

Mast cells regulate IFN- γ expression in the skin and circulating IgE levels in allergen-induced skin inflammation

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Background: Mast cells are important effector cells in IgE-mediated allergic reactions. They are present in normal skin and increased in skin lesions of patients with atopic dermatitis (AD).

Objective: We used mice deficient in mast cells (W/W^v) to assess the role of these cells in a murine model of allergen-induced skin inflammation induced by repeated epicutaneous sensitization with ovalbumin (OVA); the model exhibits many of the characteristics of AD.

Methods: Mice deficient in mast cells were sensitized with OVA. Histologic and immunohistochemical examinations, as well as measurements of IL-4 and IFN- γ mRNA, were performed on OVA-sensitized skin. Total and antigen-specific serum IgE levels were determined.

Results: Infiltration in W/W^v mice by mononuclear cells, T cells, and eosinophils in OVA-sensitized skin was comparable to that in wild-type (WT) controls. Expression of IL-4 mRNA in sensitized skin sites was similarly increased in WT and W/W^v mice. However, IFN- γ mRNA expression was significantly increased in sensitized skin of W/W^v mice but not in that of WT controls. IL-4 mRNA was readily detectable in unsensitized skin of WT controls but not in that of W/W^v mice, whereas expression of IL-12 p40 mRNA was significantly increased in unsensitized skin of W/W^v mice in comparison with WT controls. Total serum IgE levels were significantly increased after epicutaneous sensitization in W/W^v mice in comparison with WT controls.

Conclusion: These results suggest that mast cells regulate IFN- γ expression in the skin and IgE levels in the circulation in a model of allergen-induced skin inflammation with similarities to AD. This is important, given the role of IFN- γ in keratinocyte injury in AD and the role of IgE-mediated reactions in exacerbating AD. (J Allergy Clin Immunol 2002;109:106-13.)

Key words: Atopic dermatitis, mast cells, inflammation, antibodies, cytokines, in vivo animal models

Atopic dermatitis (AD) is a common pruritic inflammatory skin disease that often begins in infancy and frequently affects subjects with personal or family histories of atopic disease.¹ Examination of skin lesions in AD reveals the presence of a mononuclear cell infiltrate consisting predominantly of CD3+CD4+CD45RO⁺ T cells, monocytes-macrophages, and eosinophils. There is a significant increase in the expression of IL-4, IL-5, and IL-13 mRNA in acute lesions of AD, suggesting preferential accumulation of T_H2 cells. In chronic skin lesions, there is also increased expression of mRNA of the T_H1 cytokine INF- γ .^{2,3}

Approximately 80% of patients with AD have elevated levels of total IgE and evidence of specific IgE to a variety of food and environmental allergens.^{3,5,6} In addition, a number of studies have shown that avoidance of aeroallergens or food allergens results in clinical improvement. Several observations are consistent with the hypothesis that allergens play an important role in AD.⁷⁻¹²

Mast cells are known to be effector cells in IgE-mediated immediate hypersensitivity reactions.¹³ After an encounter with an allergen, IgE-sensitized mast cells release a broad panel of bioactive mediators, including histamine, prostaglandins, leukotrienes, and several cytokines (eg, IL-4, IL-5, GM-CSF, and TNF- α).¹⁴ Previous studies in mast cell-deficient mice have established that mast cells are important in the development of IgE-dependent cutaneous late-phase reactions.¹⁵ In some systems, allergen-induced bronchial hyperresponsiveness and airway injury have been shown to be mast cell-dependent.^{16,17} Moreover, it has been reported that mast cells play an important role in eosinophil infiltration into the lung after allergen challenge.¹⁸ In addition, it has been recently documented that histamine, through stimulation of H2 receptors, suppresses IL-12 production and stimulates the generation of IL-10.^{19,20} Such an alteration of local cytokine levels could shift the T_H1/T_H2 balance toward T_H2 dominance, the results being augmented eosinophil recruitment and allergic inflammation.

We have recently developed a mouse model of allergen-induced skin inflammation using repeated epicutaneous (EC) sensitization with ovalbumin (OVA).^{21,22}

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Abbreviations used

AD: Atopic dermatitis
EC: Epicutaneous
OVA: Ovalbumin
HPF: High-power field
W/W^v: Mast cell-deficient [mice]
WT: Wild-type

This model displays many of the features of human AD, including (1) elevated total and specific IgE and (2) a dermatitis characterized by dermal infiltration that consists of predominantly CD3⁺CD4⁺ T cells and eosinophils and by local expression of mRNA for the cytokines IL-4, IL-5, and, in some but not all strains, INF- γ .²¹⁻²³ We took advantage of the availability of mice deficient in mast cells (WBB6F1/J-Kit^W/Kit^{W-v}; W/W^v) to assess the role of these cells in allergen-induced cutaneous inflammation.

METHODS

Mice

J-Kit^W/Kit^{W-v} (WBB6F1) mice, which virtually lack tissue mast cells and possess <1.0% of the number of mast cells in the skin of congenic WBB6F1 mice,²⁴ were obtained from the Jackson Laboratory, together with congenic normal WBB6F1 wild-type (WT) mice (Bar Harbor, Me). All mice were kept in a pathogen-free environment. All procedures performed on the mice were in accordance with the guidelines of the Animal Care and Use Committee of the Children's Hospital.

EC sensitization

EC sensitization of 4- to 6-week-old female mice was performed as described previously.²¹

Antibodies

Rat and hamster mAbs were used for immunohistochemical analysis and were directed against mouse CD3 (145-2C11), CD4 (H129.19), CD8 (53-6.7), and CD45 (30-F11). The mAbs used in OVA-specific ELISA were rat antimouse IgE mAb clone R35-72 and biotin-conjugated rat antimouse IgG2a (R19-15). Total IgE measurements were performed as previously described.²¹ Anti-CD40 mAb (clone HM40-3) and all mAbs were purchased from PharMingen (San Diego, Calif).

ELISA

Mice were bled and sera were collected 1 day after the end of the series of 3 EC sensitizations. The standard PharMingen protocol for sandwich ELISA was used to quantify the total amount of IgE. OVA-specific IgG2a and IgE antibodies were measured as described previously.²¹ Results of IgG2a anti-OVA were expressed in arbitrary units per milliliter by comparison with a standard consisting of pooled sera from 10 mice immunized intraperitoneally at day 0 and day 14 with OVA in alum and bled on day 28. Results of IgE anti-OVA were expressed in nanograms per milliliter by comparison with a standard consisting of purified mouse IgE anti-OVA secreted by the hybridoma TOe (a kind gift of Dr Mamoru Kiniwa, PhD).

Histologic analysis

For histologic examination, specimens were obtained from patched areas on the skin 24 hours after the patch from the third sensitization was removed. Specimens were fixed in 10% buffered for-

malin and embedded in paraffin. Multiple 4- μ m sections were stained with hematoxylin and eosin. Individual inflammatory cell types were counted by a blinded investigator in 15 to 20 high-power fields (HPFs) at $\times 1000$ and expressed in cells per HPF. Mast cells were identified in tissue sections after staining with toluidine blue.

Immunohistologic analysis

Skin sections were embedded in Tissue-Tek oxacalctriol compound (Miles Inc, Elkhart, Ind) on dry ice. Sections of 4 μ m were prepared and stored at -80°C . Sections were stained through use of an avidin-biotin method, as described previously.²⁵ Stained cells were counted by a blinded investigator in 15 to 20 HPFs at $\times 400$.

RNA preparation, cDNA synthesis, and RT-PCR

Skin biopsy specimens and lymph nodes that drain the sensitized skin sites were obtained 24 hours after the third patch was removed and were immediately frozen in dry ice. For determination of baseline cytokine expression, skin biopsy specimens were obtained from the shaved skin of unsensitized W/W^v mice and controls. To extract the RNA, the samples were homogenized in Trizol (GIBCO BRL, Grand Island, NY) through use of a Polytron RT-3000 (Kinematica AG, Littau, Switzerland). Further RNA extraction was performed as discussed previously.²¹ cDNA was synthesized from 10 μ g of total RNA in a 40- μ L reaction mix through use of Superscript II (GIBCO BRL) for 120 minutes at 37°C . The primers used to amplify cDNA for $\beta 2$ -microglobulin IL-4, IL-10, IL-12 p40, and IFN- γ , have been described previously.^{21,26,27} To quantify mRNA, a fixed amount of reverse-transcribed cellular mRNA was coamplified in the presence of serial dilutions of a multispecific internal plasmid control (pMUS3), which contains nucleotide sequences of multiple cytokines. The results were expressed as ratios of cytokine cDNA to cDNA of the constitutively expressed $\beta 2$ -microglobulin gene.

In vitro IgE immunoglobulin synthesis

Single-cell suspensions from spleen from nonsensitized W/W^v mice and WT controls were cultured in RPMI-1640 plus 10% fetal calf serum at $2 \times 10^6/\text{mL}$. Cultures were stimulated with IL-4; as a source, we used a 1:4 dilution of conditioned medium from the ICL-6 (J558L-IL-4) cell line (a kind gift of Dr Philip Leder, MD) and LPS (20 $\mu\text{g}/\text{mL}$) or anti-CD40 mAb (1 $\mu\text{g}/\text{mL}$). IgE in the supernatants of 7-day cultures was measured by ELISA, as described previously.²⁸

Statistical analysis

Nonparametric unpaired *t* testing with Welch correction was applied to compare the different groups of mice, inasmuch as the variance was heterogeneous from one group to another. A *P* value of less than .05 was considered statistically significant.

RESULTS

Cellular infiltration in sensitized skin sites is normal in OVA-sensitized W/W^v mice

An important feature of our model of allergen-induced skin inflammation, as well as of AD, is dermal infiltration with eosinophils.²¹ We have previously shown that this is dependent on IL-4 and on IL-5, inasmuch as eosinophil infiltration was strongly reduced in the sensitized skin of IL-4^{-/-} mice and absent in the sensitized skin of IL-5^{-/-} mice.²² Because the skin is rich in mast cells and mast cells are an important source of IL-4 and IL-5,^{29,30} it is possible that they contribute to eosinophil migration

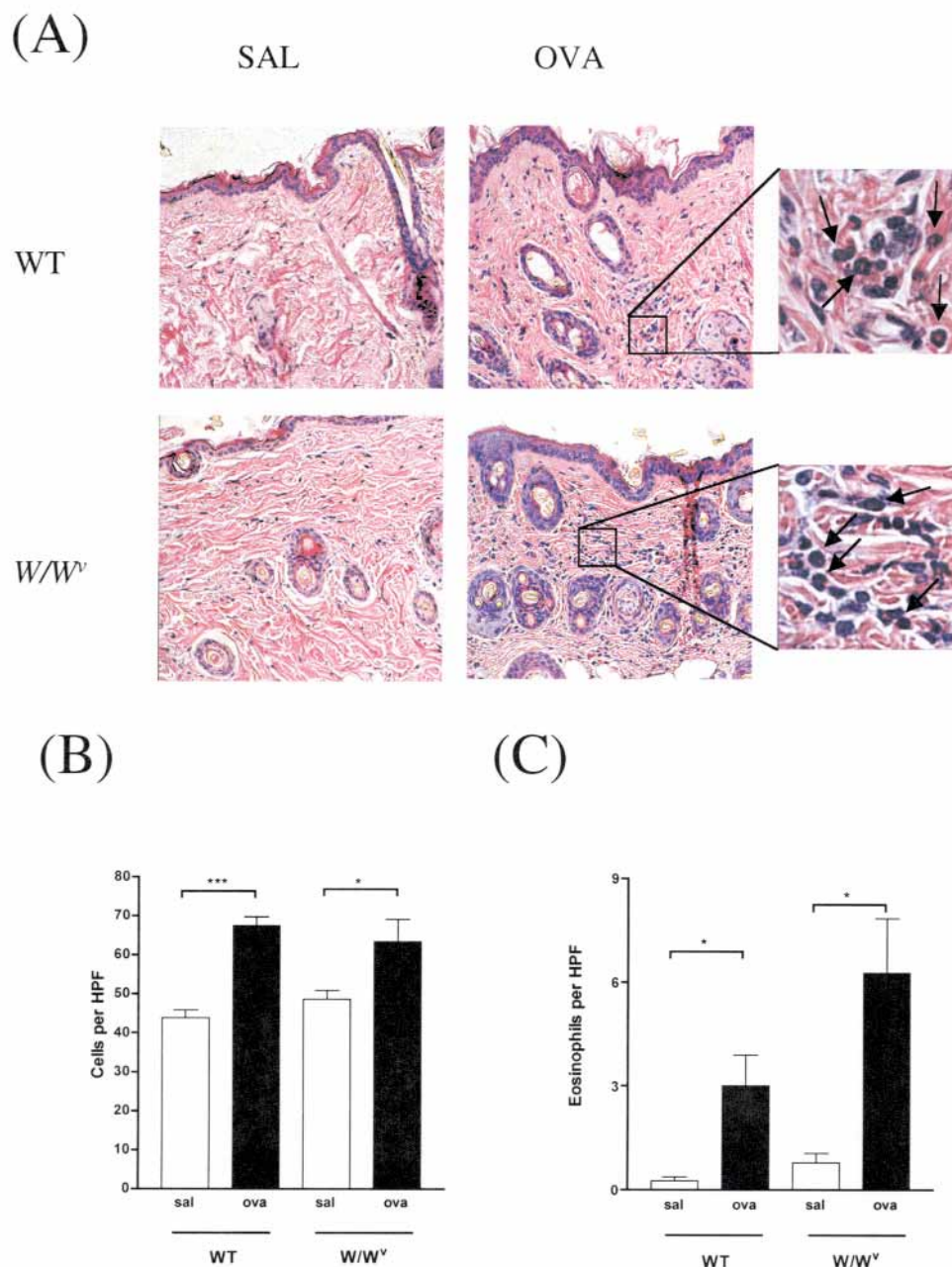


FIG 1. A, Histologic features of OVA-sensitized and saline-sensitized skin sites in *W/W^v* mice and controls. Magnification: large panels, $\times 200$; small panels, $\times 1000$. Arrowheads point to eosinophils. There is thickening of epidermal layer in the OVA-sensitized skin sites from *W/W^v* and WT mice in comparison with saline-sensitized skin sites. Infiltration of eosinophils and mononuclear cells is increased in OVA-sensitized skin sites in comparison with saline sensitization. **B,** Number of infiltrating mononuclear cells in OVA- and saline-exposed skin sites from *W/W^v* and WT mice. **C,** Number of infiltrating eosinophils. Cellular infiltrate in the dermis was assessed by examining 15 TO 20 HPFs and calculating the mean number of infiltrating eosinophils or mononuclear cells. The columns and error bars represent means $\pm$ SEMs. * $P < .05$. *** $P < .001$.

and/or survival in the skin. Fig 1, A shows that OVA-sensitized skin sites from both WT and *W/W^v* mice exhibited infiltration with eosinophils and mononuclear cells. The degree of infiltration with eosinophils and mononuclear cells was not significantly different between WT and *W/W^v* mice (Fig 1, B and C). In addition, the numbers of CD45⁺, CD3⁺, CD4⁺, and CD8⁺ cells in sensitized skin

were comparable in *W/W^v* mice and WT controls (data not shown). As expected, mast cells were readily observed in the skin of WT mice (2.7 ± 1 and 3.3 ± 0.7 cells/HPF in saline- and OVA-sensitized sites, respectively; $n = 7$ and $n = 9$, respectively) but not in the skin of *W/W^v* mice (< 0.02 mast cells/HPF in both saline and OVA-sensitized sites; $n = 7$ and 8, respectively).

Cytokine mRNA expression in sensitized skin of *W/W^v* mice

Increased expression of IL-4 mRNA in the skin is a hallmark of acute AD skin lesions, whereas IFN- γ mRNA is expressed in chronic skin lesions.² In our model of EC sensitization, IL-4 mRNA expression is uniformly increased in OVA-sensitized skin sites, whereas expression of IFN- γ mRNA is increased in some strains but not in others.^{22,23} We examined the expression of IL-4 and IFN- γ mRNA in sensitized skin sites of *W/W^v* mice using semiquantitative RT-PCR. Low levels of IL-4 mRNA were detected in saline-sensitized skin from both WT mice and *W/W^v* mice (Fig 2). After OVA sensitization, there was a significant and comparable increase in IL-4 mRNA expression in WT and *W/W^v* mice (Fig 2).

IFN- γ mRNA expression is either modestly increased or remains unchanged in OVA-sensitized skin sites from BALB/c and C57BL/6 mice.^{21,22} There was no increase in IFN- γ mRNA expression in OVA-sensitized skin sites of WT WBB6F1 mice. However, IFN- γ mRNA expression significantly increased in OVA-sensitized skin sites of *W/W^v* mice ($P < .05$). In addition, IFN- γ mRNA expression in both saline- and OVA-sensitized sites was higher in *W/W^v* mice than in WT controls. These results suggest that mast cells negatively regulate the IFN- γ response in the skin.

Unsensitized skin from *W/W^v* mice express higher levels of IL-12 p40 mRNA but lower levels of IL-4 mRNA than unsensitized skin from WT controls; the cytokine IL-12 plays a critical role in the induction of IFN- γ expression, whereas IL-4 inhibits IFN- γ expression.^{31,32} We therefore examined whether the increased IFN- γ mRNA expression in saline- and OVA-sensitized skin from *W/W^v* mice was associated with an imbalance in baseline expression of IL-12 and IL-4 in their unsensitized skin. IL-4 mRNA was readily detectable in unsensitized skin from WT WBB6F1 mice but could not be detected in unsensitized skin from *W/W^v* mice (Fig 3). In contrast, the baseline level of IL-12 p40 mRNA expression in skin from *W/W^v* mice was significantly increased in comparison with that in skin from WT controls ($P < .02$). IL-10 mRNA expression in unsensitized skin of *W/W^v* mice was comparable to that in WT controls (Fig 3). IFN- γ mRNA expression was not detectable in unsensitized skin of either strain (data not shown).

EC sensitization with OVA induces higher serum IgE levels in *W/W^v* mice than in WT controls

Mast cells secrete IL-4 and IL-13, cytokines known to be important for the development of a T_H2 -response.³³⁻³⁵ Mast cells express Fc ϵ RI IgE receptors, which might play a role in clearing circulating IgE. We compared total and OVA-specific serum IgE levels in *W/W^v* mice and WT WBB6F1 controls. Fig 4, A shows that baseline serum IgE levels were higher in *W/W^v* mice (889 ng/mL $\pm$ 13; $n = 3$) than in WT mice (519 ng/mL $\pm$ 27; $n = 3$); however,

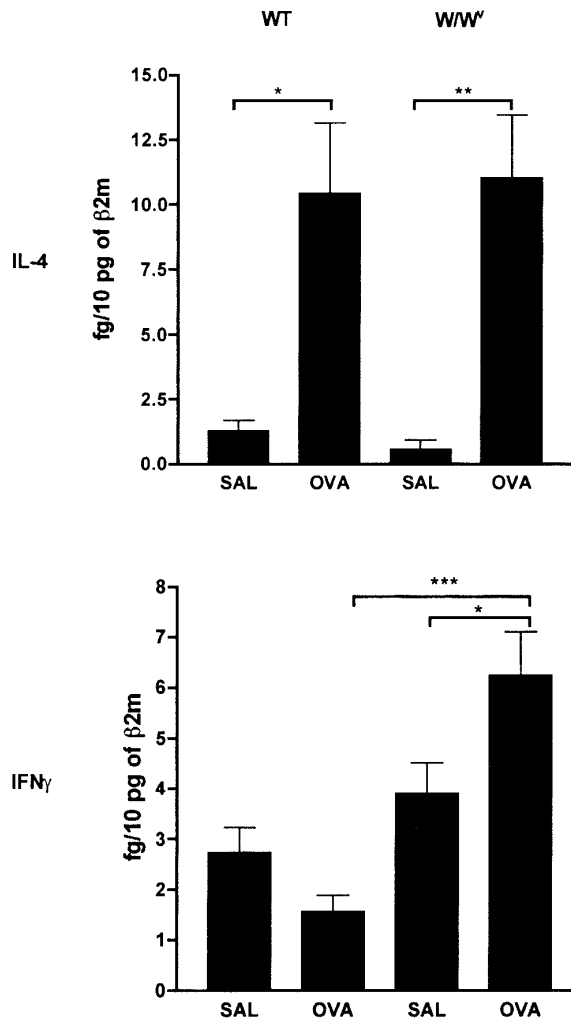


FIG 2. IL-4 and IFN- γ mRNA levels in biopsy specimens from skin sites of *W/W^v* and WT mice sensitized with OVA or saline, expressed relative to mRNA levels of β -2-microglobulin as assessed by RT-PCR analysis. These are pooled results of experiments with OVA-sensitized mice (*W/W^v* group, $n = 7$; WT group, $n = 11$) and saline-sensitized controls ($n = 9$ in both *W/W^v* and WT groups), each run being performed in duplicate. The columns and error bars represent means $\pm$ SEMs. * $P < .05$. ** $P < .01$. *** $P < .001$.

the difference was not statistically significant ($P > .05$). Serum IgE levels in both *W/W^v* and WT mice rose significantly over baseline after OVA sensitization but not after saline sensitization ($P < .004$ and $P < .03$, respectively). Serum IgE levels after OVA sensitization were significantly higher in *W/W^v* animals than in WT controls (3303 ng/mL $\pm$ 606 [$n = 11$] versus 991 ng/mL $\pm$ 169 [$n = 7$]; $P < .01$). Moreover, total IgE levels were also significantly higher in saline-sensitized *W/W^v* mice (1288 ng/mL $\pm$ 173; $n = 9$) than in similarly treated WT controls (736 ng/mL $\pm$ 94; $n = 9$; $P < .05$; Fig 4, A). Serum levels of OVA-specific IgE and IgG2a were comparable in OVA-sensitized *W/W^v* mice and WT controls (Fig 4, B and C).

We examined whether the increased IgE levels in OVA-sensitized *W/W^v* mice reflected an intrinsic increase in the ability of their B cells to undergo IgE iso-

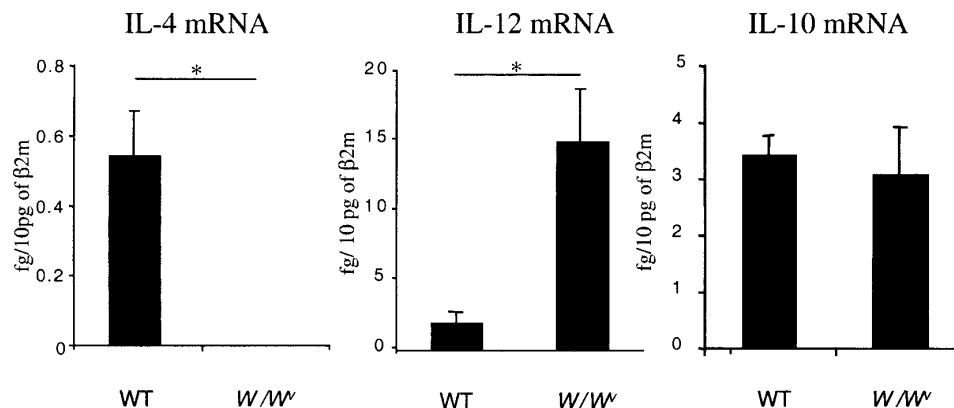


FIG 3. Baseline expression of IL-4, IL-10, and IL-12 in mRNA levels in biopsy specimens from unsensitized skin of *W/W^v* and WT mice (each group, $n = 5$), expressed relative to mRNA levels of β -2-microglobulin as assessed by RT-PCR analysis. These are pooled results of experiments with 5 *W/W^v* and 5 WT mice. The columns and error bars represent means $\pm$ SEMs. * $P < .05$. ** $P < .01$.

type switching and synthesize IgE. Splenocytes obtained from *W/W^v* mice ($n = 4$) and controls ($n = 4$) were stimulated for 7 days with IL-4 and the B-cell activator LPS or the B-cell activator anti-CD40 mAb. As expected, IL-4, together with either LPS or anti-CD40, induced IgE synthesis in B cells from WT mice (52 ± 8 and 40 ± 9 ng/mL, respectively). No significant differences in IgE synthesis were observed between splenocytes from WT mice and splenocytes from *W/W^v* mice, indicating that B cells of *W/W^v* mice have a normal ability to undergo IgE isotype switching (56 ± 5 and 31 ± 5 ng/mL for IL-4 plus LPS and IL-4 plus anti-CD40 mAb, respectively).

DISCUSSION

Infiltration of the skin with eosinophils is a hallmark of AD and of our mouse model of EC sensitization with OVA.^{21,36} Our results show that eosinophil infiltration was normal in the skin of *W/W^v* mice (Fig 1). This suggests that mast cells are not an essential source of cytokines that are known to be important for eosinophil migration and survival in the skin. In a model of allergen-induced airway inflammation, *W/W^v* mice sensitized intraperitoneally with OVA had lower numbers of eosinophils in bronchoalveolar lavage fluid and lungs than did their congenic littermates after inhaled-allergen challenge.¹⁸ In contrast, several other studies of similarly sensitized and challenged mice have found no differences in eosinophil infiltration in bronchoalveolar lavage fluid or lung tissue between *W/W^v* mice and WT controls.^{16,37} These discrepant results suggest that if mast cells play a role in eosinophil recruitment, their contribution might not be readily evident.

It has been reported that mast cells increase in the skin of *W/W^v* mice at sites of idiopathic chronic dermatitis.³⁸ There was no detectable increase in mast cells in OVA-sensitized skin sites of *W/W^v* mice. However, there was also no significant increase in mast cells in OVA-sensitized skin sites of congenic WT controls. Mice have either no detectable basophils in blood, bone marrow, or

skin³⁹ or very low levels of leukocytes with the morphology of basophils.⁴⁰ It is thus unlikely that basophils play a role in our model.

W/W^v mice exhibited an increase in IL-4 mRNA expression comparable to that observed in WT controls. This suggests that mast cells are not required for the development of a T_H2 response after EC sensitization or for the maintenance of T_H2 cytokine expression in sensitized skin. This is consistent with previous findings that IL-4 and IL-5 levels in bronchoalveolar lavage fluid as well as in airway eosinophilia fluid are comparable in a mouse model of OVA-induced airway inflammation in *W/W^v* mice and WT controls.^{41,42}

IFN- γ mRNA expression either modestly increased or remained unchanged in OVA-sensitized skin sites compared to saline-sensitized skin sites in BALB/c and C57BL/6 mice.^{21,22} The source of this IFN- γ is likely to be dependent on TCR $\alpha\beta$ cells, because IFN- γ mRNA expression is absent in OVA-sensitized skin sites of TCR $\alpha^{-/-}$ mice.²³ Very few mast cells are found to produce IFN- γ in normal skin, and there is no increase in IFN- γ -producing cells in AD lesions.⁴³ IFN- γ mRNA expression did not differ between OVA-sensitized and saline-sensitized skin sites of WBB6F1 WT mice. In contrast, IFN- γ mRNA expression significantly increased in OVA-sensitized skin sites of *W/W^v* mice. This was accompanied by an increase in IFN- γ expression in draining lymph nodes of OVA-sensitized skin in *W/W^v* mice relative to WT controls (data not shown). In addition, IFN- γ mRNA expression in skin sites of *W/W^v* mice sensitized with either saline solution or OVA was increased in comparison with similarly sensitized sites in WT controls. IFN- γ expression after saline sensitization might simply be a response to mechanical injury and/or to products of skin flora that are absorbed through the abraded skin. There was no detectable IFN- γ mRNA expression in unsensitized skin in either *W/W^v* mice or WT controls (data not shown). Taken together, the results suggest that mast cells downregulate IFN- γ mRNA expression in the skin in response to EC sensitization.

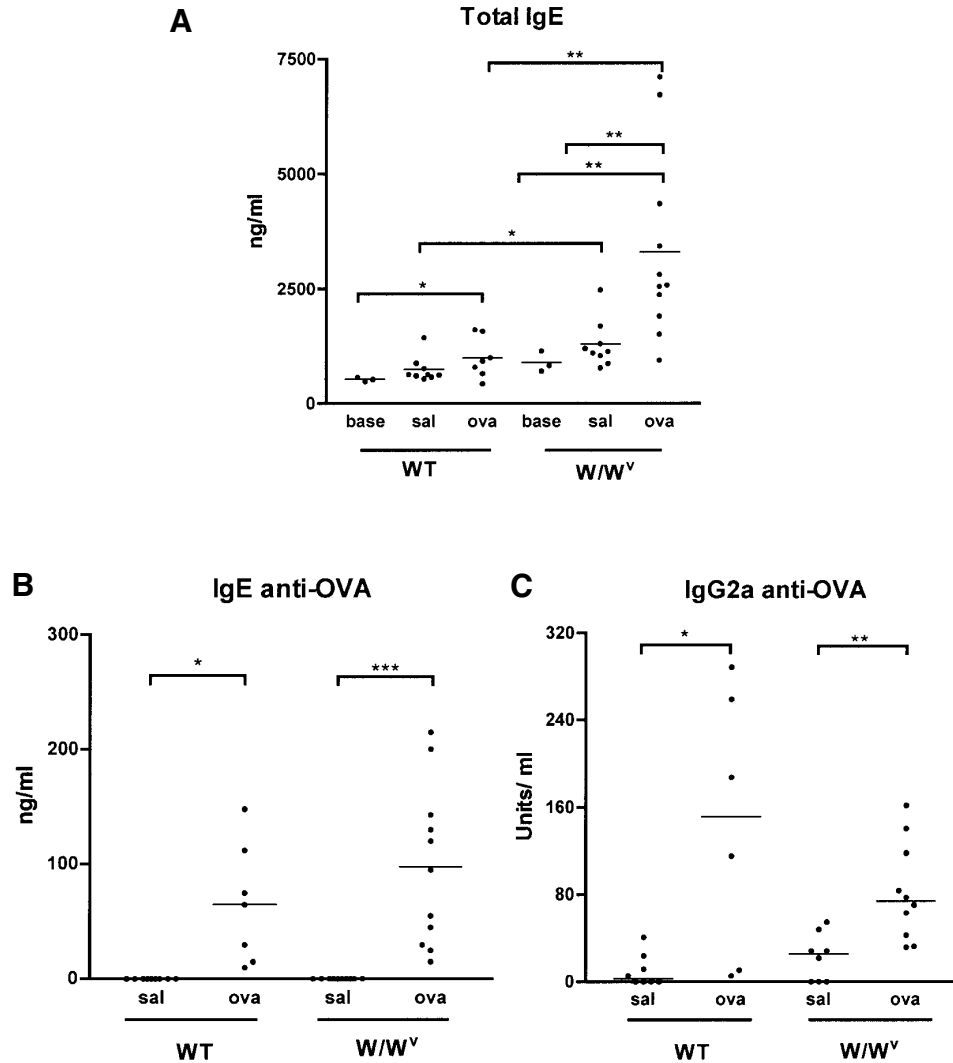


FIG 4. Serum IgE and serum antibody responses in *W/W^v* mice and controls sensitized with OVA or saline solution. **A**, Total serum IgE. Baseline IgE (BASE) and IgE after saline (SAL) or ovalbumin (OVA) sensitization. **B**, OVA-specific IgE levels, expressed in nanograms per milliliter relative to an OVA-specific mAb standard. **C**, OVA-specific IgG2a antibody levels expressed in units relative to a serum pool of intraperitoneally immunized mice run in each assay. Each dot represent 1 animal ($n = 7$ to 11 animals per group). * $P < .05$. ** $P < .01$. *** $P < .001$.

IFN- γ expression in the skin might play an important role in the pathogenesis of AD. IFN- γ enhances T cell adhesion to skin endothelium by upregulating vascular cell adhesion molecule 1 expression on endothelial cells⁴⁴ and to keratinocytes by upregulating keratinocyte intercellular cell adhesion molecule 1 expression.⁴⁵ It also inhibits keratinocyte proliferation⁴⁶ and augments keratinocyte expression of cytokines⁴⁷ and of the T_H2-selective chemokine TARC.⁴⁸ More importantly, IFN- γ upregulates Fas expression by keratinocytes, rendering them susceptible to T cell-mediated, FasL-driven apoptosis.⁴⁹

Enhanced expression of IFN- γ mRNA in sensitized skin of *W/W^v* was associated with an imbalance in baseline skin expression of the cytokines IL-4 and IL-12, which drive T_H2 and T_H1 responses, respectively.^{50,51} IL-4 mRNA was readily detectable in unsensitized skin of

WT mice but not in unsensitized skin of *W/W^v* mice, whereas expression of IL-12 p40 mRNA was significantly increased in unsensitized skin of *W/W^v* mice in comparison with WT controls (Fig 3). This suggests that mast cells are the major source of IL-4 in normal skin. However, because IL-4 mRNA expression in OVA-sensitized skin of *W/W^v* was comparable to that in OVA-sensitized skin of WT mice, infiltrating T cells are likely to be the main source of IL-4 in inflamed skin. The cell source of the increased IL-12 in the skin of *W/W^v* mice is likely to include dendritic cells⁵²⁻⁵⁵ and keratinocytes.⁵⁶ Both IL-4 and the mast cell product histamine have been reported to inhibit IL-12 gene expression.^{19,20,32} This could explain the increased baseline IL-12 p40 mRNA expression in the skin of mast cell-deficient *W/W^v* mice. IL-12 is important for T_H1 cell differentiation, is favored by IL-

12, and is inhibited by IL-4.⁵⁷⁻⁵⁹ Therefore, increased baseline expression of IL-12 and absent baseline expression IL-4 could favor a T_H1 response to EC sensitization and might account for the increased expression of IFN- γ mRNA in OVA-sensitized skin sites from W/W^v mice.

Total serum IgE is elevated in most patients with AD³⁶ as well as after EC sensitization of BALB/c and C57BL/6 mice.^{21,22} Serum IgE levels rose modestly but significantly over baseline in OVA-sensitized WBB6F1 mice. The lack of a vigorous rise in serum IgE in WBB6F1 mice is possibly due to genetic factors. In contrast, serum IgE rose markedly and significantly in W/W^v mice after OVA sensitization. Furthermore, serum IgE levels were significantly higher in sensitized W/W^v mice than in sensitized congenic WT controls (Fig 4). W/W^v mice developed higher levels of circulating OVA-specific IgE antibodies than did WT controls after EC sensitization with OVA, though the difference was not significant. This is in agreement with previous studies showing that W/W^v mice mount normal or slightly higher antigen-specific IgE responses after intraperitoneal immunization with antigen.^{37,42,60}

The increase in IgE serum levels in W/W^v was not due to an intrinsic increase in the ability of B cells to undergo IgE isotype switching. No differences in IgE synthesis were observed between splenocytes from W/W^v mice and controls after in vitro stimulation with IL-4 plus LPS or IL-4 plus anti-CD40. Furthermore, the numbers of B cells in the spleens and lymph nodes of these mice are normal (data not shown). A likely explanation for the increased IgE levels in W/W^v mice could be their secondary deficiency in high-affinity receptors for IgE (Fc ϵ RI), which are predominantly expressed on mast cells and which might normally sequester IgE from the circulation.¹⁴

The defect in W/W^v mice involves the c-kit tyrosine kinase receptor.⁶¹ Although the c-kit mutation primarily affects mast cell development, there are also decreased numbers of bone marrow granulocytes and megakaryocytes.^{62,63} It is therefore possible that other defects affect expression of cytokines and IgE in EC-sensitized W/W^v mice. To rule out this possibility, we attempted to reconstitute mast cells in these mice by intravenous infusion of cultured mast cells from congenic WT mice (25 $\times$ 10⁶ cells/mouse). Unfortunately, we obtained very low levels of skin mast cell reconstitution (<15% of WT levels after 4 and 11 weeks), and there was no correction of the increased expression of IFN- γ in OVA-sensitized skin sites. This failure might be due to poor mast cell reconstitution.

Increased IgE levels result in increased Fc ϵ RI expression and enhanced susceptibility to cytokine release by mast cells.^{64,65} Monomeric IgE was recently found to enhance the survival of mast cells,^{66,67} leading to the proposal that elevated serum IgE in atopic diseases such as AD might contribute to the increase in tissue mast cells observed in these conditions. Taken together with these findings, our results suggest a feedback circuit between numbers of tissue mast cells and levels of circulating IgE.

Our model of allergen-induced skin inflammation has many similarities to AD. Furthermore, there is strong evidence that EC introduction of allergens plays an impor-

tant role in the pathogenesis of AD.⁶⁸ The data in this study suggest that mast cells regulate IFN- γ expression in the skin and IgE levels in the circulation in response to EC immunization with allergen and are therefore directly relevant to AD. This is important, given the role of IFN- γ in keratinocyte injury in AD and the role of IgE mediated reactions in exacerbating AD.

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