

Mast cells and IgE activation do not alter the development of oral tolerance in a murine model

Matthew C. Tunis, BSc,^{a,d} Wojciech Dawicki, PhD,^{a,*} Kaitlyn R. Carson, BSc,^a Jun Wang, PhD,^{a,c,d,e} and Jean S. Marshall, PhD^{a,b,d} Halifax, Nova Scotia, and Saskatoon, Saskatchewan, Canada

Background: In addition to their well-known role as potent effector cells in patients with allergic disease, mast cells have important immunomodulatory roles regulating tolerance in allograft rejection models. The roles of mast cells in oral tolerance development have not previously been examined.

Objective: We sought to evaluate the importance of mast cells, IgE-mediated mast cell activation, and histamine receptor 1 or 2 blockade on oral tolerance development in mice.

Methods: Oral tolerance was assessed in 2 mast cell-deficient murine strains (*Kit^{W-sh/W-sh}* and *Kit^{W/W-v}* mice) and control mice. Mice were fed ovalbumin (OVA) or peanut butter for 1 week and then immunized and boosted with relevant protein antigens. Antibody responses were assessed by using ELISA. The oral antihistamines pyrilamine and ranitidine were administered during tolerance induction to OVA. IgE-mediated mast cell activation was initiated during oral tolerance induction or OVA immunization. OVA-specific regulatory T cells were assessed in the Peyer patches, mesenteric lymph nodes, and spleens by using flow cytometry after adoptive transfer.

Results: Oral tolerance was successfully induced to OVA and peanut butter in mast cell-deficient mice. *Kit^{W-sh/W-sh}* mice had higher proportions of antigen-specific regulatory T cells in the mesenteric lymph nodes than mast cell-containing control mice. However, mast cell reconstitution studies suggested this effect was mast cell independent. Oral antihistamine treatments with pyrilamine or ranitidine did not impair tolerance and neither did IgE-mediated activation.

Conclusions: Mast cells are not necessary for the induction of oral tolerance, and allergic activation of mast cells does not

impair tolerance to OVA. Oral antihistamine treatments do not disrupt the development of oral tolerance. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Mast cell, regulatory T cell, oral tolerance, antihistamine, ovalbumin, peanut, IgE

Oral immunotherapy is increasingly available as a treatment option for food allergy, with clinical trials yielding encouraging results.^{1,2} Although the complex immune mechanisms of oral immunotherapy are not completely understood, they are thought to involve a variety of cell types from both the innate and adaptive arms of the immune system, as well as structural cells, such as the intestinal epithelium.^{3,4} The functions of many immune cells can be modulated by mast cells.⁵⁻⁸ Because allergic disease involves mast cell activation and mast cell mediator blockade is frequently used in the context of oral immunotherapy, it is essential to understand what role mast cells play in the development of oral tolerance.

Mast cells are positioned close to the external environment and serve as effector cells in patients with allergic disease and in responses to pathogens.⁹ There is a growing body of work examining the interaction between mast cells, dendritic cells, and T cells and how these relationships might alter immunity to foreign antigens.^{5,6,8,10,11} Despite their strategic location in the oral mucosa and intestinal microenvironment, the role of mast cells in oral tolerance has not been investigated. Mast cells can activate T cells, with a bias toward expanding populations of regulatory T (Treg) cells *in vitro*.¹² Moreover, mast cell-deficient *Kit^{W-sh/W-sh}* mice have shown deficient Treg cell activity in an experimental autoimmune encephalomyelitis model of multiple sclerosis.¹³ Similarly, it was recently shown in a skin transplant model that mast cells are critical in extending the maintenance of peripheral tolerance and allograft survival, most likely through recruitment of Treg cells and the action of mast cell proteases.^{14,15} It has also been demonstrated that mast cell-derived IL-10 can limit inflammatory infiltrate to tissue sites in addition to inhibiting germinal center formation.¹⁶⁻¹⁸ However, the presence of other immune cell defects in both the *Kit^{W-sh/W-sh}* and *Kit^{W/W-v}* models of mast cell deficiency make interpretations of some of these studies problematic, and this issue has recently been highlighted by studies of alternate mast cell deficiency murine models that appear to have normal immune and inflammatory responses in models of multiple sclerosis and arthritis.¹⁹⁻²¹ Given this previous body of work, investigations into whether mast cells regulate oral tolerance are urgently required.

IgE-mediated mast cell degranulation has been reported to break established peripheral tolerance in a murine tissue allograft

From the Departments of ^aMicrobiology and Immunology, ^bPathology, and ^cPediatrics and ^dthe Dalhousie Inflammation Group, Dalhousie University, Halifax, and ^ethe Canadian Center for Vaccinology, Dalhousie University, IWK Health Centre, Capital Health, Halifax.

*W. Dawicki is currently affiliated with the Division of Respiriology, Critical Care and Sleep Medicine, Department of Medicine, Royal University Hospital, University of Saskatoon, Saskatoon, Saskatchewan, Canada.

Supported by AllerGen NCE, Inc. M.C.T. is supported by a studentship from the Natural Sciences and Engineering Research Council of Canada.

Disclosure of potential conflict of interest: M. C. Tunis has received research support from the Natural Sciences and Engineering Research Council of Canada. J. S. Marshall has received research support from the AllerGen Network of Centres of Excellence, Inc., and the Canadian Institutes for Health Research. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 29, 2011; revised April 4, 2012; accepted for publication April 9, 2012.

Corresponding author: Jean S. Marshall, PhD, Department of Microbiology and Immunology, Dalhousie University, 5850 College St, Room 7C, Halifax, NS B3H 4R2, Canada. E-mail: jean.marshall@dal.ca.

0091-6749/\$36.00

© 2012 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2012.04.011

Abbreviations used

BMMC: Bone marrow–derived mast cell
 CPE: Crude peanut extract
 Foxp3: Forkhead box protein 3
 GFP: Green fluorescent protein
 MLN: Mesenteric lymph node
 mMCP: Murine mast cell protease
 OVA: Ovalbumin
 pDC: Plasmacytoid dendritic cell
 PP: Peyer patch
 TNP: Trinitrophenyl
 Treg: Regulatory T

model,²² and degranulation can enhance antibody responses to both bacterial and viral antigens in the mast cell microenvironment.^{23,24} Furthermore, mast cell degranulation products interfere with the suppressive activity of natural Treg cells.²⁵ Mast cells are a major source of tissue histamine, which has multiple immunoregulatory effects.²⁶ Histamine can polarize the CD4⁺ T-cell response toward a T_H2 IL-4 phenotype through dendritic cells^{27,28} and has been shown to modulate the recruitment of both the CD8⁺ subset of dendritic cells and plasmacytoid dendritic cells (pDCs) to the draining lymph nodes.⁵ Notably, the pDC subset in the liver and other gut-associated lymphoid tissues has been implicated in the induction of immunologic tolerance through the oral route.^{29–31} Despite the known effects of histamine on DCs, oral antihistamine treatment has not been directly investigated in a model of oral tolerance. The mobilization and directed trafficking of pDCs in response to mast cells suggests another avenue by which mast cells can modulate oral tolerance.²⁵

Taken together, current evidence suggests that mast cells and their activation products have the potential to enhance immunity to food antigens and compromise oral tolerance. In the present study we sought to identify the immunologic outcomes of mast cell deficiency, IgE-mediated mast cell activation, and antihistamine treatment on oral tolerance induction and antibody production. The models of oral tolerance induction and mast cell activation used here avoid the potentially confounding variables of mast cell–mediated changes in intestinal permeability and antigen access. Oral tolerance was characterized according to several parameters, including antigen-specific serum IgE, which is an important tool in determining food allergy potential in clinical patients.³²

To our knowledge, this report is the first examination of oral tolerance in mast cell–deficient mice and assessment of the effect of mast cell degranulation on oral tolerance induction.

METHODS**Mice**

Male C57BL/6J and WBB6F1/J-*Kit*^W/*Kit*^{W-v} (*Kit*^{W/W-v}) mice were purchased from Jackson Laboratories (Bar Harbor, Me) and were normally used between 6 and 8 weeks of age. Male *Kit*^{W-sh}/HNIhrJaeBsmJ (*Kit*^{W-sh/W-sh}), B6.129P2-*Fcgr3*^{tm1Sjv}/SjvJ (*FcγRIII*^{-/-}), and C57BL/6J mice were supplied by or bred from stock obtained from Jackson Laboratories. B6.SJL-*Ptpca Pepcb*/BoyJ (CD45.1⁺) mice and B6.Cg-Tg(TcrαTerb)425Cbn/J (OT-II⁺) mice were purchased from Jackson Laboratories and crossed on site with forkhead box protein 3 (Foxp3) green fluorescent protein (GFP⁺) mice. *Kit*^{W-sh/W-sh} mice were reconstituted with cultured bone marrow–derived mast cells (BMMCs) from C57BL/6J mice by using intraperitoneal and intravenous injection of 4 × 10⁶ and 2 × 10⁶ BMMCs, respectively, and used in experiments 12 weeks later to allow mast

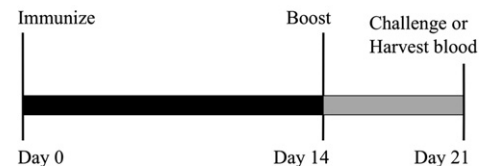
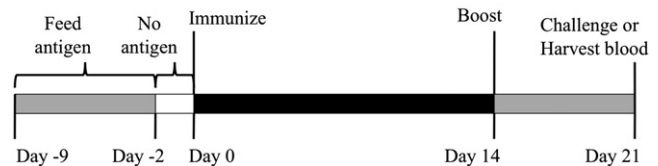
Sensitized**Tolerized**

FIG 1. Oral tolerance protocol. Schematic outline for the feeding and immunization schedule of mice. Mice were immunized and boosted with OVA or CPE, as detailed in the Methods section. Blood was harvested for antibody analysis, or mice were challenged intraperitoneally with 10 mg of OVA or 1 mg of CPE.

cells to mature *in situ*. Approval for all animal studies was granted by the Dalhousie University Committee on Laboratory Animals.

Antibodies and reagents

Anti-mouse IgG₁ and IgG_{2a} antibodies were purchased from BioLegend (San Diego, Calif). Anti-mouse IgE was purchased from eBioscience (San Diego, Calif) and Southern Biotech (Birmingham, Ala). All other antibodies were obtained from eBioscience. A murine mast cell protease (mMCP) 1 ELISA kit was purchased from eBioscience. Magnetic activated cell sorting was performed with the CD4⁺ T cell isolation kit II and CD62L (L-selectin) microbeads from Miltenyi Biotec, Inc (Auburn, Calif). Ranitidine, pyrilamine, and ovalbumin (OVA) from chicken egg white were purchased from Sigma (Oakville, Canada). Trinitrophenyl (TNP)–BSA was purchased from Biosearch Technologies, Inc (Novato, Calif). Anti-TNP IgE was enriched from TIB141 (ATCC, Manassas, Va) supernatant by means of ammonium sulfate precipitation followed by HPLC. Crude peanut extract (CPE) was purchased from Greer Technologies (Lenoir, NC).

Induction and assessment of OVA tolerance

Mice were provided 4 mg/mL OVA in drinking water *ad libitum* for 7 days, whereas a control group was given normal drinking water (Fig 1). On day -2; all mice were returned to normal water. All groups were immunized on day 0 with 50 μg of alum-precipitated OVA in PBS, except where indicated. All groups were boosted with soluble OVA (10 μg, except where indicated) in PBS on day 14, and blood samples were taken by means of cardiac puncture on day 21. In some experiments fecal samples were taken on day 21 and homogenized in PBS with 0.01% sodium azide. OVA-specific antibodies were compared between tolerized mice and corresponding control groups to assess a relative degree of tolerance induction. Significant tolerance induction in each antibody compartment was defined by statistically significant reductions in plasma OVA-specific antibody levels.

Induction and assessment of peanut tolerance

Mice were treated as detailed in Fig 1. One group of mice was fed peanut butter *ad libitum* (Kraft “All Natural Peanut Butter,” Don Mills, Canada) for 7 consecutive days, followed by 2 days of regular chow. A control group was fed regular mouse chow devoid of peanut protein. Mice consumed an average of 1.8 ± 0.07 g (SEM) of peanut butter per mouse per day, which is equivalent to 488 mg of peanut protein (n = 31). Mice were immunized with 10 μg of CPE-alum. All groups were boosted with 1 μg of CPE in 100 μL of PBS on day 14. Blood samples were taken on day 21. The degree of peanut tolerance was assessed by means of a relative comparison of peanut-specific antibodies between groups, as described for OVA tolerance induction.

Antihistamine treatment

Mice were fed either pyrilamine (0.04 mg/mL) or ranitidine (0.04 mg/mL) *ad libitum* in drinking water for 1 day before the initiation of tolerance induction. During the feeding stage of OVA tolerance induction, mice were provided with pyrilamine or ranitidine mixed in OVA-water (4 mg/mL) to provide an approximate oral dosing of 10 mg/kg/d. After 2 days of normal drinking water, mice were immunized and boosted with OVA, as described above.

Passive mast cell sensitization and activation

Mice were sensitized *in vivo* by means of intraperitoneal injection on day -9 with 4.4 μ g of anti-TNP IgE in 100 μ L of PBS. After oral treatment with OVA or normal water, mice were injected intraperitoneally with 62.5 μ g of TNP-BSA in PBS on day 0 concurrent with OVA immunization to induce a subclinical mast cell activation event; control groups were injected with BSA. Mice were later boosted with OVA in PBS on day 14.

In a second model designed to address the effects of mast cell activation during oral tolerance induction, mice were sensitized with anti-TNP IgE on days -16 and -7 . TNP-BSA or control BSA was delivered by means of intraperitoneal injection concurrent with gavage feeding of 1 mg of OVA in 100 μ L of PBS on days -9 and -3 of OVA treatment. Mice were then immunized with OVA-alum (10 μ g) on day 0 and boosted with OVA (1 μ g) on day 14.

Assessment of anaphylaxis in actively sensitized or tolerized animals

Mice were challenged intraperitoneally with 10 mg of OVA or 1 mg of CPE in 100 μ L of PBS, as indicated, on day 21 of treatment (Fig 1). Rectal temperature was measured before and 10, 30, and 50 minutes after challenge. In mice challenged with CPE, blood was collected 60 minutes after challenge, and mMCP-1 levels were assessed in plasma by using ELISA as per the manufacturer's protocol (eBioscience). Samples less than the limit of detection for the assay were assigned a value of that limit based on the standard curve (115.98 pg/mL) for the purposes of statistical analysis.

Assessment of passive IgE sensitization and subanaphylactic activation

Wild-type C57BL/6 mice were sensitized intraperitoneally with 4.4 μ g of anti-TNP IgE. Seven days later, mice were challenged by means of intraperitoneal injection of 62.5 μ g of TNP-BSA or BSA control. Blood was collected after 60 minutes for mMCP-1 analysis in plasma by using ELISA. In other experiments wild-type C57BL/6 mice were sensitized intraperitoneally with 4.4 μ g of anti-TNP IgE. Control mice received PBS injections. Eight to 10 minutes after TNP-BSA activation, mice received 1 mg of 4S fluorescein isothiocyanate–dextran intravenously. Blood and peritoneal lavage fluid were harvested 10 minutes later. Fluorescein isothiocyanate–dextran influx into the peritoneum was assessed by means of spectrofluorometry. Rectal temperature was assessed before and 10, 30, and 50 minutes after TNP-BSA administration.

Adoptive transfer and antigen-specific Treg cell assessment

Spleens and peripheral lymph nodes were removed from OT-II/CD45.1⁺/Foxp3-GFP⁺ mice. Naive CD4⁺/CD62L⁺ T cells were isolated by means of magnetic activated cell sorting, as per the manufacturer's protocol. One million naive CD4⁺/CD62L⁺ cells were injected intravenously. One day later, mice were provided with 4 mg/mL OVA *ad libitum* in drinking water; control subjects received normal drinking water. After 7 days, the Peyer patches (PPs), mesenteric lymph nodes (MLNs), and spleens were harvested. CD4⁺/CD45.1⁺/Foxp3-GFP⁺ Treg cell counts as a percentage of total CD4⁺/CD45.1⁺ T cells were determined by using flow cytometry.

Evaluation of reconstitution with BMDCs

The efficacy of peritoneal reconstitution was assessed with flow cytometry for CD117⁺/SSC^{high} cells in a 4-mL PBS lavage of the peritoneum. All

peritoneal cavities were successfully reconstituted, yielding an average of $9.18\% \pm 1.54\%$ (SEM) CD117⁺/SSC^{high} mast cells. Wild-type C57BL/6 peritoneal cavities contained $2.62\% \pm 0.25\%$ mast cells, and *Kit*^{W-sh/W-sh} mice contained $0.07\% \pm 0.02\%$ mast cells. Evaluation of reconstitution of jejunal tissues was performed by means of evaluation of mMCP-1 content, although previous studies have suggested very few mast cells at this site.³³ Briefly, jejunal tissue was sonicated in 20 mL of PBS per gram of wet tissue. The mMCP-1 ELISA was performed on supernatants. Wild-type C57BL/6 jejunum contained a mean 1215 ± 586.3 ng (SEM) of mMCP-1 per gram of tissue. *Kit*^{W-sh/W-sh} and reconstituted *Kit*^{W-sh/W-sh} jejunum contained 86.36 ± 17.79 and 37.08 ± 5.72 ng of mMCP-1 per gram of tissue, respectively.

Antigen-specific antibody ELISA

OVA-specific and peanut-specific antibodies were measured by using an antigen-binding ELISA assay. Plates were coated with anti-mouse IgE, IgG₁, IgG_{2a}, or IgA in 0.2 mol/L borate-buffered saline (pH 8.3). After washing, plates were blocked for 1 hour at room temperature in PBS with 2% BSA. Plasma or fecal samples were serially diluted in PBS with 0.2% BSA and 0.005% Tween-20 and then added to the plates and incubated at 4°C overnight. Plates were washed, and biotinylated OVA or CPE was added to the wells. Streptavidin–alkaline phosphatase and a commercial ELISA amplification system (Invitrogen, Carlsbad, Calif) were used to detect the bound biotinylated antigen. A standard positive serum sample was used on all plates. OVA-specific IgG₁ and IgG_{2a} antibody levels were determined based on titer threshold. To assess titers, the dilution curve of each sample was log transformed, and the point of intersection with a titer threshold was reported as the inverse log value. OVA-specific and peanut-specific IgE and IgA levels and peanut-specific IgG₁ and IgG_{2a} levels were compared based on OD. OVA-specific IgE standard samples were compared with a commercial OVA-specific IgE standard (Chondrex, Inc, Redmond, Wash) and reported as nanograms per milliliter based on that standard.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 4.0a software (GraphPad Software, Inc, La Jolla, Calif). Flow cytometric analysis was performed with FCS Express 3 software (De Novo Software, Los Angeles, Calif). All datasets were tested for normality. Where not normally distributed, comparisons between 2 groups were made by using the Mann-Whitney test or between multiple groups by using Kruskal-Wallis nonparametric analysis followed by the Dunn multiple comparison test. IgG₁ and IgG_{2a} titers were expressed as medians with interquartile ranges. Where normally distributed, mean nanograms per milliliter or ODs for IgE and IgA were compared between 2 groups by using the Student *t* test or between multiple groups by using 1-way ANOVA followed by the Dunnett multiple comparison test. Where variances were unequal, the Welch correction was applied to *t* tests. IgE and IgA levels were expressed as means $\pm$ SEMs. All experiments were performed in a minimum of 2 separate trials, and results were pooled for analysis.

RESULTS

Mast cell-deficient mice have normal IgE, IgG₁, and IgG_{2a} antibody responses after OVA-alum immunization

Mast cell-containing (wild-type C57BL/6) or mast cell-deficient (*Kit*^{W-sh/W-sh}) mice were sensitized to OVA (Fig 1) to assess whether the presence or absence of mast cells altered the normal antibody response to an intraperitoneal immunization. There were no significant differences observed between the OVA-specific IgE ($P = .16$), IgG₁ ($P = .0503$), or IgG_{2a} ($P = .07$) antibody responses of C57BL/6 and *Kit*^{W-sh/W-sh} mice (Table I).

TABLE I. IgE, IgG₁, or IgG_{2a} antibody responses to peritoneal immunization are unchanged in mast cell-deficient mice: anti-OVA antibody levels in plasma of sensitized mice 1 week after boost

	IgE	IgG ₁	IgG _{2a}
C57BL/6 control mice	20.14 ± 3.41	4.81 (0.01-5.71)	3.28 (0.01-4.39)
<i>Kit</i> ^{W-sh/W-sh} control mice	28.74 ± 4.11	5.53 (3.70-5.71)	3.77 (3.39-4.83)

Antibody levels of OVA-immunized and boosted control mice are expressed as mean ± SEM nanograms per milliliter for IgE or median –log titers with interquartile ranges for IgG₁ and IgG_{2a}. C57BL/6 antibody responses were compared with those of *Kit*^{W-sh/W-sh} mice by using *t* or Mann-Whitney tests: no significant differences were observed (n = 8-20).

Mast cells are not required for oral tolerance induction

Wild-type C57BL/6 and *Kit*^{W-sh/W-sh} mice were tolerized orally to OVA (Fig 1) or provided with normal drinking water. After later intraperitoneal sensitization and boost, OVA-specific IgE levels were significantly reduced in tolerized *Kit*^{W-sh/W-sh} mice ($P < .001$) and in wild-type mice ($P < .001$, Fig 2) compared with those seen in control mice. Tolerized *Kit*^{W-sh/W-sh} mice were also found to have significantly lower OVA-specific IgG₁ and IgG_{2a} levels than *Kit*^{W-sh/W-sh} control mice ($P < .01$ and $P < .01$, respectively). Plasma OVA-specific IgA levels were significantly reduced after tolerance induction to OVA in both C57BL/6 wild-type and *Kit*^{W-sh/W-sh} mice ($P < .05$ and $P < .05$, respectively; Fig 3, A). Secreted fecal OVA-specific IgA levels were also significantly reduced in tolerized *Kit*^{W-sh/W-sh} mice ($P < .05$; Fig 3, B). Oral tolerance was therefore readily induced in mast cell-deficient mice.

By employing antibody responses as a readout, the degree of tolerance was compared between wild-type and mast cell-deficient mice. OVA-specific IgE levels after sensitization were significantly lower in tolerized mast cell-deficient *Kit*^{W-sh/W-sh} mice than in tolerized wild-type mice ($P < .05$, Table II). In contrast, OVA-specific IgG₁, IgG_{2a}, or IgA levels were not significantly different between tolerized mast-cell containing and mast cell-deficient mice ($P = .24$, $P = .55$, and $P = .92$, respectively; Table II). Although *Kit*^{W-sh/W-sh} mice are understood to have few abnormalities,³³ some have been described.³⁴ To further evaluate the role of mast cells in oral tolerance, we therefore repeated these studies with a second strain of mast cell-deficient mice: *Kit*^{W/W-v} mice and their wild-type WBB6F1 control mice.³⁵ Oral tolerance was induced in both the WBB6F1 control mice and the *Kit*^{W/W-v} mice, as indicated by suppression of the IgE antibody response to OVA immunization and boost ($P < .001$ and $P < .01$, respectively; Fig 2, B). Notably, IgE levels in tolerized *Kit*^{W/W-v} mice were increased but not statistically different from those in tolerized WBB6F1 control mice ($P = .06$, Table II). OVA-specific IgG_{2a} levels were also significantly suppressed in *Kit*^{W/W-v} mice after OVA feeding ($P < .01$), but IgG₁ responses were not changed in mast cell-containing or mast cell-deficient mice ($P = .28$ and $P = .46$, respectively; Fig 2, B). OVA-specific IgA plasma responses were significantly reduced in both *Kit*^{W/W-v} and WBB6F1 control mice when tolerized ($P < .001$ and $P < .01$, respectively; Fig 3, C).

Oral tolerance to peanut was assessed as a model of a complex relevant food allergen. C57BL/6 and *Kit*^{W-sh/W-sh} mice were fed peanut butter *ad libitum* for 1 week, whereas control mice from both strains were fed regular chow. Mice were immunized and boosted with CPE-alum and CPE, respectively (Fig 1). Peanut-

specific IgE levels were significantly reduced in both peanut butter-fed groups ($P < .001$ and $P < .05$, respectively; Fig 2, C). Peanut-specific IgG₁ and IgG_{2a} levels were significantly reduced in C57BL/6 mice tolerized with peanut butter compared with those seen in untreated control mice ($P < .01$ and $P < .05$, respectively), but significant suppression of the IgG_{2a} antibody response was not observed in *Kit*^{W-sh/W-sh} mice ($P = .11$; Fig 2, C). IgG₁ responses to peanut were too low to compare in *Kit*^{W-sh/W-sh} mice. Peanut-specific IgA responses were significantly reduced in peanut butter-fed compared with control *Kit*^{W-sh/W-sh} mice ($P < .05$; Fig 3, D).

Taken together, these results demonstrate that oral tolerance can be reliably induced in the absence of mast cells, as confirmed in 2 different mast cell-deficient strains of mice and in 2 different allergenic food models. Oral tolerance induction to OVA was enhanced in 1 strain of mast cell-deficient mice (*Kit*^{W-sh/W-sh}) but not in another (*Kit*^{W/W-v}).

Physiologically relevant oral tolerance is induced by OVA or peanut butter feeding

In mice, IgG antibodies can be a major contributor to anaphylaxis.^{36,37} To assess the protective role of oral tolerance in our model, we used the FcγRIII-deficient mice to isolate the IgE-dependent anaphylactic response to OVA and better model anaphylaxis in human disease. After oral tolerance induction to OVA (Fig 1), mice were challenged intraperitoneally with soluble OVA. The OVA-tolerized FcγRIII^{-/-} mice were protected from temperature loss 30 and 50 minutes after challenge compared with the FcγRIII^{-/-} sensitized mice ($P < .01$ and $P < .01$, respectively), indicating a physiologically relevant degree of tolerance induction using this protocol (Fig 4, A). Tolerized mice from both the FcγRIII^{-/-} and C57BL/6 strains had similar OVA-specific IgE levels (C57BL/6 mice, 4.8 ± 1.1 ng/mL; FcγRIII^{-/-} mice, 3.5 ± 1.0 ng/mL), as did sensitized mice (C57BL/6 mice, 20.1 ± 3.4 ng/mL; FcγRIII^{-/-} mice, 19.1 ± 3.9 ng/mL; n = 15-20). To assess protective tolerance to peanut, wild-type C57BL/6 mice were challenged intraperitoneally with soluble CPE after tolerance induction to peanut butter (Fig 1), and anaphylactic responses were compared with those of control sensitized C57BL/6 mice. The peanut-tolerized mice were protected from temperature loss 10, 30, and 50 minutes after challenge ($P < .05$, $P < .001$, and $P < .001$, respectively), indicating physiologically relevant tolerance to peanut butter (Fig 4, B). mMCP-1 levels were measured in plasma 60 minutes after peanut challenge to confirm the involvement of mast cells in peanut-induced anaphylaxis. Peanut butter-tolerized mice had significantly lower levels of circulating mMCP-1 after peanut challenge (1.53 ± 1.17 ng/mL [SEM]) than control peanut-sensitized mice (9.17 ± 2.22 ng/mL [SEM], $P < .001$, n = 9-10).

Antigen-specific Treg cells are enhanced in *Kit*^{W-sh/W-sh} MLNs after oral tolerance induction to OVA through a mast cell-independent mechanism

The development of antigen-specific Treg cells was assessed in the PPs, MLNs, and spleen to further investigate the mechanisms by which oral tolerance to OVA was more efficiently induced in *Kit*^{W-sh/W-sh} mice. Mice received 1×10^6 naive OT-II cells by means of intravenous adoptive transfer and were provided with OVA-containing or control (OVA-free) drinking water for

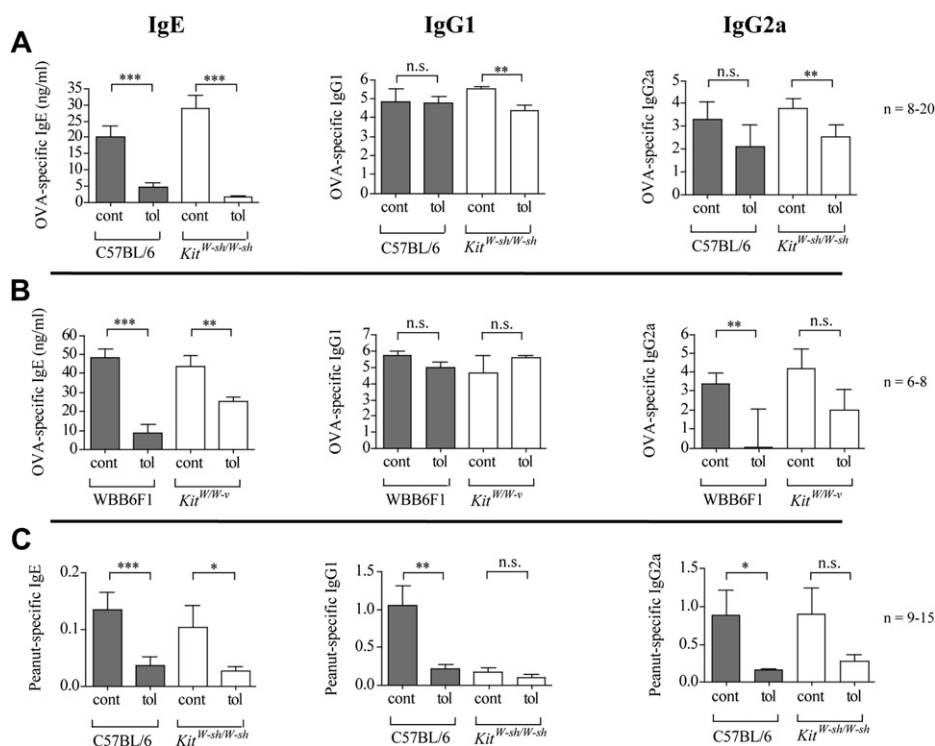


FIG 2. Mast cells are not required for oral tolerance induction. **A**, OVA-specific antibody levels were compared between OVA-fed sensitized mice (*tol*) and control sensitized mice (*cont*) in C57BL/6 or *Kit^{W-sh/W-sh}* mast cell-deficient mice to determine relative oral tolerance induction. Bars represent mean $\pm$ SEM IgE levels or median IgG₁ and IgG_{2a} levels with interquartile ranges. **B**, OVA-specific antibody levels were compared between control and tolerized *Kit^{W/W-v}* mice and control WBB6F1 littermates. **C**, Anti-peanut antibody levels were compared between peanut butter-fed sensitized mice (*tol*) and control sensitized mice (*cont*) from C57BL/6 or *Kit^{W-sh/W-sh}* mice. Bars represent mean $\pm$ SEM IgE, IgG₁, or IgG_{2a} ODs. * $P < .05$, ** $P < .01$, and *** $P < .001$. n.s., Not significant.

7 days. Lymph nodes and spleens were harvested and analyzed for OT-II Treg cells by using flow cytometry on the seventh day of treatment through analysis of both the overall numbers of OT-II cells and the number of GFP⁺ Foxp3-expressing OT-II cells. Both C57BL/6 and *Kit^{W-sh/W-sh}* mice revealed significantly higher levels of OT-II Treg cells as a percentage of total OT-II CD4⁺ cells in the MLNs after oral OVA treatment ($P < .05$ and $P < .001$, respectively; Fig 5, A and B). The MLNs of mast cell-deficient *Kit^{W-sh/W-sh}* mice contained approximately twice as many OT-II Treg cells as a proportion of the total CD4⁺ OT-II cells when compared with those of control tolerized mice ($P < .001$; Fig 5, B). However, when a similar experiment was performed with *Kit^{W-sh/W-sh}* mice systemically reconstituted with BMMCs, the OT-II Treg cell response to oral tolerance induction did not return to wild-type C57BL/6 levels (Fig 5, C). This indicates that the enhanced tolerance response by Treg cells was not mast cell dependent. Importantly, both *Kit^{W-sh/W-sh}* and C57BL/6 mice showed increased OT-II Treg cell numbers in the spleen after oral OVA treatment, demonstrating the induction of oral tolerance in both strains at the systemic level (Fig 5, B).

Interestingly, tolerized *Kit^{W-sh/W-sh}* mice contained, on average, 26% more total CD4⁺ T cells in the PPs than tolerized C57BL/6 control mice ($P < .05$; Fig 5, C). This difference in CD4⁺ levels was not observed in either the MLNs or spleen and was not specific to OT-II T cells (data not shown). When *Kit^{W-sh/W-sh}* mice were systemically reconstituted with BMMCs, the CD4⁺ compartment of the PPs returned to wild-type levels (Fig 5, C). This

highlights a previously undescribed mast cell-dependent suppression of CD4⁺ T-cell populations in the PPs. OVA treatment did not result in selected enhancement of OT-II Treg cells (Fig 5, D) in wild-type mice at this site. Instead, the PPs in tolerized mice revealed higher total OT-II CD4⁺ T-cell populations with Foxp3⁺ expression (C57BL/6 mice, $P < .05$; *Kit^{W-sh/W-sh}* mice, $P < .001$) or Foxp3[−] expression (C57BL/6 mice, $P < .001$; *Kit^{W-sh/W-sh}* mice, $P < .01$; Fig 5, D). This supports a framework of tolerance induction wherein the PPs serve as an initial site of antigen recognition and T-cell activation, whereas the MLNs are a site of robust antigen-specific Treg cell expansion and immunologic tolerance.

IgE-mediated activation of mast cells does not impede the maintenance of oral tolerance to OVA

IgE-mediated mast cell activation by food antigen in a murine model of food allergy induced the production of the antibody-promoting cytokines IL-4, IL-13, IL-6, and proinflammatory IL-17.³⁸ Furthermore, it has been shown that IgE-mediated mast cell activation can impair the maintenance of peripheral tolerance in a murine allograft model.²² We sought to examine whether a similar tolerance disruption would be observed in the case of established oral tolerance to OVA using mice passively sensitized with anti-TNP IgE antibody. Fluorescein isothiocyanate (FITC)-dextran influx into the peritoneum was assessed 15 to 20 minutes after activation in 2 separate groups of mice to confirm effective

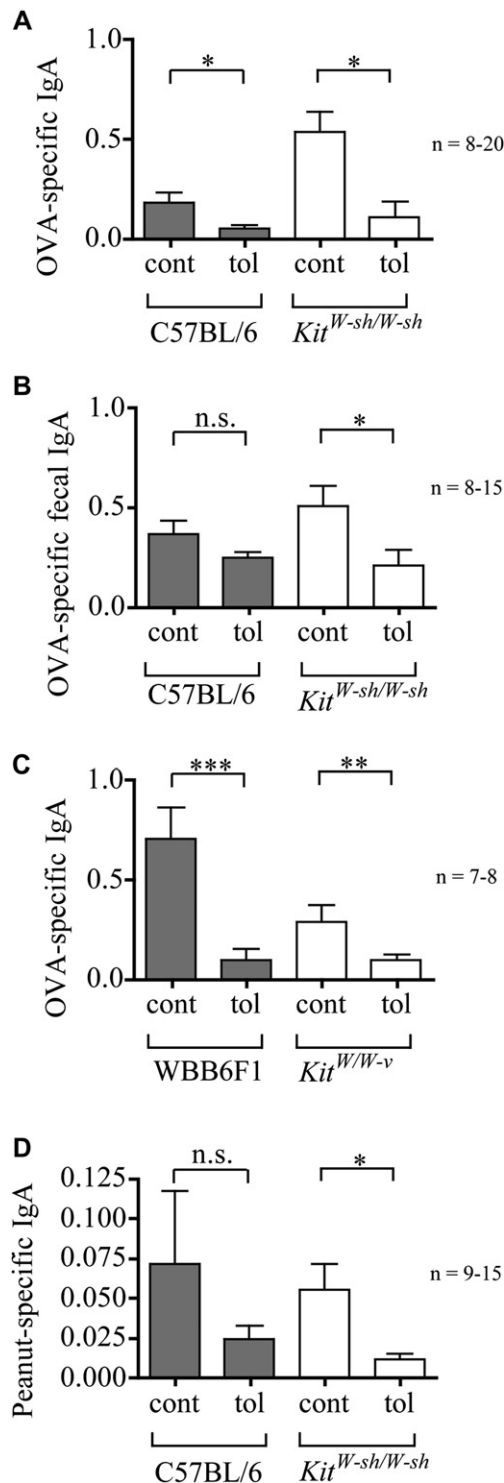


FIG 3. IgA tolerance is intact in the absence of mast cells. **A**, Mice were treated with OVA-containing drinking water (*tol*) or normal water (*cont*) for 1 week and then immunized and boosted with OVA, as detailed in Fig 1, A. OVA-specific IgA antibody levels in plasma were compared between tolerized and control *Kit*^{W-sh/W-sh} or C57BL/6 mice 1 week after boost. Bars represent mean $\pm$ SEM IgA responses. **B**, Fecal samples were assessed for OVA-specific IgA in tolerized and control mice. **C**, OVA-specific IgA levels were compared in plasma of tolerized or control mice 1 week after OVA boost in *Kit*^{W/W-v} mice and WBB6F1 littermates. **D**, Mice were fed peanut butter (*tol*) or normal chow (*cont*) for 1 week and then immunized and boosted with CPE, as detailed in Fig 1, A. Peanut-specific IgA (mean $\pm$

IgE-mediated mast cell activation in the peritoneum. IgE-mediated activation resulted in an average 31% increase of FITC-dextran influx into the peritoneum compared with that seen in unactivated control mice ($P < .05$, $n = 9-10$, data not shown).

IgE-mediated mast cell activation was also confirmed by means of ELISA for the mast cell-specific protease mMCP-1 in blood 60 minutes after activation. Sensitized mice challenged intraperitoneally with TNP-BSA showed average systemic mMCP-1 levels of 364.6 ± 91.09 pg/mL, which were significantly increased compared with those in sensitized mice injected with a BSA control (147.5 ± 26.31 pg/mL [SEM], $P < .05$, $n = 9-10$). This level of systemic mast cell activation did not induce systemic anaphylaxis, as assessed based on rectal temperature 50 minutes after TNP-BSA delivery (data not shown). Wild-type C57BL/6 mice were sensitized intraperitoneally with anti-TNP IgE to examine the outcome of IgE-mediated mast cell activation on the maintenance of oral tolerance. After 1 week of OVA tolerance induction treatment, TNP-BSA was delivered intraperitoneally to activate mast cells concurrent with OVA-alum immunization in one group of tolerized mice. A control group of tolerized mice received BSA instead of TNP-BSA. Tolerized groups were compared with sensitized control mice injected intraperitoneally with BSA during the OVA-alum immunization. The OVA-specific IgE levels of both OVA-tolerized groups were significantly lower than the levels of control OVA-sensitized mice regardless of IgE-mediated activation. Therefore the maintenance of oral tolerance was not impaired in the IgE compartment despite mast cell activation ($P < .01$; Fig 6, A).

Similarly, suppression of the OVA-specific IgA response was sustained in both tolerized groups ($P < .01$ and $P < .05$; see Table E1 in this article's Online Repository at www.jacionline.org). Tolerance was not induced in the IgG₁ compartment for wild-type C57BL/6 mice ($P = .07$, see Table E1). Interestingly, OVA-specific IgG_{2a} levels were significantly lower in tolerized mice than in OVA-sensitized mice ($P < .05$) but not when IgE-mediated activation was applied to the tolerized mice (see Table E1). Levels of OVA-specific IgG_{2a} remained significantly lower in tolerized mast cell-deficient *Kit*^{W-sh/W-sh} mice when compared with those in sensitized *Kit*^{W-sh/W-sh} control mice in the presence of TNP-BSA activation of IgE ($P < .01$, see Table E1).

IgE-mediated activation of mast cells does not alter antibody responses during oral tolerance induction to OVA

The effect of allergic activation of mast cells on oral tolerance induction to OVA was also assessed. Wild-type C57BL/6 mice were passively sensitized intraperitoneally with anti-TNP IgE. In one group of such mice, TNP-BSA was delivered intraperitoneally twice during 1 week of OVA feeding concurrent with OVA treatment by gavage, whereas a control group of animals received BSA intraperitoneally in place of TNP-BSA. Mice were immunized and boosted as described in Fig 1. Both tolerized groups produced mean OVA-specific IgE levels more than 60% lower than in the untreated sensitized control mice, although differences were not significant when compared by using ANOVA ($P = .056$; Fig 6, B). OVA-specific IgA, IgG₁, and IgG_{2a} levels were not significantly

SEM) levels were compared between the plasma of tolerized or control mice 1 week after CPE boost in the *Kit*^{W-sh/W-sh} and C57BL/6 groups. * $P < .05$, ** $P < .01$, and *** $P < .001$. n.s., Not significant.

TABLE II. Comparison of oral tolerance induction between murine strains and effect of oral antihistamine treatment: anti-OVA antibody levels in plasma 1 week after boost

	IgE	IgA	IgG ₁	IgG _{2a}
A. Comparison between tolerized C57BL/6 and <i>Kit</i> ^{W^{sh}/W^{sh}} mice (n = 8-20)				
C57BL/6 (tolerized)	4.81 ± 1.12	0.053 ± 0.014	4.77 (0.01-5.31)	2.15 (0.01-4.61)
<i>Kit</i> ^{W^{sh}/W^{sh}} (tolerized)	1.47 ± 0.56*	0.105 ± 0.081	4.35 (2.65-5.30)	2.54 (0.01-4.01)
B. Comparison between tolerized WBB6F1 and <i>Kit</i> ^{W^W/v} mice (n = 7-8)				
WBB6 (tolerized)	13.79 ± 4.26	0.093 ± 0.057	4.965 (3.95-5.52)	0.01 (0.01-4.21)
<i>Kit</i> ^{W^W/v} (tolerized)	24.07 ± 2.68	0.095 ± 0.027	5.612 (0.01-5.92)	2.00 (0.01-4.01)
C. Comparison between tolerized and antihistamine-treated tolerized groups (n = 10-20)				
C57BL/6 (tolerized)	4.81 ± 1.12	0.053 ± 0.014	4.77 (0.01-5.31)	2.15 (0.01-4.61)
C57BL/6 (tolerized) pyrilamine treated	3.87 ± 1.12	0.057 ± 0.012	4.30 (4.01-5.46)	1.79 (0.01-3.27)
C57BL/6 (tolerized) ranitidine treated	2.95 ± 0.74	0.066 ± 0.026	4.33 (0.01-5.46)	1.91 (0.01-3.83)

Antibody levels are expressed as mean ± SEM nanograms per milliliter (IgE), mean ± SEM ODs (IgA), or median -log titers with interquartile ranges (IgG₁ and IgG_{2a}). Groups were compared by using t or Mann-Whitney tests in A and B. Antihistamine-treated groups were compared with untreated control groups by using ANOVA with the Dunnett posttest (IgE and IgA) or the Kruskal-Wallis test with the Dunn posttest (IgG₁ and IgG_{2a}). Values in boldface indicate 40% or greater antibody reduction compared with the corresponding control group.

**P* < .05.

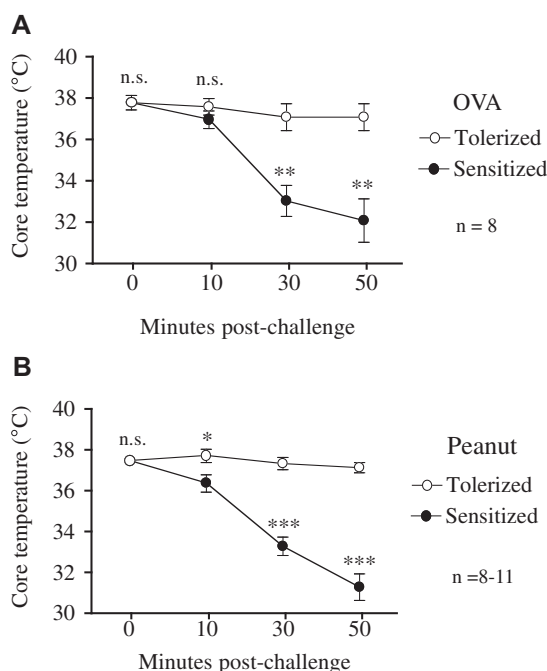


FIG 4. Physiologically relevant oral tolerance is induced by OVA or peanut butter feeding. Mean core temperature was measured before and 10, 30, and 50 minutes after systemic antigen challenge to assess the effect of oral tolerance on anaphylaxis. **A**, *FcγRIII*^{-/-} mice were challenged intraperitoneally with OVA. **B**, C57BL/6 mice were challenged intraperitoneally with CPE. Average temperatures were compared between groups at each time point by using *t* tests. Mean ± SEM temperatures are displayed. **P* < .05, ***P* < .01, ****P* < .001. n.s., Not significant.

different between control and tolerized mice when analyzed by using ANOVA (IgA, *P* = .08) or Kruskal-Wallis analysis (IgG₁, *P* = .13; IgG_{2a}, *P* = .41; see Table E1).

Oral antihistamine treatment has no effect on oral tolerance induction

Histamine is an important product of mast cell degranulation and can contribute to adverse anaphylactic reactions to oral

immunotherapy. We investigated whether histamine, acting through HRH1 or HRH2, might alter oral tolerance induction. Groups of wild-type C57BL/6 mice were orally pretreated with the HRH1 antagonist pyrilamine or the HRH2 antagonist ranitidine in drinking water for 1 day before and throughout a 1-week period of tolerance induction to OVA. Neither antihistamine treatment resulted in significant changes to OVA-specific antibody levels in tolerized wild-type mice when compared with levels in untreated tolerized mice for IgE, IgA, IgG₁, and IgG_{2a} (Table II).

DISCUSSION

The findings of this study demonstrate that mast cells are not required for the induction of oral tolerance in 2 strains of mice and in 2 model antigen systems. To our knowledge, this is the first study to directly investigate the role of mast cells in oral tolerance induction. We also observed that there were no substantial effects of oral antihistamine treatment or IgE-mediated mast cell activation on oral tolerance development, as evaluated based on the plasma IgE responses to OVA. In addition to these major findings, our studies revealed evidence for mast cell-mediated modulation of the IgG subclass response in the context of oral tolerance to OVA. We also observed enhanced Treg cell generation and oral tolerance responses in a commonly used model of mast cell-deficient mice.

In contrast to these findings, experiments with skin graft models have demonstrated that mast cells are essential for peripheral tolerance to an allograft.^{14,15} These studies investigated the maintenance of established graft tolerance but did not address the role of mast cells in the induction phase of tolerance. Differences in mast cell protease expression could provide a further reason for the divergent findings of these models. Tolerance in graft models was found to be dependent on mMCP-6,¹⁴ but mMCP-6 is not produced by mucosal mast cells, which are the most likely mast cell subset to be involved in regulating oral tolerance.³⁹

There are a number of murine models available to assess the role of mast cells in disease processes. We used 2 of the best characterized and most widely studied models: *Kit*^{W^W/v} and

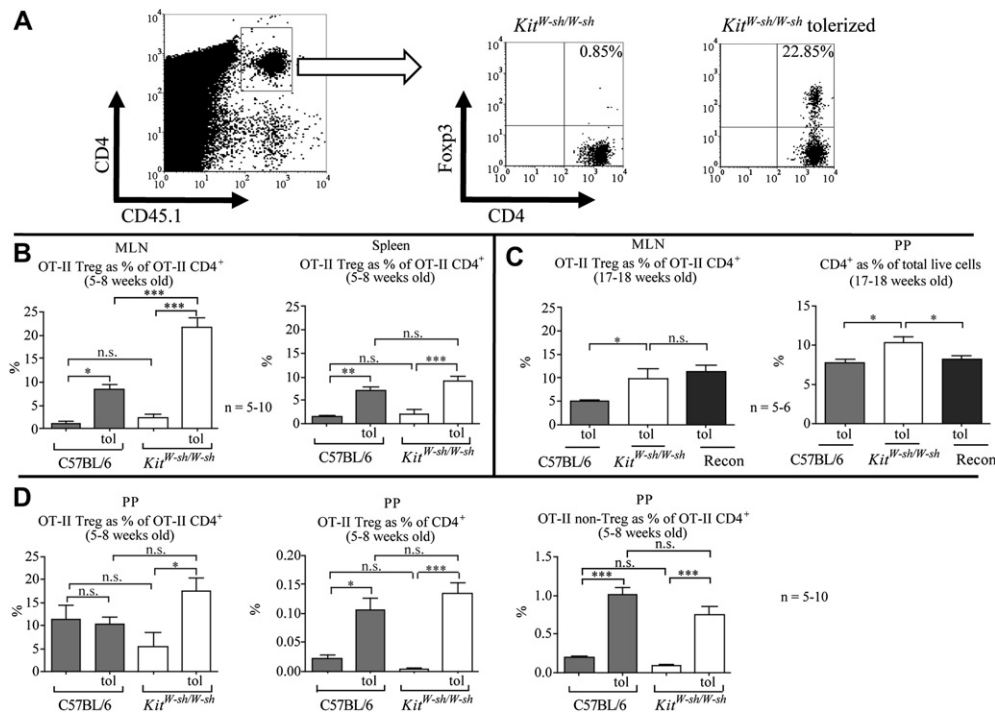


FIG 5. OT-II Treg cells are enhanced in the *Kit*^{W-sh/W-sh} MLNs after oral tolerance induction to OVA through a mast cell-independent mechanism. Naive OT-II T cells (1×10^6) were adoptively transferred to C57BL/6, *Kit*^{W-sh/W-sh}, or mast cell-reconstituted (*Kit*^{W-sh/W-sh} recon) mice. Animals were treated with OVA-containing drinking water for 7 days, and the development of Treg cells (Foxp3⁺) was compared with that seen in untreated control mice. **A**, Representative plots of CD45.1⁺/CD4⁺/Foxp3⁺ (OT-II Treg cells) as a percentage of OT-II CD4⁺ cells recovered from the MLNs of tolerized or untreated *Kit*^{W-sh/W-sh} mice. **B**, Transferred OT-II Treg cells recovered from the MLNs and spleen were compared between OVA-tolerized and untreated C57BL/6 or *Kit*^{W-sh/W-sh} mice. **C**, Tolerized C57BL/6, *Kit*^{W-sh/W-sh}, and *Kit*^{W-sh/W-sh} recon mice were compared by using analysis of OT-II Treg cells as a percentage of OT-II CD4⁺ T cells recovered from the MLNs or analysis of CD4⁺ T cells as a percentage of live cells recovered from the PPs. **D**, Transferred cells were compared in tolerized and untreated C57BL/6 and *Kit*^{W-sh/W-sh} PPs. * $P < .05$, ** $P < .01$, and *** $P < .001$. n.s., Not significant.

Kit^{W-sh/W-sh} 33,35,40,41 Although these mice come from distinct genetic backgrounds, the lack of mast cells did not impede oral tolerance development in either case. To the contrary, on feeding OVA, *Kit*^{W-sh/W-sh} mice exhibited enhanced tolerance in the IgE class and a higher proportion of OVA-specific Treg cells in the MLNs compared with those seen in wild-type control mice. These changes in MLN Treg cell numbers were not eliminated by systemic reconstitution of *Kit*^{W-sh/W-sh} mice with wild-type mast cells. Furthermore, *Kit*^{W/W-v} mast cell-deficient mice experienced successful tolerance but did not show enhanced humoral tolerance compared with matched mast cell-containing animals. These findings suggest that a mast cell-independent feature of the *Kit*^{W-sh/W-sh} mice enhances the induction, expansion, or migration of Treg cells in the MLNs and the development of oral tolerance.

It has been observed that *Kit*^{W-sh/W-sh} mice have altered crypt architecture in the jejunum, resulting in reduced intestinal permeability and antigen transit compared with that seen in C57BL/6 control animals.⁴² However, unlike the enhanced tolerance induction in *Kit*^{W-sh/W-sh} mice, these changes were eliminated by mast cell reconstitution, and therefore they are not likely responsible for the observed differences in oral tolerance.

It is possible that the observed differences in oral tolerance to OVA between *Kit*^{W-sh/W-sh} and C57BL/6 mice are a product of differences in intestinal motility or antigen access resulting from

deficiency in the interstitial cells of Cajal.³³ We observed that 5- to 8-week-old *Kit*^{W-sh/W-sh} mice consumed, on average, 26% more water than C57BL/6 control mice ($n = 8$ measurements from 2 cages per strain; $P < .01$, data not shown). However, further experiments showed that tolerance induced with 2, 4, or 8 mg/mL OVA provided *ad libitum* to C57BL/6 mice resulted in similar OT-II Treg cell levels in the MLNs ($6.60\% \pm 0.39\%$, $8.45\% \pm 1.64\%$, and $10.94\% \pm 0.78\%$ of OT-II CD4⁺ cells, respectively; $n = 5$ from 2 pooled experiments). This suggests that the dramatic differences between *Kit*^{W-sh/W-sh} and C57BL/6 mice were not due to differences in antigen dosage. Excellent intestinal and immunologic comparisons were made between the *Kit*^{W-sh/W-sh}, *Kit*^{W/W-v}, and wild-type control mice early in the development of the *Kit*^{W-sh/W-sh} model of mast cell deficiency.³³ However, a more comprehensive comparison of the distribution and phenotype of dendritic cell and T-cell subsets between these strains could yield valuable information on possible mechanisms of tolerance enhancement. Specifically, interstrain comparisons of the PPs, MSNs, and lamina propria might identify important differences that could present therapeutic targets for enhancing oral tolerance in affected patients.

Notably, since the initial description of these mast cell-deficient mice, a number of cell subsets have been described with c-kit expression that might have immunoregulatory roles. These

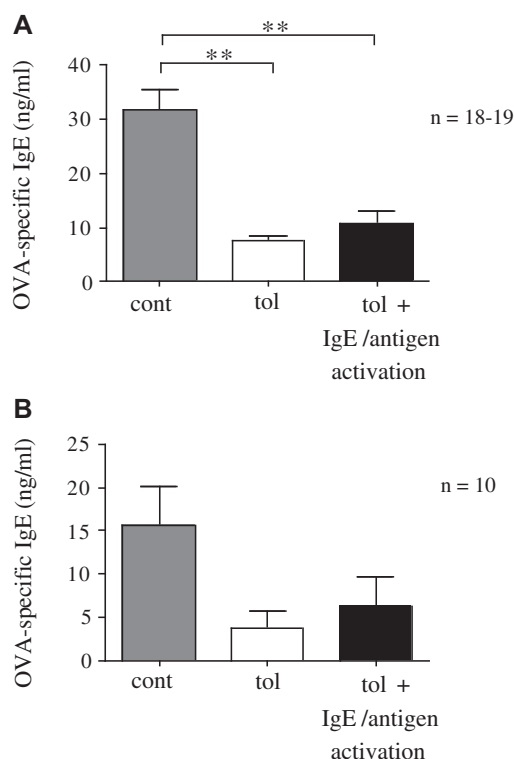


FIG 6. Oral tolerance to OVA in the context of IgE-mediated mast cell activation. **A**, Mice were sensitized with anti-TNP IgE before treatment with OVA-containing drinking water (*tol*) or normal water (*cont*). TNP-BSA or control BSA was delivered by means of intraperitoneal injection during OVA-alum immunization. OVA-specific IgE levels (means $\pm$ SEMs) were compared between groups by using ANOVA with the Dunnett multiple comparison test. $^{**}P < .01$. **B**, Mice were sensitized with anti-TNP IgE before treatment with OVA-containing drinking water (*tol*) or normal water (*cont*). TNP-BSA or control BSA was delivered by means of intraperitoneal injection during gavage treatment with OVA. *n.s.*, Not significant.

include several subsets of recently characterized innate lymphoid cells,⁴³ the multipotent progenitor type 2 cells,⁴⁴ and the B220^{low}CD19[−] regulatory cell population identified in the nasal associated lymphoid tissue.⁴⁵ These require further examination in the context of c-kit-related mast cell deficiency models.

In our studies oral tolerance to OVA was assessed by OVA-specific antibody levels and OVA-specific Treg cells but also by anaphylaxis in response to OVA challenge in Fc γ RIII^{−/−} mice. It has been well documented that basophils, macrophages, or both can bind IgG antibodies and cause IgG-mediated anaphylaxis in wild-type mice.^{36,46} We therefore used the Fc γ RIII^{−/−} mice to eliminate the contribution of IgG-mediated anaphylaxis in tolerized mice that is not known to occur in human subjects, allowing us to assess the physiologic relevance of OVA-specific IgE levels in tolerized C57BL/6 and *Kit*^{W-sh/W-sh} mice with comparable IgE levels. IgG-mediated anaphylaxis could occur in tolerized C57BL/6 or *Kit*^{W-sh/W-sh} mice,⁴⁷ but this would not reflect the significance of IgE levels and might have limited relevance to human allergic disease and the important role of mast cells in human anaphylaxis.⁴⁸

Patients with food allergy often respond to multiple allergens and might have other allergic disorders. It is therefore important to understand to what extent allergic mast cell activation can modify oral tolerance development. Studies performed in a skin graft rejection model have demonstrated that mast cell degranulation is sufficient to break peripheral tolerance and

accelerate graft rejection.²² Systemic IgE-mediated activation during a peritoneal allergen challenge did not break established oral tolerance to OVA in the IgE class (Fig 6, A). This demonstrates that oral tolerance can be resilient to the immunomodulatory events that accompany IgE-mediated activation. However, it remains possible that in a model of oral tolerance to a potent complex allergen, such as peanut, there is the potential for IgE activation to weaken tolerance. Peanut is known to have additional immunogenic and inflammatory properties, such as spontaneous complement activation, that could amplify the specific immunologic effects of IgE-mediated activation during a peanut challenge.^{49,50} Additionally, in a food allergy setting local mast cell degranulation could increase intestinal permeability; this physiologic change might interfere more directly with tolerance to food antigens.⁵¹ Although OVA-specific IgE responses were not altered in our model of IgE-mediated activation, the OVA-specific IgG_{2a} levels were enhanced by IgE-mediated activation during immunization (see Table E1). The apparent variability in these IgG_{2a} responses might reflect the variation observed in the extent of mast cell activation evaluated by mMCP-1 levels (364.6 ± 91.09 pg/mL) in IgE/antigen-activated mice. This change toward OVA-specific IgG_{2a} suggests a T_H1-polarized response and is therefore unlikely to result in allergic symptoms, but it does identify that there are significant immunomodulatory outcomes of IgE-mediated mast cell activation on the antigen response within the context of oral tolerance.

Oral antihistamines have been used clinically to control adverse reactions during rush or long-term oral immunotherapy protocols^{52,53} and have been administered extensively in other forms of allergen immunotherapy.⁵⁴ Despite their frequent use, it is unknown how some antihistamines can influence tolerance induction to a new antigen or effect on oral immunotherapy effectiveness. The administration of levocetirizine to patients undergoing honeybee venom immunotherapy did not interfere with tolerance induction and resulted in significantly more IL-10 production from allergen-specific T cells.⁵⁵ Similarly, a double-blind placebo-controlled study demonstrated that patients receiving pretreatment with the HRH1 antagonist terfenadine during bee venom immunotherapy were significantly less likely to react to a sting challenge several years later.⁵⁶ Taken together, these studies suggest that antihistamine treatment does not interfere with tolerance induction and might enhance immunotherapy to a preexisting allergic condition. This supports our finding that treatment with relatively high doses of pyrilamine (HRH1 antagonist) or ranitidine (HRH2 antagonist) did not interfere with the development of oral tolerance induction to a food antigen. However, our model did not demonstrate enhanced tolerance resulting from antihistamine treatment.

Overall, our data demonstrate that mast cells do not alter the development of oral tolerance to a food antigen, as assessed by measuring humoral IgE responses. Similarly, if mast cells are activated by IgE during allergen challenge, the maintenance of oral tolerance to the simple egg allergen OVA is unchanged in the IgE response. There is clear evidence from these and many previous studies that mast cells can play a profound immunomodulatory role in other types of tolerance and in other immune compartments, such as the IgG_{2a} response to food antigens. These data identify complexity in the oral tolerance response to activated mast cells but underscore the potential safety and usefulness of antihistamine treatment during oral tolerance approaches in clinical settings.

We thank Drs Wade Watson and Ian Haidl for helpful discussions related to this manuscript, Yisong Wei and Nong Xu for technical assistance, and Dr Manel Jordana for his assistance with anaphylaxis assessment methodology.

Key messages

- Mast cells are not necessary for the induction of oral tolerance to food antigens. *Kit*^{W-sh/W-sh} mast cell-deficient mice present abnormalities in oral tolerance induction and Treg cell development that are not mast cell dependent.
- Systemic IgE-mediated mast cell activation does not alter established oral tolerance. This suggests that the immunologic effects of mast cell activation might not reduce tolerance to foods.
- Oral antihistamine treatment does not inhibit or enhance oral tolerance induction.

REFERENCES

- Green TD, Burks AW. Oral food desensitization. *Curr Allergy Asthma Rep* 2010; 10:391-7.
- Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, et al. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J Allergy Clin Immunol* 2011;127:654-60.
- Vickery BP, Scurlock AM, Jones SM, Burks AW. Mechanisms of immune tolerance relevant to food allergy. *J Allergy Clin Immunol* 2011;127:576-86.
- Vickery BP, Burks AW. Immunotherapy in the treatment of food allergy: focus on oral tolerance. *Curr Opin Allergy Clin Immunol* 2009;9:364-70.
- Dawicki W, Jawdat DW, Xu N, Marshall JS. Mast cells, histamine, and IL-6 regulate the selective influx of dendritic cell subsets into an inflamed lymph node. *J Immunol* 2010;184:2116-23.
- Jawdat DM, Rowden G, Marshall JS. Mast cells have a pivotal role in TNF-independent lymph node hypertrophy and the mobilization of Langerhans cells in response to bacterial peptidoglycan. *J Immunol* 2006;177:1755-62.
- Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2007;212:505-19.
- Galli SJ, Tsai M. Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity. *Eur J Immunol* 2010;40:1843-51.
- Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. *Curr Opin Immunol* 2007;19:31-8.
- Sayed BA, Brown MA. Mast cells as modulators of T-cell responses. *Immunol Rev* 2007;217:53-64.
- Abraham SN, St John AL. Mast cell-orchestrated immunity to pathogens. *Nat Rev Immunol* 2010;10:440-52.
- Kambayashi T, Allenspach EJ, Chang JT, Zou T, Shoaq JE, Reiner SL, et al. Inducible MHC class II expression by mast cells supports effector and regulatory T cell activation. *J Immunol* 2009;182:4686-95.
- Li H, Nourbakhsh B, Safavi F, Li K, Xu H, Cullimore M, et al. Kit (W-sh) mice develop earlier and more severe experimental autoimmune encephalomyelitis due to absence of immune suppression. *J Immunol* 2011;187:274-82.
- de Vries VC, Elgueta R, Lee DM, Noelle RJ. Mast cell protease 6 is required for allograft tolerance. *Transplant Proc* 2010;42:2759-62.
- Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K, et al. Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature* 2006;442: 997-1002.
- Biggs L, Yu C, Fedorick B, Lopez AF, Galli SJ, Grimbaldston MA. Evidence that vitamin D(3) promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. *J Exp Med* 2010;207:455-63.
- Chacon-Salinas R, Limon-Flores AY, Chavez-Blanco AD, Gonzalez-Estrada A, Ullrich SE. Mast cell-derived IL-10 suppresses germinal center formation by affecting T follicular helper cell function. *J Immunol* 2011;186:25-31.
- Grimbaldston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* 2007;8:1095-104.
- Feyerabend TB, Weiser A, Tietz A, Stassen M, Harris N, Kopf M, et al. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. *Immunity* 2011;35:832-44.
- Katz HR, Austen KF. Mast cell deficiency, a game of kit and mouse. *Immunity* 2011;35:668-70.
- Piconese S, Costanza M, Musio S, Tripodo C, Poliani PL, Gri G, et al. Exacerbated experimental autoimmune encephalomyelitis in mast-cell-deficient Kit W-sh/W-sh mice. *Lab Invest* 2011;91:627-41.
- de Vries VC, Wasiuk A, Bennett KA, Benson MJ, Elgueta R, Waldschmidt TJ, et al. Mast cell degranulation breaks peripheral tolerance. *Am J Transplant* 2009;9:2270-80.
- McGowen AL, Hale LP, Shelburne CP, Abraham SN, Staats HF. The mast cell activator compound 48/80 is safe and effective when used as an adjuvant for intradermal immunization with *Bacillus anthracis* protective antigen. *Vaccine* 2009;27: 3544-52.
- McLachlan JB, Shelburne CP, Hart JP, Pizzo SV, Goyal R, Brooking-Dixon R, et al. Mast cell activators: a new class of highly effective vaccine adjuvants. *Nat Med* 2008;14:536-41.
- Forward NA, Furlong SJ, Yang Y, Lin TJ, Hoskin DW. Mast cells down-regulate CD4+CD25+ T regulatory cell suppressor function via histamine H1 receptor interaction. *J Immunol* 2009;183:3014-22.
- O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol* 2011;128: 1153-62.
- Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* 2001;108:1865-73.
- Caron G, Delneste Y, Roelands E, Duez C, Bonnefoy JY, Pestel J, et al. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* 2001;167:3682-6.
- Dubois B, Joubert G, Gomez de Agüero M, Gouanvic M, Goubier A, Kaiserlian D. Sequential role of plasmacytoid dendritic cells and regulatory T cells in oral tolerance. *Gastroenterology* 2009;137:1019-28.
- Goubier A, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* 2008;29:464-75.
- Matta BM, Castellana A, Thomson AW. Tolerogenic plasmacytoid DC. *Eur J Immunol* 2010;40:2667-76.
- Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: report of the NIAID-sponsored expert panel. *J Allergy Clin Immunol* 2010;126(suppl): S1-S8.
- Grimbaldston MA, Chen CC, Piliponsky AM, Tsai M, Tam SY, Galli SJ. Mast cell-deficient W-sh c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am J Pathol* 2005;167:835-48.
- Nigrovic PA, Gray DH, Jones T, Hallgren J, Kuo FC, Chaletzky B, et al. Genetic inversion in mast cell-deficient (Wsh) mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol* 2008;173:1693-701.
- Kitamura Y, Go S, Hatanaka K. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood* 1978;52:447-52.
- Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109:658-68.
- Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaR-III. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997;99:901-14.
- Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-Fc epsilonRI-IL-13 pathway. *J Allergy Clin Immunol* 2010;126:306-16, e1-12.
- Reynolds DS, Gurley DS, Austen KF, Serafin WE. Cloning of the cDNA and gene of mouse mast cell protease-6. Transcription by progenitor mast cells and mast cells of the connective tissue subclass. *J Biol Chem* 1991;266:3847-53.
- Tsai M, Grimbaldston MA, Yu M, Tam SY, Galli SJ. Using mast cell knock-in mice to analyze the roles of mast cells in allergic responses in vivo. *Chem Immunol Allergy* 2005;87:179-97.
- Galli SJ, Tsai M, Gordon JR, Geissler EN, Wershil BK. Analyzing mast cell development and function using mice carrying mutations at W/c-kit or Sl/Grf (SCF) loci. *Ann N Y Acad Sci* 1992;664:69-88.
- Groschwitz KR, Ahrens R, Osterfeld H, Gurish MF, Han X, Abrink M, et al. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc Natl Acad Sci U S A* 2009;106: 22381-6.
- Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;12:21-7.
- Saenz SA, Siracusa MC, Perrigoue JG, Spencer SP, Urban JF Jr, Tocker JE, et al. IL25 elicits a multipotent progenitor cell population that promotes T(H)2 cytokine responses. *Nature* 2010;464:1362-6.

45. Rharbaoui F, Bruder D, Vidakovic M, Ebensen T, Buer J, Guzman CA. Characterization of a B220+ lymphoid cell subpopulation with immune modulatory functions in nasal-associated lymphoid tissues. *J Immunol* 2005;174:1317-24.
46. Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, et al. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* 2008;28:581-9.
47. Arias K, Chu DK, Flader K, Botelho F, Walker T, Arias N, et al. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* 2011;127:1552-61.e1.
48. Kalesnikoff J, Galli SJ. Anaphylaxis: mechanisms of mast cell activation. *Chem Immunol Allergy* 2010;95:45-66.
49. Khodoun M, Strait R, Orekov T, Hogan S, Karasuyama H, Herbert DR, et al. Peanuts can contribute to anaphylactic shock by activating complement. *J Allergy Clin Immunol* 2009;123:342-51.
50. Shreffler WG. The perfectly potent peanut. *J Allergy Clin Immunol* 2009;123:352-3.
51. Perrier C, Corthesy B. Gut permeability and food allergies. *Clin Exp Allergy* 2011;41:20-8.
52. Itoh N, Itagaki Y, Kurihara K. Rush specific oral tolerance induction in school-age children with severe egg allergy: one year follow up. *Allergol Int* 2010;59:43-51.
53. Patriarca G, Nucera E, Roncallo C, Pollastrini E, Bartolozzi F, De Pasquale T, et al. Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment Pharmacol Ther* 2003;17:459-65.
54. Cox L, Nelson H, Lockey R, Calabria C, Chacko T, Finegold I, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol* 2011;127(suppl):S1-55.
55. Muller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Kriegel C, et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. *J Allergy Clin Immunol* 2008;122:1001-7.e4.
56. Muller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. *J Allergy Clin Immunol* 2001;107:81-6.

TABLE E1. Passive IgE-mediated systemic activation in oral tolerance induction and maintenance: anti-OVA antibody levels in plasma 1 week after boost

	IgA	IgG ₁	IgG _{2a}
A. Tolerance maintenance in wild-type C57BL/6 mice (n = 18-19)			
Control + IgE/control BSA during OVA-alum immunization	0.354 ± 0.063	5.08 (4.64-5.34)	3.58 (1.04-4.42)
Tolerized + IgE/control BSA during OVA-alum immunization	0.112 ± 0.015†	4.65 (4.17-4.88)	0.56* (0.01-3.45)
Tolerized + IgE/TNP-BSA activation during OVA-alum immunization	0.186 ± 0.044*	4.83 (4.33-5.11)	1.78 (0.01-3.63)
B. Tolerance maintenance in <i>Kit^{W-sh/W-sh}</i> mice (n = 9-14)			
Control + IgE/control BSA during OVA-alum immunization	0.387 ± 0.102	5.11 (3.62-5.47)	3.49 (1.53-4.34)
Tolerized + IgE/control BSA during OVA-alum immunization	0.082 ± 0.036†	3.66* (0.01-4.63)	0.01† (0.01-2.94)
Tolerized + IgE/TNP-BSA activation during OVA-alum immunization	0.072 ± 0.019*	3.34 (0.01-4.88)	0.01† (0.01-1.45)
C. Tolerance induction in wild-type C57BL/6 mice (n = 10)			
Control + IgE/control BSA during OVA feeding	0.122 ± 0.031	4.66 (2.78-4.96)	1.93 (0.67-2.92)
Tolerized + IgE/control BSA during OVA feeding	0.020 ± 0.004	4.76 (4.63-4.96)	1.47 (0.01-2.50)
Tolerized + IgE/TNP-BSA activation during OVA feeding	0.084 ± 0.043	4.57 (4.46-4.69)	0.95 (0.01-2.40)

Antibody levels are expressed as mean ± SEM ODs (IgE) or median –log titers with interquartile ranges (IgG₁ and IgG_{2a}). Antibody responses of tolerized groups were compared with those of control sensitized mice by using ANOVA with the Dunnett test (IgE and IgA) or the Kruskal-Wallis test with the Dunn multiple comparison test (IgG₁ and IgG_{2a}). Values in boldface indicate 40% or greater antibody reduction compared with the corresponding control group.

**P* < .05.

†*P* < .01.