News in the pathophysiology of asthma

Novine u patofiziologiji astme

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Introduction

Asthma is a complex disorder that displays heterogeneity and variability in its clinical expression both acutely and chronically. This heterogeneity is influenced by multiple factors including age, sex, socioeconomic status, race and/or ethnicity, and gene by environment interactions. Understanding the immunopathology of airways in asthma has been markedly advanced with the use of bronchoscopy and biopsy. Airway samples can then be analyzed by using histologic and immunologic methods, and identified features can be evaluated in relationship to clinical features of asthma to more fully understand the contribution of cellular and molecular events to the resulting physiology and response to treatment. It is helpful to arbitrarily consider asthma in terms of the traditional T-helper2 (Th2) inflammatory processes.

In the acute inflammatory aspects of asthma, allergen-IgE–directed processes are predominant features of airway pathology. Mast cells, Th2 lymphocytes and eosinophils are the predominant histologic features. The cytokine network associated with these processes includes IL-3, IL-4, IL-5, IL-9 and IL-13. Mast cells are important contributors to the initiation of asthma with release of acute-phase mediators, including cysteinyl leukotrienes, and also inflammatory cytokines, which serve to perpetuate inflammatory events in the airway. Subpopulations of lymphocytes polarized toward a Th2 profile further sustains the inflammatory process by the release of cytokines, including IL-4, IL-5 and IL-13. These factors serve to drive inflammation (e.g., recruitment of eosinophils) and also regulate IgE production.

Eosinophils are a characteristic feature of allergic inflammation. Eosinophils that are recruited to the airway in asthmatic subjects by the families of cytokines and chemokines [e.g., IL-5, Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) and eotaxin] undergo cell activation through processes not fully identified and release highly inflammatory mediators.

Recent years have been marked by rapid progress in understanding cellular and chemical mechanisms in the pathogenesis of asthma and other allergic disorders. Studies published in the Journal of Allergy and Clinical Immunology described advances in our knowledge of signaling molecules and pathways, cytokines and activation and tolerance in asthma and murine models of this disease. Additional studies provided novel information about the induction and regulation of allergic inflammation and the genetic determinants of asthma and responsiveness to asthma therapy.

The news in asthma genetics

Recent articles explored novel variants in candidate genes potentially involved in asthma and in the regulation of glucocorticoid responsiveness. Several studies have suggested that chromosome 19q13.1–3 contains asthma susceptibility genes. The microsatellite analyses provided tentative support for an asthma/lung function susceptibility locus, and fine mapping localized modest association to the plasma urokinase plasminogen activator receptor gene (PLAUR, also known as urokinase receptor or CD87). PLAUR SNPs in the 5' region, intron 3, and the 3' region were found to be associated with asthma and bronchial hyperresponsiveness. The same 5' region and 3' region SNPs were found to be determinants of forced expiratory volume in 1 second (FEV1) decrease in subjects with asthma. This is the first report to identify PLAUR as a potential asthma susceptibility gene. The association of PLAUR with lung function decrease supports hypothesis for PLAUR role in airway remodeling.

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Mediators production by epithelial cells repairing and the epithelial mesenchymal trophic unit activation

The bronchial epithelium is a barrier to the external environment and plays a vital role in protection of the internal milieu of the lung. It functions within the epithelial-mesenchymal trophic unit (EMTU) to control the local microenvironment and help maintain tissue homeostasis. However, in asthma, chronic perturbation of these homeostatic mechanisms leads to alterations in the structure of the airways, termed remodeling. Damage to the epithelium is now recognized to play a key role in driving airway remodeling. Several important mediators of remodeling have been identified, most notably transforming growth factor-β, which is released from damaged/repairing epithelium or in response to inflammatory mediators, such as IL-13. In summary, the cross talk between the epithelium and the underlying mesenchyme appears to be central in driving remodeling responses in asthma. The expression of the asthma susceptibility gene ADAM33 in the EMTU and its involvement with airway remodeling helps place these processes at the center of asthma pathogenesis.

In asthma there is an evidence that epithelial injury and repair are abnormal. Several studies have reported increased susceptibility to injury and abnormal repair responses, including increased expression of the epithelial growth factor receptor (EGFR) in bronchial biopsies from adults and children with asthma, as well as expression of the cyclin-dependent kinase inhibitor p21waf1. More recent studies using differentiated epithelial cultures have confirmed that damage causes release of TGF-β and have shown that co-culture of epithelial cells and fibroblasts results in sustained TGF-β release. In this coculture model, there was also marked synthesis of interstitial collagen, which was deposited in close proximity in basal surface of the epithelium, closely mirroring the thickening of the lamina reticularis seen in asthmatic bronchial biopsies.

Although the epithelium was initially considered to function solely as a physical barrier, it is now evident that it plays a central role in the Th2-cell sensitization process due to its ability to activate mucosal dendritic cells. Cytokines are inevitable factors in driving immune responses. To the list of numerous cytokines already known to be involved in the regulation of allergic reactions, new cytokines were added, such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. IgE is also a central player in the allergic response. The activity of IgE is associated with a network of proteins, especially with its high- and low-affinity receptors for immunoglobulins (Fc receptors).

Mucosal dendritic cells (DCs) are extremely efficient sentinels in the defense against antigen challenge. They are strategically positioned within the epithelium in the basolateral space, separated from the inhaled air only by the epithelium tight junction barrier. Despite the fact that most inhaled antigens are transported to the lymph nodes by DCs, the usual outcome following the inhalation of harmless protein antigens is the induction of tolerance. This is because they cannot fully activate DCs to induce an effective T-cell response. It follows that DCs have to be somehow activated to break tolerance. Conventional DCs express numerous pattern-recognition receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain and C-type lectin receptors.

As most inhaled allergens, such as those derived from cockroaches and house-dust mites, are contaminated with lipopolysaccharides (LPSs) and peptidoglycans, they can activate DCs. In fact, it was shown that the main house dust mite allergen, Der p 2, acted as a functional homologue of myeloid differentiation factor 2 (MyD2) that drove airway inflammation in a TLR4-dependent manner.

MyD2 physically associates with the extracellular domain of human TLR4 and binds the lipid A region of LPS without the need for LPS-binding protein. Given that many allergens, including Der p 2, are members of the MyD2-like lipid-binding protein family and that more than 50% of major allergens are lipid-binding proteins, such mimicry could also explain the immunogenicity of these allergens.

In the absence of contaminating TLR ligands, some allergens can activate DCs by triggering protease-activated receptors (PARs). The ligation of TLRs and PARs leads to a cascade of events that culminates in the production of chemokines that attract neutrophils, monocytes, and DCs to the airways and to the production of cytokines that can induce DC maturation and Th2 polarization. Thymic stromal lymphopoietin (TSLP), granulocytemonocyte colony-stimulating factor (GM-CSF), and interleukin (IL)-25 are among the most important mediators.

Mast cells have a key role in asthma. They are concentrated in the mucosal tissues and are recruited to the surface of the airways by stem-cell factor released from epithelial cells. In addition, CXCL8 and CXCL10, (chemokine ligands) produced by airway smooth muscle cells, are important in the recruitment of mast cells by interacting with their receptors, CXCR2 and CXCR3, respectively. Moreover, these chemokines also prime mast cells for enhanced mediator secretion. Reversely, mast cells secrete CCL19 which, through its CCR7, stimulates airway smooth muscle cell migration and contributes to smooth muscle hyperplasia.

The cross-linking of IgE-FcεRI complexes on mast cell surfaces by allergens leads, within minutes, to the so-called “early phase” of the allergic reaction, which involves their degranulation and release of histamine, tryptase and other proteases, heparin and some cytokines, which are preformed and stored in granules, as well as newly formed eicosanoids (LTC4, LTD4, LTE4, PG2, and TXA2). These mediators are potent smooth muscle contractile agents and also increase microvascular permeability. Both PGD2 and LTD4 interact with cell-surface receptors on eosinophils, macrophages, basophils, and mast cells, where they serve as chemoattractant as well as priming agents. Cytokines and chemokines liberated in this early phase initiate the “late phase”, which peaks some hours later. The inflammation that occurs in asthma is often described as eosinophilic.

Eosinophils are a rich source of granule basic proteins, such as major basic protein, eosinophil peroxidase, and eosinophil cationic protein, and also have the capacity to generate eicosanoids such as prostacyclin (PGI2) and leukotrienes. They also release potentially tissue damaging superoxide and a range of cytokines and chemokines. Eosinophil-derived neurotoxin is released by eosinophils as well. It was recently shown that it had the capacity to activate Th2-polarizing DCs by triggering the TLR2-MyD88 signaling pathway and to enhance the Th2-based immune response.

**Cytokines network**

Cytokines play a key role in orchestrating the chronic inflammation of asthma and chronic obstructive pulmonary disease (COPD) by recruiting, activating, and promoting the survival of multiple inflammatory cells in the respiratory tract. Over 50 cytokines have now been identified in asthma and COPD, but their role in the pathophysiology of these complex airway diseases is often unclear. For the purpose of this review, cytokines are classified into lymphokines (cytokines that are secreted by T cells and regulate immune responses), proinflammatory cytokines (cytokines that amplify and perpetuate the inflammatory process), growth factors (cytokines that promote cell survival and result in structural changes in the airways), chemokines (cytokines that are chemotactic for inflammatory cells) and antimicrobial cytokines (cytokines that negatively modulate the inflammatory response), although many of these functions may overlap.

**T-helper 2 (Th2) cytokines.** In patients with asthma, there is an increase in the number of CD4+ Th cells in the airways, which are predominantly of the Th2 subtype. Th2 cells are characterized by secretion of IL-4, IL-5, IL-9, and IL-13. The transcription factor GATA3 is crucial for the differentiation of uncommitted naive T-cells into Th2 cells and regulates the secretion of Th2 cytokines. There is an increase in the number of GATA3+ T cells in the airways of stable asthmatic subjects. Following ligation of the TCR and CD28 coreceptor by antigen presenting cells (APCs), GATA3 is phosphorylated and activated by p38 MAPK, resulting in translocation from the cytoplasm to the nucleus, where it activates transcription of genes characteristic of Th2 cells. Nuclear factor of activated T cells (NFAT) is a T cell-specific transcription factor and enhances the transcriptional activation of the IL4 promoter by GATA3. Finally, IL-33, a member of the IL-1 family of cytokines, promotes differentiation of Th2 cells by translocating to the nucleus and regulating transcription through an effect on chromatin structure, but it also acts as a selective chemoattractant of Th2 cells.

**T-helper 1 (Th1) and Tc1 cytokines.** The transcription factor T-bet is crucial for Th1 cell differentiation and secretion of the Th1-type cytokine IFN-gama. Consistent with the prominent role of Th2 cells in asthma, T-bet expression is reduced in T cells from the airways of asthmatic patients compared with airway T cells from nonasthmatic patients. After phosphorylation, T-bet associates with and inhibits the function of GATA3 by preventing it from binding to its DNA target sequences. In turn, GATA3 inhibits the production of Th1-type cytokines by inhibiting STAT4, the main transcription factor activated by the T-bet inducing cytokine IL-12. Th1 cells are the prominent CD4+ T cells, and Tc1 cells the predominant CD8+ T cells expressed in COPD lungs, but their role in the pathogenesis of COPD is not yet certain.

**Interleukin-12 (IL-12) and related cytokines.** They play an important role in differentiating and activating Th1 cells and is produced by activated macrophages, DCs, and airway epithelial cells. IL-12 induces T cells to release IFN-gama, which regulates the expression of IL-12 and so maintains the differentiation of Th1 cells, whereas IL-4 suppresses IL-12B expression and thus antagonizes Th1 cell differentiation.

**Th17 cytokines.** Th17 cells are a subset of CD4+ T cells that play an important role in inflammatory diseases and are regulated by the transcription factor retinoic acid orphan receptor gamma t (ROR gamma t). IL-6, IL-1B, TGF-B, and IL-23 are all involved in the differentiation of human Th17 cells. Th17 cells are increased in the sputum of individuals with asthma and Th17 cells are increased in the airways of asthmatic subjects. More work is needed to understand the role and regulation of Th17 cells in asthma and COPD, as they may provide important new targets for future therapy.

**The role of proinflammatory cytokines in asthma and chronic obstructive pulmonary disease**

Proinflammatory cytokines, such as TNF-alpha, IL-1B, and IL-6, are found in increased amounts in the sputum and bronchoalveolar lavage (BAL) fluid in individuals with asthma and COPD and amplify inflammation, in part through the activation of NF-kB, which leads to the increased expression of multiple inflammatory genes.

Many cells have the capacity to secrete TNF-alpha, including macrophages, mast cells, T-cells, epithelial cells, and airway smooth muscle cells. TNF-alpha is expressed in various cells in asthmatic airways, particularly mast cells, and may play a key role in amplifying asthmatic inflammation through the activation of NF-kB.

IL-6 often works in concert with other cytokines and provides a link between innate and acquired immunity. IL-6 is found in increased amounts in induced sputum of asthmatic patients after mast cell activation. It may play a role in the expansion of Th2 and Th17 cells and therefore have a proinflammatory effect in asthma.

Thymic stromal lymphopoietin (TSLP) is a cytokine belonging to the IL-7 family that shows a marked increase in expression in airway epithelium and mast cells of asthmatic patients.

**Growth factors**

Several cytokines implicated in airway inflammation either promote the differentiation and survival of inflamma...
tory cells or result in proliferation and/or activation of structural cells, contributing to airway remodeling granulocyte-monocyte colony-stimulating factor (GM-CSF). GM-CSF plays a role in the differentiation and survival of neutrophils, eosinophils, and macrophages and has been implicated in asthma and COPD.

It is the ligand of the c-Kit tyrosine kinase receptor SCF, which is expressed by several structural and inflammatory cells in the airways. SCF is produced by epithelial cells, airway smooth muscle cells, endothelial cells, fibroblasts, mast cells, and eosinophils. It is a critical growth factor for mast cells and promotes their generation from CD34+ progenitors.

**Neurotrophins.** They are cytokines that play an important role in the function, proliferation, and survival of autonomic nerves. In sensory nerves, neurotrophins increase responsiveness and expression of tachykinins. Nerve growth factor (NGF) may be produced by mast cells, lymphocytes, macrophages, and eosinophils as well as structural cells, such as epithelial cells, fibroblasts, and airway smooth muscle cells.

Chemokines play an important role in the recruitment of inflammatory cells from the circulation to the airways in both asthma and COPD.

**Anti-inflammatory cytokines**

Although most cytokines increase or orchestrate the inflammation process in asthma and COPD, some cytokines have inhibitory or anti-inflammatory effects. As discussed above, IL-12, through the release of IFN-γ from Th1 cells, can suppress Th2 cytokine release and allergic inflammation.

**Conclusion**

Bronchial asthma was once considered a purely allergic disorder dominated by Th2 cells, IgE, mast cells, eosinophils, macrophages and cytokines. However, it is now clear that the disease also involves local epithelial, mesenchymal and vascular events that are involved in directing allergic reactions to the lung which eventually result in remodeling of the bronchial wall. Better understanding of genetics, environmental factors, and immunopathogenesis of asthma can lead to improved therapeutic approaches.

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