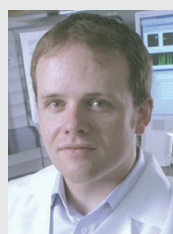


Modeling food allergy: pathways to therapy

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“As we improve our ability to understand the genetics of human diseases ... mouse models may become vital in establishing the functions, significance or mechanisms of human-associated genetic changes.”

“Why do we even need an animal model of food allergy?” This question was pitched to me at a recent meeting focused on the growing problem of food allergy. After having spent the last couple of years developing new approaches to studying food allergy and focusing on mouse models, it was an important question to hear and upon which to refocus. Considering first asthma, another highly investigated allergic disease, a plethora of animal models exist for this disease and have been subject to extensive investigations for over a decade. Clinically, however, glucocorticoids remain the mainstay therapy of choice in the USA. The discrepancies between the effectiveness of anti-IL-5 in mouse models of asthma and their lack of effectiveness in the initial clinical studies are well documented [1]. Similarly, we demonstrated that desloratadine, an inhibitor of the H1R histamine receptor, reduced allergic airway responses in the mouse [2] but antihistamines are considered ineffective for asthma therapy. Given the difficulties observed in translating from the mouse to the clinic, it would be easy to conclude that the mouse has contributed little to clinical allergy management. However, in the desire to improve quality of life and treatment options for patients, it might be easy to overlook the important roles that animal models can play in reaching these goals. The discovery of new pathways and mechanisms, or the improved understanding of those already characterized, is a vital pipeline towards the next generation of therapies and, while the murine immune system can provide discoveries

of this sort to be tested for translational importance, the translation can go both ways. As we improve our ability to understand the genetics of human diseases and identify genes or polymorphisms that may confer susceptibility, mouse models may become vital in establishing the functions, significance or mechanisms of human-associated genetic changes. In this context, a number of critical advances in the technology and techniques associated with murine models of allergy suggest that the future is likely to be considerably brighter for both asthma and food allergy research.

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For many years, a diverse array of experimental models have been used to explore the mechanisms of asthma, whereas food allergy has proved difficult to study. However, recent developments in mouse models of food allergy have provided us with new tools to better understand this disease. As with humans, mice have exquisite mechanisms that ensure tolerance to antigens passing through the intestinal system. For a number of years, cholera toxin has been successfully utilized to break through oral tolerance and promote immune responses to food proteins, such as ovalbumin (the egg protein commonly used in asthma models) [3–11]. While the coadministration of cholera toxin and food antigen elicited robust

anaphylactic responses in these studies, it fails to elicit some of the hallmarks of allergic diseases, including eosinophilia and a dominant T-helper (Th)2-type cytokine response. In addition, many of these studies relied upon responses in one specific mouse strain, C3H/HeJ mice, which are hyporesponsive to lipopolysaccharide (LPS) [12,13]. This model has been used to test the effectiveness of potential therapies, such as the herbal remedy Food Allergy Herbal Formula 2 (FAH2) [14–16], which is currently progressing towards clinical trials for the treatment of food allergy. Recent work has shown that cholera toxin may function by enhancing OX40L expression of gastrointestinal dendritic cells, thereby promoting Th2-skewed T-cell responses [17], and, while our understanding of this model improves, there have been a number of other models that have recently been described. In a unique model of allergic diarrhea, Berin and colleagues were able to demonstrate that the antigen-reactive T cells in the mesenteric lymph nodes of animals that had been sensitized and challenged orally were sufficient to migrate back to the intestine and promote intestinal responses to antigens after adoptive transfer [18]. This defined a method to specifically study the role of T cells in the symptoms of experimental food allergy and, in particular, the allergic diarrhea response. We have recently reported a new model for food allergy, whereby allergic responses to oral-administered egg (i.e., ovalbumin) or peanut were promoted by coadministration with Staphylococcal enterotoxin B (SEB) [19]. In this model, we can elicit anaphylactic responses to relatively low amounts of food antigen (100 µg), which approximately translate to dietary levels for humans. The spectrum of responses observed also includes aspects not observed with cholera toxin, such as increased eosinophilia. Interestingly, we demonstrated that the mice exhibited reduced expression of TGF-β1, a cytokine important for immune tolerance. TGF-β1 is also reduced in the mucosa of patients with food allergy [20]. Importantly, the described models are effective in standard laboratory strains of mice and open the door for investigators to examine the plethora of genetically modified mice already generated. However, as is now the case with research on asthma models, it seems likely that the future of food allergy research will encompass a variety of models, each with their own particular advantages and disadvantages, and that the critical pathways will be defined by those studies that adopt a diverse range of approaches.

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Recent advances in manipulating the murine genome have opened the door to a new era of immunological study. The coexpression of fluorescent reporter molecules with a protein of interest allows us to visualize the cells in their native state, without the need to permeabilize or for culture. In 2001, Locksley and colleagues were one of the first groups to utilize an internal ribosomal entry site (IRES) into a Th2 context, when they generated the IL-4 reporter mouse [21]. Importantly, the

IRES-driven expression of enhanced green fluorescent protein (eGFP) allowed for fluorescent visualization without altering the IL-4 molecule, which does occur with fusion molecules. Investigations using this mouse model have provided definitive evidence that mast cells, basophils and eosinophils are also capable of expressing IL-4 [22]. This approach to precisely identify cytokine-producing T cells has been applied to IL-10 [23], and other cytokine reporters are being developed. Similarly, the study of regulatory T cells (Tregs) was revolutionized by the generation of the FoxP3–eGFP mouse model. Previously, in order to distinguish Tregs from activated effector cells, which also express CD4 and CD25, investigators had to fix and permeabilize the cells to stain for the Treg-specific transcription factor FoxP3. Permeabilization, however, killed the cells and prohibited further functional analysis. Using FoxP3–eGFP mice, these cells can now be simply sorted based on the fluorescent reporter. Already, a tool to identify the relatively new Th17 cell lineage is available in the RORγt–GFP mice developed by Littman and colleagues [24], reflecting the ability of the immunologist to rapidly develop such approaches. The application of this reporter system to food allergy research can further uncover the specific roles of multiple cell types in the development of food allergy.

“...development and testing of safer immunotherapy regimens may be heavily dependent on data derived from murine models.”

In addition to reporter mice, polymorphisms that have disease associations have been engineered into mice and are an exciting new development that allows us to interrogate the functional effects of such subtle alterations. As our ability to study our own population-based genetics improves, the validation of potential risk factors and polymorphic associations will become increasingly vital. Mouse models offer an ideal approach that allows for complex systems and outcomes. This approach was elegantly shown in work examining the IL-4 receptor signaling chain. It was known that the S503P polymorphism was strongly associated with allergic disease and atrophy in patients [25–27], but several other polymorphisms were also described in this region. The functional role of this specific change was tested in the mouse by engineering an analogous amino acid alteration into the murine IL-4 receptor α-chain. The Y500F mutant displayed an enhanced allergic response in a model of allergic airway inflammation, despite the other polymorphic sites being normal [28], and thus defined this as a critical polymorphism that is functionally involved in the elevated responses in patients with the S503P genotype.

Moving forward in food allergy research, the question is: do mouse models have a role to play? I believe that the answer is a strong and justified ‘yes’. The new models being developed, their application to answer specific questions and the advances in mouse strains that specifically target immune responses are critical elements that will lead to the therapies of the future. This will most likely occur via the discovery and careful characterization of the pathways regulating oral tolerance and its loss, as

well as via the discovery of methods to safely restore tolerance, without causing anaphylaxis. Since the use of immunotherapy for food allergy cannot be recommended at this time due to the risk of adverse reactions, development and testing of safer immunotherapy regimens may be heavily dependent on data derived from murine models. The translation of defined pathways in the mouse into clinically significant pathways in humans will be the next step on the ladder to such therapies, and the tools to feed this process are increasingly exciting and the future looks promising.

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