

## REVIEW

## Experimental Models for Studying Food Allergy

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## SUMMARY

Animal models have been invaluable tools for understanding the immunologic mechanisms of IgE-mediated food allergy and for testing novel treatment options. This review summarizes commonly used murine models and discusses their advantages and shortcomings with regard to how they phenocopy the human disease.

**Immunoglobulin E-mediated food allergy is rapidly developing into a global health problem. Publicly available therapeutic intervention strategies are currently restricted to allergen avoidance and emergency treatments. To gain a better understanding of the disease pathophysiology so that new therapies can be developed, major research efforts have been put into studying food allergy in mice. Animal models should reflect the human pathology as closely as possible to allow for a rapid translation of basic science observations to the bedside. In this regard, experimental models of food allergy provide significant challenges for research because of discrepancies between the presentation of disease in humans and mice. The goal of this review is to give a summary of commonly used murine disease models and to discuss how they relate to the human condition. We will focus on epicutaneous sensitization models, on mouse strains that sensitize spontaneously to food as seen in humans, and on models in humanized animals. In summary, expanding the research toolbox of experimental food allergy provides an important step toward closing gaps in our understanding of the derailing immune mechanism that underlies the human disease. The availability of additional experimental models will provide exciting opportunities to discover new intervention points for the treatment of food allergies. (Cell Mol Gastroenterol Hepatol 2018;x:x) (Cell Mol Gastroenterol Hepatol 2018;■:■-■; <https://doi.org/10.1016/j.jcmgh.2018.05.010>)**

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The prevalence of immunoglobulin (Ig) E-mediated food allergies has increased dramatically during the last decade, with a reported prevalence of 6%–8% of children in Western countries.<sup>1,2</sup> Because of the rapid rise in the number of patients with food allergies, it has become imperative that novel treatments are developed for patients with this condition. The disease is currently managed by

allergen avoidance and treatment of accidental exposures with epinephrine. However, this management plan has its shortcomings, because approximately 40% of individuals with food allergies face accidental exposures each year, placing food anaphylaxis among the leading causes for emergency department visits in the United States.<sup>3,4</sup>

Leading emerging therapies for food allergies include oral (OIT) and epicutaneous (EPIT) allergen specific immunotherapy. Both methods aim to achieve tolerance by exposing patients to allergens at doses that stimulate an immune response without eliciting clinical symptoms of allergy. During OIT, patients are repeatedly exposed to increasing doses of allergens via the oral route. Although desensitization can be achieved, OIT patients are at risk of developing severe therapy-associated type I hypersensitivity reactions.<sup>5–7</sup> Furthermore, concerns have recently emerged with regard to the development of therapy-resistant eosinophilic esophagitis as a side effect of OIT in 2.7% of patients with IgE-mediated food allergies.<sup>8</sup> In EPIT, an allergen adsorbed epicutaneous delivery system placed on the skin is used to expose allergic individuals to the allergen. An example is Viaskin (DBV Technologies, Montrouge, France), a polyethylene membrane that has been demonstrated in mice to promote the diffusion of allergens from the surface of intact skin through to the stratum corneum and toward the epidermis.<sup>9</sup> The allergen is taken up by dermal dendritic cells and Langerhans cells, processed, and presented to T cells in the lymph nodes to elicit an immune response.<sup>10</sup> Repeated application of the allergen has been demonstrated to decrease reactivity to the allergen and to increase effector memory and naive regulatory T cells (Tregs) in the spleen.<sup>11</sup> Tregs induced from EPIT maintain their suppressive properties for a longer period after the end of the treatment than those generated from OIT. This may be because although both routes of immunotherapy promote effector memory Tregs, EPIT also

**Abbreviations used in this paper:** EPIT, epicutaneous immunotherapy; FCER1A, high-affinity immunoglobulin epsilon receptor subunit alpha; FcεRI, high-affinity immunoglobulin E receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSC, hematopoietic stem cell; Ig, immunoglobulin; IL, interleukin; LCT, long chain triglycerides; MCPT, mouse mast cell protease; MCT, medium chain triglycerides; OIT, oral immunotherapy; PBMC, peripheral blood mononuclear cell; Th, T helper; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin; WASP, Wiskott-Aldrich syndrome protein.

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promotes induction of naive Tregs that are more capable of proliferating and surviving than effector cells.<sup>11</sup> In patients with peanut allergies, 1 year of daily EPIT using 250  $\mu$ g peanut protein applied by using Viaskin increased the dose needed to elicit an allergic response by at least 10-fold from their baseline tolerable dose.<sup>12</sup> Nonetheless, there are limitations to EPIT. For some patients a 10-fold increase in tolerable challenge dose is still a minor amount of allergen that can be tolerated, treatment responses were different between adults and children, and long-term benefits of EPIT remain to be investigated. Undoubtedly, additional research is needed to improve the current immunotherapy protocols as well as to develop novel intervention strategies. For this purpose, murine models will be invaluable tools because experimental interventions in humans are unethical.

In general, mouse models have been invaluable tools to gain a better understanding of the root cause of food allergies, mediators, and effectors of the immune reaction. The great degree in overlap of the genetics and the immune system between mice and humans has allowed researchers to gain a better understanding of the pathophysiology of food allergies.<sup>13</sup> The assortment of inbred strains with varying susceptibility to the disease and the generation of gene and cell type specific knockouts have helped uncover some of the key defects in the host that promote the development of food allergies.

Ideally, murine models of food allergy should be as homologous as possible to the human disease. In a recent review, Oyoshi et al<sup>14</sup> discuss isomorphic murine models of food allergies, which encompass most models of food allergy, in which the induction of disease is under the control of the investigator. Although the cause of the sensitization is not shared between human disease and isomorphic models, these models mimic clinical symptoms and can be used for developing treatments for food allergies. In this review, we will focus on 3 categories of recently investigated models: epicutaneous sensitization models, spontaneous sensitization models, and humanized mouse models.

## Pathophysiology of Human Food Allergy as Reflected in Murine Models

There are many steps in the process of establishing oral tolerance that may be disturbed, resulting in the failure to develop oral tolerance or in the loss of oral tolerance. Epidemiologic and experimental studies have demonstrated that sensitization can occur through defects in the skin epithelium, resulting from disorders such as eczema and atopic dermatitis. This type of sensitization can be recapitulated in mice through dermal sensitization models discussed later in the review.<sup>15-18</sup> In addition to defects in the skin barrier, increase in baseline permeability of the gut due to decrease in tight junction integrity can promote the development of food allergies.<sup>19</sup> The acidic environment in the stomach helps in preventing sensitization by either degrading the allergen and/or by affecting the uptake of the allergen by immune cells.<sup>20</sup> Thus, alterations of stomach pH is a risk factor for food allergy induction as documented epidemiologically by a higher rate of sensitization among

antacid users and elevated IgE titers and T-cell reactivity when allergens are administered in conjunction with antacids in mouse models.<sup>21,22</sup> The gut microbial community and its role in food allergy have been studied extensively.<sup>23</sup> Certain class of bacteria, such as Clostridia, have been associated with the promotion of oral tolerance and protection from allergen sensitization by increasing IgA production.<sup>24</sup>

In concert with defective mechanisms in the host, allergic patients fail to develop oral tolerance to their allergen because of interplay of intrinsic and extrinsic factors that allow antigens to maintain their structural integrity until processed and presented in the context of inflammatory signals. The allergen itself can have innate adjuvant properties that promote sensitization. For example, the major peanut allergen Ara h 1 can interact with CD209 on dendritic cells, promoting phagocytosis of the allergen and subsequently leading to antigen presentation to T cells.<sup>25</sup> The presence of disulfide bonds can help allergens maintain their structure by protecting them from proteolysis and thermal degradation.<sup>26,27</sup>

Clinical features of human food allergy, such as serum sensitization, mast cell expansion, and T helper 2 (Th2)-type tissue inflammation, are properly recapitulated, albeit to varying degrees, in mouse models of food allergy. The sensitization status of mice is commonly determined by measuring allergen-specific Igs, such as IgE and IgG1, in serum. When using alum as an adjuvant during sensitization, serum IgE has up to 80% specificity for the model antigen in mice; however, in patients only a small fraction (0.1%–15%) of total serum IgE is specific to a single food allergen.

Hallmark Th2 cytokines, interleukin (IL) 4 and IL13, promote the switch of Ig production by B cells to IgE, which, in combination with IL5 and IL13, induce mast cell expansion in the affected mucosal tissues. In murine models, the expression of these cytokines is typically measured at the mRNA level from small intestinal tissue samples or at the protein level from cell suspensions of the mesenteric lymph nodes or spleens. Expansion of the mucosal mast cell compartment of the small intestine is a measure of severity of food allergy in mice and in humans. In mice, mucosal mast cells are commonly quantified by chloroacetate esterase staining. Serum mast cell protease, MCPT1, is used as a systemic readout for mucosal mast cell activation on antigen-specific IgE cross-linking.<sup>28-30</sup> Unfortunately, no human equivalent for MCPT1 exists for monitoring IgE-mediated immune activation in patients with food allergies. Other in vivo markers of mast cell degranulation include histamine and serotonin, release of calcium stores, and induction of eicosanoid metabolism.<sup>31</sup>

Some pathophysiologic aspects of human food allergy are harder to recapitulate in murine models. For instance, in patients, anaphylaxis after exposure to an allergen causes rapid and acute hypotension coupled with skin, mucosal, gastrointestinal, respiratory, or cardiovascular symptoms.<sup>32-34</sup> Oral anaphylaxis is hard to achieve in most mouse models; therefore different challenge strategies are used, and systemic anaphylaxis is commonly monitored as

the readout. In mice, the decrease in core body temperature after systemic, cutaneous, or intragastric allergen challenges is monitored by rectal or subcutaneous probes.<sup>35,36</sup> In addition, allergic diarrhea can be scored by assessing the stool viscosity and stool hemorrhage, and they have been used as another readout for intestinal anaphylactic responses.

Overall, a combination of causes ranging from the host's environment, defects in the host's barrier and immune systems, and specific properties of the allergens can lead to the development of food allergies. An appropriate model for studying food allergies must be chosen depending on the goal of the research. The best models are those that most accurately reflect the human disease phenotypes. Depending on the research question, it may be important to consider models that reflect the induction of the disease in humans, as in epicutaneous and spontaneously sensitizing models, in addition to the observed phenotypes. Furthermore, translating the findings in mice to humans is undoubtedly of utmost importance, and this is more easily achieved through humanized mice. How these models are used for food allergy studies and their strengths and weaknesses are discussed below.

## Adjuvant-free Epicutaneous Sensitization Models

Feeding of food antigens to mice generally results in oral tolerance, as it does in most humans. Classic isomorphic food allergy models therefore resort to co-administration of antigens with Th2 skewing agents such as aluminum hydroxide, cholera toxin, or staphylococcal enterotoxin B to counteract the normal tolerogenic response. This adjuvant-dependent sensitization is followed by intravenous or enteral allergen challenges.<sup>30,37–39</sup> These models have helped us understand the type 2 immune response and the transcriptional, cellular, and humoral profiles of the effector phase of food allergies.<sup>14</sup> Yet, they cannot help to accurately draw conclusions about natural mechanisms of sensitization as occurring in humans (Table 1).

Epidemiologic studies have established a strong correlation between atopic dermatitis and the development of food allergies.<sup>40</sup> In addition, studies indicate that contact of damaged and/or inflamed skin with peanut oil during childhood can result in sensitization to peanut allergens.<sup>41</sup> To investigate whether allergen exposure through skin barrier defects can bypass oral tolerance and lead to gastrointestinal allergic disorders, murine models of epicutaneous sensitization have been developed.<sup>42,43</sup> Epicutaneous sensitization can be achieved in multiple ways. Although some groups simply administer the allergen intradermally, others apply the allergen to damaged skin. Skin damage can be achieved by repeated tape stripping of the dorsal skin (after removal of the fur) or by ultraviolet radiation.<sup>44–46</sup> In both cases, mice are challenged by intragastric administration of the allergen to produce food allergy-related phenotypes. Co-administration of adjuvants with the allergen is unnecessary, because administering the allergen alone can elicit an allergen-specific humoral

response. In these skin damage models, sensitization occurs comparable to tattooing strategies that are used to improve vaccination efficiency during the induction of a protective immune response.<sup>47</sup>

As repeatedly demonstrated in intraperitoneal ovalbumin sensitization models, skin sensitization also results in the production of allergen-specific IgE and IgG1 antibodies.<sup>48</sup> Furthermore, ex vivo cultured cells isolated from the axillary, subscapular, and inguinal draining lymph nodes of ovalbumin exposed mice produced increased amounts of IL4 in the presence of the allergen.<sup>49</sup> The resulting gastrointestinal allergy has been shown to be driven by the release of thymic stromal lymphopoietin (TSLP) and IL33, two cytokines that drive the type-2 response in the skin.<sup>50,51</sup> Further studies have shown that IL33 can drive gastrointestinal allergy independent of TSLP.<sup>52</sup>

Epicutaneous models have also been used to test how maternal sensitization status affects the risk of food allergy development in progeny. Ohsaki et al.<sup>45</sup> demonstrate that when female mice are epicutaneously sensitized to ovalbumin before mating, during gestation, and during breastfeeding, their offspring are passively immunized against the same allergen. Mice weaned from ovalbumin sensitized mothers were protected from developing an allergic immune response when skin sensitized and orally challenged with ovalbumin. This protection was conferred by the transfer of IgG:allergen immune complexes, and not free allergen, via the breast milk, which contributed to increased allergen-specific Tregs in protected mice. Importantly, pups from mothers who were not sensitized to the allergen were protected from food allergy phenotypes when nursed by surrogate sensitized females.

These studies demonstrate that epicutaneous allergen sensitization can result in IgE-mediated mast cell expansion, intestinal allergy, and anaphylaxis. Thus, the models can be used to study maternal-fetal transfer of protection and to develop therapeutic strategies aimed at preventing the development of food allergy in patients with atopic dermatitis.

## Adjuvant-free Oral Sensitization or Spontaneous Sensitization Models

Mouse models that mimic spontaneous oral sensitization to food allergens likely reflect the development of food allergy in humans more accurately because they do not require an exogenous adjuvant to shape the allergic immune response to food. The food allergen-specific serum IgE titers in spontaneous models appear lower than in isomorphic models and therefore reflect the serum sensitization status of patients more closely.<sup>29</sup> To date, spontaneous models have relied on genetically modified mouse strains with mutations in genes associated with the development of allergies such as the IL4 receptor (IL4R). Spontaneous sensitization to chow or to a model allergen, such as ovalbumin or peanut, can be achieved through repeated intragastric administration without an adjuvant. A bolus challenge with the allergen produces a food allergy phenotype such as diarrhea and oral anaphylaxis (Table 1).

**Table 1.** Adjuvant-free and Spontaneously Sensitizing Murine Models of Food Allergy

| Strain                                                   | Description                                                                         | Sensitization                                                                                                       | Challenge                                            | Phenotype of allergic mice                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Reference    |
|----------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|
| Epicutaneous and adjuvant-free models                    |                                                                                     |                                                                                                                     |                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |              |
| BALB/c                                                   | Inbred strain                                                                       | Days 0, 4, 8, and 12: id 5 $\mu$ g rTLSP and 10 $\mu$ g OVA                                                         | Days 17–22: daily ig 50 mg OVA                       | <ul style="list-style-type: none"> <li>• Acute diarrhea symptoms starting from third challenge</li> <li>• Infiltration of inflammatory cells into the jejunum</li> <li>• Elevated OVA-IgE, <i>IL4</i>, <i>IL13</i>, MCPT1</li> <li>• Decrease in core body temperature</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 50           |
| BALB/c                                                   | Inbred strain                                                                       | Days 0, 3, and 6: 100 $\mu$ g OVA or peanut protein applied to tape stripped skin on a patch                        | Day 9: ig 100 mg OVA or ig 50 $\mu$ g peanut protein | <ul style="list-style-type: none"> <li>• Increase in allergen-specific IgE, <i>IL4</i>, and MCPT1 in the serum</li> <li>• Expansion of mast cells in the jejunum</li> <li>• Increase in <i>IL13</i> expression in the jejunum</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 45           |
| Intragastric and adjuvant-free models                    |                                                                                     |                                                                                                                     |                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |              |
| IL4RF709                                                 | Substitution of tyrosine (Y) for phenylalanine (F) at position 709 of IL4R $\alpha$ | Week 0: ig 5 mg OVA on days 0, 1, 2, and 7<br>Weeks 1–8: ig 5 mg OVA weekly<br>Weeks 0–8: ig 100 $\mu$ g OVA weekly | Week 9: ig 150 mg OVA<br><br>Week 9: 150 mg OVA      | <ul style="list-style-type: none"> <li>• Decrease in core body temperature</li> <li>• Diarrhea</li> <li>• Increase in serum total IgE, OVA-IgE, OVA-IgG1, and MCPT1</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 56           |
|                                                          |                                                                                     | Weeks 1–4: ig 5 mg peanut protein weekly                                                                            | Week 5: ig 100 mg peanut protein                     | <ul style="list-style-type: none"> <li>• Decrease in core body temperature</li> <li>• Edema and mast cell expansion in the small intestine</li> <li>• Increase in serum total IgE, OVA-IgE, and MCPT1</li> <li>• Specific microbiome signature associated with allergen exposure</li> <li>• Increase in serum MCPT1, IgE, and peanut-specific IgE and IgG1</li> <li>• Increase in IL-4 secreting CD4<sup>+</sup> T cells in MLNs and spleen</li> <li>• Decreased number of Foxp3<sup>+</sup> Tregs in colon</li> <li>• Allergen induced proliferation of Foxp3<sup>+</sup> Tregs ex vivo</li> <li>• Increase in <i>IL25</i> and <i>IL33</i> in the small intestine</li> <li>• Increase in ILC2 and ILC3 in MLNs</li> <li>• Increase in total serum IgE and OVA-IgE, and MCPT1</li> <li>• Allergic diarrhea</li> <li>• Intestinal mast cell expansion</li> </ul> | 36<br>58, 59 |
| WASP deficient (Was <sup>-/-</sup> on BALB/c background) | Targeted mutation in Was gene                                                       | Days 0, 5, 10, 15, 20, 25, 30, and 35: ig 5 mg OVA                                                                  | Day 49: ig 50 mg OVA                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 65           |

Table 1. Continued

| Strain                                                                                       | Description                                                                                                                | Sensitization                                                                                                                    | Challenge | Phenotype of allergic mice                                                                                                                                                                                                                                                                         | Reference |
|----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| C3H/HeJ<br>Balb/c                                                                            | Inbred strains                                                                                                             | Acute: ig 80 mg peanut protein or 60 mg OVA with MCT oil<br>Chronic: ig 80 mg peanut protein for 4 weeks                         |           | <ul style="list-style-type: none"> <li>Acute and chronic: increase in antigen-specific IgE and IgG in the serum</li> <li>Chronic: increase in <i>Ts/p</i>, <i>IL25</i>, <i>IL33</i> in the intestinal epithelium</li> </ul>                                                                        | 72        |
| Balb/c                                                                                       | Inbred strain                                                                                                              | Days 0 and 3: ig MCT administration<br>Followed by ig MCT with egg white every 2 days for 3–4 weeks or every 4 days for 5 weeks. |           | <ul style="list-style-type: none"> <li>Decrease in core body temperature</li> <li>Increase in serum egg white specific IgE, MCPT1, IL4, and IL13</li> <li>Increase in number of mast cells, eosinophils, and dendritic cells in the lamina propria</li> </ul>                                      | 73        |
| Spontaneous sensitization models                                                             |                                                                                                                            |                                                                                                                                  |           |                                                                                                                                                                                                                                                                                                    |           |
| CNS1 knockout                                                                                | Deficient in CNS1                                                                                                          | Spontaneous sensitization to chow                                                                                                | None      | <ul style="list-style-type: none"> <li>Tregs have Th2 skewed profile (GATA3<sup>+</sup>)</li> <li>Increase in Th2 cytokine production by CD4<sup>+</sup> T cells (IL4, IL5, IL13)</li> </ul>                                                                                                       | 64        |
| WASP deficient (Was <sup>-/-</sup> on C57BL/6J background)<br>Was <sup>fl/fl</sup> Foxp3-Cre | Targeted mutation in <i>Was</i> gene<br><br>Targeted mutation in <i>Was</i> gene conditionally in Foxp3 <sup>+</sup> cells | Spontaneous sensitization to wheat and soy in chow                                                                               | None      | <ul style="list-style-type: none"> <li>High serum levels of IgE and MCPT1</li> <li>Intestinal inflammation</li> <li>Expansion of mast cells in the small intestine</li> <li>Proliferation of CD4<sup>+</sup> T cells in MLNs</li> <li>Tregs have Th2 skewed profile (GATA3<sup>+</sup>)</li> </ul> | 29        |

id, intradermal; ig, intragastric; ip, intraperitoneal; ILC, innate lymphoid cell; MCPT1, mast cell protease 1; MLNs, mesenteric lymph nodes; OVA, ovalbumin; rTSLP, recombinant thymic stromal lymphopoietin; Was, Wiskott-Aldrich syndrome.

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### C.129X1-IL4 $\alpha^{tm3.1Tch}$ /J – IL4RF709 Mice

The IL4RF709 mice contain a targeted mutation in the immunoreceptor tyrosine-based inhibitory motif domain of the IL4 $\alpha$  at amino acid 709, substituting tyrosine for phenylalanine. Although a human equivalent of this mutation does not exist, it phenotypically mimics human single nucleotide polymorphisms on IL4 $\alpha$  that promote the signaling through the receptor and are associated with atopy, such as IL-4 $\alpha$  I75V and IL-4 $\alpha$  Q576R.<sup>53,54</sup> This constitutively activating mutation results in a heightened IgE response and increased inflammation after allergen exposure in an asthma model.<sup>55</sup> Food allergy phenotypes in IL4RF709 mice are similar after intragastric sensitization with or without an adjuvant.<sup>56</sup> It can therefore be concluded that constitutive activation of the IL4 $\alpha$  can circumvent the tolerance pathways by providing the IL4R signaling achieved in other models with adjuvants such as cholera toxin.<sup>57</sup> Allergic patients fail to develop oral tolerance to their allergen in part because of a defect in the generation of Tregs, which are not only vital to tolerance of the adaptive immune system but have been shown to have a direct effect on mast cells by blocking degranulation.<sup>35,58–60</sup> The IL4RF709 mice can reproduce this absence to produce allergen-specific Tregs.<sup>35</sup> In these models, the Tregs that are produced may be skewed toward a Th2 profile as monitored by the expression of the transcription factor GATA-3 and Th2 cytokines.<sup>35</sup> In addition, activation of the IL4 $\alpha$  by IL4 or a mutation has been shown to regulate human gastrointestinal mast cells by shifting their cytokine profile from a Th1 to a Th2 phenotype.<sup>61,62</sup> It was also observed that allergic IL4RF709 mice develop edema and have a greater number of small intestinal mast cells, which are located higher up in the villi closer to the lumen.<sup>56,58,59</sup>

### CNS1 Knockout Mice

The intronic Foxp3 enhancer, CNS1, is a genetic determinant for the differentiation of inducible Tregs.<sup>63</sup> CNS1 knockout in mice selectively blocks the differentiation of peripheral T cells to inducible Tregs, which leads to the spontaneous development of Th2-mediated diseases in the mucosal tissues.<sup>64</sup> CNS1 deletion increases GATA3 expression in CD4<sup>+</sup> T cells in the gastrointestinal tract, which is indicative of a Th2 skewed peripheral T-cell compartment. This Th2 environment can induce IL4, IL5, and IL13 in the Peyer's patches, mesenteric lymph nodes, and small intestinal lamina propria, which in turn contributes to the production of chow-specific serum IgE, an increase in the number of germinal center B cells, and a loss of body weight.<sup>64</sup>

### Wiskott-Aldrich Syndrome Protein Deficient (*Was*<sup>-/-</sup>) Mice

*Was*<sup>-/-</sup> mice display many phenotypes also observed in Wiskott-Aldrich syndrome protein (WASP)-deficient patients. In addition to developing colitis, both WASP-deficient patients and mice have an increase in antigen-specific antibodies against common food allergen epitopes.<sup>29</sup> Even in non-colic *Was*<sup>-/-</sup> strains, sensitization to chow,

predominantly to soy and wheat, occurs spontaneously within the first 6 weeks.<sup>65–67</sup> The intestinal sensitization in *Was*<sup>-/-</sup> mice occurs under germ-free conditions and is thus independent of the composition of the intestinal flora. This implies that no microbial danger signal for the loss of oral tolerance to food is required in this model. However, mice housed under specific pathogen-free conditions showed an overall skewed humoral response to the food antigens with higher levels of food-specific IgA, which is considered protective. Therefore, this model might help identify microbial strains that provide protective signals by altering intestinal immune responses. Observations about microbial strains as regulators of sensitization and food allergy in mice may not be transferable to humans because of species-specific differences in microbiota composition. Generally, it is fair to state that mouse models have allowed us to understand that a subject's allergic status can be altered by manipulating its gut microbial community. Targeted alterations of the microbial community can be studied with respect to their overall effect on anti-food humoral responses. In this respect, it might be necessary to focus intervention strategies on animals whose gut are colonized with microbiome of patients with food allergies.

Chow-sensitized *Was*<sup>-/-</sup> mice present with elevated serum IgE, IgG1, and MCPT1 and increased small intestinal mast cell expansion. Experiments using ovalbumin as a model antigen confirmed that the mice also sensitize to a newly introduced food allergen while already sensitized to chow.<sup>29,65</sup> Therefore, *Was*<sup>-/-</sup> mice provide a unique opportunity to study de novo sensitization on primary food exposure in a pre-sensitized and allergic host.

In contrast to cell type-specific deletions in B cells, dendritic cells, or macrophages, deletion of WASP in Tregs (*Was*<sup>fl/fl</sup>Foxp3-Cre) results in the mice spontaneously developing food allergy phenotypes more severe than that observed in the complete knockout animals. The *Was*<sup>fl/fl</sup>Foxp3-Cre mice have a Th2 skewed T-cell profile with increase in GATA3 expression in both memory T cells and Tregs as well as increased IL4 and IL13 secretion on ex vivo CD3/CD28 stimulation of CD4<sup>+</sup> T cells from mesenteric lymph nodes. This phenotype mimics the observation made in human patients with food allergies who also have Th2-skewed Treg cells.<sup>68,69</sup> *Was*<sup>fl/fl</sup>Foxp3-Cre can be viewed as a minimal genetic alteration model in which the effect of aberrant Tregs can be studied.

### Oral Sensitization Promoted by Medium Chain Triglycerides

We are discussing the medium chain triglycerides (MCT) model as a spontaneous disease model because human diet contains this type of triglycerides. However, it is important to mention that MCT could serve as an adjuvant and, therefore, the sensitization phase might be closely comparable with other types of isomorphic models that rely on adjuvant-based oral sensitization. Nonetheless, studying this model and the effect of MCT is beneficial to society because it is a component of certain infant formulas. Infants allergic to milk or at risk of developing a milk allergy are fed with

hypoallergenic formulas that are extensively hydrolyzed and contain a high content of MCT.

The role of fatty acids in the development and progression of allergic inflammation has been explored extensively.<sup>70</sup> Depending on the length of the carbon backbone, a proinflammatory or anti-inflammatory effect may be observed, because the route of absorption of the allergen and the development of tolerance to the allergen are affected by the lipid. Compared with long chain triglycerides (LCT) (fatty acids that have >12 carbon atoms), MCT (fatty acids that contain ≤12 carbon atoms) are absorbed less readily into the blood, resulting in increased antigen bound to CD11c<sup>+</sup> cells in the Peyer's patches and lower antigen concentrations in the blood and mesenteric lymph nodes. This difference in antigen localization is attributed to the formation of chylomicrons by LCT, which does not occur with MCT.<sup>71</sup> In non-genetically modified inbred strains of mice, such as C3H/HeJ and Balb/c animals, a single gavage of peanut protein with MCT results in significant increases of peanut-specific IgE and IgG in the serum compared with control animals. In addition, an increase in *Tslp*, *IL-25*, and *IL-33* expression in the small intestinal epithelial cells is also observed.<sup>72</sup> Repeated administration of egg white protein with MCT for at least 3 weeks increases total and egg white-specific IgE, increases serum mast cell activation marker (MMCP1) and Th2 cytokines (IL4 and IL13), and increases the number of mast cells, eosinophils, and dendritic cells in the lamina propria.<sup>73</sup> The MCT mediated model of food allergy can be considered an example of how a dietary component can facilitate oral sensitization to food.

In conclusion, spontaneously sensitizing models provide a unique opportunity to study the induction of food allergy. These models do not require the use of adjuvants and can be used to elicit allergy to chow antigens and may also be well-suited to define allergenic properties of different foods.

## Humanized Murine Models of Food Allergy

Novel therapeutic strategies for food allergies, such as the injection of Treg cells, anti-IgE therapy, and anti-cytokine therapy, have been explored in mouse models. Although there is a significant overlap in the disease phenotypes between mice and humans, there are inherent differences between the human and the murine immune system that limit the translation of the findings from one host to another. Humanized murine models, which contain human cell compartments, help mitigate these difficulties for testing novel food allergy treatments. In addition, transgenic mice have been established that express human proteins that are vital to the sensitization and effector phase of allergy.<sup>74</sup> The use of transgenic strains has the benefit of reducing the need to obtain tissue samples from allergic humans. Obtaining biopsies from patients with food allergies is rather limited because they do not regularly undergo invasive procedures. Humanized mice that carry human genes, cells, and tissues provide a highly sought after alternative to human tissues and cells for testing treatment ideas.<sup>75</sup>

The studies presented below make use of unique strains such as the NOD/Shi-scid IL2r $\gamma$ null (NOG), NOD/LtSz-scid IL2r $\gamma$ null (NSG), and BALB/c Rag2nullIL2r $\gamma$ null.<sup>76–81</sup> Because they lack an endogenous immune system, they can be repopulated with human hematopoietic cells to study human immune cells in an in vivo context. Overexpression of markers such as IL13 and stem cell factor by using transgenic mice promotes an allergic environment. After the induction of food allergy, these mice can generate human mast cells and food-specific human IgE (Table 2).

## Allergen-specific Immunoglobulin E Production in Human Peripheral Blood Mononuclear Cells Engrafted in NOD-scid- $\gamma$ c<sup>-/-</sup>

Weigmann et al<sup>82</sup> developed a model in NOD-SCID mice to study inflammation in the gut after engraftment of peripheral blood mononuclear cells (PBMCs) from individuals allergic to grass or birch pollen. Although these 2 allergens are not classic food allergens, they have been shown to have cross-reactivity to hazelnut.<sup>82,83</sup> Stimulation of the engrafted PBMCs by intraperitoneal injection, followed by rectal or oral challenge with the respective allergen, produced colitis. Histologic changes observed include infiltration of CD45<sup>+</sup> cells into the mucosa, thickening of the epithelial layer, and mucosal hypertrophy. After allergen restimulation, human PBMCs isolated from the mouse spleen proliferated ex vivo and produced IL4, IL13, IL10, and interferon gamma. Inflammation was primarily driven by human PBMCs because both depletion of high-affinity immunoglobulin epsilon receptor subunit alpha (FCER1A)-expressing cells before injection and treatment with omalizumab (anti-human IgE monoclonal antibody) ameliorated the allergic inflammation.

## CD34<sup>+</sup> Hematopoietic Stem Cell Engraftment Into NOG Mice Expressing Human Interleukin 3 and Granulocyte-Macrophage Colony-stimulating Factor Transgenes (NOG IL-3/GM-Tg)

Ito et al<sup>84</sup> engrafted NOG mice bearing human IL3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (NOG IL-3/GM-Tg) with human umbilical cord blood-derived CD34<sup>+</sup> stem cells. This engraftment resulted in the population of mouse blood with human myeloid cells. A population of human high-affinity immunoglobulin E receptor (FcεRI<sup>+</sup>)CD203c<sup>+</sup> cells were present in the peripheral blood, bone marrow, spleen, and broncho-alveolar lavage fluid of the transgenic animals but not in the non-transgenic mice, indicating that IL3 and GM-CSF are central in the development and expansion of functional FcεRI expressing mast cells and basophils. Passive cutaneous anaphylaxis experiments using human hapten-specific IgE and serum from pollinosis patients (high titer of antibodies against Japanese cedar pollen) resulted in cutaneous anaphylactic reactions with stronger local inflammatory response compared with non-transgenic mice.

**Table 2.** Humanized Murine Models of Food Allergy

| Strain                                                                                      | Description                                                          | Engraftment                                                                                                            | Sensitization                                                        | Challenge                                                          | Phenotypes                                                                                                                                                                                                                                                                                                                          | Reference |
|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| NOD-scid- $\gamma$ C <sup>-/-</sup> (NSG)                                                   | NOD-SCID mice with targeted mutation in IL2 receptor $\gamma$ -chain | ip: $2 \times 10^7$ PBMCs from food allergic donors with 20 $\mu$ g allergen (birch pollen, grass pollen, or hazelnut) | None                                                                 | Oral or rectal with the same allergen given during the engraftment | <ul style="list-style-type: none"> <li>Production of allergen-specific IgE</li> <li>Allergen induced proliferation and cytokine production of splenic CD4<sup>+</sup> T cells in vitro</li> <li>Inflammation in the colon – mucosal hypertrophy, enhanced wall thickening, infiltration of mucosa with mononuclear cells</li> </ul> | 82        |
| NOD/Shi-scid-IL2r $\gamma$ (null) (NOG), bearing human IL3 and GM-CSF genes (NOG IL3/GM-Tg) | NSG expressing human IL3 and GM-CSF                                  | iv: $4-5 \times 10^4$ CD34 <sup>+</sup> HSCs                                                                           | id 0.5 $\mu$ g anti-NP clgE id serum from pollinosis patients        | iv 500 $\mu$ g NP-BSA 200 JAU/mL cedar pollen extract              | <ul style="list-style-type: none"> <li>Local mast cell response</li> <li>Increase in number of mature basophils and mast cells expressing human Fc<math>\epsilon</math>RI</li> </ul>                                                                                                                                                | 84        |
| NSG-SCF/GM-CSF/IL3 (NSG-SGM3)                                                               | NSG expressing human IL3, GM-CSF, and SCF                            | Human thymus, liver, and hematopoietic stem cells                                                                      | PCA: id (ear) 10 ng anti-NP clgE<br>PSA: iv 1.6 $\mu$ g anti-NP clgE | PCA: iv 500 $\mu$ g NP-BSA<br>PSA: iv 500 $\mu$ g NP-BSA           | PCA: increase in ear thickness<br><br>PSA: decrease in core body temperature<br>human IgE dependent degranulation of mast cells                                                                                                                                                                                                     | 85        |
| NSG-SCF                                                                                     | NSG expressing human SCF                                             | iv: $5 \times 10^4-10^5$ CD34 <sup>+</sup> HSCs derived from cord blood                                                | Weeks 0–7: ig 5 mg peanut protein weekly                             | Week 8: ig feeding with 78 mg peanut protein                       | <ul style="list-style-type: none"> <li>Expansion of human mast cells in the jejunum</li> <li>Expression of human tryptase in the serum</li> <li>Allergen-specific human IgE in the serum</li> </ul>                                                                                                                                 | 86        |
| IgE <sub>R</sub> -TG                                                                        | Express human FCER1A                                                 | None                                                                                                                   | Days 0 and 14: ip 100 $\mu$ g OVA with aluminum hydroxide            | Days 28–38: ig 50 mg OVA on alternating days                       | <ul style="list-style-type: none"> <li>Increase in human IgE-bound dendritic cells in the spleen and mesenteric lymph nodes</li> <li>Decreased inflammation in lamina propria</li> <li>Decreased expression of mast cell specific proteases MCPT1, MCPT2, and CPA3</li> <li>Decreased Th2 cytokines IL4 and IL13</li> </ul>         | 97        |

BSA, bovine serum albumin; clgE, chimeric IgE; FCER1A, high affinity immunoglobulin epsilon receptor subunit alpha; Fc $\epsilon$ RI, high-affinity IgE receptor; HSCs, hematopoietic stem cells; id, intradermal; ig, intragastric; ip, intraperitoneal; iv, intravenous; JAU, Japanese allergy units; OVA, ovalbumin; NP, 4-(hydroxy-3-nitro-phenyl) acetyl; PBMCs, peripheral blood mononuclear cells; PCA, passive cutaneous anaphylaxis; PSA, passive systemic anaphylaxis; SCF, stem cell factor.

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### NSG-SCF/GM-CSF/IL3 Mice Engrafted With Human Thymus, Liver, and Hematopoietic Stem Cells

Bryce et al.<sup>85</sup> produced humanized mice by implanting thymic tissue before the injection of CD34<sup>+</sup> hematopoietic stem cells (HSCs) into NSG-SCF/GM-CSF/IL3 mice. Human mast cells were detected in multiple tissues such as the lungs, spleen, small intestine, cardiac, stomach, and skin. After passive sensitization with anti-NP chimeric IgE, both passive cutaneous anaphylaxis and passive systemic anaphylaxis can be observed in these mice. However, this model does not respond with oral anaphylaxis. These mice also provide the unique advantage of generating human mast cells that can be isolated and cultured for ex vivo experiments. Around  $1 \times 10^6$  human mast cells can be recovered from the peritoneal cavity of these mice.

### NOD.Cg-PrkdcscidIl2rgtm1Wjl Tg(PGK1-KITLG\*220)441Daw/SzJ Engrafted With CD34<sup>+</sup> Cord Blood Cells

Burton et al.<sup>86</sup> reconstituted immunodeficient mice with human hematopoietic progenitor cells (CD34<sup>+</sup> cord blood HSCs). After reconstitution, human CD45<sup>+</sup> leukocytes were circulating in the mouse blood, spleen, mesenteric lymph nodes, bone marrow, and small intestinal lamina propria. Expression of human stem cell factor transgene in NSG mice promoted human mast cell expansion, creating a pro-allergic environment. Bolus challenge with peanut allergen after repeated sensitization produced an oral anaphylactic response. It was validated that this response was mediated by human mast cells, and not host mast cells, by measuring human tryptase in the plasma. Treatment with anti-human IgE, omalizumab, protected from the anaphylaxis, further confirming that the allergic phenotype was mediated by the humanized compartment of the mouse. Importantly, this is currently the only humanized model that presents with oral anaphylaxis after peanut ingestion.

Overall, large variability in the data generated from humanized models can be observed on the basis of the route of the HSC injection (intravenous or intrahepatic), the age of the mice, the method of irradiation, and the source of the HSC (cord blood or fetal liver).<sup>87</sup> All of these factors affect the efficiency of the engraftment and the characteristics of the cells that populate the animals. Because of variations in engraftment efficiencies, large numbers of mice are needed

to achieve statistical significance in the data. Reproducibility of data between laboratories can also be affected by the variability in colony microbiota. Cell types such as erythrocytes and platelets are not humanized along with the immune cells. Platelets have been shown to contribute to allergen sensitization and challenge. For example, blocking platelets and the action of their preformed mediators can decrease sensitization and the phenotypes in response to allergen challenge.<sup>88-90</sup> It is fair to say that current humanization strategies are incomplete, and therefore certain aspects of the inflammatory cascade operating in human food allergy cannot be properly studied.

### Murine Strains Expressing Human High-affinity Immunoglobulin E Receptor, FcεRI

The high-affinity IgE receptor, FcεRI, is a multimeric immune recognition receptor that exists in a trimeric and tetrameric form. Although the tetrameric form (composed of  $\alpha$ ,  $\beta$ , and dimer of  $\gamma$  chains) is found on both human and murine IgE effector cells, the trimeric form (which lacks the  $\beta$  chain) is only constitutively expressed on human dendritic cells. However, in both humans and mice it can be induced on other cell types such as monocytes, neutrophils, and inflammatory dendritic cells in the context of infection.<sup>91-95</sup> Although the trimeric form can be induced in mice, a humanized model is necessary to understand the functional consequences of the constitutively expressed trimeric receptor on dendritic cells in humans. This is relevant to food allergy research because human dendritic cells of the esophagus and the lamina propria of the small intestine express FcεRI as their main IgE receptor.<sup>96-99</sup> Because dendritic cells are sentinel immune cells that decide whether inflammatory or tolerogenic responses are induced, the FcεRI expression pattern of the human mucosal tissues stresses the importance of studying the contributions of IgE-activated dendritic cells to mucosal allergies.<sup>100</sup>

Currently, FcεRI-humanized animals are the only established murine models for studying the role of constitutively expressed FcεRI on dendritic cells and immunologic functions of dendritic cell-bound IgE.<sup>98,99</sup> Several different strains with humanized FcεRI expression can be found in the literature (Table 3, human proteins in red). Mice expressing human FCER1A under the control of the human FCER1A promoter were generated with embryonic stem cells from Fcεr1a<sup>-/-</sup> mice (HP-TG mice).<sup>101-103</sup> In these

**Table 3.** Mouse Strains Expressing Human FCER1A

| Mouse strains                                                              | Mast cells and basophils          | Dendritic cells              | Reference |
|----------------------------------------------------------------------------|-----------------------------------|------------------------------|-----------|
| C57BL/6J                                                                   | $\alpha^{\text{mu}}\beta\gamma_2$ | No expression                |           |
| MuFcεr1a <sup>-/-</sup>                                                    | No expression                     | No expression                | 101       |
| huFCER1A <sup>HP</sup> TG × muFcεr1a <sup>-/-</sup> (HP-TG)                | $\alpha^{\text{hu}}\beta\gamma_2$ | $\alpha^{\text{hu}}\gamma_2$ | 102, 103  |
| huFCER1A <sup>(Cd11c)</sup> TG (Cd11c-TG)                                  | $\alpha^{\text{mu}}\beta\gamma_2$ | $\alpha^{\text{hu}}\gamma_2$ | 97, 104   |
| huFCER1A <sup>(Cd11c)</sup> TG × muFcεr1a <sup>-/-</sup> ( $\alpha$ DC-TG) | No expression                     | $\alpha^{\text{hu}}\gamma_2$ | 105       |

NOTE. Human proteins are in red.  
hu, human; mu, murine; TG, transgenic.

animals, FcεRI on all innate cells is a chimera of the human FcεRIα and the murine signaling units, including on dendritic cells.<sup>98,104</sup> In huFCER1A<sup>(Cd11c)</sup>TG animals (Cd11c-TG), the Cd11c promoter is used to drive the expression of human FCER1A on dendritic cells, whereas murine endogenous FCER1A is expressed on mast cells and basophils.<sup>97,104</sup> The huFCER1A<sup>(Cd11c)</sup>TG x muFcer1a<sup>-/-</sup> mice (αDC-TG) express the chimeric FcεRI on dendritic cells, but no form of the FcεRI receptor is found on other cell types.<sup>105</sup> By using this strain, one can differentiate between receptor-mediated activation of mast cells and dendritic cells. It is important to note that human and murine FcεRIα bind murine IgE with comparable affinity. In contrast, human IgE interacts only with the human receptor.

By using HP-TG mice, it has been demonstrated that FcεRI on dendritic cells facilitates the clearance of serum IgE.<sup>106</sup> In addition, a tolerogenic antigen presentation pathway was identified by using antigens fused to monomeric IgE.<sup>107</sup> The role of the dendritic cell-bound IgE pool in allergic diseases has been studied in Cd11c-TG and αDC-TG.<sup>104</sup> Unlike the tetrameric receptor, antigen-specific cross-linking of the trimeric receptor results in a regulatory anti-inflammatory signal. In food allergy models, the dendritic cell-bound IgE pool reduced inflammation in the small intestine as measured by decreased mast cell expansion and reduced expression of mast cell activation markers (Mcp1, Mcpt2, carboxypeptidase A3) and lower tissue levels of IL4, IL6, IL13, and CCL2.

On the basis of the published studies, it can be concluded that IgE/FcεRI-mediated dendritic cell activation is part of a regulatory feedback loop that restrains the allergic inflammatory response. The next important steps are to gain insights as to why IgE-mediated control mechanisms fail, how they affect sensitization to allergens, and whether they can be exploited for therapeutic purposes.

## Concluding Remarks

A critical comparison of currently available experimental models of food allergy shows that none of them reflect the pathology of the human condition completely. Adjuvant dependent models fall short in replicating the sensitization phase and loss of oral tolerance to food. Spontaneous sensitization models poorly replicate the effector phase phenotypes of individuals with food allergies as exemplified by their lack of oral anaphylaxis. Nonetheless, spontaneous models will be valuable to study the atopic march or perform maternal-fetal allergy transfer studies. Humanized mouse models also mimic human food allergies only partially, because they do not develop spontaneous disease, and most do not mount an anaphylactic response on oral challenge. Going forward, it will be important to critically evaluate all available experimental options and choose the best model for specific questions. Although few murine models can ever re-create a human disease in its entirety, comparative studies with multiple models, each with their own strengths, will allow researchers to more fully understand food allergies than a single model ever could.

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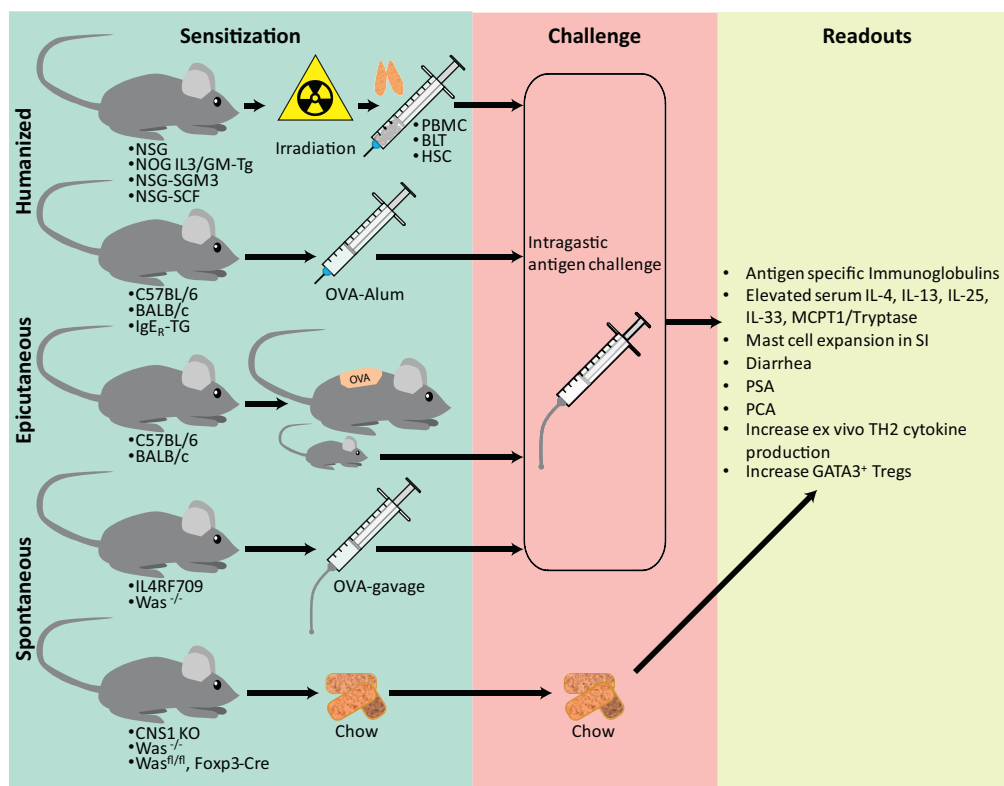
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#### Conflicts of interest

The authors disclose no conflicts.

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Supplemental Graphical Summary.