

● Asthma and Mucous Cell Hyperplasia

Asthma is characterized by episodic, variable airflow obstruction and increased responsiveness of the airways to a variety of stimuli accompanied by wheezing and sputum production. In biopsied specimens (1,2) and autopsied lungs (3,4), goblet cell hyperplasia and mucous cell metaplasia (MCM) in peripheral airways (5) are consistent pathologic characteristics of bronchial asthma. Impaired ciliary clearance leads to the



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accumulation of mucus and occlusion of airways. Pathologic examination of fatal cases of *status asthmaticus* almost always reveals accumulation of mucus and mucous plugs in airways (4). This contributes to hyper-inflated lungs that fail to collapse on opening of the pleural cavities (6). Thus, the accumulation of mucous secretions plays an important role in the occlusion of airways in asthma.

● Inflammatory Responses Induce Mucous Cell Metaplasia

A cascade of events involving the activation of many cell types including, but not limited to, eosinophils, lymphocytes, and mast cells cause an aberrant immune response resulting in allergic asthma (7). This inflammatory reaction mediates expression of mucin genes, particularly MUC5AC (8,9), synthesis of mucous glycoproteins, and MCM in the bronchiolar regions (10).

The Role of Th1 and Th2 Lymphocytes in MCM: The respiratory tract of rodents normally exhibits very few mucous secretory cells. Many inflammatory mediators that cause MCM were originally defined in mice (13) and were subsequently identified in humans (14). Therefore, the mouse provides a useful model to elucidate the mechanisms of MCM in asthma. In mouse models of asthma, systemic

sensitization to OVA followed by repeated exposure to aerosols generated from OVA solutions induce allergic inflammation and MCM (11,12).

The role of Th1 and Th2 cells in MCM was determined by adoptively transferring cultured Th1 and Th2 cells into syngeneic mice, followed by an allergen challenge (15). A marked increase in MCM was observed in mice that received Th2 cells but not in mice that received Th1 cells. In mice deficient in T-, B-, and mast cells, CD4⁺ T cells are alone sufficient to restore allergen-induced airway hyper-reactivity, allergic inflammation, and MCM (16).

The Role of IL-5 in MCM: Although transgenic mice that overexpress IL-5 in their lungs show accumulation of extensive MCM (17), Th2 transfer experiments with IL-5 (-/-) T cells (18) and MUC5AC promoter activity assays with recombinant IL-5 (19) show that IL-5 does not directly induce mucin biosynthesis and storage.

The Role of IL-4 in MCM: Transgenic mice overexpressing IL-4 in their lungs (20) or mice instilled with IL-4 (21) show MUC5AC gene expression (11) and MCM. However, Th2 transfer experiments (15) and our studies using microdissected airway segments in organ cultures (unpublished observation) show that IL-4 is not crucial for the development of MCM (Fig. 1). Somewhat contradictory to these findings, IL-4 directly induces MUC5 gene expression in normal human bronchial epithelial cells (HBEs)

(21). This apparent contradiction may be explained by the fact that HBEs not only act as target cells, responding to inflammatory mediators, but also can act as effector cells, synthesizing and releasing cytokines (22) to pathologic stimuli.

The Role of IL-13 in MCM: IL-13 confers the asthma-like phenotype and MCM through the IL-4R α chain (23) (24) utilizing the Stat 6-dependent pathway (25) (Fig. 1). IL-13 is sufficient

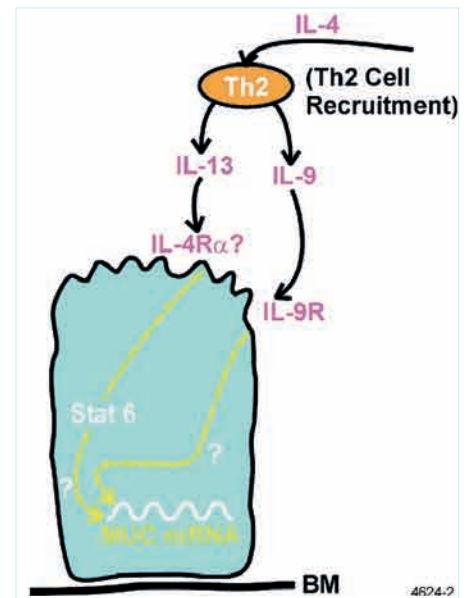


Figure 1: Mediators of mucous cell metaplasia in asthma. Th2 lymphocytes affect airway epithelial cells to induce MCM by secreting IL-9 and IL-13.

for the expression of pathophysiological features of asthma (including MCM) in a manner that is independent of IgE and eosinophils (26,9). Indeed, transgenic mice overexpressing IL-13 in their lungs show extensive MCM (27). The direct effect of IL-13 on epithelial cells to cause MCM was shown in HBEs (28,29) and in mouse bronchial segments. However, IL-13 does not induce MUC5AC in HBEs. (30). Reconstruction of Stat 6 in airway epithelial cells alone in Stat 6-deficient mice is adequate for IL-13-induced MCM (31).

The Role of IL-9 in MCM: Localized overexpression of IL-9 in

the lung also causes massive airway inflammation and MUC2 and MUC5AC gene expression (32) as well as extensive MCM in the airways (33). Evidence for the direct effect of IL-9 on airway epithelial cells was shown by recombinant IL-9 increasing MUC2 and MUC5AC transcription and mucous glycoprotein synthesis in the mouse epidermoid carcinoma cell line, NCI-H292 and HBEs (19,32) (and personal observation). MCM is absent in IL-9-deficient mice in a Th2-driven pulmonary granuloma model (34); however, the development of allergen-induced MCM is not affected in IL-9-deficient mice (35). The presence of high levels of IL-13 may have caused MCM in the allergen challenged mice in the absence of IL-9. Thus, current studies indicate that IL-9 and IL-13 may independently regulate mucin biosynthesis and storage by directly affecting airway epithelial cells (Fig. 1). Furthermore, it is not known whether IL-9 and IL-13 directly induce MUC5AC gene expression or initiate airway cell differentiation in mucous cells, which then express MUC5AC as a signature gene.

● Loss of Metaplastic Mucous Cells in Asthma

IFN γ Reduces Allergen-Induced MCM in Humans and Rodents: Although rodents are useful for the study of mechanisms of MCM development in asthma when challenged with an allergen for a limited time, rodent models of asthma differ from human asthmatics when rodents are repeatedly challenged with allergen for an extended period.

In rodents, chronic exposure to allergen causes initially induced inflammation to decrease over time (36,37); while in humans with allergic asthma, inflammation persists and is chronic during their lifetime (38). The apparent "tolerance" to allergens in rodents has been associated with termination of Th2 responses and the appearance of Th1-like conditions, and is, therefore, termed "immune deviation" (39). One could speculate that humans with allergic asthma are deficient in developing such immune deviation to specific compounds or in IFN γ signaling to reduce MCM.

The state of apparent "tolerance" to prolonged allergen challenge is considered an immune deviation because it is associated with the termination of CD4+ Th2 responses and coincident appearance

of OVA-specific IFN γ -producing CD8+ T cells (40). Apparent tolerance is also linked to T cell receptor-gamma delta+ T cells that produce high levels of IFN γ in response to OVA stimulation (41). A study reporting the reduction of MCM when Th1 cells are adoptively transferred along with Th2 cells (42) supports the involvement of IFN γ in reducing MCM. This dose-dependent inhibitory effect of Th1 cells is abolished when Th1 and Th2 cells are transferred into IFN γ receptor (-/-) mice, indicating that IFN γ receptor signaling is crucial for the inhibitory role of Th1 cells (Fig. 2) (42). Our recent studies show that IFN γ mediates the reduction of allergen-induced MCM by inducing programmed cell death (43).

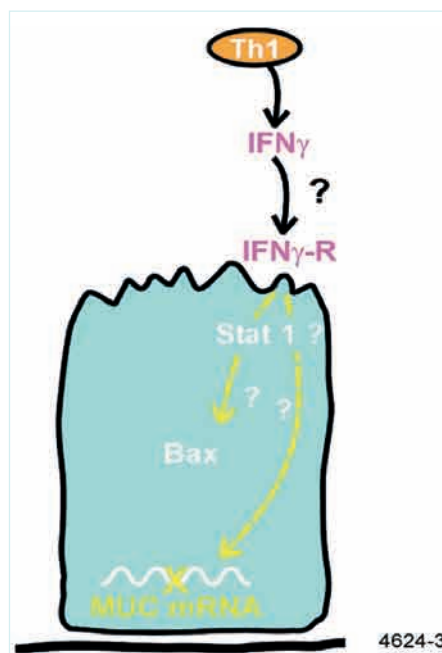


Figure 2: A possible pathway responsible for Bax expression in mucous cells after extended exposure to allergen. IFN γ is involved in inducing Bax expression.

Apoptotic Regulators in MCM: In an attempt to understand mechanisms that reduce the numbers of metaplastic mucous cells after inflammatory responses subside, we discovered that metaplastic mucous cells express regulators of apoptosis from the Bcl-2 family of proteins (44,45). The Bcl-2 family of cytoplasmic proteins can register diverse forms of intracellular damage, gauge whether other cells have provided a positive or negative death stimulus, and determine the progression or inhibition of the suicide program (46). Bcl-2, an inhibitor of apoptosis, is a member of a large group of apoptotic proteins that prevent or induce apoptosis

when overexpressed. "BH3-only" members of the Bcl-2 family respond to death signals and subsequently trigger the inactivation of the anti-apoptotic proteins (Bcl-2 or Bcl-X_L) or activation of pro-apoptotic proteins (Bax or Bak) (47). This event leads to mitochondrial membrane premeabilization, the release of cytochrome c, and the cascade of activation of caspases and DNases that are responsible for the appearance of apoptotic morphology (48). This morphology includes DNA fragmentation, chromatin condensation, membrane blebbing, cell shrinkage, and disassembly of the cell (49).

Bcl-2 is expressed in metaplastic mucous cells induced by ozone exposure in rat nasal epithelia (44) or in airway epithelia by exposure of rats to endotoxin or allergen (45). Several lines of evidence suggest that Bcl-2 is induced by inflammatory mediators and directly link its expression to the sustenance of metaplastic mucous cells. Our findings demonstrate that apoptosis plays a role in regulating MCM.

Repeated exposure of mice to allergen for a limited time is not accompanied by expression of Bax. However, exposure to allergen for prolonged periods or instillation with IFN γ causes the pro-apoptotic Bax to be expressed in metaplastic mucous cells and MCM to decrease (50). In addition, IFN γ causes Bax expression in IL-13-induced MCM in microdissected airway cultures. Instillation of IFN γ into rats that have extensive MCM also causes Bax expression in metaplastic mucous cells.

Our hypothesis is that Th2 cytokines in the lungs of asthmatics induce mucin biosynthesis and inhibit the role of Bax to induce apoptosis, while IFN γ in non-asthmatics sustains and induces Bax expression, thereby controlling the development and resolution of MCM.

IFN γ has been shown to induce apoptosis in a variety of cell types, including colon adenocarcinoma cells (51), A549 lung epithelial cells (52,53), primary human keratinocytes (54), HeLa cells (55), breast tumor cells (56), and fibroblasts (57). Although IFN γ -enhanced signaling proteins that are responsible for cell death remain largely unknown (58), various pathways appear to be involved in IFN γ -induced apoptosis. Our results with HBEs show that exposure to IFN γ induces Bax expression and caspase activation (50). Although IFN γ has induced Fas in keratinocytes (54), we did not observe

induction of Fas in HBEs exposed to IFN γ . In addition, Fas-antibody did not enhance IFN γ -induced cell death in HBEs as was found in keratinocytes. Together, these observations suggest that IFN γ may induce cell death via several different pathways.

In some instances, serum levels of IFN γ can be increased in severe asthma cases (59), and IFN γ levels in bronchoalveolar lavage fluid (BALF) are sometimes found even in mild asthma (60,61). Although these data seem paradoxical, a reasonable hypothesis would explain a failure of IFN γ in these asthma patients to drive the resolution of airway hyperreactivity and MCM. For instance, IFN γ levels may not be high enough to reverse the Bax inhibitory effects of IL-13. Supporting this hypothesis, the instillation of 100 ng IFN γ in our recent studies caused reduction of MCM while 50 ng did not (43). It is also possible that in a subpopulation of asthmatics a deficiency in the IFN γ -signaling pathway may render IFN γ incapable of inducing cell death in epithelial cells. This hypothesis is supported by recent reports that polymorphisms in genes encoding for IFN γ and IFN regulatory factor-1 (IRF-1) confer genetic susceptibility to allergic asthma in Japanese children (62) and that Stat 1 is constitutively activated in epithelial cells of asthmatics (63). These alterations may contribute to deficient signaling of IFN γ and sustained increased levels of MCM even in the presence of IFN γ . Our findings that mice deficient in Stat 1 (an obligatory protein for IFN γ signaling) could not resolve MCM after repeated and prolonged

exposure to allergen (43) support this hypothesis. These animals may represent appropriate models to study chronic inflammatory airway disease that resembles human asthma and to test immunomodulatory therapies to reverse established airway remodeling.

In summary, increased numbers of mucous cells in peripheral airways of asthmatics persist until exposure to allergen triggers airway constriction and mucous secretions causing the formation of mucous plugs. Thus, determining the mechanisms that contribute to the persistence of asthma will be useful for designing new therapies for asthma. The major reward may be to prevent death in asthmatics from severe, unstable disease. Understanding the interactions of IL-13 and IFN γ signaling pathways in the maintenance of metaplastic mucous cells will lead to novel therapeutic targets that may be exploited to reduce MCM by inducing Bax and programmed cell death.

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