

Editorials

Where are the gaps in asthma research? A counter-perspective

A few weeks ago, I naively accepted the task of writing a commentary to accompany the research priorities of asthma experts from the United Kingdom. What I didn't realize at the time was that there would be so many of them—ie, so many priorities (and experts). And while it is impressive that this is the case, I do not have nearly enough space to provide commentary on each of the directions. So instead I have taken the opportunity to look for the gaps in what has been proposed as the to-do list for understanding asthma—and to try to make the case that despite the great insight of the British perspective, there are still some missed, and potentially critical, opportunities for better understanding of this complex disease and the development of better treatment for it.

Let me set the background for any new ideas by first issuing a generic plea for future asthma research: *There is a need for a multidisciplinary and translational strategy*. Why is that important? Common diseases in general, and asthma in particular, are likely to be multifactorial and multigenic. In addition, the lung exhibits a limited set of responses, so there is significant overlap in the phenotype that is observed among different diseases. This complexity poses significant challenges for developing complete and accurate models of lung disease that can be used for understanding pathogenesis, and in particular for the current emphasis on genetic and genomic analysis. Nevertheless, a layered strategy that includes *in vitro* and *in vivo* systems can offset these limitations. *In vitro* systems have evolved from simple organ culture (eg, smooth muscle baths and tracheal tissue explants) to intricate procedures for cell culture. Culture systems now exist for the preservation of airway cell differentiation, and these systems exhibit high fidelity to behavior *in vivo*. Similarly, *in vivo* systems have evolved from traditional physiology-based models in large animals and rodents to the current studies of genetically homogeneous mouse strains as well as mice subjected to genetic modification by targeted null mutations or transgenic expression. The development of lung-specific promoter systems that can be conditionally regulated has added

another useful dimension to this approach. In addition, several tools (eg, endobronchial biopsy or brushing, bronchoalveolar lavage, and new imaging techniques) and new protocols (eg, glucocorticoid withdrawal) can directly monitor the endogenous behavior of the human airway. A well-coordinated assessment of representative *in vitro* systems, animal models, and human subjects represents a significant strength for approaches to complex lung diseases. Asthma is a perfect paradigm for the application of these principles, and this multidisciplinary, translational approach remains critical to identifying and pursuing new directions in asthma research.

Where's the alternative beef?

Given the breadth and depth of the British perspective on asthma research, where could one possibly find a gap? Let me formulate a possible fissure with a piece of data: If a count is made of the number of times that allergy or atopy is mentioned in the British approach, even discounting indirect references such as "challenge" or "tolerance" or "immunotherapy," one finds 20 such references; if, on the other hand, a count is made of the number of times that virus or epithelium is mentioned, one finds just a single instance of each—and even in these cases, the approach is not developed beyond a simple phrase. Similarly, when one tabulates the suggestions for new therapeutic approaches, it is found that only immunotherapy directed to regulating the allergic response is given significant priority. Even when other possibilities are raised (eg, pharmacogenomics), the approach is exemplified through use of a mediator of the allergic response—ie, leukotriene generation. Inasmuch as the summary is being presented in a journal of allergy, perhaps this bias is appropriate to the context. Is it, however, also appropriate, at this point, in terms of what we know about the development of asthma and in terms of our success (or lack thereof) with effective and complete treatment?

The reader has probably learned the long history of the role of allergy in asthma; if not, full reviews (from inside and outside the United Kingdom) are available to provide this information.^{1,2} Let me instead submit that several lines of evidence in model systems and in human beings raise questions about the role of allergy and the corresponding T_H2 bias as a sole explanation for asthma. For example, antigen-specific T_H2 cells are not sufficient for initiating the asthmatic phenotype in experimental models of allergy³; conversely, the phenotype might develop without notable allergic components, such as IgE production or eosinophilic inflammation.⁴⁻⁶ Perhaps these disconnects should not be surprising, given that in human subjects the

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development of allergy and the development of asthma are often dissociated as well⁷ and linkage to candidate genes for atopy might be absent in large segments of the asthmatic population.⁸ Moreover, treatment aimed at selective blockade of T_H2 pathways has not yet proved to be efficacious in asthma.⁹ Nonetheless, these discrepancies are generally ascribed to the complexity of the allergic response. Even given this diversity, however, an allergen-based proposal for pathogenesis provides little explanation for at least 2 major observations of the asthma phenotype: first, an invariant and persistent abnormality in airway epithelial programming toward an antiviral response that reflects a T_H1 bias¹⁰; second, in a related manner, the development of a chronic (likely permanent) phenotype in the face of a relatively short-term allergic response. Let me next try to address each of these issues, beginning with how the epithelial antiviral network becomes involved.

This line of reasoning originates from the concept that the adaptive immune system (manifested by the diverse repertoire of T cells and B cells) requires signals about the origin of the antigen and the type of response to be induced. Furthermore, these signals must be provided by the innate immune system. In this general context, and in the particular context of the response to inhaled agents, epithelial cells lining the airway surface represent an ideal candidate to act as a sentinel site in innate immunity. Indeed, we now recognize that epithelial-immune cell interaction depends on the expression of a subset of epithelial immune response genes under distinct transcriptional and posttranscriptional controls. At the transcriptional level, this gene network is regulated by IFN- γ signal transduction featuring the Stat1 transcription factor as a critical regulator of gene expression.^{11,12} At the posttranscriptional level, the gene network is regulated directly by viral replication (thereby bypassing the need for immune cell contributions) and is controlled by a complementary biochemical mechanism that relies on viral replication and consequent stabilization of mRNA.¹³ Remarkably, this entire antiviral network is invariably activated in otherwise healthy subjects with asthma, either under stable conditions or during flares of the disease.¹⁴⁻¹⁶ Accordingly, the usually normal response to viral infection is also a permanent signature of the abnormal response in asthma.

These findings highlight the role of epithelial reprogramming in asthma and lead to an alternative paradigm for asthma pathogenesis that includes epithelial, viral, and allergic (epi-vir-all) components.¹⁰ However, asthma is probably a lifelong abnormality and is strongly influenced by genetic background, so this revised model still must address the issues of chronicity and causality. Indeed, we now recognize that at least experimentally, viral infection can lead to a chronic asthma phenotype.¹⁷ Moreover, acute inflammation and concomitant postviral airway hyperreactivity can be genetically segregated from the persistent asthma phenotype. And while a similar response is also inducible by allergen challenge, in this case the phenotype resolves spontaneously with time. The findings establish the capacity of a single

paramyxoviral infection to cause both acute and chronic manifestations of the phenotype for hypersecretory airway disease and define the relevance of specific host defense genes in moderating the acute but not necessarily the chronic phenotype. In addition, the results provide initial evidence of the capacity for nononcogenic riboviruses (ie, asthmagenic paramyxoviruses) to irreversibly reprogram host cell behavior in a manner previously restricted to oncogenic DNA viruses.¹⁸ These findings therefore raise the possibility that asthma not only resembles a persistent antiviral response but might even be caused by it; they thus provide the experimental link between paramyxoviral infection in infancy with subsequent asthma in childhood and perhaps adulthood.

Unresolved questions for the viral cascade

The new findings indicate that the interaction between paramyxoviruses and their human hosts can cause an effective switch to the asthma phenotype. If this is the case, a new set of questions, analogous to those already posed for allergen-driven disease, now arises for virus-driven asthma (Fig 1). These new queries include the following: What is the nature of the viruses that confer the asthma phenotype? What determines the susceptibility to developing it? How is the response initiated? How is memory for the response preserved? How is this memory effectively translated by effector cells into the asthma phenotype? Let me briefly address each of these uncertainties.

- *Nature of asthmagenic viruses.* The viral organism must be equipped with specific components to confer a long-term asthma phenotype. At the least, this will require viral genes encoding for products that target infection to the airways. At a more complex level, paramyxoviruses must be equipped to cause both acute airway inflammation/hyperreactivity and chronic airway remodeling/hyperreactivity phenotypes. The acute response is driven by viral replication, but the chronic one is based on a hit-and-run strategy, because viral effects persist after clearance. Inasmuch as the 2 host responses can be genetically segregated, it is likely that they are driven by distinct viral components. Further proof of and understanding of this process will depend on the identification of specific viral gene products responsible for altering host gene expression and consequent phenotype. If specific targets can be defined, then specific therapy—even vaccination strategies—can be applied to selectively reverse or prevent the development of the chronic phenotype.
- *Host susceptibility.* Initial results indicate that the chronic response to paramyxoviral infection develops only in a specific host genetic background. Moreover, the capacity to develop distinct postviral chronic phenotypes (eg, mucus cell hyperplasia versus airway hyperreactivity) can be segregated within a single host genetic background through use of chronologic and physiologic parameters. At present, we need additional genetic and genomic analysis to determine how these chronic asthma phenotypes segregate in mice

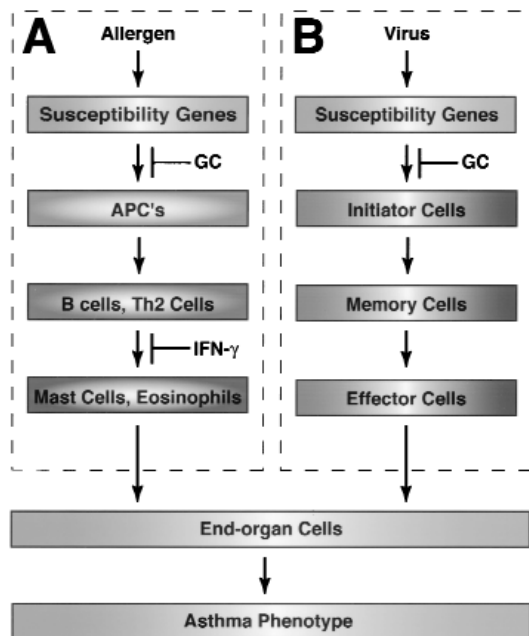


FIG 1. Scheme for parallel allergic and viral cascades for asthma pathogenesis and the research gaps in defining the virus-driven events. **A**, In genetically susceptible individuals, allergen is processed by antigen-presenting cells (APC's) and then leads to the involvement of B cells and T_H2 cells and subsequent activation of mast cells and eosinophils. These events combine to cause alterations in end-organ cells (eg, goblet cells and airway smooth muscle cells) to cause the asthma phenotype (eg, mucus hypersecretion and airway hyperreactivity). **B**, Viral replication, in susceptible individuals, can first activate host initiator cells (eg, airway epithelial cells, macrophages, and dendritic cells) and then lead to the generation of memory cells (eg, B cells and T cells) and consequent activation of effector cells (eg, virus-specific T cells and mast cells) to cause end-organ alterations that are characteristic of asthma. Allergen- and virus-driven pathways appear at least partially sensitive to downregulation by glucocorticoid (GC) at the initial as well as subsequent steps, but the allergic response appears selectively sensitive to inhibition by IFN- γ . Each of the steps in the viral pathway (including the nature of the virus) needs to be more completely defined, as outlined in the text.

and in human beings. A useful strategy will be to positionally clone the susceptibility genes for the asthma phenotype in the mouse model of viral bronchiolitis and then test for the relevance of candidate genes in mice and in human subjects. Human studies will need to be designed with the same biochemical parameters that are identified experimentally, and they will need to precisely validate and follow each parameter with and without treatment.

- **Initiation of the asthma phenotype.** Professional antigen-presenting cells serve to initiate the allergic response, and this step is followed by a series of well-defined events that result in cellular and humoral immune sensitivity. Comparable events that mediate the chronic response to viral infection still need to be determined. In particular, the possible role of the professional antigen-presenting cells (eg, dendritic cells) or nonprofessional host cells (eg, airway epithelial cells and macrophages) in triggering the alterations in the adaptive immune system still needs definition.

These studies should be aimed at the acute versus the chronic responses to viral infection and should attempt to segregate these responses on the basis of distinct molecular mechanisms. It will then be possible to develop strategies that enhance the acute antiviral events and downregulate the chronic maladaptive events that lead to the asthma phenotypes.

- **Memory for the asthma phenotype.** As noted above, the results suggest a hit-and-run hypothesis for the viral effect; even so, there must be some element of memory in the host tissue so that the phenotype switch can be preserved. Differential sensitivity to regulation by T helper cytokines (eg, IFN- γ) indicates that the memory compartment might be distinct for allergen-versus virus-induced asthma phenotypes.^{17,19} Furthermore, although the memory compartment for allergen is well defined, the corresponding compartment for virus is uncertain. A conspicuous candidate is suggested by the persistence of virus-specific T cells in the lungs,^{20,21} and subsets of this population have been incriminated as upregulating and downregulating the T_H2 features of the acute antiviral response.²²⁻²⁴ Moreover, the sensitivity of T cells and the virus-induced phenotype to glucocorticoid action reinforces T-cell candidacy for involvement.^{17,25} As noted above, however, these T-cell studies have focused on the acute response to virus, and the relevance of these cell populations (and other immune cell types) for persistent phenotypes must still be defined.
- **Effector cells of the viral response.** As was the case for the memory cell population, the identity of the effector cell population that drives the postviral chronic response also needs to be identified. For the allergic response, well-studied activities of mast cells and eosinophils, as well as $CD4^+$ T_H2 cells, lead to the generation of specific mediators that are capable of driving the asthma phenotype. The corresponding cell populations and mediators are largely undefined for the chronic asthma phenotype that develops in the setting of viral infection. As noted above, it appears unlikely that there will be complete overlap between the 2 cascades. Thus, identifying the effectors of the viral response is likely to provide distinct therapeutic strategies that have not yet been targeted for development. In this case too it is critical to define effectors of the acute versus the chronic response to successfully achieve increased antiviral immunity but decreased virus-induced asthma. Each of these endpoints could prove effective for disease intervention.
- **Changes in end-organ cells.** For both allergen- and virus-driven events, the precise consequences for end-organ cells in the airway need to be determined. In particular, the cellular and molecular events that mediate the development of mucus cell metaplasia (leading to hypersecretion) and smooth muscle hypertrophy/hypercontractility (leading to airway hyperreactivity) need to be defined. Only slight progress has been made in this area, leaving us with few drugs that selectively target these events in a disease-modifying manner. Virtually no

options exist for effective downregulation of mucus production, yet this is a major source of morbidity in populations with asthma, bronchitis, or bronchiolitis. Similarly, although we can block smooth muscle contraction effectively with β -adrenergic agonists, we have no means of regulating intrinsic aspects of abnormal smooth muscle behavior, such as hypertrophy, hyperplasia, and abnormal intracellular or paracellular signaling.

SUMMARY

Asthma is characterized by abnormal immune cell accumulation and activation in the airways and consequent dysfunction of specialized parenchymal cells. To date, research strategies aimed at defining asthma pathogenesis have focused on the hypothesis that this altered state is a consequence of an excessive allergen-driven response. However, this view might not fully account for the development of asthma and thus might not lead to curative therapeutics. In this context, it is relevant to consider that asthma might share with paramyxoviral infection a propensity to activate a network of epithelial immune response genes that are part of the innate immune response. More recent results extend this concept by establishing the capacity of a single transient paramyxoviral infection to permanently change airway epithelial behavior and reactivity in a pattern that is remarkably similar to one in asthma and that overlaps with other hypersecretory diseases. The teleology of this chronic response is uncertain, but it might represent an evolving but maladaptive attempt to improve antiviral host defense. Further studies are now required to precisely identify the cellular and molecular events responsible for chronic airway remodeling and hyperreactivity in response to paramyxoviral infection. Definition of the cellular and molecular pathways for viral reprogramming of airway behavior in isolated cell and mouse models will eventually translate into an understanding of how these pathways work in adult and pediatric populations with chronic airway disease, in which the link between viral infection and disease appears strong. Indeed, initial results indicate that viral infection in a specific genetic background might lead to chronic dysfunction of host cell behavior that overlaps with but does not depend on allergy, thereby providing a road on which to drive new disease-modifying strategies.

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