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Dual Role of Toll-Like Receptors in Asthma and Chronic Obstructive Pulmonary Disease

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Abstract—During the last decade, significant research has been focused on Toll-like receptors (TLRs) in the pathogenesis of airway diseases. TLRs are pattern recognition receptors that play pivotal roles in the detection of and response to pathogens. Because of the involvement of TLRs in innate and adaptive immunity, these receptors are currently being exploited as possible targets for drug development. Asthma and chronic ob-

structive pulmonary disease (COPD) are chronic inflammatory airway diseases in which innate and adaptive immunity play an important role. To date, asthma is the most common chronic disease in children aged 5 years and older. COPD is prevalent amongst the elderly and is currently the fifth-leading cause of death worldwide with still-growing prevalence. Both of these inflammatory diseases result in shortness of breath, which is treated, often ineffectively, with bronchodilators and glucocorticosteroids. Symptomatic treatment approaches are similar for both diseases; however, the underlying immunological mechanisms differ greatly. There is a clear need for improved treatment specific for asthma and for COPD. This review provides an update

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on the role of TLRs in asthma and in COPD and discusses the merits and difficulties of targeting these proteins as novel treatment strategies for airway diseases. TLR agonist, TLR adjuvant, and TLR antagonist therapies could all be argued to be effective in airway disease

management. Because of a possible dual role of TLRs in airway diseases with shared symptoms and risk factors but different immunological mechanisms, caution should be taken while designing pulmonary TLR-based therapies.

I. Introduction

Since 1960, the prevalence of airway diseases has increased markedly in the Western world (Devenny et al., 2004). To date, asthma is the most common chronic inflammatory disease in children older than 5 years of age. More than 150 million people worldwide are diagnosed with asthma, predominantly in developed and Westernized countries (Waite et al., 1980). Asthma is characterized by episodes of reversible airway narrowing, bronchial hyperresponsiveness, chronic pulmonary inflammation, and airway remodeling.

The highest ranked airway disease among adults is chronic obstructive pulmonary disease (COPD¹). COPD is defined as a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is, in contrast with asthma, not fully reversible (Table 1). The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases. (Rabe et al., 2007). This late onset-disease was in 2001 the fifth-leading cause of death in developed countries and the sixth-leading cause in lower income countries (Lopez et al., 2006). COPD is still a growing cause of morbidity and mortality, and it is estimated to become the third leading cause of death worldwide by 2020 (Buist et al., 2007; Rabe et al., 2007).

The first step in COPD management is the reduction of risk factors (Rabe et al., 2007). Smoking cessation is currently the only effective therapy for a decline of COPD progression (Donnelly and Rogers, 2003). Inhaled long-acting bronchodilators in combination with glucocorticosteroids form the basis of symptomatic treatment in COPD

¹Abbreviations: BAL, bronchoalveolar lavage; CCL2, monocyte chemoattractant protein-1; CCL20, macrophage inflammatory protein 3- α ; CCL3, macrophage inflammatory protein-1 α ; CD44, major cell receptor for hyaluronan; COPD, chronic obstructive pulmonary disease; CpG, cytosine-guanine repeat; CXCL8, chemoattractant interleukin-8; DAMP, damage associated molecular pattern; DC, dendritic cell; dsRNA, double-stranded RNA; ECM, extracellular matrix; GAG, glycosaminoglycan; GM-CSF, granulocyte macrophage-colony-stimulating factor; HA, hyaluronic acid or hyaluronan; HDM, house dust mite; HEK, human embryonic kidney; HMGB1, high-mobility group protein B1; HSP, heat shock protein; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; LRR, leucine-rich repeat domain; MMP, matrix metalloproteinase; NK, natural killer; ODN, oligonucleotide; OVA, ovalbumin; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood derived monocyte; poly(I:C), polyinosine-polycytidylic acid; ROS, reactive oxygen species; SLIT, sublingual immunotherapy; SNP, single nucleotide polymorphism; Th, T helper; TIR, intracellular Toll/IL-1 receptor domain; TLR, Toll-like receptor; TNF, tumor necrosis factor.

and asthma patients. Roflumilast, a long-acting phosphodiesterase-4 inhibitor, acts as an anti-inflammatory agent and is currently approved for the treatment of severe COPD and for reducing exacerbations (Diamant and Spina, 2011; Kobayashi et al., 2011). Roflumilast is still under development for the treatment of asthma. Even though the immunological mechanisms underlying asthma and COPD are very different, which will be discussed in more detail in sections III.A and IV.A, the treatment approaches are very similar. None of the existing medications have been proven to diminish the long-term reduction in lung function. Moreover, long-term oral glucocorticosteroid therapy causes significant side effects such as muscle weakness and a decrease in muscle functionality. The long-term administration of long-acting β 2 adrenergic agonists has been linked to increased risk of mortality and desensitization of the β 2 receptor (Charlton, 2009; Weatherall et al., 2010). Furthermore, in 50% of the patients, the disease is poorly controlled by current therapy (Gamble et al., 2009). Because of the global increase in airway disease prevalence and rising health costs, there is a clear interest in finding novel targets that could lead to the development of new respiratory drugs.

The respiratory tract is constantly open to elements from the environment. The architecture of the upper airways (i.e., the airway epithelium, lung surfactant, and mucociliary escalator) forms the first line of pulmonary defense. Inflammatory responses can be initiated to cope with trespassers. However, to preserve the lungs' gas-exchange function, tissue homeostasis should be maintained. Epithelial cells and immune cells are equipped with diverse pattern recognition receptors that can initiate an appropriate immune response by sensing molecules derived from bacteria and viruses [e.g., microbial-associated molecular patterns, pathogen-associated molecular patterns (PAMPs), or by sensing endogenously derived molecules (e.g., damage-associated molecular patterns, DAMPs)]. Toll-like receptors (TLRs) are a class of pattern-recognition receptors that play a key role in innate and adaptive immunity. During the last decade, significant research has been focused on the role of TLRs in the immune control of the airways and in the pathogenesis of airway diseases (Akira, 2003; Takeda et al., 2003; Chaudhuri et al., 2005; Sabroe et al., 2005; Pandey and Agrawal, 2006). TLRs are currently being exploited as possible targets for drug development (Mbow and Sarisky, 2005; Pandey and Agrawal, 2006; Romagne, 2007). TLR-specific treatment can be classified in three clusters (Chaudhuri et al., 2007): agonists (induce protective immunity), agonist adjuvants [antitumor treatment, T helper (Th) 1/Th2 bal-

TABLE 1
Airway disease classification

Disease and Severity	Postbronchodilator FEV1/FVC	FEV1 Predicted %	Symptoms
COPD			
Stage I: mild	<0.7	≥80	Chronic cough, sputum production may be present
Stage II: moderate	<0.7	50–80	Shortness of breath, cough, and sputum production
Stage III: severe	<0.7	30–50	Greater shortness of breath, reduced exercise capacity, fatigue, repeated exacerbations
Stage IV: very severe	<0.7	<30	Chronic respiratory failure (PaO ₂ < 8 kPa, PaCO ₂ > 6.7 kPa at sea level)
Asthma			
Intermittent	≥0.7	≥80	Shortness of breath, chest tightness, tachycardia, wheezing less than once a week
Mild	≥0.7	≥80	Symptoms intermittent less than once a week, more than once per day
Moderate persistent	≥0.7	60–80	Symptoms intermittent daily
Severe persistent	≥0.7	<60	Symptoms intermittent daily associated with night-time symptoms

FVC, forced vital capacity (volume of air that can forcibly be exhaled after full inhalation); FEV1, forced expiratory volume in 1 s (maximum volume of air that can be exhaled in 1 s); predicted, the percentage FEV1 compared with average population with the same age, sex, height, and body weight; PaO₂, arterial partial pressure of oxygen; PaCO₂, arterial partial pressure of CO₂.

ance and vaccination], and antagonists (down-regulation of excessive inflammation). For each cluster, relevant data for airway diseases will be discussed in this review. Moreover, this review provides an update on the role of TLRs in asthma and in COPD and discusses the merits and difficulties of targeting these proteins as novel treatment strategies for airway diseases.

II. The Toll-Like Receptor Family

A. Toll-Like Receptor Function, Structure, and Ligands

TLRs are named after the *Drosophila melanogaster* “Toll” receptor. Toll receptors in insects mediate dorsoventral patterning, cellular adhesion as well as immune responses against microbial products (Hashimoto et al., 1988). A family of mammalian proteins was discovered that share structural and functional similarities with the *D. melanogaster* Toll receptor, and they were thus named “Toll-like” receptors (Medzhitov et al., 1997; Rock et al., 1998; Takeda et al., 2003). So far, 11 human TLRs have been identified. TLRs are characterized by a diverse extracellular, leucine-rich repeat (LRR) domain and a less diverse intracellular Toll/interleukin (IL)-1 receptor (TIR) domain (Akira, 2003). The various LRR domains are involved in the recognition of exogenous compounds such as viral and bacterial products. In addition to their protective role against microbial infections, it is also becoming clear that TLRs exhibit homeostatic roles by sensing endogenously derived materials. One such molecule is the breakdown product of the extracellular matrix (ECM) component hyaluronan (HA). The recognition of different forms of HA seems to be required for epithelial integrity maintenance in health and for epithelial survival and proliferation after injury (Jiang et al., 2005). The latter is in turn necessary for restoration of normal tissue architecture (Jiang et al., 2005; O’Neill, 2005; Sabroe et al., 2008). Table 2 summarizes current described exogenous and endogenous TLR ligands and synthetic compounds that are currently under development for the treatment of inflammatory disorders (Basith et al., 2011).

TLR2 and TLR4 can be classified as lipid-recognizing receptors. Lipids that activate TLR2 and TLR4 include

lipopolysaccharide (LPS), an outer cell wall component of Gram-negative bacteria (Sukkar et al., 2006) and lipoproteins, respectively. TLR5 primarily recognizes the protein flagellin, which is a structural component of flagellated bacteria. Nucleic acids derived from viruses or bacteria are recognized by TLR3 and TLR7–9 (Takeda et al., 2003). For research purposes, synthetic analogs of identified natural ligands are often used to trigger TLRs. TLR3 activation for example can be induced by polyinosine-polycytidylic acid [poly(I:C)], which is a synthetic analog of viral double-stranded RNA (dsRNA). TLR9 recognizes various types of unmethylated cytosine-guanine (CpG) repeat dideoxynucleotides (Davis, 2000; Zuany-Amorim et al., 2002). Synthetic CpG ODNs are analogs for unmethylated bacterial DNA. TLR10 is the latest discovered human TLR. It is an orphan receptor, and it has no rodent homolog. The ligand of TLR10 has not been identified yet.

The efficacy of TLR ligands is partly dependent on their ability to induce TLR conformational change possibly followed by TLR homo- or heterodimerization. Most TLRs are homodimeric; however, TLR2 can form heterodimers with TLR1 and TLR6 upon recognition of different structures of lipopeptides and lipoproteins (Hasan et al., 2005; Revets et al., 2005; Into et al., 2007; Wilde et al., 2007; Ospelt and Gay, 2010). Furthermore, TLR10 forms heterodimers with TLR1 and TLR2 (Hasan et al., 2005; Ospelt and Gay, 2010).

B. Toll-Like Receptor Signaling

Upon activation, TLRs set in motion different downstream signaling cascades. TLR downstream signaling is complex and beyond the scope of this review. Detailed overviews of TLR signaling can be found in various reviews written by Akira and colleagues (Akira, 2003; Takeda et al., 2003; Takeda and Akira, 2004; Kaisho and Akira, 2006). In brief, downstream signaling is dependent on the adaptor molecules that are recruited to the intracellular TIR domain (Akira, 2003). All TLRs (except TLR3) signal via the shared MyD88 adaptor molecule followed by subsequent association with the tumor necrosis factor receptor-associated factors or with kinases such as the IL-1RI-

TABLE 2
TLR ligands and clinically relevant TLR modulators

TLR	Exogenous and Endogenous Ligands	TLR Modulators under Clinical Development ^d	
		Agonists	Antagonists
TLR1	Bacterial lipopeptides		
TLR2	Bacterial lipoproteins and glycolipids Endogenous HMGB1, HSP70, EDN, HA, HS		OPN-305 (antibody; inflammation, autoimmunity, ischemia/ reperfusion, preclinical)
TLR2/TLR1	Bacterial diacyl lipopeptides		
TLR2/TLR6	Bacterial triacyl lipopeptides		
TLR3	Viral double-stranded RNA	AMP-516 (rintatolimod; viral infections, phase II) Poly I:C (vaccine adjuvants, phase III) Pollinex Quattro (allergy, phase III)	
TLR4	Bacterial LPS Endogenous HMGB1, HSP60, HSP70, EDN, HA, HS, Fibrinogen, S100 protein		NI-0101 (antibody; acute and chronic inflammation, preclinical)
TLR5	Bacterial flagellin	Vax102, flagellin.HuHA, and flagellin.AvHA fusion proteins (vaccine adjuvants: bacterial, viral infections, phase I)	
TLR6	Bacterial triacyl lipopeptides Fungal zymosan		
TLR7	Viral single-stranded RNA	AZD8848 (asthma and allergic rhinitis, phase IIa) ^b R-848 (resiquimod) (infectious diseases, phase II) R-848 (resiquimod) (infectious diseases, phase II)	
TLR8	Viral single-stranded RNA	ISS1018 (adjuvant allergy, phase II) ^c AVE675 (asthma and allergic rhinitis, phase I) ^d IMO-2134 (allergy, asthma, phase I) ^e SAR-21609 (asthma) ^d	
TLR9	Bacterial and viral CpG-DNA		
TLR10	Unknown		
TLR11	Profilin		

EDN, eosinophil-derived neurotoxin; HA, hyaluronan; HS, heparan sulfate.

^a Data from Basith et al. (2011).

^b Ikeda et al. (2010).

^c Friedberg et al. (2009).

^d Heijink and Van Oosterhout (2006); Parkinson (2008).

^e Kline and Krieg (2008).

associated protein kinases and the transforming growth factor B-activated kinase. TLR3 signals via the TIR-domain-containing adapter-inducing interferon- β (IFN- β) adaptor molecule, which is shared with TLR4. The downstream TLR signaling cascade leads to gene transcription and subsequently the production of various chemokines and cytokines, which results in specific cellular responses.

C. Toll-Like Receptor Expression in the Respiratory Tract

TLRs exhibit diverse cell- and stimulus-specific patterns of expression in different tissues. Real-time quantitative PCR showed that all TLR genes are expressed in human lung tissue (Zarembler and Godowski, 2002). Studies using goat material confirmed high expression of TLRs in mammal lung tissue (Tirumurugaan et al., 2010). TLRs are localized either on cell surfaces or in cellular compartments. For TLR3, TLR7, TLR8, and TLR9, most studies point toward a cellular endosome-restrictive expression pattern. However, conflicting data do exist. Table 3 summarizes TLR protein expression on airway epithelial cells, airway smooth muscle cells, and inflammatory cells. Because of the limited availability of specific anti-TLR Abs, it is still technically challenging to detect protein TLR levels on relatively rare cell populations in the lungs. Cell types

derived from other compartments may not always reflect expression profiles that are seen in the airways. For example, one study showed a decreased TLR2 expression on alveolar macrophages derived from cigarette smokers and patients with COPD compared with healthy subjects (Droemann et al., 2005). On the other hand, other investigators reported an up-regulation of TLR2 on peripheral blood derived monocytes (PBMCs) from patients with COPD (Pons et al., 2006). This shows a discrepancy between alveolar monocytes and PBMCs. Distinct responses of lung and spleen dendritic cells (DCs) to CpG ODNs can also be explained by differences in TLR9 expression profiles on the respective DCs (Chen et al., 2006).

Cellular expression could be influenced by microenvironmental conditions, which is a topic that is starting to get more attention. TLR4 surface expression is down-regulated upon short-term exposure to cigarette smoke medium, which can be explained by internalization of the receptor (Sarir et al., 2009). TLR expression may be different in activated and nonactivated inflammatory cells.

Changes in TLR expression throughout the course of a disease is also an issue of increasing interest. TLR4 expression on monocytes, lymphocytes, and DCs in patients with asthma has been shown to be significantly lower compared with the control subjects, which will be further

TABLE 3
TLR protein expression

	Airway Epithelium ^a	Human Airway Smooth Muscle ^b	Monocyte ^{c,d}	Human Neutrophil ^{e,f}	Lymphocytes and DCs ^g	Human Eosinophil ^f	Mast Cell ^{g-i}	Human Platelet ^j
TLR1	Apical		Yes	Low ^e	Ubiquitous	Surface		N.D.
TLR2	Apical	Surface/intracellular	Low on alveolar macrophage surface	Intermed. ^e	DC	Surface/intracellular	Rat mast cells ^h	Surface and cytoplasm
TLR3	Intracellular and apical	High on surface		N.D. ^e /Low ^f	Immature DCs	N.D.	P815 mast cells ^g	
TLR4	Basolateral and (sub)-apical	Not detectable	Similar on monocyte and alveolar macrophage surface	Low ^e	DCs	Surface/intracellular	Rat mast cells ^h and mice PMCs ⁱ	Surface and cytoplasm
TLR5	Basolateral and apical		Yes	High ^e	Immature DCs NK cells T cells	Surface/intracellular		
TLR6				Intermed. ^e		Surface/intracellular		N.D.
TLR7			Yes	Low ^e	B cells NK cells	Surface/intracellular	P815 mast cells ^g	
TLR8			Yes	Low ^e	NK cells T cells	N.D.		N.D.
TLR9	Apical		High on alveolar macrophage surface	High ^e	Precursor pDCs, B cells	Intermediate	P815 mast cells ^g and mice PMCs ⁱ	Surface and cytoplasm
TLR10					Precursor pDCs, B cells			

N.D., not detectable.

^a Greene and McElvaney (2005).^b Sukkar et al. (2006).^c Muzio et al. (2000).^d Juarez et al. (2010).^e Koller et al. (2009).^f Wong et al. (2009).^g Yang et al. (2009).^h Konopka et al. (2010).ⁱ Matsushima et al. (2004).^j Cognasse et al. (2005).

discussed in section III.C. (Lun et al., 2009). Bronchoalveolar lavage (BAL) cells obtained from patients with COPD, on the other hand, showed increased TLR4 expression, which could promote airway neutrophilic inflammation (Pace et al., 2011).

In Lun et al. (2009) and Pace et al. (2011), patient material was used, but because of the difficulty in obtaining human material, animal disease models are more often used to analyze TLR expression profiles. Differences exist between murine and human TLRs; hence, extrapolation to human diseases of findings derived from animal studies should be done with care. One should also take into account that expression profiles alone do not fully explain TLR function. TLR knockout mice, TLR agonists, and TLR antagonists could provide additional information on disease-related TLR function. In the next sections, the role of TLRs in asthma in COPD and in healthy tissue homeostasis will be discussed in more detail.

III. The Role of Toll-Like Receptors in Asthma

A. Asthma Risk Factors and Pathophysiology

Asthma prevalence has increased very rapidly during the past decades in many different regions worldwide. This widespread and rapid increase in prevalence makes it unlikely that genetic changes play a predominant role

in the increased asthma incidence (Asher et al., 1998). A family history of atopy, rhinitis, and eczema, however, has been associated with asthma (King et al., 2004). Asthma can be classified as allergic or nonallergic asthma (Beasley et al., 2000). More than 50% of adult asthma cases and 80% of childhood asthma cases are of the allergic subtype (Knudsen et al., 2009). Risk factors for allergic asthma include allergens such as house dust mites (HDM), pollen, and pet fur components (Devereux, 2006). On the contrary, nonallergic asthma symptoms are induced by nonspecific triggers, which have not yet been defined. However, nonallergic asthma has been associated with early life and current environmental exposures such as pets in the first year of life and unsatisfactory school cleaning (Janson et al., 2007). On the other hand, a decline in exposure to a wide range of microorganisms as a result of improved hygiene and widespread antibiotic use has also been associated to increased asthma prevalence. This finding has led to the hygiene hypothesis, which states that lack of exposure to microbial agents at early childhood contribute to increased susceptibility to the development of allergic diseases (Strachan, 2000).

The asthmatic inflammation is defined in part by a deregulated pattern of antigen-specific CD4⁺ Th2 cells

(Fig. 1). Th2 cells induce a particular pattern of cytokine production: IL-4, IL-5, and IL-13 (Robinson et al., 1992; Krug et al., 1996; Wills-Karp, 2004; Barnes, 2008a). IL-5 is the main cytokine involved in the differentiation of eosinophils from bone marrow precursor cells and prolongs eosinophil survival. Th2 cells direct B cells to produce antigen-specific IgE antibodies (Lambrecht et al., 2000; Chaudhuri et al., 2005; Schröder and Maurer, 2007; Lambrecht, 2005; Hammad et al., 2002; Barnes, 2010; Wills-Karp, 2004).

Mast cells are important in the acute as well as the adaptive asthmatic response (Schröder and Maurer, 2007; Kambayashi et al., 2009). Antigen-specific IgE can bind to the high-affinity receptors (FcεRI), resulting in rapid activation of the mast cell upon re-exposure to the same antigen. Activated mast cells secrete granular stored and de

novo synthesized mediators, such as histamine, serotonin, prostaglandins, leukotrienes, tumor necrosis factor (TNF)-α, IL-4, IL-5, IL-9, and IL-13. The release of vasoactive mediators increases the vascular permeability, allowing the flow of inflammatory mediators, eosinophils, and more antigen-specific Th2 cells into the antigen-encountered site, resulting in bronchoconstriction (Hines, 2002; Barnes, 2008b; Kambayashi et al., 2009). IgE antibodies initiate most cases of allergic asthma. The mechanisms initiating nonallergic asthma are not well defined, whereas similar inflammatory changes occur in both forms of asthma (Frew, 1996). Activation of residential mast cells (acute response) and infiltrated Th2 cells and eosinophils (late response) will together lead to airway narrowing because of smooth muscle constriction, mucus hypersecretion, and mucosal edema (Barnes, 2008b). Chronic inflam-

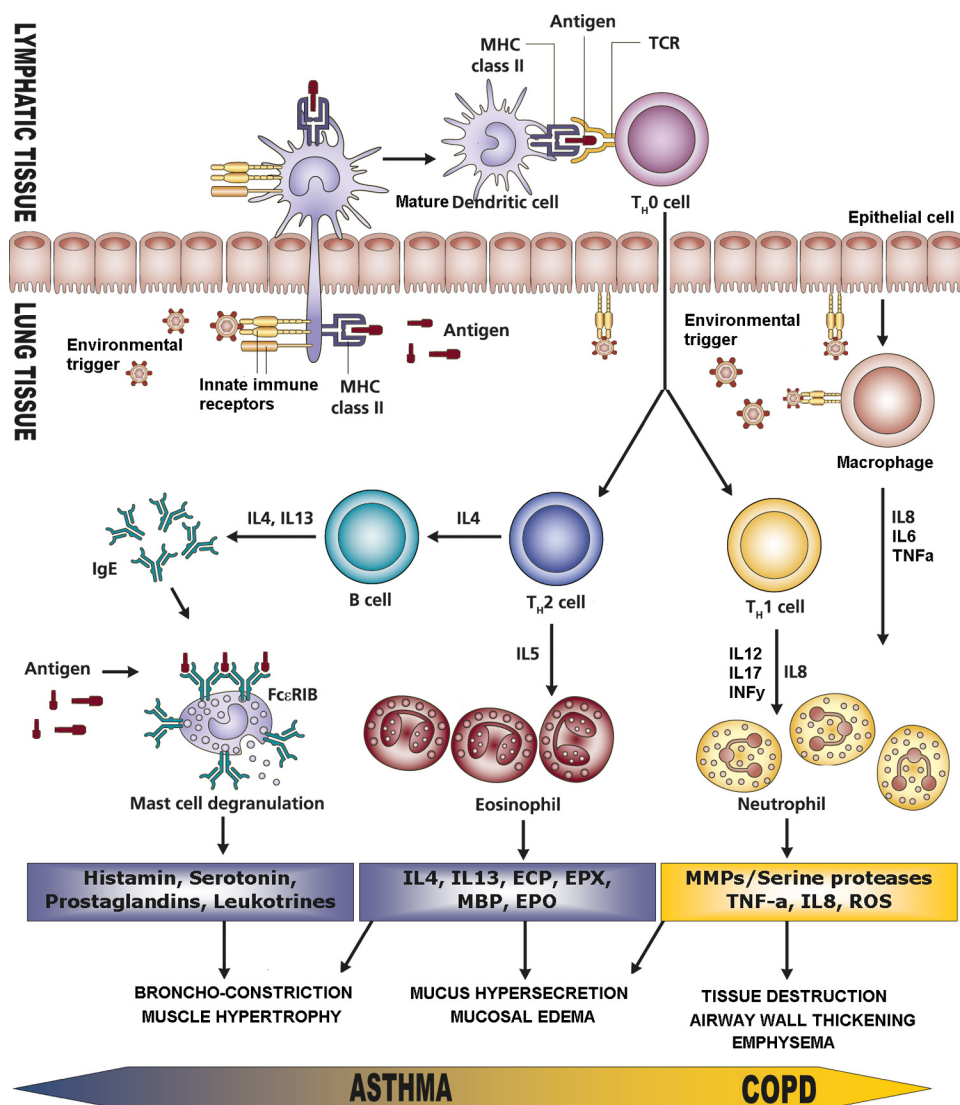


FIG. 1. Asthma and COPD have different immunological mechanisms. The pathologic features of asthma are defined by eosinophilic infiltration and mast cell degranulation. Dendritic cells are involved in the direction of naive T cells into either Th2 or Th1 cells in asthma and COPD, respectively. Environmental triggers in COPD recruit neutrophils via activated epithelial cells and macrophages. Asthma disease progression leads to mucus hypersecretion, mucosal edema, bronchoconstriction, and muscle hypertrophy. COPD progression leads to airway wall thickening and emphysema. One cannot draw a strict line between asthma and COPD. Patients may have characteristics of both pathologic conditions (blue/yellow arrow). MHC, major histocompatibility complex; TCR, T cell receptor; T_H, T helper cell; FcεRI, high-affinity receptor for IgE; ECP, eosinophil cationic protein; EPX, eosinophil protein X; MBP, major basic protein; EPO, eosinophil peroxidase; MMP, matrix metalloproteinase.

mation in asthma leads to characteristic structural changes such as collagen deposition at the basolateral side of the epithelium (basement membrane thickening), angiogenesis, and smooth muscle hypertrophy and hyperplasia (Barnes, 2008b).

B. Toll-Like Receptor Polymorphisms

A German case-control study was carried out in which functional genetic variants in TLR1–10 genes were evaluated for their association with different asthma phenotypes in children (Kormann et al., 2008). Single-nucleotide polymorphisms (SNPs) in the genes for TLR1 (intron and coding sequence regions leading to change in amino acids), TLR6 (promoter region leading to changes in transcription factor binding), and TLR10 (coding sequence region leading to changes in amino acids), showed protective effects on atopic asthma. The TLR1, TLR6, and TLR10 SNPs reduced the risk for atopic asthma by almost half. It is noteworthy that all these receptors are able to form heterodimers with TLR2. The protective effects of the TLR1, TLR6, and TLR10 SNPs that were evaluated in this study can be explained by gain of function as a result of increased TLR mRNA and protein expression, which correlated with proinflammatory and Th1 cytokine IFN- γ expression levels and reduced Th2-associated IL-4 production after *in vivo* stimulation with respective TLR ligands. Increased TLR protein expression due to SNPs in the *TLR1* and *TLR6* genes was shown to be cell type-dependent with higher increases on B cells compared with monocytes (Kormann et al., 2008).

One nonsynonymous TLR6 SNP located in the coding region and resulting in amino acid variation at position 249 in the extracellular domain of the TLR6 protein (S249P) has been significantly associated with protection from asthma in African Americans and shows a similar trend in European Americans in a case-control disease-association study (Tantisira et al., 2004). This association is consistent with the “hygiene hypothesis,” because genetic variation in TLR6 may predispose an individual toward a greater risk of infection, whereas at the same time, variation may encode for a protective effect toward asthma. In another study, the 249Ser allele was weakly but significantly associated with childhood asthma indicating conflicting data (Hoffjan et al., 2005).

TLR10 is highly polymorphic. In 47 subjects (24 African American and 23 European American), 78 SNPs of TLR10 were found. From these SNPs, two SNPs in coding sequences (1031G>A, 2322A>G) showed significant association with asthma in a case-control study in European American subjects, including 517 asthma cases and 519 control subjects (Lazarus et al., 2004). SNPs in the *TLR10* gene on the other hand have been associated with protective effects on atopic asthma. TLR10 SNPs have been linked to proinflammatory and Th1 cytokine IFN- γ expression and reduced Th2 associated IL-4 production (Kormann et al., 2008). To date, the available data are contra-

dictory and more information on loss or gain of function as a result of SNPs could clarify the reported heterogeneity.

A family-based association analysis has identified TLR7 and TLR8 as novel risk genes in allergic phenotypes, including asthma. Significant associations were observed between asthma and rs179008 SNP affecting TLR7 processing and rs5741883 SNP affecting TLR8 splicing (Møller-Larsen et al., 2008). Moreover, the function of TLR7 was reduced in adolescents with asthma compared with healthy individuals (Roponen et al., 2010).

In summary, genetic variation leading to TLR1 and TLR6 gain of function has been associated with protective effects in asthma. TLR10 polymorphisms were postulated to be both protective as well as hazardous for the development of asthma, and more insight in TLR loss or gain of function could clarify this heterogeneity. TLR7 and TLR8 SNPs leading to reduced function were identified as novel risk genes for the development of allergic asthma.

C. Toll-Like Receptor-Induced Disease Aggravation

The function of the distinct TLRs in allergic airway inflammation is in part mediated by the activation of cells of the innate immune system. DCs and mast cells are the major players in allergic asthma. Mast cell activation and subsequent secretion of proinflammatory cytokines and chemokines is directly induced by TLR ligands without the induction of degranulation and arachidonic acid metabolism (McCurdy et al., 2001; Hines, 2002; Kulka et al., 2004; Matsushima et al., 2004; Qiao et al., 2006; Zaidi et al., 2006; Kambayashi et al., 2009; Mrabet-Dahbi et al., 2009). Immature pulmonary DCs express TLRs (Table 3), and these cells become activated during infection or inflammation leading to up-regulation of costimulatory molecules. The role of TLRs during virally or bacterially induced disease exacerbations will be discussed in detail in section V.B. TLR-primed DCs may present an allergen to effector T cells initiating proliferation of these cells, and the release of Th2 cytokines, such as IL-4, IL-5, and IL-13. The release of these cytokines causes eosinophil proliferation and infiltration and enhanced mucus production by goblet cells resulting in chronic airway inflammation (Chaudhuri et al., 2005; Schröder and Maurer, 2007) (Lambrecht et al., 2000; Hammad et al., 2002; Lambrecht, 2005). The functional involvement of TLR4 in the development of allergic asthma has been extensively studied. TLR4 expression in irradiated chimeric mice is necessary for DC activation and the priming of T helper responses to HDMs in the asthmatic lung (Hammad et al., 2009). Moreover, administration of the TLR4 antagonist, an underacylated form of *Rhodobacter sphaeroides* LPS, by inhalation at the time of HDM injections reduced the features of asthma. Mice treated with this TLR4 antagonist showed reduced eosinophilia and lymphocytosis, reduced levels of Th2 cytokines IL-5, IL-13, and IFN- γ in the BAL fluid, decreased goblet cell hyperplasia and also lower airway hyper-responsiveness (Hammad et al., 2009). HDM has been suggested to induce asthma via TLR4 triggering of airway neutrophils

and monocytes (Li et al., 2010). A role for TLR4 in asthma aggravation has also extensively been studied using the TLR4 agonist LPS. It is proposed that the dose of LPS determines which type of immune response (Th1 or Th2) will be induced. A combination of the allergen ovalbumin (OVA) with a low-dose LPS enhanced eosinophilic and neutrophilic lung inflammation accompanied by high levels of Th2 cytokines; IL-4, IL-5, and IL-13 and mucus hypersecretion in asthmatic mice (Dong et al., 2009). A high LPS dose, however, induced a Th1 response with recruitment of neutrophils into lung tissue and increased IFN- γ levels in BAL fluid. In another study, the expression of TLR4 mRNA in alveolar macrophages showed no correlation with the dose of LPS and an up-regulation of TLR4 was observed in the lungs of all asthmatic mice regardless of the LPS dose received (Dong et al., 2009). TLR4 expression on monocytes, lymphocytes, and DCs in patients with asthma has been shown to be significantly lower compared with the control subjects. Moreover, the ex vivo production of TNF- α , IL-10, and IL-1 β by PBMCs stimulated by LPS was also significantly lower in patients with asthma. The observed reduction in TLR4 activation may lead to reduced release of Th1-related cytokine IL-1 β and anti-inflammatory cytokine IL-10 and thereby contribute to the immunological mechanisms of asthma (Lun et al., 2009).

There is also rising evidence for a functional role of TLR3 in the pathogenesis of severe asthma. Human thymic stromal lymphopoietin is expressed in the lungs of patients with asthma and activates a specific subset of DCs (CD11c⁺) giving rise to proallergic T-cell responses (Ying et al., 2005; Liu et al., 2007b; Tanaka et al., 2009). TLR3 ligands in combination with thymic stromal lymphopoietin were shown to activate human DCs and thereby promote the differentiation of Th17 cells (Shannon et al., 2008; Tanaka et al., 2009). Th17 cells are a unique subset of CD4 T cells producing the highly inflammatory cytokines IL-17 and IL-25. These cytokines have been implicated in pathogenesis of inflammatory disorders including asthma (Tesmer et al., 2008; Wakashin et al., 2008; Cosmi et al., 2011). TLR3 activation could thus contribute to the pathogenesis of severe asthma via the induction of Th17 cells. Moreover, stimulation of human alveolar smooth muscle cells with the TLR3 ligand induces the release of the chemokine interleukin 8 (CXCL8) (Sukkar et al., 2006). Furthermore, dsRNA was an effective activator in an airway epithelial cell line as well as in human primary bronchial epithelial cells. Activation of TLR3 by dsRNA increased the expression of TLR3 and triggered intense expression of CXCL8, CCL20 (macrophage inflammatory protein 3- α), and granulocyte macrophage-colony-stimulating factor (GM-CSF). CCL20 and GM-CSF may play a role in the recruitment and maturation of immature DCs. These data are consistent with data from murine models of airway inflammation and suggest a pro-inflammatory role of TLR3 agonists (Sha et al., 2004). Reported mediators, such as CXCL8 and GM-CSF play an important role in COPD, and it could thus be argued that these effects are not asthma-specific.

Figure 3 summarizes TLR-induced inflammation as seen in asthma and COPD.

D. Immune Modulation in Asthma

A lot of attention has been given to immune modulating properties of TLRs in respect to allergic asthma. Redirecting an allergic Th2 response by triggering a Th1 response via TLR activation has been postulated as an approach to treat patients with asthma. To date, TLR9 is in this respect one of the most extensively studied TLRs. In the specific context of asthma, synthetic TLR9 ligands (CpG ODNs) were shown to be beneficial in various rodent and primate models of asthma and have also shown positive results in a number of early human clinical trials (Table 2) (Hayashi and Raz, 2006; Vollmer and Krieg, 2009). These CpG ODN motifs activated NK cells via stimulation of DCs, leading to the induction of a Th1 response. The latter is characterized by the production of IFN- γ , as well as IL-10 and IL-12, and counterbalances the allergic Th2-dominated phenotype (Feleszko et al., 2006; Uematsu and Akira, 2006; Kline, 2007). In section VII, TLR9 will be discussed more extensively in relation to preclinical and clinical evidence of its beneficial use as a TLR-targeted therapy for the treatment of allergic symptoms.

TLR2 activation inhibits the mite allergen-induced Th2 response (Taylor et al., 2006). Intranasal challenge of OVA-sensitized mice with the allergen combined with a TLR2/4 agonist resulted in suppression of airway inflammation represented by decreased airway eosinophilia and Th2 cytokines (Revets et al., 2005; Fuchs and Braun, 2008). In addition, TLR2/1 and TLR2/6 heterodimers are able to skew a Th1/Th2 balance toward a Th1 response (Kormann et al., 2008). TLR2/1 binds specifically to triacetylated lipopeptides, whereas TLR2/6 binds to diacetylated lipopeptides (Hornung et al., 2002; Ospelt and Gay, 2010). Intratracheal treatment of asthmatic mice with a TLR2/6 agonist in combination with the Th1-cytokine IFN- γ resulted in reduction of airway hyper-responsiveness, eosinophilia, and Th2 cytokines IL-5 and IL-13 in the BAL fluid (Weigt et al., 2005; Fuchs and Braun, 2008; Fuchs et al., 2010). Consistent with this, a TLR2/6 agonist reduced eosinophilic infiltration in a murine model of chronic allergic airway inflammation in which mice were intranasally sensitized to Timothy grass pollen antigens (Fuchs et al., 2010).

Conflicting evidence is found on the effect of TLR2/1 agonists in mice models of allergic asthma. TLR2/1 ligands were found to reverse established airway inflammation in a murine model of OVA-induced asthma (Patel et al., 2005; Fuchs and Braun, 2008). In contrast, another study showed an increased Th2 response and thus allergic asthma aggravation in mice immunized with OVA allergen in combination with a TLR2/1 agonist (Redecke et al., 2004; Fuchs and Braun, 2008).

Murine models of allergic asthma have thus shown that TLR2 homo- and heterodimer agonists have the potency to both inhibit and promote the development of allergic im-

immune responses (Fuchs and Braun, 2008). The dual role of TLR2 in asthma seems to depend partly on which TLR2 heterodimer is addressed. Moreover, the agonist dose is likely to play a role in the eventual effect. As mentioned in section III.C, the dose of the TLR4 agonist LPS determines which type of immune response (Th1 or Th2) will be induced. Low-dose LPS in combination with OVA induced a Th2-type cytokine profile and enhanced eosinophilic and neutrophilic lung inflammation and mucus hypersecretion. In contrast, a high LPS dose induced a Th1 response with recruitment of neutrophils into lung tissue and increased IFN- γ levels in BAL fluid (Dong et al., 2009). This dose dependence may also account for other TLR agonists.

The synthetic compound S28463 (resiquimod, R-848), a TLR7-TLR8 ligand in clinical phase II for the treatment of hepatitis C virus infection (Table 2), prevents chronic asthma-induced airway remodeling in rats. S28463 inhibited the development of the airway remodeling features such as goblet cell hyperplasia and increased airway smooth muscle mass. The protein expression of both Th1 cytokine IFN- γ as well as Th2 cytokines IL-4, IL-5, and IL-13 was reduced in the lungs of rats (Camateros et al., 2007). Activation of TLR7 in early life, however, seems to promote the development of Th2 cells resulting in allergic airway inflammation upon allergen challenge in later life (Phipps et al., 2009).

IV. The Role of Toll-Like Receptors in Chronic Obstructive Pulmonary Disease

A. Chronic Obstructive Pulmonary Disease Risk Factors and Pathophysiology

COPD is caused by inhalation of noxious particles and gases. Cigarette smoke is the best-studied risk factor for COPD. Cigarette smoke contains more than 4500 components in its gaseous and particulate phase encompassing a major source of particles, free radicals, and reactive chemicals. The particulate and oxidant burden on the lungs can induce immune cell activation and damage through multiple mechanisms. Reactive oxygen species (ROS) can oxidize cellular lipids and DNA, and it can inactivate proteins such as α 1-antitrypsin. Oxidative stress regulates specific signal transduction pathways and histone modifications that are involved in lung inflammation (Rahman and Adcock, 2006). Moreover, CD8 T-cell proliferation potentiated by cigarette smoke primed DCs is mediated via ROS generation (Mortaz et al., 2009a). The prevalence of COPD among nonsmokers is also considerable, especially in developing countries (Salvi and Barnes, 2009). Among the worldwide population of patients with COPD, 25 to 45% of patients have never smoked, which indicates that smoking alone does not fully explain disease prevalence. There is increasing evidence for the association between the burning of biomass fuel and the development or aggravation of COPD (Shrestha and Shrestha, 2005; Orozco-Levi et al., 2006; Liu et al., 2007a; Mattson et al., 2008). Exposure to indoor air pollutants as a consequence of burning biomass,

derived from plant or animal sources, for cooking and heating purposes contributes greatly to the COPD prevalence in developing countries such as India, China, and Guatemala (Smith, 2000; Díaz et al., 2007; Liu et al., 2007a). Biomass fuels have a low combustion efficiency resulting in higher pollution compared with more efficient fuels, such as modern gas or kerosene. Biomass fuel is especially hazardous because half the world population is exposed to it (Salvi and Barnes, 2009).

Upon activation of epithelial cells and macrophages by noxious particles or gases, these cell types will release pro-inflammatory cytokines such as IL-6 and TNF- α , and chemoattractants such as CXCL8 and mononuclear cell attractants CCL2 (monocyte chemoattractant protein-1) and CCL3 (macrophage inflammatory protein-1 α) that recruit neutrophilic granulocytes and macrophages, respectively (Hogg et al., 2004). Activated neutrophils are very efficient in the defense against invading pathogens by releasing inflammatory mediators and serine proteases (such as cathepsin K, matrix metalloproteinases, and elastase) and by releasing ROS (Fig. 1). Inflammatory responses may be further amplified by reactive nitrogen species generated by nitric oxide produced by a wide variety of cell types under oxidative stress conditions (Ricciardolo et al., 2004). Although the lung epithelium and lung lining fluid are equipped to cope with oxidative stress via the excretion and presence of nonenzymatic and enzymatic antioxidants, an overzealous neutrophil and mucophage response might overwhelm these defenses leading to lung tissue damage and even alveolar tissue breakdown (Tetley, 2002; Thorley and Tetley, 2007; Stämpfli and Anderson, 2009; Tzortzaki and Siafakas, 2009).

In contrast to asthma, COPD is characterized by a predominance of CD8-positive cytotoxic T cells and a Th1-cell response, which is typified by the production of IL-8, IL-12, IL-17, TNF- α , and INF- γ (Barnes, 2004b; Hodge et al., 2007a). A complete overview of mediators that are involved in COPD can be found in a detailed review by Barnes (2004b). There is increasing evidence that airway diseases are associated with systemic manifestations. COPD is linked to cardiovascular disease, skeletal muscle dysfunction, systemic inflammation, nutritional abnormalities, and weight loss (Agusti et al., 2003; Gan et al., 2004; Barnes and Celli, 2009). This review, however, focuses primarily on the pulmonary angle of airway diseases.

Chronic bronchitis and emphysema are typical characteristics of severe COPD. Chronic bronchitis is caused by reoccurring activation of the airway epithelium and of circulating immune cells by noxious particles or gases resulting in chronic inflammation and mucus accumulation. Progression of bronchitis may cause irreversible morphological changes of the peripheral air spaces of the lung. For example, the walls of the bronchi and bronchiole can become thickened as a result repair and remodeling processes (Hogg et al., 2004). In addition, persistent inflammation in the adjacent air spaces of the respiratory bronchioles, alveolar ducts, and alveoli may cause destruc-

tion of alveolar septa. This results in a permanent increase in the size of the air spaces, also known as emphysema, which leads to a reduced gas exchange area and thus low arterial partial pressure of oxygen (PaO_2) and high arterial partial pressure of carbon dioxide (PaCO_2) (Table 1). Emphysema develops more rapidly in patients with anti-1-antitrypsin deficiency (NHLBI Workshop, 1985). Anti-1-antitrypsin is a protease inhibitor that is important in maintaining the lung parenchymal integrity. Insight into the role of this protease inhibitor in COPD has led to the hypothesis that an imbalance between ECM degrading enzymes and proteins that oppose this activity underlies the early development of emphysema (Gooptu et al., 2009; Marciniak and Lomas, 2009). Polymorphisms in other genes that are involved in ECM homeostasis as well as genes that are involved in the production of detoxifying enzymes such as glutathione transferase, heme oxygenase 1, superoxide dismutase, and possibly others are also likely to be involved in the COPD disease pathology (Marciniak and Lomas, 2009).

B. Cigarette Smoke-Induced Toll-Like Receptor Activation

As mentioned previously, the best described risk factor of COPD is cigarette smoke. Many of the cigarette smoke components modulate the function of immune cells after in vivo as well as in vitro administration. Cigarette smoke components activate and increase the numbers of alveolar macrophages (Sarir et al., 2010). In concert with activated epithelium, this will lead to mucus hypersecretion and poor mucociliary function and subsequent inflammation that may lead to tissue damage. The cigarette smoke-induced inflammatory response encompasses macrophage, neutrophil, monocyte, DC, and T-lymphocyte-attracting factors and the secretion of proinflammatory mediators, ROS, and proteolytic enzymes, all of which are important in COPD (Fig. 1) (Reynolds et al., 2006; Xu et al., 2009).

TLR2, TLR4, and TLR9 have already been linked to cigarette smoke-induced inflammation (Fig. 3), among which the function of TLR4 has most extensively been studied. Karimi et al. (2006) demonstrated for the first time that TLR4 is involved in cigarette smoke-induced cytokine production (Karimi et al., 2006). Later, Sarir et al. (2009) showed that TLR4 surface expression is down-regulated upon short-term cigarette smoke medium exposure, which can be explained by internalization of TLR4. The subsequent intracellular TLR4 expression might be further up-regulated as a result of an increase in TLR4 mRNA (Sarir et al., 2009). Other research has confirmed an involvement of TLR4 in cigarette smoke-induced CXCL8-dependent lung inflammation using epithelial cells (Pace et al., 2008) and TLR4 knockout mice (Maes et al., 2006). In the latter study, neutrophil, DC, and lymphocyte levels in BAL were decreased in TLR4-deficient mice compared with wild-type mice upon subacute cigarette smoke exposure. Evidence for a role of TLR4 in smoking-related COPD also comes from a recent population-based

study in which the association between common TLR polymorphisms (TLR2-R753Q, TLR4-D299G, and TLR4-T399I) and the development of COPD was investigated in a group of 240 heavy smokers (Speletas et al., 2009). Dysfunctional polymorphisms of TLR4-T399I can contribute to the development of COPD in smokers, which could be explained by an increased susceptibility to infections. The D299G TLR4 polymorphism, which also results in LPS hyporeactivity, however, has been hypothesized to decrease COPD severity (Sabroe et al., 2004). More research is warranted to elucidate the role of TLR polymorphisms in COPD. TLR4 deficiency in mice results in the spontaneous development of lung emphysema (Zhang et al., 2006). This indicates an involvement of TLR4 in normal tissue homeostasis, which will be discussed in more detail in section VI. Taken together, current knowledge shows that TLR4-mediated inflammation triggered by environmental components as well as defective TLR4 function in health and in disease may all contribute to COPD pathology.

TLR2 has been linked to COPD because of its altered expression and function as a result of cigarette smoke exposure. Droemann et al. (2005) showed a decreased TLR2 expression on macrophages derived from cigarette smokers and patients with COPD compared with healthy subjects. Furthermore, TLR2 mRNA and protein expression was not increased after LPS stimulation of macrophages obtained from smokers and patients with COPD in contrast to healthy nonsmokers. However, Pons et al. (2006) did report an up-regulation of TLR2 on monocytes from patients with COPD. This might seem to conflict with the findings from Droemann et al. (2005); however, cells used in Pons study were PBMCs, suggesting a difference between alveolar and systemic effects in terms of TLR expression.

More recently, Mortaz et al. (2010) elaborated on the role of human TLR9 in cigarette smoke-induced signaling. They demonstrated in vitro that TLR9 is involved in cigarette smoke-induced CXCL8 production by plasmacytoid DCs and neutrophils. More evidence for a role of TLR9 activation in cigarette smoke-induced inflammation originates from a study using TLR9 human embryonic kidney (HEK)-transfected cell lines. Cells that lack TLR9 show reduced cigarette smoke medium-induced CXCL8 production. Targeting synthetic CpG ODN to the mice lung, as well as intraperitoneally, gives rise to increased neutrophilic lung and systemic inflammation together with an enhanced lung permeability (Knuefermann et al., 2007; Tasaka et al., 2009). CpG ODN-induced inflammation has been suggested to be fully dependent on TNF- α and to be rodent-specific as a result of high expression of TLR9 in the rodent lung (Campbell et al., 2009). On the other hand, studies in horses revealed that TLR9 expression in the lung can be up-regulated by LPS treatment. Hence, TLR9 expression and thus response to CpG ODNs might increase because of specific costimulatory patterns in these nonrodent mammals (Schneberger et al., 2009). As well as other TLRs, Table 3 summarizes the human pulmonary

expression patterns of TLR9. The involvement of TLR9 in COPD is currently being investigated in vivo.

C. Lung Microbiome and Toll-Like Receptors

Although the healthy lung has often been regarded as sterile, there is increasing support for a lung microbiome (Sze et al., 2011). Global or microanatomical changes of the bacterial communities in the airways could be involved in COPD progression (Sethi et al., 2002; Erb-Downward et al., 2011). Cigarette smoking and airway obstruction are related to increased bacterial colonization of the distal airways (Zalacain et al., 1999).

As early as 1999, Hasday et al. (1999) showed that LPS is present in the main stream, and to a lesser extent in the side stream, of cigarette smoke. More recently it has been shown that tobacco contains bacteria that could be the source for LPS and possibly other bacterial residue components in cigarette smoke (Pauly et al., 2010; Sapkota et al., 2010). This finding gives new insight into the role of TLRs in COPD. Not only noxious particles and gases but also microbial components may contribute to cigarette smoke-induced immune responses that lead to disease initiation and aggravation. Depending on ligand dose, TLR activation could lead either to inflammation or to tolerance. It could be speculated that long-term exposure to low-dose LPS present in cigarette smoke may lead to LPS tolerance and subsequent induced risk for a bacterial infection. Cigarette smoke does lead to impaired innate lung defense, thereby giving rise to increased risk for microbial colonization, which is known as the vicious circle hypothesis (Sethi and Murphy, 2008). Moreover, increased susceptibility to infections, as seen in patients with COPD, could be the result of cigarette smoke-induced mucus hypersecretion and poor mucociliary clearance, giving rise to an environment that favors bacterial growth. Bacteria-induced disease exacerbations in relation to TLRs will be discussed in the next section.

V. The Role of Toll-Like Receptors in Airway Disease Exacerbations

A. Exacerbation: Causes and Symptoms

Asthma and COPD are both chronic inflammatory diseases with episodes in which disease symptoms worsen. An exacerbation involves a need to change a patient's regular medication due to worse symptoms than normal day-to-day variation. Inhaled bronchodilators in combination with glucocorticosteroid are currently the most effective treatment for exacerbations (Rabe et al., 2007). When there are clear signs that the exacerbation is caused by an infection, antibiotic treatment can be beneficial. Although changes in a patient's symptoms possibly followed by hospitalization and the requirement for additional medication are well recognized, there is still no clear definition of a disease exacerbation (Pauwels, 2004). An acute asthma attack is often associated with allergen exposure (Fig. 2) or other

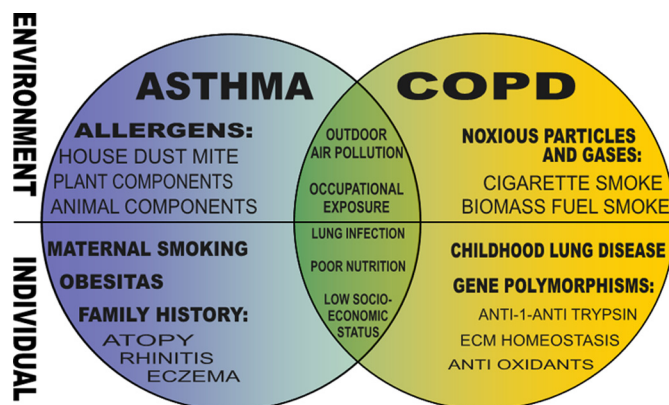


FIG. 2. Airway disease risk factors. The pathologic features of asthma and COPD are influenced by several environmental and personal risk factors. Shared environmental risk factors are outdoor air pollutants and occupational exposures. Shared personal factors that may lead to disease development or progression are lung infections, poor nutrition, and low socioeconomic status.

types of triggers, such as cold, in which the patient experiences acute shortness of breath, chest tightness, a rapid heart rate (tachycardia), and wheezing (Table 1). The acute asthma attack can be treated with bronchodilators and is different in this respect from an exacerbation. The time course of a disease exacerbation, in both asthma and COPD, is on the order of 2 weeks, during which disease symptoms worsen throughout the first week followed by a week of recovery after a change in medication (Tattersfield et al., 1999; Pauwels, 2004). Exacerbations are more frequent in more severe asthma and COPD classifications (Table 1). Symptoms include increased inflammatory infiltrates, increased sputum production, increased cough, and wheeze. Lung function and quality of life significantly declines as a result of frequent exacerbations (Seemungal et al., 1998; Donaldson et al., 2002). The majority of COPD exacerbations are associated with viral and bacterial respiratory tract infections (Seemungal et al., 1998, 2001; Patel et al., 2002; Papi et al., 2006). Asthma exacerbations are predominantly associated with allergen and/or viral exposure rather than bacterial infections (Pauwels, 2004). Besides bacteria- or viral-induced exacerbations, airway diseases may be additionally aggravated by air pollutant exposure. TLRs play an important role in defense and it can thus be hypothesized that poor TLR function contribute greatly to pulmonary disease exacerbation. Reduced TLR function could cause disease worsening because of comprised sensing of bacterial or viral components (Laan et al., 2004; Kulkarni et al., 2010; Eddleston et al., 2011; Manzel et al., 2011) as well as a reduced ability to phagocytose bacteria (Hodge et al., 2007b; Phipps et al., 2010) and apoptotic cells (Hodge et al., 2003; Richens et al., 2009). Overactive TLR triggering, on the other hand, could result in excessive inflammation and subsequent lung tissue damage. A fast recovery of an exacerbation could thus minimize tissue destruction, and the subsequent remodeling induced

long-term decline in lung function. To date, only a few studies have addressed the role of TLRs in disease exacerbations.

B. Toll-Like Receptors in Defense against Pulmonary Infection

TLR2 and TLR4 have been linked to bacteria-induced COPD exacerbations. Tokairin et al. (2008) showed that animals with elastase-induced lung emphysema had a significantly increased inflammatory response to streptococcal infection compared with nonemphysematous animals because of up-regulated TLR2 and -4 expression on alveolar macrophages. The role of TLR3 in the exacerbations of pulmonary diseases has been studied in murine airway inflammation models as well as in vitro studies. Intranasal administration of a synthetic TLR3 ligand poly(I:C) into wild-type mice resulted in up-regulation of gene expression of TLR2, TLR3, TLR7, and TLR9 as well as up-regulation of chemokines, cytokines, and signaling molecules in the lung (Stowell et al., 2009). In addition, poly(I:C)-treated mice showed a significant increase in the total cell number, especially in neutrophils, in the BAL fluid. Moreover, poly(I:C) stimulation induced bronchial epithelial cell hypertrophy in wild-type mice leading to impairment of the pulmonary function in those mice. However, in TLR3 knockout mice poly(I:C)-induced inflammatory cell influx was attenuated and the mice were protected from bronchial epithelial cell hypertrophy and changes in the lung function. These data suggest that viral activation of TLR3 can play a critical role in exacerbation of respiratory diseases (Stowell et al., 2009). Existing infections in patients with COPD aggravate viral susceptibility, partly as a result of increased TLR expression in the lung. As such, *Haemophilus influenza* infection increases lung cell TLR3 and intercellular adhesion molecule 1 expression, which potentiates a subsequent rhinovirus infection (Sajjan et al., 2006).

TLR7 and -8 both induce neutrophil activation and CXCL8 production upon viral infection, which could lead to disease exacerbations (Wang et al., 2008). Virus-induced disease exacerbations can be further potentiated by oxidative stress encouraged TLR triggering (Yanagisawa et al., 2009). Both the synthetic TLR7 ligand imiquimod (R-837) and the TLR9 ligand CpG activate human eosinophils from allergic patients and healthy subjects (Månsson and Cardell, 2009). The TLR responses of eosinophils were more pronounced in allergic patients, and activation of TLR7 and TLR9 resulted in the activation of eosinophils at several levels, including prolonged survival, enhanced migration, and induction of CXCL8 release. These data suggest that during viral respiratory infections, TLR7 and TLR9-mediated activation of eosinophils may contribute to allergic exacerbations. Even though TLR9 has been extensively studied for allergic asthma treatment, CpG ODN may contribute to disease exacerbations.

C. Pollutant-Induced Disease Aggravation

Environmental factors play a key role in asthma and COPD (Fig. 2). Traditionally, COPD has been linked to noxious particles, whereas asthma is linked to allergens. However, many environmental pollutants, originating from traffic and industry, are associated with the aggravation of both asthma and COPD (Pope et al., 1995; Anderson et al., 1997; Künzli et al., 2000; Mortimer et al., 2002). Occupational exposures that aggravate airway diseases include (in)organic dusts from crop and animal farming; chemicals released in leather, food, and rubber industrial settings; metal fumes; and stone dust derived from coal, rock, concrete or brick (MacNee and Donaldson, 2000; Trupin et al., 2003; Schikowski et al., 2005; Salvi and Barnes, 2009). Five to 10% of new-onset asthma cases in adults are work-related (Kogevinas et al., 1999).

The abnormal immune response to inhaled triggers (smoke, allergen, environmental pollutants) encompasses disturbed clearance mechanisms and antigen presentation. Alveolar macrophages play a key role in the removal of particulates from the airways. Moreover, macrophages play a significant role in the regulation of local immune responses via secretion of mediators that influence surrounding cells. In contrast to macrophages in other tissues, the alveolar macrophage has a less prominent antigen-presenting function (Chelen et al., 1995). DCs are the key pulmonary antigen-presenting cells. Because naive helper T cells cannot recognize antigens alone, presentation of an antigen is essential for adaptive immunity, and DCs thus form an important bridge between innate and adaptive immunity in asthma and in COPD. Immature DCs are efficient in the uptake of antigens, which will cause maturation and subsequent DC migration to the draining lymph nodes where the antigen is presented to precursor Th cells (Lambrecht et al., 2001). Depending on the type of antigen and on other costimulatory factors, the naive T lymphocyte can differentiate and mature into different kinds of Th phenotypes. Disturbed macrophage and DC function could lead either to asthma or to COPD, depending on the predominant trigger.

Noxious particles and gases could have an adjuvant potential for an infectious or an allergic response (de Haar et al., 2006). Diesel particles amplify responses to microbial agonists and alter the nature of the inflammatory milieu induced by TLR agonists (Chaudhuri et al., 2010). Ambient particulates and diesel exhaust particles and carbon black also activate pulmonary dendritic cells, giving rise to T-cell proliferation and Th2-type cytokine secretion (Bezemer et al., 2011). Furthermore, in both asthma and COPD, the pollutants may trigger goblet cell hyperplasia and mucus hypersecretion, causing airway narrowing and poor clearance function. Particles can act as a carrier for allergens and microbes, thereby contributing to airway disease symptoms (Ormstad et al., 1998; Li et al., 2010). Organic dust has been linked to increased TLR2 gene and protein expression on cultured bronchial epithelial cells

derived from hog confinement workers who are at risk of developing COPD (Bailey et al., 2008). Diesel exhaust particles may aggravate asthma and COPD symptoms and give rise to exacerbations (Takano et al., 1997; van Vliet et al., 1997; Nel et al., 1998; Ulvestad et al., 2000; Hart et al., 2006). TLR4 point mutant mice, compared with wild-type mice, show decreased lung inflammation and neutrophil influx in BAL upon exposure to diesel exhaust particles (Inoue et al., 2006). TLR2 and TLR4 are also involved in the immune response against fine and coarse air-pollution particles (Shoenfelt et al., 2009). Inhaled ozone, another risk factor for airway diseases, alters the distribution of TLR4 on alveolar macrophages and increases the functional response of alveolar macrophages to endotoxin (Hollingsworth et al., 2007). Ozone is thus able to modulate innate immune responses via altered TLR4 expression and function.

Cigarette smoke components may cause reduced TLR expression and function, which can thus act as an immunosuppressive (Barnes, 2004a; Stampfli and Anderson, 2009). Mortaz et al. (2009b) showed down-regulation of the release of IFN- α and other proinflammatory cytokines by plasmacytoid DCs upon cigarette smoke medium exposure. Cigarette smoke may increase susceptibility to a bacterial or viral infection, thereby giving rise to disease exacerbations.

Decreased functional response of TLR5 has been demonstrated in patients with asthma compared with healthy subjects. Ex vivo flagellin-stimulated production of TNF- α , IL-10, and IL-1 β by PBMCs was significantly lower in patients with asthma. In addition, the expression of TLR5 was significantly decreased in monocytes, lymphocytes, and DCs of patients with asthma. The observed reduction in TLR5 activation may lead to reduced release of Th1-related cytokine IL-1 β and anti-inflammatory cytokine IL-10, thereby contributing to the immunological mechanisms of asthma (Lun et al., 2009).

VI. The Role of Toll-Like Receptors in Tissue Homeostasis

TLR signaling is part of the maintenance of homeostasis between damage and repair mechanisms (O'Neill, 2005; Zhang and Schluesener, 2006). Patients suffering from asthma and COPD show increased ECM turnover and increased deposition of ECM components in their lungs (Noble and Jiang, 2006). The role of ECM in health and disease has received a lot of attention in recent years (Teder et al., 2002; Jiang et al., 2007; Papakonstantinou and Karakiulakis, 2009). ECM components are significantly involved in maintaining tissue structure and function of the lung and in the modulation of inflammatory responses. ECM mainly consists of glycosaminoglycan (GAG) molecules. There are two main types of GAGs: non-sulfated GAGs, which remain noncovalently attached to cells (HA), and sulfated GAGs (heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratin sulfate),

which remain attached to cells via their covalent binding to a protein core that extends beyond the cell membrane. HA is the most abundant nonsulfated GAG in the lung ECM. In patients with mild to severe COPD, enhanced levels of HA were detected in the sputum, which correlates with disease severity and with markers of inflammation. In addition, lungs of patients with severe COPD showed an increased expression of hyaluronidase, suggesting an enhanced HA turnover (Dentener et al., 2005). Animals exposed to cigarette smoke display an acute increase in alveolar and bronchial deposition of HA that correlates with changes in genes associated with HA modulation (Bracke et al., 2010). Both TLR2 and TLR4 have been associated with the regulation of lung injury and repair via recognition of HA and HA fragments (Jiang et al., 2005). Conflicting evidence exists in the literature regarding the critical receptors for mediating the response to HA and HA fragments. CD44 is the major cell receptor for HA and is present on both hematopoietic cells as well as parenchymal cells such as epithelial cells and fibroblasts (Teder et al., 2002); (Aruffo et al., 1990). Although CD44-TLR2 receptor complexes play a protective role in TLR-mediated inflammation, this is not dependent on HA binding (Kawana et al., 2008). CD44-MD2-TLR4 receptor complexes are involved in the biological activity of low-molecular-weight HA. The presence of CD44 however, is not critical and may function more to enhance or stabilize the interaction between HA and TLR4 (Taylor et al., 2007). In addition, TLR4 but not TLR2 or CD44 binding of HA fragments is able to induce DC maturation and to initiate an inflammatory response (Termeer et al., 2002). In contrast, the inflammatory response by DCs to low-molecular-weight HA is critically dependent on TLR2, and this can be blocked by the addition of high-molecular-weight HA (Scheibner et al., 2006). These data suggest that the inflammation-induced turnover of high-molecular-weight HA into low-molecular-weight HA might influence tissue homeostasis in which TLRs could play a role (O'Neill, 2005; Bollyky et al., 2007; Jiang et al., 2007; Bollyky et al., 2009).

Patients suffering from asthma and COPD have increased numbers of apoptotic cells in their lungs. Increased apoptosis together with impaired clearance mechanisms contribute to disturbed tissue homeostasis (Kasahara et al., 2001; Vandivier et al., 2002, 2006; Hodge et al., 2003, 2007b; Richens et al., 2009). Apoptotic cells, when not cleared fast enough, may lose cell wall integrity, thereby releasing intracellular components that can function as alarm signals. Polly Matzinger (Oppenheim and Yang, 2005) was the first to suggest that the immune system might become activated by self-generated alarm signals, which are also referred to as DAMPs (Oppenheim and Yang, 2005). DAMPs were originally described as any molecule that is not normally exposed during, after, or because of injury or damage (Seong and Matzinger, 2004). DAMPs can be subdivided into molecules from microbial origin, such as the above-mentioned PAMPs, and endogenous DAMPs (Oppenheim and Yang, 2005). Endogenous

DAMPs with an adjuvant activity are rapidly released in response to infection or injury and are active as highly purified molecules. These DAMPs have chemotactic and activating properties on cells of the innate immune system at physiological levels. Inhibition of these endogenous DAMPs will modulate the biological activity of the dead or injured cell (Oppenheim and Yang, 2005; Bianchi, 2007; Kono and Rock, 2008). DAMPs that contribute to the pathogenesis of airway diseases and that are reported to use TLR signaling to mediate their biological activity are summarized in Table 2 (for a more complete overview regarding DAMPs and receptors involved, see Bianchi, 2007; Rubartelli and Lotze, 2007; Kono and Rock, 2008; Carta et al., 2009).

One of the best known DAMPs released by apoptotic cells is the high-mobility group protein B1 (HMGB1) (Bianchi, 2007; Lotze et al., 2007; Kono and Rock, 2008). HMGB1 is both a nuclear factor and an excreted protein. Inside the nucleus, it is loosely bound to chromatin; outside, it is bound with high affinity to the receptor for advanced glycation end products and functions as a potent mediator of inflammation and cell migration (Yang et al., 2005; Rauvala and Rouhiainen, 2010). In patients with COPD, HMGB1 expression is correlated with inflammatory and clinical parameters (Ferhani et al., 2010). TLR2 and TLR4 have been suggested as receptors for HMGB1 and mediate its inflammatory action by stimulating neutrophils, monocytes, and macrophages to secrete proinflammatory mediators (Park et al., 2004; Yang and Tracey, 2010). When added intraperitoneally, HMGB1 elicits an inflammatory response that was ameliorated in TLR4 knockout mice and also enhanced in TLR2 knockout mice (van Zoelen et al., 2009). In contrast to the response in primary cells, when HMGB1 was added to HEK-TLR transfected cells, only TLR2 transfected cells responded to HMGB1 with increased CXCL8 production (Yu et al., 2006). Conflicting data found increased nuclear factor κ B activity in TLR2- as well as in TLR4-HEK-transfected cells. In addition, by using fluorescent resonance energy transfer techniques and immunoprecipitation, it was shown that HMGB1 was bound to TLR2 and TLR4 (Park et al., 2006). HMGB1 nucleosome complexes have inflammatory activities via its interaction with TLR2 (Urbonaviciute et al., 2008).

Heat shock proteins (HSPs) are a family of proteins that are essential for maintaining normal cell function by assisting in folding, assembly, and translocation of newly synthesized proteins. Under normal physiological conditions, HSPs are expressed at low levels. Upon cellular stress, the expression of HSPs is markedly increased (Lindquist, 1986; Hartl and Hayer-Hartl, 2002). HSPs have been shown to be potent activators of the innate immune system by mediating cytokine function (Wallin et al., 2002; Tsan and Gao, 2004). Serum levels of the 27-, 70-, and 90-kDA HSPs can be correlated to disease progression in patients suffering from COPD (Hacker et al., 2009). Plasma and induced sputum levels of HSP70 correlate

with disease severity in patients with asthma and might provide a diagnostic tool for the diagnosis of asthma (Hou et al., 2011). TLR4 and TLR2 have both been implicated as receptors for endogenous HSPs (Asea, 2008). TLR4 has been shown a key receptor mediating the interaction of a HSP70 family member, Hsp70L1, with DCs and subsequently enhancing the induction of Th1 immune response (Fang et al., 2011). Further research is needed to establish the role of elevated serum and sputum HSP levels in the pathogenesis of COPD and asthma.

S100 proteins are multifunctional signaling proteins that are involved in the regulation of diverse cellular processes such as contraction, motility, cell growth, differentiation, cell cycle progression, transcription, and secretion. Marenholz et al. (2004) provide an excellent overview of the diversity of S100 family members and their functions. Clinical data from BAL fluid obtained from patients with lung disorders revealed that concentrations of calgranulin A (S100A8) and calgranulin B (S100A9) were elevated in smokers with COPD versus asymptomatic smokers. Moreover, S100A8 protein levels were increased when asymptomatic smokers were compared with nonsmokers. In contrast, however, no difference in S100A8, S100A9 levels were detected when induced sputum of patients with COPD was compared with sputum of healthy subjects (Lorenz et al., 2008). S100A8 and S100A9 have been linked to TLR 4 signaling (Foell et al., 2007; Ehrchen et al., 2009).

It seems unlikely that the different extracellular and intracellular derived DAMPS (e.g., ECM components, HBMG1, HSPs, S100 proteins) and PAMPs (e.g., viral and bacterial components) are recognized by the same extracellular LRR domain. Many of the proposed TLR binding molecules failed to demonstrate biological activity in subsequent studies wherein the molecule was rigorously purified (Tsan and Gao, 2009; Erridge, 2010). Further research is thus necessary to unravel the true importance of the various described ligands. Intrinsic to the function of many endogenous ligands (i.e., in their normal physiological state), it could be argued that the endogenous molecules are very "sticky" and therefore facilitate TLR interaction of the contaminating PAMPs (Seong and Matzinger, 2004; Bianchi, 2009; Erridge, 2010). In theory, as suggested by Clett Erridge, upon sterile tissue damage, the released endogenous molecules could bind and trap circulating PAMPs and lower the threshold of cellular responsiveness, adding to the inflammatory status. That would indicate that PAMPs are the actual mediators of inflammation, whereas DAMPs function as initiators and facilitators of inflammation (Erridge, 2010).

VII. Toll-Like Receptors as Therapeutic Targets

A. Toll-Like Receptor Activation: Agonists and Adjuvant Therapy

Synthetic TLR activators that are currently under clinical development for the treatment of asthma, allergies, and infections are summarized in Table 2 and include TLR3, TLR4, TLR5, TLR7, and TLR9 agonists (Basith et

al., 2011). It is well established that TLRs play a crucial role as a first line of defense against microbial infection (Akira and Takeda, 2004). Both Asthma and COPD are chronic lung diseases with episodes of disease worsening, as discussed in section V. Inflammation has a key role in both diseases, mainly during the exacerbation status, whereas symptoms such as shortness of breath remain after exacerbations. TLR agonist or adjuvant therapy could be successful treatments during bacteria- or virus-induced disease exacerbations. Effective defense mechanisms during infection together with a fast recovery will minimize the duration of disease worsening. Triggering of TLR by using agonists may boost the protective inflammatory response that destroys pathogens and protects the host. Indeed, it has been shown that TLR3 and TLR9 agonists help in protection against lethal seasonal influenza virus infections (Wong et al., 2009). It should be noted that such therapies might amplify unwanted tissue destructive inflammation. TLR antagonist therapy could thus be posed to restore homeostasis after the infection has been cleared. In this respect, it is of huge importance to use proper timing and to get insight into the pulmonary microbial conditions.

In addition to the induction of protective immunity during infection, TLR activation may also modulate existing inflammatory disorders. Certain TLR activators have the potential to reverse Th2-type responses to allergens and thus restore the balance of the immune system (Hussain and Kline, 2001). TLR agonists that induce a Th1 response could lead to neutrophilic inflammation in addition to the suppressive effects on Th2. While using this approach, proper dosing and timing should be very tightly controlled. CpG ODNs are the most extensively studied synthetic TLR ligands in preclinical models of allergic asthma as well as in the clinical setting. Klinman et al. (1996) reported that CpG ODNs induce the production of IL-6, IFN- γ , and IL-12 by NK cells, B cells, and CD4⁺ T lymphocytes both in vivo and in vitro. CpG ODN induces IL-12 secretion by activated macrophages and other antigen-presenting cells. IL-12 in turn stimulates the secretion of IFN- γ by NK cells and T-lymphocytes, resulting in a Th1 type of inflammatory response. The stimulation of regulatory T-cell production has been reported after DC stimulation with CpG ODN via enhanced IL-12 and IL-10 production (Jarnicki et al., 2008). Indoleamine 2,3-dioxygenase, the rate-limiting enzyme of tryptophan, is induced by TLR9 ligands and mediates in part the anti-inflammatory responses reported to be caused by TLR9 agonist administration.

Preclinical models of asthma have demonstrated that CpG ODNs are potent inhibitors of atopic responses, suppressing Th2 cytokine (Chu et al., 1997) and reducing airway eosinophilia (Sur et al., 1999), systemic levels of IgE, and bronchial hyper-reactivity (Hussain and Kline, 2001). CpG ODN are effective in such models to prevent the development of airway remodeling by reducing goblet cell hyperplasia, subepithelial fibrosis, airway hyper-reactivity, peribronchial smooth muscle layer thickening,

peribronchial fibrosis, mucus production, peribronchial myofibroblast accumulation, and levels of the profibrotic cytokine transforming growth factor- β 1 (Chu et al., 1997; Sur et al., 1999; Hussain and Kline, 2001). In established asthma, CpG ODN can reverse manifestations of disease, both when used alone or in combination with allergen immunotherapy. Early clinical trials have had mixed results. In an allergic rhinitis immunotherapy study, a significant benefit was found when CpG ODNs were conjugated to ragweed allergen by shifting a Th2-dominant response to a Th1-dominant response (Simons et al., 2004). However, only limited efficacy was seen when administered before allergen challenge in patients with asthma (Gauvreau et al., 2006). In clinical studies, the administration of CpG ODN did not result in adverse effects. Initial phase II studies with TLR9 vaccines conjugated to a ragweed allergen demonstrated that they reduce symptoms of allergic rhinitis during the ragweed season; consequently less allergy medication was prescribed (Creticos et al., 2006). Th1-skewing was noted in the same study population (Tulic et al., 2004). Further study of CpG ODNs for the treatment of asthma and other atopic disorders is warranted by existing data (Fonseca and Kline, 2009).

Sublingual immunotherapy (SLIT) is another novel approach for the treatment of allergic asthma (Broide, 2009). SLIT provides an oral route of administration for an allergen to induce tolerance to inhaled allergens. A candidate adjuvant for SLIT is the TLR2 agonist Pam3CSK4. Sublingual administration of Pam3CSK4 together with the antigen in BALB/c mice sensitized to OVA dramatically decreases airway hyper-responsiveness and OVA-specific Th2 responses in cervical lymph nodes (Goldman, 2008; Lombardi et al., 2008). Studies of SLIT in allergic rhinitis demonstrated that it reduces symptoms and medication use and is associated with a low incidence of systemic allergic reactions.

B. Toll-Like Receptor Inhibition: Antagonist Therapy

TLR inhibitors that are currently under clinical development for the treatment of inflammation, autoimmunity, and acute and chronic inflammation are summarized in Table 2 (Basith et al., 2011). Antagonist therapy could down-regulate excessive inflammation, which could be beneficial during sterile inflammation or in autoimmunity by reducing or inhibiting unwanted inflammation. Antagonist therapy could be achieved by blocking the interaction between the TLR and the disease-related ligand or by blocking downstream signaling molecules. Studies using TLR knockout or TLR signaling molecule knockout mice could provide useful information in this respect. To our knowledge, so far no studies have addressed a potential role for TLR antagonists in the treatment of asthma or COPD specifically. One could argue that inflammation induced by bacteria-derived molecules such as LPS or CpG-DNA motifs or by endogenously derived molecules, in the absence of a bacterial infection, would trigger unwanted inflammation, thereby increasing the risk for tissue dam-

age. If part of the cigarette smoke mediated inflammation were triggered by LPS, CpG-DNA, or endogenous molecules via TLR activation, one could argue that TLR4 and TLR9 antagonists would lessen the cigarette smoke-induced abnormal inflammatory response. However, in cases where a bacterial infection is present, this approach could lead to disease aggravation and should be avoided.

TLR antagonists have been discussed for the treatment of cardiovascular diseases: atherosclerosis, thrombosis, myocardial remodeling, ischemic reperfusion injury, and valvular disease (Lin et al., 2009). TLR2 and TLR4 activation may modulate immune responses that potentially lead to the development of cardiovascular diseases. The beneficial effect of statins in the treatment of cardiovascular disorders may partly be explained by TLR4 antagonism, because statins have been shown to inhibit TLR4-mediated inflammatory responses, and statins negatively regulate monocyte TLR4 signaling (Lin et al., 2009). Cardiovascular disorders are associated with lung diseases, especially COPD, and TLR4 antagonist therapy could thus be argued to be beneficial for the treatment of cardiovascular effects in patients with COPD. However, antagonist therapies may impair local immune defenses, and in multifactorial diseases such as COPD, caution should thus be taken (Sabroe et al., 2008).

C. Probiotics in Airway Diseases

Probiotics are "living microorganisms which, when administered in adequate amounts, confer a health benefit to the host" (World Health Organization, 2001). Probiotics in the form of Gram-positive bacteria contain TLR ligands and can thus potentiate a TLR driven response as well as shifting the balance from a Th2 response to a Th1 response. Probiotics have been shown to differentially induce a regulatory T-cell response in humans (de Roock et al., 2010; Fink, 2010) and induce the expression of FoxP3 in intestinal lamina propria cells in a murine asthma model (Hong et al., 2010). It has been stated that there is substantial evidence from clinical studies to suggest a role of probiotics in the prevention and management of allergy (Kalliomäki et al., 2010; Rijkers et al., 2010). Evidence for the potential use of probiotics in the treatment of allergy arises predominantly from clinical studies with children suffering from atopic disease. It has been shown that the composition of the gut microbiota differs between healthy and allergic infants and in countries of high and low allergy prevalence (Björkstén et al., 1999, 2001; Kalliomäki et al., 2001; Watanabe et al., 2003; Penders et al., 2007). More than 25 randomized, double-blind controlled clinical trials have been conducted to study the effects of various probiotics on treatment and prevention of allergic diseases. A systematic review of randomized controlled trials with probiotics for the treatment of allergic rhinitis and asthma has been published (Vliagoftis et al., 2008). Overall, probiotics were shown to have beneficial effects on patients with

allergic rhinitis by reducing symptom severity and reducing their medication use.

Preclinical data from an OVA-sensitized allergic mouse model showed that live and heat-killed *Lactobacillus reuteri*, but not *Lactobacillus salivarius*, significantly attenuated airway hyper-responsiveness and the influx of eosinophils to the airway lumen and BAL fluid of antigen-challenged animals, but there was no change in eotaxin or IL-10 (Forsythe et al., 2007). These responses were dependent on TLR9, because ingestion by TLR9 knockout mice did not result in attenuation of eosinophil influx, BAL cytokine levels or airway hyper-responsiveness to methacholine (Forsythe et al., 2007). There is still a need for more studies investigating the possible role of TLR in the complex working mechanisms of probiotic strains that are effective in the prevention or treatment of asthma.

COPD is associated with increases in weight loss and with a possible imbalance of the intestinal microbiota. Antibiotic treatment is often prescribed to patients with COPD during disease exacerbations, which might affect the intestinal microbiota. Probiotic treatment could thus be argued to be beneficial for patients with COPD by restoring the intestinal disturbance. However, the faecal microbiota in patients with COPD with a history of frequent antibiotic use could not be altered by multispecies probiotic intake in a randomized placebo-controlled double-blind study (Koning et al., 2010). On the contrary, studies from our group do show protective effects of different pro- and prebiotic strains; *Bifidobacterium breve* and *Lactobacillus rhamnosus*, on the development of lung emphysema and heart hypertrophy in LPS-induced COPD mouse models (van Bergenhenegouwen et al., 2011; Verheijden et al., 2011).

VIII. Concluding Remarks

TLRs play a key role in innate and adaptive immunity, and these proteins could be powerful targets for immune manipulating. This review provides an in-depth update on the role of TLRs in the asthma and in COPD. The main difficulty of targeting these proteins as novel treatment strategies for airway diseases exists in the dual role that TLRs have in health and in disease. Because of the broad function of TLRs in innate and adaptive immune responses and in tissue homeostasis, TLR targeting for one purpose could influence its other functions. Proper understanding of disease conditions and timing of TLR-based therapies and TLR dose should be tightly controlled. One should also carefully consider the impact on established homeostatic systems elsewhere in the body, especially when TLRs are targeted systemically. In this review, we focused on airway diseases, but the role of TLRs has been implicated in many diseases that involve innate and adaptive immunity. Hence, TLR signaling has been linked to autoimmune diseases and several other immune-mediated inflammatory diseases (Rifkin et al., 2005; Marshak-Rothstein, 2006; Drexler and Foxwell, 2010; Ospelt and

Gay, 2010). In a healthy person, there is a balance between the protective inflammatory host response and inhibition of an excessive autoimmune response. This balance might be disturbed during disease and is another reason for having a good understanding of the molecular mechanisms regarding TLR activation (Liew et al., 2005). Disease may result from overactive TLR signaling triggered by harmless molecules or may result from insufficient TLR signaling during viral or bacterial infections leading to poor defense mechanisms. Both ways could contribute to disease pathology, and for therapeutic implications, it is of huge importance to understand the causal relationships underlying disease symptoms. TLR functionality is complicated by the fact that the TLRs often act together by forming homodimers or heterodimers with other TLRs or with costimulatory molecules expanding the range of the TLR ligands, which have to be studied to elucidate the functional role of the TLRs in disease pathogenesis. Moreover, a combination of stimuli may have different effects than purified stimuli. Although purified TLR agonists provide useful research tools for understanding TLR signaling, this does not fully explain TLR function upon relevant environmental exposures. Few studies have addressed the relationship between TLR-induced disease pathogenesis and disease-related risk factors such as house dust mites and cigarette smoke. Better understanding of the interaction between relevant environmental exposures and TLR signaling, together with improved characterization of underlying mechanisms that lead to airway diseases, could help in building a solid basis for TLR directed therapeutic implications.

Another merit of targeting TLRs for airway disease management lies in the fact that the etiology of asthma and COPD are not fully understood. Restoring a Th2-type response by inducing a Th1 response via TLR2, TLR4, or TLR9 (Table 2) might seem a good solution for the treatment of asthma; however, one could easily end up with a situation in which one inflammatory disorder is replaced by another. Activation of TLR2, TLR4, and TLR9 has been implicated in the pathogenesis of COPD as discussed in section V and in Fig. 3. Although the inflammatory responses that underlie the difficulties of breathing differ greatly between asthma and COPD, one cannot draw a strict line between these diseases. The mechanism of asthma and COPD both involve an exaggerated under-controlled immune response to inhaled triggers. The reason for the abnormal immune responses in COPD and asthma can only partly be explained by genetic predisposition. Parts of the asthma and COPD disease pathology may coexist in the same patient. Eosinophilic infiltration for example does occur in COPD; however, in contrast to asthma, it is believed to play a minor role in the overall pathology (Lacoste et al., 1993). The neutrophil on the other hand has a less dominant role in asthma compared with COPD. Gibson et al. (2001) reported a common non-eosinophilic pattern in induced sputum of nonsmoking adults with severe asthma, which indicates differing mech-

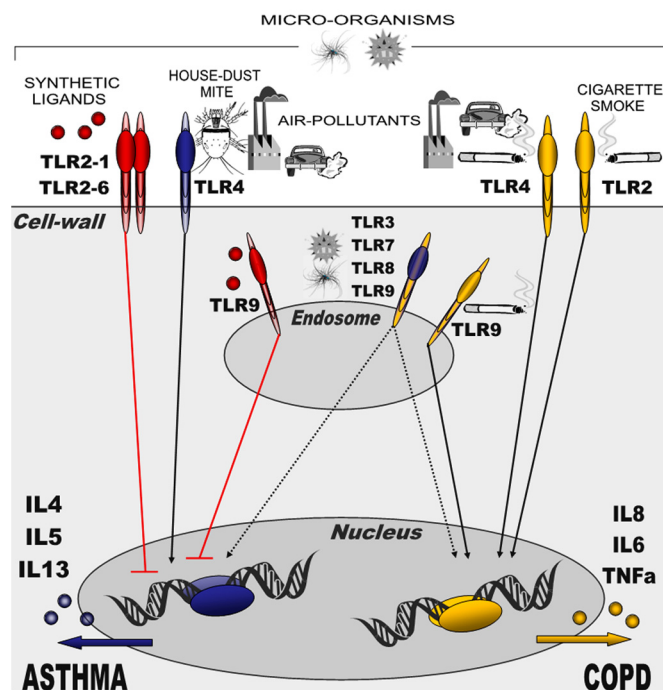


FIG. 3. Dual role of environmental-induced TLR activation in asthma and COPD. Environmental factors activate gene transcription via TLR signaling pathways, which results in disease-specific cytokine secretion. TLR2, TLR4, and TLR9 are linked to cigarette smoke-induced COPD, which can be worsened by air-pollutants (indicated in yellow). House dust mite and pollutants can aggravate asthma via TLR4 (indicated in blue). TLR3, TLR7, TLR8, and TLR9 are involved in virus- and bacteria-induced disease exacerbations (indicated in yellow/blue). Insufficient or excessive signaling will lead to disease worsening (dashed line). TLRs on cell walls are also involved in microbial defense. TLR ligands are listed in Table 2. Allergic asthma can be redirected from Th2 toward Th1 by agonist adjuvancy via TLR9 and via TLR2 homo- and heterodimers with TLR1 and TLR6 (indicated in red). Agonists that are currently under development for the treatment of allergic disorders include TLR4, TLR7, and TLR9 agonists (Table 2).

anisms underlying adult asthma. Over an entire life span, the immune system changes and, as such, a Th2-type asthma phenotype at a young age may shift toward a predominant Th1 phenotype in later life. This shift can occur as a result of changes in environmental stimuli such as viral infections or by the ageing process itself (Smith et al., 2001; Kim et al., 2008). The Dutch hypothesis states that both asthma and COPD share common origins with differences in phenotypic presentation related to the course of the disease or to the interaction between host and environmental factors (Orie et al., 1961; de Marco et al., 2011). Because of shared risk factors (Fig. 2) and shared symptoms (Table 1), misclassification of airway diseases is still a well known phenomenon (Mannino and Buist, 2007; Dirven et al., 2010). Misdiagnosis could be a bottleneck for the success of TLR adjuvant therapy for the treatment of allergic asthma by causing serious disease progression when administered to a patient at risk for the development of COPD. Because of the heterogeneous nature of airway diseases diagnostic tools should thus be improved along with TLR target research.

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