Chapter 8

Summary, general discussion and future perspectives
SUMMARY

Exposures to the farm environment protect against allergic disorders while they may induce nonallergic lung disease. The aim of this thesis was to assess the mechanisms underlying these paradoxical effects of farm exposure. To study allergic asthma, house dust mite mouse (HDM) models of asthma were used, whereas nonallergic asthma was investigated in a mouse model of farm dust exposure and in occupationally exposed subjects.

Chapter 2 describes a study in which both the protective effect of farm dust exposure as well as its deleterious effect in inducing nonallergic lung disease were investigated. The question whether farm dust from different types of farm (cattle and pig farms; onion and flower bulb industries) would induce different responses in mice and occupationally exposed subjects was addressed. All different types of farm dust extract protected mice equally against all measured parameters of HDM-induced allergic lung inflammation, namely levels of Th2 cytokines and eosinophils in the lungs, HDM-specific IgE levels in serum and airway hyperresponsiveness. Furthermore, all types of farm dust extract induced IL-17 and IL-1β in mice lungs, which were accompanied by mixed inflammatory infiltrates and higher numbers of neutrophils in the lungs. Agricultural workers had higher frequencies of activated (CD69+) T helper and cytotoxic T cells spontaneously expressing IL-17 or IFNγ, and lower frequencies of activated T helper cells expressing IL-4 as compared to non-exposed healthy controls. There were no differences in regulatory T cell frequencies between agricultural workers and non-exposed controls.

Chapter 3 shows whether a healthy worker survival effect could explain the finding that high microbial exposures is associated with less atopy in agricultural workers. This question was addressed in a five-year follow-up study, comparing baseline general characteristics, respiratory health, atopy and endotoxin exposure of agricultural workers followed up, versus workers lost to follow-up. Additionally, health status of participants who at follow-up had changed to jobs with lower exposure were compared to those with jobs with similar or higher exposure at follow-up. No major healthy worker survival effect was found, although some differences were observed between subjects included in follow-up and those lost to follow-up. Those lost to follow-up were older, had a lower peak expiratory flow, and were less often raised on a farm. Agricultural industry workers lost to follow-up with a farm childhood had more often self-reported allergy, but this was not observed for total IgE, atopy, HDM IgE, and grassmix IgE or respiratory symptoms. Comparing participants with lower exposure at follow-up with participants with similar or higher exposure at follow-up, no differences were found for any of the studied characteristics.

In Chapter 4 results are reported with regard to the effect of fluticasone propionate treatment on nonallergic lung inflammation and hyperresponsiveness induced by farm dust extract. Furthermore, in this study parameters that best predicted airway hyperresponsiveness (AHR) were investigated in the models of allergic and nonallergic asthma. Fluticasone propionate treatment reduced airway hyperresponsiveness and levels of CXCL1, IL-1β and IL-17 whereas it did not affect neutrophil counts, IL-6 or TNFα levels.
In Chapter 5 a study is described in which macrophage phenotypes were characterized and quantified in three models of HDM-induced asthma (14, 21, and 24 days). M1, M2, and anti-inflammatory (or M2-like) phenotypes were identified by means of immunohistochemistry according to the expression of interferon regulatory factor 5 (IRF5), YM1, and IL-10, respectively. Higher percentages of eosinophils were found in HDM-exposed mice compared to control but there were no differences between HDM models. T cell numbers were higher after HDM exposure and were the highest in the 24-day HDM protocol. Higher numbers of M2 macrophages after HDM exposure correlated with higher eosinophil numbers. Numbers of M1 macrophage correlated negatively with numbers of M2 macrophages. Lower numbers of anti-inflammatory macrophages were found after HDM exposure and these correlated negatively with M2 macrophages. Furthermore, female mice had more HDM-induced eosinophilia, effector T cells, regulatory T cells and higher levels of HDM-specific IgE as compared to male mice.

In Chapter 6 macrophage phenotypes were characterized in established models of allergic and nonallergic asthma, by means of flow cytometry and immunohistochemistry. Both models showed an increase in total numbers of macrophages, alveolar macrophages and activated alveolar macrophages after FDE and HDM exposure. The activation of macrophages, however, diverged depending on the exposure and inflammatory status of the tissue. While in allergic asthma M2-dominant macrophages predominated in the lungs, in nonallergic inflammation M1-dominant macrophages were more prevalent. Anti-inflammatory macrophages were higher in control mice than in HDM- or FDE-exposed mice. Furthermore, in a macrophage cell line, FDE exposure exerted a direct effect on macrophages, inducing exclusively M1-dominant activation.

Chapter 7 describes whether FDE exposure would induce phenotypical changes in polarized macrophages, and whether these phenotypical changes could provide possible mechanisms by which FDE exposure protects against the development of Th2 responses. FDE exposure blocked the effects of IL-13 on RAW macrophages, preventing M2 polarization. Furthermore, high doses of FDE induced IL-10 expression by anti-inflammatory macrophages.
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GENERAL DISCUSSION

Immunological and inflammatory responses to farm exposures

Protection against Th2 responses

Several hypotheses have been proposed to explain the recent rise in the prevalence of asthma and allergies, such as air pollution, obesity, tobacco smoke exposure, or the lack of microbial exposures [1]. The concept that increased microbial exposure in early life may prevent the development of asthma and allergies was first coined by Strachan in 1989 [2] and since then it has been subject of much research and debate in the literature. According to this hypothesis, children with fewer siblings are less exposed to microbes and therefore are more susceptible to develop asthma and allergic diseases. In agreement with this hypothesis, several epidemiological studies have shown that farmer’s children, who are highly exposed to microorganisms, have a low prevalence of asthma and allergies [3-8]. This protective effect has also been shown in adult farmers [5,6,9-12] and is particularly strong for animal contact. Since keeping animals is associated with high microbial exposure, and consequently high exposure to endotoxin, a cell wall component of Gram negative bacteria that can be easily measured, an important role for endotoxin exposure on the protective effect of farm exposures has been proposed [13]. Although exposure to other bioaerosol components such as fungal spores and ammonia have also been shown to be negatively associated with allergic asthma in farmers [5], most studies have focused on the role of endotoxin and have shown inverse association between endotoxin exposure and allergies and asthma [9,11,12,14]. A potential bias in these epidemiological studies is the healthy worker survival effect, a circumstance in which people who are sensitive to an exposure will change their jobs to work in lower exposed or unexposed jobs. In chapter 3 we showed that agricultural workers lost to follow-up did not have more atopic sensitization or respiratory symptoms compared to agricultural workers followed up. This indicates that health selection did not occur in the studied occupational population of Dutch farmers. Our data shows that FDEs obtained from different types of farms (cattle and pig farms and onion and flower bulb industries) and thus containing different endotoxin content, protected equally against HDM-induced allergic lung inflammation. A threshold of endotoxin exposure needed for protection may have been achieved in all FDEs; or it may also indicate that other components besides endotoxin, such as peptidoglycan (a component of the cell wall of Gram positive bacteria), β(1-3)-D-glucans and extracellular polysaccharides from fungi may be important in the protection against Th2 responses, and that the complexity of the settled dust should be considered. Importantly, it has been proposed that the protective effect of farming is mainly based on the microbial diversity found in rural farming sites [7]. Analysis of bacteria-specific 16S rRNA isolated from children’s mattress dust samples in rural regions revealed the presence of several microorganisms that were inversely associated with asthma, atopic sensitization and hay fever, suggesting that specific microbes might have allergo-protective properties [15]. Studies in OVA and HDM mouse models for asthma have shown similar results as ours regarding the protective effect of exposure to dust extract [16], polysaccharides [17] and specific Gram positive and Gram negative bacteria [18-20] isolated from farm cowsheds. Mechanisms underlying the allergy protection in these studies included upregulation of Th1 [18,19] or tolerogenic dendritic cells [16,17], as reflected by increased IFNγ and IL-10,
respectively, or even a generalized T-cell suppression [20]. Since we have not found increased levels of IFN\(\gamma\) or IL-10 in our studies, but rather found an increase in lung Th17 cells, the mechanisms of protection in our model remain to be elucidated and may be related to innate immunity, which plays an important role in activating the antigen-specific T cell response [21].

It has been shown that maternal exposure to different farm animals, thus a microbial rich environment, was associated with increased gene expression of Toll-like receptor (TLR)2, TLR4 and CD14 in blood cells of children [22]. Furthermore, in vitro studies have shown that FDE exposure of monocyte-derived dendritic cells and macrophages affects maturation and function of these cells [23,24]. In this thesis, we show that FDE exposure indeed affects macrophage activation, inducing classical or M1 activation in vivo and in vitro (chapter 6), inducing IL-10 production of anti-inflammatory macrophages, and blocking IL-13-induced M2 activation in vitro (chapter 7). Furthermore, in mice, concomitant HDM+FDE exposure abolished the HDM-induced high levels of YM1 in serum and numbers of M2 macrophages in the lungs (figure 1A and B). It is still an ongoing debate whether M2 macrophages contribute to development of asthma or are a consequence of the increase in IL-4 and IL-13 in the lung microenvironment in asthma. In chapter 5, we showed that numbers of M2 macrophages correlated with numbers of eosinophils in HDM models of allergic asthma. Further studies targeting M2 macrophages will reveal whether inhibiting activation of this macrophage phenotype can protect against asthma development, thus representing a possible mechanism explaining how farming exposures protect against allergic asthma.

![Figure 1: A) YM1 levels in serum and B) YM1 volume percentage in lung tissue of mice exposed 4 times a week for 5 weeks to PBS, HDM, FDE from pig farms and the combination of HDM+FDE.](image)

**Promotion of the IL-17 response**

Despite the indications that exposure to a farm environment protects against allergic asthma and atopy development, it is well known that these same exposures can cause or exacerbate respiratory diseases, including nonallergic asthma. We have shown in chapters...
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2 and 4 that FDE exposure induced proinflammatory cytokines IL-6, IL-17A, IL-1β, neutrophil and Th17 cell infiltration in the lungs, as well as airway hyperresponsiveness (in chapter 4). Furthermore, we showed that agricultural workers have higher percentages of Th1 and Th17 cells and lower percentages of Th2 cells in peripheral blood than healthy non exposed controls. These results indicate a shift in the immune system as a result of farm-related exposures.

Besides Th17 cells, other cell types also produce IL-17, such as γδ T cells, natural killer cells, innate lymphoid cells, neutrophils and Tc17 cells. We found that agricultural workers had higher numbers of Th17 and Tc17 cells as compared to non-exposed controls, but other sources of IL-17 were not investigated in these subjects. In our mouse study, double immunohistochemical stainings showed that all IL-17-producing cells were CD3+, some CD4+IL-17+ and others CD8+IL-17+, but no IL-17+ γδ T cell was found in our model. Naïve T cells differentiate into Th17 cells when antigen is presented by dendritic cells in the presence of TGFβ and IL-6. This differentiation is mediated by the transcription factors RORγt and RORα. Effector Th17 cells typically produce IL-17A, IL-17F, IL-21 and IL-23. IL-21 is an autocrine cytokine that potentiates Th17 differentiation, while IL-17A, IL-17F and IL-23 are effector cytokines that stimulate mucosal epithelium [25].

The role of IL-17 and Th17 cells in allergic asthma has not yet been fully elucidated, with IL-17 in some studies favoring and in other studies protecting mice from the disease [26,27]. These discrepancies can be explained by the timing of neutralization of administration of IL-17, since a protective role for IL-17 has been shown only during the challenge phase [27]. IL-17 has been shown to contribute to asthma by promoting proliferation [28], migration [29] and contractility [30] of airway smooth muscle cells, inducing mucus production [31] and by stimulating airway epithelium to produce CXCL1, a chemokine that attracts neutrophils [32-34]. Although the presence of IL-17 in BALF and sputum of allergic and mild asthmatics has been reported [35,36], IL-17 is often related to neutrophilic and severe asthma and has often been linked to corticosteroid resistance [32,33,37]. Sun et al. demonstrated that IL-17A protein levels are increased in induced sputum of patients with severe asthma as compared to healthy controls and it correlates with airway neutrophilia [38]. Recently, Raedler and collaborators reported a shifted immunity towards IL-17 in patients with neutrophilic asthma [21,39].

In agreement with our findings, exposures to farm environment have been reported to induce IL-17 in the lungs of mice and human subjects [40-42]. The mechanisms by which this occurs is however yet to be elucidated. IL-17 is important in host defense against extracellular pathogens, thus considering that farm dust and the farm environment in general is rich in bacterial and fungal products, the induction of IL-17 production probably starts as a protective mechanism, that when deregulated can contribute to nonallergic lung disease. During fungal infection, CD11b+ dendritic cells are required for optimal Th17 differentiation, due to their IL-6 and IL-23 production [43]. Dectin-1, a receptor that recognizes β-(1-3)-glucans from fungal wall, promotes Th17 differentiation by inhibiting Th1 differentiation and upregulating RORγt in T cells [44]. Regarding bacterial products, it has been shown that the TLR4 pathway induces IL-23, which triggers IL-17 release by CD4+ and CD8+ cells following Klebsiella pneumoniae infection [45]. Detection of both endotoxin and bacterial DNA by TLR4 and TLR9, respectively, is required for optimal IL-17 production, indicating a cooperative role of TLR4 and TLR9 in innate immune responses against gram negative bacteria [46]. Interestingly, classical activation of lung macrophages was also
dependent of concomitant TLR4 and TLR9 stimulation [46]. This clearly deserves further attention in our FDE model, since we have demonstrated increased IL-17 response and classical macrophage activation. In addition, peptidoglycans, present in the cell wall of Gram positive bacteria, which activate TLR2, were also reported to induce IL-17 and TH17 cells in mice lungs [40] and TLR2-deficient mice showed less lung neutrophilia after FDE exposure than wild type mice, demonstrating an important role for TLR2 pathway in farm dust-induced lung pathology [47]. Importantly, treatment of monocyte-derived dendritic cells with Staphylococcus sciuri W620 (a microbe found in farm environments and associated with protection against asthma and atopy), induced IL-23 production, while inhibiting IL-12 production, through TLR2 activation [20]. IL-23 is important for the terminal differentiation and clonal expansion of Th17 cells [25]. In Mycobacterium tuberculosis infection, Dectin-1 and TLR4 are responsible for IL-17A induction, which was dependent on IL-β [48], while TLR2 signaling helps to maintain Th17 at the site of infection [49]. Dissecting TLR and other pattern recognition receptors such as dectin-1 expression and activation in different cells types in the FDE model can help us understand the mechanisms of IL-17 release in farm exposures. Furthermore, it might be warranted to investigate the lung inflammatory response after farm dust exposure in mice deficient in IL-17R signaling, to achieve better understanding of the role of IL-17 in farm-related pulmonary diseases.

**Airway hyperresponsiveness (AHR) in allergic and nonallergic asthma**

*Measuring AHR*

Airway hyperresponsiveness (AHR) is the excessive response of the airways to a variety of stimuli and is considered a cardinal feature of asthma, being required for asthma diagnosis. AHR can be measured in patients by spirometry before and after inhalation of increasing doses of constricting agents. Methacholine (MCh) and histamine are frequently used to induce bronchoconstriction. Both compounds exert a direct effect on receptors on airway smooth muscle cells (muscarinic and H1 receptors, respectively), inducing contraction and causing airway narrowing. Adenosine 5'-monophosphate (AMP) provocation also induces bronchoconstriction, but in an indirect manner, mainly via stimulation of mast cells. In response to AMP, primed mast cells release histamine, prostaglandins and leukotrienes which subsequently cause airway smooth muscle contraction. Since provocation with AMP exerts its effects via mast cells, which are primed for histamine release in allergic asthmatics, it is thought that provocation with AMP more closely reflects airway inflammation and AHR in allergic asthma than MCh or histamine provocation [50,51].

Measurement of AHR in mice clearly presents challenges due to the small size of their airways. Nevertheless, considerable progress has been made in developing valid and suitable measures of AHR in mice, with the development of several different invasive and noninvasive lung function techniques to characterize the phenotype of experimental models of lung disease. There are advantages and disadvantages to be considered in both invasive and noninvasive methods, and it is important to keep in mind that there is a correlation between the invasiveness of a technique and its accuracy [52]. Invasive methods are more sensitive and allow specific analysis of pulmonary mechanisms, with bypass of upper airway resistance and controlled ventilation, but are time consuming;
require anesthesia, tracheotomy expertise, and sacrifice of mice when the measurement is concluded. Noninvasive methods are easy to perform, allow longitudinal measurements in the same mouse, with normal breathing of conscious mice without need for anesthesia. However, these methods are more prone to artifacts due to movements or changes in room temperature and upper airway resistance such as changes in glottal and nasal openings which can divert the results, resulting in difficulties in achieving reproducible consistency [53]. In this thesis, both a noninvasive method (chapter 2) and an invasive method (chapter 4) were used for AHR measurement, which showed conflicting results regarding FDE-induced AHR. In chapter 2, we used whole body plethysmography and due to the high variation of the results within the groups, we had to conclude that FDE exposure did not induce AHR. In chapter 4, pulmonary resistance was measured using the FlexiVent (Scireq), which showed more sensitivity, and less variation within the groups, allowing the detection of AHR induced by FDE, which is less prominent than HDM-induced AHR. A disadvantage of this method that should be noted is that some HDM-exposed mice did not resist the higher doses of MCh and died before the end of the measurement.

**Underlying mechanisms of AHR**

Even though AHR is required for the diagnosis of asthma, its presence is not specific for asthmatics since it may occur in other diseases such as atopic rhinitis and chronic obstructive pulmonary disease (COPD). An important question is which parameters determine the presence and severity of AHR in allergic and nonallergic asthma. We addressed this question in chapter 4, and found that, within the measured parameters, levels of TNFα and IL-6 were the best predictors of AHR in allergic asthma, while IL-1β and IL-17 contributed the most to AHR in nonallergic asthma. Although AHR is often considered to be a consequence of airway inflammation, the role of airway smooth muscle and of neuronal control in AHR should not be neglected. It has been proposed that AHR has two components: an acute and reversible component, which can be induced by certain exposures and is related to airway inflammation, and a persistent component, related to the chronicity of the disease and airway remodeling [54].

In allergic asthma, the relationship between AHR and airway inflammation is convincing. Numerous studies have linked the level of AHR to numbers of inflammatory cells, particularly eosinophils and mast cells, in BALF and blood of allergic asthmatics [55-57]. The role of eosinophils in allergen-induced AHR has also been investigated at the level of Th2 cytokines. Inhalation of recombinant IL-5 increased eosinophils in sputum and MCh responsiveness in subjects with mild allergic asthma [58]. In the ovalbumin (OVA) model of asthma, intranasal administration of anti-IL-5 before OVA challenge prevented eosinophil infiltration and AHR. Interestingly, in the same study, anti-IgE treatment had no effect on eosinophil recruitment or AHR, suggesting that T cell cytokine production and AHR occur independently of IgE [59]. Additionally, anti-IL-4 [60] and anti-13 [61] treatments have also been reported to inhibit allergen-induced AHR. Although preliminary results with recombinant human IL-4 receptor treatment seemed promising, its efficacy could not be demonstrated in phase III trials [62]. Likewise, anti-IL-5 treatment in mild asthmatics had no effect on AHR to histamine, despite decrease in sputum eosinophils [63]. Interestingly, in our HDM model of asthma, we found that AHR was more strongly correlated with proinflammatory cytokines TNFα and IL-6, than with Th2-related cytokines or numbers of
eosinophils. In agreement to our findings, AHR in asthmatic subjects has been shown to be associated with levels of TNFα in induced sputum, in a stronger manner than sputum eosinophil counts [64]. In addition, it has been reported that TNF-α promotes an increase in the contractility of human bronchial tissue in vitro, thereby possibly inducing AHR [65]. Furthermore, serum levels of interleukin-6 (IL-6) are higher in asthmatics in comparison to healthy controls and even higher during exacerbations [66]. These data, together with our results indicate that it is difficult to predict the presence and severity of AHR only considering markers of Th2 inflammation, and that other inflammatory parameters also contribute to AHR, in some cases, even more than Th2-related inflammatory cells and cytokines.

The mechanisms of AHR in nonallergic asthma are even less well understood than those of allergic asthma. In chapter 6, we show that nonallergic AHR did not correlate with lung neutrophilia and that fluticasone propionate treatment effectively suppressed AHR while numbers of neutrophils in BALF and lung tissue remained high. Similar results were found in smoking asthmatics after fluticasone propionate therapy and in COPD patients after smoking cessation [67], suggesting a dissociation between lung neutrophilia and nonallergic AHR. IL-1β is known to be involved in the differentiation of Th17 cells, and has also been implicated in AHR development in animal models [68,69]. Recently, it has been shown that treatment with an IL-1β antagonist prevents viral-induced bronchoconstriction in parainfluenza-infected guinea pigs [70]. Literature also points to the contribution of IL-17 to AHR in asthma. A positive correlation between IL-17 levels in sputum and AHR to methacholine in asthma patients has been shown [71]. In experimental models driven by HDM or ozone, IL-17 contributes to remodeling by promoting fibroblast proliferation [72] and by counteracting the anti-inflammatory role of regulatory T cells [73]. Furthermore, it has recently been shown that IL-17A produced by Th17 cells drives HDM- and OVA-induced AHR in mice and exerts a direct effect on airway smooth muscle cells from mice and men, increasing contractility [30]. Our results add to the amount of results that suggest an important role for IL-1β and IL-17 in nonallergic asthma, which deserves further future investigation. Contrary to our expectations, AHR in our model of nonallergic lung inflammation was suppressed by fluticasone propionate treatment. Future studies will reveal whether IL-1β and IL-17 play a role in corticosteroid resistant AHR.

In addition to the episodic AHR that is related to exposures, most chronic asthmatics exhibit a persistent level of AHR that relates to structural changes in the airway wall collectively referred to as airway remodeling, and that are probably best reflected by ARH to direct-acting stimuli such as methacholine and histamine [54]. These changes include collagen deposition on the basement membrane, increased airway smooth muscle layer, changes in extracellular matrix composition and goblet cell hyperplasia, leading to increased cell wall thickness and increased mucus production. In our study, no differences were found in collagen III deposition and airway smooth muscle layer thickness between the different exposure groups, possibly because our models are too short for the development of these alterations which are related to chronicity of asthma. Numbers of goblet cells were however increased in the airways of both FDE- and HDM-exposed mice, as compared to controls (data not shown). Unfortunately, since remodeling parameters were studied in a different set of experimental mice than AHR, the contribution of goblet cells to AHR could not be determined. Our data show that asthma phenotypes are heterogeneous, and that many inflammatory pathways may operate simultaneously in a
web of interactions leading to asthma symptoms. An approach including different parameters should provide a more comprehensive picture of the disease process [74].

**The multiple faces of macrophages – what should we call them?**

Macrophages were originally characterized as immune effector cells, part of the first line of defense against invaders. In the past decades however, the long ignored roles of macrophages on homeostasis and tissue repair have gained more and more attention. In the late 1970s, the antimicrobial function of macrophages has been recognized [75]. In an attempt to mirror the Th1/Th2 cell paradigm, macrophages have been classified as M1 and M2 phenotypes. In this classification, the M1 denomination was reserved for classically activated macrophages, with potent microbicidal properties, and the M2 denomination for alternatively activated macrophages, associated to Th2 responses. However, soon enough the idea has become clear that macrophage activation occurs in a spectrum and cannot be easily projected in two different groups and the M2 denomination expanded to include all other macrophage subsets, with similarities regarding expression of some receptors, but with marked different physiology [76]. The result was the subdivision of M2 macrophages in M2a, M2b and M2c, or the use of the term “M2-like” to denominate anti-inflammatory macrophages (in contrast to the M2 denomination which was reserved to macrophages involved in Th2 responses and wound healing). At this point many different nomenclatures have been proposed to designate macrophage subsets, generating confusion and difficulties in macrophage research, not only due to differences in nomenclature but also in activation markers.

In this thesis, the nomenclature of macrophage phenotype has evolved according to the knowledge in the macrophage research field. In chapter 5, we used the nomenclature proposed by Mosser and Edwards [77] and Sica and Mantovani [78], as this was the most accepted nomenclature at the time. According to this nomenclature, macrophage phenotypes were classified in M1 (induced by TNFα or IFNγ and LPS), M2 (induced by IL-4 and IL-13) and M2-like (induced by corticosteroids, IL-10 or prostaglandin 2) phenotypes. In chapter 6, the terminology “anti-inflammatory macrophage” was used instead of “M2-like macrophages”. In chapter 7, we used the most recently proposed nomenclature which was put forward in an attempt to standardize the codification of macrophage subsets. In this new classification, the authors propose that researches describe different phenotypes according to the markers they express (or absence of expression), preferably using a combination of markers to define macrophage populations. In in vitro studies, the stimulations used should be properly described and a nomenclature linked to the activation standards should be adopted, i.e., M(IFNγ), M(IL-4), M(IL-10) for macrophage phenotypes induced by IFNγ, IL-4, and IL-10 respectively [79]. Such a system avoids the complexity of contrasting classifications and that different laboratories have different activation definitions, allowing new activation conditions to be compared with these core examples. In the future, as more macrophage populations are explored ex vivo, more information will help understand the nature of in vivo macrophage activation.
FUTURE PERSPECTIVES

The studies presented in this thesis suggest several possibilities for further research on the mechanisms of occupational, nonallergic asthma. The involvement of IL-17 in nonallergic asthma has gained much attention in the last years. Indeed, we showed increased IL-17, Th17 and Tc17 cells in lungs of mice with FDE-induced nonallergic lung pathology and AHR, and increased numbers of Th17 and Tc17 cells in PBMCs of occupationally exposed subjects. Studies using the FDE model in mice lacking IL-17R signaling may reveal the contribution of IL-17 to the FDE-induced lung pathology. Although studies have reported IL-17 to be resistant to corticosteroid treatment, we showed that 3-week treatment with fluticasone propionate significantly abolished FDE-induced IL-17 levels and AHR. Further studies of fluticasone treatment in symptomatic occupationally exposed subjects are needed to confirm whether fluticasone propionate treatment is also effective in humans. Besides IL-17, we have demonstrated that IL-1β is strongly correlated with nonallergic AHR, while lung neutrophilia seems to be dissociated from it. Further investigation on the mechanisms by which IL-1β contributes to AHR could be of interest. In addition, it would be interesting to know whether AHR in the nonallergic model persists after neutrophil depletion.

The exact mechanisms by which exposures to the farm environment protect against allergic disorders remain to be elucidated. Recent literature suggests that this protection is mainly based on the microbial diversity found in the farm environment and that combination of signaling through multiple pathways may enhance protective effects inducing a shift of the immune response towards allergen tolerance [20]. Recent studies on airway and gut microbiome have shown that the environment is an important modifier of the composition of the host’s microbial communities. Direct and frequent contact with our cohabitants (human and animal) significantly shapes the composition of our microbiome [80]. There are some indications that the microbial community of the lower airways differs between asthmatics and controls [81]. Whether these differences are cause or consequence of the disease remain yet to be elucidated. Studies of airway and gut microbiomes of farmers and non-exposed controls would provide more insight on the effect of farm environments on the microbiome and its consequences for disease development.

This thesis emphasizes the importance of recognizing asthma as a heterogeneous disease and identifying the different phenotypes. We show that environmental exposure protects against allergic asthma, while it can induce a nonallergic form of the disease. Although classification may sometimes be difficult in human patients, with overlap of some parameters, we demonstrate marked differences in immune responses in models for allergic and nonallergic asthma and suggest new targets for future research. Not only Th2 x Th17 cells and eosinophil x neutrophil inflammatory preponderance in the lungs, but also macrophage subsets and parameters associated with AHR were different according to the asthma phenotypes. Regarding macrophages, a clear shift in balance was shown, from the predominance of IL-10+ macrophages in homeostasis, to a predominance of CD206highYM1+ macrophages in allergic asthma, and MHC class IIhighIRF5+ macrophages in nonallergic asthma. These findings could be the basis of future studies in which the functional roles of these macrophage phenotypes in allergic and nonallergic asthma are further characterized and potential targets to modulate these diseases are identified.
In conclusion, we have shown that exposures to farm dust extracts in mice and occupational exposure to farm environment induce a shift in the immune system towards Th1 and Th17 cells and MHC class II$^{\text{high}}$IRF5$^+$ macrophages (or M1 macrophages). This shift may protect against Th2-related disorders, such as allergic asthma, but is associated with risk to develop nonallergic asthma. Furthermore, we propose that IL-1β and IL-17 can play an important role in the development and severity of nonallergic airway hyperresponsiveness. Future research still needs to explore these pathways as potential targets to modulate nonallergic AHR.
In conclusion, we have shown that exposures to farm dust extracts in mice and occupational exposure to farm environment induce a shift in the immune system towards Th1 and Th17 cells and MHC class II high IRF5+ macrophages (or M1 macrophages). This shift may protect against Th2-related disorders, such as allergic asthma, but is associated with risk to develop nonallergic asthma. Furthermore, we propose that IL-1β and IL-17 can play an important role in the development and severity of nonallergic airway hyperresponsiveness. Future research still needs to explore these pathways as potential targets to modulate nonallergic AHR.

References


Summary, general discussion and future perspectives


