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Cellular network in airways inflammation and remodelling

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Summary Chronic inflammation and airway remodelling are two key steps in asthma pathophysiology. The development of chronic airway inflammation depends upon the continuous recruitment of inflammatory cells from the bloodstream towards the bronchial mucosa and by their subsequent functional activation. The release of inflammatory mediators by activated cells contributes to the generation of a complex network which involves mobile inflammatory cells and structural cells such as epithelial cells, fibroblasts and myofibroblasts. This network is responsible for the amplification and persistence of the inflammatory process as well as for the development of a peculiar microenvironment which can directly modulate the survival of inflammatory cells in the inflamed airways. Increased cellular recruitment and activation, enhanced cell survival and cell:cell interactions are therefore the key steps in the development of chronic airway inflammation in asthma and represent the major causes for tissue damage, repair and remodelling. © 2002 Elsevier Science Ltd

INTRODUCTION

The inflammation of chronic asthma appears to be far more complex than eosinophilic inflammation alone. All airway cells are involved and become activated including T-cells, eosinophils, mast cells, macrophages, epithelial cells, fibroblasts and even bronchial smooth muscle cells. They play an effector role by the release of pro-inflammatory mediators, cytotoxic mediators and cytokines resulting in vascular leakage, hypersecretion of mucus, smooth muscle contraction and epithelial shedding and bronchial hyperresponsiveness. They are also involved in the regulation of airway inflammation and initiation of the process of remodelling by the release of cytokines and growth factors. This review will focus on (i) the acute and (ii) the chronic inflammation of asthma and (iii) the remodelling process.

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MECHANISMS OF AIRWAY INFLAMMATION

Immunological mechanisms involved in airway inflammation

In many cases, especially in children and young adults, asthma is associated with atopy through IgE-dependent mechanisms. The population risk for atopy as contributing to the asthma phenotype has been estimated at ~40% in both children and adults.[1]

The immune system can be separated into antibody-mediated and cell-mediated processes. B lymphocytes produce and secrete specific antibodies, whereas T lymphocytes, in addition to controlling B lymphocyte

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function, have proinflammatory actions through cytotoxic activity ($CD8^+$) and the secretion of cytokines. A pivotal step in the generation of an immune response is the activation of T lymphocytes by antigen presented by accessory cells involving the major histocompatibility complex (MHC class II molecules).

Dendritic cells (DCs) are the primary antigen presenting cell (APC) in the airways. They form an extensive network of interdigitating cells located beneath the airway epithelium. From there they migrate to local lymphoid collections under the influence of granulocyte macrophage colony stimulating factor (GM-CSF), a cytokine released from activated epithelial cells, myofibroblasts, T cells and mast cells. Following antigen uptake, DCs move into lymphocyte-enriched regions where, under the influence of additional cytokines, they mature into effective antigen presenting cells.[2] Dendritic cells can drive the polarisation of naiive T cells (Th_0) towards the Th_2 cells, which secrete cytokines, encoded in a cluster on chromosome $5q_{31-33}$ — the IL-4 cluster. This process directs the immune response in asthma. Dendritic cells increase with asthma severity and decrease with corticosteroid therapy.[3]

APCs and allergen-specific T lymphocytes are responsible for the switching of B lymphocyte synthesis from IgG and IgM to allergen-specific IgE. This process engages CD40 and CD40-ligand on B and T lymphocytes, respectively and is mediated by interleukin (IL-)4 and IL-13.

In addition to IgE involvement, after antigen presentation Th_2-like lymphocytes release cytokines causing accumulation and activation of eosinophils. Cytokine production requires a second signal provided by the engagement of CD80 or CD86 on APCs and CD28 on T cells. Under certain circumstances, a second adhesion molecule CTLA-4 may be induced on APCs which, on engaging with CD28, results in loss of T cell immunocompetency (anergy) or in programmed cell death (apoptosis).[4]

Once the airways are sensitised, re-exposure to the same antigen causes further activation of specific T lymphocytes. These have the capacity to stimulate IgE production and to attract and activate other leukocytes. Through the latter mechanism, T cells may directly increase the growth, activation and survival of mast cells. In this sense, activated T lymphocytes are proinflammatory cells in their own right by releasing cytokines of the IL-4 gene cluster, GM-CSF, IL-3, IL-4, IL-5, IL-9 and IL-13.[5] These have pronounced effects on inflammatory cells, including eosinophils which dominate the airway inflammatory infiltrate in asthma.[6]

Activated T cells initiate and propagate inflammation of the airways and, therefore, participate directly in the pathological events responsible for exacerbations of asthma.[6] In mice and humans, at least two distinct T-helper (Th), $CD4^+$ lymphocyte subtypes have been characterised on the basis of their cytokine production profile.[7] Although both T lymphocyte subtypes secrete IL-3 and GM-CSF, the Th_1 subtype preferentially produces IL-2. IL-2 stimulates T lymphocyte proliferation and interferon-gamma (IFN-gamma), which inhibit B lymphocyte activation and IgE synthesis by B lymphocytes and tumour necrosis factor beta (TNF-beta).[7] These cytokines are responsible for the development of the classic delayed-type or cell mediated hypersensitivity reaction.

The Th_2 subtype preferentially secretes IL-4, IL-5, IL-9, and IL-13. Through the specific actions of these cytokines on B lymphocytes, mast cells, and eosinophils, Th_2 lymphocytes may lead to the characteristic inflammatory response of asthma.[7] In this way, Th_2 lymphocytes are believed to be responsible for immediate-type (type 1) hypersensitivity reactions associated with allergic diseases and asthma.[7]

Unlike $CD4^+$ T cells, most $CD8^+$ cytotoxic T lymphocytes recognise endogenous antigens, usually presented in the context of MHC class I molecules. This type of cell-mediated immunity is involved mainly in the response to intracellular infectious agents including viruses and tumours. There is some evidence that these cells may also be involved in the inflammatory response in some forms of late onset asthma, including isocyanate sensitivity. T lymphocytes are capable of modulating eosinophil adherence, locomotion, and activation and of stimulating these cells to cause tissue damage. An increased number of activated $CD25^+$ (IL-2 receptor expressing) T lymphocytes, activated eosinophils and mast cells have been observed in patients with asthma. The presence of activated lymphocytes and eosinophils in bronchial biopsies of atopic and non-atopic patients with asthma suggests a T-lymphocyte–eosinophil interaction is important, a hypothesis further supported by the finding of cells expressing IL-5 in bronchial biopsies of atopic patients with asthma. IL-5 is an important regulating cytokine for eosinophils and its level of expression in the airway mucosa of patients with asthma correlates with markers of T lymphocyte and eosinophil activation.[6]

Intrinsic non-allergic asthma

Intrinsic asthmatics have negative skin tests and no clinical or family history of atopy. Serum total IgE levels frequently fall within the normal range and there is no evidence of specific IgE antibodies directed against common allergens. These patients are usually older than their allergic counterparts, often with a more severe clinical course which follows a history of a respiratory virus infection. There is a preponderance of females and the association of nasal polyps and aspirin sensitivity. The Swiss SAPALDIA survey of 8357 adults, aged 18–60 years revealed that a third of all asthmatics could be categorised as non-allergic.

Ever since the first description of intrinsic asthma, there has been debate about its relationship to atopy. One suggestion is that intrinsic asthma represents a form of autoimmunity, or autoallergy, triggered by infection (because a respiratory illness often precedes its onset). Some have suggested that intrinsic asthmatics are allergic to an as yet undetected allergen although, in the absence of IgE, this seems unlikely. It has also been suggested that even if intrinsic asthma has a different clinical profile it does not appear to be a distinct immunopathological entity in showing a Th_2 cytokine profile with accompanying inflammatory cells.[8] A small proportion of non-atopic asthma may have its origins in the workplace following unrecognised non-IgE sensitisation to reactive chemicals.

THE CELLULAR NETWORK IN ASTHMA

Constitutive cells of airway inflammation

In asthma, normal resident cells of the airways generate an array of cytokines which may contribute to the chronicity of airway inflammation characteristic of human asthma, as opposed to allergen-sensitised animal models. Epithelial cells, fibroblasts, myofibroblasts and smooth muscle cells play an important role in airway inflammation asthma. Prominent among these are bronchial epithelial cells which play a sentinel role in supporting airway inflammation and remodelling in asthma.[9]

Epithelial cells in asthma express several membrane markers, including adhesion molecules and release a wide spectrum of molecules participating in airway repair (including autacoid mediators fibronectin, growth factors, cytokines and chemokines).[9,10]

Similarly, fibroblasts, myofibroblasts, smooth muscle and vascular endothelial cells are important sources of cytokines and growth factors capable of supporting Th_2 mediated inflammation. Fibroblasts play a key role in the remodelling and inflammatory processes. They produce collagen, reticular and elastic fibres as well as proteoglycans and glycoproteins of the amorphous extracellular matrix (ECM).[11] Their biological activity is regulated by a range of cytokines and growth factors. Although they are regarded as fixed cells of ECM, they retain the capacity for growth and regeneration and may evolve into various cell types, including smooth muscle cells, to become myofibroblasts.

Myofibroblasts contribute to tissue remodelling by releasing ECM components such as interstitial collagens Types I, III, V, fibronectin and laminin and producing growth factors for blood vessels (ET-1, VEGF), nerves (NGF) and smooth muscle (TGF-β, PDGF). Increased numbers of myofibroblasts are found in asthmatic airways and their number has been correlated with the thickness of the basement reticular membrane.[12] Following bronchial allergen challenge, myofibroblast numbers in the submucosa increase, possibly by migrating from deeper in the airway wall. It appears that the ability of myofibroblasts to promote tissue remodelling is influenced by the degree of activation and damage of bronchial epithelial cells releasing profibrogenic growth factors, such as basic fibroblast growth factor, platelet-derived growth factor, insulin growth factor (IGF-1), TGFβ2 and endothelin-1.[9,10] While there is clear evidence for impaired epithelial proliferative responses in asthma and increased expression of inhibitors of cell cycling, the precise molecular mechanisms have not yet been elucidated.

Smooth muscle cells have been implicated as *in vitro* inflammatory cytokines in asthma.[13] In addition to contractile responses and mitogenesis, airway smooth muscle cells have synthetic and secretory potential with the release of RANTES. They may participate in chronic airway inflammation by interacting with both Th_1- and Th_2-derived cytokines to modulate chemoattractant activity for eosinophils, activated T lymphocytes and monocytes/macrophages. Smooth muscle cells also have the potential to alter the composition of the ECM environment and orchestrate key events in the process of chronic airway remodelling.[14] What is not known is whether these events occur in the fully established asthmatic airway since observations have relied on *in vitro* studies involving culture of cells that may present precursors which are pleiotrophic.

Inflammatory cells

Eosinophils

In chronic asthma, eosinophils are found in increased numbers[15] usually located beneath the basement membrane. They are in an activated state. Most asthmatics, including those with mild asthma, have eosinophils in their bronchi and there is an association between their activation, the severity of the asthma and the degree of bronchial hyperresponsiveness.

Eosinophils possess a wide array of biological properties due to their release of toxic granule proteins, oxygen free radicals, eicosanoids (sulphido-peptide leukotrienes), PAF, Th_2 cytokines and growth factors. Activated eosinophils may initiate contraction of human bronchial smooth muscle, increase vascular permeability and induce airway hyperresponsiveness. However, recent observations show that anti-IL-5, which blocks monoclonal antibodies, when administered for up to 16–20 weeks reduces blood and sputum eosinophils to almost undetectable levels but has no effect on the early and late asthma reactions (EAR/LAR), on BHR or on clinical outcome measures. These findings question the role of the eosinophil as a pro-inflammatory cell in asthma, especially when similar reductions in eosinophil count are

produced by exogenous IL-12 or IFN-γ without any evidence of physiological or clinical benefit.[16]

Mast cells

Mast cells are found in the bronchi of normal subjects and asthmatics.[17] They are often shown to be degranulated in the airways of asthmatics both in the stable phase of the disease and, to a greater extent, following allergen challenge.[18] In addition to releasing autacoid mediators, airway mast cells are an important source of neutral proteases, especially tryptase.

Neutrophils

Neutrophils can release a wide variety of enzymes including ECM degrading proteases and elastase, oxygen free radicals and cytokines such as IL-1, TNF-α and IL-6. Neutrophils are increased in the airways of patients with chronic and severe asthma during respiratory virus exacerbations or after exposure to air pollutants[19] suggesting they might participate in airway inflammatory and remodelling responses.

Macrophages

Airway macrophages have the potential to secrete a wide variety of products many of which play a major role in injury and repair.[20] Tissue macrophages can synthesise and secrete plasminogen activator and a group of metalloproteinases having the capacity to degrade various extracellular matrix macromolecules including elastin.[21] Macrophages may also be involved in the regulation of airway remodelling through the secretion of growth factors such as PDGF, basic fibroblast growth factor (b-FGF) or TGFβ.[22]

FROM CHRONIC INFLAMMATION TO AIRWAY REMODELLING

Remodelling is defined in the *Concise Oxford Dictionary* as 'model again or differently, reconstruct.' This is a critical aspect of wound repair in all organs and represents a dynamic process which associates matrix production and degradation with reaction to an inflammatory insult,[23] leading to a normal reconstruction process (model again) or a pathological one (model differently). Structural remodelling in airway diseases was initially proposed to describe changes induced in endothelial cells and extracellular matrix by injury to the pulmonary circulation. It was then extended to many other pathological situations including asthma.[24]

The links between allergen exposure and remodelling are not yet resolved. The airways are continuously exposed to several triggers including allergens, pollutants, tobacco smoke, viruses and bacteria. These agents are able to stimulate immune and inflammatory responses in the airways which can be sustained by the release of chemokines and Th_2-like cytokines.

Pathological characteristics of airway remodelling

Airway remodelling is heterogeneous, leading to changes in connective tissue deposition and to permanently altered airway structure through a dynamic process of migration, differentiation and maturation involving structural cells.[25] Several features of airway remodelling can be seen in asthma:

The basal lamina is of normal thickness but thickening of the lamina reticularis is a characteristic feature, occurring early in the disease process. It consists of a plexiform deposition of interstitial collagens I, III and V and fibronectin. These proteins are produced by activated myofibroblasts derived from the attenuated fibroblast sheath beneath the epithelium.[12] The combination of epithelial damage, reduced epithelial repair, over-production of profibrotic growth factors (e.g. TGF-β) and proliferation and differentiation of fibroblasts into myofibroblasts is thought to form the pathogenesis of the thickened lamina reticularis.

Changes in the homeostasis of the ECM may play a role in the pathogenesis of airway remodelling. The extracellular matrix is a dynamic structure and a balance between synthesis and degradation is required for the maintenance of its homeostasis. Metalloproteases selectively degrade ECM components. They have been implicated in angiogenesis and in smooth muscle hyperplasia. They also play a role in the trafficking of inflammatory and structural cells. Metalloproteinase (MMP)-9 is the major MMP expressed in bronchoalveolar lavage (BAL) fluid and bronchial biopsies in asthma. An overproduction of TIMP-1 is characteristic of patients with stable asthma[26] while an excess of MMP-9 is found in patients with uncontrolled asthma or during an asthma exacerbation.[27]

While the inflammatory process results from a complex interaction between various cell types, components of the ECM also interact with inflammatory cells. Among their many effects, including formation of a reservoir for cytokines and growth factors, proteoglycans and glycosaminoglycans serve as traps for water. They cause resistant swelling and as ligands for cell adhesion molecules on inflammatory cells, including integrins or homing receptors can influence cell trafficking.[28] In addition, metalloproteases (MMPs) selectively degrade ECM components and have been implicated in angiogenesis and in smooth muscle hyperplasia by cleaving growth factors such as IGF. MMPs also play a crucial role in the trafficking of inflammatory and structural cells.

Hypertrophy and hyperplasia of airway smooth muscle, goblet cells and submucous glands[29] have been all reported in asthmatic bronchi, especially in chronic and severe disease. These changes are driven by growth factor released from the abnormal epithelium as well as from infiltrating inflammatory cells.

The airways display various structural alterations that can contribute to an increase in airway wall thickness and to a residual airway obstruction which may even be detected in asymptomatic patients. The effect on airflow is compounded by the presence of increased mucus secretion, an inflammatory exudate and reduced surfactant which adds to those factors favouring airway closure.

AIRWAY INFLAMMATION AND REMODELLING AND NATURAL HISTORY OF ASTHMA

Although there are no longitudinal studies starting in infancy, it is thought that such a decline may start at a very early stage in the natural history of the disease. It is also conceivable that lung function decline is due to the underlying inflammation and remodelling of the airways. However, our knowledge on the natural history of lung growth and senescence in individuals with asthma is still poor. Lung growth appears to be relatively normal in most children with asthma but is reduced throughout childhood and adolescence in those with severe and persistent symptoms.[30] It is not known if this reflects a failure to reach full growth in relation to bronchoconstriction or whether the lungs are congenitally small.[31] Approximately 50% of children with asthma still have respiratory symptoms in adult life.[32]

Lung immunopathology in children

Most information about the underlying inflammatory process in children comes from bronchial biopsy studies in children in remission[33] or with chronic cough for 3 months and with lower respiratory illness.[34] In the first study,[33] the histopathological and ultrastructural features were compared with lung biopsies from age-matched children who died from asthma attacks. The typical findings in the latter were the high prevalence of peribronchial eosinophils and focal denudation of the mucosa. The histological examination in both studies shows the same macroscopic changes with mucus plugging, thickening of the basement membrane and peribronchial smooth-muscle hypertrophy. The microscopic picture shows characteristic massive infiltration of eosinophils and mast cells in different stages of degranulation. Moreover, the luminal surfaces show goblet cell hyperplasia and ciliary abnormality. The second study[34] used bronchial biopsies to confirm the decreased numbers of ciliated cells which were inversely correlated with the degree of inflammation. This was supported by spreading oedema and an increased area of intercellular spaces with high numbers of inflammatory cells. Longitudinal studies in children who develop asthma show that airway remodelling develops early.[7,8] Thus, higher numbers of activated (EG_2+ cells) eosinophils in the lamina propria and a greater thickness of subepithelial lamina reticularis was found in those who subsequently had asthma. These findings suggest airway remodelling, such as the lamina propria thickness, is already established before the first symptoms and it seems to evolve independently of eosinophilic inflammation into an irreversible component of airflow limitation. There was no correlation between the duration of the symptoms before the bronchial biopsy and the histological changes. If it is accepted that inflammation underlies the disease, a new strategy of early intervention needs to be established to modify the natural course of the disease.

Immunopathology in infants

Complex interplay between genetics and environment plays a central role in asthma development. A genetic susceptibility in a patient with asthma is related to alterations in the expression of specific genes which contribute to phenotypic and functional changes of epithelial cells. The abnormal expression of these proteins, which are important during embryogenesis, may play a role in the initiation of the disease. Potential early life influences on subsequent disease have been derived from the knowledge of growth and structural development of lung. During early phases of gestation (53 days), the airway smooth muscle completely covers the branching epithelial tubules and an abundant neural plexus covers the smooth muscle. The rapid rate of formation of new alveoli during the fetal period continues throughout the first 2 years of life. The inappropriate development of the respiratory tract or changes in airway epithelial phenotype, possibly resulting from altered gene expression, may be associated with abnormal function and disease. In particular, it is likely that alteration of the injury–repair cycle of bronchial epithelial cells may lead to the persistent activation of those specific clusters of genes encoding for molecules involved in wound healing processes. In this regard, it is important to note that wound healing is characterised by the production of fetally important components (Ln subchains α_2 and $\beta 2$, tenascin, bombesin-like peptides) and growth factors whose expression and down-regulation occurs in a spatial–sequential fashion. The loss of such dynamic regulation of gene expression and silencing during early life may interfere with the development of the repair process within the airways and contribute to chronic production of ECM proteins and of growth factors characterising airway inflammation and remodelling in asthma.

CONCLUSION

Studies of airways in chronic asthmatics using bronchoscopic methods and induced sputum have provided much data. Within the past 20 years this has led to a better understanding of the mechanisms of inflammation and pathogenesis of asthma. Experiments such as bronchial challenge provide valuable insights into the allergic inflammatory response but we still do not understand how this may lead to a remodelling process. The recent availability of genetically altered animals lacking genes for selected cytokines and growth factors, so-called 'knock-out' mice, and those whose genes have been enhanced, so-called 'knock-in' mice, may prove useful in the elucidation of the role of individual factors to the overall inflammatory cascade and the remodelling process.

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