Toll-Like Receptors and Airway Inflammation

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ABSTRACT
The respiratory tract opens to the external environment at the oral side edge, and the other edge of the respiratory tract connects to the closed space (alveoli), and so to preserve the sterility in the terminal respiratory tract is critical for protection against pathogens. The recognition machinery for the invasion of microbes is indispensable for the preservation of the sterility in the lungs. Our general understanding of how microbes are recognized by the innate immune system has increased considerably over the past several years, and the contribution of Toll-Like Receptors (TLRs) to innate immunity is now well documented. In the meantime, it has come to understand that many inflammatory processes may depend on TLR signaling, it has been considered to be involved in the pathogenesis of airway inflammatory diseases such as airway infections, bronchial asthma, and occupational airway diseases. In this review, we focus on physiological roles of TLRs in defense mechanisms of the airways, and pathophysiological roles on airway diseases.

KEY WORDS
airway inflammation, bronchial epithelial cells, innate immune

INTRODUCTION
The alveoli of the lungs that facilitate and are thereby a vital part of the gas exchange process: they are the terminal of the respiratory tree. To carry out efficient gas exchange, the respiratory system and the circulatory system get to within a few microns distance of each other. Alveolar type-I epithelial cells, which occupy 90% of the surface area of the alveoli, are specialized in facilitating the gas exchange process, and therefore they do not have a significant role to play in defending against infections. These particular features of the organizational structure of the alveoli are a disadvantage in terms of the defense against infections, as the invasion of microbes into the alveolar area facilitates the entry of pathogens into the circulatory system, which can result in sepsis and thereby incur the risk of death.

For these physiological and anatomical reasons, the lungs are kept aseptic via various protective mechanisms. The respiratory tract opens to the external environment at the oral side edge, and the other edge of the respiratory tract connects to the closed space (alveoli), in a semi-closed circuit, and so sterility in the respiratory tract increases incrementally from the upper to the lower airway. Neither the trachea nor the relatively large bronchi are aseptic; moreover, because microbes enter frequently from the oral cavity, these organs have to protect themselves from microbes largely through actions like mucous secretions, or mucociliary movements. The final bifurcation of the bronchi is the alveolar bronchi-ole where the bronchi and the alveoli coexist; it is believed that this area, corresponding to the entrance of the alveoli, is maintained aseptic. The recognition machinery for the invasion of microbes is critical to preserve the aseptic condition.

TLR-MEDIATED INNATE IMMUNE RECOGNITION
Innate immunity refers to a built-in defense system of the host to resist the invasion of microbes. The recognition of microbes by an innate immune system has been considered to play a key role in the maintenance of the aseptic condition. Our general understanding of how microbes are recognized by the innate immune system has increased considerably over the past several years, and the contribution of Toll-
Like Receptors (TLRs) to innate immunity is now well documented.1-5 The discrimination between “self” and “nonself” by TLRs as sensors of pathogen molecules, is a key mechanism of the immune system response. For mammals to be able to detect pathogens, a common strategy used by multiple TLR family members is the targeting of constituents as an indicator of infectious non-self. It now seems that many inflammatory processes may depend on TLR signaling.6,8 To date, about 15 mammalian TLRs have been reported.12,24 For example, TLR4 recognizes lipopolysaccharide (LPS) a common constituent of the cell wall of gram-negative bacteria and TLR2 recognizes peptidoglycan a common constituent of gram-positive cell walls, both of which are critical for the innate immune response against bacterial invasion.1,5 TLR3, TLR7, TLR8, and TLR9 recognize pathogen nucleic acids, such as viral RNAs and bacterial DNA.1-5 TLR activation results in the engagement of signaling intermediates, including the following: myeloid differentiation factor-88 (MyD88); Toll-interleukin (IL)-1 receptor (TIR)-associated protein (TIRAP, also known as MAL); Toll receptor-associated activator of interferon (TRIF); Toll receptor-associated molecule (TRAM); IL-1 receptor-associated kinases (IRAK) and tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6).1,5 RNA virus infections such as respiratory syncytial virus (RSV) or influenza virus, are major causes of virus-induced airway inflammation.9-11 Detection of the “molecular signature” for RNA viruses has been attributed to TLR3, TLR7, and TLR8. Double-stranded RNA (dsRNA) is a molecular pattern which is associated with viral infection, because it is produced by most viruses at some point during their replication process.12-16 TLR3 has been shown to recognize dsRNA, and the binding of dsRNA to TLR3 activates signaling to induce anti-viral responses.17 TLR7 and TLR8 have been described as mediating the immune response to guanine and uracil (GU)-rich single-stranded RNA (ssRNA) viruses.18-20 TLR3, TLR7, and TLR8 have been shown to be endosomally located.21,22 TLR3 is also constitutively expressed on the surface of airway epithelial cells.23 The surface expression of TLR3 recognizes extracellular dsRNA but if TLR3 expression is blocked, the antiviral response to the RNA virus is inhibited which leads to an increase in viral replication and release.23 TLR3, TLR7, and TLR8 can each signal independently of MyD88, via a Toll/ interleukin 1 (IL-1) receptor domain containing adaptor-inducing beta interferon (IFN-β) (TRIF); this independent signaling, leads to NF-kB activation and production of type-I interferon which plays an important role in the innate immune response against viral infection.13,16

Not only innate immune responses, different types of adaptive immune responses are also induced via several TLRs expressed on dendritic cells or monocytes. Functioning of the adaptive immune system is based on a diverse set of rearranged T cell receptors and B cell receptors that recognize a wide variety of antigens. Although T and B lymphocytes express receptors of enormous diversity, their activation depends on signals derived from the innate immune system. Immature DCs residing in the periphery are incapable of inducing T cell priming, and TLRs on DC play important role on the DC maturation. When TLRs on DC recognize their ligands, the initiate signaling that lead to DC maturation cause up-regulation of MHC molecules loaded with pathogen-derived peptides and surface expression of co-stimulatory molecules, such as CD80 and CD86. The TLR-mediated control of co-stimulatory molecule expression on DC plays a crucial role in T cell activation.24

**TLRs IN BRONCHIAL EPITHELIAL CELLS**

The lung have multiple regulatory mechanisms to maintain the aseptic condition of the terminal airway, and the alveolar macrophages, which specialize in monitoring microbes in the alveoli, provide complete protection by triggering a strong inflammation reaction when microbes are found.25,26 On the other hand, regarding the monitoring of microbes in the bronchi, bronchial epithelial cells are on front-line of defense against infections.27-30 Most TLRs expression in airway epithelial cells have been documented in research using either cell lines, primary cells or tissues, but their exact expression patterns and expression levels are still to be elucidated. From the aforementioned differences in the levels of sterility, the central bronchi and the peripheral bronchi seems to have different roles in terms of how they monitor microbes; however, there have been few reports up until now that have shown any notable differences in TLR expression level between the central bronchial epithelium and the peripheral bronchial epithelium. Additionally, there have been few reports that verify any apparent differences in responsiveness to the TLR ligand in cultured bronchial epithelial cells derived from the central airway or the peripheral airway. The culture experiments, using bronchial epithelial cells, showed differences in responsiveness to the LPS between the bronchial epithelium and the monocyte/macrophage lineage. For the macrophages and monocytes, the LPS response was observed with only a minimal nM of concentration. On the other hand, for the tracheal epithelium, the response required more than 100 times that minimal concentration, thus showing a clear difference in sensitivity to LPS.27,36,37 As to responses to the peptidoglycan and dsRNA, which are ligands within the same TLR, such a significant difference in sensitivity has not been observed.38-40 It is believed that this difference stems from the different roles of the tracheal epithelial cells and the monocyte/macrophage series in monitoring microbes. There are some differences in patterns of the se-
created cytokine response to TLR ligands between the bronchial epithelium and macrophages. For example, TNF is a representative cytokine that is produced from alveolar macrophages when they are stimulated with TLR, but there is little evidence to indicate that the differentiated bronchial epithelial cells can produce TNF. TNF itself has strong inflammation induction effects as well as tissue damage effects. The fact that bronchial epithelial cells do not produce TNF can be rationalized by the fact that the main purpose of the monitoring of microbes by the bronchial epithelial cells is not to induce inflammations strong enough to cause tissue destruction but rather to achieve the recruitment of neutrophils by chemotaxis as an initial response to the entry of microbes.

**ALLERGIC AIRWAY INFLAMMATION AND TLRS**

Bronchial asthma is a complex inflammatory disease of the airways that is associated with bronchial hyperreactivity, airway obstruction, and increased mucus production. The imbalance between TH1 and TH2 lymphocytes with a predominant TH2-type immune response is a central component in the regulation and perpetuation of the asthma pathology. The release of TH2 cytokines including IL-4, IL-5, and IL-13 in response to allergen exposure induces recruitment and, activation of eosinophils, mast cell activation, and the switch to IgE production by B cells in asthma. The inverse relationship between microbial load in childhood and later development of allergic diseases has led to the hygiene hypothesis. This hypothesis was first proposed by David P. Strachan in an article published in the British Medical Journal in 1989. This hypothesis has now been developed to an understanding that frequent exposure to microbial products results in a predominant TH1 phenotype, whereas a lack of such interactions could promote TH2-driven allergic diseases. This “hygiene hypothesis” proposes that infections acquired early in life may protect children against asthma. Gram-negative bacteria, mycobacteria and many viruses strongly induce TH1 responses. These pathogens, or their purified components, have been shown to prevent TH2-type responses in animals. The hygiene hypothesis is strengthened by certain epidemiological evidence and by the recent discovery that polymorphisms in CD14, an LPS coreceptor, are associated with an increased asthma risk in children. These genetic variants of CD14 may reduce the intensity of LPS responses, thereby reducing development of TH1-type immunity, LPS though appears to have paradoxical roles depending on the timing and the context of the LPS exposure. Although early exposure to LPS (or other TLR ligands) can decrease the incidence of atopic asthma in later life, many reports have demonstrated an increase in allergen-induced asthma severity following exposure to LPS. Viral infection during the first 3 years of life greatly enhances the risk of asthma in children, suggesting that specific interactions between respiratory allergies and viral infections in the respiratory tracts exists; especially, RSV infection in respiratory viral infections, in early childhood, might enhance the development of airway allergen sensitization. A replication intermediate of RSV is dsRNA, and several studies have revealed that TLR3 and dsRNA lead to the induction of genes related to the pathogenesis of bronchial asthma. Thymic stromal lymphopoietin (TSLP) has been shown to be highly involved in the pathogenesis of inflammatory diseases in general. High TSLP expression has been found in the skin of patients with acute and chronic atopic dermatitis, while TSLP is not detectable in normal skin or in non-sional skin of atopic dermatitis patients. In addition, TSLP has been found to be increased in asthmatic airways. High amounts of TSLP have been found in bronchoalveolar lavage fluid in a mouse asthma model and lung-specific TSLP transgenic mice show airway inflammation including massive infiltration of inflammatory cells, goblet cell hyperplasia, and airway hyper responsiveness, whereas mice lacking the TSLPR exhibit strong TH1 responses and fail to develop an inflammatory lung response to antigens. A recent study revealed that TSLP strongly and significantly induced TLR3 ligand and that the combination of IL-4 and dsRNA synergistically enhanced TSLP production in airway epithelial cells. This indicates that respiratory viral infection and the recruitment of TH2 cytokine-producing cells may amplify TH2 inflammation via the induction of TSLP into the asthmatic airway.

**ENVIRONMENTAL AIRWAY INFLAMMATION AND TLRS**

Environmental substances reportedly have a complex effect upon the airway, and epidemiologic studies have shown that asthma-related hospital emergency room visits increase during periods of increased levels of particulate matter less than 10 micrometers in diameter (PM10). Residual oil fly ash (ROFA) produced from the combustion of residual fuel oil significantly increases the ambient air pollution. In human airway epithelial cells, ROFA has been shown to induce inflammatory cytokines, such as IL-6, IL-8, and TNF-α. Inhalation of ROFA into the airways of experimental animals, as surrogates, has been an acceptable model to study the biologic effects of PM10 air pollution. Furthermore, ROFA reportedly causes increased airway hyperreactivity, neutrophilic inflammation, and hyperpermeability in animal models and can amplify allergic inflammation in mouse models of asthma. Cho HY et al. have reported that significant interstrain (genetic) variation was observed in ROFA-induced lung inflammation and hyperpermeability phenotypes. C3H/HeJ (HeJ) mice were most
resistant to the ROFA-induced airway inflammation responses. They observed that ROFA-induced lung injury was significantly greater in CH3/OuJ mice compared with HeJ mice. CH3/OuJ mice have normal TLR4, on the other hand, CH3/HeJ mice have a missense mutation in the tlr4 gene, resulting in non-functional TLR4. They also found that ROFA significantly enhanced transcript and protein levels of lung TLR4 in OuJ but not in HeJ mice, and that they observed greater activation of downstream signal molecules, such as MYD88, TRAF6, IRAK-1, NF-kappaB, MAPKs, and AP-1 was observed in OuJ mice than in HeJ mice before the development of ROFA-induced airway injury.

Ozone is another ubiquitous urban air pollutant that can significantly contribute to increased pulmonary morbidity and mortality in human populations. Despite the demonstrated clinical relevance of ambient ozone, the biological mechanisms of ozone-induced airway inflammation have remained to be elucidated. Humans exposed to ozone develop neutrophilic inflammation, increased expression of proinflammatory cytokines, and decrements in pulmonary function. Interestingly, TLR4 level has been reported to be strongly linked to the sensitivity of the airway to ozone. This evidence indicates that TLR4 might be a candidate susceptibility gene for environmental airway diseases.

REFERENCES

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