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Regulation of Mucous Cell Metaplasia in Bronchial Asthma

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Abstract

Mucous cell metaplasia (MCM), defined by the appearance of mucous cells in airways where mucous cells were not present, is a consistent pathologic characteristic in the peripheral airways of bronchial asthma. Under mild inflammatory conditions MCM occurs as a result of pre-existing airway epithelial cells (AECs) starting to express mucin genes and differentiating into mucous cells. Under extensive inflammatory responses, AECs proliferate, and the development of MCM involves the differentiation of pre-existing and proliferating cells into mucous cells. Epithelial cell numbers per mm basal lamina are increased by approximately 30%. IL-13 is the central cytokine that is responsible for MCM in asthma through GABA-R- and STAT6-mediated mechanisms involving the calcium-activated chloride channel CLCA. IL-13 is also responsible for the proliferation of AECs by causing cells to produce TGFα that acts on the epidermal growth factor (EGF) receptor. Normally, resolution of MCM involves two distinct mechanisms. 1) Some of the metaplastic mucous cells stop the synthesis of mucus and dedifferentiate into Clara or serous cells to reconstitute the epithelium. 2) When proliferation of epithelial cells had occurred, approximately 30% of metaplastic cells are eliminated during the resolution process. Thus, a safe approach to reducing IL-13-induced MCM would involve blocking mucous synthesis and storage, blocking secretion of stored mucus, and eliminating hyperplastic mucous cells. Understanding the molecular mechanisms of each of these processes is necessary for developing effective therapies for reducing mucous hypersecretion in asthma and leading to a repaired epithelium.

Keywords

mucous cell metaplasia; lung pathogenesis; hyperplasia of airway epithelium; programmed cell death; inflammation; Th2 cytokines; allergy and asthma; proliferation and cell cycle

Introduction

Epithelia lining the skin, the digestive and the reproductive tracts, or the airways have similarities because of their role in maintaining a barrier function. However, upon closer inspection, the functions of the various epithelia are vastly different, because they are exposed to different types of environment. The airway epithelium is primarily exposed to the air we breathe, while the epithelia lining the skin or the digestive tract are constantly exposed to UV light or chemicals contained within foods and other items that we ingest, respectively. Therefore, the airway epithelium has developed various mechanisms to sense

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bacterial or viral infectious agents and environmental pollutants that are suspended as aerosols and to mount appropriate actions for protection and cleaning. Contact with these environmental agents elicits increased secretion and synthesis of mucus that traps and clears the insult and can cause the epithelium to elicit an inflammatory response that inactivates infectious agents or clears particles.

The airway epithelial surface is covered by mucus that is secreted by mucus-producing goblet cells and by mucous and serous cells in the submucosal glands. The sticky nature of mucus is suited to trap particles and help remove them out of the tracheobronchial tract with the help of ciliated cells. In humans, the site of most mucous hypersecretion is the large airways. Whether the source of the major part of secreted mucus stems from the surface epithelial goblet cells or the submucosal glands is not clear because of limitations in real-time measurement capabilities of synthesized and secreted mucus at rest or under inflammation conditions. However, detailed morphometric analysis of the amount of sustainable stored secretory product in the tracheobronchial airways of the Rhesus monkey reveals at least twice as much mucus in the surface epithelium as in the submucosal glands [1]. In the lower airways of the Rhesus monkey, as in the majority of other animal species, glands are absent, and surface epithelial mucous cells are the sole source of mucus.

In the lungs of mice and humans, the airways are lined by a mixed population of ciliated and nonciliated epithelial cells. In mice, the nonciliated cells comprise 50–70% of the total epithelial population throughout the entire conducting airway network [2]. In naïve mice, the majority of these nonciliated cells are Clara cells, and there are few or no mucous cells in airways distal to the trachea. In healthy humans, there are few Clara cells in the large conducting airways, but their numbers progressively increase in more distal airways [3]. Normally, mucous cells are absent or sparse in airway less than 2 mm in diameter [4].

Mucous cell metaplasia (MCM) is defined by the appearance of mucous cells in airways where mucous cells were not present [5]. Cells that populate the small airways, primarily Clara cells, can proliferate and start to express mucin genes in response to inflammatory responses. AECs that start to store mucins are defined as mucous cells. Following inflammatory responses, mucin gene expression can be regulated by increased transcription [6], [7], [8] or by increased mRNA stabilization [9], [10]. Increased levels of mucin mRNAs translate into increased levels of mucin apoproteins that are glycosylated and constitute the stored mucosubstances.

MCM and Pathological Conditions in Asthma

A history of sputum production is independently associated with an accelerated rate of decline in forced expiratory volume in one second (FEV_1) [11]. Furthermore, hypersecretion of mucus plays a central role in the pathogenesis of severe airway obstruction and asphyxiation in fatal attacks of asthma [12]. Asthmatic patients who died suddenly and unexpectedly show luminal occlusion by mucous plugs, plasma exudates, and inflammatory cells in the peripheral airways of autopsied lungs [13]. The blockage of the airway is enhanced by the sticky nature of mucus trapping inflammatory cells that release DNA and further increase its viscosity [14]. The majority of the airways of asphyxiated asthmatic

individuals are blocked, not because of airway constriction, but because of widespread, contiguous plugs of a gelatinous substance composed of mucin gene products. However, the mechanisms governing hypersecretion of mucus in asthma are poorly understood. Aikawa et al. [4] were the first to report that in severe asthmatics goblet cell hyperplasia is increased 30–fold when comparing patients with bronchial asthma who died of non-acute asthma attack to patients with bronchial asthma who died of severe acute asthma attack. It is now well established from biopsied specimens [15], and autopsied lungs [12], [13], that mucous cell hyperplasia and MCM in peripheral airways [16] are consistent pathologic characteristics of bronchial asthma.

MCM in Asthma—A Dysregulation of an Innate Protective Response?

MCM is an integral part of the defense system in the airways to help the production of increased levels of mucus to neutralize and remove injurious particles or infections with the help of ciliary action. In rats and mice, MCM can occur as a result of pre-existing Clara or serous cells that begin to express Muc5ac in the absence epithelial cell proliferation [17], [18]. Another study [19] reported that 10% of mucous cells proliferate in conjunction with a compensatory decrease in the number of Clara and ciliated cells. MCM is an integral part of the defense system and helps the necessity of producing increased levels of mucus to neutralize and help remove injurious particles or infection with the help of ciliated cells.

While MCM can arise by non-mucous cells starting to differentiate into a mucin-storing and -secreting cells, an extensive inflammatory response can also cause epithelial cells to proliferate [20], [18]. Epithelial cells undergo proliferation in remodeling airways [21], [22], [23], [24], and the extent of proliferation appears to be dependent on the extent of injury and inflammatory response associated with the length of allergen exposure. Proliferation of epithelial cell is also relevant for human asthma because an increased cellular proliferation in the airway epithelia is found in subjects with severe asthma, as compared with subjects with mild asthma or normal non-diseased control subjects [25].

Under conditions where extensive inflammation occurs the epithelial layer may be disrupted and these responses may initiate the proliferation of epithelial cells to close the injury-induced gap in the epithelium [26], [16], [27], [28]. Therefore, MCM can occur as a result of pre-existing and proliferating cells differentiating into mucous cells [18], [29], [30]. The number of epithelial cells per mm basal lamina increases by approximately 30–40% following repeated allergen exposure over 4–5 d [30]. The increase in epithelial cells essentially constitutes the increase in the number of mucous cells, suggesting that newly formed cells are mucus-secreting cells. Several studies have shown that secretory cells are the cells that proliferate following injury to the epithelium [31], [32], [33], [34], [18], suggesting that AECs capable synthesizing and secreting mucus may also be the first to proliferate and enhance protection to the epithelium. This response may be explained by the effort of the epithelium to produce sufficient mucus for protection from further injury.

IL-13 is necessary for the development of MCM in asthma [35] by activating the IL-13 receptor alpha 1 together with the IL-4 receptor alpha [36]. The role of IL-9 in independently inducing MCM or in conjunction with IL-13 has been debated for a long time

[37], [6], [38]. However, several studies now support the idea that IL-13 either produced by epithelial cells or by Th2 cells is the primary regulator of MCM *in vivo*. The blockade of IL-13 in IL-9-overexpressing mice completely prevents the development of MCM [39]. All other Th cytokines depend on IL-13 to induce MCM and IL-13 acts, not through intermediate inflammatory cells, but on structural cells within the lung, likely the airway epithelium itself [40]. The potency of IL-13 is shown, requiring its complete blockade for a significant reduction in mucus production [41], [42].

Ligation of the IL-13α1/IL-4R receptor complex by IL-13 results in activation of JAK1 and Tyk-2 kinases, and monomers of STAT6 bind through their Src homology 2 domains to phosphorylated tyrosine residues of IL-4Rα. After phosphorylation, STAT6 dimerizes and translocates to the nucleus to activate promoter regions regulated by IL-13. Studies with *STAT6*$^{-/-}$ mice have shown that STAT6 is necessary for IL-13 to induce MCM [43]. The direct effect of IL-13 on epithelial cells was confirmed when reconstitution of STAT6 only in epithelial cells was sufficient for IL-13 to induce MCM in the absence of inflammation [40]. Despite the importance of IL-13 in inducing MCM the precise mechanisms by which it causes MCM remain unclear. However, a recent study found that IL-13 enhances expression of both the γ-aminobutyric acid (GABA) receptor and the GABA synthetic enzyme signaling pathway in the airway epithelium [44], [45]. Blockade of the GABA pathway inhibited the development of IL-13 or allergen-induced MCM. Therefore, it appears that GABA has a role in stimulating the metaplastic transition from the non-secretory cell type to a secretory one. Whether this pathway is independent from the IL-13 receptor and STAT6-mediated pathway remains to be determined but these findings are starting to unravel the complex mechanistic pathways by which IL-13 may be acting to induce MCM.

In murine models of asthma, this MCM is associated with the induction of Muc5ac mRNA expression [8]. In the mouse models, MUC1, MUC2 and 3 mRNA levels are not affected, but IL-13-induced MCM is primarily driven by the expression of Muc5ac. However, the events connecting IL-13R activation to Muc5ac gene expression are incompletely defined, but preliminary work with inhibitors indicates requirements for MEK/ERK, p38MAPK, and PI3K activation in cultured cells [46]. Whether these signaling proteins are mediators for the expression and activation of the calcium-activated chloride channel (CLCA) protein family, which are also key molecules for the development of MCM in asthma is unclear. The murine CLCA3, the mouse homologue of human CLCA1, is induced by IL-13 and suppressing mCLCA3 inhibits the development of MCM in the mouse model of asthma while overexpression of this gene induces the expression of MUC5AC in human airway cell lines [47]. In addition, mCLCA3 gene transfer to mouse airway epithelium was sufficient to induce MCM, and expression of hCLCA1 is significantly upregulated in patients with bronchial asthma compared with control subjects as determined by *in situ* hybridization and immunohistochemical analysis [48]. In light of all this supporting evidence for mCLCA3 to be crucial for the development of IL-13-induced MCM, it was an unexpected surprise that allergic challenge with ovalbumin or intranasal administration of IL-13 produced a robust allergic response in both *mCLCA3*$^{+/+}$ and *mCLCA3*$^{-/-}$ mice [49]. These unexpected findings suggested that other compensatory mechanisms may be present and other CLCA family members may also mediate IL-13-induced MCM [50]. Despite all these studies to unravel the role of the CLCA family, how hCLCA1 fits within the signaling pathway of the

IL-13-induced mucin gene expression is still not understood. However, NF-κB, important in pseudomonas-induced MCM [51] was excluded from being involved in the development of IL-13-induced MCM [42].

IL-13 also directly induces proliferation of normal human airway epithelial cells (NHBE) cells, as reflected by tritiated thymidine uptake and cell counts in differentiated normal human bronchial epithelial cells in culture [52]. The proliferative effects of IL-13 are mediated through the epidermal growth factor receptor (EGFR), as proliferation was attenuated by AG1478, an EGFR tyrosine kinase inhibitor. IL-13 induces release of TGFα from the epithelial cells that, in turn, binds via an autocrine/paracrine-type action to the EGFR, initiating proliferation [52]. However, which pathways and cell cycle regulatory proteins are activated or induced to drive cell proliferation in NHBEs and other AECs is largely unknown. In addition, whether proliferating cells in airway epithelia are always programmed to immediately produce and store mucus or whether the pathways that induce cell proliferation or mucus gene expression are distinct pathways is not well-understood.

Recent studies have started to address whether the proliferative responses may be driven by pathways that are different from those driving mucous cell differentiation. In rats, IL-13-induced MCM required signaling through EGFR, because selective EGFR tyrosine kinase inhibitors prevented IL-13-induced MCM in a dose-dependent manner [53]. In addition, how EGFR signaling is involved in the IL-13 signaling through IL-13 and IL-4Rα was clarified by a mouse model of viral infection that showed ciliated cells proliferate and persistent activation of EGFR signaling prevents apoptosis of ciliated cells, presumably through the PI3/Akt pathway [54]. In that setting, ciliated cells transdifferentiate into goblet cells establishing MCM in the presence of IL-13. Under those conditions, blockage of the EGFR signaling prevents virus-induced increases in ciliated and goblet cells because ciliated cells undergo apoptosis [54]. Inhibition of IL-13 signaling reduces synthesis and storage of mucus and increases the number of ciliated cells, suggesting that it does not affect the cell death of ciliated cells. These findings suggest that EGFR sustains ciliated cell hyperplasia while IL-13 causes the transdifferentiation of ciliated cells to mucous cells. Future studies are needed to define the interaction of these pathways in various model systems representing allergen-induced MCM.

Resolution of MCM

Normally, MCM is transient and spontaneous resolution of MCM has been observed after cessation of allergen exposure. In BALB/c mice, when MCM develops as a result of a single allergen challenge in the absence of cell proliferation, the mucous cell phenotype resolves after 3-4 weeks [17]. Resolution occurs over a course of 10 d when the same mouse strain is repeatedly exposed to allergen to increase MCM and the epithelial cell numbers per mm basal lamina [55]. For C57Bl/6 mice repeatedly exposed to allergen for 5 d, approximately 10-11 d are necessary for the resolution of MCM in the absence of further allergen challenge [56].

Depending on the frequency and concentration of allergen AECs proliferate in C57Bl/6 mice [19]. This defensive response is normally transient and resolved in the absence of further

challenge so that the original epithelium is restored in which none or very few mucous cells remain [56].

When the number of mucous cells/mm basal lamina is subtracted from the total epithelial cell number at various time points during the resolution of epithelial cell hyperplasia the number of mucous cells is reduced, while the number of non-mucous cells remains largely unaffected indicating that the increase in total epithelial cell numbers is entirely composed of hyperplastic mucous cells [29]. The resolution of MCM involves various mechanisms. First, some of the mucous cells appear to transdifferentiate into non-mucus cells. This change must involve reducing mucus synthesis and possibly differentiating into ciliated [54] or serous cells (personal unpublished observation). This process of transdifferentiation could be due to the decline in cytokines and other inflammatory mediators responsible for mucin gene expression and the presence of a combination of inflammatory mediators stimulating the differentiation of these cells into another epithelial cell phenotype. Second, the resolution of MCM involves the reduction of approximately 30% of AECs being removed from the epithelium. Because all of these cells represent mucus-producing cells, this mechanism may account for the reduction of mucus production by at least one-third. This resolution is in part orchestrated by the Bcl-2 family of proteins such that anti-apoptotic proteins are downregulated allowing the pro-apoptotic members to elicit cell death and reduce the number of hyperplastic epithelial cells [57]. Whether the decline in specific cytokine levels causes the downregulation of anti-apoptotic proteins or an increase in pro-apoptotic regulators, and thereby the cell death of hyperplastic epithelial cells is being investigated.

Resolution of MCM also occurs when mice are exposed to the allergen continuously for prolonged periods [56]. In rodents, chronic exposure to an allergen initially induces inflammation, which decreases over time [58], [59]; while in humans with allergic asthma, inflammation persists and is chronic during their lifetime [60]. This transition from antigen sensitization to immunological tolerance is accompanied by a shift in the lymphocyte content in the lung tissue and bronchial lavage fluid (BALF) [61], [58]. Antigen-specific regulatory T cells are believed to produce IL-10 transiently and inhibit the asthma phenotype during the development of tolerance [62], [63]. Prolonged exposure of mice to an allergen causes the cytokine IL-13 which is secreted by T helper cell type 2 (Th2) to decrease and IFNγ, which is secreted by Th1 cells, to increase in the BALF [64]. IFNγ mediates the reduction of IL-13-induced MCM by inducing programmed cell death [56].

Resolution of MCM during prolonged exposure to allergen involves an active process that requires cell death-inducing conditions. The experimental role of IFNγ signaling in the resolution process of MCM was identified when instillation of IFNγ enhanced resolution of allergen-induced MCM by causing cell death in airway mucous cells. The importance of IFNγ in resolving MCM was confirmed by the finding that mice deficient in IFNγ (unpublished personal observation) and STAT1 [56], [65], a protein that plays an obligated and dedicated role in mediating IFNγ-dependent responses, were unable to resolve MCM during prolonged exposure to allergen. The main cell type that undergoes cell death in allergic mice is the mucous cell. However, our studies demonstrate that growing NHBE cells are susceptible to IFNγ-induced cell death regardless of whether they express mucin genes or not. Interestingly, non-proliferating NHBE cells are generally unaffected by IFNγ [56].

The mechanism for the selective susceptibility of proliferating cells to IFNγ is not clear. However, the reason why mucous cells are reduced during the resolution process may be due to their hyperplastic nature that makes them a target of IFNγ. Overall, these findings suggest that IFNγ causes a reduction of MCM through the apoptosis of hyperplastic cells, which restores the normal number of cells in the epithelium.

Classic apoptotic epithelial cells are not obvious during the recovery of the airway epithelium. Whether some of the metaplastic cells slough off during this process is still under investigation. It is possible that selected cells are removed by extrusion and involve detachment from the basement membrane, which is also called anoikis [66], thereby being sloughed off before the classic apoptotic morphology appears.

IFNγ-Induced Cell Death and Check Points Controlling this Pathway in AECs

Many studies have shown that T lymphocytes, neutrophils, and eosinophils undergo cell death in massive numbers within 3–6 h after cytokine deprivation or stimulation with death agonists [67]. However, it is widely recognized that epithelial cells in general and AECs in particular are resistant to cell death [57].

Two major characteristics define IFNγ-induced cell death in AECs: 1) Only proliferating AECs are sensitive and die, while confluent AECs are resistant to IFNγ-induced cell death [56]. The sensitivity of only proliferating cells may ensure the selective removal of hyperplastic cells during the resolution of MCM and that the majority of AECs, which are quiescent and in the resting G0 phase of the cell cycle, are protected. This selective cell killing provides a mechanism to restore the epithelial structure without affecting the rest of the airway epithelium. **2**) Cell death induced by IFNγ treatment is not evident until after 3 d of treatment. The role of this slow execution of the cell death process may be to allow neighboring cells time to close the resulting gap and maintain an intact barrier function.

The apparent requirement of 2–3 d for IFNγ-induced cell death suggests that apoptosis in AECs is mediated by an indirect pathway, i.e., the cells must produce the factor that induces cell death. Alternatively, the delayed cell death could stem from checkpoints that are in place to abrogate direct activation of the death signals. AECs may have developed signaling pathways that allow activation of pro- and anti-apoptotic pathways in response to an injury stimulus, making the cell very refractory to cell death unless the anti-apoptotic factors are overwhelmed by a sustained death stimulus. Such complex activation of checkpoints allows the airway epithelium to resist massive cell death and fulfill its ancient role as a crucial innate protector from environmental insults.

In the context of apoptosis to restore homeostasis in hyperplastic epithelium, it remains unclear how the epithelium determines the number of cells that must be eliminated to restore the original condition. Is it possible that the epithelial cells that populate the epithelium signal through gauging the intercellular pressure that there is not enough room. Furthermore, it is unknown whether the discarded cells represent abnormal or damaged epithelial cells and whether there are signals that determine which cells should be discarded from the

airway epithelium to restore the normal proportions of epithelial cell types so that the remaining cells differentiate into serous, Clara, ciliated, and mucous cells to reconstitute the proportions found in normal epithelia.

One of the regulators of cell death during the resolution of MCM is Bax that translocates to the ER and causes calcium release [64], [65]. The percentage of Bax-immunopositive mucous cells increases from approximately 3 to 25%, while the number of metaplastic mucous cells decreases [64]. Overall, approximately 25–35% of mucus cells express Bax after repeated exposure to allergen for 15 d. Trifilieff et al. [27] found that by 3 d post allergen exposure, approximately 30% of epithelial cell nuclei are BrdU-positive, a marker for cells that undergo DNA synthesis during the cell cycle. Taken together, the observed Bax positivity in approximately 25–35% of mucus cells during the resolution of allergen-induced GCM suggests that the Bax-positive mucous cells may represent cells that must be eliminated to reconstitute the original cell number of the repaired epithelium.

Sustained MCM in Asthma – Possible Therapies to Reduce Mucous Hypersecretion

It is generally believed that MCM in asthma does not resolve because of persistent inflammation in diseased airways that ensures the continuous presence of IL-13, the central regulator of MCM. Several studies have reported that IL-13 is present in the lungs of asthmatics at concentrations that are sufficient to induce cell proliferation and MCM. In patients with asthma, IL-13 levels are increased only upon allergen challenge to concentrations ranging from 0.4–3 ng/ml [68], sufficient to cause proliferation of NHBE cells [52]. IL-13 levels are found to be higher even in the sputum of mild asthmatics than in non-asthmatic controls [69]. Therefore, the presence of this cytokine may be the main reason for sustained MCM in asthma.

Given the importance of IL-13 in the development of MCM, efficient therapies for reducing mucous hypersecretion need to be based at least in part on the understanding of how the overabundance of IL-13 in the lungs of asthmatics is sustained. Dysregulation of IL-13 production in asthma may occur due to polymorphisms in the IL-13 gene that have been shown to be associated with the asthmatic phenotype [70], [71]. Several studies have demonstrated that blocking IL-13 and its signaling proteins reduce or eliminate the development of MCM in animal models of asthma [35], [54]. Clinical trials with IL-13 inhibitors will define the importance of this approach in reducing MCM in human asthma. Because CLCA proteins are critical for IL-13-induced MCM, while CLCA1 expression may not be a selective determinant of MCM so that shared homologies between CLCA family members may still represent a useful target for focused therapeutic intervention in hypersecretory airway disease.

The biosynthesis of mucus can be blocked by inhibiting the signaling proteins for mucin gene expression or blocking the glycosylation process. Inhibition of MUC mRNA biosynthesis using MAP kinase inhibitors has been proposed to inhibit mucin biosynthesis [51]. However, for developing effective therapies directed toward reducing the biosynthesis of mucins understanding the molecular mechanisms of the IL-13-mediated increase in

MUC5AC gene expression and/or mRNA stabilization needs to be expanded. In addition to the biosynthesis of mucin glycoproteins, increased mucous hypersecretion requires proteins such as myristolated, alanine-rich C-kinase substrate (MARCKS) that is a target for phosphorylation by protein kinase C [72]. A MARCKS-related peptide was successfully used to block mucus hypersecretion in a mouse model of asthma [73], and human studies are under way to determine whether this approach will be clinically relevant.

N-acetyl cysteine has been used to reduce the viscosity of mucins so that the airways are not blocked by this viscous substrate [74]. However, in asthma it is the acute secretion of mucus in areas where MCM is prevalent that causes blockade of the airways and airway obstruction. This approach may only be suitable for the acute condition and not a treatment for the chronic presence of MCM.

Because surface epithelial mucous cells represent a great potential for secretion of mucosubstances [16], it is important to investigate how their numbers are regulated in airway epithelia. Therefore, reducing MCM based on the understanding of AEC apoptosis is regulated may also be highly effective in reducing mucous hypersecretion in asthma.

There is the possibility that the normal resolution of MCM is disrupted in patients with asthma. One could also speculate that humans with allergic asthma are deficient in developing such immune deviation to specific compounds or in IFNγ signaling to reduce MCM. In some instances, serum levels of IFNγ can be increased in severe asthma cases [75], and IFNγ levels in BALF are sometimes found even in mild asthma [76], [77]. Although these data seem paradoxical, IFNγ levels may not be high enough to increase the pro-apoptotic Bcl-2 family members. Supporting this hypothesis, the instillation of 100 ng IFNγ causes the reduction of MCM, while 50 ng does not [56]. Furthermore, polymorphisms in genes encoding for IFNγ and IFN regulatory factor-1 (IRF-1) confer genetic susceptibility to allergic asthma in Japanese children [78] and Stat 1 is constitutively activated in epithelial cells of asthmatics [79]. Therefore, it is also possible that in a subpopulation of asthmatics a deficiency in the IFNγ-signaling pathway may render IFNγ incapable of inducing cell death in epithelial cells and in sustaining MCM. In that context, initiating cell death of hyperplastic AECs using pro-apoptotic Bcl-2 family members as part of a general therapeutic effort to reduce inflammation may be highly effective. Chronic inflammation in animal models needs to be established to adequately address these complex issues of MCM in asthma.

In summary, the amount of mucus in the airways of asthmatics may be controlled at various levels. First, the biosynthesis of mucins including the transcription of mucin genes followed by translation and posttranslational glycosylation, can be crucial stages for controlling the amount of mucin synthesized. Second, the secretion of the synthesized mucins may be suppressed by affecting the mechanisms underlying the transport and release of vesicles containing the mucins. Third, the number of mucus-producing cells can be reduced by initiating cell death by driving the proapoptotic pathways that are involved in the normal resolution of airway epithelial hyperplasia and thereby help reconstitute the normal epithelium.

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Abbreviations:

AECs	airway epithelial cells
BALF	bronchial lavage fluid
CLCA	calcium-activated chloride channel
FEV	forced expiratory volume
GABA	γ-aminobutyric acid
MARCKS	myristolated, alanine-rich C-kinase substrate
MCM	mucous cell metaplasia
NHBEs	normal human airway epithelial cells

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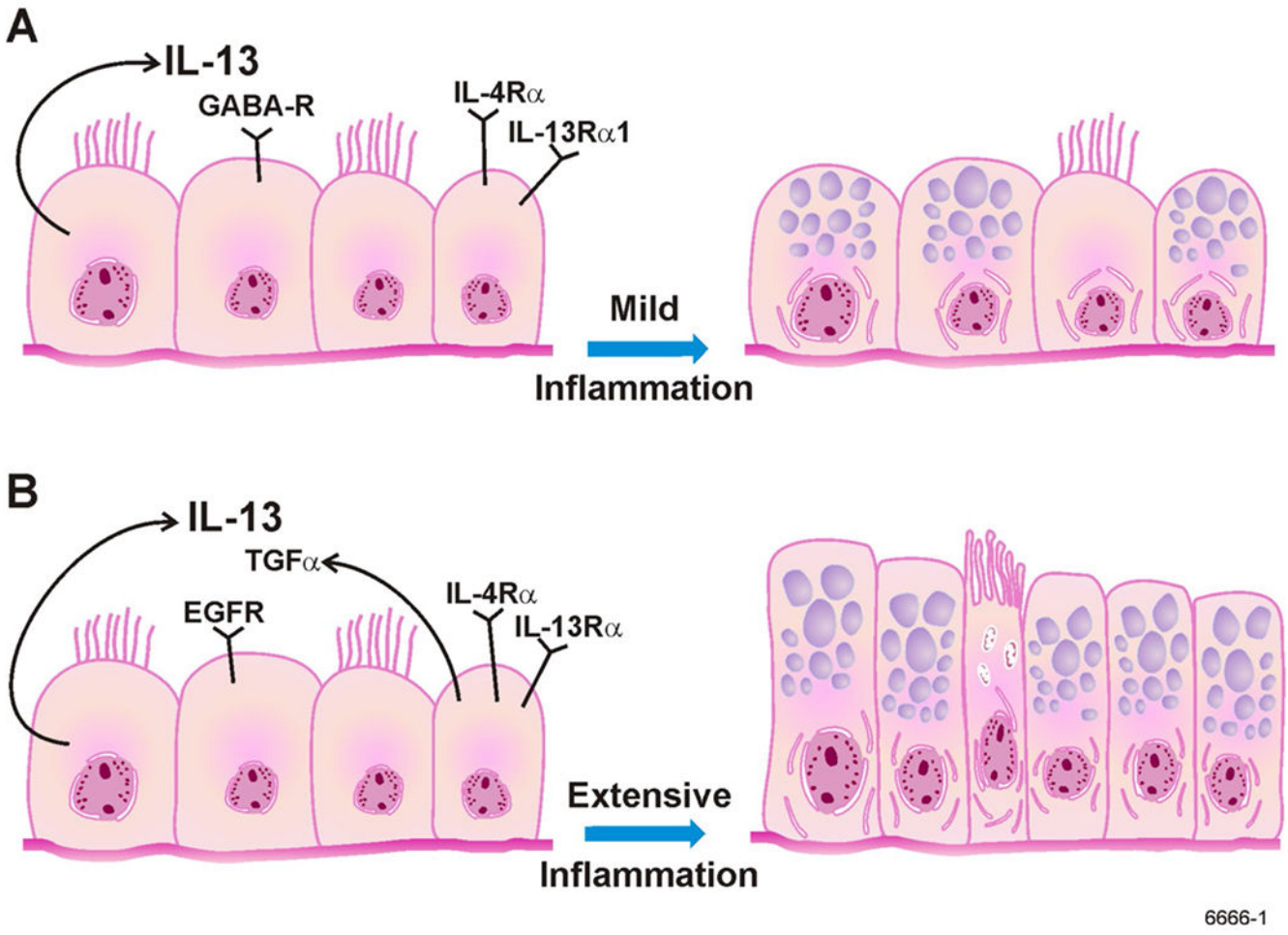

Fig. 1 (A):

Under conditions of mild inflammation, MCM is induced when AECs start to produce and store mucous proteins. IL-13 that is produced by AECs acts through IL-4α and IL-13α1 and on GABA-R to increase mucin gene expression and protein synthesis. (B): Under conditions of extensive inflammation, MCM is induced by AECs undergoing proliferation and storing MCM. IL-13 causes proliferation of AECs through EGF-R/TGFα-dependent mechanisms.