

REVIEW ARTICLE

Novel insights into mechanisms of food allergy and allergic airway inflammation using experimental mouse models

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To cite this article: Corazza N, Kaufmann T. Novel insights into mechanisms of food allergy and allergic airway inflammation using experimental mouse models. *Allergy* 2012; DOI: 10.1111/all.12065.

Keywords

animal models; asthma; food allergy; Immunoglobulin E; innate immunity.

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Accepted for publication 02 October 2012

DOI:10.1111/all.12065

Edited by: Thomas Bieber

Abstract

Over the last decades, considerable efforts have been undertaken in the development of animal models mimicking the pathogenesis of allergic diseases occurring in humans. The mouse has rapidly emerged as the animal model of choice, due to considerations of handling and costs and, importantly, due to the availability of a large and increasing arsenal of genetically modified mouse strains and molecular tools facilitating the analysis of complex disease models. Here, we review latest developments in allergy research that have arisen from *in vivo* experimentation in the mouse, with a focus on models of food allergy and allergic asthma, which constitute major health problems with increasing incidence in industrialized countries. We highlight recent novel findings and controversies in the field, most of which were obtained through the use of gene-deficient or germ-free mice, and discuss new potential therapeutic approaches that have emerged from animal studies and that aim at attenuating allergic reactions in human patients.

As with most human diseases, animal models have tremendously advanced our better understanding of key events of allergic disease development and have helped to identify novel therapeutic targets. However, it is clear that there is hardly an animal model that can recapitulate all features of a particular human disease in its entirety. With each model having its advantages and disadvantages, precise knowledge of its limitations, careful data interpretation, and the combination of various models is crucially important when attempting to generalize and translate experimental findings into the context of human disease (1, 2).

Abbreviations

AAI, allergic airway inflammation; alum, aluminum hydroxide; BLG, β -lactoglobulin; DAMP, damage-associated molecular pattern; mDC, myeloid dendritic cell; GC, glucocorticoid; HDM, house dust mite; HIF-1, hypoxia-inducing factor 1; IgA/-E, immunoglobulin A/-E; IL-1 α /- β , interleukin 1 alpha/beta; IL-4/-5/-17/-33, interleukin-4/-5/-17/-33; IL1R1, interleukin 1 receptor type I; OVA, chicken egg ovalbumin; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; TNF α , tumor necrosis factor alpha; TGF β , tumor growth factor beta; TLR, Toll-like receptor; Treg, regulatory T cell; WT, wild type.

Food allergy and allergic asthma represent major health problems, particularly in industrialized countries, with incidences increasing (3, 4). In allergy research, particularly research concerning food allergy, asthma, and anaphylaxis, where experimentation on humans often bears too high risks, great advancements in understanding pathogenesis have been achieved in animal models. Also, novel approaches of potential therapeutic benefit can be safely tested. This is particularly true for food allergy, where little is known about the development of disease and much efforts are ongoing to find non-allergen-specific treatments (5).

Even though intensive attempts are undertaken to recapitulate allergic asthma in animal models, asthma seems to be a uniquely human disease (6). However, many important insights into the development of asthma-like conditions have been obtained using animal models of allergic airway inflammation (AAI) (5).

The mouse has long emerged as prime model organism for fundamental biomedical research, including research on allergies and acute and chronic inflammation. Besides the low cost of maintenance and short breeding times, many well-characterized inbred mouse strains, suitable for specific applications, a vast and increasing arsenal of genetically modified

strains, and molecular and immunological tools are available to the scientific community. Over the last years, many interesting studies based on *in vivo* allergy models in the mouse have been published in leading journals on experimental allergy research, including 'Allergy'. Here, we are highlighting recent novel insights into the mechanisms of food and AAI that have been obtained from mouse experimentation and describe novel potential therapeutic targets that have been proposed through these reports.

Food allergy

Immunoglobulin E (IgE)-mediated food allergy represents a growing concern among industrialized countries, and it is estimated that it currently affects up to 4% of the population of westernized countries and 6–8% of young children (7). In contrast to the increasing number of treatments for airway and cutaneous allergies, the therapeutic approach for food allergy remains very limited. Several efforts, most of them relying on *in vivo* experimentation in the mouse, have been made in the last few years aiming to understand which food antigens are immunogenic and induce an IgE response and why and how immunological leakiness responsible for the failure of normal oral tolerance develops (8). Multiple methods are used to trigger food allergy in the mouse. The main differences consist of the food antigen (or model allergen) used and the strategy to sensitize the animal prior to oral challenge (Fig. 1).

The importance of antigen exclusion through IgA

In healthy persons, the gut has developed a complex immune system able to discriminate between pathogens and harmless food antigens to avoid uncontrolled immune response to food antigens and components of the commensal microbiota.

Mucosal immunity protects the epithelial barrier by two main mechanisms: firstly, by immune exclusion of foreign antigens through secretory antibodies and secondly, by anti-inflammatory tolerance mechanisms. Efficient exclusion of foreign antigens requires polymeric antibodies, that is, dimeric IgA and pentameric IgM. Among the antibody isotypes, IgA represents the most abundant immunoglobulin produced in mammals and is predominantly secreted across the mucosa. At these frontlines, IgA, which is constantly exposed to pathogenic and harmless microbes as well as food antigens, provides immunological defense by transporting antigens across the intestinal layer to the lumen. Thus, IgA has been hypothesized to play a protective role in the context of food allergy. Frossard et al. demonstrated that in mice tolerized to the milk allergen β -lactoglobulin (BLG), intestinal IgA levels were elevated compared with naïve or sensitized animals (9). However, Karlsson et al. recently showed that mice deficient for polymeric immunoglobulin receptor (pIgR), which cannot export secretory SIgA or SIgM, were hyperreactive to intradermal ovalbumin (OVA) challenge. Oral tolerance induced by OVA suppressed IgG1 levels in sensitized *pIgR*-deficient mice, protected mice against anaphylaxis and prohibited delayed-type hypersensitivity toward OVA (10). These observations demonstrate that antigen exclusion at least in this model does not represent a critical parameter for the development of food allergy, suggesting that uncontrolled responses to food antigen in the gut must be coordinated by other factors.

Regulatory mechanisms controlling oral tolerance

Recently, multiple regulatory cells that control immune responses and maintain intestinal homeostasis and tolerance have been identified. Intestinal tolerance is strictly controlled by specific interactions between the innate and adaptive immune system. In this context, dendritic cells (DC) in the

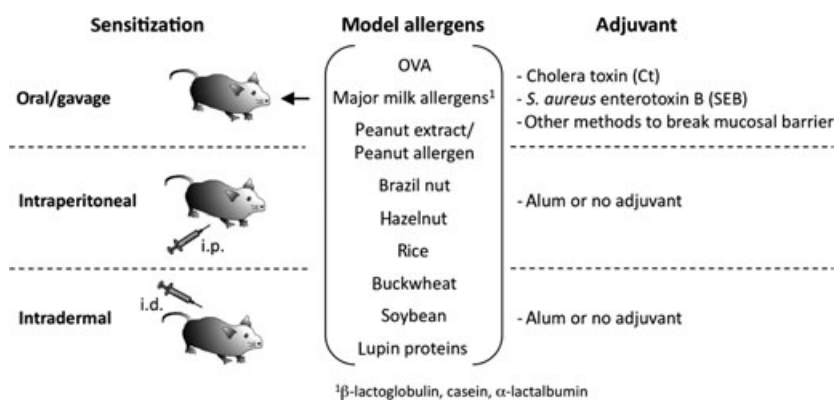


Figure 1 Food allergy models in the mouse. Many different mouse models for food allergy are in use. The biggest differences are the use of model allergens and the sensitization strategy prior to oral challenge [reviewed in (67–69)]. For oral sensitization, addition of an adjuvant (or other method to manipulate the intestinal epithelium) is needed in most cases to break tolerance in the gut. Cholera toxin (Ct) is most commonly used. However, *Staphylococcus aureus*

enterotoxin B (SEB) has been shown to be effective as adjuvant and may be clinically much more relevant for human food allergy (70). Alternatively, mice are systemically sensitized (i.p. or i.d.) prior to oral challenge, resulting in anaphylactic reactions. Systemic sensitization models in the presence or absence of adjuvant (most commonly alum) are established.

intestinal mucosa represent crucial mediators, driving T-cell polarization toward Th1 and regulatory phenotypes. Different DC populations have been characterized in the intestine of mice based on the expression of CD11c, CD11b, CD8a, CD103, and CXCR1. The CD11c⁺CD103⁺ DC subset has raised particular interest due to its important role in activating regulatory T cells. Upon contact and phagocytosis of antigen, CD11c⁺CD103⁺ DC migrate from the intestinal mucosa to mesenteric lymph nodes and start to produce retinoic acid and TGF β , thereby inducing Foxp3⁺ regulatory T cells involved in tolerance induction (11). By exposing mice to the mucosa adjuvant cholera toxin in combination with peanut extract, Smit and colleagues demonstrated a profound shift of DC populations in intestinal tissue toward a more inflammatory and less regulatory phenotype (12). Sensitization to peanut allergen in fact increased conventional CD11b⁺ DC in intestinal tissues and reduced the CD103⁺ subset. Moreover, the authors demonstrated that expansion of all DC subsets by Flt3L significantly reduced allergic reaction toward peanut allergen as demonstrated by reduced levels of peanut-specific IgG1, IgE, and Th2 cytokines. Surprisingly, even though CD11c⁺CD103⁺ DC have been involved in tolerance induction by activating regulatory T cells, it appears that, in this model, the protective effect of Flt3L preventing food allergy seems to be largely dependent on the expansion of the plasmacytoid DC subset (CD11c⁺B220⁺mPDCA⁺) (12).

Among the numerous T-cell subsets in the intestinal tract, Foxp3⁺CD4⁺CD25⁺ regulatory T cells (Treg) have been the most investigated cell type in the induction of oral tolerance. Foxp3⁺ Treg can be further subdivided into natural Foxp3⁺ Treg (nTregs) and induced Foxp3⁺ Treg (iTregs). Whereas nTregs are negatively selected in the thymus, iTregs are generated in peripheral organs from naïve CD4⁺ T cells. Hadis et al. demonstrated that gut-homing Treg are generated in the mesenteric lymph nodes by interacting with antigen presenting CD103⁺ DC in the presence of TGF β and retinoid acid (11). Once activated in mesenteric lymph nodes, they migrate back to the intestinal mucosa, in particular to the lamina propria, where they are able to expand and to induce oral tolerance. The requirement of Treg in dumping food allergy has been investigated in many experimental models, and several strategies are now in the developing phase to redirect Th2 immune responses toward Th1–Treg responses. In this respect, Curotto de Lafaille et al. showed, using a mouse model in which nTreg but no iTreg can be generated, that oral tolerance to OVA cannot be induced in the absence of iTreg (13). In line with these findings, Adel-Patient et al. demonstrated that systemic sensitization and elicitation of allergic reactions were completely inhibited in mice upon gavage with the major cow milk allergen native β -lactoglobulin (14). Induction of tolerance correlated with expansion of Foxp3⁺CD4⁺ T cells and depletion of Treg resulted in impaired oral tolerance (14). Similarly, Yamashita and colleagues provided evidence that adoptive transfer of mesenteric lymph nodes (or Treg) isolated from mice orally pretreated with OVA prevented recipient mice from anaphylactic responses (15).

Although intestinal epithelial lymphocytes (IEL) represent the first line of defense against luminal antigen, only few reports examined the role of these particular cell subsets in food allergy. Among the IEL populations in mice, $\gamma\delta$ T cells represent a considerable proportion of IEL. These cells are located in the paracellular space between intestinal epithelial cells and are thus ideally positioned to effectuate a crosstalk between intestinal epithelial cells and immune cells in the lamina propria. By performing depletion experiments, it was demonstrated that $\gamma\delta$ T cells participate in the induction of oral tolerance (16, 17). Cardoso et al. now showed that a peanut sensitization and challenge model in mice is accompanied by intestinal epithelial damage and reduced number of $\gamma\delta$ T cells (18). In line with these results, Bol-Schoenmakers reported that $\gamma\delta$ T cells have the potential to suppress Th2-dependent IgE responses without affecting IgG responses to inhaled antigens. Induction of food allergy by the administration of peanut proteins together with cholera toxin resulted in a profound decrease in the percentage of $\gamma\delta$ T cells in intestinal tissues at the initial phase of sensitization and depletion of $\gamma\delta$ T cells resulted in elevated allergic responses toward peanut allergen (19).

Immune modulation through commensal flora

Another essential aspect of oral tolerance is the commensal bacteria flora. Experimental animal models highlight the importance of immune modulation exerted early in life by commensal bacterial flora in the gastrointestinal tract, directing the development of tolerance. Geuking and colleagues have recently demonstrated in experiments using germ-free mice that intestinal regulatory T cells are induced and activated by commensal bacterial colonization (20). The activation of intestinal Treg is thereby intrinsic and not due to differences in the flora composition and is mandatory for efficient CD4⁺ T-cell homeostasis. This work strongly supports the existence of a direct link between commensal flora and the development of Tregs, and currently, several therapeutic strategies employing probiotic mixtures are being tested to modulate the intestinal immune system. On this basis, Schiavi et al. demonstrated that oral administration of the probiotic mixture VSL3 to shrimp tropomyosin-sensitized mice was effective in redirecting allergen-specific Th2-polarized immune responses toward Th1–Treg responses (21). Similarly, oral administration of bifidobacteria have been shown to have the capacity to suppress the skewed Th2 response in mice sensitized to OVA, increasing the number of Treg and IL-10-positive cells (22). Meanwhile, it is assumed that the intestinal flora is mandatory to maintain not only the intestinal immune homeostasis but also represents an important factor for the development of immune homeostasis at other peripheral sites. Studies employing germ-free mice have shown that the intestinal microbiota shapes the profile of the immune system outside the gut. For instance, Olszak et al. illustrated that exposure to microbes during early childhood is associated not only with protection from gut-associated diseases such as inflammatory bowel disease (IBD) but also from development of asthma (23). The authors nicely show

that in germ-free mice, invariant natural killer T (iNKT) cells accumulate in the colonic lamina propria and lung, resulting in increased morbidity in models of IBD and allergic asthma as compared with specific pathogen-free mice. Colonization of neonatal but not adult germ-free mice with a conventional microbiota protected the animals from mucosal iNKT accumulation and related pathology (23).

This mutual relationship between the intestinal commensal microbiota and the host immune system develops early in life. Thus, prevention strategies intervening at early stage of development seem promising to modulate immune responses with persistent effects. Schwarzer and colleagues recently demonstrated that neonatal colonization of mice with *Lactobacillus plantarum* producing an aeroallergen redirects allergen-specific Th2-polarized immune responses toward Th1–Treg responses (24). It can be concluded that immune responses to commensal microbiota and food antigen induced early in life represent crucial events to direct the immune response toward tolerance and that this will condition the development of uncontrolled immune response against harmless antigens.

Allergic airway inflammation

Allergic asthma is among the most common chronic diseases in Western countries, with incidences further increasing (25, 26). Asthma is characterized by reversible airway obstruction and chronic inflammation of the respiratory tract, correlating with increased infiltration and activation of immune cells (DC, Th2-polarized T cells, eosinophils, and mast cells) and increased concentrations of largely Th2-type inflammatory cytokines, including IL-4 and IL-5. IL-4 induces the production of allergen-specific IgE by B cells, whereas IL-5 has a major role in recruiting and activating eosinophils (25, 26).

Asthma seems to be a disease restricted mostly to humans. Very few animals spontaneously develop asthma-like conditions (6). The mouse is by far the most commonly used animal model used to mimic various features of asthma by inducing conditions of allergic airway inflammation (AAI). Several methods have been developed in the last decades to mimic allergic inflammatory responses in the airways of mice, with the aim to understand cardinal features of the development of allergic asthma. This is facilitated by the vast arsenal of genetically modified mouse strains available. The Balb/c strain is a strain of choice in allergy research because of its tendency to develop Th2 immune responses (26). As many transgenic mice are generated on (or backcrossed onto) a C57BL/6 genetic background, the C57BL/6 strain is increasingly also used in experimental allergy research, even though it is less prone to develop Th2-biased immune responses.

Mouse models of allergic asthma are typically induced by sensitizing animals to a foreign antigen by intraperitoneal injection, in the presence or absence of an adjuvant. After the sensitization period, the mice are challenged with the antigen directly in the lung (aerosol inhalation of antigen) or the nose (Fig. 2). Airway inflammation is subsequently elicited. Important variations of the method include the use of different model antigens and adjuvants as well as different

entry routes for antigen delivery during the sensitization phase. Intraperitoneal delivery of the antigen predominates, as does the use of the adjuvant aluminum hydroxide (alum). However, it has become clear that different entry routes as well as use of other adjuvants (or absence of adjuvants; see below) may significantly alter immune responses (26). Regarding the model antigen, the majority of studies have been performed using chicken egg ovalbumin (OVA). Of note, OVA lacks allergenic activities that are known for clinically relevant allergens in humans (26). However, using OVA has its advantages, as elegant molecular and immunological tools are available, including transgenic mice bearing T cells expressing OVA-specific T-cell receptors. Nevertheless, studies relying on more clinically relevant antigens, such as extracts prepared from house dust mite (HDM), birch pollen or *Aspergillus*, are on the rise. Another important difference to asthma in humans, which is a chronic disease, is that most mouse asthma models induce an acute form of airway inflammation, as approaches with longer antigen exposure in mice largely fail to induce chronic inflammation (6).

Chemotaxis of dendritic cells to sites of lung inflammation

Allergic airway inflammation in mice is typically characterized by the infiltration of the airway wall by Th2-type lymphocytes, eosinophils, and mast cells, all contributing to the inflammatory phenotype. Th2 polarization is thereby initiated by myeloid dendritic cells (mDC), which effectuate the link between the adaptive and the innate immune system and have been shown to be essential for AAI (27, 28). During AAI, mDCs are actively recruited to inflamed airways through chemotactic agents, including molecules released from damaged lung tissue. Extracellular ATP, released in the airways of asthmatic patients or mice in asthmatic models, has been shown to be a chemoattractant essential in recruiting and activating mDC and maintaining asthmatic airway inflammation (29). Müller et al. now identified the P2Y₂R purinergic receptor as the ATP receptor subtype to be crucially involved in that process, mediating the ATP-triggered recruitment of mDC as well as eosinophils (30).

Role of the inflammasome in allergic airway inflammation?

Dendritic cells express a large arsenal of pattern recognition receptors (PRR) and are capable of potentially activating their inflammasome (31). The role of inflammasomes and the resulting interleukin-1 β (IL-1 β) and IL-18 production in AAI are controversially discussed. High extracellular concentrations of ATP are a known activator of the NLRP3 inflammasome (32). A prerequisite to inflammasome activation by ATP, or other inflammasome triggers, is a preceding priming event, such as exposure to bacterial endotoxins (LPS) or other PRR agonists, leading to NF- κ B-mediated transcriptional induction of pro-IL-1 β (31). Activation of the inflammasome during the course of allergic airway disease has been shown to be a crucial event in the activation mDCs and, consequently, in the modulation of adaptive immune response. It still remains obscure, however, how an allergenic antigen can

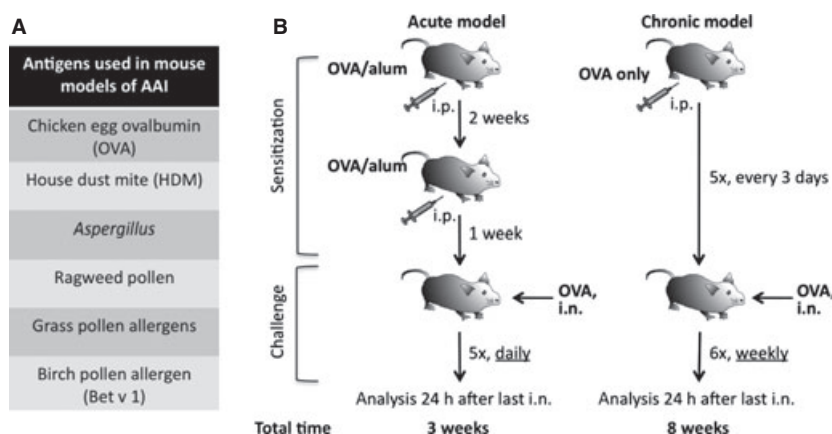


Figure 2 Mouse models of allergic airway inflammation (AAI). (A) Antigens commonly used as model allergens to trigger AAI. Several transgenic mouse strains and molecular tools facilitate the analysis of OVA-induced AAI. However, OVA lacks clinical relevance for human asthma and more relevant and naturally occurring antigens are increasingly used. (B) Typical example of acute and chronic AAI models triggered by sensitization and challenge with OVA [as

described by Allen et al. (35)]. In the acute model, an adjuvant (typically aluminum hydroxide, alum) is co-administered during the sensitization phase to enhance the antigenicity of the antigen. Chronic AAI is elicited in the absence of adjuvant. Alternative models to OVA-induced AAI follow similar schemes but rely on more clinically relevant antigens, such as HDM or *Aspergillus*, or a combination of multiple antigens.

trigger the activation of the inflammasome. OVA is the most commonly used model antigen to trigger AAI in mice. It has been suggested that bacterial endotoxin contamination of OVA preparations is needed to trigger an effective Th2 response upon inhalation of antigens in murine models of asthma (33). These observations indicated that activation of the inflammasome via endotoxins such as LPS could represent an important factor in the OVA asthma model. However, the role of the caspase-1 inflammasome, with the production of biologically active IL-1 β , in the development of asthma remains controversial. Besnard et al. reported a requirement for the NLRP3 inflammasome and subsequent IL1R1 signaling in an adjuvant-free OVA model of acute AAI (34). The authors showed that impaired Th2 responses, eosinophil infiltration and lung inflammation occur in the absence of NLRP3. It was consequently proposed that NLRP3 contributes to DC function and the induction of Th2 responses and may thus represent a potential novel therapeutic target in conditions of allergic lung inflammation or allergy in general. Interestingly, Besnard et al. also showed that the critical role of IL1R1 in allergic inflammation is dependent not only on IL-1 β but also on IL-1 α , which is active in its immature form without the need of being processed by the caspase-1 inflammasome.

In another report, Allen and colleagues compared wild type with *Nlrp3*^{-/-} mice in four different allergic asthma models, including acute (alum-dependent) or chronic (alum-independent) OVA models as well as acute and chronic house dust mite (HDM)-based models (35). In contrast to Besnard et al., Allen et al. found no role for the NLRP3 inflammasome in the development of AAI in any of these models (35). In agreement with Besnard et al., Allen et al. did find a significant reduction in the levels of the proinflammatory cytokines IL-13 and IL-33 in alum-independent models in the absence of

NLRP3 compared with WT controls. The study by Allen et al. supports a previous report by Kool and colleagues who reported a NLRP3 inflammasome and IL-1 signaling-independent role for uric acid in the induction of Th2 responses in OVA and HDM lung inflammation models (36). How may these different findings be accounted for? Allen et al. raise the interesting possibility that, similarly to the gut, where the composition of the host microbiome is well known to have tremendous impact on the phenotypic outcome of disease models, the host microbiome within the lung could similarly contribute to the diverse outcomes in *Nlrp3*^{-/-} mice upon development of AAI (35).

Interleukin-33 - a new therapeutic target within the IL-1 family?

Interleukin-33 (IL-33) is a newly described member of the IL-1 family that is expressed by many cell types upon pro-inflammatory stimulation. In contrast to IL-1 β , IL-33 does not need to be processed by inflammasomes but rather seems to be released during tissue injury, thereby acting as an alarmin [reviewed in (37, 38)]. IL-33 induces the production of Th2 cytokines via the IL-33 receptor, which is widely expressed, including expression on mast cells and Th2 cells (37). Upon release from damaged cells in a setting of airway inflammation, IL-33 can promote the severity of inflammation by stimulating the differentiation of IL-5- and IL-13-producing Th2 cells through its effect on lung DC differentiation and recruitment (38). IL-33 is thus currently discussed as a potential therapeutic target in allergic diseases, including asthma and anaphylactic shock. IL-33 can further exacerbate airway inflammation through its activating action on mast cells. In the presence of IgE, IL-33 directly induces degranulation of mast cells, and experiments in mice indicate that

IL-33 has an important role in anaphylactic shock in the presence of IgE (39). Komai-Koma et al. have recently shown that IL-33 triggers IgE synthesis, mast cell degranulation, and systemic anaphylaxis via IL-4 (40). Anti-IL-33 antibody treatment has been shown to be effective in mouse models of OVA-induced AAI (41, 42). The therapeutic potential of anti-IL-33 antibody was recently further underlined in an experimental OVA-based mouse model of allergic rhinitis (43). Serum total and OVA-specific IgE were significantly reduced in anti-IL-33-treated animals, along with decreased eosinophil infiltration and lower IL-4, IL-5, and IL-13 levels in BAL fluid (43). Similar results were obtained using a soluble form of the IL-33 receptor ST2 (IL1RL1) in an OVA mouse model of AAI (44). Yin and colleagues further provided experimental proof that adenovirus-mediated delivery of soluble ST2 is effective in the mouse in diminishing AAI, underlining the therapeutic potential of interfering with IL-33/ST2 signaling (45).

TNF antagonism to treat allergic airway inflammation?

Targeting TNF α is being discussed as a means to fight symptoms of severe (refractory) asthma, particularly in patients that do not, or only poorly, respond to glucocorticoids (46). The classical OVA or HDM sensitization/challenge models lead to elevated TNF α levels in the BAL fluids of mice during AAI. Choi et al. recently reported TNF α also to be a key player in the Th2 response to inhaled OVA induced by simultaneous administration of viral PAMP double-stranded RNA mimetic, poly(I:C) (47). It was shown in the mouse that anti-TNF α treatment could reduce allergen-induced airway inflammation in HDM and OVA models. A beneficial effect of infliximab, a TNF α blocking antibody, was further demonstrated in an OVA/alum rhinitis model (48). However, it was already suggested that anti-TNF α therapies might be more effective in the symptomatic treatment of severe asthma (49–51). The pathogenesis of severe asthma is still poorly understood, and improved animal models for severe asthma are needed (6). Even though TNF antagonism led to some promising results in clinical trials targeting patients with severe asthma, a marked heterogeneity in response was observed, and it was concluded that the efficacy needed to be fine-balanced against present risk factors concerning the safety of chronic anti-TNF therapies [reviewed in (52)].

Other novel insights and potential targets for asthma treatment

Through their widespread action of downregulating inflammatory responses, inhaled glucocorticoids (GC) have a central place in the symptomatic treatment of asthma. However, long-term GC treatment increases the risk of systemic absorption and adverse side effects. Hostettler and colleagues described for the first time local GC production in the lung tissue of mice (53). Interestingly, local GC synthesis in the lung seems to be defective in models of airway hypersensitivity (53). It remains to be tested whether GC production is

similarly defective in asthma patients and whether absence of local GC production contributes to pathogenesis or persistence of disease (54). If affirmative, stimulation of the local GC production in the lung of affected patients would constitute an attractive novel therapeutic approach in the treatment of allergic airway diseases.

There are indications that hypoxia-inducing factor 1 alpha (HIF-1 α) plays crucial roles in immune and inflammatory responses, partially by upregulating vascular endothelial growth factor (VEGF) (55, 56). Huerta-Yepez et al. showed that HIF-1 α and VEGF levels are increased in asthma and rhinitis patients as well as in mice subjected to an OVA model of AAI (57). The authors provide evidence that HIF-1 contributes to the development of AAI and suggest that specific inhibitors of HIF-1 may be effective in treatment of asthma (57). In a follow-up study, it was shown that HIF-1 contributes to AAI partially by regulating the expression of the chemokine CCL-2 (58). Insulin-like growth factor 1 (IGF-1) can stimulate HIF-1 activity and increase the secretion of VEGF (59, 60). In a recent study by Kim and colleagues, the beneficial effect of exogenously added recombinant IGF-binding protein 3 (IGFBP3) in attenuating OVA-induced airway hyperresponsiveness was demonstrated in C57BL/6 mice (61).

Eosinophils are important effector cells in allergic lung inflammation and are known to be sensitive to undergo apoptotic cell death in response to stimulation with the death ligand FasL (also called CD95L) (62, 63). Sharma et al. investigated the effect of FasL neutralization in a mouse asthma model of *Aspergillus fumigatus* (Af) sensitization and challenge-induced airway eosinophilia (64). The authors reported that FasL neutralization increases eosinophilic inflammation, further underlining that FasL plays an active role in the resolution of activated eosinophils at sites of inflammation in affected airways (65, 66).

Conclusion

Even though the application routes and the antigens employed in animal models to investigate the mechanisms of the development of allergic diseases did not significantly change in the last decade, the growing number of genetically modified mouse strains and the development of germ-free mice considerably increased the understanding of the events causing allergic reactions. Further knowledge will doubtlessly be gained in the future from these models, as well as from other recently developed transgenic strains, including mice harboring cell-type-specific gene deletions or lacking specific subsets of immune cells. Importantly, intense experimentation in animal models not only opened the door to knowledge and new potential therapeutic targets but also increased the debate whether and which models are best suited to get novel mechanistic insights into a particular human disease. As with all animal models, there is hardly a perfect model to fully mimic a condition of human disease. However, accurate analyses and careful interpretations of published reports by the scientific community will contribute in helping to translate obtained results into the human situation.

Acknowledgments

Research in the authors' laboratories is supported by fellowships and grants from the Swiss National Science Foundation (to TK), Novartis Foundation for Medicine and Biology (to NC, TK) and the 3R Research Foundation (to TK).

Conflict of interest

The authors declare no conflict of interest.

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