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REVIEW ARTICLE

Viral Infection in Asthma

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ABSTRACT
In bronchial asthma, respiratory virus infection involves several issues: 1) respiratory virus infection in infancy is a risk factor for, and may predispose to, the development of asthma later in life; 2) respiratory virus infection is associated with the acute exacerbation of bronchial asthma; and, 3) glucocorticosteroids (GC) are not adequate for controlling asthma-related symptoms upon respiratory virus infection. Various cells, inflammatory mediators and cytokines participate in the production of airway inflammation upon respiratory virus infection. Bronchial epithelial cells are a site of infection and replication of respiratory virus. They actively participate in the production of airway inflammation: 1) they produce various proinflammatory cytokines, chemokines and mediators; and, 2) they undergo apoptosis, thereby impairing the repair process. It is therefore important to understand the role of bronchial epithelial cells in the pathophysiology of bronchial asthma. In this review, the interaction between viral infection and asthma is discussed to elucidate the role of bronchial epithelial cells in viral infection.

KEY WORDS
airway inflammation, asthma, remodeling, virus

INTRODUCTION

Rhinovirus, respiratory syncytial virus (RSV), metapneumovirus, influenza virus, and parainfluenza virus infections in bronchial asthma patients have been implicated in several issues.[1,2] Firstly, respiratory virus infection is associated simultaneously with the etiopathogenesis and the acute exacerbation of bronchial asthma.[3,4] Recurrent severe respiratory virus infections in infancy are acknowledged as a risk factor for, and may well predispose the patient to, the development of asthma later in life. Secondly, bronchial asthmatics have increased susceptibility to respiratory virus infections presenting more respiratory symptoms, combined with reductions in pulmonary functioning, than normal subjects who are similarly infected.[3,5] Thirdly, glucocorticosteroids (GC) are widely used for the management of bronchial asthma because of their anti-inflammatory effects; however; the efficacy of GC on respiratory virus infection-induced wheezing episodes and on the acute exacerbation of bronchial asthma in general, is insufficient.[6,7] Other therapeutic options should therefore be investigated.

Bronchial epithelial cells are found at the site of respiratory virus infection and replication. They are involved in the production of airway inflammation upon viral infection by their expression of proinflammatory cytokines, chemokines and mediators, and also by the fact that they can be responsible for the loss of the epithelial barrier function.[8-11] In this review, the interaction between viral infection and asthma is discussed to elucidate the role of bronchial epithelial cells.

DEVELOPMENT OF ALLERGY AND ASTHMA

In infancy, acute episodes of wheezing, which are predominantly caused by respiratory virus infections, are an important cause of death.[12] Respiratory virus infection in infancy also implies an increased risk of the later development of asthma. Immunological responses at birth may determine and predict a risk of acute episodes of wheezing.[13-15] Interferon-(IFN) γ production by cord blood lymphocytes stimulated with RSV or phytohemaggulutinin (PHA) is associ-

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ated both with anti-viral activity and the rate of wheezing in the first year of life.[15] There have been several risk factors associated with the development and persistence of asthma documented, including the following: low birth weights; reduced lung functioning; exposure to second hand smoke; a maternal history of allergy; elevated serum IgE levels; and, the presence of atopy.[16-19] Respiratory virus infection in early life, particularly in infancy, is an important risk factor for the development of asthma later in life. There are several reasons why respiratory virus infection influences asthma development. Firstly, infancy is a period of time which is characterized by lung development, including alveolar multiplication and airway growth, and it is also a time of heightened susceptibility to viral infection;[13,14,20] therefore, viral infection could adversely affect lung development.[21] Secondly, epidemiological studies have shown that severe and/or repeated respiratory viral infections in the lower respiratory tract in infancy influences immune responses. The interaction between respiratory viral infection and allergen exposure in Th-1 or Th-2 polarized responses has been extensively investigated in animal models of allergic asthma and child asthmatics.[12,13,22-25] It is not clearly understood, but in general, timing and severity of viral infection determines Th-1 or Th-2 polarized responses.[12,13,22-25] For instance, when RSV infection and allergen exposure are concurrent, Th-2 responses are enhanced; however, if RSV infection precedes allergen exposure, Th-2 responses are not enhanced.[25]

ACUTE EXACERBATION

Several factors, including microbes such as respiratory viruses, allergens, environmental pollutants, occupational irritants, medications such as aspirin and mental stress are known to cause the acute exacerbation of bronchial asthma[26-28]. In school age children, about 80% of acute exacerbation is associated with viral infection. In adults, although the rates of detection and isolation of viruses varies widely in different studies, respiratory viral infection is the most common cause of acute exacerbation.[26-28] Bronchial asthmatics have generally more susceptibility to respiratory virus infection. They are more likely to develop lower respiratory symptoms and these symptoms are likely to persist for long periods of time.

Mechanisms involved in the respiratory virus infection-triggered acute exacerbation of bronchial asthma have been extensively investigated. Bronchial epithelial cells are the site of viral infection and replication. Cellular responses and damage to epithelial cells upon viral infection are known to be involved in and possibly trigger the acute exacerbation of bronchial asthma.[26-28] A variety of proinflammatory cytokines, chemokines and proallergic cytokines are up-regulated during the acute exacerbation of asthma. Cellular profiles and cytokine patterns in the airways in virus-triggered acute exacerbation cases differ from those in allergen-triggered exacerbation cases.[26-28] In virus-triggered acute exacerbation, there are the following: bronchial mucosa neutrophilia and eosinophilia; up-regulation of CXCL8 (interleukin-8; IL-8) and its receptors; up-regulation of CXCL5 (epithelial cell-derived neutrophil attractant 78), and its receptors; CXCR1; and, CXCR2. CCL5 (RANTES) is also up-regulated.[29-31] By contrast, there is bronchial mucosa eosinophilia and increased IL-5-positive cells in allergen-triggered acute exacerbation.[27] The mechanisms might be different between them. For instance, airway epithelial cells are the primary target cells for viruses, since respiratory viruses infect and replicate in airway epithelial cells; however, for allergens, bronchial epithelial cells are not the primary target cells. It is also important for allergens to pass through the epithelial cell barrier and reach the internal milieu of the airway.

TOLL-LIKE RECEPTOR

The toll-like receptor (TLR) plays an important role in pathogen recognition and in innate immunity. Airway epithelial cells constitutively express the ten members of the TLR family and their ligands which have been identified.[32] Among these 10, the biological functions of TLR2, TLR3 and TLR4 and their roles in airway inflammation have been well documented. TLR3 binds double strand RNA (dsRNA) synthesized in virus-infected cells. This binding of dsRNA to TLR3 induces the production of various proinflammatory cytokines, chemokines and interferon (IFN).[33]

TLR EXPRESSION

TLR3 is constitutively expressed, but up-regulated by microbe infections including bacteria and viruses.[34] Recently it was discovered that *H. influenzae* infection potentiates airway epithelial cell responses to rhinovirus by expressing both the intracellular adhesion molecule-1 (ICAM-1) and TLR3.[35] Histamine also up-regulates TLR3.[36] Thus, local inflammation influences TLR3 expression. In addition, RSV infection sensitized airway epithelial cells to bacterial endotoxins and lipopolysaccharides (LPS) via the up-regulation of TLR4 expression.[37] TLR4, which is a ligand for LPS, also recognizes viral motifs including the F protein of RSV.[38] Results from the data on the impaired host defense against RSV infection in TLR4-deficient mice have shown that TLR4 plays an important role in RSV infection.[39] Recently, Tulic M *et al.* have shown that children, who demonstrate expression of certain TLR4 genotypes, may have more susceptibility to RSV infection.[40] Since repeated and severe respiratory virus infections, including RSV, is suggested to be a risk factor for developing bronchial asthma, it is necessary to clarify whether children with certain TLR4 genotypes would develop bronchial asthma.

DEFICIENT INNATE IMMUNITY AGAINST VIRUS INFECTIONS

As for the responsiveness of bronchial epithelial cells to respiratory virus infections, asthmatics have been shown to have impaired IFN production of their bronchial epithelial cells; moreover, their bronchial epithelial cells demonstrate a decrease in IFN-β expression and cell apoptosis occurs upon rhinovirus infection which results in increased virus replication occurring.[41] Rhinovirus load in asthmatic bronchial epithelial cells correlated significantly with abnormal pulmonary function. Thus, increased susceptibility to rhinovirus infection and virus replication in the bronchial epithelial cells in asthmatics may be closely linked to the development of not only bronchial asthma symptoms but also to bronchial asthma itself. Recently, deficient IFN-λ production and increased virus replication in the bronchial epithelial cells from asthmatics also has been proven.[42] Rhinovirus infects the cells via ICAM-1 and subsequently enters the cells. After entry, dsRNA is synthesized in the cells. ICAM-1-mediated signals and TLR3-mediated signals are both able to induce IFN production.[43] Blocking of TLR3 results in a decrease in antiviral activity against rhinovirus infection.[44] Differences in TLR3 expression, on airway epithelial cells and polymorphisms in genes encoding TLR3, might be a potential mechanism which can account for the impaired interferon responses to viral infection; however, there have been no reports to support this hypothesis. Further studies should be pursued to elucidate the mechanisms involved in impaired IFN expression upon rhinovirus infection.

EPITHELIAL CELLS IN AIRWAY INFALAMMATION AND REMODELING

The intact epithelial cells, and the tight junctions between them, form a barrier between the external and internal milieu of the airway. This physiological barrier prevents invading environmental agents from transferring from the lumen to the interstitium via the paracellular route and thusly it prevents them from contacting subepithelial basement membranes.[45] In addition to their function as a physiological barrier, they produce airway secretory proteins, which clear out inhaled agents, and cytokines, which attract and activate inflammatory cells to facilitate them to work on the host defense.[9-11,46] Thus, bronchial epithelial cells play a pivotal role in the host defense and in the normal homeostasis of the internal milieu of the respiratory system, while epithelial cells are actively involved in the production of airway inflammation and remodeling: 1) they produce various cytokines and mediators; 2) they undergo injury and their repair process is impaired; and, 3) they lose or become deficient as regards the function of the epithelial tight junction.[9-11,46] Why does airway inflammation and remodeling occur in bronchial asthmatics when they inspire environmental agents and gases but not in nonasthmatics? A reasonable answer could be that the bronchial epithelial cells of asthmatics have several different characteristic features.

There are several reports describing the different degrees of susceptibility of bronchial epithelial cells to various stimuli in asthmatics. The bronchial epithelial cells of asthmatics produce more cytokines in response to DEP and have more susceptibility to oxidant-induced injuries;[9-11,46] this indicates that the characteristic features of bronchial epithelial cells might be closely related to chronic airway inflammation. These results were obtained from the bronchial epithelial cells of adult asthmatics. Airway inflammation and treatment could modify the characteristic features of the bronchial epithelial cells and it is important to determine when these characteristic features occur and how inherited factors contribute to these characteristic features. A recent study has demonstrated that the bronchial epithelial cells from asthmatic children also have intrinsic biochemical and functional differences from normal healthy controls.[47] The interaction between genetic and environmental factors may contribute to the pathogenesis of bronchial asthma; therefore, genetic factor(s) predisposing susceptibility of bronchial epithelial cells to various stimuli should be clarified.

CYTOKINE PRODUCTION AND MEDIATOR RELEASE

Bronchial epithelial cells are the site of viral replication and actively participate in airway inflammatory responses by their expression of various cytokines and mediators. Various inflammatory and immunocompetent cells, including macrophages, dendritic cells, lymphocytes, mast cells, eosinophils and neutrophils, are involved in the production of allergic airway inflammation and remodeling in bronchial asthma patients. Several factors stimulate bronchial epithelial cells to produce proinflammatory cytokines, chemokines and arachidonic acid metabolites: environmental factors, including air pollutants; alterations in airway osmolarity and temperature; and, microbes including viruses. These cytokines and mediators recruit inflammatory and immunocompetent cells into the sites of airway inflammation.[9-11,46]

There are a variety of cytokines and chemokines which influence cell functions. Eotaxin/CCL11 and RANTES/CCL5, stem cell factor (SCF) and thymic stromal lymphopoietin (TSLP), IL-8/CXCL8, ENA-78/CXCL5 and neurotropin, TARC, MDC and IP-10, and B-cell activating factors are all produced by bronchial epithelial cells stimulated by proinflammatory stimuli and microbes, including respiratory virus infection, and they recruit and activate eosinophils, mast cells, neutrophils, Th2 cells and B cells, respectively.[48-55] These cells influence each other and orchestrate the cascade like production of airway in-

flammation. Mast cells and eosinophils are found in increased numbers in the airways of bronchial asthma patients and they play an important role in the production of airway inflammation and remodeling, although recent data has brought into question the pivotal role of eosinophils in airway hyperresponsiveness and the clinical features of asthma.[56] Neutrophils are found in increased numbers in the airways of bronchial asthma patients, particularly in instances of severe asthma and at the acute exacerbation phase.[57] Thus, epithelial cell-derived cytokines and chemokines participate in recruiting and activating inflammatory and immunocompetent cells. Recruited cells, in turn, influence epithelial cell functions. For instance, eosinophil-secreted products, including eosinophil cationic protein (ECP), major basic protein (MBP), eosinophilic peroxidase (EPO), and neutrophil-secreted products, including neutrophil elastase, injure epithelial cells and increase epithelial permeability.[58,59] IL-17A, which is mainly produced by activated T cells synergistically, enhances rhinovirus-induced IL-8 and human beta-defencin-2 (HBD-2) production by bronchial epithelial cells.[60] IL-8 recruits neutrophils and HBD-2 recruits immature dendritic cells and memory T cells into the airways. Dendritic cells play an important role in immunologic and allergic responses including in antigen recognition. Thus, the interaction between epithelial cells and various other cells orchestrates the production of airway inflammation and could lead to an acceleration of airway inflammation.

There are two major arachidonic acid metabolic pathways: the cyclooxygenase (COX) pathway; and, the 5-lipoxynase (5-LO) pathway. COX-1 and COX-2, two isoforms of COX, catalyze the synthesis of prostaglandin (PG) H_2 from arachidonic acid. PGH_2 is then metabolized to various PGs, such as PGE_2, PGD_2, PGI_2, and thromboxane A_2 (TxA_2). COX-1 is constitutively expressed, but COX-2 is inducibly expressed. 5-LO generates cysteinyl-LTs (cys-LTs) and LTB4. Individual PGs have different roles in allergic inflammation, such as inducing and/or regulating the inflammation. Cys-LT, LTC4, LTD4 and LTE4 cause bronchoconstriction, mucosal edema, mucus hypersecretion and eosinophils chemotaxis. LTB4 induces neutrophil chemotaxis. In allergic inflammation, proallergic cells, mast cells and eosinophils basically synthesize and release PGs and LTs. Recently, the expression of COX-2 and 5-LO in bronchial epithelial cells expression has been shown. Upon respiratory infection, respiratory virus infection and dsRNA upregulation, COX-2 expression occurs and the subsequent release of PGs and LTs occurs.[61,62] Thus, arachidonic acid metabolites released from bronchial epithelial cells upon viral infection participate in the acute exacerbation of bronchial asthma.

THYMIC STROMAL LYMPHOPOIETIC

TSLP was originally identified as supporting the maturation and differentiation of B cells and the proliferation of T cells. In addition to its known activities, new roles for TSLP in allergic inflammation have recently been documented. TSLP activates myeloid dendritic cells to polarize Th2 responses. TSLP which is a novel IL-7-like cytokine was originally identified in the supernatants produced from the mouse thymic stromal cell lines.[63] TSLP supports the maturation and differentiation of B cells and the proliferation of T cells. In addition to its function on pre-B cell maturation, TSLP is shown to function as a Th2-polarized immune response through the activation of myeloid dendritic cells. TSLP activates immature myeloid dendritic cells to up-regulate co-stimulatory molecules such as CD40, OX40 and CD80 in order to acquire the capacity to induce Th2 responses. TSLP can stimulate the differentiation and activation of immature dendritic cells to induce the expression of Th2 cell attracting chemokines such as CCL17 and CCL 22. Native CD4+ T cells primed by TSLP-stimulated dendritic cells produce proallergic cytokines such as IL-4, IL-5 and IL-13, and tumor necrosis factor (TNF) -α, but little or no IL-10 or IFN-γ. Thus, TSLP is able to trigger dendritic cell-mediated Th2 immune responses.[64-66]

The role of TSLP in allergic inflammation has been explored. The critically important role of TSLP in the triggering and the production of allergic inflammation in asthmatics is supported by the following data: 1) TSLP mRNA expression is increased in asthmatic airways and it correlates with Th2-attracting chemokine expression and with disease severity[67]—in mice, TSLP expression in the lungs of mice with antigen-induced asthma is increased; 2) TSLP receptor-deficient mice had much lower Th2 responses and considerably reduced allergic airway inflammations in the airways;[68] 3) lung-specific expression of TSLP transgene induces allergic inflammation of the airways with eosinophilia, goblet cell hyperplasia and subepithelial fibrosis;[68] 4) TSLP is able to trigger dendritic cell-mediated Th2 immune responses;[64-66] and, 5) TSLP induces IL-5, IL-13, IL-6, GM-CSF, CXCL8 and CCL1 production by mast cells, although TSLP did not induce mast cell degranulation or lipid mediatory release.[64-66]

TSLP is expressed in human skin keratinocytes, bronchial epithelial cells, smooth muscle cells and lung fibroblasts, but not in most hematopoietic cells, including B cells, T cells, natural killer cells, macrophages, monocytes, dendritic cells, and neutrophils. Airway epithelial cells express and produce TSLP mRNA and protein in response to proinflammatory cytokines, including IL-1 and TNF-α, and proallergic cytokines, including IL-4 and IL-13;[51] furthermore, TLR ligands, peptidoglycan (PGN), dsRNA, and rhinovirus infections are able to induce TSLP production

by airway epithelial cells.[50,51] In addition to TSLP-triggered dendritic cell-mediated Th2 immune responses, since TSLP activates mast cells to produce Th2 type cytokines, epithelial cell-derived TSLP upon respiratory virus infection may play an important role in the initiation and promotion of allergic inflammation independent of the Th2-type T cell and IgE-dependent mechanism. TSLP also might also be involved in the possible mechanism involved in the acute exacerbation of asthma upon viral infection.

EPITHELIAL BARRIER FUNCTION AND PERMEABILITY

There are two major routes for environmental agents and microbes to reach the internal milieu of the airway. Injuring epithelial cells and disrupting epithelial tight junctions, resulting in increased epithelial permeability, allows the environmental agents to invade the internal milieu.

EPITHELIAL CELL APOPTOSIS, REMOVAL OF DYING CELLS AND REPAIR

Innate immunity and adaptive immunity co-operate in the host defense against respiratory virus infections. Major host defense mechanisms against respiratory virus infections are as follows: 1) antiviral cytokines including IFN; 2) cell lysis by cytotoxic T cells and natural killer T cells; 3) cell apoptosis to abort the virus propagation process; 4) immunoglobulin; 5) surfactant and defensin; and, 6) barrier function of bronchial epithelial cells.[69-72] The antiviral response by expressing IFN is important for protecting cells against viral infections and replication. Cell apoptosis is a critical process for the pathogenesis of certain diseases. In the case of respiratory virus infections, apoptosis and phagocytosis, of apoptotic cells by phagocytes named as efferocytosis, are helpful defense mechanisms in the host for avoiding virus propagation. Appropriate and successful epithelial cell apoptosis in virus-infected cells is a limiting factor for viral replication and spreading progeny viruses to neighboring cells to abort infection.[69,70] Dying cells should be cleared from the site of inflammation, since viruses are able to survive and replicate in even dying cells and dying cells are also able to release inflammatory mediators. Recently, it has been emphasized that effective efferocytosis is important to clear dying cells and to terminate the inflammation process without inflammatory mediator releases.[73] Thus, appropriate and successful epithelial cell apoptosis, and effective effecrocytosis are critical for avoiding virus propagation and eliminating viruses, and for terminating inflammation at the sites of the inflammation. Clinical data on the persistence of rhinovirus mRNA in nasal wash, after asthma exacerbation in children, is correlated with disease severity; this could support the biological significance of epithelial apoptosis and efferocytosis.[74]

Molecular mechanisms involved in apoptosis have been extensively investigated. A variety of stressful factors, including respiratory virus infections, could induce oxidative stress in the bronchial epithelial cells. Influenza virus infection activates apoptosis-regulating kinase-1 (ASK-1), which is a mitogen-activated protein kinase (MAPK); this kinase is activated by oxidative stress. Influenza virus infection-induced cell apoptosis is induced through caspase-3 activation and N-acetyl-cysteine (NAC) attenuation.[75] Although epithelial cell apoptosis and efferocytosis for aborting virus infections is an important host defense mechanism against respiratory virus infections, epithelial cell injury often results in a loss of the epithelial cell barrier function; therefore, the process of repairing epithelial cells is a crucial step for triggering the recovery of an injured airway.

CELL INJURY, APOPTOSIS AND THE REPAIR PROCESS

Asthmatic bronchial epithelial cells are more susceptible to oxidant-induced injuries and also to an oxidant-induced increase in permeability.[11,45] *In vitro* studies have shown that asthmatic bronchial epithelial cells have an increased number of apoptotic cells compared to healthy control subjects. Respiratory virus infections are able to induce oxidative stress; therefore, it might be possible that repeated viral infections and an impaired repair process combined could participate in the increased number of apoptotic cells in asthmatics.

Cell growth/proliferation is regulated by cell-growth/proliferation signals and cell cycle inhibition signals. The bronchial epithelial cells from adult asthmatics have shown high expression levels of epidermal growth factor receptor (EGFR) and cyclin/cyclin-dependent kinase (CDK) inhibitor $p21^{waf}$, and low expression levels of cell proliferation marker Ki67 in association with concurrent epithelial stress and injury.[11] In childhood asthma, the bronchial epithelial cells from mild or moderate asthmatics, aged 5–15 years, have shown similar findings with their bronchial epithelial cells as the characteristics of adults.[76] This dysregulation of the expression of cell-growth/proliferation signals and cell cycle inhibition signals could be associated with an impaired repair process. Although epithelial cell apoptosis in virus-infected cells is a self-limiting factor for viral replication and the spreading of progeny virus infected cells to neighboring cells, repeated cell apoptosis might result in the production and progression of airway remodeling. After bronchial epithelial cell injury, a wound-repair process, to cover the wounded surface, occurs. During this wound-repair process, proliferating bronchial epithelial cells express and produce profibrogenic cytokine transforming growth factor-β (TGF-β).[8,11,45] TGF-β has been shown to enhance RSV replication in bronchial epithelial cells.[77] Thus,

repeated infection could accelerate the airway remodeling process.

EPITHELIAL TIGHT JUNCTION

Tight junctions located in the apicolateral cell membranes of epithelia form a barrier between adjacent cells. The epithelial barrier function mediated by a tight junction is regulated by the tight junction proteins including claudin-1, claudin-4, occuludin, ZO-1 and junctional adhesion molecule-A.[11,45,78] Functions of these peripheral cell proteins are regulated by a complex mechanism, but proinflammatory cytokines, oxidants and thrombin down-regulate the functions of bronchial epithelial junction proteins.[79,80] The bronchial epithelial cells of asthmatics are more susceptible to oxidant-induced injury and to ozone- or nitrogen dioxide-induced increased permeability.[11,45] Rhinovirus infections impair the bronchial epithelial barrier function possibly through virus infection-induced oxidative stress,[81] although it has not been shown that respiratory virus infections *per se* could alter the bronchial epithelial barrier function. Thus, respiratory virus infections which result in increased epithelial permeability allow allergens and other environmental agents to invade and reach the internal milieu. Pharmacological modulation of the epithelial barrier function by increasing the expression and the function of junctional proteins is quite an important strategy for the host defense mechanism. Azithromycin, a macrolide antibiotic, inhibits the expression of ICAM-1, a receptor for the rhinovirus, and it has been shown to reduce the acute exacerbation of asthma. Transepithelial electrical resistance (TER) is an index of epithelial permeability. Increases in TER imply decreases in epithelial permeability. Azithromycin increases the TER of bronchial epithelial cells by changing the processing of tight junction proteins.[82] Azithromycin-mediated processing of tight junctional proteins may have a beneficial effect in preventing the acute exacerbation of asthma, since cell integrity is important for the host defense against microbes, including virus infections, by preventing the entry of microbes.

Filament aggregating protein (filaggrin), which is produced in the basal epidermal layer of the skin during the terminal differentiation of keratinocytes, aggregates at the epidermal cytoskelton to form a dense-lipid matrix that is cross-linked by transglutaminases to form a cornified cell envelope. This structure, which forms the skin barrier, regulates permeability of the skin and prevents epidermal water loss and it also prevents the entry of allergens, infectious organisms and toxic materials into the host. In atopic dermatitis, the loss of the skin barrier function, because of inherited reduction or loss of filaggrin expression, has been shown to predispose the host to the development of this disease.[83] In bronchial asthma, immunohistochemical staining of bronchial mucosa from bronchial asthmatics and normal healthy subjects, obtained via biopsy, has demonstrated that filaggrin immunoreactivity is not detectable in biopsy specimens.[84] This data does not support the hypothesis that loss or deficiency of filaggrin expression in the bronchial mucosa is associated with bronchial asthma. On the other hand though, Palmer C *et al*. have shown that filaggrin mutations are associated with increased asthma severity.[85] Little is known about the regulation of fillagrin expression. Th2-type cytokines, IL-4 and IL-13 down-regulate filaggrin expression in skin keratinocytes, but IFN-γ is up-regulated.[86] Since it is not conclusively proven whether fillagrin is expressed in bronchial epithelial cells or not, the effect of respiratory virus infections on fillagrin expression is not able to be determined.

VIRUS INFECTIONS AND AIRWAY REMODELING

Respiratory virus infections may predispose to airway remodeling of bronchial asthma. Rhinovirus infection induces the production of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2) and transforming growth factor-β by bronchial epithelial cells, which function in both the angiogenesis and the fibrosis processes.[87] Rhinovirus infection induces cell cytotoxicity and thereby reduces the cell proliferation rate resulting in an impaired repair process of the bronchial epithelial cells.[88] Thusly, rhinovirus infection in bronchial epithelial cells may contribute to the development of airway remodeling. Severe and repeated respiratory virus infections, including RSV infection during infancy, is associated with the development of subsequent bronchial asthma later in life. In animal models of asthma, RSV infection in allergen-exposed mice develop profound airway inflammation and remodeling compared to only allergen-exposed mice.[89]

SIGNAL TRANSDUCITON

Respiratory virus infection and dsRNA induce the production of various cytokines, chemokines and mediators by bronchial epithelial cells. Much effort has been expended to try to clarify the role of intracellular signaling molecules in eliciting cellular responses upon virus infection. The mammalian mitogen-activated protein kinase (MAPK) superfamily has been molecularly characterized as follows: extracellular signal-regulated kinase (Erk); p38 MAP kinase; and, c-Jun-NH_2-terminal kinase (JNK). p38 MAP kinase and JNK were originally identified as playing a role in apoptosis and cytokine expression, whereas Erk plays a central role in cell proliferation and differentiation. Recent studies have suggested that each of these kinases has some element of overlapping biological activity.[90] The influenza virus infection induces p38 MAP kinase and JNK to produce RANTES in the bronchial epithelial cells,[91] and rhinovirus in-

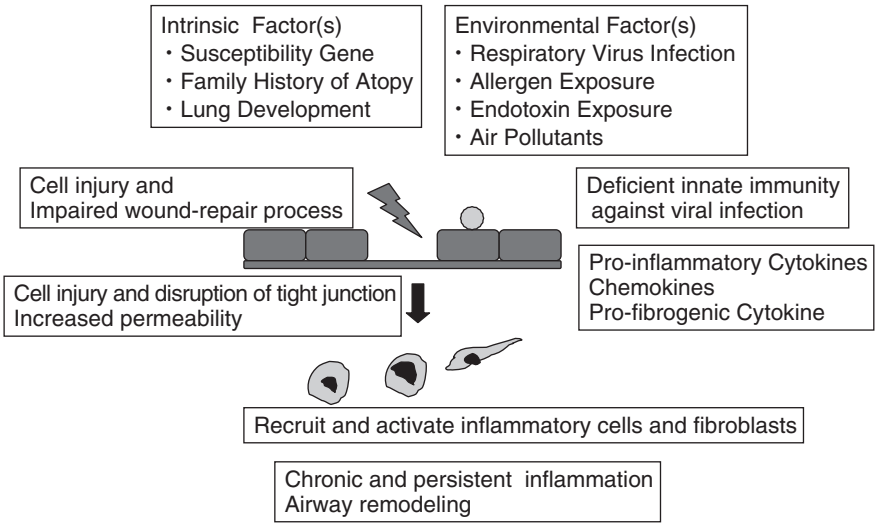

Fig. 1 Respiratory virus infection influences asthma pathogenesis. The interaction between environmental factors and intrinsic factors plays an important role in the pathogenesis of bronchial asthma. Respiratory virus infection is associated with the etiopathogenesis and the acute exacerbation of bronchial asthma. Bronchial epithelial cells are at the site of respiratory virus infection and replication. Respiratory virus infection induces the production a variety of cytokines, and causes the injury epithelial cell and the disruption of tight junction. As consequence, airway inflammation and airway remodeling process could occur.

fection induces p38 MAP kinase to produce cytokines including IL-1, IL-6, IL-8 and GM-CSF.[92] ICAM-1 ligation due to rhinovirus infection activates phosphatidylinositol 3-kinase (PI3-K) to regulate viral endocytosis and produce cytokines and chemokines including IL-8.[43,93,94] Syk is diffusely distributed in the cytosol. Ligation of ICAM-1 due to rhinovirus recruits Syk into the plasma membrane and enhances the Syk-ICAM-1 association, resulting in Syk phosphorylation. Syk phosphorylation leads to the activation of p 38 MAP kinase and IL-8 expression in bronchial epithelial cells.[95] Syk is well known to be an important regulator in the Mast cell functions and Syk inhibitor is clinically effective for the treatment of allergic rhinitis.[96] It is therefore logical to expect that Syk inhibitors could work to regulate rhinovirus-induced airway inflammation and asthma-related symptoms.

THERAPEUTIC STRATEGIES

Antiviral agents against rhinovirus and other respiratory viruses have been developed and are quite important to effectively cure infection-related respiratory symptoms in infants and to prevent the development of bronchial asthma later in life. Three main regimens are used in the management of bronchial asthma: inhaled corticosteroids (ICS); a combination of ICS and long-acting β2 agonists (LABA); and, cysteinyl leukotriene (CysLT1) receptor antagonist (LTRA). ICS is effective at reducing asthma-related symptoms and the frequency of acute exacerbation; however, ICS, and even systemic GC, are not sufficient to reduce acute wheezing. The combination of ICS and long-acting β2 agonists (LABA) synergistically suppress VEGF, EGF-2 and various other chemokines produced by bronchial epithelial cells;[97] furthermore, the combination of ICS and LABA, as a maintenance and reliever therapy, is reported to have an advantage as it reduces the frequency and severity of acute exacerbation in children with asthma.[98] Although the role of respiratory virus infection during childhood in the development of asthma and airway remodeling in later life needs to be clarified, children with asthma having repeated wheezing episodes caused by respiratory virus infection should be treated with a combination of ICS and LABA.

LT and CysLTs are released into the site of inflammation during respiratory virus infection and cause airway inflammation and bronchoconstriction.[99,100] LTRA reduced the clinical symptoms subsequent to RSV infection in patients 3 to 36 months of age hospitalized with RSV bronchiolitis.[101] LTRA reduced asthma exacerbations in 2- to 5-year old children with intermittent asthma over 12 months of treatment.[102] Short-course treatment with LTRA, for at least 7days, introduced at the first sign of an asthma episode, resulted in a modest reduction in asthma-related symp-

toms and parental work absence in 2- to 14 year old children with intermittent asthma.[103] LTRA is thusly useful for the treatment of viral infection-induced asthma-related symptoms. Early intervention of ICS combined with LTRA could prevent the development of asthma, although the effect of LTRA on airway inflammation, and on the structural changes induced by viral infection, has yet to be determined. In addition to pharmacological treatments, an anti-inflammatory therapy targeting intracellular signaling molecules and transcription factors has been investigated:[104,105] it is expected to be approved for use as an inhibitor for the proinflammatory transcription factor, nuclear factor kappa B, to control airway inflammation caused by a respiratory virus infection.

Genetic and environmental factor interaction is critical for the pathogenesis of bronchial asthma. Infection by a respiratory virus, including rhinovirus and respiratory syncytial virus, is common in young children; however, determining who will develop bronchial asthma later in life is not possible to predict yet. Several gene variations, such as IL-4 and IL-4α receptor and chemokine receptor CCR5, have been shown to be associated with the susceptibility and severity of respiratory virus infection.[106,107] It has yet to be clarified whether children who have these variants will develop bronchial asthma or not; it seems though that these children would be good candidates for treatment with anti-RSV antibody.[108]

SUMMARY

Recurrent and severe respiratory virus infection in infancy is a risk factor for, and may predispose to, the development of asthma later in life. Recurrent and severe respiratory virus infections may result in epithelial cell injury. Injured and stressed epithelial cells trigger the process of airway remodeling through activation of an epithelial-mesenchymal tropic unit (Fig. 1). Histopathological studies have revealed that bronchial epithelial cell damage and the remodeling process occur early in the course of childhood asthma.[109] Respiratory virus infection might be a major cause of airway inflammation and remodeling which could occur early in childhood, although further study should be conducted to clarify the precise mechanism of how respiratory virus infections contribute to the production of airway inflammation and remodeling early in childhood.

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