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Animal models of asthma: Reprise or reboot?

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ABSTRACT

Animal models of disease represent the pinnacle of hierarchical research efforts to validate targets and compounds for therapeutic intervention. Yet models of asthma, particularly in the mouse, which, for practical reasons, has become the *sine qua non* of asthma research, have been a bone of contention for decades. With barely a nod to their limitations and an extensive history of translational failures, they continue to be used for target identification and to justify the clinical evaluation of new compounds. Recent improvements – including sensitization directly to the airways; use of more relevant allergens; development of a chronic rather than short-term condition; utilization of techniques to measure lung function beyond uninterpretable measures of airway hyperresponsiveness – are laudable but cannot bridge the chasm between the models and the myriad complexities of the human disorder and multiple asthma endophenotypes. While further model developments are necessary, including recognition of key environmental factors beyond allergens, the judicious integration with newer ex vivo and in vitro techniques, including human precision-cut lung slices, reprograming of patient-derived induced pluripotent stem cells and fibroblasts to epithelial and smooth muscle cells, and use of other clinical samples to create a more holistic depiction of activities, might improve their translational success.

Contents

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1. Asthma: a complex immune disorder

Asthma is a chronic respiratory disorder where the main characteristic is variable and recurring episodes of airflow obstruction causing impaired breathing, which are reversible [1]. From this common trait, asthma rapidly becomes more complex in its definition. Aside from symptoms associated with narrowing of the airways – shortness of breath, wheezing, and chest tightness – the airways are inflamed and become hyper-responsive to various bronchoconstrictor agents and noxious stimuli (termed *airway hyperreactivity* or AHR). The pattern of features can vary between patients, as can their severity, ranging from mild asthma with occasional symptoms, to severe forms where the symptoms are persistent and impact daily life [2]. However, even patients with mild disease can suffer from severe acute attacks called exacerbations, where there is a rapid deterioration in lung function that is unresponsive to regular treatment. Over the long term airway remodeling with thickening of the epithelium and underlying smooth muscle can also lead to a fixed (non-reversible) obstruction of airflow [1]. Moreover, these different features of asthma, while being inter-related and acting in concert to create the disorder, probably have discrete causal mechanisms since they exhibit differential responses to different medications. For example, β2-adrenoceptor sympathomimetics dilate the airways but do not affect airway inflammation and do not suppress, and indeed may even increase, the incidence of asthma exacerbations [3]. In contrast, the anti-IgE monoclonal antibody omalizumab reduces exacerbations with relatively small effects on airflow [4].

Adding to the complex etiology of asthma is the recognition that there are both genetic and environmental factors that play significant roles in disease causality and occurrence. Twin studies indicate that genetics and environment each contribute about 50% of the risk to develop the disorder [5]. Simplistically, a principal viewpoint in the first half of the 20th century was that a large part of asthma was psychosomatic (even recommending treatment with a few whiffs of chloroform to relax the patient, or, the opposite approach, to take them toward the dentist's home to stress them into recovery). In contrast, the second half of the 20th century promoted the concept that asthma is an allergic disorder, and that the vast majority of asthma patients were atopic. More recently it has been recognized that approximately 50% of the US population in general is atopic, and since ~50% of the asthma population is also atopic it appears that it is not atopy per se that is the problem, but the asthmatic's response to particular allergens [6,7]. While up to half of asthma is still ascribed to atopy, there has been increased recognition of the contribution by other environmental and life-style factors such as pollution, cigarette smoke, chemicals, viruses, and diet. In contrast to the traditional view that the lower airways represent a sterile environment, a disordered airway microbial milieu might also contribute to the disease [8,9]. Changes to the gut microbiome also influence immune function [10]. As stated by Holmes et al. [11] "The realization that factors other than atopy are implicit in asthma has significant implications for disease modeling and drug development", but these have yet to be realized to any substantial measure.

Studies in the ovalbumin-sensitized and challenged mouse (OVA-mouse) model of asthma (see Section 4.1.), iterated with clinical observations, have played a large role in defining the term "asthma" as synonymous with an immune T helper 2 (Th2) cell-mediated disorder [12,13]. According to this paradigm, allergic sensitization and subsequent challenge results in T cell activation and transformation to a Th2 phenotype. The release of the Th2 pattern of cytokines, including interleukin (IL)-4, IL-5, IL-13, and IL-9, promote airway inflammation rich in eosinophils, which are considered responsible for the asthmatic response. The sequence of events and the importance of dendritic cells, Th2 cells and eosinophils to the allergic response, have been mapped out primarily in this mouse model, utilizing a variety of cell, gene and pharmacologic techniques resulting in a detailed model of pathways leading to airway inflammation and AHR, becoming the dominant mechanism of asthma over the last 30 years. While it is now recognized that the original concept of asthma as solely a Th2-mediated disorder is overly simplistic, and other T cell repertoires are also incriminated, including Th1, Th17, regulatory T (Treg) cells, cytotoxic CD8+ T cells, natural killer T cells (NKT) and γδT cells [12], nevertheless, murine asthma models have directed attention to discrete pathways and components of the immunological response deemed critical for the asthmatic response, and continue to be the primary model used to evaluate new therapies.

2. Heritability of asthma

The heritability of asthma is estimated to be 40–60% [5]. Genetic linkage studies initiated in 1986 to identify the genetic basis of asthma reported associations with a substantial number of genes that made apparent mechanistic sense based on prevailing wisdom from mouse models and clinical observations, such as IL4, IL13, IL4RA, FCER1B and CD14 [14]. In 2007 genetic linkage studies were superseded by genome-wide association studies (GWAS), resulting in most of the previously reported genetic associations disappearing [15]. However, there is not full agreement even between the major GWAS, while the genes that have been identified account for only a trivial portion of asthma heritability. Even the long recognized association with HLA could not be ascribed to a single genotype. GWAS have differentiated genetic variants associated with asthma from those associated with atopy, indicating the two are not synonymous [16].

A principal finding, since it has been replicated independently in several studies, is that SNPs located on chromosome 17q21 close to the genes for ORMDL3 (orosomucoid-like 3) and GSDML (gasdermin-like) indicate asthma susceptibility, particularly in childhood asthma [16,17]. This region seems to be associated with chronic inflammation since it has also been implicated in ulcerative colitis, Crohn's disease and Type I diabetes by GWAS [18]. ORMDL3 had not been considered as a mediator of asthma previously, and its relevance was not immediately clear. A subsequent investigation found that ORMDL3 expression in airway epithelium is induced in sensitized mice by exposure to ovalbumin or the cytokines IL-4 and IL-13, in a STAT-6-dependent manner [19]. ORMDL3, in turn, regulates activation of the transcription factor ATF6, linked to SERCA-2b and implicated in airway remodeling; while ORMDL3 also activates several other genes encoding certain metalloproteases, chemokines and other factors. Whether it subserves a key role in human asthma remains to be determined.

GWAS have also identified SNPs in regions implicated in Th2 signaling, including the RAD50-IL-13 locus, IL33 and IL1RL1/ST2, while rare variants in the coding exons and flanking regions of candidate genes in the EVE consortium GWAS implicated IL12RB1 [15,17]. Data from the Gabriel GWAS was used to provide evidence for an association between asthma and the gene for the transcription factor TCF3, which binds β-catenin and is a downstream effector of the *Wnt* signaling pathway [15].

Based on the results from a recent GWAS assessing genes associated with lung function in asthmatics it has been proposed that variants in Th2 pathway genes – IL13, IL33, TSLP and IL1RL1 – are associated with asthma susceptibility, while variants in Th1 or IL-12 cytokine family genes (including IL12A, IL12RB, STAT4, and IRF2 among others) are related to lung function and airway remodeling [20].