General discussion

Primary immunodeficiency diseases (PIDs) are generally characterized by an increased risk of infectious complications due to specific inborn errors in immune cell function. However, patients with PIDs also present with features of significant immune dysregulation, often leading to autoimmunity, autoinflammation, (hematological) malignancies and allergic disorders.[1-3] Previously, immunodeficiency and autoimmunity were considered to be mutually exclusive conditions. Increased understanding of the complex immune regulatory and signaling mechanisms involved, coupled with the application of genetic analysis, reveals more and more the relation between primary immunodeficiency syndromes and autoimmune diseases.[4] Monogenic defects can cause rare diseases that predominantly present with autoimmune symptoms or recurrent infections (immunodeficiency). However, it has been increasingly recognized that various genetic variant give rise to a clinical phenotype of both immunodeficiency and autoimmunity. Also other features of immune dysregulation, like (hematological) malignancy, may be attributed to genetic variants found in PIDs. As an example, a major complication of activated phosphatidylinositol 3-kinase-δ (PI3K-δ) syndrome (APDS) is malignancy (especially B-cell lymphoma), which is the result of uncontrolled PI3K-δ activity in lymphocytes.[5]

The first aim of this thesis was to gain more insight in the nature and prevalence of infectious and noninfectious skin disorders and manifestations within the atopic syndrome, including atopic dermatitis (AD), food allergy (FA), asthma and allergic rhinitis (AR), in patients with PIDs. Recognition of these symptoms could facilitate earlier diagnosis of PIDs. As skin disorders, including AD, and atopic manifestations were demonstrated as frequently occurring symptoms in PIDs, it suggests that immune dysregulation also plays a role in the multifactorial pathogenesis of AD and the atopic syndrome. Moreover, common pathways appear to be involved in the pathogenesis of these disorders and PIDs. Therefore, this thesis aimed to identify these common pathways of immune dysregulation in PIDs, atopy and AD. Knowledge on immune dysregulation processes in PIDs was used to gain more insight in the pathogenesis of AD and the atopic syndrome, in which endotypes were identified. Moreover, the antibody response and microbiome in AD were studied to better understand the interaction between various pathogenic factors in AD and immune dysregulation. Based on the predominance of *S. aureus* in the skin microbiome of AD patients, a clinical trial was performed using an endolysin selectively targeting *S. aureus*. In the following paragraphs the main findings of this thesis are summarized and discussed.

MAIN FINDINGS OF THE THESIS

Skin disorders are prominent clinical features in primary immunodeficiency diseases

PIDs are characterized by an increased risk of infections, autoimmune disease, autoinflammatory complications, malignancy and allergic disorders. In Chapter 2 of the thesis it was demonstrated that skin disorders are also frequently occurring symptoms in patients with PIDs, based on a systematic literature analysis of 67 mainly cross-sectional studies. A complete spectrum of skin disorders was composed, categorized in 15 main groups of cutaneous manifestations, across 30 PID phenotypes. Presence of skin manifestations characteristic for ataxia-telangiectasia (AT), hyper immunoglobulin (Ig) E syndrome (HIES), selective IgA deficiency (SIgAD), autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) and chronic granulomatous disease (CGD) have been identified. For example, skin abscesses were found to be a well-defined characteristic for HIES and CGD, and alopecia and skin pigmentation disorders for SIgAD and APECED. In various PIDs skin disorders were already known to be among the clinical characteristics and frequently occurring manifestations, such as telangiectasia in AT and granuloma and skin abscesses in CGD. However, this thesis also reveals infectious and noninfectious skin disorders of which an association with a PID was not generally recognized. Examples include alopecia in SIgAD and hypopigmented macules in AT. Although various skin conditions were described in patients with a PID, the prevalence of these skin conditions in a matched control group remains largely unknown. Interpretation of the data should, therefore, be done with caution. Nonetheless, the high prevalence of skin conditions in PIDs suggests that these could be used as an additional warning sign to raise suspicion for underlying PID. Linking skin disorders to PIDs provides a valuable tool that could raise PID awareness in clinical practice.

Skin infections and nail disorders are warning signs for primary immunodeficiency diseases

In Chapter 3.1 of the thesis, a Dutch cohort of 45 pediatric patients and 207 adult patients with a PID and 56 unaffected partner-controls was studied using a questionnaire. A history of skin disorders, in particular skin infections and nail disorders, was more frequently reported in adult PID patients (80.2%) when compared with adult partner-controls (41.1%, $p<0.001$). As far as we know this was the first study evaluating the nature and prevalence of skin disorders in a mainly Western population of PID patients. Data retrieved from this cohort of PID patients were mostly consistent with the data summarized in our systematic review (Chapter 2), which included mainly data derived from Middle-Eastern countries. However, in the Dutch patient cohort viral and bacterial skin infections, erythematous skin lesions and skin rashes were more frequently reported than in current literature.

Skin disorders were found to develop earlier in life in adult patients with PIDs (mean age 20.9 (SD 22.0) years) when compared with unaffected partner-controls (mean age 33.7 (SD 26.6) years, p=0.02) suggesting an association between the skin condition and immune dysfunction in PID. Moreover, skin disorders preceded a diagnosis of PID on average 20.3 (SD 20.5) years in adult patients, whereas classical PID symptoms, such as respiratory tract infections, developed on average 15.9 (SD17.7) years before the PID diagnosis in this patient cohort. Based on these results, skin disorders frequently appeared to be presenting symptoms, while they are not included in the warning signs for PIDs.

Current literature appointed eczematous dermatitis as common finding and presenting clinical manifestation among PIDs, especially in rare PIDs which are not well represented in our cohort.[6] In our study, (atopic) dermatitis also was the most commonly reported presenting skin disorder in PID patients. However the prevalence did not significantly differ from partner-controls (29.5% vs. 25.0%, respectively) and even less adult patients reported (atopic) dermatitis as first developed skin disorder compared with partner-controls (15.0% vs. 52.2%, respectively). Moreover, the prevalence of a history of (atopic) dermatitis in both adult patients and partner-controls included in this study corresponds to the lifetime prevalence of atopic dermatitis in the Dutch population, which is up to 25% in children and 1-7% in adults.[7,8] Therefore, we feel that (atopic) dermatitis is not a distinctive presenting manifestation in the development of PIDs in general. By contrast, we hypothesize that (a combination of) other skin manifestations, like skin infections and nail disorders (prevalence in adult patients vs. adult controls 61.4% vs. 17.9%, p<0.001, and 38.6% vs. 7.1%, p=0.005, respectively), are more useful in raising suspicion of an underlying PID in addition to the presence of the currently used warning signs for PIDs.[9]

Staphylococcus aureus-targeting treatment in patients with primary immunodeficiency diseases could be beneficial in reducing severity of skin disease

Staphylococcus (*S.*) *aureus* is involved in the pathogenesis of many common infectious skin disorders (i.e. *S. aureus*-induced skin infections) and the inflammatory skin disorder AD. In patients with PIDs, only a few studies reported culture-proven *S. aureus*-associated skin disorders (Chapter 2).[6,10-12] These skin disorders included a papulopustular eruption, eczematous dermatitis, (cold) abscesses and wounds in HIES[13-16], skin infections in Comèl Netherton syndrome[17] and suppurative dermatitis in patients with CGD.[18] Furthermore, colonization with *S. aureus* was found to be significantly correlated with skin disease severity in PID patients.[19]

In our Dutch population of adult PID patients, including mostly predominantly antibody deficiencies (PADs), a positive *S. aureus* culture at a single time point was found in 40.0%

(12/30) of the skin disorders with suspected *S. aureus*-related etiology (Chapter 3.1). These positive cultures originated from dermatitis lesions, ecthyma and folliculitis. The prevalence of lesional *S. aureus* in our PID population was lower than the prevalence of lesional *S. aureus* in patients with AD (70%).[20] *S. aureus* colonization in less than half of the clinical suspected *S. aureus*-related skin lesions in our study could reflect intermittent carriage of *S. aureus*.[21] We suppose that repetitive culturing during clinical examination to identify skin lesions colonized with *S. aureus* can contribute in selection of PID patients, which might have benefit from *S. aureus*-targeting treatment to reduce skin disease severity.

Atopic manifestations are underestimated clinical features in primary immunodeficiency diseases

The systematic review presented in Chapter 2 of the thesis shows that original data on atopic manifestations in PIDs are limited, as studies comprise small patient samples and the diagnosis of atopic manifestations is generally not based on diagnostic tests. Although atopic manifestations are described in various PID phenotypes, presence of these manifestations is mainly reported in immunodeficiencies affecting both cellular and humoral immunity (combined immunodeficiencies; CIDs), like DOCK8 deficiency, and CIDs with associated or syndromic features, such as Comèl Netherton syndrome.[6,22] In addition, atopic manifestations have also been reported in PADs, like SIgAD, with a prevalence comparable to the general population.[23-28]

In Chapter 3.2 we showed that all atopic manifestations, including AD, FA, asthma and AR, were highly prevalent in 47 children and 206 adults with a PID based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and diagnostic criteria or tests.[29] Using the questionnaire, we found in adult patients a self-reported prevalence of 49.5%, 10.7%, 55.7% and 49.8% for AD, FA asthma and AR, respectively. Remarkably, a discrepancy was shown between the prevalence of AD based on patient-reported skin disorders (Chapter 3.1) and the ISAAC questionnaire (Chapter 3.2). Self-reported ever experienced atopic manifestations were significantly more common in adult PID patients when compared with 56 adult partner-controls. Although our study population mainly involved patients with PADs and CIDs, atopic manifestations were reported in a large spectrum of PIDs across the various phenotypes, which is in contrast with previous literature (Chapter 2). The prevalence of FA, asthma and AR based on diagnostic test results was significantly lower than the prevalence reported by adult patients (FA 4.8%, asthma 16.4% and AR 19.2%). This discrepancy could be due to overreporting of clinical symptoms related to atopy, the relapsing-remitting course of atopic manifestations over time, or because PID patients are known to commonly have asthma-like airway complaints without of a positive diagnostic test.[30] Unfortunately, data on diagnostic tests from the control group or another

reference population were not available. Nonetheless, estimates of atopic manifestation prevalence in PID patients were provided in this study that can be used for future studies.

Compared with our review (Chapter 2), in which diagnosis of atopic manifestations was generally based on medical records, we found a significantly higher prevalence of atopic manifestations in patients with a PAD and comparable numbers of patients with atopic manifestations in CIDs. The exact mechanism underlying the development of atopic manifestations in PIDs remains to be elucidated. Nonetheless, the pathogenic pathway involved in the atopic syndrome, in which T lymphocytes play a central role, could suggest that patients with a PID affecting cellular immunity are more prone to develop atopic manifestations.[31] However, it is known that also in PADs T lymphocyte abnormalities are found, which could contribute to development of the atopic syndrome in these patients.[32,33] According to our data, we propose early evaluation of atopic manifestations in patients with CIDs and PADs to prevent clinical deterioration. Future studies should focus on identification of specific atopic characteristics of PIDs to evaluate whether they could serve as a potential warning sign for an underlying PID.

New clustering algorithm shows endotypes within the atopic syndrome based on expression profiles of immune cell lineages

As mentioned in the introduction of the thesis, the atopic syndrome has a heterogeneous clinical presentation, which probably is the result of an interaction between physiological, biological, immunological and genetic mechanisms.[34] Atopic manifestations are prevalent comorbidities in PIDs, suggesting that genetic defects in pathways that are involved in monogenetic PIDs could also play a role in the pathogenesis of the atopic syndrome.[6,35] In Chapter 4 we defined subclasses within the atopic syndrome via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages. We identified 160 atopy-related genes, of which 22 genes were overlapping with disease-causing genes of monogenic PIDs.[36] Seven distinct clusters within the atopy-related genes were identified. The overlapping genes involved in both the atopic syndrome and PIDs were bundled in two of the atopy-related gene clusters suggesting that these endotypes of the atopic syndrome could be associated with the predisposition to develop a PID. However, these data should be confirmed *in vivo* as atopy-related variants in the overlapping genes might differ from disease-causing variants resulting in of PID.

Based on the known atopy-related genes and corresponding expression profiles of immune cell lineages, T helper (Th) lymphocytes seem to play a crucial role in the pathogenesis of the atopic syndrome, which is in accordance with previous findings. Disturbed T lymphocyte function has been described in AD, FA, asthma and AR.[37-43] This finding supports the hypothesis that changes in the immune system underlie and could be involved in the

pathogenesis of the atopic syndrome next to genetic mechanisms, which have previously been reported in literature.[44-53]

Patients with atopic dermatitis have an increased IgE response against *Staphylococcus aureus*

In Chapter 5 of the thesis a systematic review on the antibody response against *S. aureus* was performed to gain more insight in how the immune system of AD patients counteracts the bacterium. This might help us to better understand the role of *S. aureus* in AD pathogenesis, as well as the mechanisms by which *S. aureus* causes inflammation. We significantly more often found an IgE response against *S. aureus* immune modulating superantigens (33% for staphylococcal enterotoxin (SE) A and 35% for SEB) in patients with AD as compared with healthy controls (pooled odds ratio with 95% confidence interval 8.37 (2.93-23.92) for SEA and 9.34 (3.54-24.93) for SEB). Subgroup analyses to explain the high heterogeneity in the pooled analyses suggest that the antibody response is dependent on the method of antibody identification (ELISA vs. RIA) and the geographical region of the study centre (Asia vs. Europe) in accordance with the study of Taylor *et al*.[54] The increased IgE response against immune modulating antigens suggests that *S. aureus* encourages epithelial damage via direct T lymphocyte stimulation and subsequent T lymphocyte proliferation and cytokine release.[55-57] Nonetheless, it is unclear whether the increased IgE response is specific for patients with AD as comparable studies in other *S. aureus*-related diseases (i.e. *S. aureus* bacteraemia, folliculitis) are not available. Furthermore, it is unknown whether the elevated IgE response in AD is a consequence of the increased skin permeability, abundance of SEA and SEB carrying *S. aureus* strains or an altered immune response against *S. aureus*. For example, the anti-SEA and anti-SEB IgE responses could be the result of increased expression of these antigens by AD-specific *S. aureus* strains, indicating SEA and SEB expression as possible bacterial mechanisms to aggravate or even initiate inflammation in AD. However, previous studies describe absence of a prevailed *S. aureus* genotype, suggesting that the IgE response is the result of immune dysregulation in AD in contrast to colonization with an AD-specific *S. aureus* strain.[58-61]

Targeted intervention against *Staphylococcus aureus* had no effect on symptoms of atopic dermatitis

The effect of long-term treatment of AD with an endolysin that selectively targets *S. aureus* was studied using a double-blind, vehicle-controlled design and described in Chapters 6.1 and 6.2 of this thesis. Over the 12-week intervention period (corresponding to 8400 days for 100 patients), patients in the endolysin group used a topical corticosteroid (TCS) for 1889 (45.0%) days compared with 1566 (37.3%) days in the vehicle group. There was no statistically significant difference in the probability of TCS use per day between the groups nor a difference in reduction of clinical disease severity scores. These data

are in accordance with data from a Cochrane review showing no significant effect of short-term nonspecific anti-*S. aureus* therapy (e.g. antibiotics and antibacterial therapeutic clothes) in patients with noninfected AD.[62] We could not confirm the positive results of other longer-term studies. However, these studies used broad-spectrum antimicrobials and mainly included patients with signs of bacterial infection.[63] Patients with clinically infected AD were excluded from our study. A possible effect of anti-*S. aureus* endolysins in patients with clinically infected AD should be determined in future studies.

Several other hypotheses could explain our results, which has implications for future studies. First, use of triamcinolone in the run-in phase resulted in a decrease in AD severity, which could have masked a possible benefit of endolysin treatment. Second, anti-*S. aureus* treatment might not be suitable for all patients with AD because it is a heterogeneous disease, indicating the need for subphenotyping. Since only 56% of our study population had two consecutive positive *S. aureus* skin cultures (indicating persistent colonization) before start of the intervention, the target population that would probably benefit the most from endolysin treatment was small.

However, the most likely reason for the absence of a significant reduction in TCS use in this study might be that there was no difference in *S. aureus* load reduction between the endolysin and vehicle treated groups determined by semi-quantitative culture and qPCR. Possibly, patients were recolonized with *S. aureus* from the nose since 73% of them were nasal *S. aureus* carriers.[64,65] Furthermore, cetomacrogol used as basis of the cream could have formed a barrier on the skin that prevents the endolysin to reach and subsequently kill *S. aureus*. Some reduction in *S. aureus* load could, however, have been expected in both treatment groups due to the use of corticosteroids and emollients in this study, which both have shown to reduce the *S. aureus* load on the skin.[66-70] Nonetheless, it is unclear whether complete eradication of *S. aureus* is required for clinical improvement as a case series showed a clear clinical improvement without significant *S. aureus* reduction using a qualitative culture in clinically infected AD.[71]

In conclusion, endolysin treatment as studied in this randomized controlled trial did not contribute to unraveling the contribution of the microbiome in the pathogenesis of AD due to absence of a clinical effect on AD and reduction of *S. aureus* load. Nonetheless, endolysin treatment was well tolerated and this study provides estimates of AD symptoms, use of TCSs and the percentage of persistent *S. aureus* carriers that can be used for future clinical studies.

IMMUNE DYSREGULATION: A MODEL FOR RESEARCH ON PRIMARY IMMUNODEFICIENCY DISEASES, SKIN DISORDERS AND ATOPY

Primary immunodeficiency disease as immune dysregulation disorder

PIDs are characterized by a compromised or entirely absent function of a part of the immune system, which makes people vulnerable for infections. Therefore, PIDs are generally considered to be immunodeficiency diseases, as implied by its name. However, there is increasing evidence that non-infectious complications, including autoimmune and autoinflammatory complications, (hematological) malignancies and allergic disorders, are involved in PIDs as well.[1-3] Autoimmune disorders, such as type 1 diabetes mellitus, rheumatoid arthritis and psoriasis, are the result of an immune response directed against normal parts of the body, termed auto-antigens. In autoinflammatory disorders, like familiar mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), the innate immune system is abnormally activated, leading to recurrent episodes of fever and inflammation.[72] Both autoimmune and autoinflammatory conditions are characterized by disruption of the normal function of the immune system, also called immune dysregulation. Interestingly, different forms of immune dysregulation, both as primary or as accompanying problem, next to the immunodeficiency seem to occur in one and the same PID. Therefore, PIDs should be considered as immune dysregulation disorders instead of immunodeficiency diseases.[73]

Immune dysregulation in skin disorders and atopy

Immune dysregulation also plays a role in the multifactorial pathogenesis of skin disorders and atopy.[74] For example, chronic inflammation caused by different triggers, such as biological agents (e.g. bacteria, viruses), physical agents (e.g. UV radiation) and immunologic disorders (e.g. PIDs), is suggested as one of the hallmarks in skin carcinogenesis.[75] In AD, a number of immunological abnormalities have been described, such as increased serum IgE levels, elevated Th2-type cytokines in acute lesions and Th1-type cytokines in chronic lesions, and decreased expression of antimicrobial peptides.[76] The immunological dysregulation might precede barrier changes (inside-out hypothesis) or could be the effect of barrier dysfunction and subsequent penetration of environmental stimuli into the skin (outside-in hypothesis).[77] It could be suggested that immune dysregulation is primary affected in AD as not all patients with AD have a genetic polymorphism in one of the barrier genes (e.g. *FLG*, *KIF3A*, *OVOL1* and *ADAMTS*).[78,79] The fact that a significant amount of genes involved in AD is associated with immune dysregulation further supports a genetic predisposition of immunological alterations in AD.[44] More than half of the patients with AD will develop asthma and other allergic diseases (i.e. FA and AR) involved in the atopic syndrome, which indicates that both local and systemic immunity are involved.[80,81] In a Dutch study group

of children with moderate-to-severe AD more than 60% of the patients had a diagnosis of FA and even more than 80% of the patients were diagnosed with asthma and AR.[82] The pathway involved in the atopic syndrome could be characterized by auto-allergy, in which atopy seems to stand at the boundary between allergy and autoimmunity. Herein, the presence of IgE antibodies against self-proteins is an important pathogenic feature, emphasizing the role of immune dysregulation in the atopic syndrome.[83-85]

Spectrum of immune dysregulation

The spectrum of clinical features associated with PIDs has broadened due to the recent identification of many novel causative genes.[86] Patients with a PID and noninfectious complications are increasingly recognized with features of immune dysregulation, including autoimmunity, inflammation, lymphoproliferation and malignancy.[87] Depending on the pathways involved and the corresponding number and functionality of immune cells involved, the clinical manifestations of diseases involving immune dysregulation are characterized by a higher susceptibility to infections or, on the other hand, an increase in manifestations of autoimmunity.[4] Moreover, it has been increasingly recognized that various genetic variants give rise to a clinical phenotype of both immunodeficiency and autoimmunity. One could visualize immune dysregulation as a spectrum with infections on one end and autoimmunity on the other end. PIDs can present with a variety of symptoms covering the entire spectrum (Figure 1). Within PIDs, for example, patients with severe combined immunodeficiency (SCID) are particularly susceptible to opportunistic infections, whereas patients with APECED usually present with autoimmune manifestations in the endocrine glands.

INFECTIONS **AUTOIMMUNITY**

Figure 1. Spectrum of immune dysregulation

Patients with (mild) AD and other atopic manifestations are characterized by an infectious phenotype as the immune system in these diseases is directed against external factors. On the other hand, patients with increased severity of atopic manifestations may be characterized by autoimmunity as levels of IgE autoantibodies have been shown to be associated with disease severity.[84]

Overlap in immune dysregulation: a model for future studies

Taking the hypothesis of a spectrum one step further, different variants of immune dysregulation result in a diversity of clinical manifestations within the same patient. We found that 80% percent of the patients with a PID has a history of skin disorders and around

one third suffered from AD during life, which was higher as compared with controls. Furthermore, atopic manifestations were found significantly more prevalent in PID patients than in a matched control group. These results show a clinical overlap in presence of skin disorders, atopic manifestations within the atopic syndrome and PIDs. Expression profiles of immune cell lineages substantiated a genetic overlap between (monogenic) PIDs and endotypes within the atopic syndrome as well. Therefore, we propose a model that assumes overlap between immune dysregulation disorders (PID, skin disorders and atopy) (Figure 2). AD is a clinical example of the overlap between skin disorders and the atopic syndrome, in CGD both (non-atopy related) skin disorders and PID are involved and HIES is an example of disease in which all three components are affected.

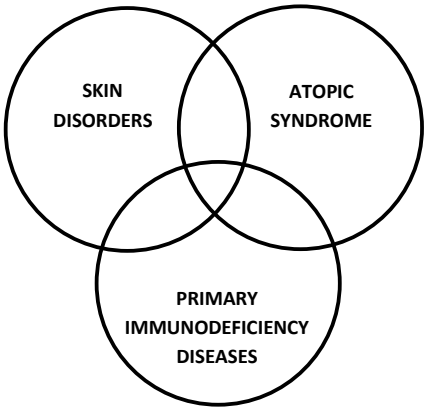

Figure 2. Overlap in immune dysregulation

Presence of manifestations of immune dysregulation could fluctuate throughout life, suggesting immune dysregulation has a dynamic spectrum of diseases involved. Our assumption emphasizes that more attention should be paid to simultaneous presence of multiple immune dysregulation disorders in clinical care. The model as shown in Figure 2 should be used both in clinical care and for research purposes. Caretakers should consider other immune dysregulation disorders in patients who present with one of the disorders. In this context, detailed registration of these clinical manifestations of each of the components within the model could give further insight in the clinical overlap of immune dysregulation manifestations within a patient (group). Additionally, the model can help design future studies getting more insight in the pathogenesis and role of immune dysregulation within the associated diseases.

CLINICAL IMPLICATIONS OF RESULTS OF THE THESIS

The diagnostic delay of primary immunodeficiency diseases might be reduced by using skin symptoms as warning signs

As mentioned in the introduction of the thesis, the delay between onset of the first symptoms and diagnosis of PIDs in the Netherlands is up to 14.5 years and might result in a reduced quality of life.[88-93] In our questionnaire-based studies (Chapters 3.1 and 3.2), patients reported a diagnostic delay up to 15.9 years from the first classical PID symptom,

such as respiratory tract infections. Skin disorders were shown to precede the diagnosis of PIDs for many years. Moreover, patients with a PID appear to develop skin disorders at a younger age compared with people without a PID.

Within the spectrum of PIDs, skin infections and nail disorders, were significantly more prevalent in adult patients compared with partner controls. In addition, a history of asthma and AR was reported significantly more often by adult patients than controls. Although (atopic) dermatitis was the most frequently noted skin disorder in patients, is was not found to be a specific skin condition related to PIDs.

Although current literature appointed eczematous dermatitis as common finding and presenting clinical manifestation among PIDs, we feel that reduction of the diagnostic delay of PIDs in general could be achieved by recognition of non-dermatitis-like skin disorders, like skin infections and/or nail disorders in addition to the presence of the currently used warning signs for PIDs. Moreover, data shown in this thesis indicate that atopic manifestations, of which at least asthma and AR, might serve as a potential warning sign for an underlying PID as well. To further discriminate between the different types of PIDs based on patients' clinical symptoms, we composed an overview of PIDs per skin disorders that could serve as a valuable support tool for PID awareness in clinical practice and for registries (Chapter 2). However, skin disorders as well as atopic manifestations are also frequently described in the general population.[6] Therefore, it is of importance to realize that presence of specific skin symptoms alone does not necessarily point towards a (specific) PID. Nonetheless, recognition of specific cutaneous manifestations in combination with other clinical features suggestive of an immunodeficiency, like the warning signs for PIDs, should raise awareness to an underlying PID and may facilitate earlier diagnosis of PIDs.[9] Moreover, identification of specific atopic characteristics of PIDs in future studies could further increase the suspicion of an underlying PID. In this context, the diagnosis of suspected PIDs or PID-classes based on clinical symptoms, for example multiple skin disorders and recurrent upper airway infections, could be confirmed with laboratory tests. Using the multi-stage diagnostic protocol of de Vries[3] or the phenotypic approach for PID classification and diagnosis by Bousfiha *et al.*[94], a first diagnostic step in case of a supposed antibody deficiency or neutropenia could be blood count and differentiation, IgG, IgA, IgM and IgE. In case of a possible combined immunodeficiency disease, these tests should be supplemented by lymphocyte subpopulations.

Extended registration of clinical features will improve the diagnostic and therapeutic processes in patients with primary immunodeficiency diseases

Data from our review and questionnaire-based studies (Chapters 2, 3.1 and 3.2) demonstrate a high prevalence of skin disorders and atopic manifestations in patients with

a PID. However, most PIDs are rare and a reliable prevalence of skin disorders and atopic manifestations in PIDs can only be obtained by reporting these clinical features in larger cohorts. The international PID database of The European Society for Immunodeficiencies (ESID) registers, among others, data on warning signs of PIDs. These currently applied warning signs focus on the presence of infectious skin disorders. Noninfectious cutaneous symptoms and atopic manifestations are not included. Based on data of studies included in this thesis, showing skin disorders and the atopic syndrome as highly prevalent and potentially distinctive symptoms of PIDs, we suggest to collect more detailed data on all skin disorders and atopic manifestations in the ESID registry in order to improve the diagnostic and therapeutic processes in patients with PIDs. In this context, higher number of data on these manifestations reported per PID might further improve our composed support tool for PID awareness in clinical practice, creating the possibility to better discriminate between PID phenotypes based on cutaneous and/or atopic symptoms. Furthermore, extensive registration of these symptoms in patients with a PID might provide reliable data on the prevalence of cutaneous and atopic manifestations of rare PID phenotypes. Second, collection of data on cutaneous *S. aureus* carriage on skin lesions in PIDs might identify patients that could have benefit from *S. aureus*-targeting treatment to reduce skin disease severity.[19] In this thesis, a discrepancy between the self-reported prevalence of atopic manifestations and the prevalence based on diagnostic criteria or tests was demonstrated indicating the need for registration of test results as well. For example, the standardized use of skin biopsies to confirm the diagnosis of cutaneous manifestations histopathologically should be considered, as clinically comparable skin lesions could demonstrate different histopathological images in patients with PIDs due to an altered immune system.[95] In addition, we recommend having skin conditions diagnosed by a dermatologist within the diagnostic process to increase its reliability.

RECOMMENDATIONS FOR FUTURE STUDIES

Genome sequencing should be further implemented in the diagnostics of primary immunodeficiency diseases

The last two decades there has been an exponential growth in genome sequencing with immense increase in speed and efficiency concomitantly with reduction in cost. Genome sequencing has proved its usefulness in diagnostics for PIDs and is the gold standard in most of the (monogenic) PID diagnoses, but is currently not used as first-line diagnostic. As genome sequencing becomes cheaper and more convenient, both supply and demand will rise. Subsequently, sequencing will be used as standard in care for patients with a PID, which will shorten the diagnostic delay and reduce chronic deterioration due to PID-associated symptoms. By identification of genetic variants in patients with PIDs, we would

be able to correlate clinical features, including cutaneous and atopic manifestations, to specific genetic variants. Moreover, sequencing could identify patients based on detected genetic variants, that require additional specialist care or screening because of an increased risk of associated comorbidities (e.g. dermatologist because of high probability of development of skin disorders, pulmonologist because of association with asthma or other airway complaints). Routine diagnostic genetic testing of patients with a CVID phenotype was recently suggested to improve diagnostics in these patients.[96]

Clustering of atopy-related genes might unravel the heterogeneous presentation of the atopic syndrome

Currently, atopic patients have already been stratified based on clinical and immunological characteristics, including the type of immune response involved.[97] In this thesis, subclasses within the atopic syndrome were for the first time defined via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages of healthy mice, as reported in Chapter 4. These gene expression profiles may not be identical to those in (atopic) humans and may explain why we could not cluster all human atopy-related genes including *FLG*, which is an important atopy gene based on the number of atopy-related variants (n=62). Furthermore, data from mice cannot directly be applied for subgrouping of the atopic syndrome in humans. Therefore, the genetic material of large cohorts of patients with the atopic phenotype should be sequenced to investigate whether atopy clusters could be generated based on the gene expression profiles of immune cell lineages of atopic human. If clusters could be identified, it would be of interest to correlate these genetic endotypes to atopic phenotypes and, secondly, to study the overlap with disease causing genes of monogenic PIDs. Subsequently, in atopic patients with an endotypic profile associated with PID (based on overlapping genes) referral to an immunologist may be considered in case of warnings signs of a PID.

Overlap in genes involved in atopy and primary immunodeficiency diseases identifies (new) therapeutic targets for the atopic syndrome

In PIDs and the atopic syndrome common pathogenic pathways seem to be involved. Therefore, several gene-targeted and/or pathway-targeted treatment strategies for PIDs could be of clinical benefit in the atopic syndrome as well. The clustering model as shown in Chapter 4 creates possibilities for identification of novel therapeutic targets for subgroups of the atopic syndrome, leading to more personalized and targeted treatment. For example, *STAT3* and *CTLA4* polymorphisms were found to be both involved in atopy and PID (HIES, STAT3 gain-of-function disease and large granular lymphocytosis, and CTLA4-deficiency, respectively). Jakinibs, such a tofacitinib and ruxolitinib, were described as safe and effective treatment modalities in patients with a *STAT3* gain-of-function variant to treat the autoimmune and autoinflammatory manifestations.[98] In patients with a CTLA4-

deficiency, treatment with a CTLA4 mimetic (e.g. abatacept) was shown to improve patient's autoimmune symptoms.[99] Furthermore, abatacept is registered and used as therapy for autoimmune diseases, like rheumatoid artritis.[100] Based on their mode of action, these therapies could also be effective in patients with (severe) atopic manifestations and an autoimmunity phenotype, based on genetic variants in these genes.

The Th lymphocyte mediated pathway was found to be most often involved in the atopic syndrome. Acute AD lesions and FA, asthma and AR are predominantly characterized by a Th2 response with production of, among others, interleukin (IL)-4 and IL-13.[37-43] Dupilumab is a human monoclonal antibody that blocks IL-4 and IL-13 signaling by binding to the IL-4 receptor alpha chain, modulating Th2-mediated inflammation.[101] Blockade by dupilumab of these key drivers of Th2-mediated inflammation has already proved effective in AD, but could potentially reduce symptoms of other atopic manifestations (i.e. FA, asthma and AR) as well.

Using the antibody response against *Staphylococcus aureus* to gain insight in the pathogenesis of atopic dermatitis

In Chapter 5 of the thesis we studied the humoral antibody response against *S. aureus* antigens and found an increased IgE response against immune modulating antigens suggesting *S. aureus* encourages epithelial damage via direct T lymphocyte stimulation.[55-57] To investigate further the role of *S. aureus* and the immune response against this bacterium in the AD pathogenesis, future studies should focus on other antibody subtypes than the IgE mediated response and other *S. aureus* antigens, like microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), membrane-damaging molecules, housekeeping antigens and other types of immune modulating proteins. Totté *et al.* showed that the IgG mediated immune response against immune modulating (non-superantigen) *S. aureus* antigens was associated with the disease severity in children with AD.[102] It can be argued that children with more severe AD have an altered immune response against *S. aureus* antigens or the association might be the result of a higher *S. aureus* load in children with more severe AD. However, in the latter hypothesis the IgG response against all *S. aureus* antigens would be increased instead of a subset. Following the inside-out hypothesis, in which immunologic dysregulation precedes barrier changes, alterations in the immunologic function of AD patients may allow or stimulate cutaneous colonization of *S. aureus*, which expresses several antigens resulting in persistence or exacerbation of AD symptoms.[77] On the other hand (outside-in hypothesis), *S. aureus* strains involved in AD may express more immune modulating antigens, which leads to a more severe AD phenotype and immunologic activation. In this context, it would be of interest to study whether AD patients are colonized by specific *S. aureus* strains. However, previous studies describe absence of a prevailed *S. aureus* genotype, which suggests that a primary immune

dysregulation precedes and stimulates microbial alterations, including abundance of *S. aureus*, within the pathogenesis of AD.[58-61]

Pitfalls in the design of a randomized controlled trial to study new antimicrobial treatment options in patients with atopic dermatitis

In Chapter 6.1 and 6.2 of the thesis we studied a targeted intervention directed against *S. aureus* using an endolysin. However, endolysin treatment as studied in this randomized controlled trial did not contribute to unraveling the contribution of the microbiome within the pathogenesis of AD due to absence of a clinical effect on AD and reduction of *S. aureus* load. According to the limitations in the design of our study, it might be relevant to investigate the effects of a targeted anti-*S. aureus* treatment using a wash-out period, in which topical corticosteroid and other (systemic) AD medications are not allowed, as their (long-term) effect on AD severity may mask an additional effect of the *S. aureus* targeting therapy. Furthermore, inclusion of patients based on two consecutive positive *S. aureus* cultures or clinically infected AD seems to be important to select a more appropriate population for *S. aureus* targeted therapy.

It can be doubted whether endolysins are the most convenient treatment strategy to target the microbiome based on results of our study. However, antibiotics are no suitable alternative as they induce bacterial resistance and have influence of the commensal flora. On the other hand, different promising (non-antibiotic) treatment strategies for modulation of the microbiome, including monoclonal antibodies and probiotics, are under development at the moment, but are not yet available for clinical use.

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