

Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment

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Prospective birth cohort studies tracking asthma initiation and consolidation in community cohorts have identified viral infections occurring against a background of allergic sensitization to aeroallergens as a uniquely potent risk factor for the expression of acute severe asthma-like symptoms and for the ensuing development of asthma that can persist through childhood and into adulthood. A combination of recent experimental and human studies have suggested that underlying this bipartite process are a series of interactions between antiviral and atopic inflammatory pathways that are mediated by local activation of myeloid cell populations in the airway mucosa and the parallel programming and recruitment of their replacements from bone marrow. Targeting key components of these pathways at the appropriate stages of asthma provides new opportunities for the treatment of established asthma but, more crucially, for primary and secondary prevention of asthma during childhood.

Asthma is a common disease affecting millions of children and adults. In its chronic form, asthma has a complex pathology that is characterized by airway inflammation accompanied by alterations in patterns of vascularization, innervation and airway smooth muscle growth and disturbances of the epithelial-mesenchymal trophic unit throughout the conducting airways¹. These changes collectively compromise an individual's capacity to maintain normal respiratory functions, particularly when exposed to ubiquitous airborne environmental stimuli. This pathological state stems from repeated episodes of airway inflammation and ensuing cycles of tissue repair and regeneration occurring over a period of years (Fig. 1, top). This results in cumulative structure and function changes in the lung that eventually exceed critical thresholds beyond which the 'persistent asthma phenotype' comes to represent the new norm (Fig. 1, bottom).

In terms of asthma therapeutics, although effective symptom-relieving treatments are available, attempts to develop disease-modifying drugs have met with limited success. This slow pace of drug development is a reflection of the complexity of the underlying pathogenic mechanisms of asthma. However, this complexity is not universally acknowledged, and in the quest for new drugs, the strong tendency is still to view the disease process in unidimensional terms and focus exclusively on established asthma. This view is exemplified by the approach to the most common form of this disease, atopic asthma, in which the identity of the perceived primary pathogenic mechanism is enshrined in the name of the disease itself. Superficially, it seems that it could not be more straightforward:

atopic sensitization leads to airway inflammation, which results in asthma. This sequence of events is readily demonstrable in experimental models, findings from which have driven the development of a range of therapeutic agents, such as a monoclonal antibody (mAb) to interleukin-5 (IL-5), that are effective in blocking their specific allergy-associated targets in short-term trials in subjects with established atopic asthma but do not achieve long-term disease modification. We do not share the view held by some in this field that the underlying experimental approach to understanding asthma pathogenesis that led to the development of these drugs is flawed; however, we believe that much of the knowledge gained from the experimental approach continues to be applied clinically in suboptimal contexts. In this regard, we share the growing view that whereas aeroallergen sensitization is one of the strongest asthma risk factors, it rarely *per se* leads directly to persistent asthma. Instead, this sensitization most frequently acts in synergy with other proinflammatory environmental cofactors, most notably respiratory viral infections, to drive disease development. The nature of the interactions between antiviral and atopic pathways and how they may be potentially targeted to treat and/or prevent asthma is the focus of this review.

Mechanisms driving asthma initiation: birth cohort studies

The potential role of respiratory viral infections as an initiating factor in asthma pathogenesis has been recognized for many years², but a more comprehensive understanding of the magnitude of the impact of these infections has only become evident relatively recently in light of emerging results from long-term birth cohort studies in the United States^{3,4}, Australia^{5,6} and Europe⁷ that span decades (reviewed in ref. 8).

These studies have collectively showed that whereas wheeze is common during infancy—particularly in association with respiratory infection—expression of this phenotype is usually transient and resolves spontaneously by age ~3 years (Box 1). In a subset of children, however, wheeze consolidates into a persistent clinical pattern that is indicative of early

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onset asthma, and this phenotype is strongly associated with early sensitization to aeroallergens. In addition, lower respiratory viral infections (LRIs) can predispose to the development of early asthma, conferring a slightly higher risk than atopy alone. However, the most severe childhood asthma, and the type that confers the highest ensuing risk for progression to persistent asthma, is encountered when LRIs occur against a background of pre-existing aeroallergen sensitization^{5,6,8–10}, in particular during the period when postnatal lung growth and differentiation are proceeding most rapidly (**Box 1**). Under these circumstances, affected children are tenfold to 30-fold more likely to develop persistent and/or severe disease^{6,9–11}, and this outcome is a reflection of both the LRI frequency and intensity and the amounts of aeroallergen-specific IgEs that are present at the time of these episodes⁵.

A key underlying issue is the relative strength of the associations between different respiratory viruses and early asthma pathogenesis. Earlier studies emphasized the key role of respiratory syncytial virus (RSV)¹², however, recent attention has shifted toward rhinovirus^{3,10,13}, especially the frequently encountered rhinovirus type C¹⁴. However, RSV is a major pathogen in infants¹⁵, and the relative roles of these viruses in driving asthma beyond childhood are not completely understood¹⁶.

Progression of asthma beyond early childhood

The question of what sustains asthma progression beyond the preschool years is central to the development of improved treatments, and cohort studies have again been instructive in this area. The strength of the association between atopy and asthma in schoolchildren was first recognized in the 1980s, but the clinical relevance of this association was not initially widely appreciated. These findings have proven to be extremely robust over time, as exemplified by recent studies in teenagers, particularly the clear quantitative associations between the strength of T helper type 2 (T_H2) cell immunity against indoor aeroallergens and asthma severity¹⁷. This connection, however, is not absolute—whereas the majority

of teenagers with asthma are atopic, only a minority of these individuals with atopy develop asthma¹⁷. This suggests that although atopy manifesting as an inhalant allergy constitutes a strong risk factor for asthma in this age group, it is usually insufficient to trigger full-blown disease on its own, and additional cofactor(s) must be involved.

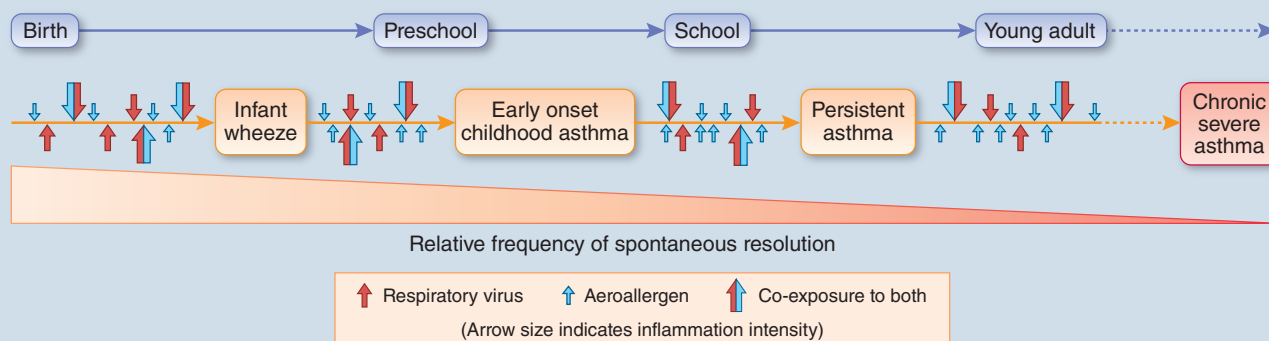
Epidemiological clues as to the nature of these cofactor(s) have come from studies on environmental triggers that precipitate wheezing attacks. Controlled exposure of aeroallergen-sensitized individuals with asthma to aerosolized allergens can provoke wheeze accompanied by T_H2 cell-associated airway inflammation¹⁸, mirroring the murine asthma models. However, the extent to which direct aeroallergen-induced triggering explains spontaneous asthma attacks is unknown, as a clear clinical history of acute asthma after allergen exposure is rarely obtained. At the severe end of the asthma exacerbation spectrum, as exemplified by episodes that are associated with school absenteeism or hospital admission (reviewed in ref. 8), the most notable clinical feature is the concomitant presence of viral LRI. A variety of evidence suggests that this susceptibility is determined in part by aberrations in host antiviral immunity involving type 1 and/or type 3 interferons (IFNs)^{19–22}. However, it is noteworthy that >80% of affected children are also atopic, and this comorbidity is a prominent feature of severe asthma exacerbations among adults as well⁸. This pattern is also reflected in children with less severe asthma, among whom the severity and duration of respiratory-virus-associated illnesses and the accompanying loss of asthma control are associated with an underlying atopy¹¹. Emerging evidence suggests that additional features of these more severe asthma episodes are covert interactions between virally-triggered and allergen-triggered inflammatory pathways, as discussed below.

Viral-induced perturbation of immunological homeostasis

As noted above, viral infections perturb a range of mechanisms that are central to the maintenance of immunological homeostasis in the airways.

BOX 1 Initiation, progression and persistence of asthma in individuals with atopy

Normal lung function develops along percentiles^{76,77} in a fashion similar to normal height, and disturbance to lung growth in early life can alter lung function permanently. The relevant lung growth processes, which proceed most rapidly during the first 2–3 years of life, include (i) gross structural alterations, such as progressively increasing airway diameter; (ii) more subtle processes, including alveolarization and associated changes in the airway-lining epithelia; and (iii) establishment of neural control of airway smooth muscle and local irritant receptor systems⁷⁸. It has become apparent that environmental exposures that interfere with normal lung growth during this crucial period have the potential to limit an individual's capacity to attain optimal respiratory function^{79,80}. The result of this interference is that the lung growth trajectory of an affected individual is dislodged, potentially permanently, onto a lower percentile line, and, thus, the individual is at an increased risk for asthma. A classic example is maternal tobacco smoke exposure during pregnancy that results in reduced respiratory function in her offspring at birth, which subsequently 'tracks' into later childhood and adulthood. Additionally, postnatal inflammatory insults to the growing lung that are associated with viral infection⁴ and acute asthma exacerbations⁸¹ also exert potent deleterious effects on the development of healthy respiratory function that can persist into later life. The precise mechanisms underlying this tracking phenomenon are probably multifactorial in nature and may include tissue remodeling changes associated with alterations in airway epithelial and myelofibroblast populations (the epithelial-mesenchymal trophic unit^{82,83}).



A key cell population in this regard comprises the airway mucosal dendritic cells (AMDCs), which express the immature antigen-surveillance phenotype that they normally maintain until migration to the regional lymph nodes (RLNs), where they deliver a signal that is weakly T_H2 polarized that precedes the induction of tolerance²³. During the course of viral infection, murine respiratory tract dendritic cell populations go through a variety of changes that potentially influence the tonus of the local immune surveillance mechanisms. One such change is the accumulation of plasmacytoid dendritic cells (pDCs)²⁴, which normally comprise a minor subpopulation in resting airway mucosa. These cells are postulated to play a crucial part in mucosal tolerance²⁵ and are also a major source of the IFNs that mediate the primary defense against viral infection.

A feature of ongoing immune surveillance within the airway mucosa is the orderly turnover of resident myeloid dendritic cells (mDCs), as replacements for emigrating antigen-bearing mDCs trafficking to the RLNs are continuously recruited from bone marrow²³. In rats, this process is markedly accelerated during parainfluenza infection²⁶. Comparable AMDC mobilization has been confirmed in mouse models^{24,27} and in virally infected children²⁸. Notably, expansion of the AMDC population persists well beyond parainfluenza clearance²⁶, and similar findings apply to RSV infection^{24,27,28}. The mechanistic basis for these persistent post-viral effects on AMDCs remains to be determined.

It is noteworthy that marked changes have been observed in other lung cell populations in infected mice that also persist after viral clearance²⁹. Prominent among these populations are macrophages expressing the 'alternative activation' phenotype that is dependent on IL-4 or IL-13, and similar cells have been reported in lung biopsies from a small sample of patients with asthma and chronic obstructive pulmonary disease²⁹ and from bronchoalveolar lavage fluid derived from subjects with pulmonary fibrosis³⁰. More detailed study of the activation phenotype of lung macrophages in individuals with asthma after exacerbation merits high priority given the long life span of these cells and, thus, their potential to influence the local tissue milieu for prolonged periods after a transient activation event.

Linking antiviral and atopic inflammatory pathways

Recent investigations have suggested that in addition to changes in the population dynamics of lung myeloid cell populations, viral infections may also profoundly influence their functional phenotype. Of particular relevance to this discussion are findings from Grayson and colleagues showing type 1 IFN-dependent induction of the high-affinity IgE receptor (FcεR1) on lung dendritic cells in the wake of murine parainfluenza infection^{31,32}. The authors suggested that subsequent crosslinking of this receptor by viral-specific IgE may lead to the chemokine (C-C motif) ligand 28 (CCL28)-mediated recruitment and subsequent local activation of virus-specific T_H2 memory cells, thus providing a secondary source of proinflammatory cytokines in the already inflamed airways. The potential implications of a similar mechanism operating against a background of conventional aeroallergen sensitization were not explored in this model. However, a more recent study on the effects of influenza in mice sensitized to house dust mites confirmed upregulation of FcεR1 in infected lung tissues and linked this upregulation with an increased responsiveness to concomitant exposure to aerosolized house dust mites, which manifested as enhanced T_H2 cell-associated airway inflammation and decreased lung function³³.

Severe asthma exacerbations in children: recent insights

As has been previously noted⁸, a small subset of asthma exacerbations in children are sufficiently severe to require hospitalization, and the children with these severe exacerbations typically have ongoing high-level inhalant allergy together with a concurrent respiratory viral infection. We recently

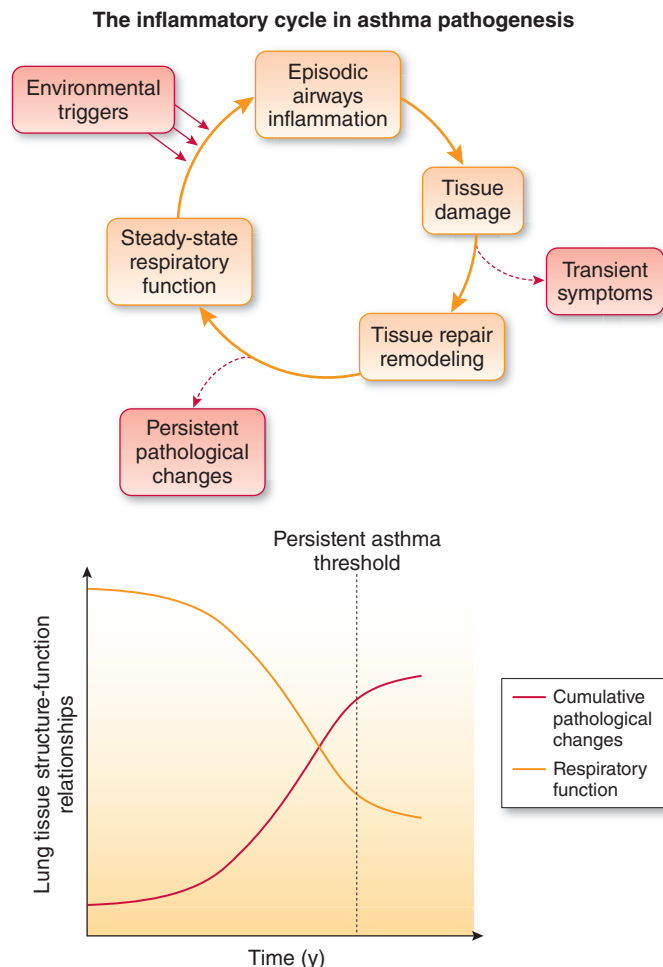


Figure 1 The inflammatory cycle in asthma pathogenesis. Asthma development is driven by repeated cycles of inflammation triggered by airborne irritant stimuli (top). Symptoms are initially intermittent and are associated with acute inflammation and edema and intermittent airway narrowing. Over time, the resolution of inflammation between clinically apparent episodes of asthma becomes less complete. Persistent inflammation leads to repeated cycles of tissue repair and regeneration, which may themselves be aberrant, and can lead to pathological changes that persist for long periods. As these changes accumulate, they lead to progressive deterioration in respiratory function (bottom). Once these changes exceed a critical threshold, they may not be reversible and may result in persistent asthma, with persistent symptoms that are not easily controlled by currently approved medications.

studied a cohort of such subjects³⁴ using an approach that was derived from earlier literature, which established that challenge sites in the lung signal to the bone marrow to recruit replacements for the myeloid cells that are engaged in antigen clearance during inflammatory episodes³⁵. This process also involves selective preprogramming of effector functions that are dictated by the mix of incoming inflammatory signals reaching the bone marrow, thus equipping emigrating cells to deal optimally with the specific challenge involved³⁶. Sampling blood-borne cell populations during inflammation as opposed to at baseline may provide some insight into the nature of the signals emanating from the challenge site and the resultant functional programming of migratory effector cells without directly accessing the challenge site itself.

Adopting this approach, we profiled circulating peripheral blood mononuclear cells from children with atopy who were hospitalized for severe

viral-induced asthma using a combination of microarray, quantitative RT-PCR and flow cytometry, comparing samples collected at admission to those collected during the subsequent convalescence³⁴. The most prominent exacerbation-associated signatures in these subjects were within circulating myeloid populations (monocytes, mDCs and pDCs) and were indicative of IL-4- or IL-13-dependent activation and type 1 IFN signaling. Moreover, strong quantitative relationships were observed between the expression of individual genes and the intensity of the exacerbation³⁴. These included *CCR2*, encoding chemokine (C-C motif) receptor 2, a major chemokine receptor directing migration to inflamed lung³⁷, together with *IL13RA2* (encoding IL-13R) and *CD1d*; upregulation of these genes was reproduced *in vitro* by the incubation of monocytes with recombinant IL-4 or IL-13 (ref. 34). The myeloid exacerbation signature also included FcεR1-γ, which was inducible *in vitro* by type 1 IFN, and parallel upregulation of FcεR1-α was shown both on monocytes and on the major dendritic cell subsets using flow cytometry³⁴.

The cytokine signals that are relevant to this systemic mechanism are probably transmitted from the airways to the bone marrow in a cell-associated form (Fig. 2). Indeed, the bone marrow is now recognized as a key repository for migratory T memory cells³⁸, and the *in situ* activation of these cells by immigrant antigen-bearing dendritic cells has also been formally shown³⁹. Additionally, preactivation of these T memory cells may occur at sites of allergic inflammation, as has been inferred in human studies that identified IL-5-secreting T_H2 cells in bone marrow aspirates immediately after bronchial allergen challenge¹⁸. Type 1 IFN-dependent preprogramming of lung-homing leukocytes in bone marrow has also been shown during respiratory viral infection⁴⁰, and although the source of the IFN signal was not identified, the pathway through which tissue-derived dendritic cells can bypass the RLNs and traffic directly to bone marrow³⁹ may be involved.

It is noteworthy that FcεR1-α is known to be constitutively hyperexpressed on myeloid cells in individuals with atopy⁴¹, and the translocation to the cell surface and stabilization of FcεR1-α as functional FcεR1-αγγ complexes is limited by the availability of the γ chain^{42,43}. Consequently, upregulation of the γ chain gene in myeloid cells from individuals with atopy by type 1 IFN, as was shown in our *in vitro* studies³⁴, provides a potential mechanism for increasing the overall expression of functional FcεR1, similar to what has been reported in the atopic asthma mouse model^{32,33} (Fig. 2). If this is the case, viral-triggered release of type 1 IFN in the infected airway may initiate this cascade through direct effects on resident AMDCs, and this cascade may be further amplified as inflammation associated with T_H2 cells develops in the mucosa and the resultant cell trafficking delivers the optimal combination of cytokine signals required to drive expression of both the FcεR1-α and γ chain genes in the myeloid precursor compartment. This mechanism may explain the presence in the circulation of monocytes and dendritic cells bearing functional FcεR1 in conjunction with gene signatures that are indicative of biomarkers of the alternative activation phenotype, which includes the FcεR1 γ chain itself plus the chemokine receptors required to facilitate their homing to the lung and airways to replenish local populations³⁴. This systemic mechanism may function in virally infected children with atopy in parallel with the locally operating mechanisms described in murine parainfluenza models that stimulate the expression of the same range of T_H2-associated phenotypic markers on resident lung and airway myeloid cells^{29,31,32}.

One consequence of FcεR1-αγγ upregulation on AMDCs in virally infected individuals with atopy is suggested by observations relating to the pathogenesis of atopic dermatitis. Notably, studies on lesional sites in atopic dermatitis have indicated that if local dendritic cells armed with functional FcεR1 are coexposed to specific IgE and allergen, their capacity to capture, process and present activation signals to T_H2 memory cells is markedly amplified^{44,45}. The preconditions of local availability of specific

IgE and the relevant allergen would probably be met in the airways of many exacerbation-prone children with asthma among whom presensitization to perennial aeroallergens is common⁸. If both the specific IgE and relevant allergen are present, increased FcεR1-αγγ expression on the AMDCs triggered by virus should result in enhanced T_H2 cytokine release in the airway mucosa, increasing local inflammation in the infected airway (Fig. 2). A further consequence of increased FcεR1-αγγ expression may be a prolongation of the initiating infection itself through T_H2 cell-mediated antagonism of antiviral immunity, mirroring 'immune evasion' strategies that are used by a range of other pathogens^{46,47}. This conclusion is consistent with a recent report on sputum-derived cells collected from children during severe viral-induced asthma exacerbations indicating the upregulation of T_H2-associated gene signatures, including *Fcer1a*, *IL5* and *IL13* with reciprocal downregulation of the T_H1-like/cytotoxic effector pathway⁴⁸. It is also pertinent to mention a recent report that found that triggering of FcεR1 on pDCs attenuates their capacity to produce type 1 IFNs⁴⁹, thereby blunting the primary antiviral defense.

Protection against the spread of pathogens beyond the initial infection site is the primary role of type 1 IFNs, and the LRIs that are associated with asthma exacerbations reflect an initial failure of these primary defenses. Airway epithelial cells from individuals with asthma reportedly have a reduced capacity for the production of type 1 and type 3 IFNs^{20,21}, which may increase these individuals' susceptibility to infection. However, in terms of asthma exacerbations, the implications of variations in IFN-response capacity may be more complex and context dependent, given that several IFN-producing cell types (epithelia, neutrophils and pDCs) may be involved at various stages in the exacerbation cycle. Additionally, we have shown that although IFN-α efficiently activates myeloid FcεR1-γ gene transcription if present at sufficiently high concentrations, it can also provide feedback inhibition of IL-4- or IL-13-mediated FcεR1 α-chain induction³⁴, which is consistent with a longstanding reports in the literature regarding the allergy-antagonistic effects of this cytokine. This suggests that the same type 1 IFN signal that potentially initiates this FcεR1-dependent cascade through direct effects on resident AMDCs may eventually terminate the bone-marrow-dependent amplification loop that sustains the response, provided it accumulates in sufficient quantities in the bone marrow.

FcεR1 expression on myeloid precursors during the 'atopic march'

Whereas the overall scenario described above (Fig. 2) may be restricted to the more severe manifestations of virus-associated asthma, the underlying mechanism involving communication between atopic lesional sites and myeloid precursor populations seems to operate covertly at lower intensities across a broad atopic disease spectrum. Three observations are consistent with this suggestion. First, blood monocytes from individuals with atopy constitutively hyperexpress FcεR1-α at levels that correlate strongly with their serum IgE titers⁴¹, which can now be understood as precursor activation through a chronic drip feed of T_H2 cytokine signals from sites of allergic inflammation to the bone marrow. Second, IL-5 signals triggered in the airways by aeroallergen challenge of individuals with atopic asthma¹⁸ (and sensitized mice⁵⁰) leads to the activation of eosinophils in bone marrow and their subsequent trafficking back to the lung. And third, findings have indicated that during active flares of atopic dermatitis, rhinitis and atopic asthma, FcεR1-α expression is upregulated on dendritic cells in the respective target tissues (relative to when disease is quiescent) and that this transient upregulation is mirrored in dendritic cell populations in unaffected tissues in the same subjects⁵¹; this 'spillover' phenomenon is explicable via the operation of the atopic lesion-bone marrow axis, given that dendritic cell populations in all tissues are continuously renewed from the same precursor compartment.

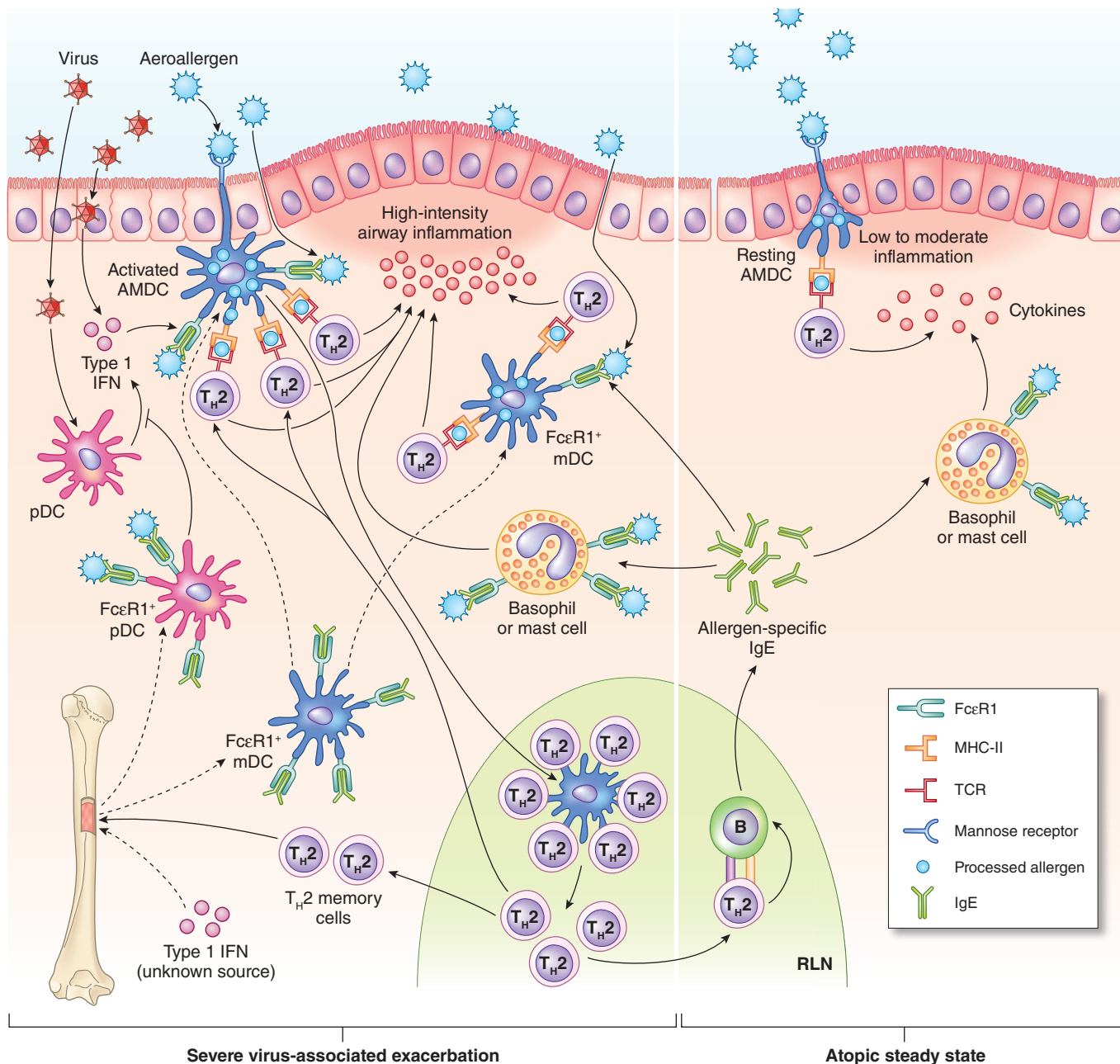


Figure 2 Cellular processes underlying virus-associated atopic asthma exacerbations. In individuals with stable atopic asthma, ongoing aeroallergen exposure sustains a state of relatively low level T cell-mediated and granulocyte-mediated inflammation in the airway epithelium that is punctuated occasionally by episodes of moderate intensity inflammation, for example, when aeroallergen exposure levels 'spike'; however, the potential for the local expression of high-intensity T_H2 cell-mediated inflammation is normally limited by restriction of the functional phenotype of the resident AMDCs to antigen uptake and processing with minimal presentation. This ongoing 'steady state' is perturbed by the local release of type 1 IFN in response to viral infection, which sets in motion a cascade that is initiated by the upregulation of turnover and FcεR1 expression on resident AMDCs, leading to increased recruitment and local (mucosal) activation of T_H2 effectors from the RLN and the subsequent expansion of T_H2 memory cell clones in the RLN by migrating AMDCs. The ensuing translocation of cytokine signals to the bone marrow by migrating T_H2 memory cells together with a source of type 1 IFN results in the release into the circulation of functionally upregulated myeloid populations, which home to the lung to further amplify local inflammatory responses. An additional short-term consequence is attenuation of local type 1 IFN production by pDCs through activation of their surface FcεR1, potentially contributing to viral persistence. The kinetics of this overall process are incompletely understood and may be highly variable; in particular, upregulation of myeloid cell populations in the lung and airways that persists long after viral clearance has been reported in both human and experimental systems, and the underlying mechanisms for persistence are unknown. B, B cell; MHC-II, major histocompatibility complex type 2.

These observations, together with those discussed in the previous section, suggest a plausible mechanism for what has been termed the 'atopic march', which is the characteristic pattern of atopic disease development during childhood involving the progression from initial atopic dermatitis

and/or food allergy to rhinitis and eventually asthma⁵² (Fig. 3). The operation of this axis provides a means by which active atopic disease in one organ system can facilitate the expression of disease in a secondary (previously unaffected) tissue by enhancing the efficiency of

the functions of local antigen-presenting cells through myeloid FcεR1 upregulation, thus optimizing the conditions for the local activation of allergen-specific T_H2 memory cells in the secondary tissue. The example in **Figure 3** draws on the literature showing, first, that aeroallergen sensitization in the absence of asthma symptoms is the rule rather than the exception¹⁷ and, second, that allergic rhinitis is a strong risk factor for subsequent asthma⁵². In the scheme shown, active expression of allergic inflammation in the nose (rhinitis) increases the likelihood that inhalation of an aeroallergen will trigger clinically relevant levels of T_H2 cytokine secretion in the conducting airway mucosa by indirect upregulation of local FcεR1-dependent functions of the antigen-presenting cells. Over time, this may accelerate the progression toward expression of persistent airway disease (asthma), which is dependent on accumulation of pathological changes above a critical threshold in target tissues (**Fig. 1, bottom**).

It is worth noting that a related mechanism has been proposed based on murine experimental atopic dermatitis studies showing aggravation of asthma-like manifestations in the airways through hyperproduction of thymic stromal lymphopoietin (TSLP) by epidermal keratinocytes^{53,54}; recent evidence suggests that this aggravation may also be mediated by effects in the bone marrow, possibly involving the stimulation of basophil hematopoiesis⁵⁵.

The respiratory microbiome: a role for bacteria in asthma?

Opportunistic bacterial infections have long been recognized as potential contributors to the morbidity associated with viral LRIs, and this issue has arisen again in relation to the link between respiratory infections and asthma in light of two lines of recent evidence. First, using traditional culture methodology, it has been shown that early nasopharyngeal colonization with bacterial pathogens is associated with an increased risk for childhood asthma⁵⁶. Recently, the application of sensitive metagenomic technology has revealed the presence in airway aspirates and tissue samples of a hitherto unrecognized microbiome comprising multiple bacterial strains^{57,58}. These findings relate both to healthy individuals and individuals with asthma. Although bacterial diversity and density are increased in individuals with asthma, these are nevertheless multiple species that are also present in healthy individuals, suggesting that efficient homeostatic mechanisms must operate continuously in the lower respiratory tract to maintain a local steady state. Nevertheless, the constant presence of bacteria on the airway surface, particularly in atopic asthma, in which disturbance of epithelial integrity secondary to local inflammation is a characteristic feature, poses a series of quandaries relating to pathogenesis which have yet to be addressed.

From an immunological perspective, one window into the underlying relationships between a host and its respiratory microbiome is through an assessment of humoral immunity to index organisms, and some recent studies on the responses to protein antigens from *Haemophilus* and *Streptococcus* have provided interesting pointers for the future. Children who develop early asthma and atopy have lower serum concentrations of bacterial-specific IgG1 relative to the overall population^{59,60}, as do

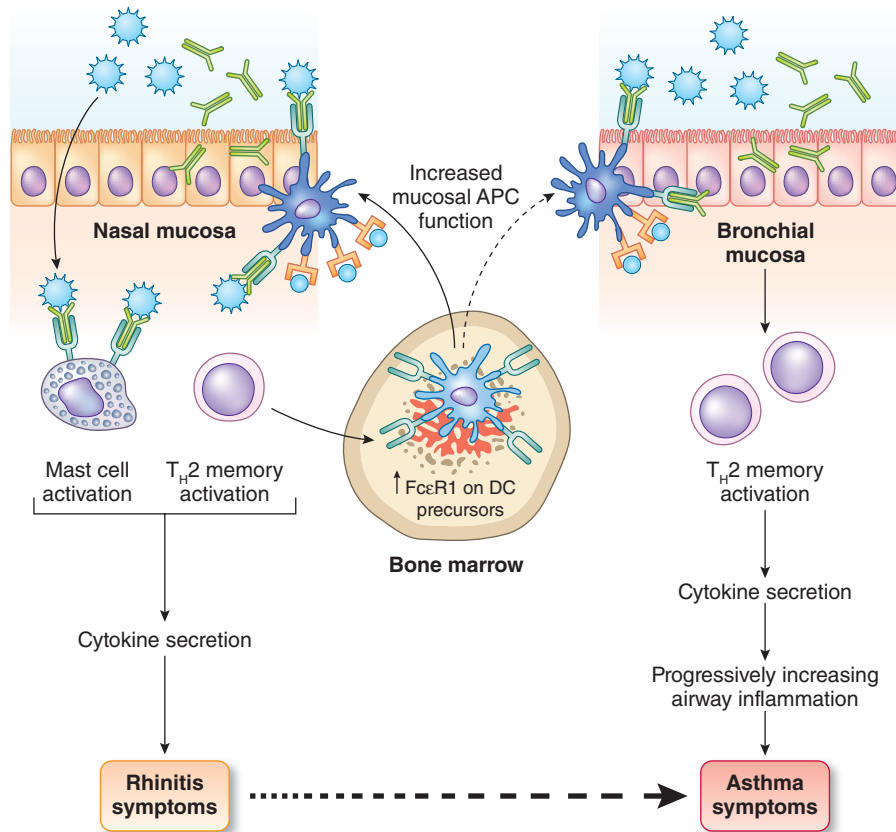


Figure 3 The atopic march unmasked? Findings summarized in the text provide a plausible mechanism for a longstanding but poorly understood clinical paradigm in the allergy field, notably the atopic march, which is exemplified by progression from allergic rhinitis to asthma. DC, dendritic cell.

children who are susceptible to viral-triggered asthma exacerbations⁶¹. Reduced IgG1 concentrations in this context suggest an increased susceptibility to transmucosal bacterial incursion and may also connote underlying deficiencies in immune surveillance mechanisms. In this context, it is pertinent to note reports suggesting enhanced susceptibility to invasive pneumococcal disease in individuals with asthma^{62,63}.

Providing a second line of evidence, IgE responses against *Haemophilus* and *Streptococcus* are found in virtually all teenagers and, surprisingly, associate with a reduced susceptibility to asthma⁶⁴; follow-up studies in 5-year-old subjects indicated a similar relationship⁶⁰. Based on an extensive literature from other areas of inflammation biology, it seems probable that this bacterial-specific IgE may be a surrogate for underlying T_H2 cell immunity, especially for specific T_H2 memory cells producing IL-4 or IL-13. In this context, one of the most potent inflammatory responses involves interactions between bacteria and tissue macrophages, triggering the secretion of cytokines including IL-1 and tumor necrosis factor α (TNF-α), and this process is strongly inhibited by IL-4 or IL-13 (reviewed in ref. 64). Recirculating bacterial-specific T_H2 memory cells may serve this purpose in airway tissues, mitigating the inflammatory responses against low-level bacterial incursions across inflamed airway epithelial barriers that may be a relatively common occurrence. In this regard, it should be noted that bacterial-specific IgE concentrations increase in the wake of viral-associated asthma exacerbations⁶⁰, which is consistent with 'boosting' by an incoming bacterial antigen.

The question of why IgE against *Haemophilus* and *Streptococcus* do not apparently provoke mast-cell-mediated inflammation may reflect the fact that the relevant antigens are only released as membrane-associated

complexes that are accessible to phagocytes but are unsuitable for FcεR1 crosslinking. In contrast, IgEs against *Staphylococcus* superantigens that are secreted in a soluble form associate strongly with airway inflammation in the same children (reviewed in ref. 64).

New opportunities for prevention and treatment of asthma

Efforts over the last 20 years to develop improved therapeutics for atopic asthma have focused primarily on T_H2 effector mechanisms in subjects with established disease. These include biologics and small-molecule antagonists specific for T_H2 cytokines and the IgE antibody itself. Many of these drugs are highly effective at blanketing their respective targets, but in the contexts in which they have been tested in clinical trials, none have provided evidence of long-term disease modification. Part of the problem is that the pathological processes described in **Figure 1** become progressively less reversible over time. Additionally, it is becoming apparent that subphenotypes of established asthma exist that vary subtly with respect to the relative contributions of different inflammatory pathways to pathogenesis^{65,66}. We argue, however, that an equally crucial issue is the lack of alignment of therapeutic strategies with the growing body of epidemiological and clinical information on how patterns of disease expression evolve over time under the influence of different classes of environmental triggers and, in particular, how interactions between the ensuing pathological processes contribute to symptom severity at different stages of disease. Bringing these issues to the forefront of the considerations regarding to target identification and clinical trial design provides new perspectives on drug development in asthma that might logically lead to new approaches to treatment and, more crucially, to asthma prevention (**Box 2**). Prominent among these approaches are a series of strategies based on using existing drugs in nonstandard clinical contexts that are outside the current treatment paradigms. From these we nominate below a high-priority list of treatment options that, based on available safety and efficacy data, are amenable to immediate testing.

Treatment of established atopic asthma. The most crucial targets in established asthma are the acute exacerbations triggered by a virus and/or aeroallergen. Current clinical practice involves the use of inhalers to relieve symptoms of airway narrowing together with nonspecific anti-inflammatory drugs (typically corticosteroids). However, this approach is essentially empirical and takes no account of the efficiency of these drugs in controlling individual rate-limiting steps in the cycle that drives the exacerbation. The scheme in **Figure 2** and the proof-of-concept studies cited in **Box 2** provide a rationale for the use of anti-IgE and a topical IL-4/IL-13Rα antagonist in this context, specifically targeting AMDC-driven or FcεR1-facilitated aeroallergen presentation and the ensuing activation of T_H2 memory cells in the airway mucosa and RLNs; these are the key steps in the underlying inflammatory cascade, and blocking either step sufficiently early in the cycle (meaning close to symptom onset) may halt its progression toward bone marrow amplification. Later in the cycle, an IL-4/IL-13Rα antagonist given systemically may be the ideal candidate drug to truncate the downstream events in the bone marrow, notably the IL-4- or IL-13-dependent generation and programming of CCR2⁺ lung-homing alternatively activated macrophages and the FcεR1^{hi} replacements for airway dendritic cells.

Halting the progression of atopic asthma during childhood. Atopic asthma progresses through recognizable stages during childhood (**Box 1**), and there is growing interest in early identification of children already on a trajectory toward asthma to institute treatments to reduce the frequency of the inflammatory episodes driving disease progression and consolidation. The priority targets are as follows.

(i) Virus-associated exacerbations. The most persuasive recent data supporting this approach are from the US Inner City Asthma Consortium showing that children on monthly anti-IgE therapy had significantly

reduced rates of exacerbations, particularly during the common cold season⁶⁷. The seasonality of respiratory viral infections (especially rhinovirus) leaves open the strong possibility of ‘common cold season only’ prophylactic treatment of high-risk individuals with atopy as a viable public health measure. An additional possibility meriting testing is treatment during the common cold season with oral immune enhancer (IE) preparations⁶⁸, particularly those can stimulate mucosal regulatory T (T_{reg}) cell activity^{69,70}, such as those described in **Box 2**.

(ii) Inhalant allergy: halting the atopic march. The burgeoning area of immunotherapy focuses primarily on ‘curing’ allergic diseases. However, in light of the evidence that during childhood one form of allergy can lead to another (shown in the link between allergic rhinitis and subsequent asthma in adults and children^{71,72} and **Fig. 2**), early immunotherapy in appropriately selected subjects will also probably be useful for preventing disease progression, and this idea is supported by proof-of-concept studies on asthma prevention in children with rhinitis⁷³. Notably, the key cellular process that is probably driving this example of the atopic march, namely mucosal dendritic cell trafficking, is highly sensitive to topical corticosteroids⁷⁴; therefore, despite the failure of long-term topical steroid use by inhaler to ameliorate established wheeze⁷⁵, nasal steroids merit testing as an asthma prophylactic in the specific context of children with allergic rhinitis who are asthma free.

The ‘holy grail’: primary prevention of asthma. The ultimate goal is to identify high-risk subjects in the infancy to preschool age range and institute measures to subvert atopic and viral-associated inflammation early enough to block the initiation of disease. As noted in **Box 1**, the wheezy phenotype shows a high degree of plasticity at these early ages but becomes progressively less reversible thereafter, marking this period as a potentially ideal therapeutic window for long-term disease modification. The atopy pathway is already being tackled using prophylactic immunotherapy (**Box 2**). However, it is not yet possible to conduct trials for anti-IgE in this context because safety data is lacking for this age group. However, this agent merits testing as soon as these data become available in light of a recent report showing the quantitative nature of the relationship between aeroallergen-specific IgEs and LRIs in early asthma development. Notably, the strength of the synergism between IgEs and LRIs increased linearly with antibody titers across the full range of LRI frequencies, even at IgE concentrations below the conventional sensitization thresholds.

Tackling the viral pathway in this young age group has been problematic. Whereas an effective antibody treatment for RSV exists (**Box 2**), it has not yet been made available for testing in this clinical context, despite strong support (which we endorse) from senior investigators in this field¹⁶. Alternative approaches include the use of preparations from the IE class, given its potential for reducing both infection frequency and intensity in preschoolers⁶⁸, and, potentially, type 1 IFNs.

Future priorities

The cycles of episodic airway inflammation (**Fig. 2**) are driven by migrating T_H2 memory cells and myeloid cells responding to chemokine gradients through specific receptors such as CCR2 and the chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRT_H2); targeting these players should be given high priority. Slightly over the horizon, rapid developments in relation to the airway microbiome are already creating expectations that antibacterials will soon join antivirals on the asthma agenda, despite justifiable concerns related to bacterial drug resistance. In our view, the key challenge is not the production of safe drugs that are effective against their designated targets (this will undoubtedly be achieved) but rather is to ensure that the clinical testing programs do justice to the upstream research and development efforts that led to their production. Achieving this for asthma will require a more disciplined approach to drug testing strategies than has been used in the past,

BOX 2 The development of rational strategies for asthma treatment and prevention

Respiratory infection resistance

Antivirals. A monoclonal antibody (mAb) for the treatment of RSV is available and has been shown to markedly reduce severe infections in infants⁸⁴.

Orally delivered immune enhancers (IE). This area is dominated by probiotics, and this approach has the potential for the mitigation of respiratory infection severity in children⁸⁵. A related class of therapeutics derived from mixed bacterial extracts has been used extensively to enhance infection resistance in patients with chronic respiratory diseases, for example, chronic obstructive pulmonary disease^{86,87} and has been shown more recently to reduce the frequency and intensity of respiratory infections in young children^{68,88}.

Type 1 IFN. The use of IFN therapy for the prevention of viral infection has a checkered history, particularly in terms of side effects. In a recent development, a nasal spray containing low-dose recombinant IFN- α -2b reduced influenza, parainfluenza and adenovirus infections in military recruits⁸⁹.

Airway inflammation

A topical antagonist to IL-4/IL-13R α . Studies with an aerosolized antagonist reported significant reduction in the T_H2 cell-dependent late-phase response to aeroallergen challenge in individuals with atopic asthma⁹⁰.

MAb to IgE. An mAb to IgE has been tested in a variety of clinical contexts since its initial release over 10 years ago, but the clinical effects achieved in conventional trial designs have been modest; recent studies have shown that continued use of these mAbs can reduce the frequency of exacerbations^{67,91,92}.

T_{reg} cell stimulants. An unexpected recent finding in recent preclinical studies in rodent asthma models suggests that a major target for one class of the immune enhancer preparations is the expansion of mucosal-homing T_{reg} cell population(s) that dampen airway inflammation^{69,70}. This may explain their effects in reducing the clinical symptoms associated with respiratory infection⁶⁸.

Myeloid cell activation in bone marrow

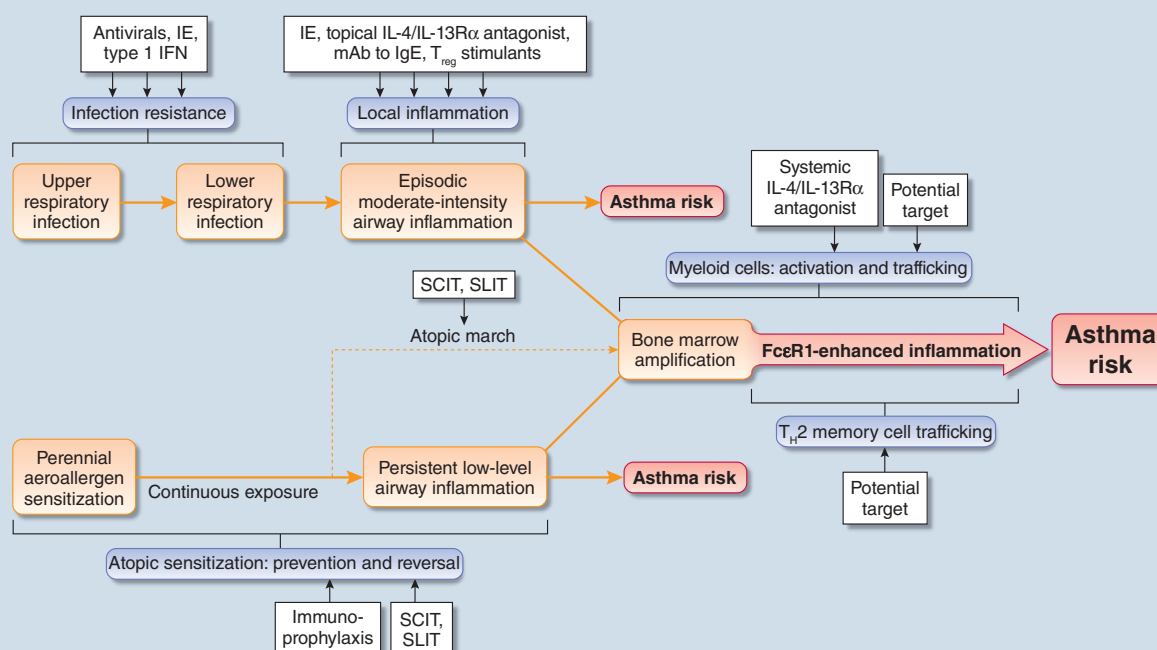
A systemic antagonist to IL-4/IL-13R α . The agent detailed in reference 90 was equally potent in reducing the asthma late-phase response when delivered systemically in a form that would be bioavailable in bone marrow (and is also probably effective in airway RLNs).

The atopy pathway

The concept of immunoprophylaxis (primary prevention of sensitization) in infants and young children with oral or sublingual allergen is currently being tested in trials sponsored by the US National Institutes of Health; extensive literature exists on immunotherapy for sensitized children and adults, in particular, proof-of-concept studies showing that desensitization of children with rhinitis who are allergic to pollen using subcutaneous immunotherapy (SCIT) prevents the subsequent development of asthma⁷³ and studies on recently developed sublingual immunotherapy (SLIT⁹³).

Gaps in the armor

Currently unavailable but highly relevant are drugs targeting the trafficking of myeloid and T_H2 cells. Also, as noted previously, this inflammatory cycle also has the potential for progression to a later phase that involves autocrine production of IL-13 by alternatively activated macrophages that may persist beyond viral clearance²⁹, and this cycle is potentially targetable with an mAb to IL-13 (ref. 94).



with a sharper focus on the clinical context within which individual targeted mechanisms contribute to symptoms at different stages of disease. In terms of existing drugs, specifically those targeting IgE and IL-4/IL-13R α , we have only scratched the surface with respect to their optimal clinical use in asthma treatment, particularly from the perspective of preventing progression to a chronic state.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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