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The high affinity IgE receptor (FcεRI) expression and function in airway smooth muscle

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ABSTRACT

The airway smooth muscle (ASM) is no longer considered as merely a contractile apparatus and passive recipient of growth factors, neurotransmitters and inflammatory mediators signal but a critical player in the perpetuation and modulation of airway inflammation and remodeling. In recent years, a molecular link between ASM and IgE has been established through Fc epsilon receptors (FcεRs) in modulating the phenotype and function of these cells. Particularly, the expression of high affinity IgE receptor (FcεRI) has been noted in primary human ASM cells *in vitro* and *in vivo* within bronchial biopsies of allergic asthmatic subjects. The activation of FcεRI on ASM cells suggests a critical yet almost completely ignored network which may modulate ASM cell function in allergic asthma. This review is intended to provide a historical perspective of IgE effects on ASM and highlight the recent updates in the expression and function of FcεRI, and to present future perspectives of activation of this pathway in ASM cells.

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1. Introduction

Asthma is among the commonest chronic conditions in Western countries, although the global prevalence ranges from 1 to 18% of the population in different countries [1]. With an estimated global burden of 300 million people, asthma is responsible each year for 250,000 premature deaths, as well as 20 million lost working days [2]. Due to this global impact, major efforts are required to understand the mechanisms of this devastating chronic airway disease.

Asthma is clinically characterized by airway obstruction, enhanced bronchial responsiveness and airway inflammation [3–5]. The inflammatory part of this disease includes an increased infiltration of activated T lymphocytes, eosinophils, mast cells, and neutrophils within the airway lumen and bronchial sub-mucosa [3,6]. Extensive evidence from human and animal studies suggest that CD4+ T cells are the major cell type involved in regulation of airway inflammation through the expression of Th-2 type cytokines [7,8].

Although the role of lymphocytes and inflammatory cells is undeniable, tissue-forming structural cells have an inherent role in secretion of multiple inflammatory mediators and in maintaining chronic allergic inflammation. Airway smooth muscle

(ASM) cells are thought to be the principal effector component of airway narrowing. Due to intrinsic phenotype plasticity, airway myocytes exhibit a multifunctional behavior and are shown to be actively involved in airway inflammation and fibrosis [9]. Emerging data suggests that ASM cells can contribute directly to asthma pathogenesis by their capacity to express cell adhesion and co-stimulatory molecules, and by secreting multiple proinflammatory cytokines and chemokines that may instigate or perpetuate airway inflammation and the development of airway remodeling *in vivo* [9–12]. Extensive studies in last two decades have added to our knowledge of the biology of this important cell type and defined a new role of ASM in allergic asthma. Of special interest, it has been suggested that ASM cells may contribute to airway inflammation and airway remodeling by mechanisms depending on Fc epsilon receptor (FcεR) activation, reviewed in Refs. [13,14]. Recent intriguing data expands the role of these receptors, particularly FcεRI, on smooth muscle activation and its functional consequences in allergic asthma as presented in next sections.

2. FcεR network and allergic asthma

Asthma resulting from immunological reactions is referred to as allergic asthma and is mostly initiated by immunoglobulin E (IgE) antibodies. Eighty percent of childhood asthma and >50% of adult asthma is reported to be allergic [15,16]. IgE is overproduced in

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allergic individuals in response to pollens (trees, grasses and weeds), dust mites, pet dander, occupational substances and, sometimes foods [17,18]. Normal levels of IgE in newborns are considered to be about 0.8–1.2 ng/ml, which tend to gradually increase up to about 25–100 ng/ml in adolescence [19]. In adulthood, mean serum IgE levels are around 150 ng/ml. In allergic individuals, serum IgE levels may reach over ten times the normal, to about 35,000 ng/ml (or 35 µg/ml) [18,20], whereas allergen specific IgE may be over 1000 times higher than the detection limit in normals and tend to associate with disease symptoms [21]. Furthermore, in subjects with asthma or hay fever, IgE may be detected only in secretions from target organs, suggesting the occurrence of local IgE synthesis [18].

Allergic individuals respond to environmental allergens with an immediate (type I) hypersensitivity reaction [3]. Allergens bind to the basophil or mast cell-bound specific IgE and trigger cross-linking of the high affinity Fc receptor for IgE (FcεRI) [22–24]. This cross-linking incites a cascade of events resulting in cell degranulation and the release of inflammatory mediators such as histamine, leukotrienes and multiple cytokines and chemokines [22]. Altogether, these mediators orchestrate the early inflammatory response along with mucosal edema and smooth muscle contraction, resulting in allergic diseases such as asthma.

So far, two types of IgE Fc receptors have been identified in human: the low affinity FcεRII/CD23 [25,26] and the high affinity receptor FcεRI [14,22]. Since the focus of this review is FcεRI, recent reviews are recommended for updates on the structure and function of FcεRII/CD23 [14,17,27].

3. The high affinity receptor for IgE: FcεRI

FcεRI is a member of the multi-chain immune recognition receptor family, first described as a tetrameric complex (αβγ2) [22]. The α chain is a member of the immunoglobulin superfamily, and contains the binding site for IgE [22]. The β subunit with its four transmembrane domains separating N- and C-terminal cytoplasmic tails, functions to amplify signaling response [28–30]. The two disulfide linked γ-subunits are the members of the γ/ζ/η family of antigen receptor subunits, comprise essentially of a transmembrane region and cytoplasmic tail, and are critical for FcεRI signaling [22]. Both β- and γ-chains are responsible for the downstream propagation of signaling through the phosphorylation of their immunoreceptor tyrosine based activation motif (ITAM) [14,22].

The structure of FcεRI varies according to the species. In mice, FcεRI-β chain is a requisite for cell surface expression so that the FcεRI is expressed as αβγ2 tetramers exclusively on mast cells and basophils, while in humans FcεRI can be expressed as an αβγ2 or αγ2 complex depending on cell type [14,22]. The tetramer (αβγ2) form is expressed on the classical effector cells of allergic reactions such as mast cells and basophils [24,31], although evidence implicates this form of receptor expression on allergic asthmatic neutrophils [32], eosinophils, platelets [33–35], and airway smooth muscle cells (see next sections). The trimeric (αγ2) form of the receptor is expressed on antigen presenting cells such as Langerhans cells, peripheral blood DCs, and monocytes [35,36].

Particularly in mast cell and basophils, signaling through FcεRI is one of the most critical and well-studied phenomenon and offers novel targets of therapeutic intervention in allergic diseases [31,37,38]. Cross-linking of FcεRI through IgE-bound multivalent antigens commences a series of biochemical events beginning with the Src family protein tyrosine kinase Lyn that phosphorylates the ITAM motifs of FcεRI-β and -γ chains [22,39]. ITAM phosphorylation then serves as a docking site for another Src homology domain 2 (SH2) containing kinase, spleen tyrosine kinase (Syk) which

eventually leads to a conformational change of Syk leading to increase in its enzymatic activity [40]. Syk can autophosphorylate at tyrosine residues which then leads to downstream propagation of signals [41]. Activated Syk then mediates phosphorylation of a number of proteins including linker for activation of T cells (LAT), SH2-containing leukocyte specific protein of 76 KDa (SLP-76), Vav, phospholipase C-γ1 (PLC-γ1) and PLCγ2 [39]. FcεRI aggregation was shown to activate Fyn leading to the adapter protein Grb2-associated binder-like protein 2 (Gab2) tyrosine phosphorylation [42]. Gab2 binds to the p85 subunit of phosphatidylinositol 3-kinase (PI3K) leading to PIP3 production and recruitment of pleckstrin homology (PH) domains to the membrane, i.e. Btk, PLCγ1, PLCγ2, and phosphoinositide-dependent kinase 1, eventually resulting in release of Ca²⁺ from internal stores. Data from other reports have also demonstrated that FcεRI activation-induced early events lead to the activation of ERK-associated MAP kinases (Vav, Raf1, MEK) and Rac/Rho GTPases (Rac/Rho) [43,44]. FcεRI cross-linking also leads to the phosphorylation and degradation of IκB which then allows the release and nuclear translocation of the NF-κB pathway. Some other signaling molecules involved in this pathway include PKCβ, CARMA1, Bcl10, and MALT1. The activation of transcription factors NFAT and NF-κB, and (Erk, JNK and p38) MAPK pathways [43,45] leads to the synthesis of several cytokines. Protein kinase C (PKC) and MAPKs also act on the cytosolic PLA2 which releases arachidonic acid that is metabolized by the cyclooxygenase or the lipoxygenase pathways to synthesize the inflammatory mediators, prostaglandins, and leukotrienes [14,31].

4. Early evidence of IgE effects on ASM sensitization

Prausnitz and Kustner were first to recognize that the allergen sensitivity can be transferred to non-allergic individuals through the transfer of serum from an atopic subject [46]. Subsequent studies showed that the exposure to serum from asthmatic subjects caused an increase in mediator release from isolated cells [47], and initiated allergen-induced bronchoconstriction in isolated human airways [48]. However, the detailed studies to understand the contractile properties of ASM in asthma began with a report from Antonissen and colleagues (1979), in which a canine (dog) model of asthma was used. Sensitization of dogs with dinitrophenol and ovalbumin (DNP-OVA) injections intraperitoneally lead to the production of anti-DNP IgE antibodies. Interestingly, the tracheal smooth muscle (TSM) from sensitized animals exhibited significantly greater shortening velocity and an increased isotonic shortening at any given load after maximal electrical field stimulation (EFS) [49]. This study led to the idea of passive sensitization of isolated bronchial tissue with serum from atopic individuals (containing high levels of IgE), summarized in Ref. [50]. Interestingly, similar to the canine study, passive sensitization of isolated human airways also enhanced the smooth muscle shortening velocity and capacity in response to EFS, and augmented the myogenic responses [51,52]. From atopic serum sensitization studies, it was suggested that serum IgE levels play an important role in smooth muscle hyperreactivity [50,53]. Indeed, the bronchial hyperresponsiveness has been shown to be associated with serum IgE levels and transferable by IgE-rich serum from asthmatic to non-asthmatic individuals [53,54]. Moreover, IgE was proposed to cause smooth muscle contractile function through binding to the smooth muscle membrane inducing subsequent hyperpolarization [55]. Finally, the pre-incubation of an IgE neutralizing antibody (anti-IgE, 17–9), which reduces free IgE to undetectable levels, prevented the IgE-dependent passive sensitization of human airways and subsequent allergen-induced contractile responses *in vitro* [56].

Although numerous studies proposed an association between IgE and ASM, a clear molecular link between IgE and ASM was

missing. Only recently these cells were recognized with a capacity to express both low affinity FcεRII/CD23 and high affinity FcεRI [14]. In attempts to investigate the mechanisms underlying altered ASM responsiveness in atopic-sensitized state, Hakonarson and colleagues first showed that ASM tissues of both human and rabbit origin constitutively express FcεRII/CD23 and Fc receptors for IgG; FcγRIII (CD16), FcγRII (CD32), and FcγRI (CD64). The FcεRII/CD23 expression was upregulated in presence of atopic (IgE-rich) serum or IgE-immune complexes. Interestingly, FcεRII/CD23 activation by IgE-immune complexes or atopic serum induced 'pro-asthmatic' like changes in ASM responsiveness, such as increased contractility and attenuated relaxation [57,58]. Moreover, Rhinovirus (RV) and atopic sensitization displayed cooperative effects in enhancing the FcεRII/CD23 expression and subsequent induction of 'pro-asthmatic' like changes in ASM, potentially by ICAM-1-coupled LFA-1 signaling in ASM itself [59]. The same group later showed that the IgE sensitization of ASM cells leads to autocrine release of IL-13 and IL-5, which may underlie the altered ASM responsiveness including heightened agonist-induced constrictor responsiveness and impaired β₂-agonist-mediated relaxation [13,60,61]. Belleau and colleagues (2005) also showed constitutive FcεRII/CD23 expression in human ASM cells which was further upregulated by IL-4, GM-CSF, or both. Moreover, the cytokine-upregulated FcεRII/CD23 was also accompanied by changes in cell morphology such as depolymerization of actin fibers, cell spreading, and membrane ruffling, and an overall increase in cell volume and protein content, suggesting hypertrophy of ASM [62]. Collectively, ASM cells are now known to express both low affinity FcεRII/CD23 [58] and high affinity FcεRI receptors [14,63]. However, FcεRI binds to IgE with more than 1000-fold higher affinity than FcεRII/CD23 [64] and therefore FcεRI is a likely determinant of majority of IgE effects.

5. IgE exposure of ASM *in vivo*

Although a direct evidence of IgE gaining access to smooth muscle compartment *in vivo* is not available, there could be a number of instances wherein IgE either in free or antigen/allergen-bound form could plausibly bind to ASM. For instance, local IgE expression has been demonstrated *in vivo* in the airways of atopic subjects [17]. Studies in atopic asthma patients have clearly shown a preponderance of IgE expression in bronchial mucosal surfaces with a distribution pattern from proximal towards distal airways, however none or undetectable IgE levels in lung parenchyma [65]. In fact, the larger size of IgE (similar to IgM but larger than IgA or IgG) and heavy glycosylation has been suggested to limit the diffusion of IgE across tissue compartments, which may eventually retain the IgE in sites where it is produced, i.e. mucosal/sub-mucosal regions [65], where smooth muscle also reside. Furthermore, the observations showing an overall low concentration of IgE in serum (0.004% of total immunoglobulins), and its prolonged half-life in tissue compared to serum (~14 days versus 3 days, respectively) suggest that (i) the immune surveillance by IgE happens mainly in tissue and (ii) prolonged half-life facilitates long-lasting IgE-mediated immune responses within bronchial mucosa [17,18,65]. Moreover, in the classical models of allergic sensitization of airways [66], IgE produced in regional/local lymph nodes is believed to diffuse locally and enter the lymphatic vessels, bloodstream and finally to get distributed systemically. Subsequent access to the interstitial fluid leads IgE to bind to FcεRI on airway mucosal tissue-resident mast cells. Since mast cells have been shown to infiltrate smooth muscle layers and affect ASM function in asthma through IgE-dependent activation of former [67,68], it is highly likely and worth considering that the ASM gets sensitized in a fashion similar to mast cells. Recent data from animal studies have provided insights about the transport of IgE and IgE-allergen

complexes to sub-epithelial tissue. For instance, a splice variant of the IgE receptor, expressed by intestinal epithelial cells, was shown to bind and transport allergens into sub-epithelial tissue [69]. In fact, there is evidence for IgE-allergen transport through intestinal epithelial cells in large quantities without changing their structure [70]. Although these studies were limited to intestinal mucosa, a similar possibility of IgE/IgE-allergen transport to sub-epithelial zone in airways cannot be denied. More recently, house dust mite allergens were shown to disrupt the cell–cell contacts and to create gaps in airway epithelial cell barrier, by involving the activity of protease activated receptor (PAR)-2, which may facilitate allergens to infiltrate into sub-epithelial tissue [71].

6. Expression and regulation of FcεRI in ASM cells

Early studies from our lab showed the expression of transcripts of α, β, and γ subunits of FcεRI in human ASM cells. The surface expression of IgE-binding subunit FcεRI-α was also shown by flow cytometry, besides the detection of its protein expression by immunoprecipitation (IP)-coupled-Western blotting (WB), immunocytochemistry, and immunohistochemistry. Even though the FcεRI-α chain immunoreactivity was shown in ASM bundles of allergic asthma patients [63], the successful detection of FcεRI expression in ASM cells has been a matter of debate. Whilst an increase in the number of anti-IgE positive cells was observed within smooth muscle layer, the prominent cell type was suggested to be the mast cells [72]. Another group could not detect the expression of FcεRI-α or FcεRII/CD23 in non-asthmatic human ASM cells. Although a weak signal for FcεRII/CD23 and the common Fcγ subunit mRNA was observed, authors could not detect the former at protein level [73]. Moreover, IgE or antigen-IgE was not observed to induce any mediator release in human ASM cells [73], completely in contrast to multiple other studies [57,63,74–77]. As discussed above, multiple studies have established the constitutive, cytokine or allergic serum-inducible expression of FcεRII/CD23 in both asthmatic and non-asthmatic human ASM cells. While human serum has been proposed to augment the synthetic functions and mitogenesis of human mesenchymal cells [78,79], the use of fetal bovine serum (FBS) in culture of ASM cells has been proposed to partly underlie the inconsistencies in detection of FcεRs in some of the studies [73]. The discrepancies observed in detection of FcεRI may be explained through multiple factors that need to be considered in assessing this critical receptor, some of which were discussed by us recently [80]. Studies [57,73] that failed to detect FcεRI expression in ASM have concluded on the basis of RT-PCR, immunohistochemistry (IHC) and flow cytometry. Failure to detect FcεRI by IHC can be explained by some earlier reports. It has been known for a long time that auto-antibodies to IgE and FcεRI-α chain are produced and bound to FcεRI on cell surface in many autoimmune and allergic diseases including chronic urticaria, allergic rhinitis, autoimmune arthritis, and allergic and non-allergic asthma [81–84]. At least one third of anti-FcεRIα autoantibodies are competitive with IgE [85,86]. Although their role in pathogenesis of allergic diseases is a matter of debate, autoantibodies to FcεRI-α chain or IgE may mask the detection of FcεRI or IgE bound to FcεRI [86–88].

The use of IHC and flow cytometry techniques in detecting FcεRI in mesenchymal cells has some limitations. Assessing the surface expression of FcεRI is challenging in adherent (ASM) cells which are generally trypsinized prior to detection of FcεRI surface expression. Trypsin has been shown to degrade the cell surface receptors [89,90], and therefore jeopardize the detection of FcεRI receptor on cell surface. Moreover, considering the low abundance of FcεRI on ASM cells [63], analysis by flow cytometry may not be appropriate in its detection. Instead, approaches using high amount of cellular protein such as Western blotting combined with or without

immunoprecipitation may prove adequate in detecting FcεRI in ASM [63,74,75]. An interesting parallel can be drawn from a recent study by Wang et al [91] in which authors for the first time detected the FcεRI-α chain expression in human vascular (aortic) smooth muscle (VSM) and endothelial cells by immunoblotting. Results of this study fully support the arguments presented above since authors initially failed to detect the FcεRI-α chain band in their cell lysates but could detect it once they increased the amount of VSM cell lysate analyzed by immunoblot. Moreover, a strong upregulation with interferon-γ (IFN-γ) was detected in VSM and endothelial cells, again suggesting a likelihood of FcεRI regulation by local inflammatory milieu. Furthermore, a modest degree of immunoreactivity for FcεRI-α chain was also detected within α-smooth muscle actin region of human vascular tissue. This could be an important study in opening a whole new area of investigation in which IgE can potentially affect the cells of non-inflammatory phenotype and modulate the biological function of vascular tissue beyond classical allergic response [91]. In addition, thorough investigation of the function of IgE–FcεRI network in vascular tissue is warranted in context of pulmonary vascular biology.

Extensive studies in inflammatory cells suggest that the amount of FcεRI present on cell surface determines the effector functions of these cells and thus the intensity of allergic reaction [92]. IgE was found to be the principal factor governing surface FcεRI-α surface expression in mast cells, basophils and neutrophils, and in the amplification of subsequent functions such as cell degranulation, inflammatory mediator release, and antigen presentation by APCs [93–97]. Although IgE induced a slight increase in FcεRI-α transcript in ASM cells, we did not observe any change in its protein expression (N.S. Redhu and A.S. Gounni, unpublished observations). Another group also did not find the regulation of FcεRI-α chain by its ligand IgE [75], altogether suggesting that different regulatory mechanisms may exist in ASM cells.

Besides the ligand itself, various other factors control the regulation of FcεRI-α expression. These include β- and γ-subunits of FcεRI, cytokines, and growth factors present in local milieu [92,93]. Indeed, FcεRI expression in ASM cells can potentially be augmented by proinflammatory (TNF) and Th2 (IL-4) cytokines [74]. A microarray-based study also suggests an IL-13-inducible FcεRI-α mRNA expression in ASM cells [98]. These studies essentially support a regulatory nature of FcεRI in ASM cells. As we showed recently [74], Th2 cytokine IL-4, and proinflammatory cytokines TNF and IL-1β induced a remarkable increase in FcεRI-α transcripts whereas only IL-4- and TNF-induced effect translated into FcεRI protein. These findings indeed partly corroborate a recent study on distal promoter of human FcεRI gene wherein IL-4 was found to enhance the intracellular expression of FcεRI-α chain [99].

In dendritic cells (DCs), FcεRI-γ chain serves as a limiting factor in modulating the FcεRI-α surface expression. However, unlike DCs, we did not find any change in FcεRI-γ chain expression in response to different cytokine stimulations, though TNF induced a slight increase in transcript levels [74]. Since the β-chain has been shown to enhance the expression and function of FcεRI-α chain in mast cells [30,100], additional regulatory mechanisms of FcεRI-α chain expression in ASM may also exist which require further investigation. Collectively, similar to inflammatory cells, FcεRI expression in ASM is highly regulated which should be considered while assessing by various approaches.

7. FcεRI-mediated release of cytokines and chemokines in ASM cells

IgE was initially thought to activate only inflammatory cells which subsequently activate the ASM via release of cytokines and growth factors. However, in contrast to being just passive recipients

of inflammatory cell input, ASM has recently been demonstrated to respond directly to inhaled extrinsic environmental factors such as dust, microbes, gases, and intrinsic factors such as cytokines and immunoglobulins including IgE and IgGs, reviewed recently in Refs. [101,102]. In very early studies, IgE sensitization of ASM through FcεRI/CD23 was shown to cause autocrine release of IL-13 and IL-5, which may underlie the altered ASM responsiveness [13,57,58].

FcεRI activation following IgE/anti-IgE cross-linking induced IL-4, IL-5, IL-13, and CCL11/eotaxin-1 release in human ASM cells. FcεRI cross-linking by IgE/anti-IgE also induced a rapid and transient increase in intracellular Ca²⁺, a likely determinant of ASM contractility and subsequent AHR (Fig. 1). Particularly, a blocking antibody for FcεRI-α chain abrogated the release of these mediators, suggesting that these effects were FcεRI-mediated [14,63]. Mimicking of asthmatic airway milieu with proinflammatory and Th2 cytokines enhanced the expression of FcεRI-α chain, and TNF presensitization of human ASM amplified the IgE-induced release of chemokines such as CCL11/eotaxin-1, CCL5/RANTES, CXCL8/IL-8, and CXCL10/IP-10 [74]. Mechanistic analysis further showed that the expression of these chemokines was mediated through promoter activation by IgE sensitization. Lentiviral-shRNA-transduced approach showed that the transcriptional activation of these chemokine promoters was abrogated in ASM cells deficient in Syk, a crucial kinase in FcεRI signal transduction [31]; therefore, our results argued for an FcεRI-specific effect of IgE in ASM cells [74]. Another study showed that the IgE/anti-IgE treatment of human ASM cells leads to modest levels of matrix metalloprotease 1 (MMP-1) production which may contribute to airway inflammatory and remodeling responses [68]. Roth and Tamm (2010) recently showed that the ASM cells from asthma patients showed *de novo* synthesis, and release of higher levels of pro-asthmatic mediators such as IL-4, IL-6, IL-8/CXCL8, and TNF compared with the ASM cells obtained from COPD patients or from healthy controls [75]. An abstract study utilizing PCR gene array also showed the expression of chemokines such as IL-8/CXCL8, CXCL6, CXCL1, and CXCL2 in response to IgE in human ASM cells [77]. Collective data thus far suggests that IgE can induce a plethora of cytokine and chemokine mediators in ASM (Fig. 1). Moreover, these mediators are also known to be produced by ASM cells *in vivo* [9], which may augment the recruitment of multiple inflammatory cells such as eosinophils, basophils, neutrophils, monocytes, DCs, and Th2 lymphocytes. The accumulation of these inflammatory cells may release granular enzymes, proteases, and other mediators thus contributing to the development of airway inflammation, AHR, and tissue injury.

8. FcεRI-induced TSLP in inflammatory airway immune response

One of the recently discovered mediators that are pivotal in shaping allergic immune responses is thymic stromal lymphopoietin (TSLP). TSLP was recently found to be necessary and sufficient to drive Th2 cytokine-mediated airway inflammation in murine models of asthma. Lung-specific expression of a TSLP transgene induced allergic airway inflammation characterized by a massive infiltration of inflammatory cells, goblet cell hyperplasia, and sub-epithelial fibrosis, with increased serum IgE levels [103]. On the contrary, mice lacking the TSLPR failed to develop asthma in response to inhaled antigen (ovalbumin plus alum), probably due to the inability of CD4⁺ T cells to respond to TSLP, as reconstitution with TSLPR-positive T cells restores the aspects of the inflammatory disease [104]. The findings from these animal models are closely related to human asthmatic subjects where higher concentrations of TSLP have been detected in the lungs, correlating with Th2

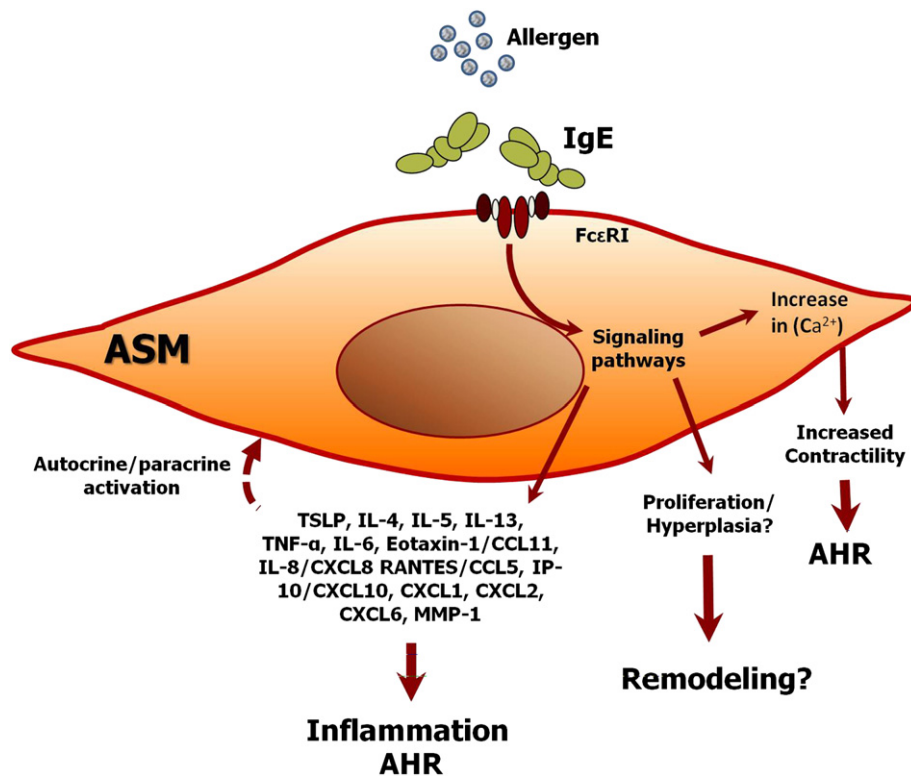


Fig. 1. FcεRI activation-induced effector functions on ASM. FcεRI activation on ASM by IgE (allergen-dependent or -independent) leads to proinflammatory mediator and intracellular Ca^{2+} release which may eventually contribute to airway inflammation, and AHR. Unpublished data from our group and animal models also suggest that IgE can directly induce ASM cell proliferation (hyperplasia) which may account for part of the ASM remodeling observed in allergic asthma.

attracting chemokines and disease severity [105,106]. Given the emerging role of TSLP in allergic inflammatory diseases [107–109] and the ‘inflammatory-like’ nature of ASM [9,10,110], it is plausible that ASM may contribute to airway inflammatory response via production of, and/or responsiveness to TSLP. Indeed, a constitutive, TNF or IL-1 β -inducible, and *in vivo* expression of TSLP was demonstrated in ASM of COPD patients [111]. Besides MAPKs [111], NF- κ B, and AP-1-like transcription factors were found to be critical in TNF-induced TSLP release in ASM cells [112,113]. Importantly, IgE also induced the TSLP transcription and protein release in ASM cells through the activation of NF- κ B and AP-1 transcription factors [76]. Syk played a crucial role in IgE-induced TSLP expression since Syk inhibition by pharmacologic or genetic approaches attenuated the TSLP promoter activity. In support of our observations, human ASM tissue from asthma patients was recently shown to express TSLP *in vivo* [114]. In contrast to a distinct correlation demonstrated between lung TSLP expression and asthma severity [106], ASM-specific TSLP expression was increased only in mild-moderate disease but not in severe asthma [114]. Authors reasoned for the relatively attenuated TSLP expression in ASM bundles of severe asthma patients to a response to high dose inhaled corticosteroids which may require further investigation [114]. Regardless, IgE-induced TSLP expression in ASM clearly points towards a plausible vicious cycle of allergic airway inflammation mediated by TSLP [76].

Interestingly, the expression of a heterodimeric TSLP receptor (TSLPR- consisting of IL-7R α chain and a common- γ receptor like TSLPR chain) was also reported in ASM cells *in vitro* and in smooth muscle bundles of allergic asthma patients. TSLPR activation on ASM by recombinant human TSLP induced a significant release of CCL11/eotaxin-1, CXCL8/IL-8 chemokines, and proinflammatory cytokine IL-6 [115]. These findings were recently confirmed by

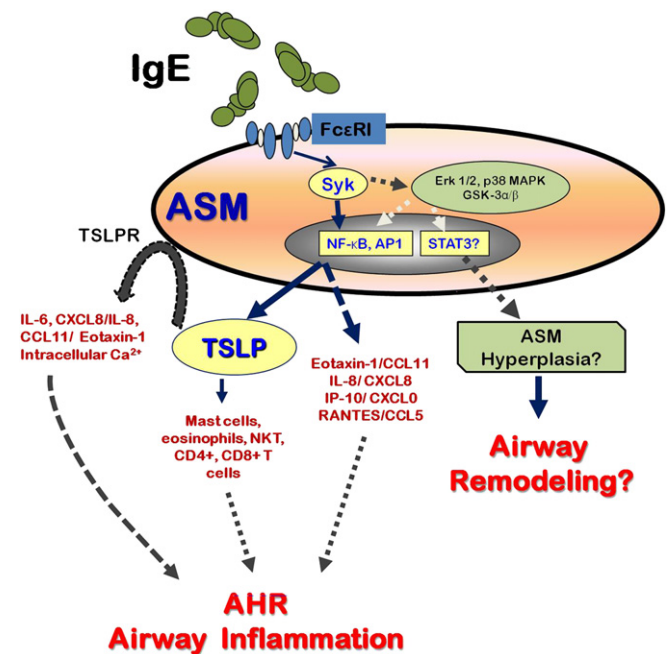


Fig. 2. Proposed model of IgE sensitization-induced signaling events in ASM. FcεRI activation by IgE leads to Syk activation which can activate Erk1/2 and other MAPKs culminating in NF- κ B, AP-1, and/or STAT3 activation. Eventually, the NF- κ B and AP-1 transcription factors may translocate to the nucleus and induce proinflammatory mediator expression such as CC and CXC chemokines, and TSLP. Various MAPKs and STAT3 may also cooperate to regulate ASM cell growth eventually contributing to part of the airway remodeling.

others [116] wherein authors also showed TSLPR upregulation by cigarette smoke extract (CSE) exposure. Interestingly, TSLP stimulation of ASM cells led to enhanced $[Ca^{2+}]_i$ responses to bronchoconstrictor agonists, a critical determinant of ASM contractility [116]. Collectively, these studies suggest that ASM cells are a rich source and target of TSLP, and uncover a novel role of TSLP in modulating the function of ASM that may eventually contribute to airway inflammation and bronchoconstriction in a TSLP/TSLPR-dependent autocrine/paracrine manner (Fig. 2) [113].

It is a matter of great debate whether FcεRI activation on ASM can directly modulate the immune response within the airways. Our data has provided some leads in unraveling the IgE-induced expression of pro-allergic TSLP in ASM [76]. Notably, TSLP can induce the maturation of dendritic cells (DCs) which in effect can induce naïve CD4⁺ T cells to differentiate into Th2 cells *in vitro* and *in vivo*. DCs do this potentially via upregulation of OX40L on their surface in presence of TSLP and absence of IL-12, which triggers the inflammatory Th2 differentiation following an OX40L-OX40 interaction between DC and T cells [103,104,117]. In addition, ASM itself can express enhanced OX40L in inflammatory milieu [118], and ASM has been shown to possess the molecular machinery for antigen presentation (MHC I and II, co-stimulatory molecules, and cellular adhesion molecules) [119–121]. In light of these observations, the role of ASM in antigen presentation to T cells through the modulation of DC function or through TSLP-OX40L-OX40 interaction between T cells and ASM itself is enticing to explore. Therefore, understanding the role of ASM-produced TSLP in this Th2 differentiation process may establish a direct role of ASM in airway immune responses.

9. FcεRI-mediated ASM growth?

Airway remodeling is the cardinal feature in most allergic asthma patients. Despite a good control of inflammation, most asthma medications fail to reduce airway remodeling [122]. ASM hyperplasia and hypertrophy are thought to be the leading causes of ASM remodeling, besides attenuated cellular apoptosis and fibrocytes/myocytes migration mechanisms [123]. Particularly, asthmatic ASM proliferate faster, partly due to the lack of C/EBPα transcription factor expression [124]. BAL fluid from asthmatic subjects exerted mitogenic effects on ASM cells [125], while it is known that BAL from asthmatics contains high levels of IgE upon segmental allergen challenge [126]. Since IgE was recently shown to exert pro-survival effects in inflammatory cells such as mast cells, monocytes, and asthmatic neutrophils [127–130], it is enticing to speculate that IgE may modulate ASM growth directly. Indeed, IgE caused a significant increase in DNA synthesis and cell number through Syk and STAT3 activation in ASM cells (N. S. Redhu and A. S. Gounni, manuscript submitted). At least in a murine model of chronic asthma, anti-IgE treatment decreased the thickness of ASM layer and peribronchial fibrosis compared with the untreated mice; implicating that (i) IgE could perhaps be one of the factors inducing ASM remodeling *in vivo*, and (ii) targeting IgE/FcεR network on ASM with anti-IgE represents a novel approach in reducing airway remodeling [131]. In this line, anti-IgE asthma therapy, Omalizumab/Xolair® has been proposed to provide some benefits in overall airway tissue remodeling [122].

10. FcεRI activation-induced contraction of ASM?

A careful comparison suggests that the FcεRI-induced signaling resembles greatly with agonist-induced contraction in ASM. Activation of FcεRI triggers multiple signaling pathways in inflammatory cells including phosphorylation of ITAM motifs of FcεRI-β and

–γ chains by Lyn kinase, and the activation of Syk via its recruitment to FcεRI [39,132]. Activation of Syk is critical for FcεRI downstream signal propagation including phosphorylation of PLCγ, and calcium mobilization [39]. Our early report indicates that the activation of FcεRI leads to marked transient increase in intracellular Ca^{2+} concentration in ASM cells [133]. Similarly, it is well known that smooth muscle contraction is triggered by an increase in intracellular Ca^{2+} in response to bronchospastic triggers/contractile agonists [134]. Contractile agonists bind to Gq protein coupled receptors (GPCR) resulting in PLC activation. The PLC in turn catalyzes the hydrolysis of PIP-2 to IP-3 and diacylglycerol (DAG). IP-3 mediates the initial release of calcium from intracellular stores as a result of its binding to receptors on sarco-endoplasmic reticulum [134]. In FcεRI-bearing cells particularly basophils, one of the best characterized signaling pathways involves Syk-mediated phosphorylation of PLCγ which leads to an increase in intracellular Ca^{2+} and activation of the calcium/calmodulin-dependent serine/threonine phosphatase calcineurin [22,135]. In ASM cells, the elevation in intracellular Ca^{2+} concentration activates the calcium/calmodulin-sensitive myosin light chain kinase (MLCK), followed by phosphorylation of the regulatory myosin light chain (MLC) and subsequent initiation of cross-bridge cycling between myosin and actin [136]. Increased smooth muscle (sm) MLCK content associated with enhanced ASM contraction has been demonstrated in passively sensitized bronchus [137], human ASM cells from asthmatic subjects [138], and ragweed-sensitized canine ASM [139]. These data suggest that high levels of IgE present in atopic/asthmatic serum may influence ASM contractile apparatus. Although a recent study suggests that IgE/anti-IgE induces a modest expression of matrix metalloproteinase 1 (MMP-1) in ASM cells, authors did not observe any effect on ASM contraction [68], and concluded that pathways other than MMP-1 may also be required. At least in our data, IgE appears to increase the abundance of smMLCK in ASM cells through FcεRI activation (J. Balhara and A. S. Gounni, manuscript in preparation). Further studies are underway to ascertain the signaling mechanisms involved in FcεRI activation of ASM cells which may influence ASM cell contraction. Taken together, these data indicate that the FcεRI activation in ASM cells can modulate calcium signaling and thus may promote a hyperresponsive phenotype of ASM cells (Fig. 1).

11. IgE-induced signaling and proposed model of FcεRI activation in ASM

IgE induces multiple signaling pathways in inflammatory cells such as mast cells and basophils. These signaling mechanisms differ in IgE 'sensitization' versus 'cross-linking' models. However, some of the signaling molecules activated in response to IgE are common in both models [129,140]. In our efforts to understand the function of FcεRI activation in human ASM cells, we investigated some of the putative signaling mechanisms in parallel. IgE-activated ASM cells showed Syk requirement to induce synthetic (release of CC/CXC chemokines and TSLP) and mitogenic function (unpublished observations and [74,76]). Although various tyrosine kinases have been implicated in FcεRI-mediated signaling, only the lack of Syk results in complete inhibition of cell degranulation and cytokine release [31,141]. Syk-deficient cells are defective in most of the signaling events that occur downstream of FcεRI activation, and Syk-/- mast cells fail to activate NFAT or NF-κB transcription factors [31]. In fact, novel Syk inhibitors are in clinical development for the treatment of airway diseases [37], with preliminary evidence of success in controlling the allergen-driven symptoms in human subjects [38]. Our genetic ablation and biochemical data not only validates these observations and establish an upstream signaling target in ASM [74,76], it also provides an evidence of FcεRI-specificity of

IgE-induced ASM activation since Syk serves as a signature pathway of FcεRI activation [14,22,39,92] (Fig. 2).

IgE sensitization also induced phosphorylation of MAPK and GSK-3α/β, and the transcription factor STAT3 (N.S. Redhu and A.S. Gounni, manuscript submitted). Other groups have also shown an IgE-induced activation of Erk1/2 and p38 MAPK in ASM synthetic response [75]. Moreover, we have also shown recently that IgE-induced TSLP expression in ASM requires NF-κB and AP-1 activation [76].

Collective data so far suggest that IgE can activate ASM cells in both antigen-independent [74–76] and antigen-dependent manner [14,63]. However, detailed studies are required to fine-tune the IgE-induced mediator release in ASM. A putative signaling network induced by IgE binding to FcεRI is proposed in Fig. 2.

12. Concluding remarks and future directions

IgE–FcεRI network is now widely studied in various contexts beyond allergic inflammation, and there is already some evidence of FcεRI transcripts expression in the cells of non-hematopoietic origin such as mouse neurons [142], mouse melatonin-secreting pinealocytes from pineal gland [143], mouse and human aortic smooth muscle cells, and endothelial cells in acute myocardial infarction [91], and human intestinal and airway epithelial cells [144,145], thus suggesting an evolving role of IgE–FcεRI complex interaction beyond the ‘classical’ inflammatory cells. Cumulative data reviewed here suggest a rational *in vivo* interaction of ASM with IgE/IgE-allergen complexes/FcεRs and supports the above mentioned emerging role of this pathway in ASM phenotype and function. Moreover, a therapeutic humanized monoclonal anti-IgE antibody, omalizumab/Xolair® (Genentech/Novartis, South San Francisco, CA, and Tanox, Inc., Houston, TX) is already in clinic and has shown promise in regulating IgE-induced ASM function [75], discussed in a separate review in this issue.

Collectively, while substantial efforts have been invested in examining the FcεRI activation on immune cells, only limited reports are available to address its function on ASM cells. Although FcεRI expression in ASM bundles of allergic asthma patients was shown earlier, there is a stern need to evaluate the association between FcεRI expression in ASM tissue and severity of allergic asthma and COPD in large population. Sincere efforts are needed to understand the composition, dynamics, and effector functions of FcεRI activation in ASM. It is of critical importance to investigate the role of individual Fcε receptors (FcεRI, FcεRII/CD23) activation and their contribution to ASM function in airway inflammation, AHR, and remodeling in allergic asthma.

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