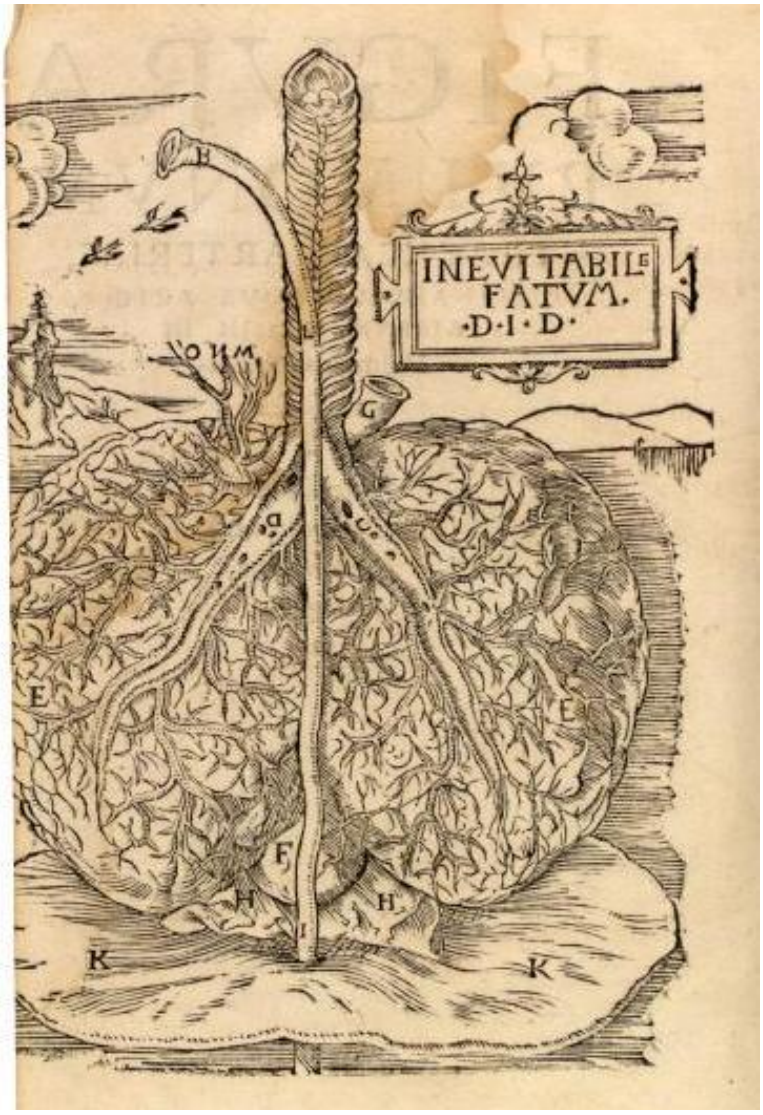
The role of the airway epithelium in the modulation of the immune response towards LPS in asthma



Simke Waijer
02-07-2009

university of groningen

umcg

The role of the airway epithelium in the modulation of the immune response towards LPS in asthma

Bachelorthesis

Name:	Simke Waijer
Student number:	1615157
Date:	02-07-09
Department:	Dept Pathology and Medical Biology, UMCG
Supervisor:	H.I. Heijink

Figure: Respiratory system by Johann Dryander, Anatomiæ 1537

Abstract

Asthma is a chronic lung disease, hereby patients have recurrent episodes of reversal airway obstruction. During these episodes patients suffer from shortness of breath, coughing and chest tightness.
A T helper 2 (Th2) reaction to inhaled allergens is associated with asthma and allergy. Possibly, there is an imbalance between the T helper 1 (Th1), Th2, T helper 17 (Th17) and regulatory T cells (Treg) in asthmatic patients, with a shift towards a Th2 response.
According to the hygiene hypothesis, frequent infection during childhood can prevent the development of asthma. Frequent bacterial challenge can possibly promote a shift towards the Th1 immune response.
The first site of contact with an allergen is the airway epithelium. The airway epithelium is now seen as central player in the Th2 immune response by influencing the function of dendritic cells (DC). DC are professional antigen presenting cells (APC) that can activate naïve Th cells. Different cytokine signals released by airway epithelial cells can modulate and activate DC, DC then polarize naïve Th cells to become Th1, Th2 or Treg effector cells.
Lipopolysaccharide (LPS), is a component of the cell wall of gram-negative bacteria. There are conflicting results about the role of LPS is asthma, it is not known whether LPS protect from the development of asthma or promotes the development of asthma. The dose of LPS may be an important factor, because different doses of LPS give different Th cell responses.
The airway epithelium can bind LPS on their toll like receptors (TLR). This activates airway epithelial cells and induces the release of different cytokines. These cytokines can prime DC to induce Th cells polarization, towards either a Th1, Th2 or Treg immune response.
In this essay, the central question is whether the airway epithelium releases different cytokines upon stimulation by different doses of LPS. A high dose of LPS could stimulate the release of Th1/Treg promoting cytokines, a low dose of LPS may stimulate the development of asthma by promoting the release of Th2 inducing cytokines by the epithelium.
According tho this hypothesis, a low dose of LPS has been shown to induce the release of Th2 stimulating cytokines (e.g. IL-33) by the airway epithelium in mice. In addition, a Th17 cell attracting chemokine (CCL20) was released after LPS stimulation of an intestinal epithelial cell line (m-IC_{cl2})
A high dose of LPS causes the release of Th1 attracting chemokines (CCL5, CXCL10 and CXCL11) by a airway epithelial cell line (BEAS-2B). These chemokines attract Th1 cells to the airways. Also, the release of a Th17 attracting chemokine (CCL20) was shown. In mice, stimulation with a high dose of LPS stimulated the release of IL-33, probably by the airway epithelium. IL-33 stimulates a Th2 immune response.
The release of cytokines by the airway epithelium after stimulation of LPS is not yet fully understood. A low dose of LPS did induce the release of Th2 stimulating cytokines, as was expected, but there was also production of Th17 attracting chemokines.
Overall, LPS can probably stimulate the release of cytokines and chemokines by the airway epithelium and thereby influencing the Th cell proliferation. Also, the dose of LPS may influence this reaction. Therefore, LPS may play a role in the development of asthma. However, it is not yet known which cytokines are released by the epithelium upon stimulation with different doses of LPS.

Table of contents

1. Introduction

Asthma

Asthma is a chronic lung disease, hereby patients have recurrent episodes of reversal airway obstruction. [1, 2] Asthma is highly prevalent, worldwide there are 300 million people suffering from asthma. In the last decades there is an increase in the prevalence of asthma, this increase is especially seen in the western world. [3, 4]
Asthma is characterized by periods with no symptoms, followed by periods with increased symptoms, called exacerbations. During an exacerbation patients have more symptoms, like shortness of breath, prolonged expiration, coughing, and wheezing. Exacerbations can be achieved by contact with allergen and by infections. [3, 5] Infections can be caused by viruses, like a common cold, caused by rhinoviruses.[5] During exacerbations there are high numbers of lymphocytes and eosinophils seen in the lungs. [6] Recurrent episodes of airway inflammation can lead to scar tissue formation and remodeling of the airways, resulting in a decreased long function. [7]
Asthma is characterized by airway inflammation, airway remodeling and bronchial hyperreactivity of the airways. The chronic inflammatory process underlies the hyperresponsiveness, bronchoconstriction and mucus hypersecretion seen in asthma. [1, 2] Bronchial hyperreactivity is the increased constriction of smooth muscles in the bronchia in response to non-specific stimuli like fog, cold air and physical exercise. [8]
The most common form of asthma is allergic asthma. In allergic asthma the airway inflammation is caused by an immunologic reaction to inhaled allergens. Allergens that cause allergic asthma are for instance house dust mite, pollen and animal dander. [9, 10] There is not much known about non-allergic asthma, therefore this essay will focus on the allergic form of asthma.
There are several types of T helper lymphocytes; T helper 1 cells (Th1), T helper 2 cells (Th2), regulatory T cells (Treg) and T helper 17 cells (Th17). Asthma is caused by a Th2-mediated immune response to allergens. [11]
A summary of the allergic cascade will be discussed below.

Induction of the allergic reaction
Inhaled antigen is taken up by antigen presenting cells (APC), like dendritic cells (DC). After antigen uptake by APC, the antigen is processed and proteolyzed into small peptides. These peptides bind to the major histocompatibility complex (MHC)-II molecules and are presented to naïve Th cells. Presenting of antigen by APC in combination with costimulation with costimulatory molecules, like B7-1 and B7-2 induces Th cell activation. [12]
Th2 cells are central in the pathogenesis of asthma. After presenting antigen to naïve Th cell by DC, T cells derived from asthmatic patients differentiate mainly into Th2 effector cells. [7]
The type of Ig produced by B cells is influenced by the Th cell subset. Th2 cells can induce isotype switching in B cells by producing cytokines (interleukin(IL)-4 and IL-13) and costimulation with CD40 ligand. The isotype produced by B cells switches towards the production of allergen specific IgE. IgE is released into the blood and can bind to Fc receptors on mast cells. [12-15]

Early-phase asthmatic reaction
Mediators released during the induction of the allergic reaction cause the early phase asthmatic reaction (EAR). [15] Re-exposure to antigen causes mast cell degranulation. Pro-inflammatory molecules, like histamine, prostaglandines, leukotrienes and cytokines, are then released by mast cells. These mediators induce constriction of airway smooth muscle cells, increased mucus production, enhanced airway hyperresponsiveness, increase vascular permeability and promote the recruitment of inflammatory cells. [12, 15]

Late-phase asthmatic reaction

The EAR is 4-6 hour later followed by the formation of the late-phase asthmatic reaction (LAR). During the EAR mediators are released that induce the recruitment of inflammatory cells, like Th2 cells, eosinophils, macrophages, basophils, neutrophils and epithelial cells. [12]

During the LAR there is excessive airway inflammation, resulting in airway remodeling. Airway remodeling is characterized by airway wall thickening, subepithelial fibrosis, goblet cell hyperplasia (increase in cell number) and hypertrophy (increase in cell size) and epithelial hypertrophy. Goblet cells are the mucus producing cells in the airway epithelium, hyperplasia of goblet cells results in increased mucus production. [12, 15]

Besides having an important role in the induction phase, Th2 cells are also important for ongoing allergic inflammation. Th2 cells are important for many of the features of asthma [14], an overview of the functions of Th2 cells is given in figure 1.

Th2 cells can produce IL-5, IL-3 and granulocyte-macrophages colony-stimulating factor (GM-CSF), which induce the recruitment and proliferation of eosinophils. IL-4, IL-13 and tumor necrosis factor (TNF) can prime the vessel walls for inflammatory cell recruitment.[14] IL-9 and IL-13 can change the excitability of bronchial smooth muscles, this causes bronchial hyperreactivity. [14] Cytokines like IL-5, IL-9, IL-13 an TNF are responsible for airway-wall remodeling.[14] Th2 cells are also important for epithelial-cell damage and for goblet cell hyperplasia seen in asthma. [14] (Figure 1)

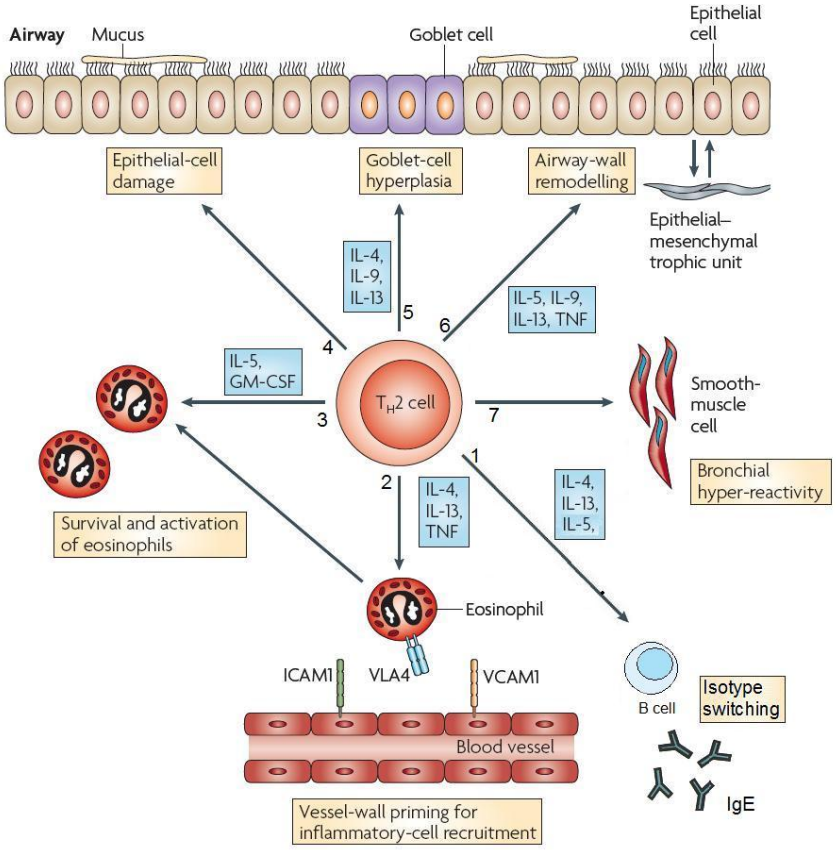


Figure 1. The important role for Th2 cells in the pathogenesis of asthma. 1. IL-4, IL-5 and IL-13 produced by Th2 cells induce isotype switching in B-cells, allowing the production of IgE. **2**. Th2 cells produce IL-4, IL-13 and TNF, this primes the vessel walls for inflammatory cell recruitment. **3.** IL-5, IL-3 and GM-CSF recruit eosinophils to the airways. **4.** Th2 cells can induce epithelial-cell damage. **5.** Th2 cells produce IL-4, IL9 and IL-13, these cytokines play a role in mucus hypersecretion by causing goblet cell hyperplasia. **6.** Th2 cells produce cytokines (IL-6, IL-9, IL-13 and TNF) that are involved in airway remodeling. **7.** Bronchial hyperreactivity is mediated by IL-9 and IL-13. *Figure modified from Hammad et al. 2008.* [14]

Hygiene hypothesis

The hygiene hypothesis tries to explain the increase in prevalence of asthma. The hygiene hypothesis states that allergic diseases can be prevented by infections in early childhood. [16] The increased hygiene standards and the increased availability of antibiotics seen in the western world have resulted in a decreased number of infections. According to the hygiene hypothesis, this decreased number of infections during childhood is correlated with a higher prevalence of asthma. [16, 17]
Frequent microbial infections are thought to skew the immune system to a Th1 response. Possibly, the decreased infection rate during childhood causes the immune system to shift towards a Th2 response and thus promote the development of asthma. [7, 11, 18]

Lipopolysacharide (LPS) is a component of the wall of gram-negative bacteria. LPS is a endotoxin and induces strong immune responses.[7] LPS is commonly found in the environment, like in house dust mite (HDM).[7, 19]
There are conflicting results about the role of LPS is asthma. [7] Some clinical studies indicate that LPS is protective for the development of asthma. [20], while other clinical studies indicate that LPS promotes the development of asthma. [21]
The exact role of LPS in asthma remains unknown, but there are indications that different doses of LPS have different effects.[22] A low dose of LPS, in combination with allergen, can stimulate a Th2 response and eventually asthma exacerbation. Contradictory, a high dose of LPS can stimulate a Th1 response, and thus prevent the development of asthma. [22, 23]

In this essay the interaction between epithelial cells and DC will be described. The central question is what DC activating cytokines are released by airway epithelial cells upon LPS exposure and what the effects are for the T cell polarizing function of DCs.
The hypothesis is that a high dose of LPS can induce a Th1 or Treg response and a low dose of LPS can induce a Th2 response, by the release of different cytokines and chemokines by the airway epithelium.

2. Immune response in asthma

2.1. adaptive immune response in asthma

Th1, Th2, Treg and Th17 cells produce different cytokines and have different effector functions. [11] The Th1 cells are characterized by the secretion of IL-2, interferon(IFN)-γ, TNF-α and TNF-β, and are involved in the cell-mediated immune defence against intracellular pathogens. [1, 6] Th2 cells produce IL-4, IL-5, IL-9 and IL-13, Th2 cells are associated with the immune defence against parasites and worms. However, the Th2 response also mediates allergic inflammation and asthma [1, 6, 11] The Th1 and Th2 response can counteract each other. [11, 14]
Tregs have an immunosuppressive function and have a different cytokine profile compared to Th1 and Th2 cells. Tregs can inhibit the immune response and can induce tolerance. Treg are thought to do so by the secretion of IL-10 and transforming growth factor (TGF)-β. [11, 14, 24] There are different types of Tregs. The first type are the naturally occurring Tregs, these Tregs are formed in the thymus. There are also induced Tregs, these cells are differentiated from T helper cells by stimuli like TGF- β. [11]
An new subset of Th cells, Th17 cells, was recently described. Th17 cells secrete IL-17, IL-17F and IL-22. Il-17 is involved in the defence against bacterial infection. Th17 cells have been associated with auto-immune diseases. [25] Th17 cells may also be related to asthma, recently was shown that Th17 cells can enhance Th2 mediated eosinophilic airway inflammation in a mice model of asthma. [26, 27]
In asthma there may be an imbalance between the different T helper cell subsets, as enhanced infiltration of Th2 cells and production of Th2 cytokines has been observed in the airways of asthmatic patients. On the other hand, Th1 cells are the dominant Th cells in te blood of healthy individuals. [6, 28-30] Also, a lower frequency of Treg cells in the blood of allergic individuals has been observed.[14, 30] Therefore, the balance of effector Th2 cells and Th1/Treg cells is probably important for the induction of allergy and asthma. [1, 6, 14]

2.2. Dendritic cells (DC)

DC are professional APC, laying alongside the airway epithelium. This way they can encounter pathogens or antigens immediately.[31]
Before encountering with an antigen, the DC are immature. Immature DC can capture antigen, but for presenting antigen to naïve Th cells, a maturation process is needed. [31-33] After recognizing an antigen on their toll like receptors (TLRs), DC become activated. Activated DC can upregulate chemokine receptor (CCR) 7. CCR7 is a homing receptor, it facilitates the migration of DC towards the draining lymph nodes. [31-33] During this migration, DC mature. The maturation of DC is characterized by losing of their endocytic/phagocytic capacity. Also, DC upregulate MHC-II and costimulatory molecules, like B7 and CD40. Matured DC can present antigen to naïve T cells. [31-33] For activation of naïve Th cells, three signals are needed, the first signal contains presenting of an antigen by MHC-II. Signal 2 is costimulation by costimulatory ligands on the APC. Signal 3 are cytokines released by DC. [31-33]

Th cell polarization by DC

After recognizing an pathogen, DC can initiate the immune response by activating naïve T cells. It is important that DC initiate the right response, a Th1 response against intracellular bacteria or viruses and a Th2 response against helminthes and worms. 'Self' antigen and harmless antigens (allergens) should induce tolerance. [31, 34] It is unclear why allergic individuals induce a Th2 response to an allergen. [14] Especially signal 3, thus the release of cytokines by DC, can skew Th cell responses. By releasing different cytokines (signal 3) DC can give different Th cell polarizing signals. DC can induce a Th2 response, which is associated with allergy and asthma. But DC can also induce tolerance against an antigen, by polarizing the immune response towards a Treg cell subset. (figure 2) [31, 34].

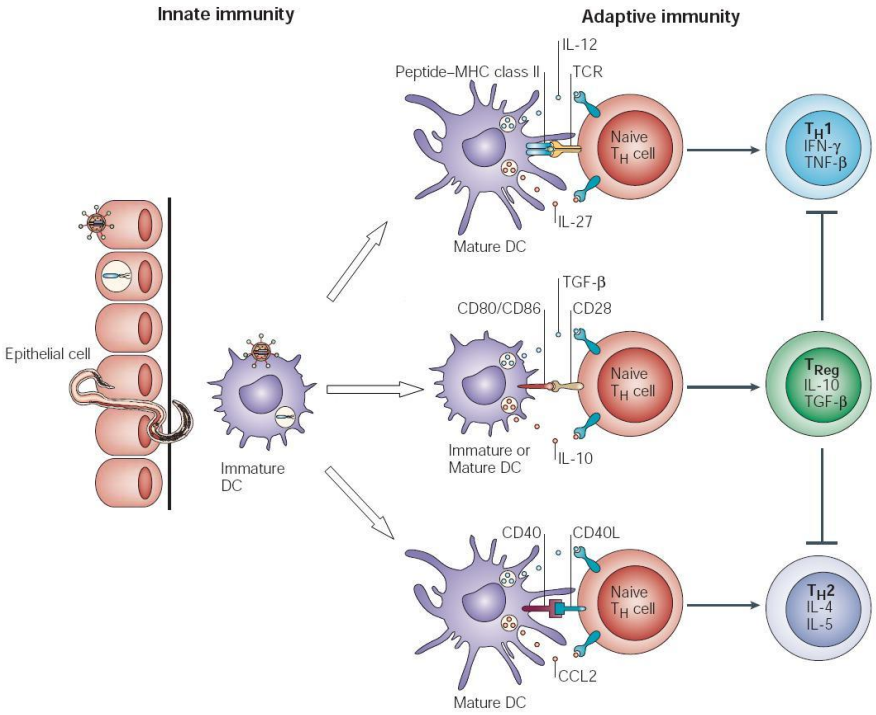


Figure 2. DC can skew the immune response towards different Th cell subsets.
DC can release different cytokines and thereby give different Th cell polarizing signal. Th1 cells are related to cellular immunity against intracellular pathogens. Treg cells can inhibit the immune response and induce tolerance. Th2 cells are related to humoral immunity, but also with allergy and asthma. *Figure modified from Kapsenberg et al. 2003.* [34]

3. Role of airway epithelium in the immune response

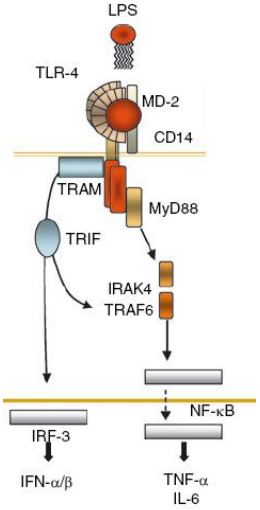
3.1. Airway epithelium

The airway epithelium lays at the interface between internal milieu and external milieu and is the first site of contact for pathogens, allergens and toxicants. The airway epithelium is more than a physical barrier, the airway epithelium plays a role in controlling many airway functions. The airway epithelium is important for regulating the fluid balance, metabolism and the regulation of airway smooth muscle function. [14, 35, 36] Also, the airway epithelium can bridge between the innate immune system and adaptive immune system by the production of cytokines and chemokines. The epithelium also has a secretory function, it can produce growth factors and bronchoconstricting peptides and is the source of proinflammatory mediators. Growth factors are responsible for the remodeling seen in asthmatic airways. [14, 35, 36]
There are many different epithelial cell types, these epithelial cell populations vary as a function of airway level. Epithelial cells can be classified in three categories; basal, ciliated and secretory cells. Columnar ciliated epithelial cells are the most common epithelial cells, they have numerous cilia on the cell surface. The function of the ciliated epithelial cells is to transport mucus from the lung to the throat. Mucous cells and Clara cells are both secretory cells. Mucous cells (goblet cells) produce mucus, mucociliary clearance clears inhaled particles inhaled from the lungs. Clara cells are found in the bronchiolar airways and produce surfactant. Basal cells are firmly attached to the basement membrane and play a role in the attachment of more superficial cells. [36, 37]
The airway epithelium may play an important role in the pathogenesis of asthma. [36] The airway epithelium is asthmatic patients has increased susceptibility to injury. In asthma, the epithelium has structural damage and goblet cell-hyperplasia. Also, the epithelium layer has a higher permeability, this way allergens can directly contact and activate DC and other immune cells laying alongside the epithelium. [14, 36, 37]

3.2. Recognition of allergen by epithelium

The airway epithelium can recognize pathogens by pattern recognition receptors (PRRs), these receptors recognize common motifs displayed by pathogens and allergens. Receptors expressed on epithelial cell are, for example TLRs and proteases-activated receptors (PAR). [7] Enzymatically active allergens can be recognized by the airway epithelium by their PARs. For example, PARs recognize Dermatophagoides pteronyssinus (Der p) 1, which is the major allergen present in house dust mite. [14] After recognition of enzymatically active allergen by epithelial cells, downstream signaling pathways, like NF-κB, are activated. This may lead to the production of proinflammatory cytokines and chemokines. [14]

Toll Like Receptors (TLR)
TLRs are a family of PRRs and can detect a wide array of pathogens, likes bacteria, viruses, parasites and fungi. [7] Also, epithelial cells can recognize antigen by TLRs.[14] During an infection, airway epithelial cells may upregulate TLR expression on the apical surface. [38]
TLR-4 can recognize LPS in combination with binding to the adapter molecule MD-2, the coreceptor CD-14 and LPS-binding protein (LBP). [7] This can cause the activation of two signaling transduction pathways, MyD88 and TRIF-related adapter molecule (TRAM). This eventually causes the activation of the transcription factors NF-κB and Interferon regulatory factor (IRF)-3. These transcription factors can promote the transcription of cytokines. [7, 38] (Figuur 3)



Figuur 3. Binding of LPS to TLR-4 induces two signaling pathways.
LPS can bind TLR-4 in combination with binding to MD-2, CD-14 and LBP. This can cause the activation of two signaling transduction pathways; MyD88 that leads to translocation of NF-κB to the nucleus and TRAM that leads to the activation of IRF-3. *Figure modified from Schroder and Maurer 2007.* [7]

3.3. Interaction between airway epithelium and DC

The epithelium is now seen as central player in the Th2-mediated inflammation by influencing the function of DC. [36] Epithelial cells bind incoming pathogens by their TLRs and subsequently produce different chemokines and cytokines. This can cause the recruitment and activation of airway DC. [14] Chemokines, like CC chemokine ligand (CCL)20 are produced by epithelial cells to attract DC to the site of inflammation. [14] CCR2 [39] and CCR6 [40] are chemokine-receptors found on DC, binding of this receptor by their ligands causes the attraction of DC. [14]
Different cytokine signals produced by epithelial cells can modulate DC, so they can prime naïve Th cells to become Th1, Th2 of Treg effector cells. [14, 35] This way, DC can bridge between innate immunity and adaptive immunity. [31-33]

3.4. Immune modulation by the airway epithelium

Airway epithelial cells produce different cytokines that can activate DC, to prime Th cell differentiation towards different Th-cell subsets. Cytokines produced by the epithelium are summarized in Table 1. Also, the airway epithelium can release chemokines that attract different subsets of Th cells to the airways. (Table 2)

Cytokines produced by airway epithelial cells
Thymic stromal lymphopoietin (TSLP) is a cytokine that is released by epithelial cells. TSLP can activate immature DC, and stimulate them to become strong inducers of a Th2 response. [14, 35]
Studies in mice showed that TSLP is a key factor in promoting a Th2 immune reaction. Mice with TSLP overexpression in lung epithelial cells had a major DC-driven Th2 immune response, while TSLP receptor deficient mice had a reduced Th2 response. [41]
Other studies also showed that TSLP is an important factor in the DC-driven development of a Th2 response. In the airways of humans with asthma, a higher level of TSLP was found. [14] Also there is genetic association between the risk of becoming allergic to inhaled antigens an polymorphisms in the TLSP receptor gene IL7R. [14]
TSLP, released by the airway epithelium can bind to the IL-7 receptor or TSLP receptor on DC. This causes immediate changes in DC, leading to the production of CCL11, CCL17 and CCL22. These chemokines directly recruit Th2 cells to the airways. [14] TSLP also causes activation and maturation of DC.[14] During maturation DC migrate to the lymph nodes and

upregulate a co-stimulatory molecule, OX40L. TSLP induced maturation does not give the production of Th1 cell promoting cytokine IL-12, this might be an explanation for the observed Th2 polarization. [14] Maturated DC can induce polarization toward Th2 cells, this is dependent of OX40L. [14] TSLP might also directly promote the production of Th2-associated cytokines by Th2 cells.[14]
Summarizing, TSLP released by epithelial cells can activate DC. DC can then polarize the Th-cell subset towards a Th2 immune response.

GM-CSF is released by epithelial cells after exposure to antigen. GM-CSF is a growth and activation factor for DC. [35]. Epithelial cells produce GM-CSF after contact with proteolytic allergen, like the enzyme Der p 1 [14]
Mice that overexpressed GM-CSF by intranasal infection with an adenovirus construct expressing GM-CSF, had a promoted DC dependent Th2 response after inhalation of a model allergen. (ovaalbumin, OVA) There was a higher Il-4 and Il-5 production and eosinophilia observed in the lung, this indicates a Th2 response. [14, 42].

TNF and IL-1β function upstream of the inflammatory cascade induced in asthmatic lungs. TNF and IL-1β synergize with TSLP to induce a Th2-associated cytokine production by mast cells. TNF can also induce TSLP production by epithelial cells. [14] Airway epithelial cells produce TNF after recognition of allergen, this can break tolerance by activating DC. Besides, recognition of a peptide by PAR2 in combination with the harmless OVA antigen leads to the production of TNF by epithelial cells. This may cause DC activation and Th cell polarization towards a Th2 response. [14]

Osteopontin is released from epithelial cells and can promote Th2 sensitization, but the exact mechanism is still unknown. [14] In the airways of asthmatic patients there is a higher osteopontin expression in bronchial epithelial cells. [43] It is likely that osteopontin alters the balance between tolerogenic and immunogenic DC. The effects of osteopontin of Th cell polarization by DC seems to be dependent on the pase of the immune response. [43]

IL-33 is a IL-1 family cytokine and is produced by epithelial cells, fibroblasts and smooth muscle cells. IL-33 induces Th2 responses and suppresses Th1 responses. DC are activated by IL-33 through binding of IL-33 to the receptor ST2. Activated DC promote a Th2 response, characterized by the release of IL-5 and IL-13. But there is no IL-4 production. [44]

Il-25 can be produced by airway epithelial cells and is associated with a Th2 immune response. [33, 45] Overexpression of IL-25 in the airway epithelium in mice resulted in increased asthma symptoms. Above that, blockade of IL-25 resulted in reduced asthma symptoms.[45] Also, Th2 cell polarization by TSLP-matured DC is induced by IL-25. [14, 46]

IFN-β causes the upregulation of IFN-γ by DCs. This causes Th cell polarization towards the Th1 subset. [47]. Infection with rhinoviruses is a common cause for asthma exacerbations. Primary bronchial epithelial cells were used to measure IFN-β after infection with a rhinovirus strain. Bronchial epithelial cells derived from asthmatic individuals produced less IFN- β after this infection, compared with normal controls. This indicates that there is a difference in the reaction of the airway epithelium to a trigger, like an infection. [48]

Chemokines produced by airway epithelial cells

Chemokines can induce the attraction of different type of Th cells. Chemokines produced by the epithelium include CCL20, CCL5, CXC chemokine ligand (CXCL)1/-3/-5/-8 and CXCL10/-11. (Table 2)

Epithelial cells can upregulate CCL20 production. CCL20 is an agonist for CCR6, which is found on immature DC and on TH17 cells. [25, 49] Th17 cells migrate to tissues where CCL20 is expressed. [50]

CCL5 is produced by epithelial cells and DC. CCL5 is a CCR5 ligand, and can thus bind to CCR5 on activated and memory T cells, monocytes an immature DC. There is an association found between CCL5 and Th1 immune responses, possibly CCL5 can preferentially attract Th1 cells. This is consistent with the finding that CCR5 is found more on Th1 cells compared to Th2 cells. [51]

CXCL10 and CXCL11 are recognized by the receptor CXCR3 [52]. Th1 cells, express CXCR3 and are attracted by CXCL10/-11. [53]

CXCL1/-3/-5 and -8 are neutrophil specific chemokines, CXCR2 is a ligand for these chemokines. [54, 55] CXCL8 is the strongest neutrophil recruiting chemokine and is also associated with bronchoconstriction, oedema and neutrophilia.[54]

Table 1. overview of cytokines produced by airway epithelial cells.

Cytokine:	**Mode of action:**	**Th cell polarization:**
TSLP	DC activation	Th2
GM-CSF	DC activation	Th2
TNF and IL-1β	DC activation, synergy with TSLP	Th2
Osteopontin	Alters balance between tolerogenic and immunogenic DC	Th2
IL-33	DC activation	Th2
IL-25	Unknown	Th2
IFN-β	Upregulation of IFN-γ by DCs	Th1

Table 2. overview of chemokines produced by airway epithelial cells.

Cytokine:	**Mode of action:**	**Attraction of Th cell subset**
CCL20	Ligand for CCR6	Th17
CCL5	Ligand for CCR5	Th1
CXCL1/-3/-5/-8	Ligand for CXCR2. Neutrophil recruiting cytokines	-
CXCL10/-11	Ligand for CXCR3	Th1

4. Hygiene Hypothesis

The hygiene hypothesis was firstly proposed by Strachan in 1989. Strachan suggested that "allergic diseases were prevented by infections in early childhood". These infections can be the result of unhygienic contact with older siblings. Also the increased hygiene standards and the increased availability of antibiotics might have decreased the number of infections, this might be correlated with a higher prevalence of asthma. [16, 17]
The relationship between infections and the prevalence of asthma might be caused by a shift in type of immune response, towards Th2 cells by infrequent infections. [18] Frequent microbial infections are thought to prevent the development of asthma by skewing the immune system to a Th1 response. The other way around, less frequent infections and insufficient challenging of a Th1 immune response skew the immune system toward a Th2 response, which favors the development of asthma. [7]

4.1. Epidemiological evidence

Increased exposure to infections could suppress allergy and asthma. However, epidemiologic studies on the hygiene hypothesis and asthma show conflicting results. [17]
A number of studies showed that children growing up on a farm have a decreased incidence of asthma and allergy, this protection from the development of asthma continues into adulthood. Children growing up on a farm are exposed to a diverse microbial environment, for instance in animal sheds, hay lofts and by eating certain foods like unpasteurized milk. Children growing up in neighboring villages that were less exposed to farming activities were not protected from the development of asthma. [17, 56]
Recent studies confirmed the inverse relationship between contact with other children at young age and asthma, allergen sensitization or hay fever. Contact with other children is thought to increase the number of infection. [57]
Day care attendance in early life has been inversely associated with asthma in some studies. Children that attend day care have increased contact with other children. In a birth cohort study of children with a parental history of atopy, day care attendance in early life was inversely correlated with asthma at age six. However, children with a maternal history of asthma were not prevented from asthma by day care attendance. [57-59]
In 1989 Strachan already associated the number of siblings and hay fever. [16]
However, there are also conflicting findings regarding the relation between number of siblings and asthma. A study of 18.530 European adults showed that an increased number of siblings was associated with decreased odds of hay fever but increased odds of asthma. [57, 59]
Summarizing, epidemiological studies on the hygiene hypothesis are still controversial. Some studies support the hypothesis, while other studies contradict the hygiene hypothesis.

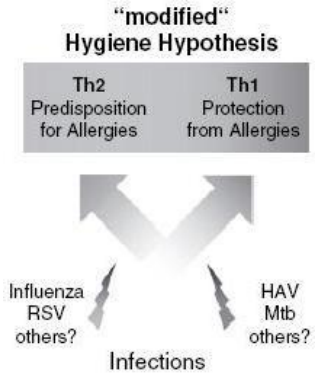


Figure 4. The modified hygiene hypothesis. Recent data support a modified hygiene hypothesis, where some infections promote the development of asthma and others inhibit the development of asthma. *Figure from Schroder and Maurer.* 2007. [7]

4.2. Infections and asthma

According to the hygiene hypothesis, frequent infections at young age can prevent the development of asthma. Indeed, infections with hepatitis A have been shown to inhibit asthma by favoring a Th1 immune response. Also infections with *Mycobacterium tuberculosis* have been inversely related to atopic diseases. [7]
However, some infections can increase the frequency of asthma. For example respiratory infections, caused by respiratory syncytial virus (RSV) or Influenza virus, favor the development of asthma. [7] Also, infections with rhinoviruses are suggested to induce asthma. [60, 61] In addition, bacterial airway infections, like *Mycoplasma pneumonia and Chlamydia pneumonia* have been repeatedly associated with the development of asthma. [60]
These conflicting observations have led to the formation of a modified hygiene hypothesis. [7] The modified hygiene hypothesis states that some infections, like RSV, can induce asthma or allergies by promoting a Th2 immune responses. [7] While other infections, like Hepatitis A promote the induction of a Th1 immune response, and thus prevent the development of asthma. [7] (Figure 4)

4.3. LPS / TLR-4 signaling in epithelium

LPS can be recognized by TLRs on the surface of innate immune cells and epithelial cells. [7, 38] (Figure 3)
LPS is potentially protective against the development of asthma. It is thought that LPS exposure early in life can prevent from asthma, by inducing strong Th1 inflammatory responses. [62]
Indeed, the level of LPS contamination in HDM was found to be higher in environments that were shown to prevent the development of asthma, like in HDM samples of big families, in daycare centers and also in the houses of farmers. [62] A cross-sectional survey in Germany and Switzerland showed a dose-dependent inverse relationship between exposure to house dust endotoxin and the frequency of atopic asthma and allergy. [20, 62]
However, there are also conflicting results on the role of LPS in the prevention of asthma. a prospective study compared the incidence of allergic sensitization and allergic diseases in relation to exposure to house dust endotoxin in Swedish and Estonian children. The level of endotoxin was higher in the Estonian households, compared to the Swedish households. However, only in the Swedish children an inverse relationship was seen between the levels of house dust endotoxin in the first year of life and asthma and allergic sensitization at the age of 2 years. It is unclear why there is no relationship seen in the Estonian children. [62, 63]
Because of these conflicting results, the role of LPS in the development of asthma remains not fully understood..
Recent studies showed that the dose op LPS determines whether a Th1 or Th2 immune response will be induced. Airway sensitization was induced with OVA. OVA in combination with a high dose of LPS (100 µg) induces a Th1 response, probably in combination with regulatory responses. Conversely, airway sensitization with OVA and a low concentration LPS (100 ng) gives rise to a Th2 immune response. A very low concentration LPS (<1 ng) in combination with airway sensitization with OVA induces tolerance, and thus a Treg response. [22, 23, 64]

The central question of this essay was what DC activating cytokines are released by airway epithelial cells upon LPS exposure and what the effects are for the T cell polarizing function of DCs. The hypothesis is that a high dose of LPS can induce a Th1 or Treg response and a low dose of LPS can induce a Th2 response, by the release of different cytokines and chemokines by the airway epithelium. Cytokine release by the airway epithelium upon LPS exposure will be discussed in the next chapter.

5. Role of the epithelium in the modulation of the immune response towards LPS

The underlying mechanism of different Th cell polarization by different doses of LPS remains unknown. Possibly there is an important role for the airway epithelium, which can bind LPS by their TLRs. This activates airway epithelial cells and induces the release of different cytokines. These cytokines can prime DC to induce Th cells polarization, towards a Th1, Th2 or Treg immune response. [14]
There are several studies that agree with this mechanism. Hammad et al showed that TLR4 triggering on lung structural cells, but not on DC, is necessary and sufficient for DC activation in the lung and for priming of Th responses to HDM and LPS. For this study irradiated chimeric mice with TLR4 expression on lung structural cells, but not on DC were used. [33]
Moreover, Zoumpopoulou et al showed maturation of bone-marrow derived dendritic cells after indirect activation trough the epithelial layer with *Escherichia coli* and LPS. [65]
Activation of airway epithelial cells by different doses of LPS may give different cytokine signals, which will be discussed below.

5.1. High dose of LPS

A high dose of LPS (100 µg), was previously shown to induces a Th1 response. [22]
A relatively high dose (10 µg) of LPS induces IL-33 production in mice. [33] IL-33 induces Th2 responses and suppresses Th1 responses. [44]
A recent study looked at the cytokine and chemokine response to airborn particulate matter (PM), that is found in air pollution. The effect of different PM components on a human epithelial cell line (bronchial derived BEAS-2B) was measured. They also looked at LPS found in PM, therefore they stimulated BEAS-2B cells with 10 µg of LPS and measured expression of inflammation-related genes. [54] This dose of LPS induced a more than 10-fold upregulation of the chemokine CCL20, CCL20 is a ligand to CCR6 on immature dendritic cells. It can induce the migration of DC towards the epithelium. [54] CCL20 can also attract Th17 cells towards the site of infection. [25] LPS also induced a more than 2-fold upregulation of the chemokines CCL5, CXCL10 and CXCL11. [54], these chemokines are all associated with the attraction of Th1 cells. [34, 51, 53] There was also a upregulation of neutrophil recruiting chemokines (CXCL8, CXCL1, CXCL3 and CXCL5). [54]

5.2. Low dose of LPS

A Th2 response was induced by a low dose of LPS (100 ng). [22]
A low dose (100 ng) LPS induces IL-33 production in mice. [33] IL-33 induces Th2 responses and suppresses Th1 responses. [44]
Zoumpopoulou et al used LPS to measure the production of chemokines by intestinal epithelial cells, therefore they used a intestinal epithelial cell line (m-IC_{cl2}). A dose of 10 ng/ml LPS induced the production of CXCL2 and CCL20. [65] CCL20 is associated with a Th17 response. [25] CXCL2 is an attractant for neutrophils. [66]

5.3. Very low dose of LPS

A dose of less than 1 ng LPS has been previously shown to induce a Treg response. [22] Up till now there are no studies that looked at this dose of LPS and cytokine expression by epithelial cells.

5.4. Varying LPS contaminations in HDM

HDM is a major allergen for patients with asthma. Allergens found in HDM are Der p 1, Der p 2 and Der f 2. HDM is also contaminated with LPS from colonizing bacteria. [64]
Triggering of TLR4 by HDM caused the production of GM-CSF, IL-33, TSLP and IL-25 in mice. The induction of these cytokines strongly depends on TLR-4 expression, and are probably produced by epithelial cells. [33] The HDM extract contains LPS, but this contamination was below the doses that were previously used to induce Th2 responses to OVA (1,05 ng/mg extract HDM). [33] Because triggering of LPS alone (GM-CSF, IL-1B) gives a different cytokine pattern compared to triggering with HDM (GM-CSF, IL-33, TSLP and IL-25.), the cytokine response seen after triggering with HDM cannot be entirely due to LPS contamination of HDM. [33]
Recently was shown that the HDM allergen Der p 2 promotes LPS induced TLR-4 signaling. Der p 2 has structural and functional homology with MD-2, the LPS binding component of the TLR-4 complex. Der p 2 facilitates signaling through direct interaction with the TLR-4 complex. Der p 2 in combination with extremely low (pg) LPS contamination causes a Th2 inflammation. These results indicates that Der p 2 can shift the LPS response curve into the Th2-inducing range. [64]
Therefore, a low dose of LPS in combination with HDM allergen Der p 2 may cause the release of Th2 polarizing cytokines by epithelial cells.

5.5. TLR independent epithelial activation

HDM can also activate epithelial cells independent of TLR, by binding the PAR2 receptor. Der p 1 and Der p 9 have been found to promote the release of IL-9, IL-6 and GM-CSF from bronchial epithelial cell lines and human bronchial epithelial cell cultures (HBEC) . [67] This process is highly dependent of binding of Der p 1 to the receptor PAR2 on epithelial cells. [14] Another study showed that Der p 1 increases the release of IL-8 and IL-1β from HBEC cultures. [68]
Recognition of allergen by PAR2, in combination with OVA, could break inhalation tolerance and stimulate the production of TNF by epithelial cells. TNF induces DC maturation and Th cell polarization, towards a Th2 response. [14]

6. Discussion

LPS exposure is correlated with the development of asthma. The airway epithelium is thought to play a central role in asthma, by inducing pro-inflammatory and anti-inflammatory cytokines. These cytokines can stimulate DC, DC can then induce Th cell polarization. Polarization towards Th2 cells is associated with asthma. [7]
According to the hygiene hypothesis frequent infections can prevent the development of asthma. The type of infection can influence the Th response.[7]
Also LPS, a component of the cell wall of gram negative bacteria, is associated with the development of asthma. The dose of LPS determines which Th response will be induced. A high dose of LPS induces a Th1 response, a low dose LPS induces a Th2 response and a very low dose induces a Treg response. [22, 23]
Possibly, LPS can activate the airway epithelium and stimulate the release of different cytokines. These cytokines can induce different Th cell polarization by DC. Therefore, you would expect the release of different DC-activating cytokines upon the stimulation with different doses of LPS. [14, 35]
According to this hypothesis, a low dose of LPS did induce the release of Th2 stimulating cytokines (IL-33) by the airway epithelium. However, also a Th17 stimulating chemokine (CCL20) was released. [22, 33, 65]
Th1 attracting chemokines (CCL5, CXCL10 and CXCL11) were released after stimulation with a high dose of LPS. This dose also promoted the release of a Th17 stimulating chemokine (CCL20) and the release of a Th2 stimulating cytokine (IL-33) by the airway epithelium. [22, 33, 54]
The release of cytokines by the airway epithelium after stimulation of LPS is not yet fully understood. A low dose of LPS may stimulate a Th2 response by selectively promoting the production of Th2 inducing cytokines or chemokine, while there is still some Th17 promoting cytokine production. This may influence the balance in Th cells and induce the development of asthma.
LPS is also found in HDM, this is usually a very low dose and far below the dose that is used to induce Th2 polarization. However, triggering of the airway epithelium with HDM promotes the release of Th2 polarizing cytokines.[33] These results might be explained by a recent study that showed that a HDM allergen can shift the LPS response curve into the Th2-inducing range.[64]
TLR4 triggering, for example by LPS, on epithelial cells of the lung can activate airway epithelial cells and induce DC activation. Triggering of PAR2 by allergens found in HDM also induces the release of Th2 promoting cytokines by the airway epithelium. [14, 67, 68]
It seems that the airway epithelium can activate or attract Th cells directly or by the activation of DCs. Chemokines and cytokines produced by the airway epithelium play a central role in this concept. How LPS can induce the release of different cytokines, and thus stimulate different Th responses is not fully understood. More research is needed to understand the response of the airway epithelium to LPS.
Besides the effect of LPS on the cytokines produced by the airway epithelium there might also be a difference in the reaction of the *asthmatic* airway epithelium compared to 'normal' epithelium. Possibly, the asthmatic airway epithelium reacts differently on stimuli, like LPS, compared to the airway epithelium of healthy individuals. This was also shown with rhinoviruses, the asthmatic airway epithelium produces less IFN- β after infection compared to normal epithelium. [48]
Overall, LPS can probably stimulate the release of cytokines and chemokines by the airway epithelium and thereby influencing the Th cell proliferation. Also, the dose of LPS may influence this reaction. Therefore, LPS may play a role in the development of asthma. It is not yet known which cytokines are released by the epithelium upon stimulation with different doses of LPS.

7. References

1. Sel, S., M. Wegmann, S. Bauer, H. Garn, G. Alber, and H. Renz, *Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10.* J Immunol, 2007. **178**(12): p. 7805-13.
2. Bousquet, J., P.K. Jeffery, W.W. Busse, M. Johnson, and A.M. Vignola, *Asthma. From bronchoconstriction to airways inflammation and remodeling.* Am J Respir Crit Care Med, 2000. **161**(5): p. 1720-45.
3. Dougherty, R.H. and J.V. Fahy, *Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype.* Clin Exp Allergy, 2009. **39**(2): p. 193-202.
4. Eder, W., M.J. Ege, and E. von Mutius, *The asthma epidemic.* N Engl J Med, 2006. **355**(21): p. 2226-35.
5. Murray, C.S., A. Simpson, and A. Custovic, *Allergens, viruses, and asthma exacerbations.* Proc Am Thorac Soc, 2004. **1**(2): p. 99-104.
6. Herrick, C.A. and K. Bottomly, *To respond or not to respond: T cells in allergic asthma.* Nat Rev Immunol, 2003. **3**(5): p. 405-12.
7. Schroder, N.W. and M. Maurer, *The role of innate immunity in asthma: leads and lessons from mouse models.* Allergy, 2007. **62**(6): p. 579-90.
8. Meurs, H., R. Gosens, and J. Zaagsma, *Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models.* Eur Respir J, 2008. **32**(2): p. 487-502.
9. Afshar, R., B.D. Medoff, and A.D. Luster, *Allergic asthma: a tale of many T cells.* Clin Exp Allergy, 2008. **38**(12): p. 1847-57.
10. Novak, N. and T. Bieber, *Allergic and nonallergic forms of atopic diseases.* J Allergy Clin Immunol, 2003. **112**(2): p. 252-62.
11. Ozdemir, C., M. Akdis, and C.A. Akdis, *T regulatory cells and their counterparts: masters of immune regulation.* Clin Exp Allergy, 2009. **39**(5): p. 626-39.
12. Verstraelen, S., K. Bloemen, I. Nelissen, H. Witters, G. Schoeters, and R. Van Den Heuvel, *Cell types involved in allergic asthma and their use in in vitro models to assess respiratory sensitization.* Toxicol In Vitro, 2008. **22**(6): p. 1419-31.
13. El Biaze, M., S. Boniface, V. Koscher, E. Mamessier, P. Dupuy, F. Milhe, M. Ramadour, D. Vervloet, and A. Magnan, *T cell activation, from atopy to asthma: more a paradox than a paradigm.* Allergy, 2003. **58**(9): p. 844-53.
14. Hammad, H. and B.N. Lambrecht, *Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma.* Nat Rev Immunol, 2008. **8**(3): p. 193-204.
15. Bloemen, K., S. Verstraelen, R. Van Den Heuvel, H. Witters, I. Nelissen, and G. Schoeters, *The allergic cascade: review of the most important molecules in the asthmatic lung.* Immunol Lett, 2007. **113**(1): p. 6-18.
16. Strachan, D.P., *Hay fever, hygiene, and household size.* BMJ, 1989. **299**(6710): p. 1259-60.
17. von Mutius, E., *Allergies, infections and the hygiene hypothesis--the epidemiological evidence.* Immunobiology, 2007. **212**(6): p. 433-9.
18. Schroder, N.W. and M. Arditi, *The role of innate immunity in the pathogenesis of asthma: evidence for the involvement of Toll-like receptor signaling.* J Endotoxin Res, 2007. **13**(5): p. 305-12.
19. Peterson, R.D., P.E. Wicklunds, and R.A. Good, *Endotoxin Activity of a House Dust Extract.* J Allergy Clin Immunol, 1964. **35**: p. 134-42.
20. Braun-Fahrlander, C., J. Riedler, U. Herz, W. Eder, M. Waser, L. Grize, S. Maisch, D. Carr, F. Gerlach, et al., *Environmental exposure to endotoxin and its relation to asthma in school-age children.* N Engl J Med, 2002. **347**(12): p. 869-77.
21. Park, J.H., D.R. Gold, D.L. Spiegelman, H.A. Burge, and D.K. Milton, *House dust endotoxin and wheeze in the first year of life.* Am J Respir Crit Care Med, 2001. **163**(2): p. 322-8.

22. Dong, L., H. Li, S. Wang, and Y. Li, *Different doses of lipopolysaccharides regulate the lung inflammation of asthmatic mice via TLR4 pathway in alveolar macrophages.* J Asthma, 2009. **46**(3): p. 229-33.
23. Eisenbarth, S.C., D.A. Piggott, J.W. Huleatt, I. Visintin, C.A. Herrick, and K. Bottomly, *Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen.* J Exp Med, 2002. **196**(12): p. 1645-51.
24. Akdis, M., *Healthy immune response to allergens: T regulatory cells and more.* Curr Opin Immunol, 2006. **18**(6): p. 738-44.
25. Yamazaki, T., X.O. Yang, Y. Chung, A. Fukunaga, R. Nurieva, B. Pappu, N. Martin-Orozco, H.S. Kang, L. Ma, et al., *CCR6 regulates the migration of inflammatory and regulatory T cells.* J Immunol, 2008. **181**(12): p. 8391-401.
26. Wakashin, H., K. Hirose, Y. Maezawa, S. Kagami, A. Suto, N. Watanabe, Y. Saito, M. Hatano, T. Tokuhisa, et al., *IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice.* Am J Respir Crit Care Med, 2008. **178**(10): p. 1023-32.
27. Wakashin, H., K. Hirose, I. Iwamoto, and H. Nakajima, *Role of IL-23-Th17 cell axis in allergic airway inflammation.* Int Arch Allergy Immunol, 2009. **149 Suppl 1**: p. 108-12.
28. Shirai, T., K. Suzuki, N. Inui, T. Suda, K. Chida, and H. Nakamura, *Th1/Th2 profile in peripheral blood in atopic cough and atopic asthma.* Clin Exp Allergy, 2003. **33**(1): p. 84-9.
29. Robinson, D.S., Q. Hamid, S. Ying, A. Tsicopoulos, J. Barkans, A.M. Bentley, C. Corrigan, S.R. Durham, and A.B. Kay, *Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma.* N Engl J Med, 1992. **326**(5): p. 298-304.
30. Umetsu, D.T., J.J. McIntire, O. Akbari, C. Macaubas, and R.H. DeKruyff, *Asthma: an epidemic of dysregulated immunity.* Nat Immunol, 2002. **3**(8): p. 715-20.
31. Schuurhuis, D.H., N. Fu, F. Ossendorp, and C.J. Melief, *Ins and outs of dendritic cells.* Int Arch Allergy Immunol, 2006. **140**(1): p. 53-72.
32. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.J. Liu, B. Pulendran, and K. Palucka, *Immunobiology of dendritic cells.* Annu Rev Immunol, 2000. **18**: p. 767-811.
33. Hammad, H., M. Chieppa, F. Perros, M.A. Willart, R.N. Germain, and B.N. Lambrecht, *House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells.* Nat Med, 2009. **15**(4): p. 410-6.
34. Kapsenberg, M.L., *Dendritic-cell control of pathogen-driven T-cell polarization.* Nat Rev Immunol, 2003. **3**(12): p. 984-93.
35. Lambrecht, B.N. and H. Hammad, *The other cells in asthma: dendritic cell and epithelial cell crosstalk.* Curr Opin Pulm Med, 2003. **9**(1): p. 34-41.
36. Knight, D.A. and S.T. Holgate, *The airway epithelium: structural and functional properties in health and disease.* Respirology, 2003. **8**(4): p. 432-46.
37. Crystal, R.G., S.H. Randell, J.F. Engelhardt, J. Voynow, and M.E. Sunday, *Airway epithelial cells: current concepts and challenges.* Proc Am Thorac Soc, 2008. **5**(7): p. 772-7.
38. Chaudhuri, N., S.K. Dower, M.K. Whyte, and I. Sabroe, *Toll-like receptors and chronic lung disease.* Clin Sci (Lond), 2005. **109**(2): p. 125-33.
39. Robays, L.J., T. Maes, S. Lebecque, S.A. Lira, W.A. Kuziel, G.G. Brusselle, G.F. Joos, and K.V. Vermaelen, *Chemokine receptor CCR2 but not CCR5 or CCR6 mediates the increase in pulmonary dendritic cells during allergic airway inflammation.* J Immunol, 2007. **178**(8): p. 5305-11.
40. Yang, D., O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. Shogan, M. Anderson, J.M. Schroder, J.M. Wang, et al., *Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6.* Science, 1999. **286**(5439): p. 525-8.
41. Zhou, B., M.R. Comeau, T. De Smedt, H.D. Liggitt, M.E. Dahl, D.B. Lewis, D. Gyarmati, T. Aye, D.J. Campbell, et al., *Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice.* Nat Immunol, 2005. **6**(10): p. 1047-53.

42. Stampfli, M.R., R.E. Wiley, G.S. Neigh, B.U. Gajewska, X.F. Lei, D.P. Snider, Z. Xing, and M. Jordana, *GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice.* J Clin Invest, 1998. **102**(9): p. 1704-14.
43. Xanthou, G., T. Alissafi, M. Semitekolou, D.C. Simoes, E. Economidou, M. Gaga, B.N. Lambrecht, C.M. Lloyd, and V. Panoutsakopoulou, *Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets.* Nat Med, 2007. **13**(5): p. 570-8.
44. Rank, M.A., T. Kobayashi, H. Kozaki, K.R. Bartemes, D.L. Squillace, and H. Kita, *IL-33-activated dendritic cells induce an atypical TH2-type response.* J Allergy Clin Immunol, 2009. **123**(5): p. 1047-54.
45. Angkasekwinai, P., H. Park, Y.H. Wang, S.H. Chang, D.B. Corry, Y.J. Liu, Z. Zhu, and C. Dong, *Interleukin 25 promotes the initiation of proallergic type 2 responses.* J Exp Med, 2007. **204**(7): p. 1509-17.
46. Wang, Y.H., P. Angkasekwinai, N. Lu, K.S. Voo, K. Arima, S. Hanabuchi, A. Hippe, C.J. Corrigan, C. Dong, et al., *IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells.* J Exp Med, 2007. **204**(8): p. 1837-47.
47. Le Bon, A. and D.F. Tough, *Links between innate and adaptive immunity via type I interferon.* Curr Opin Immunol, 2002. **14**(4): p. 432-6.
48. Wark, P.A., T. Grissell, B. Davies, H. See, and P.G. Gibson, *Diversity in the bronchial epithelial cell response to infection with different rhinovirus strains.* Respirology, 2009. **14**(2): p. 180-6.
49. Roggen, E.L., M. Lindstedt, C. Borrebaeck, and G.R. Verheyen, *Interactions between dendritic cells and epithelial cells in allergic disease.* Toxicol Lett, 2006. **162**(1): p. 71-82.
50. Wang, C., S.G. Kang, J. Lee, Z. Sun, and C.H. Kim, *The roles of CCR6 in migration of Th17 cells and regulation of effector T-cell balance in the gut.* Mucosal Immunol, 2009. **2**(2): p. 173-83.
51. Luther, S.A. and J.G. Cyster, *Chemokines as regulators of T cell differentiation.* Nat Immunol, 2001. **2**(2): p. 102-7.
52. Liu, L., M.K. Callahan, D. Huang, and R.M. Ransohoff, *Chemokine receptor CXCR3: an unexpected enigma.* Curr Top Dev Biol, 2005. **68**: p. 149-81.
53. Balestrieri, M.L., A. Balestrieri, F.P. Mancini, and C. Napoli, *Understanding the immunoangiostatic CXC chemokine network.* Cardiovasc Res, 2008. **78**(2): p. 250-6.
54. Ovrevik, J., M. Lag, J.A. Holme, P.E. Schwarze, and M. Refsnes, *Cytokine and chemokine expression patterns in lung epithelial cells exposed to components characteristic of particulate air pollution.* Toxicology, 2009. **259**(1-2): p. 46-53.
55. Fox, S.E., W. Lu, A. Maheshwari, R.D. Christensen, and D.A. Calhoun, *The effects and comparative differences of neutrophil specific chemokines on neutrophil chemotaxis of the neonate.* Cytokine, 2005. **29**(3): p. 135-40.
56. Schaub, B., R. Lauener, and E. von Mutius, *The many faces of the hygiene hypothesis.* J Allergy Clin Immunol, 2006. **117**(5): p. 969-77; quiz 978.
57. Ramsey, C.D. and J.C. Celedon, *The hygiene hypothesis and asthma.* Curr Opin Pulm Med, 2005. **11**(1): p. 14-20.
58. Celedon, J.C., R.J. Wright, A.A. Litonjua, D. Sredl, L. Ryan, S.T. Weiss, and D.R. Gold, *Day care attendance in early life, maternal history of asthma, and asthma at the age of 6 years.* Am J Respir Crit Care Med, 2003. **167**(9): p. 1239-43.
59. Svanes, C., D. Jarvis, S. Chinn, E. Omenaas, A. Gulsvik, and P. Burney, *Early exposure to children in family and day care as related to adult asthma and hay fever: results from the European Community Respiratory Health Survey.* Thorax, 2002. **57**(11): p. 945-50.
60. Schroder, N.W., *The role of innate immunity in the pathogenesis of asthma.* Curr Opin Allergy Clin Immunol, 2009. **9**(1): p. 38-43.
61. Bartlett, N.W., R.P. Walton, M.R. Edwards, J. Aniscenko, G. Caramori, J. Zhu, N. Glanville, K.J. Choy, P. Jourdan, et al., *Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation.* Nat Med, 2008. **14**(2): p. 199-204.
62. Eder, W. and E. von Mutius, *Hygiene hypothesis and endotoxin: what is the evidence?* Curr Opin Allergy Clin Immunol, 2004. **4**(2): p. 113-7.

63. Bottcher, M.F., B. Bjorksten, S. Gustafson, T. Voor, and M.C. Jenmalm, *Endotoxin levels in Estonian and Swedish house dust and atopy in infancy.* Clin Exp Allergy, 2003. **33**(3): p. 295-300.
64. Trompette, A., S. Divanovic, A. Visintin, C. Blanchard, R.S. Hegde, R. Madan, P.S. Thorne, M. Wills-Karp, T.L. Gioannini, et al., *Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein.* Nature, 2009. **457**(7229): p. 585-8.
65. Zoumpopoulou, G., E. Tsakalidou, J. Dewulf, B. Pot, and C. Grangette, *Differential crosstalk between epithelial cells, dendritic cells and bacteria in a co-culture model.* Int J Food Microbiol, 2009. **131**(1): p. 40-51.
66. Boehler, A., X.H. Bai, M. Liu, S. Cassivi, D. Chamberlain, A.S. Slutsky, and S. Keshavjee, *Upregulation of T-helper 1 cytokines and chemokine expression in post-transplant airway obliteration.* Am J Respir Crit Care Med, 1999. **159**(6): p. 1910-7.
67. King, C., S. Brennan, P.J. Thompson, and G.A. Stewart, *Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium.* J Immunol, 1998. **161**(7): p. 3645-51.
68. Rusznak, C., R.J. Sapsford, J.L. Devalia, S.S. Shah, E.L. Hewitt, A.G. Lamont, R.J. Davies, and S. Lozewicz, *Interaction of cigarette smoke and house dust mite allergens on inflammatory mediator release from primary cultures of human bronchial epithelial cells.* Clin Exp Allergy, 2001. **31**(2): p. 226-38.