over an 18 month period in children with CD treated with 6-MP and the control group. Usage of the chimeric monoclonal antibody to TNF-alpha has been shown to be effective in children with CD.[25-34] In this patient group growth data are encouraging, but more long term follow-up data are not yet available.[33,34] In children with CD, enteral feeding has been shown to improve growth velocity.[15,17,35-40] Nonetheless, long term usage of enteral feeding in children with IBD is exceptional, and it is unknown whether the improvement of growth early in the treatment does result in a better adult height. Finally, on the premises that surgery results in a clearly better control of disease, surgery (including colectomy) may be necessary in order to obtain catch-up growth in patients with IBD in case of persistent growth failure.[10,11,41-48]
Reports on adult height demonstrate that in patients with childhood-onset UC adult height was normal.[11,23] In patients with childhood-onset CD, data on adult height are conflicting. Two studies reported that despite the high prevalence of growth failure in children with CD, adult height was normal.[9,22] Other studies showed that almost 20% of the patients with childhood-onset CD had an adult height 8 cm or more below their TH (i.e. below the TH range).[10,11,13] In one of the studies, deficits in final adult height was correlated with jejunal disease and growth failure at the time of diagnosis.[13] To a certain extent, delayed maturation and delayed puberty seem to compensate for the period of poor growth earlier in life.[11]

The aim of this study was to assess growth failure at time of diagnosis, progression of linear growth in the years after diagnosis and final adult height in patients with childhood-onset IBD.

Patients and Methods

Patients diagnosed with childhood-onset IBD, born after 1960, were included in this retrospective study. Patients were included from the department of Pediatric Gastroenterology and the department of Gastroenterology and Hepatology of two academical centers in the Netherlands. All patients were diagnosed according to the established criteria, based upon clinical, radiological, endoscopic and/or histological findings.[4] Data collected from the medical records were age at diagnosis, diagnostic delay (defined as the duration of symptoms preceding a definite diagnosis), disease localization, disease activity, medication and co-existence of other medical conditions such as celiac disease and chromosomal abnormalities. Pubertal stage at onset of disease was defined by means of chronological definitions: pre-pubertal onset of disease was defined as onset before the age of 13 years in boys, and before the age of 11 years in girls.

Heights

The heights of patients were collected from the patient's medical record. These heights were measured by a physician or nurse. When heights were lacking from the age of 18 years and onwards, or when the parental height was lacking, adults with childhood-onset IBD treated within the University MC St Radboud received a questionnaire in order to obtain the TH and the adult height. TH was calculated by means of the following formulas: Male TH = [paternal height + (maternal height + 13)] / 2 (in cm), and Female TH = [maternal height + (paternal height – 13)] / 2 (in cm). If the TH was available, adult height was corrected for TH. All height data were converted to standard deviation scores (SDS) using the 1997 TNO/LUMC Growth-investigation and/or the patients respective TH.

Statistical analysis

Where possible mean, standard deviation and range were calculated. Pearson´s correlations were analyzed between adult height and possible etiological factors.

Results

Patient Characteristics

A total of 318 patients with childhood-onset IBD were included. Patient characteristics are shown in Table 1. The study included 178 patients with CD, 131 patients with UC and 9 patients with unclassified colitis. At the Radboud University MC 155 patients were included and 163 patients were included at the Erasmus University MC. A total of 162 patients already reached adulthood (> 18 years of age).

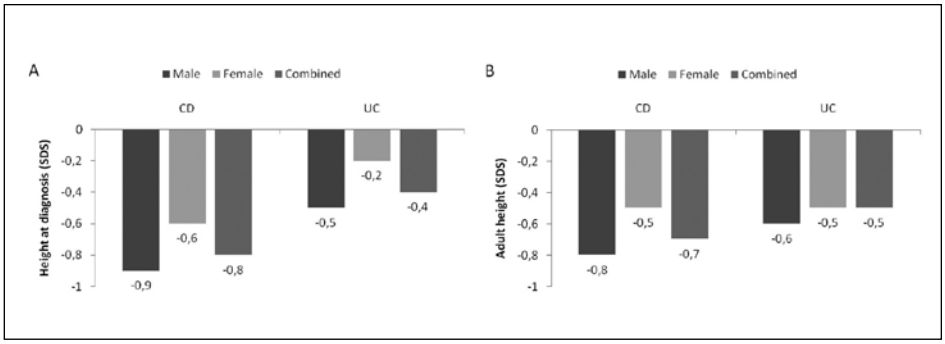
Table 1: Patients characteristic of 318 patients with childhood-onset IBD. An artificial division is shown in patients still < 18 years of age (at the end of follow-up) and patients already > 18 years of age.

	Patients (n=318)	Children (n=156)	adults (n=162)
Age diagnosis (yrs; mean/range)	12 (1-17)		
Age follow-up (yrs; mean/range)	21 (1-59)	14 (1-17)	27 (19-59)
Crohn's disease	178 (56%)	81 (52%)	97 (60%)
Ulcerative colitis	131 (41%)	67 (43%)	64 (40%)
Unclassified colitis	9 (3%)	8 (5%)	1 (1%)
Male	165 (52%)	77 (49%)	88 (54%)
Caucasian	287 (90%)	135 (87%)	152 (94%)
Prepubertal onset	143 (45%)	93 (60%)	50 (31%)
Medication used at study entry:			
mesalazine / 5-ASA	280 (88%)	127 (81%)	153 (94%)
Azathioprine	192 (60%)	111 (71%)	81 (50%)
Prednisone	238 (75%)	112 (72%)	126 (78%)
Infliximab	53 (17%)	37 (24%)	16 (10%)
Needing operation	56 (18%)	19 (12%)	37 (23%)

Height at Diagnosis

As shown in Figure 1A and Table 2, data on height at diagnosis were available in 215 of the 318 patients (67%). Height at diagnosis (expressed in SDS) was significantly lower ($p<0.01$) in children with CD compared to UC; at diagnosis, the mean height SDS was -0.8 in CD patients (range -3.1 to 1.5) and -0.4 in UC patients (range -3.5 to 2.5). Both in CD and UC patients, height SDS at diagnosis was more negative in males than in females, however this difference was not statistically significant ($p=0.15$ and $p=0.23$ respectively). The diagnostic delay was not significantly different ($p=0.06$) in patients with CD compared to UC (with a diagnostic delay of 1.1 and 0.7 years respectively). There was a significant negative relationship ($p<0.01$) between the height at diagnosis and the diagnostic delay, in patients with childhood-onset CD and UC. Both parental

Figure 1: Figure 1A demonstrates the mean height SDS at diagnosis in children with CD (n=118) and UC (n=89). Figure 1B demonstrates the mean height SDS in adults with childhood-onset CD (n=67) and UC (n=30). Adult height was measured at a mean age of 23 years (range 18 - 46).



height and height at diagnosis were available in 133 IBD patients. Corrected for the TH, the mean height SDS at diagnosis was -0.9 in CD and -0.5 in UC. After correction for the TH, the height at diagnosis was still significantly correlated to the diagnostic delay.

Table 2: height at diagnosis of 118 pediatric CD patients and 89 pediatric UC patients (compared to the general Dutch population)

Height (in SDS)	Dutch population	UC patients	CD patients	UC+CD patients
> 2	2 %	1 %	0 %	0 %
1 to 2	16 %	11 %	5 %	7 %
0 to 1	32 %	30 %	19 %	26 %
-1 to 0	32 %	24 %	34 %	29 %
-2 to -1	16 %	27 %	31 %	29 %
< -2	2 %	7 %	11 %	9 %

Change in Height before and after diagnosis

Both in CD and UC, growth was analysed in the years preceding diagnosis. In CD patients, mean height SDS decreased from -0.5 to -0.8 in the three years preceding diagnosis. In UC patients no such decrease was observed. As is demonstrated in figure 2, there is hardly catch-up growth in CD and UC patients the first 5 years of treatment. This does not mean that catch-up growth was absent in every patient. Individual patients did have complete catch-up growth, as is demonstrated in Appendix A.

In order to study catch-up growth in a more detailed way, subgroups of patients were analyzed separately. Figure 3 demonstrates the growth of subgroups of patients (CD and UC) in the first 5

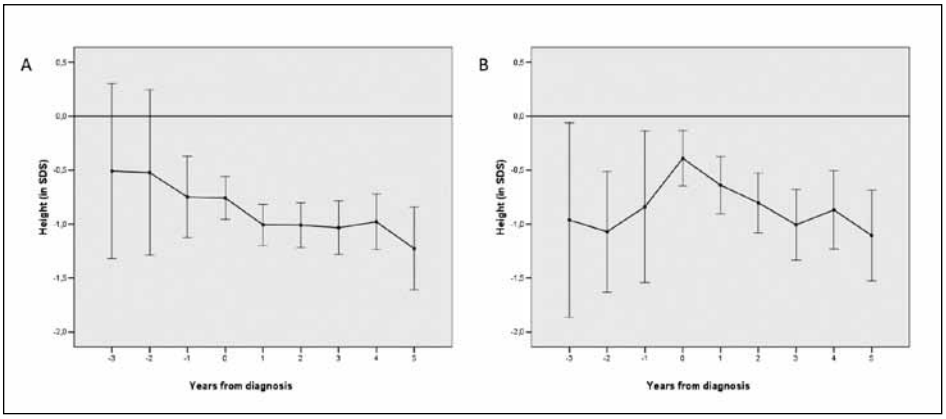


Figure 2: Figure 2A demonstrates the mean height SDS of children with CD (n=139), in the years before and after diagnosis (t=0). Figure 2B demonstrates the mean height SDS of children with UC (n=112).

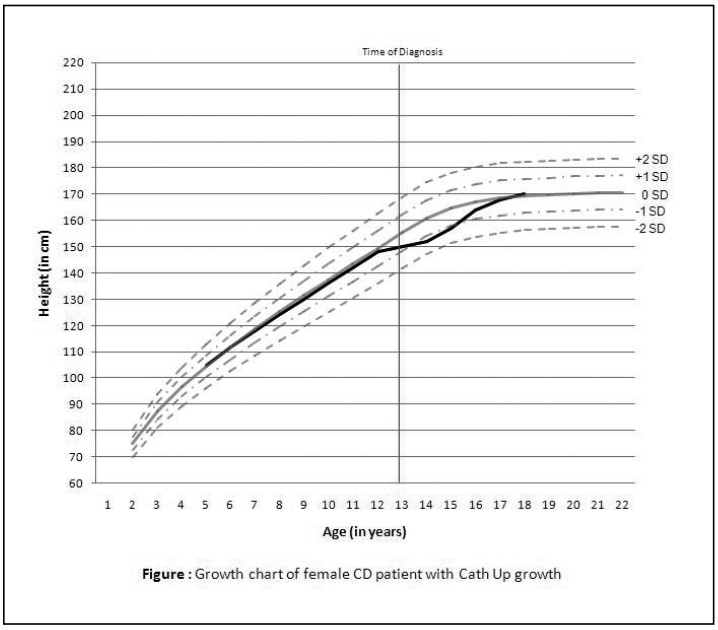


Figure : Growth chart of female CD patient with Cath Up growth

Appendix A: The growth chart of this patient with Crohn's disease shows complete catch-up growth.

years after diagnosis. There is catch-up growth in subgroups of patients, but that this catch-up growth is delayed and needs a period of at least 5 years. Remarkably, UC patients with a height SDS at diagnosis < -2 were prone for persistent growth failure. These patients (n=6), with a left-sided colitis (n=2) or extensive colitis (n=4), were resistant to therapy. In the follow-up, colectomy was performed in 3 out of 6 patients, 2 patients treated with infliximab were non-responsive.

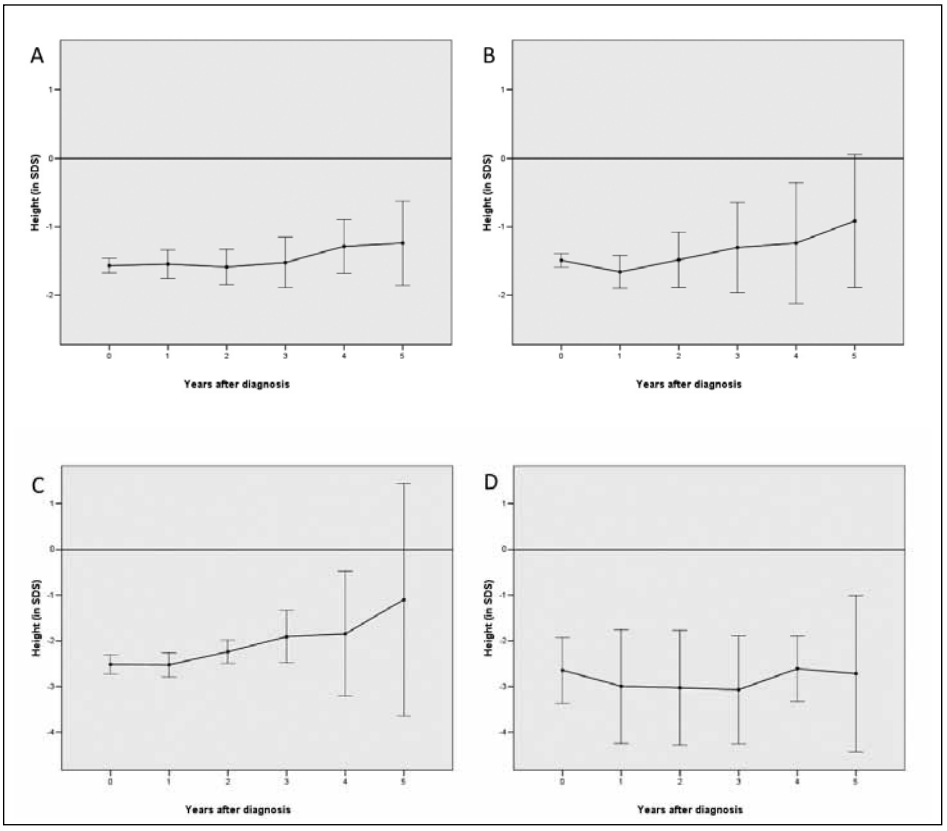


Figure 3: Figure 3A demonstrates linear growth of children with CD and a height SDS at diagnosis between -2 and -1 (n=33); the mean height SDS at diagnosis was -1.6 improving to a mean height SDS of -1.2 five years later. Figure 3B demonstrates linear growth in children with CD and a height SDS at diagnosis < -2 (n=10); the mean height SDS at diagnosis was -2.5 improving to a mean height SDS of -1.1 five years later. Figure 3C demonstrates linear growth in children with UC and a height SDS at diagnosis between -2 and -1 (n=24), the mean height SDS at diagnosis was -1.5 improving to a mean height SDS of -0.9 five years later. Figure 3D demonstrated linear growth in children with UC and a height SDS at diagnosis < -2 (n=5); the mean height SDS at diagnosis was -2.6 remaining almost unchanged (-2.7) five years later.

Adult height

Adult height was studied in the patients with childhood-onset IBD that reached the age of 18 years and onwards. The results are shown in Figure 1B and Tables 3 and 4. The mean adult height SDS was -0.7 in CD patients (range -2.8 to 1.4) and -0.5 in UC (range -2.7 to 1.1). This difference was no longer significant (p=0.48). The mean adult height of males, in CD and UC, and the mean adult height of females were not significantly different (p=0.22 and p=0.03 respectively). In CD, mean adult heights were 5.0 cm below the TH in males (range -17.0 to +5.5), and 2.4 cm below the TH in females (range -16 to +9.5). In UC, mean adult heights were 3.6 cm below the TH for

males (range -8.0 to +12.5), and 3 cm below the TH for females (range -13.0 to +5.5). When we compare adult height with height at diagnosis, both expressed in SDS, the number of patients with a height SDS < -2 decreased. However, the percentage of patients with a height SDS below 0 (i.e. below the median of the general Dutch population) remained about 75% at an adult age and at diagnosis in CD. In UC, the percentage of patients with a height SDS below 0 increased from 58% at diagnosis to 69% at an adult age.

Table 3: Target height (TH) and adult height in patients with childhood-onset IBD, with the general Dutch population as a reference

Height in cm	Male	Female
adult height general Dutch population (mean)	184.0	170.6
TH CD patients (mean)	*183.9*	*170.4*
TH UC patients (mean)	*184.0*	*170.5*
adult height CD patients (mean)	178.3	167.6
adult height UC patients (mean)	180.1	167.5

Table 4: adult height in patients with childhood-onset CD (n=67) and UC (n=30).
The general Dutch population is used as a reference.

Height (in SDS)	Dutch population	UC patients	CD patients	CD+UC patients
> 2	2 %	0 %	0 %	0 %
1 to 2	16 %	3 %	6 %	5 %
0 to 1	32 %	27 %	16 %	20 %
-1 to 0	32 %	43 %	42 %	42 %
-2 to -1	16 %	23 %	31 %	29 %
< -2	2 %	3 %	4 %	4 %

Risk factors for short stature at adulthood

Possible etiological factors for growth failure were studied including height and age at diagnosis, diagnostic delay, disease localization, disease activity, medication and other medical conditions like celiac disease and chromosomal abnormalities. In CD and UC, there was a significant correlation (r^2=0.33; P<0.001) between adult height and height at diagnosis (Figure 4). As stated above, there was a significant negative relationship between the height at diagnosis and the diagnostic delay in CD and UC. Other factors mentioned above did not have a significant correlation with growth failure. In CD, a total of 39 children was treated with anti tumor necrosis factor (infliximab). Figure 5 demonstrates the cumulative growth chart of these patients, including height SDS at

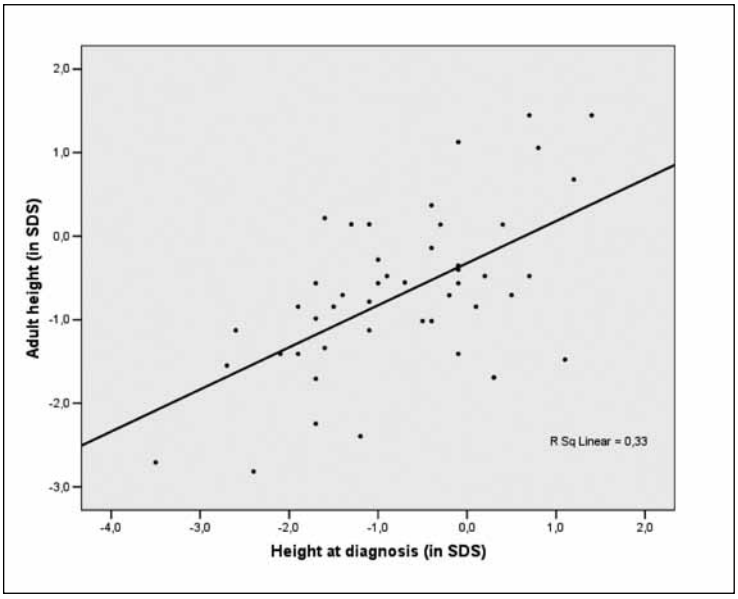
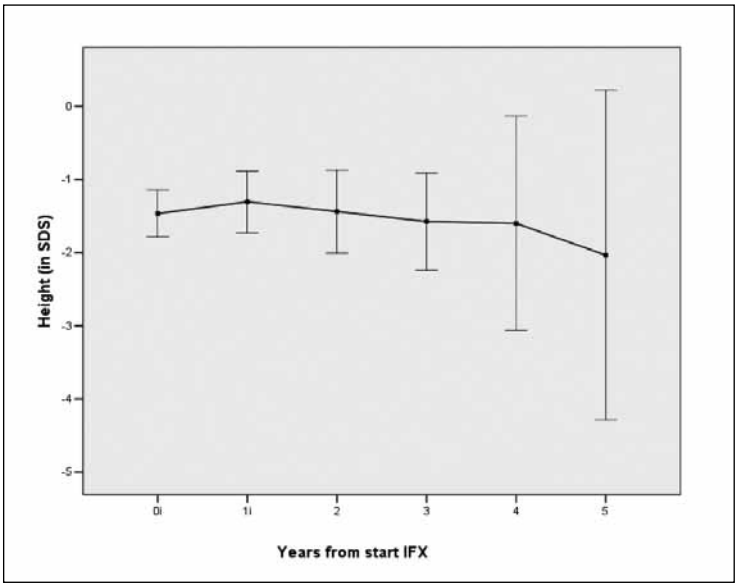


Figure 4: In CD and UC, there was a significant correlation (r2=0.33; P<0.001) between adult height (expressed in SDS) and height at diagnosis (expressed in SDS).

Figure 5: The cumulative growth chart of patients treated with anti tumor necrosis factor (infliximab; n=39) is demonstrated, including height SDS at diagnosis, at start of infliximab and during treatment. Height SDS did not clearly improve in the patients treated with infliximab.



diagnosis, at start of infliximab and during treatment. Height SDS did not clearly improve in the patients treated with infliximab. In many of the patients with jejunal CD, data on adult height were missing, therefore we were not able to correlate jejunal disease with growth failure in CD. Finally, in the group of patients with an adult height SDS equal or lower than -1, one patient was diagnosed with Down's Syndrome.

Growth after the age of 18 years

From the age of 18 years and onwards, growth data were available in 98 out of 162 IBD patients. In these 98 adult patients, 9 patients demonstrated linear growth after the age of 18. The data of these 9 patients, all diagnosed with CD, are shown in Table 5. The mean age at diagnosis was 14 years (range 11-17), with a mean height SDS of -0.6 (range -1,9 to 0,7). The mean increase in height after the age of 18 years was 5.9 cm (range 2-13). The age at which these patients reached their final adult height varied from 23 to 25 years.

Table 5: Characteristics of 9 CD patients with growth in height after the age of 18 years

	Gender	Age at diagnosis	Pubertal Onset	Height at 18 yrs (cm)	FAH (cm)	Age at FAH (yrs)	Growth after 18 yrs (cm)
1	M	11	Yes	163.5	176.5	23	13
2	F	17	No	177	180	23	3
3	M	15	No	168.5	178	25	9.5
4	M	14	No	166.5	176	>24	9.5
5	M	15	No	173.5	178	24	4.5
6	M	15	No	178	180	20	2
7	M	12	Yes	188	192	21	4
8	M	13	No	177.5	179	24	1.5
9	M	14	No	178	185	24	7

FAH = final adult height; TH = target height; M = male; F = female

Discussion

At diagnosis, height SDS was significantly lower in children with CD than in children with UC. In both groups, height SDS at diagnosis was significantly negatively correlated with the diagnostic delay. This is compatible with another study, and could be explained by a longer exposure to the negative effects of IBD on growth in children.[13] The difference in height between CD patients and UC patients could be due to several factors. There was a small (although not significant) difference in the diagnostic delay between CD patients and UC patients. In CD patients more patients had inflammation of the small intestine resulting in malabsorption, maldigestion and a more depressed appetite. In children with CD, a more pronounced derangement of the inflammatory cascade could be present with more resistance to the effects of growth hormone.[18-21] Finally, genetic circumstances related to CD may result in more pronounced growth failure.

In another bowel disease with growth failure, namely celiac disease, cumulative growth charts demonstrated complete catch-up in height the first 2 to 3 years after diagnosis (on a glutenfree diet).[49] In this study, in children with IBD, cumulative growth charts demonstrated little or no catch-up growth the first 4 to 5 years of treatment. This difference is somewhat remarkable. It could be that growth failure is more pronounced in celiac disease, resulting in better catch-up growth. In IBD, lack of catch-up growth in the first years of treatment could also be explained by flare-ups of disease activity, or by disease localisation, medication and a higher percentage of patients with delayed growth, delayed puberty and/or delayed maturation. Results from earlier studies are inconsistent. One study demonstrated a correlation between growth failure and jejunal disease.[13] Another study stated that the use of corticosteroids *in puberty* was the only factor influencing adult height in patients with childhood-onset IBD.[22] Motil et al. stated that not the use of steroids, but higher or uncontrolled disease activity would be a major cause for less growth potential.[8] In this study, we were not able to find a significant correlation between the lack of catch-up growth and variables such as age at diagnosis, disease localization, disease activity, medication or other medical conditions such as celiac disease or chromosomal abnormalities. In our study, the number of children with CD treated with enteral nutrition was limited. In CD, enteral nutrition as primary treatment promotes growth.[15,17,35-40] However, whether such improvement in growth velocity in the first period of disease will result in normal height at an adult age is unknown. Finally, and perhaps most importantly, lack of catch-up growth in pediatric IBD patients could be related to a genetic predisposition.

Our study demonstrates that especially children with UC and severe growth retardation (i.e. height SDS < -2) were prone for persistent growth failure. In order to prevent persistent growth failure in this particular group of patients more aggressive therapy will be necessary including colectomy.[10,11,41-48]

In patients with childhood-onset IBD, the mean adult height for males and females was 2.8 to 5.6 cm below the TH. This difference seems to be relatively small, on the other hand, 69% of the adults with childhood-onset UC and 77% of the adults with childhood-onset CD had an adult height

below average (i.e. below 0 SDS, compared to the general Dutch population). In correspondence with another study, adult height was significantly correlated with height at diagnosis.[13] Growth failure at the moment of diagnosis is the most important predictor for growth failure at later age.[13] Data from our retrospective study did not demonstrate improvement in adult height SDS in patients treated with immune-suppressives (such as 6-MP or azathioprine) or infliximab. Since treatment such as infliximab is limited to a selected group of patients, this comparison may be biased by differences in disease activity and severity, including steroid-dependency and treatment-resistance.

Several CD patients still had important (catch-up) growth until adulthood, even until their early twenties. This could be the result of delayed growth, delayed puberty and/or delayed maturation. We were not able to correlate this, since Tanner-staging or determination of the bone age had not been performed routinely in our patients. In our institutions, adult height was no longer recorded after transition to the gastroenterologist. As a pediatric gastroentertologist we should provide our colleagues with information about height and whether further linear growth could be expected or not. Hereby, actual height, target height, Tanner staging and bone age are important indicators. In some of the patients also the department of gastroenterology should monitor linear growth, since it may be the only sign of disease activity in patients.

References

1. Auvin S, Molinie F, Gower-Rousseau C, et al. Incidence, Clinical Presentation and Location at Diagnosis of Pediatric Inflammatory Bowel Disease: A Prospective Population-Based Study in Northern France (1988-1999). J Ped Gastroenterol Nutr 2005;41:49-55.
2. Jenkins H R. Inflammatory bowel disease. Arch Dis Child 2001;85:435–7.
3. Perminow G, Frigessi A, Rydning A, et al. Incidence and clinical presentation of IBD in children: Comparison between prospective and retrospective data in a selected Norwegian population. Scand J Gastroenterol 2006;41:1433-9.
4. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). Inflammatory Bowel Disease in Children and Adolescents: Recommendations for Diagnosis—The Porto Criteria. J Ped Gastroenterol Nutr 2005;41:1-7.
5. De Mesquita MB, Civitelli F, Levine A. Epidemiology, genes and inflammatory bowel diseases in childhood. Dig Liver Dis 2008;40:3-11.
6. Van der Zaag-Loonen HJ, Casparie M, Taminiau JA, et al. The incidence of pediatric inflammatory bowel disease in the Netherlands : 1999-2001. J Ped Gastroenterol Nutr 2004;38:302-7.
7. Kanof ME, Lake AM, Bayless TM. Decreased height velocity in children and adolescents before the diagnosis of Crohn's disease. Gastroenterology 1988;95:1523-7.
8. Motil KJ, Grand RJ, Davis-Kraft L, et al. Growth failure in children with inflammatory bowel disease: a prospective study. Gastroenterology 1993;105:681-691.

9. Griffiths AM, Nguyen P, Smith C, et al. Growth an clinical course of children with Crohn's disease. Gut 1993;34:939-43.
10. Markowitz J, Grancher K, Rosa J, et al. Growth failure in pediatric inflammatory bowel disease. J Ped Gastroenterol nutr 1993;16:373-380.
11. Hildebrand AM, Karlberg J, Kristiansson B. Longitudinal growth in children and adolescents with inflammatory bowel disease. J Ped Gastroenterol Nutr 1994;18:165-173.
12. Wine E, Reif SS, Leshinsky-Silver E, et al. Pediatric Crohn's Disease and Growth Retardation: The Role of Genotype, Phenotype, and Disease Severity. Pediatrics 2006;114:1281-1286.
13. Sawczenko A, Ballinger AB, Savage MO, Sanderson IR. Clinical Features Affecting Final Adult Height in Patients With Pediatric-Onset Crohn's Disease. Pediatrics 2006;118:124-9.
14. Pfefferkorn M, Burke G, Griffiths A, et al. Growth abnormalities persist in newly diagnosed children with crohn disease despite current treatment paradigms. J Ped Gastroenterol Nutr 2009;48:168-74.
15. Newby EA, Sawczenko A, Thomas AG, Wilson D. Interventions for growth failure in childhood Crohn's disease. Cochrane Database Syst Rev 2005 Jul 20;(3):CD003873. Review.
16. Ballinger AB, Camach-Hübner C, Croft NM. Growth failure and intestinal inflammation. QJM 2001;94:121-5.
17. Heuschkel R, Salvestrini C, Beattie RM, et al. Guidelines for the management of growth failure in childhood inflammatory bowel disease. Inflamm Bowel Dis 2008;14:839-49.
18. Shamir R, Phillip M, Levine A. Growth retardation in pediatric Crohn's disease: pathogenesis and interventions. Inflamm Bowel Dis 2007;13:620-8.
19. Murch SH, Lamkin VA, Savage MO, et al. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. Gut 1991;32:913–7.
20. De Benedetti F, Alonzi T, Moretta A, et al. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 1997;99:643–50.
21. Ballinger AB, Azooz O, El-Haj T, et al. Growth failure occurs through a decrease in insulin-like growth factor 1 which is independent of undernutrition in a rat model of colitis. Gut 2000;46:694–700.
22. Alemzadeh N, Rekers-Mombarg LT, Mearin ML, et al. Adult height in patients with early onset of Crohn's disease. Gut 2002;51:26-9.
23. Ferguson A, Sedgwicj DM. Juvenile onset inflammatory bowel disease: height and body mass index in adult life. BMJ 1994;308:1259-1263.
24. Markowitz J, Grancher K, Kohn N, et al. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. Gastroenterology 2000; 119:895–902.
25. Hyams JS, Markowitz J, Wyllie R. Use of infliximab in the treatment of Crohn's disease in children and adolescents. J Pediatr 2000;137:192–6.
26. Kugathasan S, Werlin SL, Martinez A, et al. Prolonged duration of response to infliximab in early but not late pediatric Crohn's disease. Am J Gastroenterol 2000;95:3189–94.
27. Stephens MC, Shepanski MA, Mamula P, et al. Safety and steroid-sparing experience using infliximab for Crohn's disease at a pediatric inflammatory bowel disease center. Am J Gastroenterol 2003;98:104–11.

28. Baldassano R, Braegger CP, Escher JC, et al. Infliximab (REMICADE) therapy in the treatment of pediatric Crohn's disease. Am J Gastroenterol 2003;98:833–8.
29. De Ridder L, Escher JC, Bouquet J, et al. Infliximab therapy in 30 patients with refractory pediatric crohn disease with and without fistulas in The Netherlands. J Ped Gastroenterol Nutr 2004;39:46-52.
30. Hyams J, Crandall W, Kugathasan S, et al.; REACH Study Group. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology 2007;132:863-73.
31. De Ridder L, Rings EH, Damen GM, et al. Infliximab dependency in pediatric Crohn's disease: long-term follow-up of an unselected cohort. Inflamm Bowel Dis 2008;14:353-8.
32. Hyams JS, Lerer T, Griffiths A, et al.; for the Pediatric Inflammatory Bowel Disease Collaborative Research Group. Long-term outcome of maintenance infliximab therapy in children with Crohn's disease. Inflamm Bowel Dis 2009;15:816-22.
33. Cezard JP, Nouaili N, Talbotec C, et al. A prospective study of the efficacy and tolerance of a chimeric antibody to tumour necrosis factors (remicade) in severe pediatric Crohn's disease. J Ped Gastroenterol Nutr 2003;36:632–6.
34. Walters TD, Gilman AR, Griffiths AM. Linear growth improves during infliximab therapy in children with chronically active severe Crohn's disease. Inflamm Bowel Dis 2007;13:424-30
35. Sanderson IR, Udeen S, Davies PS, et al. Remission induced by an elemental diet in small bowel Crohn's disease. Arch Dis Child 1987;62:123–7.
36. Belli DC, Seidman E, Bouthillier L, et al. Chronic intermittent elemental diet improves growth failure in children with Crohn's disease. Gastroenterology 1988;94:603–10.
37. Thomas AG, Taylor F, Miller V. Dietary intake and nutritional treatment in childhood Crohn's disease. J Ped Gastroenterol Nutr 1993;17:75–81.
38. Papadopoulou A, Rawashdeh MO, Brown GA, et al. Remission following an elemental diet or prednisolone in Crohn's disease. Acta Pediatr 1995;84:79–83.
39. Wilschanski M, Sherman P, Pencharz P, et al. Supplementary enteral nutrition maintains remission in paediatric Crohn's disease. Gut 1996;38:543–8.
40. Heuschkel RB, Menache CC, Megerian JT, Baird AE. Enteral nutrition and corticosteroids in the treatment of acute Crohn's disease in children. J Ped Gastroenterol Nutr 2000;31:8-15.
41. Castile RG, Telander RL, Cooney DR, et al. Crohn's disease in Children: assessment of the progression of disease, growth, and prognosis. J Ped Surg 1980;15:462-9.
42. Alperstein G, Daum F, Fisher SE, et al. Linear growth following surgery in children and adolescents with Crohn's disease: relationship to pubertal status. J Pediatr Surg 1985;20:129-33.
43. Davies G, Evans CM, Shand WS, Walker-Smith JA. Surgery for Crohn's disease in childhood: influence of disease and operative procedure on outcome. Br J Surg 1990;77:891–4.
44. Evans CM, Kirk JM, Savage MO, Walker-Smith JA. Growth after gut resection for Crohn's disease. Arch Dis Childh 1991;66:370.
45. Griffiths AM, Wesson DE, Shandling B, et al. Factors influencing the postoperative recurrence of Crohn's disease in childhood. Gut 1991;32:491–5.

46. Nicholls S, Vieira MC, Majrowski WH, et al. Linear growth after colectomy for ulcerative colitis in childhood. J Ped Gastroenterol Nutr 1995;21:82-6.
47. El-Baba M, Lin CH, Klein M, Tolia V. Outcome after surgical intervention in children with chronic inflammatory bowel disease. Am Surg 1996;62:1014-7.
48. Sentongo TA, Stettler N, Christian A, et al. Growth after intestinal resection for Crohn's disease in children, adolescents, and young adults. Inflamm Bowel Dis 2000;6:265–269.
49. Damen GM, Boersma B, Wit JM, Heymans HS. Catch-up growth in 60 children with celiac disease. J Ped Gastroenterol Nutr 1994;19:394-400.

CHAPTER 10

Summary and discussion

Summary and discussion

Crohn's disease (CD) and ulcerative colitis (UC), the two main subtypes of inflammatory bowel disease (IBD), are chronic relapsing inflammatory disorders of the gastrointestinal tract that have a peak age of onset in the second decade of life in children.

In **Chapter 1** – the introduction and outline of this thesis – genetics, immunopathogenesis, epidemiology, clinical presentation, diagnostics and histology are discussed in children with IBD, as well as differences in early and late onset IBD. Nowadays, there is compelling evidence that CD and UC are related polygenic diseases. Many CD susceptibility genes have been identified including *Nod2/CARD15*, *ATG16L1, IRGM, IL23R, IL12B, JAK2, STAT3, NKX2-3, TNFSF15, PTPN22, ICOSLG, CDKAL1, ITLN1, CCR6* and *ECM1*. In UC, there is a known contribution of the major histocompatibility complex, and UC susceptibility genes include *STAT3, IL12B, IL18RAP, JAK2, MST1, ECM1*, *NELL1, MDR1, LYRM4, CDKAL1, BSN, NKX2-3, HERC2, CCNY* and *PTPN2*. Two major pathogenic themes are important, at first, a critical contribution of defects in innate immunity, with polymorphisms in *Nod2* genes and the autophagy genes *ATG16L1* and *IRGM* in CD, and secondly, defects in the adaptive immune system, resulting in alternative signalling in the interleukin(IL)-12/IL-23 axis and the induction of IL-17 expression by T_H17 cells. Defects in the innate immunity may result in alterations in the recognition and intracellular processing of bacterial components and an impaired capacity to eliminate intracellular pathogens.
In general, dysregulation of the normally controlled immune response towards commensal bacteria, in genetically susceptible individuals, drives IBD. Defects may include a diminished expression of defensins (i.e. antimicrobial peptides) by the Paneth cells and disruptions in the epithelial-cell barrier function. Epithelial cells and antigen-presenting cells (APCs) such as the dendritic cells make contact with the microbial world, and the APCs will migrate to the mesenteric lymph nodes, where they promote the differentiation of naive T cells into effector T cells and regulatory T cells. In the past, analysis of the mucosal T cell phenotype largely supported the concept of CD as a T_H1 disease, driven by IL-12 and characterized by the production of the signature cytokine interferon-gamma, and UC as an atypical T_H2 disease characterized by production of cytokines such as IL-13. Nowadays, we know that the cytokine milieu secreted in part by the dendritic cells skews the differentiation of naive $CD4^+$ T cells not only in T_H1 and T_H2 cells, but also in T_H17- and regulatory T-cell subsets. The discovery of the T_H17 lineage, driven by IL-23 (as well as IL-6 and transforming growth factor beta) and characterized by IL-17 production, may even have a key role in intestinal inflammation in CD (as is discussed in chapter 5).
In the Western world, the incidence rate of IBD in children is increasing, while the incidence rate of infectious diseases is decreasing. In Western Europe, the incidence rate of IBD in children has increased to 5.2 new cases per 100.000 children per year.
The most frequent symptoms of IBD in childhood are abdominal pain, diarrhoea, rectal bleeding and weight loss. Nevertheless, in children with UC 1 out of 4 patients lack diarrhoea en 1 out of

6 patients lack bleeding. In children with CD, the symptoms mentioned above are even less frequently present, and patients may present with lethargy, anorexia, nausea, vomiting, oral ulcers, anemia, uveitis, arthralgia and arthritis, perianal abscesses, ulcers or fistulas, constipation, soiling, growth failure, delayed puberty and skin manifestations such as erythema nodosum.
From chapter 2 and onwards a number of themes are studied in children with IBD, including (1) the immunological activity of buccal epithelium, (2) the role of the dendritic cells, (3) the value of non-invasive markers of inflammation in the blood and stool of children suspected of having IBD, (4) common features, overlap and essential differences in pediatric granulomatous inflammatory bowel diseases, (5) histological criteria in children and adults with IBD, and (6) growth in patients with childhood-onset IBD.

In **Chapter 2** we demonstrate that buccal epithelium is immunological active in children with CD, even in the absence of oral lesions. In pediatric CD, buccal epithelial cells (BECs) exhibit an enhanced production of the chemokines CXCL-8, CXCL-9, and CXCL-10, compared with controls, with pediatric UC and with adult-onset CD. Remarkably, the production of CXCL-8 by BECs correlates with the erythrocyte sedimentation rate in the blood. *In vitro* stimulation with Toll-like receptor (TLR)-2 and TLR-4 agonists results in a further increase of chemokine production exclusively in BECs derived from pediatric CD patients. These cells demonstrate a lower threshold for microbial stimulation. Compared to children with UC, CXCL-8 production by stimulated monocyte-derived dendritic cells was equal to that of children with CD. Therefore, the enhanced chemokine production by BECs in pediatric CD appears to be restricted to cells derived from the epithelial barrier. We hypothesize that the assessment of chemokine production by BECs may have potential as a new, rapid and noninvasive test for screening and classification of IBD in children.

It has been suggested that CD patients have a general defect in the innate mucosal immune response, including a diminished CXCL-8 production. In **Chapter 3**, in a letter, we come to a different conclusion. Various studies demonstrate an increased number of neutrophils in mucosal tissue rather than a diminished neutrophilic influx. Intestinal epithelial cells, both in UC and in CD, are an important source of CXCL-8 production. Together with our data from chapter 2, we postulate that a diminished CXCL-8 production does not necessarily represent a general mucosal paradigm for CD. We argue that results from one study cannot be extrapolated to other involved immune cells or to specific patients such as children with CD.

In **Chapter 4**, we confirm that, in pediatric CD, BECs have a significant increase in the production of CXCL-8 and -9 in response to the TLR-agonists lipopolysaccharide or zymosan. Again, BECs in pediatric UC were not responsive to these stimuli. In this study more patients were included than in the study displayed in chapter 2. We demonstrate that the enhanced production of chemokines by BECs in pediatric CD is not associated with one of the three well known *Nod2*-polymorphisms. Therefore, differences in the production of chemokines by BECs may be based on the

presence of other genes involved in innate immunity or on differences in the constitution of the local microbial flora.

In **Chapter 5**, in the introduction, the immunopathogenesis of IBD is discussed. Nowadays, we know that the cytokine milieu secreted in part by the dendritic cells skews the differentiation of naïve $CD4^+$ T cells not only in T_H1 and T_H2 cells, but also in T_H17- or regulatory T-cell subsets. In early disease, T cell clones from the colonic mucosa of pediatric CD demonstrate a strongly polarized T_H1 response, expressing high levels of the IL-12 receptor b2 subunit and IFN-gamma. In established disease, T cell clones produce higher amounts of the immunoregulatory cytokines IL-10 (and IL-4). In response to an appropriate stimulus, T cell clones derived from early disease have the ability to upregulate markedly the secretion of IL-4 and IL-10, while T cell clones in established disease demonstrate a relative insensitivity. The question is: what will determine the development of these typical effector T cells, the expression of specific T cell receptors or the production of differentiating cytokines by antigen presenting cells? Therefore, we asses TLR-driven cytokine responses from monocyte-derived dendritis cells in children with IBD. We study whether the intrinsic ability of these dendritic cells to secrete IL-12 and IL-23 is related to the type of IBD, duration of disease (early versus late) or treatment.
Our results demonstrate that the production of IL-12 and IL-23 by monocyte-derived dendritic cells was highly variable in children with CD and UC. There is no relation between disease activity, disease location, medication and the levels of either IL-12 or IL-23. There is no difference in cytokine production by monocyte-derived dendritic cells when we compare early with late IBD (for CD as well as for UC). There is a clear dose-dependent relationship between the production of IL-12 or IL-23 and the used microbial ligands. These data indicate that neither of the specific IBD subsets (CD or UC) is associated with a "hardwired" predisposition for IL-12 or IL-23 production. We were not able to find any relationship between IL-12 and IL-23 levels, confirming that the production of these two cytokine family members is differentially regulated. We conclude that the intrinsic capacity of monocyte-derived dendritic cells to produce IL-12p70 and/or IL-23 is not associated with a specific subtype or stage of inflammatory bowel disease. We therefore hypothesize that the presence of specific mucosal T effector cells identified in CD or UC results from the local amount of particular microbial ligands and T-cell expression of specific receptors such as IL-12beta2 and IL-23R.

In **Chapter 6**, the value of non-invasive markers of inflammation (in the blood and stool) are discussed in children suspected of having IBD. Positive markers of inflammation will support the need for more invasive investigations and can be used to predict disease activity. Anemia and thrombocytosis are general indicators of IBD. In active CD the erythrocyte sedimentation rate is often elevated. In unclassified colitis, ASCA and p-ANCA can be of value in order to categorize the disease as either CD or UC. The presence of these antibodies seems to be related to age of presentation, localization of disease, result of treatment and outcome. Fecal alpha-1-antitrypsin

is mainly increased in CD, but is not clearly related to disease activity. Determination of fecal calprotectin in IBD seems to be more reliable, though in adults fecal calprotectin levels may be increased in colorectal carcinoma and NSAID-induced enteropathy. Fecal lactoferrin does not discriminate between CD and UC, but is related to disease activity.

In **Chapter 7** overlap, common features and essential differences are discussed in pediatric granulomatous inflammatory bowel diseases such as chronic granulomatous disease (CGD), sarcoidosis, Crohn's disease (CD), abdominal tuberculosis and the Hermansky-Pudlak syndrome. Depending on the mode of presentation, overlap in clinical presentation of pediatric granulomatous inflammatory bowel disease may be substantial. Chronic granulomatous disease (CGD) may present with obstruction of the gastrointestinal tract (including esophageal strictures and dysmotility, delayed gastric emptying and small bowel obstruction), failure to thrive, granulomatous colitis, perianal abscesses and hepatic granulomas and/or abscesses. Anemia, thrombocytosis, elevated CRP and ESR and hypoalbuminemia are nonspecific and may occur in any of the granulomatous inflammatory bowel diseases. In histology macrophages with cytoplasmic inclusions will be rather specific in CGD colitis. Sarcoidosis may present with abdominal pain or discomfort, diarrhoea, weight loss, growth failure, delayed puberty, erythema nodosum, arthritis, uveitis and/or hepatic granulomata. In 55% of the patients angiotensin-converting enzyme will be elevated; non-caseating epitheloid granulomata will be unspecific. In sarcoidosis, bronchoalveolar lymphocytosis and abnormalities in pulmonary function are reported, as well as in CD and CGD. Importantly, CD patients may present with granulomatous lung disease, fibrosing alveolitis and drug-induced pneumonitis. In addition, sarcoidosis and concomitant gastrointestinal CD have been reported in patients, as well as coexistence of CD and sarcoidosis in siblings. Common susceptibility loci have been indentified in CD and sarcoidosis, while CD and CGD share defects in the defence mechanisms against different microbes. In chapter 7, common features and essential differences are discussed in clinical presentation, laboratory results, radiology, endoscopy and histology of these granulomatous inflammatory bowel disease, as well as in abdominal tuberculosis and Hermansky-Pudlak syndrome. Instructions for specific diagnosis and respective treatments are provided.

It is important to establish a correct diagnosis of CD and UC as treatment options, course of disease and prognosis differ. Recent guidelines focusing on histological criteria of IBD are mainly derived from adult patients. In **Chapter 8**, we study the histological criteria discriminating best between pediatric CD and UC. Also differences in histology between pediatric-onset and adult-onset IBD are studied. In children, histology of the most affected biopsies of the colon demonstrate that crypt distortion, basal plasmacytosis and transmucosal distribution of inflammation are frequently present both in CD and UC and therefore not discriminative. Strong arguments for pediatric CD are discontinuous, focal or patchy chronic inflammation, focal crypt epithelial polymorphs and focal crypt irregularity; epitheloid granulomas are present in 46% of the patients. Strong arguments for pediatric UC are diffuse crypt architectural irregularity, diffuse chronic inflammation and dif-

fuse crypt epithelial polymorphs. Crypt branching, crypt abscesses and irregular surface or villous transformation are more frequently present in pediatric UC than in CD. In UC, crypt branching and distortion are more frequently present in children, while mucin depletion is more frequently present in adults. In CD, polymorph exudates and epitheloid granulomas are more frequently present in children, while basal plasmacytosis is more frequently present in adults. In CD, chronic inflammation is mostly focal in adults and focal, patchy or diffuse in children. Also histology of the ileum and the upper GI-tract demonstrate differences in pediatric- and adult-onset IBD, which is discussed in chapter 8. In conclusion, our study confirms that there are differences in histology of biopsies of the gastrointestinal-tract in pediatric- versus adult-onset IBD of which the pathologist and pediatric gastroenterologist should be aware.

In children, growth failure is a unique feature of IBD. Treatment aimed to reverse growth failure is not always successful. This outcome seems to be influenced by the degree of growth failure at diagnosis, use of corticosteroids and jejunal disease. On the other hand, delayed maturation and delayed puberty seem to compensate for the period of poor growth earlier in life. In **Chapter 9** we study growth failure at the time of diagnosis, progression of linear growth in the years thereafter and adult height in a large cohort of patients (n=318) with childhood-onset IBD. At diagnosis, patients have a mean height standard deviation score (SDS) of -0.8 in CD and -0.4 in UC, which is significantly different ($p<0.01$). Height at diagnosis correlates significantly to the diagnostic delay ($p<0,001$). In the first 4 to 5 years of treatment catch-up growth is often disappointing. Especially children with UC and severe growth retardation (height SDS < -2) are prone for persistent growth failure. At an adult age, the mean height SDS is -0.7 in CD and -0.4 in UC. Adult height significantly correlates with height at diagnosis ($p<0,001$). There is no correlation between adult height and the localization of disease, disease activity or therapy. There is no clear improvement in height (expressed in SDS) in the group of patients treated with anti-tumor necrosis factor (infliximab; n=39). Several CD patients still have important growth (up to 13 cm) after the age of 18 years, until their early twenties. In conclusion, height at diagnosis (expressed in height SDS) is the most important predictor of adult height.

Samenvatting en discussie

Dit proefschrift is gewijd aan onderzoek naar chronische ontstekingsziekten van de darm bij kinderen. In het proefschrift zal ik de Engelse afkorting IBD (Inflammatory Bowel Disease) gebruiken wanneer we spreken van chronische ontstekingsziekten van de darm. De twee belangrijkste subtypen van IBD zijn de ziekte van Crohn (Crohn's disease, CD) en colitis ulcerosa (ulcerative colitis, UC). Het betreft chronische ontstekingsziekten van de darm die verlopen met periodes van klachten (exacerbatie) en klachtenvrije episodes (remissie). Bij kinderen treedt de ziekte vooral op in het 2e decennium van het leven.

In **Hoofdstuk 1** – de introductie en omlijning van dit proefschrift – bespreken we de genetische achtergrond, immunopathogenese, epidemiologie, klinische presentatie, diagnostiek en histologie van kinderen met IBD. Daarbij worden opvallende verschillen benoemd tussen IBD bij kinderen en IBD bij volwassen. In de Westerse wereld neemt de incidentie van de IBD bij kinderen toe, terwijl de incidentie van infectieziekten afneemt. De incidentie van IBD bij kinderen bedraagt op dit moment circa 5.2 nieuwe patiënten per 100.000 kinderen per jaar in West-Europa. Bij CD en bij UC zijn vele polymorphismen beschreven. Bij CD zijn dit polymorphismen in de genen *Nod2/CARD15*, *ATG16L1, IRGM, IL23R, IL12B, JAK2, STAT3, NKX2-3, TNFSF15, PTPN22, ICOSLG, CDKAL1, ITLN1, CCR6* en *ECM1*, bij UC zijn dit polymorphismen in de genen *STAT3, IL12B, IL18RAP, JAK2, MST1, ECM1*, *NELL1, MDR1, LYRM4, CDKAL1, BSN, NKX2-3, HERC2, CCNY* and *PTPN2*. Daarnaast is er bij UC een belangrijke bijdrage vanuit het 'major histocompatibility complex'. Bij IBD zijn (genetische) defecten beschreven in de 'innate immunity' en defecten in de 'adaptive immunity'. Polymorphismen in *Nod2* genen en de autophagie genen *ATG16L1* en *IRGM* zoals bij CD kunnen leiden tot veranderingen in de herkenning en verwerking van microbiële componenten en minder mogelijkheden om intracellulaire pathogenen te elimineren. Defecten in de 'adaptive immunity' kunnen resulteren in een alternatieve signalering via de interleukine (IL)-12 en IL-23 as, en de inductie van T helper 17 (T_H17) cellen die IL-17 tot expressie brengen. In het algemeen wordt gesteld dat, in genetisch gevoelige personen, de gecontroleerde immuunrespons op commensale microben is verstoord bij patiënten met IBD. Deze defecten kunnen o.m. berusten op een verminderde expressie van defensines (antimicrobiële peptiden) door de cellen van Paneth en op een defecte barrière functie van de laag epitheelcellen. Epitheelcellen en antigeen-presenterende cellen (APCs) zoals de dendritische cellen maken contact met de microbiële wereld. APCs migreren naar mesenteriale lymfeklieren, waarbij zij de differentiatie van naïeve T cellen naar effector T cellen en regulatoire T cellen promoten. Analyse van mucosale T cellen ondersteunden het concept dat CD een T_H1 ziekte is, aangestuurd door IL-12 en gekarakteriseerd door de productie van interferon-gamma, en UC een atypische T_H2 ziekte gekarakteriseerd door de productie van cytokines zoals IL-13. Vandaag de dag weten we dat de cytokines die worden gesecreteerd door o.m. de dendritische cel differentiatie van naïeve T cellen induceert, niet alleen in T_H1 en T_H2 cellen, maar ook in T_H17 cellen en regulatoire T-cellen. Er zijn aanwijzingen dat T_H17 cellen, aan-

gestuurd door IL-23 (evenals IL-6 en 'transforming growth factor beta') en gekarakteriseerd door de productie van IL-17, zelfs een sleutelrol kunnen vervullen in darmontsteking bij CD. De meest frequente klachten van IBD bij kinderen zijn buikpijn, diarree, rectaal bloedverlies en gewichtsverlies. Desalniettemin heeft 1 op de 4 kinderen met UC geen diarree en heeft 1 op de 6 kinderen geen rectaal bloedverlies. Bij kinderen met CD treden bovenstaande klachten minder vaak op, deze patiënten kunnen zich presenteren met lethargie, anorexie, misselijkheid, spugen, orale aften, bloedarmoede, uveitis, gewrichtspijn of –ontsteking, perianale abcessen, zweren of fistels, obstipatie, encopresis, afbuiging van de lengtegroei, verlate puberteit en huidafwijkingen zoals erythema nodosum. Vanaf hoofdstuk 2 en verder worden een aantal onderwerpen bestudeerd bij kinderen met IBD. Deels betreft dit translationeel onderzoek. Het omvat de volgende onderwerpen: (1) de immunologische activiteit van wangslijmvlies (als spiegel voor darmslijmvlies), (2) de rol van de dendritische cel, (3) de waarde van niet-invasieve 'markers' van ontsteking in het bloed en in de faeces van kinderen die IBD kunnen hebben, (4) gedeelde en gemeenschappelijke kenmerken van verschillende granulomateuze ontstekingsziekten van de darm, evenals essentiele verschillen, (5) histologische criteria van IBD bij kinderen en volwassenen, en (6) lengtegroei bij kinderen met IBD.

In **Hoofdstuk 2** laten we zien dat wangslijmvlies van kinderen met CD immunologisch actief is, zonder orale laesies. Cellen van het wangslijmvlies van kinderen met CD hebben een verhoogde productie van de chemokines CXCL-8, CXCL-9, and CXCL-10, vergeleken met gezonde individuen, met kinderen met UC en met volwassenen met CD. Het was hierbij opmerkelijk dat de productie van het belangrijkste chemokine, namelijk CXCL-8, door de cellen van het wangslijmvlies correleerde met een ontstekingsgraadmeter in het bloed, namelijk met de bezinking. Alleen bij kinderen met CD resulteert stimulatie met Toll-like receptor (TLR)-2 en TLR-4 agonisten, *in vitro*, in een verdere toename van de productie van chemokines door het wangslijmvlies. Blijkbaar hebben deze cellen een lagere drempel voor microbiële stimulatie. Het bestuderen van de CXCL-8 productie door dendritische cellen (verkregen uit mononucleaire cellen) liet zien dat deze productie gelijk is voor kinderen met CD en UC. Derhalve lijkt het aannemelijk dat de verhoogde productie van chemokines door cellen van het wangslijmvlies van kinderen met CD is beperkt tot de epitheelcellen. In theorie kan het vaststellen van de productie van chemokines door het wangslijmvlies een nieuwe, snelle en niet-invasieve test zijn voor screening en classificatie van IBD bij kinderen.

Er is gesteld dat patiënten met CD een belangrijk defect hebben in de 'innate' immuunrespons van het slijmvlies, inclusief een verminderde CXCL-8 productie. In **Hoofdstuk 3**, in een brief in de Lancet, laten we zien dat we deze conclusie niet delen. Meerdere studies tonen aan dat het slijmvlies van de darm bij ontsteking juist een toegenomen aantal neutrofielen bevat. Epitheelcellen van de darm zijn bij IBD een belangrijke bron van CXCL-8 productie. Mede door de resultaten uit hoofdstuk 2 zijn we van mening dat een verminderde productie van CXCL-8 niet noodzakelijk een algemeen geldend paradigma betekent voor patiënten met CD. We beargumenteren dat de

resultaten van 1 studie niet geëxtrapoleerd kunnen worden naar andere cellen van het immuunapparaat of naar specifieke patiënten zoals kinderen met CD.

In **Hoofdstuk 4**, bevestigen we dat, bij kinderen met CD, cellen van het wangslijmvlies een belangrijke toename in de productie van CXCL-8 en -9 laten zien in reactie op de TLR-agonisten lipopolysaccharide en zymosan. Dit was niet het geval bij stimulatie van cellen van het wangslijmvlies van kinderen met UC. In hoofdstuk 4 werden meer patiënten geïncludeerd dan in hoofdstuk 2 en waren meer cellen beschikbaar voor stimulatietesten. Daarnaast laten we zien dat de toegenomen productie van chemokines door de cellen van het wangslijmvlies niet geassocieerd is met een van de drie bekende *Nod2*-polymorphismen. In theorie kan de toegenomen productie van chemokines door cellen van het wangslijmvlies gebaseerd zijn op de aanwezigheid van andere polymorphismen in genen verantwoordelijk voor de 'innate immunity' of op verschillen in de samenstelling van de locale microbiële flora.

In **Hoofdstuk 5**, in de introductie, worden de huidige inzichten besproken betreffende de immunopathogenese van IBD. We weten dat het cytokine milieu, ten dele bepaald door de dendritische cellen, de differentiatie van naïeve $CD4^+$ T cellen niet alleen stuurt in de richting van T_H1 en T_H2 cellen, maar ook in de richting van T_H17-cellen of regulatoire T-cellen. Bij aanvang van CD bij kinderen, laten de T cellen uit het slijmvlies van de dikke darm een sterk T_H1 reactie zien, met hoge expressie van IL-12 receptor beta 2 subunit en interferon-gamma. Bij patiënten met CD waarbij de ziekte al enige tijd aanwezig is, produceren de T cellen hogere hoeveelheden van immunoregulatoire cytokines zoals IL-10 (en IL-4). Bij aanvang van ziekte zijn T cellen in staat om de secretie van IL-4 en IL-10 aanzienlijk te verhogen bij een juiste prikkel, terwijl cellen verkregen bij ziekte die al enige tijd bestaat relatief ongevoelig zijn voor eenzelfde prikkel. De vraag die resteert is de volgende: wat bepaalt de ontwikkeling van typische effector T cellen, de mate van expressie van specifieke T cel receptoren of de productie van verschillende cytokines door APCs?
Om dit te beantwoorden onderzochten we de cytokine productie van dendritische cellen (verkregen uit mononucleaire cellen) van kinderen met IBD in antwoord op TLR-agonisten. We bestuderen of de intrinsieke mogelijkheid van deze dendritische cellen om IL-12 en IL-23 af te scheiden in relatie staat tot het type IBD, de duur van de ziekte of de behandeling. Onze resultaten tonen dat de productie van IL-12 en IL-23 door de dendritische cellen zeer variabel is bij kinderen met IBD. Er is geen relatie tussen de mate van productie van IL-12 of IL-23 enerzijds en ziekteactiviteit, lokalisatie van ziekte, of medicatie anderzijds. Er is geen verschil in de cytokine productie door de dendritische cellen van patiënten bij aanvang van ziekte of bij reeds lang bestaande ziekte, niet bij CD en niet bij UC. Er is een duidelijke dosisafhankelijke relatie tussen de productie van IL-12 of IL-23 en de gebruikte microbiële liganden. Er was geen indicatie voor een meer selectieve productie van IL-12 of IL-23 bij CD of UC. De productie van IL-12 en IL-23 verloopt onafhankelijk van elkaar, immers we konden geen enkele relatie aantonen in de productie van deze beide cytokines. We concluderen dat de intrinsieke capaciteit van de dendritische cellen om IL-12 en/of IL-23 te pro-

duceren niet geassocieerd is met een specifiek subtype of stadium van IBD. Het is goed mogelijk dat de aanwezigheid van specifieke mucosale T effector cellen in CD en UC het gevolg is van de locale hoeveelheid van microbiële liganden en van T cel expressie van specifieke receptoren zoals IL-12 receptor beta2 en IL-23R.

In **Hoofdstuk 6**, wordt ingegaan op de waarde van niet-invasieve (in het bloed en in de faeces) 'markers' van ontsteking van kinderen verdacht voor IBD. Positieve 'markers' van ontsteking maken de noodzaak van meer invasief onderzoek groter en kunnen ziekteactiviteit voorspellen. Bloedarmoede en thrombocytose zijn algemene indicatoren van IBD. Bij kinderen met CD en ziekteactiviteit is de bezinking (in het bloed) vaak verhoogd. Wanneer een chronische colitis niet is te classificeren, endoscopisch en histologisch, kan de aan- en/of afwezigheid van ASCA en p-ANCA van waarde zijn om de colitis te classificeren als CD of UC. Verder zijn er aanwijzingen dat de aanwezigheid van deze antilichamen gerelateerd is aan de leeftijd bij diagnose, lokalisatie van de ziekte, resultaat van behandeling en de prognose. In de faeces is het alpha-1-antitrypsine vooral verhoogd in het geval van CD, maar deze verhoging is niet duidelijk gerelateerd aan ziekteactiviteit. In het geval van IBD lijkt de bepaling van calprotectine in de faeces meer te relateren aan ziekteactiviteit, met de kanttekening dat, bij volwassenen, een verhoogde uitscheiding van calprotectine in de faeces ook kan optreden bij het gebruik van niet steroïde anti-inflammatoire middelen (leidend tot enteropathie) en in het geval van een colorectaal carcinoom. Lactoferrine in de faeces is weliswaar gerelateerd aan ziekteactiviteit, maar discrimineert niet tussen CD en UC.

Hoofdstuk 7 betreft een 'review' van granulomateuze ontstekingsziekten van de darm bij kinderen. Het omvat chronisch granulomateuze ziekte (chronic granulomatous disease (CGD)), sarcoidose, CD, abdominale tuberculose en het Hermansky-Pudlak syndroom. Het hoofdstuk is gebaseerd op klinische problemen die we ondervonden bij patiënten met overlap tussen sarcoidose en CD, en overlap tussen CD en het Hermansky-Pudlak syndroom. In dit hoofdstuk worden gedeelde en gemeenschappelijke kenmerken van de verschillende granulomateuze ontstekingsziekten van de darm besproken, evenals essentiële verschillen. CGD kan zich presenteren met obstructie van het maagdarmkanaal (inclusief slokdarmstricturen en dysmotiliteit, een vertraagde maaglediging en obstructie van de dunne darm), 'failure to thrive', granulomateuze colitis, perianale abcessen and granulomen of abcessen in de lever. Bloedarmoede, thrombocytose, een verhoogde bezinking en C-reactive protein, en hypoalbuminemie zijn niet specifiek en kunnen bij ieder van de granulomateuze ontstekingsziekten van de darm optreden. Wanneer er histologisch macrofagen zichtbaar zijn met cytoplasmatische inclusies duidt dit op CGD colitis. Sarcoidosis kan zich presenteren met buikpijn, diarree, gewichtsverlies, afbuiging van de lengtegroei, verlate puberteit, erythema nodosum, arthritis, uveitis en/of granulomen van de lever. Bij 55% van de patiënten met sarcoidose is het 'angiotensin-converting enzyme' verhoogd. Niet-verkazende granulomen in het epitheel zijn onvoldoende specifiek. Een afwijkende longfunctie en bronchoalveolaire lymfocytose zijn beschreven bij sarcoidose, maar ook bij CGD en CD. Patiënten met CD kunnen zich

presenteren met granulomateuze longziekte, fibroserende alveolitis en geneesmiddelgeïnduceerde pneumonie. Sarcoidose en aansluitend gastrointestinale CD zijn gerapporteerd in patiënten, evenals co-existentie van sarcoidose en CD bij broers en zussen. Voor wat betreft de genen hebben sarcoidose en CD gemeenschappelijke 'susceptibility' loci, terwijl bij CD en CGD defecten zijn beschreven in de afweermechanismen tegen verschillende microben. In het algemeen zijn in dit hoofdstuk gedeelde en gemeenschappelijke kenmerken en essentiële verschillen beschreven in klinische presentatie, bloeduitslagen, radiologie, endoscopie, histologie en behandeling van deze granulomateuze ontstekingsziekten van de darm, evenals die van abdominale tuberculose en het Hermansky-Pudlak syndroom. Instructies voor het stellen van de juiste diagnose en de behandeling worden weergegeven.

Het is belangrijk om de subtypen van IBD te kunnen benoemen daar de keuze in behandeling, het beloop en de prognose verschillen kent. Recente richtlijnen met verdieping in de histologische criteria van IBD zijn vooral verkregen uit onderzoek naar volwassen patiënten met IBD. In **Hoofdstuk 8** bestuderen we de histologische criteria die CD en UC bij kinderen onderscheiden. Ook worden de histologische verschillen bestudeerd tussen de wijze waarop IBD zich presenteert bij kinderen en bij volwassenen. Histologie van de meest aangedane biopten van de dikke darm bij kinderen toont dat crypt distorsie, basale plasmacytose en transmucosale distributie van ontsteking even frequent voorkomen bij CD als bij UC, hierin is geen onderscheid. Belangrijke argumenten voor CD bij kinderen zijn discontinue, focale of pleksgewijze chronische ontsteking, focale onregelmatigheid van de crypten en focale aanwezigheid van epitheliale polymorphonucleaire cellen in de crypten; granulomen in het epitheel worden gezien bij 46% van de patiënten. Belangrijke argumenten voor UC bij kinderen zijn diffuse onregelmatigheden in de architectuur van de crypten, diffuse chronische ontsteking en diffuse aanwezigheid van epitheliale polymorphonucleaire cellen in de crypten. Cryptvertakkingen, cryptabcessen, een onregelmatig oppervlak of villeuze transformatie zijn meer frequent aanwezig bij kinderen met UC dan bij CD. Wanneer we de verschillen bestuderen in histologische kenmerken tussen kinderen en volwassenen met IBD is op te merken dat bij UC, cryptvertakkingen en crypt distorsie meer frequent voorkomen bij kinderen en dat bij volwassenen meer frequent sprake is van mucinedepletie. Bij kinderen met CD zijn polymorfe exsudaten en epitheliale granulomen meer frequent aanwezig dan bij volwassenen, terwijl bij volwassenen basale plasmacytose meer frequent optreed. Bij kinderen met CD is de chronische ontsteking focaal, pleksgewijs of diffuus, terwijl dit bij volwassen patiënten met CD dit meestal focaal is. In dit hoofdstuk worden ook verschillen besproken in histologie van het ileum en de bovenste tractus digestivus bij kinderen en volwassen patiënten met IBD. In conclusie, deze studie bevestigd dat er verschillen zijn in histologie van biopten van de tractus digestivus bij kinderen en volwassen patiënten met IBD, waarvan de patholoog-anatoom en de kinderarts MDL zich bewust horen te zijn.

Een afbuigende lengtegroei is een uniek verschijnsel van IBD bij kinderen. Behandeling gericht op het opheffen van deze groeiachterstand zijn niet altijd succesvol. Dit lijkt te worden beïnvloedt door de mate van afbuiging van lengtegroei op het moment van diagnose, gebruik van corticosteroïden en ziekteactiviteit in het jejunum. Anderzijds lijkt een vertraagde maturatie en vertraagde puberteit te compenseren voor de periode van verminderde lengtegroei eerder in het leven. In **Hoofdstuk 9**, in een grote groep van patiënten (n=318) met IBD op de kinderleeftijd, bestuderen we afbuiging van de lengtegroei op het moment van diagnose, progressie van de lengtegroei in de jaren daarna, en de eindlengte van volwassen patiënten met IBD (waarbij de ziekte zich op de kinderleeftijd presenteerde). Op het moment van diagnose hebben de patiënten een lengte standaard deviatie score (SDS) met een mediaan van -0.8 in het geval van CD en -0.4 in het geval van UC, hetgeen significant verschillend is ($p<0.01$). De lengte bij diagnose correleert significant ($p<0,001$) met het 'diagnostic delay', oftewel het verschil in tijdsduur tussen het moment van optreden van klachten en het moment van het stellen van de diagnose. De eerste 4 tot 5 jaren van behandeling is de mate van inhaalgroei vaak teleurstellend. Het is hierbij opvallend dat inhaalgroei niet of nauwelijks aanwezig is bij kinderen met UC en ernstige groeivertraging (een lengte SDS van kleiner dan -2). Op de volwassen leeftijd (ouder dan 18 jaar), bedraagt de lengte SDS bij CD -0.7 en -0.4 bij UC. Wanneer de lengte wordt uitgedrukt in SDS correleert de eindlengte op de volwassen leeftijd significant met de lengte op het moment van diagnose ($p<0,001$). Er is geen samenhang tussen de eindlengte (op de volwassen leeftijd) en de lokalisatie van ziekte, ziekteactiviteit of ingestelde therapie. Ook bij de patiënten die behandeld worden met 'anti-tumor necrosis factor' (infliximab; n=39) is er geen duidelijke verbetering in lengte (tijdens de behandeling) uitgedrukt in SDS. Een aantal patiënten met CD hebben belangrijke lengtegroei (met een toename van maximaal 13 centimeter) na de leeftijd van 18 jaar. De lengtegroei kan vorderen tot in de eerste jaren van het 3^{e} decennium. Concluderend, de lengte bij het stellen van de diagnose IBD bij kinderen, uitgedrukt in SDS, is de meest belangrijke factor bepalend voor de eindlengte (op volwassen leeftijd).

Curriculum Vitae

Gerard Damen werd geboren te Oosterhout (Noord-Brabant) de datum 13 april 1964. Zijn V.W.O. diploma behaalde hij aan het Monseigneur Frencken College te Oosterhout in 1982. Aansluitend startte hij met de studie geneeskunde aan de Universiteit van Amsterdam. Na het behalen van het arts-examen, was hij dienstplichtig en werkte hij als eerste-luitenant arts bij het Provonciaal Geneeskundig Detachement in Deventer. Tijdens zijn studie en de dienstplicht verrichtte hij onderzoek naar inhaalgroei bij kinderen met coeliakie (Prof. H.S.A. Heymans en Prof. J.M. Wit) en naar inflammatoire darmziekten bij kinderen (Prof. H.A. Büller). In 1992 werd hij aangesteld als arts-assistent kindergeneeskunde in het Erasmus MC - Sophia Kinderziekenhuis te Rotterdam. In dit ziekenhuis werd hij opgeleid tot kinderarts (opleiders Prof. H.K.A. Visser en Prof. H.A. Büller). Voor de niet-academische periode was hij iets meer dan een jaar werkzaam in het Medisch Centrum Rijmond Zuid (destijds Zuiderziekenhuis) te Rotterdam (opleider dr. R. Sukhai). Nadien werkte hij gedurende 2 jaren als algemeen academisch kinderarts / chef de clinique op de medium care van het Erasmus MC - Sophia Kinderziekenhuis (hoofd Medium Care dr. P.C.J. de Laat). In 2001 startte de opleiding tot kinderarts MDL (voorheen kindergastro-enteroloog) in het Erasmus MC - Sophia Kinderziekenhuis, met dr. M. Sinaasappel als opleider en dr. J. Bouquet als leermeester. In 2004 was hij projectleider bij het ontwikkelen van de interactieve onderwijs Cd-rom ‘Kinderen, voeding en ziekte’ (voorzitter Prof. M. van de Bor). In 2004 was hij werkzaam in het laboratorium Kindergeneeskunde van het Erasmus MC, aldaar startte het werk voor dit proefschrift. Leidend in dit onderzoek waren Edward Nieuwenhuis, Hankje Escher en Janneke Samsom. Sinds 2005 is Gerard Damen werkzaam als kinderarts MDL in het UMC St Radboud te Nijmegen.

Gerard Damen is getrouwd met Francis van Kol. Ze hebben twee kinderen, Casper en Carlijn.

Publications this thesis

Damen GM, Nieuwenhuis EES. Niet-invasieve markers bij inflammatoire darmziekten op de kinderleeftijd. Tijdschr Kindergeneeskunde 2005;73:17-21

Damen GM, Hol J, de Ruiter L, Bouquet J, Sinaasappel M, van der Woude J, Laman JD, Hop WC, Buller HA, Escher JC, Nieuwenhuis EE. Chemokine production by buccal epithelium as a distinctive feature of pediatric Crohn disease. J Ped Gastroenterol Nutr 2006;42:142-9

Van Lierop PP, Damen GM, Escher JC, Samsom JN, Nieuwenhuis EE. Defective acute inflammation in Crohn's disease. Lancet 2006;368:578

Damen GM, van Lierop P, de Ruiter L, Escher JC, Donders R, Samsom JN, Nieuwenhuis EE. Production of IL12p70 and IL23 by monocyte-derived dendritic cells in children with inflammatory bowel disease. Gut 2008;57:1480

Damen GM, van Krieken JHJM, Hoppenreijs E, van Os E, Tolboom JJM, Warris A, Yntema J, Nieuwenhuis EES, Escher JC. Overlap, common features and essential differences in pediatric granulomatous inflammatory bowel disease. Accepted by J Ped Gastroenterol Nutr

Damen GM, Van Krieken JHJM , De Jong D, Van Rheenen R, Nieuwenhuis EES, Escher JC. Differences in histology of biopsies of the gastrointestinal-tract in pediatric- and adult-onset inflammatory bowel disease. Submitted

Damen GM, Van Rheenen R, Tolboom JJM, Otten BJ, de Jong D, van der Woude CJ, Nieuwenhuis EES, Escher JC. Catch-up growth is often lacking in patients with childhood-onset Inflammatory Bowel Disease. Submitted

Other publications

Damen GM, Boersma B, Wit JM, Heymans HS. Catch-up growth in 60 children with celiac disease. J Ped Gastroenterol Nutr 1994;19:394-400

Damen G, de Klerk H, Huijmans J, den Hollander J, Sinaasappel M. Gastrointestinal and other clinical manifestations in 17 children with congenital disorders of glycosylation type Ia, Ib, and Ic. J Ped Gastroenterol Nutr 2004;38:282-7

De Ridder L, Rings EH, Damen GM, Kneepkens CM, Schweizer JJ, Kokke FT, Benninga MA, Norbruis OF, Hoekstra JH, Gijsbers CF, Escher JC. Infliximab Dependency in Pediatric Crohn's Disease: Long-term Follow-up of an Unselected Cohort. Inflamm Bowel Dis 2008;14:353-8

Van Dommelen P, Grote FK, Oostdijk W, de Muinck Keizer-Schrama SM, Boersma B, Damen GM, Csizmadia CG, Verkerk PH, Wit JM, van Buuren S. Screening rules for growth to detect celiac disease: a case-control simulation study. BMC Pediatr 2008;8:35

Den Otter SCM, Hoevenaars BM, Winterdijk P, Swinkels DW, Damen G, Ven Heijst AFJ. Neonatale hemochromatose als oorzaak van leverfalen. Tijdschr Kindergeneeskunde 2009;77:165-9

Van Os E, Schrama M, De Herder WW, Damen GM. Abdominal pain and cystic lesions in the liver of an adolescent: Zollinger-Ellison syndrome. Submitted

Dankwoord

Zonder de hulp van een groot aantal collegae was dit proefschrift niet tot stand gekomen. Ik wil graag een aantal mensen bedanken.

De meest belangrijke persoon voor het tot stand komen van dit proefschrift is Edward Nieuwenhuis. Je begeleidde me van het eerste tot en met het laatste uur. Jij kwam in het laboratorium Kindergeneeskunde / Kindergastro-enterologie op het moment dat ik behoorde te starten met de wetenschappelijke stage van mijn fellowship Kindergastro-enterologie. Met een open vizier was je bereid een 'oudere jongere' te begeleiden bij het verrichten van onderzoek en je dacht direct in hoofdstukken van een proefschrift. Mijn enorme dank voor dit vertrouwen en voor je steun. Ik heb zeer veel van je geleerd. Wanneer ik een stukje heb opgepikt van je wijze van schrijven en taalgebruik ben ik tevreden.

De inhoud van de eerste hoofdstukken betrof basaal wetenschappelijk werk. Derhalve wil ik Lilian de Ruiter, laborante binnen het laboratorium Kindergeneeskunde / Kindergastro-enterologie, hartelijk danken. Je hebt een clinicus met twee linker handen wegwijs gemaakt binnen een laboratorium. Je stond altijd voor me klaar en ik heb veel van je geleerd. Dank voor je tomeloze inzet en je geduld.

Naast Edward was ook Hankje Escher zeer belangrijk bij het tot stand komen van dit proefschrift. In de eerste hoofdstukken hielp je me met de inclusie van de patiënten, in de latere hoofdstukken werd je bijdrage meer en meer belangrijk. Dank voor je vertrouwen en voor je geduld. Je hebt me sterk geholpen met het doen van onderzoek en het schrijven van papers.

Binnen het Sophia Kinderziekenhuis wil ik de medewerkers danken die me hielpen met de inclusie van de patiënten. Marianne en de anderen wil ik danken voor de bloedafnames en de keren dat ik jullie lastig viel op de polikliniek.

De leiding van de afdeling kindergeneeskunde wil ik danken. Allereerst Hans Büller. Reeds in Amsterdam werkte je enthousiasme voor de kindergeneeskunde aanstekelijk. Je hebt me geholpen in de eerste stappen van mijn carrière. Later kwamen onze wegen weer samen in Rotterdam. Je stond me toe om te starten met het fellowship Kindergastro-enterologie en je hebt me gesteund op het moment dat ik door moest pakken met de wetenschap. Zonder je steun had mijn carrière anders kunnen lopen! Bert van der Heijden wil ik danken voor zijn rol als promotor. Ik begrijp dat ik je heb verrast als 'oudere jongere'. Ook Ronald de Groot wil ik danken. Vanuit Nijmegen stond je me toe te werken aan de laatste hoofdstukken van dit proefschrift. Ik kende je al vanuit Rotterdam, ook jouw enthousiasme voor de kindergeneeskunde is en blijft aanstekelijk. Ik heb veel van je geleerd. Bij de leidinggevenden behoort vanzelfsprekend Prof.dr. H.K.A. Visser. Ik wil u danken

voor het feit dat ik in Rotterdam mocht komen werken, en voor de opleidingsplaats die ik kreeg. Ik heb veel geleerd en met veel plezier gewerkt in het Sophia Kinderziekenhuis.

Binnen de verschillende laboratoria van de Erasmus-toren wil ik de nodige personen danken. Lilian heb ik al genoemd. Janneke Samsom wil ik danken voor de steun en de adviezen die ik van je kreeg op het moment dat je werkzaam werd binnen het laboratorium. Voor een aantal hoofdstukken werkte ik nauw samen met Jeroen Hol en Pieter van Lierop. Ik heb de samenwerking met jullie beiden zeer gewaardeerd en ik wil jullie danken voor jullie bijdrage. Dit omvatte o.m. het wassen en stimuleren van wangslijmvlies, het verversen van medium, het in leven houden van dendritische cellen, het voeren van discussie, het leveren van opbouwende kritiek en zeker ook gezelligheid. Jeroen, wanneer je het laboratorium binnen kwam werd het levendig. Jullie rol in dit proefschrift is belangrijk. Bart Lambrecht en Hamida Hammad, dank voor de kennis die jullie met me wilden delen voor wat betreft het werk aan dendritische cellen. Een zeer belangrijk onderdeel van mijn proefschrift. Ik heb hieraan met plezier gewerkt door jullie hulp. Jon Laman en Marianne van Meurs, dank voor de steun die ik kreeg in het leren van de begrippen van immunofluorescentie. De deur stond wijd open, ik heb dit zeer gewaardeerd. Wim Hopman en Rogier Donders, dank voor jullie werk, geduld en steun inzake de statistiek. Zonder jullie hulp is publiceren een stuk lastiger. Ytje, Rolien, Dicky, en de vele anderen uit het laboratorium, ik wil jullie danken voor jullie gastvrijheid en hulp. Ik kon altijd op jullie terugvallen. Theo, het spelen met de taal zal ik missen. Binnen de kindergeneeskunde is er een prettige samenwerking tussen de verschillende laboratoria. Ik wil de collegae van de kinderoncologie danken voor het feit dat ik steeds in jullie laboratorium mocht kruipen en voor de hulp die ik van jullie kreeg. Ik heb dit zeer gewaardeerd.

Janneke van der Woude en Dirk de Jong, ik wil jullie beiden danken voor jullie bijdrage in de hoofdstukken. Jullie steun was onontbeerlijk. De deur stond wijd open. Met collegae als Janneke en Dirk blijkt samenwerking tussen verschillende centra prima te verlopen. Han van Krieken, ik wil je hartelijk danken voor je bijdrage in de hoofdstukken m.b.t. de histologie. Ook jij speelde een belangrijke rol en ik heb veel van je geleerd. Ik voelde geen drempel om bij je binnen te lopen, waarvoor mijn dank. Barto Otten, dank voor je bijdrage inzake groei en inhaalgroei. Je had een belangrijk aandeel in dit hoofdstuk. Het is uitermate prettig werken met je, dank voor je steun. Ronald van Rheenen (de enthousiaste co-assistent), je hebt hard gewerkt en een uitstekende bijdrage geleverd in de hoofdstukken. Hiervoor mijn dank. Ik zie je toekomst met vertrouwen tegemoet. Michelle Borm, dank voor het verrichten van het genetisch onderzoek bij de kinderen met IBD.

De opponenten wil ik danken voor het feit dat jullie plaats willen nemen in de promotiecommissie. Ik voel me vereerd. Het lijkt op een weerzien met ‘oude bekenden’, doch ik weet dat ik me terdege moet voorbereiden.

Ik wil Mrace (Medische Research Advies Commissie van het Erasmus MC) danken voor de subsidie

die ik kreeg voor het onderzoek. Ik wil de sponsoren hartelijk danken voor het mogelijk maken van de publicatie van dit proefschrift.

Verder wil ik deze mogelijkheid gebruiken om mensen te danken die belangrijk waren in mijn carrière. Enkelen zijn in bovenstaande nog niet genoemd. Peter de Laat wil ik danken voor het delen van zijn kennis met me. Je hebt me een beter kinderarts gemaakt. Je was en bent voor mij een voorbeeld van een inspirerend en compleet kinderarts. Maarten Sinaasappel en Jan Bouquet wil ik danken voor de mogelijkheid die ik kreeg om kinderarts MDL te worden. Ik heb genoten van de opleiding en het persoonlijke contact. Jules Tolboom, je hebt me de mogelijkheid gegeven om geruime tijd een dag in de week te werken aan mijn promotieonderzoek. Ik heb dit zeer gewaardeerd. Het was een fantastische tijd om samen met jou, op één kamer, te werken binnen het UMC St Radboud.

Wim van Kol (schoonvader), Erik de Lorme en NPN drukkers wil ik danken voor de professionele en fraaie vormgeving van dit proefschrift. Wim en Diny, dank voor de overnachtingen die ik nodig had wanneer ik vanuit Nijmegen nog eens het laboratorium indook. Vooral ook dank voor het feit dat ik met jullie dochter in het huwelijk mocht treden, een absolute eer!

Mijn ouders wil ik danken voor de mogelijkheid die zij me gaven om geneeskunde te studeren. Een keuze die jullie altijd hebben gesteund. De werklust en de vasthoudendheid heb ik van jullie. Mijn broer wil ik danken voor zijn adviezen. Doordat ook jij geneeskunde studeerde heb je me waardevolle adviezen gegeven op momenten dat in mijn carrière keuzes moesten worden gemaakt.

Mijn vrouw, Francis, en mijn kinderen, Casper en Carlijn, wil ik danken voor de tijd die ik van hen kreeg om aan onderzoek te werken. Met jullie is mijn leven evenwichtig en compleet. Jullie liefde is grenzeloos. In mijn carrière was en is jullie bijdrage onmisbaar. Ik hou van jullie.