

Mast cell–derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B

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Allergic contact dermatitis, such as in response to poison ivy or poison oak, and chronic low-dose ultraviolet B irradiation can damage the skin. Mast cells produce proinflammatory mediators that are thought to exacerbate these prevalent acquired immune or innate responses. Here we found that, unexpectedly, mast cells substantially limited the pathology associated with these responses, including infiltrates of leukocytes, epidermal hyperplasia and epidermal necrosis. Production of interleukin 10 by mast cells contributed to the anti-inflammatory or immunosuppressive effects of mast cells in these conditions. Our findings identify a previously unrecognized function for mast cells and mast cell–derived interleukin 10 in limiting leukocyte infiltration, inflammation and tissue damage associated with immunological or innate responses that can injure the skin.

Although the factors that promote innate or acquired immune responses have been investigated intensively, less is known about natural mechanisms that can limit the magnitude or duration of responses to external environmental stimuli. The production of anti-inflammatory cytokines, particularly interleukin 10 (IL-10), has been identified as a mechanism that can diminish the extent or persistence of some acquired or innate immune responses^{1,2}. IL-10 is well-suited for such a function, because it can inhibit the production of proinflammatory cytokines and chemotactic factors for granulocytes and T cells and the proliferation of CD4⁺ T cells^{1,2} and CD8⁺ T cells^{1,3}.

The mechanisms that regulate IL-10 production during innate or acquired immunity and the critical cellular sources of IL-10 in such settings are, however, not fully understood. Many cell types represent potential sources of IL-10, including macrophages, B cells, T helper type 2 cells, regulatory T cells and, in the skin, keratinocytes^{1,2}. *In vitro* studies have shown that mast cells also can secrete IL-10 (refs. 4,5). Although the relevance of mast cells as a source of IL-10 is not clear, evidence suggests that mast cells can have immunoregulatory functions, in addition to their better known proinflammatory functions, during acquired and innate immunity^{4,5}.

Here we use genetically mast cell–deficient mice, some selectively engrafted with wild-type or genetically altered (for example, *IL10*^{−/−}) mast cells, to find answers to two related questions. First, can mast cells limit the extent of the leukocyte infiltration, inflammation and tissue pathology associated with examples of innate or acquired immune responses to common environmental stimuli? Second, if mast cells do accomplish such functions, to what extent do such

functions reflect the ability of mast cells to produce IL-10? We investigate two mouse models of severe skin inflammation that can be associated with substantial tissue damage: a model of acquired immunity, cutaneous contact hypersensitivity^{6–9}; and ultraviolet B (UVB) irradiation of the skin, which induces innate inflammatory responses at sites of irradiation^{10,11}. Notably, there is evidence that IL-10 can reduce the inflammation associated with each of these responses^{12,13}. We show that mast cells and mast cell–derived IL-10 can substantially reduce the extent and duration of the T cell and other leukocyte infiltration, inflammation and tissue damage associated with these responses.

RESULTS

Mast cells and mast cell–derived IL-10 limit contact hypersensitivity

Allergic contact dermatitis, also known as contact hypersensitivity (CHS), can develop in response to exposure to plants of the *Toxicodendron* genus (Anacardiaceae family), including poison oak, poison ivy and poison sumac, as well as cashew, mango and Japanese lacquer trees¹⁴. CHS in response to poison oak (*T. diversilobum*) or poison ivy (*T. radicans*) is mediated by T lymphocytes specific for catechols (such as heptadecylcatechol or pentadecylcatechol) present in urushiol, the allergen-containing sap of such plants¹⁵. In humans^{15–17} and mice^{8,9}, antigen-specific CD8⁺ T cells are thought to be effectors of CHS induced by urushiol. CHS in response to 2,4-dinitrofluorobenzene (DNFB) in mice is also mediated by DNFB-specific CD8⁺ T cells^{6–9,18}.

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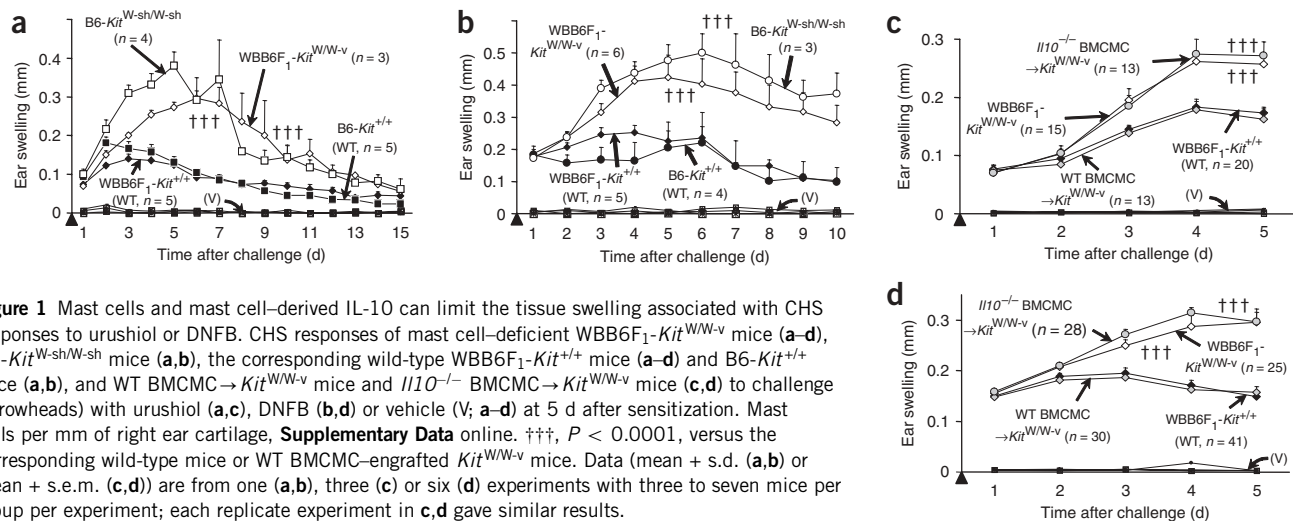


Figure 1 Mast cells and mast cell-derived IL-10 can limit the tissue swelling associated with CHS responses to urushiol or DNFB. CHS responses of mast cell-deficient WBB6F1-*Kit*^{W/W-v} mice (**a-d**), B6-*Kit*^{W-sh/W-sh} mice (**a,b**), the corresponding wild-type WBB6F1-*Kit*^{+/+} mice (**a-d**) and B6-*Kit*^{+/+} mice (**a,b**), and WT BMCMC→*Kit*^{W/W-v} mice and *Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice (**c,d**) to challenge (arrowheads) with urushiol (**a,c**), DNFB (**b,d**) or vehicle (V; **a-d**) at 5 d after sensitization. Mast cells per mm of right ear cartilage, **Supplementary Data** online. †††, *P* < 0.0001, versus the corresponding wild-type mice or WT BMCMC→*Kit*^{W/W-v} mice. Data (mean ± s.d. (**a,b**) or mean ± s.e.m. (**c,d**)) are from one (**a,b**), three (**c**) or six (**d**) experiments with three to seven mice per group per experiment; each replicate experiment in **c,d** gave similar results.

CHS responses occur when a subject that has been sensitized to an allergen such as urushiol or an experimental hapten such as DNFB¹⁸ is re-exposed to (or 'challenged' with) the same agent, typically after 5 d or longer. This exposure triggers an inflammatory cascade involving the infiltration of primed antigen-specific T cells into the challenged site, the recruitment of other inflammatory cells, and tissue edema⁸. CHS in response to urushiol in humans, however, can also include the development of intensely pruritic vesicular skin lesions at 8–48 h after exposure that can remain for up to 3 weeks¹⁴. In severe cases, lesions can develop frank necrosis, with ulceration of the epidermis and the development of notable epidermal hyperplasia^{14,19}.

Although the importance of mast cells in the elicitation of CHS was once controversial^{20,21}, studies have shown that mast cells can augment the magnitude of the tissue swelling and leukocyte infiltration associated with some^{20,22,23} but not all^{21,24,25} models of CHS in mice. In each of those studies, however, CHS reactions were measured for only 24 h, 48 h or, in one, 72 h (ref. 21) after hapten challenge. Because we wanted to evaluate whether mast cells might influence the long-term consequences of CHS responses, including their resolution, we examined CHS reactions to urushiol or DNFB for up to 15 d after a single epicutaneous challenge with hapten. We are not aware of studies of DNFB-induced CHS in mice that have examined any intervals greater than 24 h after challenge.

Beginning 3 d after challenge with urushiol (**Fig. 1a**) or DNFB (**Fig. 1b** and **Supplementary Fig. 1** online), mast cell-deficient WBB6F1-*Kit*^{W/W-v} or C57BL/6-*Kit*^{W-sh/W-sh} (B6-*Kit*^{W-sh/W-sh}) mice showed much stronger CHS responses than did the corresponding wild-type mice. In mast cell-deficient mice, tissue swelling increased progressively and peaked at about 5–7 d after hapten challenge. To assess whether the different responses of wild-type and *Kit*^{W/W-v} mice specifically reflected the lack of mast cells in *Kit*^{W/W-v} mice, rather than other c-Kit-related differences^{5,26}, we examined *Kit*^{W/W-v} mice engrafted with bone marrow-derived cultured mast cells (BMCMCs) from wild-type (*Kit*^{+/+}) mice (WT BMCMC→*Kit*^{W/W-v} mice)²⁶. The swelling associated with CHS responses to urushiol (**Fig. 1c**) or DNFB (**Fig. 1d** and **Supplementary Fig. 1**) in WT BMCMC→*Kit*^{W/W-v} mice resembled that in wild-type mice. Experiments with WT BMCMC→*Kit*^{W/W-v} mice or *Kit*^{W/W-v} mice engrafted with *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice) showed that the ability of mast cells to produce IL-10 contributed significantly to their ability to limit

and to hasten resolution of the ear swelling associated with CHS responses to either urushiol or DNFB (**Fig. 1c,d** and **Supplementary Fig. 1**). At 5 d after hapten challenge, reactions in mast cell-deficient *Kit*^{W/W-v} mice or *Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice, in contrast to those in wild-type mice or in WT BMCMC→*Kit*^{W/W-v} mice, were associated with substantial dermal leukocyte infiltration, hyperplasia and focal ulceration of the epidermis, and expansion and/or edema of the dermis (**Fig. 2** and **Table 1**).

We obtained similar findings when we repeated the experiments with mast cell-deficient *Kit*^{W-sh/W-sh} mice, the corresponding wild-type B6 mice, and WT (B6)→*Kit*^{W-sh/W-sh} mice or *Il10*^{-/-} BMCMC→*Kit*^{W-sh/W-sh} mice (**Supplementary Figs. 2 and 3** online). Thus, our work with two types of genetically mast cell-deficient mice indicates that mast cells can substantially limit the magnitude of CHS responses to urushiol or DNFB. In addition, the ability of mast cells to limit the intensity of CHS reactions has a biologically relevant consequence in that focal necrosis and ulceration of the epidermis occurred in association with CHS responses in most of the mast cell-deficient mice but in none of the corresponding wild-type or WT BMCMC→*Kit*^{W/W-v} or *Kit*^{W-sh/W-sh} mice (**Table 1** and **Supplementary Fig. 3**).

We used flow cytometry and histological analysis to characterize leukocyte and mast cell populations at sites of CHS. At 5 d after challenge, the ear skin of DNFB-challenged *Kit*^{W/W-v} mice and *Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice had more granulocytes (particularly Gr-1^{hi} granulocytes, which are thought to be activated granulocytes²⁷) and Gr-1⁺ activated macrophages (in some settings of inflammation, F4/80^{hi} activated macrophages are also Gr-1^{hi}; ref. 28) and more CD8⁺, CD4⁺, CD4⁺CD25⁺ and CD4⁺CD25⁺CD62L⁻ T cells than did that of wild-type mice or WT BMCMC→*Kit*^{W/W-v} mice (**Fig. 3a-d** and **Supplementary Fig. 4** online). *Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice had numbers of dermal mast cells at sites of mast cell injection similar to those of WT BMCMC→*Kit*^{W/W-v} mice, but not as high as those of wild-type mice (**Fig. 3e**). Only wild-type mice had a significant increase in mast cells at sites of CHS (**Fig. 3e**). Both wild-type and WT BMCMC→*Kit*^{W/W-v} mice, however, had significantly less epidermal hyperplasia at sites of CHS than did *Kit*^{W/W-v} mice or *Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice (**Fig. 3f**).

Although monocytes, macrophages, dendritic cells and some T cell populations (such as CD4⁺ or CD4⁺CD25⁺Foxp3⁺ T regulatory cells or type 1 regulatory cells) can also secrete IL-10 (ref. 1), *Kit*^{W/W-v}

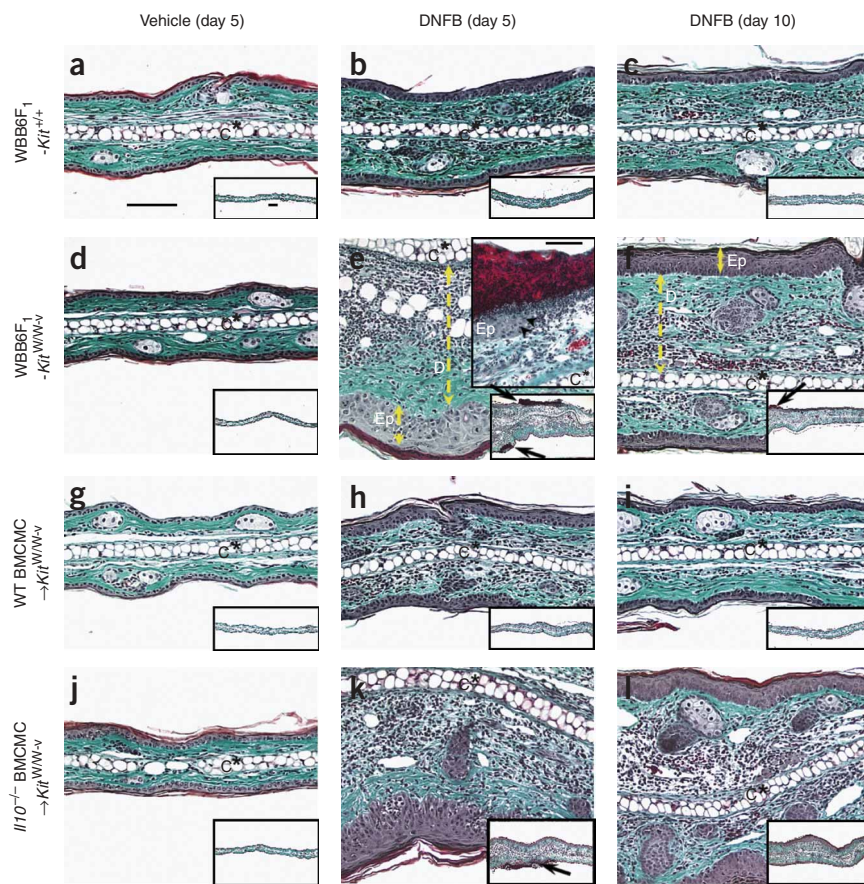


Figure 2 Mast cells and mast cell-derived IL-10 can limit the skin pathology associated with CHS responses to DNFB. Cross-sections of ears obtained from WBB6F1-*Kit*^{+/+} (wild-type) mice (a–c), mast cell-deficient *Kit*^{W/W-v} mice (d–f), WT BMCMC → *Kit*^{W/W-v} mice (g–i) and *Il10*^{−/−} BMCMC → *Kit*^{W/W-v} mice (j–l) at 5 d after challenge with vehicle only (100% acetone; a,d,g,j) or 0.2% DNFB (b,e,h,k) or 10 d after challenge with 0.2% DNFB (c,f,i,l); sections were stained with Masson's trichrome. C*, cartilage. Double-headed arrows indicate thickness of dermis (D) or epidermis (Ep); arrows in insets indicate sites of ulcers with adherent exudate (red); arrowheads (e, top inset) indicate the border between intact epithelium and (on the right) complete loss of epithelium at the base of an ulcer covered by an exudate. Scale bar, 100 μm. Results are representative of six different experiments with four to seven mice per group in each.

CHS responses to urushiol of *Fcer1g*^{−/−} BMCMC → *Kit*^{W/W-v} mice also included more infiltrating leukocytes of various subpopulations than did those of wild-type mice, although sometimes not as many as those of *Kit*^{W/W-v} mice (Supplementary Fig. 5 online).

In DNFB-induced CHS, mast cell expression of Fcγ also contributed significantly to the ability of mast cells to suppress the swelling associated with the response in the first 5 d after DNFB challenge (Fig. 4d).

Mechanisms of mast cell activation that

mice and *Il10*^{−/−} BMCMC → *Kit*^{W/W-v} mice (which had more macrophages and T cells at reaction sites than did wild-type or WT BMCMC → *Kit*^{W/W-v} mice; Fig. 3b,c) had significantly lower concentrations of IL-10 in DNFB-treated ear skin 3 or 5 d after hapten challenge than did wild-type or WT BMCMC → *Kit*^{W/W-v} mice (Fig. 3g). These findings show that mast cells represent a quantitatively important source of IL-10 in this condition.

Mast cells are activated through the Fcγ in CHS

To assess a potential mechanism by which mast cells might be activated to produce IL-10 (and perhaps other anti-inflammatory or immunosuppressive mediators) in our models of CHS, we tested *Kit*^{W/W-v} mice engrafted with BMCMCs deficient in the common γ-chain of Fc receptors (Fcγ; called 'Fcer1g^{−/−} BMCMCs' here). In the mouse, the Fcγ chain is shared by FcεRI (the high-affinity receptor for immunoglobulin E (IgE))⁵ and FcγRIII (an IgG receptor that can bind antigen-IgG1 immune complexes)²⁹. In some acquired immune responses, some mast cell functions may reflect the activation of mast cells through FcγRIII, such as activation by immune complexes of antigen and antigen-specific IgG1 antibodies^{30,31}. It has also been reported that, when transferred intravenously into naive recipient mice before sensitization, purified IgG fractions obtained from mice 25 d after sensitization with urushiol can suppress CHS responses to urushiol³².

Mice genetically deficient in Fcγ (with all cell types lacking Fcγ) showed exacerbated CHS responses (Fig. 4a,b) compared with the responses of wild-type mice. *Fcer1g*^{−/−} BMCMC → *Kit*^{W/W-v} mice (in which only mast cells lacked Fcγ) had CHS responses to urushiol similar in magnitude to the responses of *Kit*^{W/W-v} mice (Fig. 4c). The

are independent of the Fcγ may also occur in CHS reactions to DNFB, however. Thus, ear swelling was significantly less in *Fcer1g*^{−/−} BMCMC → *Kit*^{W/W-v} mice than in *Kit*^{W/W-v} mice (Fig. 4d), and epidermal thickness and numbers of activated granulocytes, activated macrophages, and CD8⁺ and CD4⁺ T cells in hapten-treated ears of *Fcer1g*^{−/−} BMCMC → *Kit*^{W/W-v} mice were intermediate between those of WT BMCMC → *Kit*^{W/W-v} mice and *Kit*^{W/W-v} mice (Supplementary Fig. 6 online). We obtained similar findings in experiments assessing tissue swelling responses to urushiol or DNFB in wild-type, *Kit*^{W-sh/W-sh}, WT BMCMC → *Kit*^{W-sh/W-sh} and *Fcer1g*^{−/−} BMCMC → *Kit*^{W-sh/W-sh} mice (data not shown). Focal full-thickness epidermal necrosis and ulceration developed in association with CHS reactions

Table 1 Epidermal necrosis in CHS responses to DNFB

Time after DNFB challenge	Mice with full-thickness epidermal necrosis	
	5 d	10 d
WBB6F1- <i>Kit</i> ^{+/+}	0/19	0/5
WBB6F1- <i>Kit</i> ^{W/W-v}	8/10*	2/3*
WT BMCMC → <i>Kit</i> ^{W/W-v}	0/16	0/3
<i>Il10</i> ^{−/−} BMCMC → <i>Kit</i> ^{W/W-v}	11/15*	2/3*

Values indicate mice whose ears showed full-thickness epidermal necrosis and, in some cases, ulcers, as assessed by histology at 5 or 10 d after challenge with DNFB (mice with necrosis/total mice). *, *P* < 0.05, wild-type (*Kit*^{+/+}) mice or WT BMCMC → *Kit*^{W/W-v} mice versus the corresponding mast cell-deficient *Kit*^{W/W-v} mice or *Il10*^{−/−} BMCMC → *Kit*^{W/W-v} mice. Data are from three experiments with similar results on day 5 (three to six mice per group per experiment) or one experiment on day 10 (three or five mice per group).

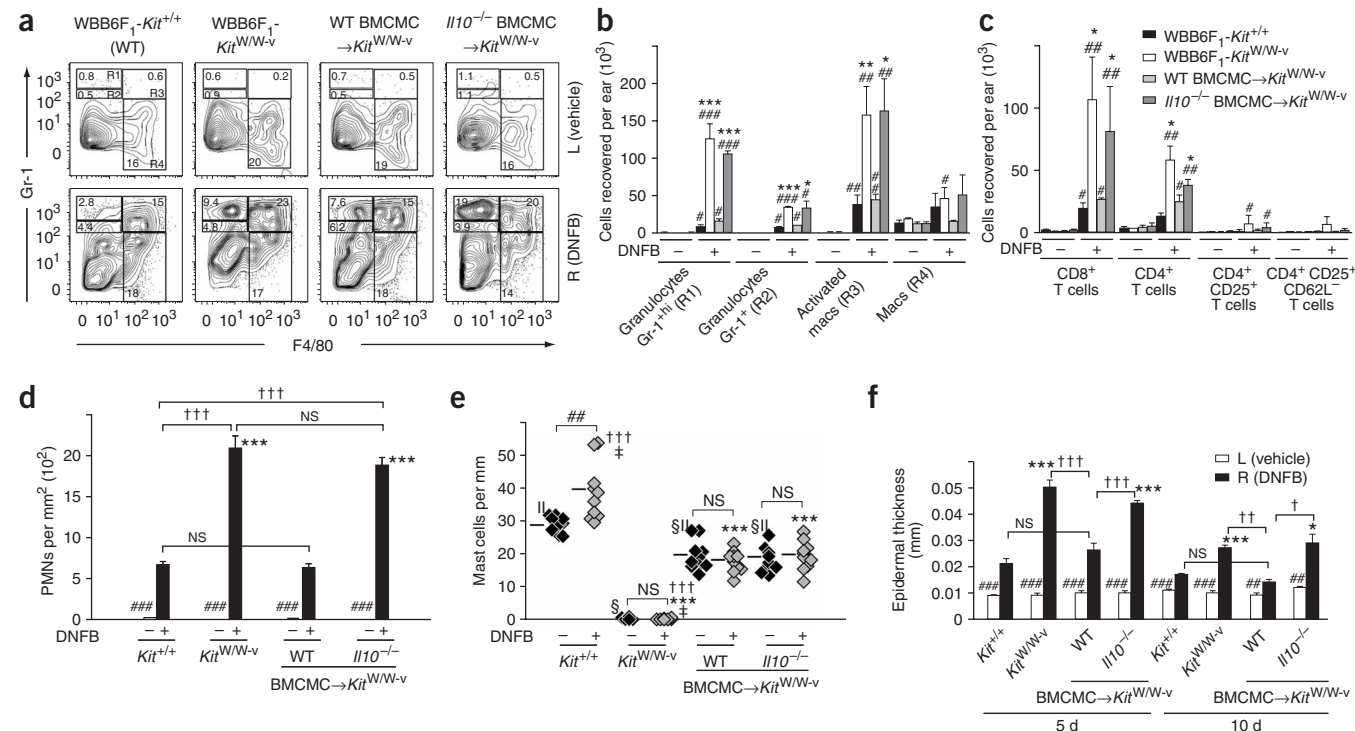


Figure 3 Mast cells and mast cell-derived IL-10 can limit the number of leukocytes and epidermal thickening at sites of CHS responses to DNFB. **(a–c)** Analysis of granulocytes, activated macrophages (F4/80^{hi} Gr-1^{hi}) and T cells in ear skin at 5 d after challenge with 0.2% DNFB (+) or vehicle (–). **(a)** Flow cytometry of cells from left (L) and right (R) ears. **(b)** Granulocytes (gated population R1, Gr-1^{hi}; R2, Gr-1^{hi}) and macrophages (macs; R3, Gr-1^{hi}; F4/80⁺; R4, F4/80⁺) in individual ears (mean + s.d.). **(c)** Flow cytometry of pooled T cells (T cell flow cytometry, **Supplementary Fig. 5**; total recovered viable cells in ears treated with vehicle or DNFB, **Supplementary Data** online). **(d)** Granulocytes (polymorphonuclear neutrophils; PMNs) per mm² in ear pinnae stained with hematoxylin and eosin. In **b–d**, *, $P < 0.01$; **, $P < 0.001$ and ***, $P < 0.0001$, versus the corresponding wild-type (*Kit*^{+/+}) mice or WT BMCMC → *Kit*^{W/W-v} mice; #, $P < 0.05$; ##, $P < 0.01$ and ###, $P < 0.001$, right versus left ears for the same group of mice. **(e)** Dermal mast cells. †, $P < 0.0001$ or §, $P < 0.0001$, versus left (vehicle-treated) ears of *Kit*^{W/W-v} mice or wild-type (*Kit*^{+/+}) mice, respectively. **(f)** Epidermal thickness at 5 or 10 d after challenge with DNFB or vehicle. **(g)** Concentration of IL-10 (per mg total protein) in ears at 3 or 5 d after challenge with DNFB or vehicle. In **d–g**, *, $P < 0.01$; **, $P < 0.001$ and ***, $P < 0.0001$, versus the corresponding wild-type (*Kit*^{+/+}) mice; †, $P < 0.01$; ††, $P < 0.001$ and †††, $P < 0.0001$, versus the corresponding WT BMCMC → *Kit*^{W/W-v} mice. In **e,g**, ‡, $P < 0.01$, *Kit*^{W/W-v} mice versus the corresponding *Il10*^{–/–} BMCMC → *Kit*^{W/W-v} mice. In **d–g**, #, $P < 0.01$; ##, $P < 0.001$ and ###, $P < 0.0001$, right versus left ears in the same group of mice. NS, not significant ($P > 0.05$). Data (mean + s.d. (**b,g**) or mean + s.e.m. (**c,d,f**)) are from one mouse per group from one experiment with three mice per group with similar results for all mice in each group (**a**); one experiment with three (**b**) or three to six (**f,g**) mice per group; four experiments with similar results (**c**; cells pooled from the ears of four to five mice per group in each); four experiments with similar results (**d**; nine to eleven ears per group); or two of six experiments with similar results, each of which included all four groups of mice with three to seven mice per group per experiment (**e**; ten mice per group).

to DNFB or urushiol in most *Fcer1g*^{–/–} BMCMC → *Kit*^{W/W-v} mice (8 of 14) but in none of the corresponding wild-type mice (0 of 27) or WT BMCMC → *Kit*^{W/W-v} mice (0 of 23; $P < 0.05$, *Fcer1g*^{–/–} BMCMC → *Kit*^{W/W-v} mice versus the corresponding WT BMCMC → *Kit*^{W/W-v} mice). On the B6 background, *Kit*^{W-sh/W-sh} mice and *Fcer1g*^{–/–} BMCMC → *Kit*^{W-sh/W-sh} mice showed similar high rates of focal epidermal ulceration in association with CHS responses to either of the contact allergens tested (data not shown).

Mast cell-deficient *Kit*^{W/W-v} mice and *Fcer1g*^{–/–} BMCMC → *Kit*^{W/W-v} or *Il10*^{–/–} BMCMC → *Kit*^{W/W-v} mice had serum titers of DNFB-specific IgG1 and IgE similar to those of wild-type and WT BMCMC → *Kit*^{W/W-v} mice (**Supplementary Table 1** online). The CHS reactions of *Kit*^{W/W-v} mice and *Fcer1g*^{–/–} BMCMC → *Kit*^{W/W-v} or *Il10*^{–/–} BMCMC → *Kit*^{W/W-v} mice contained more macrophages than did those of wild-type mice (**Fig. 3b** and **Supplementary**

Figs. 5a and **6c**); such macrophages, like mast cells, express FcγRs that can be ligated with IgG to promote IL-10 production³³. The amount of IL-10 protein per mg total protein in ears, however, showed little or no increase in CHS sites compared with vehicle-treated sites in *Kit*^{W/W-v} mice (**Fig. 3g**), *Kit*^{W-sh/W-sh} mice (**Supplementary Fig. 7** online) or *Il10*^{–/–} BMCMC → *Kit*^{W/W-v} mice (**Fig. 3g**).

To evaluate whether mouse mast cells activated through FcγRIII can produce IL-10, we stimulated BMCMCs *in vitro*³⁴. It has been shown that incubation of BMCMCs with IL-4 leads to upregulated surface expression of FcγRIII and enhances the ability of such cells to release tumor necrosis factor (TNF) and other mediators after aggregation of FcγRIII (ref. 34). We confirmed that preincubation of BMCMCs with IL-4 enhanced the ability of the cells to release TNF after aggregation of FcγRIII (ref. 34) and found that such BMCMCs

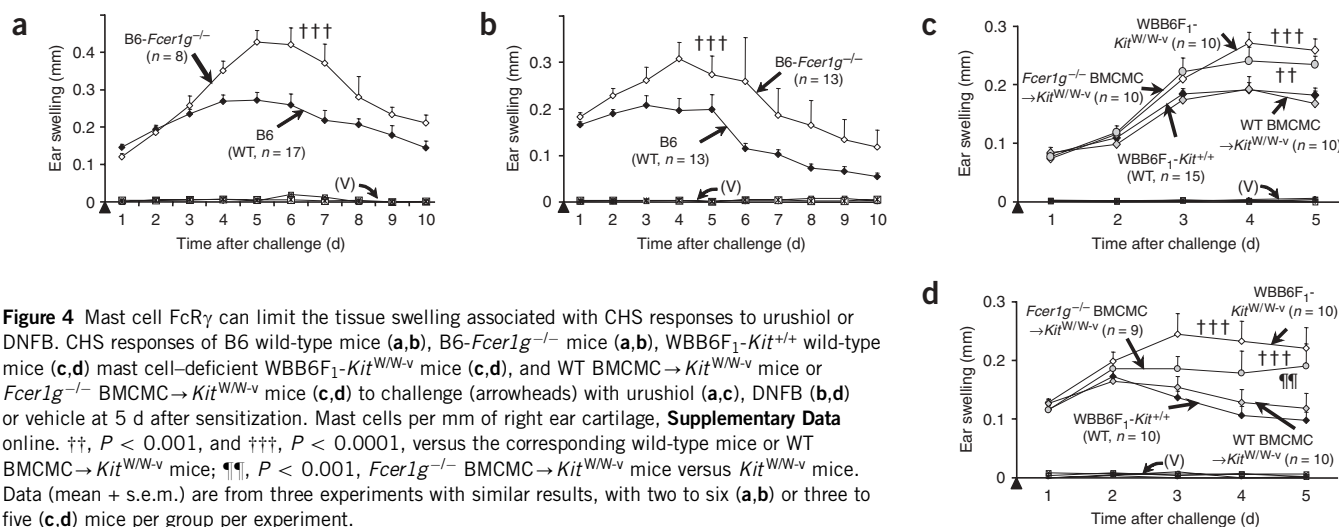


Figure 4 Mast cell Fc γ R can limit the tissue swelling associated with CHS responses to urushiol or DNFB. CHS responses of B6 wild-type mice (**a,b**), B6-*FcεR1*^{-/-} mice (**a,b**), WBB6F1-*Kit*^{+/+} wild-type mice (**c,d**) mast cell-deficient WBB6F1-*Kit*^{W/W-v} mice (**c,d**), and WT BMCMC → *Kit*^{W/W-v} mice or *FcεR1*^{-/-} BMCMC → *Kit*^{W/W-v} mice (**c,d**) to challenge (arrowheads) with urushiol (**a,c**), DNFB (**b,d**) or vehicle at 5 d after sensitization. Mast cells per mm of right ear cartilage, **Supplementary Data** online. ††, $P < 0.001$, and †††, $P < 0.0001$, versus the corresponding wild-type mice or WT BMCMC → *Kit*^{W/W-v} mice; ¶¶, $P < 0.001$, *FcεR1*^{-/-} BMCMC → *Kit*^{W/W-v} mice versus *Kit*^{W/W-v} mice. Data (mean + s.e.m.) are from three experiments with similar results, with two to six (**a,b**) or three to five (**c,d**) mice per group per experiment.

also secreted IL-10 (**Supplementary Fig. 8** online). These results indicate that aggregation of their Fc γ RIII can induce mouse mast cells to secrete IL-10.

Function of antigen-specific IgG1

To elucidate the function of antigen-specific IgG1 in limiting CHS-induced skin pathology, we developed a passive transfer model of CHS in B6 mice deficient in recombination-activating gene 2 (B6-*Rag2*^{-/-} mice). In this model, administration of an antigen-specific IgG1 monoclonal antibody (mAb), but neither an antigen-specific IgE mAb³⁵ nor a control IgG1 mAb of irrelevant antigen specificity³⁶, suppressed many features of the hapten-induced cutaneous responses elicited in mice that received antigen-specific T cells (**Supplementary Methods** and **Supplementary Figs. 9** and **10** online). Notably, several features of our passive transfer model of CHS in response to DNFB in B6-*Rag2*^{-/-} mice were very similar to those of c-Kit-mutant mast cell-deficient mice. Compared with hapten-challenged wild-type mice, hapten-challenged mast cell-deficient mice or B6-*Rag2*^{-/-} mice that received sensitized T cells without concomitant administration of antigen-specific IgG1 mAb showed greater tissue swelling (**Supplementary Fig. 9a**), greater epidermal thickening and epidermal necrosis (**Supplementary Figs. 9c** and **10d**) and more intense infiltrates of (host) granulocytes and macrophages and (donor) T cells (**Supplementary Figs. 9d–f** and **10d**). In addition, focal full-thickness necrosis of the epidermis and/or ulceration occurred at 5 d after challenge with DNFB in 10 of 12 B6-*Rag2*^{-/-} mice treated with a control IgG1 mAb to ovalbumin and in 2 of 3 B6-*Rag2*^{-/-} mice treated with an antigen-specific IgE mAb to dinitrophenol (DNP), but this occurred in only 2 of 14 wild-type mice and 1 of 13 B6-*Rag2*^{-/-} mice treated with an antigen-specific IgG1 mAb to DNP ($P < 0.05$, B6-*Rag2*^{-/-} mice treated with control IgG1 mAb to ovalbumin versus the corresponding wild-type or B6-*Rag2*^{-/-} mice treated with antigen-specific IgG1 mAb to DNP (χ^2 test)). These findings support the conclusion that antigen-specific IgG1 can have effects that suppress features of T cell-dependent cutaneous inflammation, including the epidermal necrosis associated with these responses.

Mast cells limit responses to chronic UVB irradiation

To assess whether mast cells and mast cell-derived IL-10 might have anti-inflammatory functions in conditions other than CHS, we investigated an example of an innate tissue response to a ubiquitous

environmental stimulus: UVB irradiation. Excessive exposure to solar ultraviolet radiation (particularly UVB or UVA radiation with wavelengths between 280 and 320 nm or 320 and 400 nm, respectively) causes various acute and chronic photocutaneous reactions with several deleterious effects, including sunburn and photoaging (accelerated aging of the skin caused by environmental exposure to ultraviolet irradiation)^{10,37–40}. Mice treated with regimes of multiple low-dose exposures to UVB irradiation over several weeks to months develop more mast cells at the irradiated site^{10,11}, a feature also present in photoaged human skin^{10,40}. However, studies have linked mast cells and their products to promotion of the inflammation and tissue changes of photoaging^{10,11,40}.

To test whether mast cells contribute to the pathology noted in chronically UVB-irradiated skin, we exposed the ears of wild-type mice, mast cell-deficient *Kit*^{W/W-v} mice, WT BMCMC → *Kit*^{W/W-v} mice and *Il10*^{-/-} BMCMC → *Kit*^{W/W-v} mice to one minimal erythema dose of UVB every 2 d for a total of 15 exposures (total cumulative dose, 30 kJ/m²). *Kit*^{W/W-v} mice and *Il10*^{-/-} BMCMC → *Kit*^{W/W-v} mice developed more notable ear erythema and tissue damage (**Supplementary Fig. 11** online) and significantly greater ear thickness (**Fig. 5a** and **Supplementary Fig. 12** online) after UVB irradiation than did the corresponding wild-type mice or WT BMCMC → *Kit*^{W/W-v} mice, even though both groups of BMCMC-engrafted *Kit*^{W/W-v} mice had similar numbers of mast cells in the ear pinnae (**Fig. 5b**). As expected, the smallest responses to UVB irradiation occurred in wild-type mice, the only group of mice with extensive skin pigmentation (these mice are black, unlike any of the other groups, which essentially lack skin melanocytes because of their *Kit* mutations^{26,41}).

Chronic UVB irradiation resulted in a significant increase in the numbers of skin mast cells in both wild-type mice and *Kit*^{W/W-v} mice (which nonetheless remained profoundly mast cell deficient compared with all other groups of mice in this experiment; **Fig. 5b**). In contrast, CHS responses to DNFB were associated with significant increases in the numbers of dermal mast cells in wild-type mice but not in *Kit*^{W/W-v} mice (**Fig. 3e**). These findings suggest that chronic UVB irradiation results in the expression of factors that can promote local mast cell development and/or survival in the context of profound c-Kit deficiency. Notably, neither CHS in response to DNFB nor chronic UVB irradiation was associated with a significant increase in the number of mast cells in the ear pinnae of

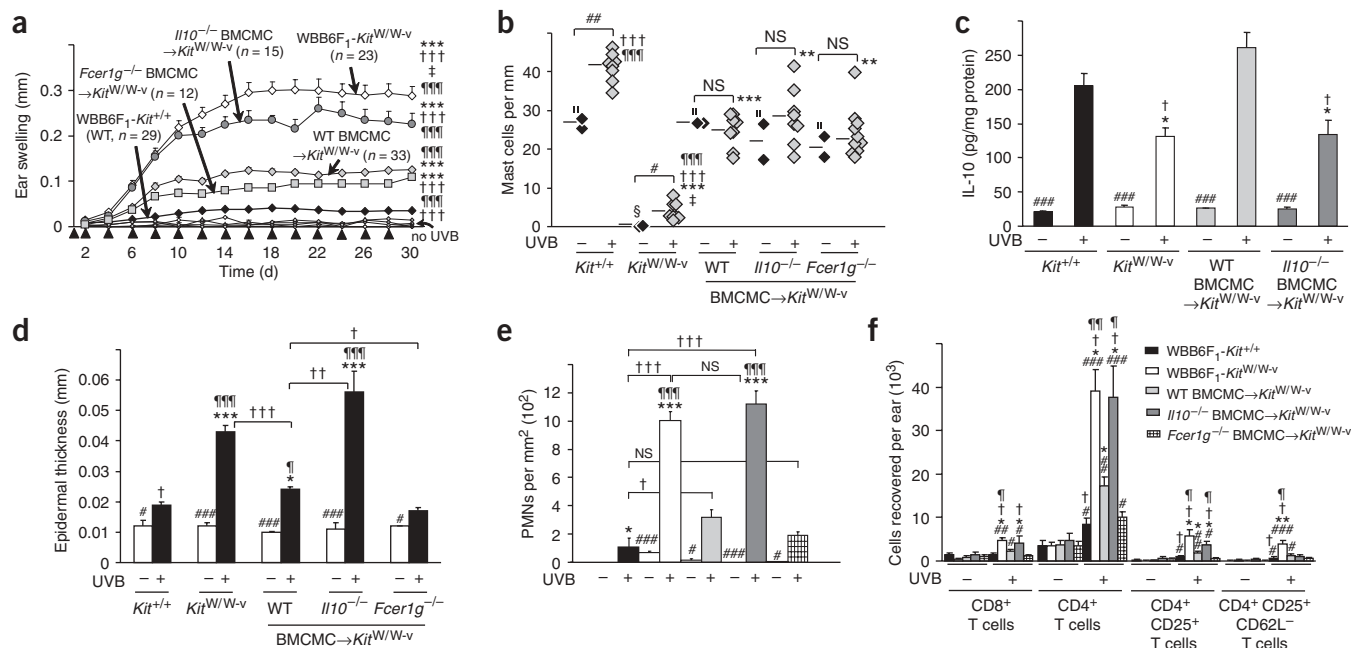


Figure 5 Mast cells and mast cell-derived IL-10, but not mast cell Fc γ R, can limit the tissue swelling, epidermal thickening and leukocyte recruitment associated with chronic low-dose UVB irradiation. (a) Ear swelling in WBB6F₁-*Kit^{W/W-v}* (wild-type) mice, mast cell-deficient *Kit^{W/W-v}* mice, WT BMCMC $\rightarrow$ *Kit^{W/W-v}* mice, *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice and *Fcer1g^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice after 15 exposures to 2 kJ/m² of UVB (each arrowhead indicates a single exposure). (b) Dermal mast cells. $\ddagger$, $P < 0.05$, or $\S$, $P < 0.05$, control ears (no UVB treatment) versus control ears of mast cell-deficient *Kit^{W/W-v}* mice or wild-type (*Kit^{+/+}*) mice, respectively. (c-f) IL-10 (per mg total protein; c), epidermal thickness (d), polymorphonuclear neutrophils (e), and CD8⁺ and CD4⁺ T cells (f) in ear skin after chronic UVB irradiation or no UVB exposure (flow cytometry, **Supplementary Data** online). *, $P < 0.01$; **, $P < 0.001$ and ***, $P < 0.0001$, versus the corresponding UVB-irradiated wild-type mice; $\ddagger$, $P < 0.01$; $\S$, $P < 0.001$ and $\P$, $P < 0.0001$, versus the corresponding UVB-irradiated *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice; $\P$, $P < 0.01$; $\P\P$, $P < 0.001$ and $\P\P\P$, $P < 0.0001$, versus the corresponding UVB-irradiated *Fcer1g^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice. #, $P < 0.01$; ##, $P < 0.001$ and ###, $P < 0.0001$ (b-d), or #, $P < 0.05$; ##, $P < 0.01$ and ###, $P < 0.001$ (e,f), control ears (no UVB treatment) versus UVB-irradiated ears in the same group of mice. Data (mean $\pm$ s.d. (c,g)) or mean $\pm$ s.e.m. (a,d-f) are from three to five experiments with similar results (a; three to seven mice per group per experiment); one (UVB -) or two (UVB +) of four to six experiments with similar results for each of the ten groups, with two to five mice per group per experiment (b); two (UVB -) or 10 (UVB +) mice per group; one experiment (c; three to four mice per group); two experiments with similar results (d; three to four mice per group per experiment); three experiments with similar results (e; six to seven mice per group); or five experiments with similar results (f; ears pooled from four to five mice per group per experiment).

Kit^{W/W-v} mice that had been engrafted with various populations of BMCMCs, even though these BMCMCs expressed wild-type c-Kit (Figs. 3e and 5b).

At sites of chronic UVB irradiation, as at sites of DNFB-induced CHS (Fig. 3g), there was significantly more IL-10 per mg total protein in ear skin of WT BMCMC $\rightarrow$ *Kit^{W/W-v}* mice and wild-type mice than in that of *Kit^{W/W-v}* mice or *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice (Fig. 5c). Similarly, UVB irradiation resulted in significantly greater epidermal thickness (Fig. 5d), more granulocytes, activated macrophages and some T cell populations (Fig. 5e,f, and **Supplementary Figs. 13 and 14** online), and significantly more epidermal hyperplasia (Fig. 6), in the skin of *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice than in that of WT BMCMC $\rightarrow$ *Kit^{W/W-v}* mice. UVB irradiation resulted in full-thickness epidermal necrosis in 7 of 11 *Kit^{W/W-v}* and 6 of 10 *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice, but in 0 of 14 wild-type or 12 *Fcer1g^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice tested, and in only 1 of 16 WT BMCMC $\rightarrow$ *Kit^{W/W-v}* mice (Table 2; $P < 0.05$, wild-type (*Kit^{+/+}*) or WT BMCMC $\rightarrow$ *Kit^{W/W-v}* mice versus the corresponding *Kit^{W/W-v}* or *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice). In contrast to our findings for CHS, the ability of adoptively transferred mast cells to limit several features of UVB-induced responses did not require that these cells expressed Fc γ R (Fig. 5a,d-f, and **Supplementary Figs. 13 and 14**).

We monitored the ear swelling responses of a subgroup of these mice (Fig. 5a) for 20 d after cessation of UVB treatment (**Supplementary Fig. 12**). Although the responses of *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice were very similar to those of *Kit^{W/W-v}* mice during the 30-day period of UVB treatment, the reactions of the *Il10^{-/-}* BMCMC $\rightarrow$ *Kit^{W/W-v}* mice resolved more rapidly than those of the *Kit^{W/W-v}* mice after UVB treatment was stopped (Fig. 5a and **Supplementary Fig. 12**). This finding indicates that mast cells can contribute to the resolution of the UVB-induced responses at least in part by mechanisms that are independent of their ability to produce IL-10.

DISCUSSION

Our findings here have identified previously unknown functions for mast cells in limiting the magnitude and duration of two examples of CHS (in response to urushiol or DNFB) and an example of an innate response to a ubiquitous environmental stimulus (chronic low-dose UVB irradiation). Mast cell production of IL-10 at the affected site represents an important mechanism by which mast cells limit the pathology associated with each of these responses, and our data indicate that mast cells and mast cell-derived IL-10 may influence the extent and duration of CHS or UVB-induced responses in part by

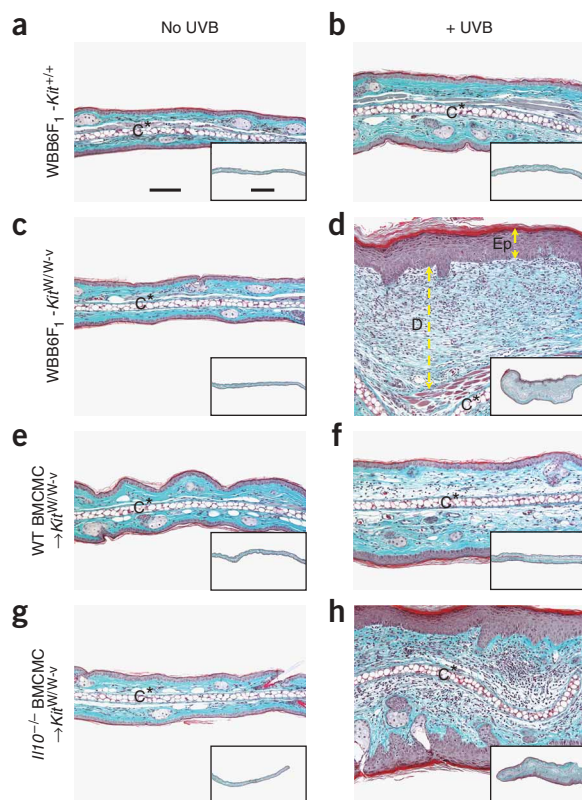


Figure 6 Mast cells and mast cell-derived IL-10 can limit the pathology associated with chronic low-dose UVB irradiation of ear skin. Cross-sections of ears obtained from WBB6F1-*Kit*^{+/+} (wild-type) mice (**a,b**), *Kit*^{W/W-v} mice (**c,d**), WT BMCMC → *Kit*^{W/W-v} mice (**e,f**) or *I110*^{-/-} BMCMC → *Kit*^{W/W-v} mice (**g,h**) at 24 h after the final exposure to 2 kJ/m² of UVB (15 exposures, 2 d apart; **b,d,f,h**); sections were stained with Masson's trichrome. Mice that did not receive UVB irradiation were also killed for analysis of skin histology at the completion of the experiment (**a,c,e,g**). Double-headed arrows indicate thickness of dermis (D) or epidermis (Ep). Scale bars, 100 μm or 1,000 μm (inset). Results are representative of four different experiments, each with three to six mice per group.

The idea that IgG1 antibodies rather than IgE antibodies are important for mast cell anti-inflammatory function in this setting is supported by the findings obtained with our model of passive transfer of CHS reactivity to *Rag2*^{-/-} mice and by studies of C57BL/6J-*Fcgr3*^{-/-} mice, whose cells do not bind IgG1 through FcγRIII but bind IgE through FcεRI. *Fcgr3*^{-/-} mice and *Fcgr3*^{-/-} BMCMC → *Kit*^{W/W-v} mice showed enhanced CHS responses to DNFB that were very similar to those of C57BL/6J-*Fcer1g*^{-/-} and *Kit*^{W/W-v} mice, respectively (data not shown). Finally, BALB/c-*Fcer1a*^{-/-} mice, in which IgE cannot bind through FcεRI, did not differ substantially from congenic wild-type mice in the tissue swelling associated with DNFB-induced CHS (data not shown).

Our findings collectively suggest that in mice that have been sensitized epicutaneously to some haptens, the development of an antigen-specific IgG1 response can result in enhanced Fcγ-dependent mast cell production of IL-10, which represents a mast cell-dependent mechanism for limiting the extent and duration of such CHS reactions. However, we found that several aspects of the pathology associated with DNFB-induced CHS in *Fcer1g*^{-/-} BMCMC-engrafted mast cell-deficient mice were intermediate in magnitude between those of WT BMCMC-engrafted mast cell-deficient mice and mast cell-deficient mice. This indicates that mast cells may be activated to express anti-inflammatory and/or immunosuppressive functions in this setting by at least one additional mechanism that is not mediated through Fcγ.

Although the mechanism by which mast cells are induced to secrete IL-10 in sites chronically exposed to UVB remains to be determined, other studies have proposed that UVB irradiation can induce mast cells to undertake immunosuppressive functions. For example, it has been reported that the mast cell-derived mediators histamine²⁵ and TNF⁴² contribute to the local and systemic immunosuppression of CHS responses that can be found in mice exposed to a single high dose²⁵ or three to four low doses⁴² of UVB irradiation. IL-10 also has been indicated as a mediator of the systemic immunosuppression noted after a single high dose of UVB irradiation (30 kJ/m² (ref. 43) or 15 kJ/m² (ref. 13)), and IL-10-expressing cells can be detected by immunohistochemistry in mouse skin after UVB irradiation⁴³. Although these IL-10 immunoreactive cells were not identified, it will be useful to determine whether mast cell-derived IL-10 can contribute to UVB-induced systemic immunosuppression.

Although we have not investigated the function of mast cell-derived IL-10 in other contexts, involvement of both mast cells and IL-10 has been indicated in two different models of immunosuppression^{44,45}. The bites of mosquitoes of the genus *Anopheles* can impair the development of antigen-specific T cell responses in a model of delayed-type hypersensitivity to ovalbumin in mice, and this phenomenon requires the presence of mast cells in the bitten skin⁴⁴. Mast cells are also required for optimal expression of tolerance to skin allografts, a model of peripheral tolerance that depends on CD4⁺CD25⁺FoxP3⁺ regulatory T cells⁴⁵. IL-10 has been associated with the mechanism of

regulating the numbers of leukocytes at these sites. In CHS, which is known to be a T cell-mediated response^{6-8,18}, the effects of mast cells on T cell trafficking, survival, proliferation and/or function may be particularly important. Regardless of how mast cells and mast cell-derived IL-10 produce anti-inflammatory or immunosuppressive effects in these settings, it is clear that the ability of mast cells to limit these responses is biologically important. Indeed, mast cells can mostly suppress the substantial epidermal thickening and the development of areas of full-thickness epidermal necrosis and ulceration that occur at sites of CHS or chronic UVB irradiation in mice lacking either mast cells or mast cell-derived IL-10.

In CHS, but not in chronic UVB exposure, the ability of mast cells to limit the responses reflects, at least in part, activation of the cells through Fcγ. Our *in vitro* data suggest that the crucial Fcγ-dependent mechanism may be the induction of mast cell secretion of IL-10 by IgG1 immune complex-induced aggregation of FcγRIII.

Table 2 Epidermal necrosis after UVB irradiation

UVB-treated mice	Mice with full-thickness epidermal necrosis
WBB6F1- <i>Kit</i> ^{+/+}	0/14
WBB6F1- <i>Kit</i> ^{W/W-v}	7/11*
WT BMCMC → <i>Kit</i> ^{W/W-v}	1/16
<i>I110</i> ^{-/-} BMCMC → <i>Kit</i> ^{W/W-v}	6/10*
<i>Fcer1g</i> ^{-/-} BMCMC → <i>Kit</i> ^{W/W-v}	0/12

Values indicate mice whose ears showed evidence of full-thickness epidermal necrosis and, in some cases, ulcers, as assessed by clinical observation and/or histology during or at the end of the period of UVB exposure (mice with necrosis/total mice). *, *P* < 0.05 wild-type (*Kit*^{+/+}) mice, WT BMCMC → *Kit*^{W/W-v} mice or *Fcer1g*^{-/-} BMCMC → *Kit*^{W/W-v} mice versus the corresponding mast cell-deficient *Kit*^{W/W-v} mice or *I110*^{-/-} BMCMC → *Kit*^{W/W-v} mice. Data are from three experiments with similar results (three to six mice per group per experiment).

immunosuppression in each of these studies, acting either in the lymph nodes draining the mosquito-bitten skin⁴⁴ or at the site of tolerant skin grafts⁴⁵. The critical cellular sources of IL-10, however, were not identified in either of those studies^{44,45}. In addition to investigating whether mast cells represent an important source of IL-10 in these settings, it will be useful to assess whether mast cells and mast cell-derived IL-10 have effects on regulatory T cell numbers, phenotype and/or function in models of CHS or chronic UVB irradiation.

Our findings have shown that mast cell-derived IL-10 can be essential to the ability of the host to limit many features of the pathology associated with some acquired immune or innate responses to environmental stimuli, but they indicate that, particularly in chronic exposure to UVB, other mast cell products also may have substantial anti-inflammatory functions *in vivo*. Although we used mice in our studies here, CHS in response to urushiol¹⁴ and other haptens⁴⁶, and chronic skin damage due to UVB irradiation^{10,40,47} represent substantial and common medical problems. Thus, it will be useful to assess whether the immunosuppressive or anti-inflammatory functions of mast cells and mast cell-derived IL-10, in these or other conditions, will provide opportunities for the prevention or treatment of such disorders.

METHODS

Mice. Female B6 (CD45.2⁺) mice, *Il10*-targeted IL-10-deficient B6 *Il10*^{tm1Cgn} (B6-*Il10*^{-/-}) mice, FcγRIIIα-deficient B6 *Fcgr3*^{tm1Sjv} mice and c-Kit-mutant genetically mast cell-deficient (WB/ReJ-*Kit*^{W/J} × C57BL/6J-*Kit*^{W-v/J})F₁ *Kit*^{W/W-v} (WBB6F₁-*Kit*^{W/W-v}) mice and their congenic normal wild-type (WBB6F₁-*Kit*^{+/+}) littermates, were from Jackson Laboratories. Genetically mast cell-deficient B6-*Kit*^{W-sh/+} mice were used as breeding pairs to produce mast cell-deficient *Kit*^{W-sh/W-sh} mice and their normal congenic wild-type littermates. Both adult *Kit*^{W/W-v} mice and adult *Kit*^{W-sh/W-sh} mice have a profound deficiency of mast cells, with less than 1.0% of the wild-type number of mast cells in the dermis^{26,48–50}. Adult *Kit*^{W/W-v} and *Kit*^{W-sh/W-sh} mice also differ in other aspects of phenotype (for example, *Kit*^{W/W-v} mice^{41,51}, but not *Kit*^{W-sh/W-sh} mice²⁶, are anemic and have been reported to have fewer granulocytes in the bone marrow). All experiments used age-matched female mice of at least 8 weeks of age. FcγR-deficient mice on the B6 background (B6.129P2 *Fcgr1g*^{tm1Rav} mice, backcrossed 12 generations) were from Taconic. B6-*Rag2*^{-/-} mice (CD45.1⁺) and B6 mice (CD45.1⁺), in addition to BALB/c *Fcrla*^{-/-} mice (backcrossed more than ten generations)⁵², were bred at the Stanford University Medical Center animal care facility. Experiments were in compliance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised, 1996) and with the approval of the Stanford University Committee on Animal Welfare.

Adoptive transfer of BMCMCs into mast cell-deficient mice. As described²⁶, BMCMCs were obtained by culture of bone marrow cells from femurs of female mice for 4–6 weeks in 20% (vol/vol) medium conditioned by the growth of the WEHI-3 mouse myelomonocytic cell line (containing IL-3); at this time over 95% of the cells were identified as mast cells by May-Grunwald-Giemsa staining and by flow cytometry. For mast cell-engraftment studies, BMCMCs derived from B6 *Kit*^{+/+} mice (WT BMCMCs), B6 *Il10*^{-/-} mice (*Il10*^{-/-} BMCMCs), B6 *Fcrlg*^{-/-} mice (*Fcrlg*^{-/-} BMCMCs) or B6 *Fcgr3*^{-/-} mice (*Fcgr3*^{-/-} BMCMCs) were transferred by intradermal injection (two injections into each ear; 1 × 10⁶ cells in 50 μl DMEM per injection) into female *Kit*^{W-sh/W-sh} or *Kit*^{W/W-v} mice 4–6 weeks of age. Experiments (CHS or UVB exposure) were initiated 6–8 weeks after intradermal transfer of BMCMCs.

Urushiol- or DNFB-induced CHS. Mice were sensitized on the shaved abdomen with 30 μl of 20 mg/ml of urushiol or 0.5% (vol/vol) DNFB (Sigma) in a vehicle of 100% acetone. At 5 d after sensitization, mice were challenged with 20 μl of vehicle (100% acetone) alone to the left ear (10 μl to each side) and 5 mg/ml of urushiol or 0.2% (vol/vol) DNFB (in 100% acetone) to the right ear

(10 μl to each side). Ear thickness was measured before and at several intervals after hapten challenge with a micrometer (Ozaki MFG).

Ultraviolet irradiation. A bank of six Philips FS40T12 lamps (Ultraviolet Resources International), emitting a broad 270- to 380-nm band of ultraviolet light with peak emission at 310 nm comprising about 65% of the energy emitted, was used to irradiate mice in individual compartments of Perspex cages. The intensity and spectral output of the UVB lamps were measured with an International Light IL1400 spectrophotometer. A new sheet of clear PVC plastic film (0.23 mm thick) was taped to the top of each Perspex cage before irradiation to screen wavelengths below 290 nm. Lamps were placed 15 cm above the cages. Mice were conscious and had full range of movement during irradiation. For chronic exposure, both ears of were irradiated with a dose of 2 kJ/m² of UVB every 2 d for 15 exposures (total cumulative dose, 30 kJ/m² of UVB). Ear thickness was measured with a micrometer before each dose of UVB irradiation. Mice were killed by CO₂ inhalation at 24 h after the final UVB exposure, and samples of ear tissue were obtained for histology, flow cytometry and IL-10 measurement.

Determination of minimal erythema dose in mast cell-deficient WBB6F₁-*Kit*^{W/W-v} mice. The inflammatory response induced by UVB irradiation in mast cell-deficient WBB6F₁-*Kit*^{W/W-v} mice was assessed by evaluation of erythema at 24 h after a single exposure of 1, 2, 3 or 4 kJ/m² of UVB. Three mice were assessed per dose. As described⁵³, erythema of ears and shaved back skin (an area of about 8 cm²) was evaluated by ‘visual scoring’ and was assigned grades according to the following pre-established standard scale: 0, no erythema; 0.5, slight redness without defined margins; 1, distinct homogeneous redness with defined margins; 2, frank redness; 3, intense redness; and 4, very intense redness. A value of 1 corresponds to one minimal erythema dose; that is, the dose of ultraviolet irradiation that produces a pale pink color with distinctly defined borders at 24 h after irradiation. In albino mast cell-deficient WBB6F₁-*Kit*^{W/W-v} mice, a single exposure of 2 kJ/m² of UVB to ears or shaved back skin was determined to be one minimal erythema dose.

Histology and quantification of mast cells and granulocytes and epidermal thickness. Mice were killed by CO₂ inhalation and samples of ear pinna were fixed in 10% (vol/vol) buffered formalin and were embedded in paraffin (ensuring a cross-sectional orientation), and then sections 4 μm in thickness were cut. Ear sections were stained with Masson’s trichrome or 0.1% (vol/vol) toluidine blue, pH 1, for the detection of mast cells (cytoplasmic granules appear purple), or with hematoxylin and eosin for the detection of polymorphonuclear neutrophils. Sections were ‘coded’ so the evaluator was not aware of their identity. Ear pinna mast cells were counted in 8–12 consecutive fixed fields, each with dimensions of 760 × 1,020 μm, with a 10× microscope objective (final magnification, ×100), and mast cells per horizontal ear cartilage field length (mm) were counted by computer-generated image analysis (NIH Image J software, version 1.29x). The entire length of a strip of skin extending from the base to the tip of the ear pinna (about 8–13 mm) was quantified. After intradermal engraftment of BMCMCs, *Kit*^{W-sh/W-sh} or *Kit*^{W/W-v} mice had mast cells from the base to the tip of the ear pinnae in an anatomical distribution similar to that of native mast cell populations in the corresponding wild-type mice. Ear pinna polymorphonuclear neutrophils in the dermis between the epidermis and cartilage were counted in ten random fixed fields, each with dimensions of 75 × 100 μm, under oil immersion with a 100× microscope objective (final magnification, ×1,000), and polymorphonuclear neutrophil numbers were quantified per mm² of dermis. For quantification of epidermal thickness, images of 12–30 consecutive microscopic fields (each with dimensions of 380 × 510 μm) of toluidine blue-stained cross-sections of ear skin from each mouse (three to six mice per group), perpendicular to the epidermis, were obtained with a 20× microscope objective (final magnification, ×200). For each image, eight randomly selected measurements of the distance between the stratum corneum and the bottom of the basal layer were recorded; epidermal thickness is expressed as mean + s.e.m. per mm of ear cartilage.

BMCMC stimulation and cytokine measurement. After 6 weeks of culture in medium containing WEHI-3-conditioned medium, BMCMCs were cultured for 4 d at a density of 5 × 10⁶ cells per ml in DMEM (Sigma) containing 10% (vol/vol) FCS with recombinant mouse IL-3 (5 ng/ml; R&D systems) plus

recombinant mouse IL-4 (20 ng/ml; PeproTech) as described³⁶. BMCMCs were centrifuged for 5 min at 4 °C at 250g, were resuspended at a density of 5×10^5 cells per ml in DMEM containing 1% (vol/vol) BSA (Sigma), were incubated for 10 min at 4 °C with 10 µg/ml of mAb to CD16 and CD32 (affinity-purified rat antibody to mouse CD16 and CD32; clone 93; eBioscience), and then were incubated for 24 h at 37 °C with 2.5 µg/ml of goat F(ab')₂ antibody to rat IgG (112-006-072; Jackson ImmunoResearch) to induce aggregation of FcγRIII on the mast cell surface. IL-10 and TNF in the supernatants were measured by enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions (IL-10 mouse kit from PeproTech and TNF OptEIA set mouse TNF (mono/mono) kit from BD Biosciences).

Statistical analysis. Analysis of variance for repeated measures was used to assess differences in ear swelling for groups of mice in the CHS assay or chronic UVB regime. All other data were tested for statistical significance with the unpaired, two-tailed, Student's *t*-test or with the χ^2 test for comparisons between groups of the occurrence of focal epidermal ulceration or percent cell populations obtained by flow cytometry. *P* values of less than 0.05 were considered statistically significant. Unless otherwise specified, data are presented as mean $\pm$ s.e.m. or mean + s.e.m.

Passive transfer of CHS responses to DNFB, measurement of IL-10 in ear skin lysates and of DNP-specific and total IgE and IgG1 serum concentrations, and flow cytometry of leukocytes in ear skin. These procedures are described in the **Supplementary Methods**.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

M.A.G. did all experiments and together with S.J.G. conceived and designed the study, interpreted the findings and wrote the manuscript; S.N. provided expertise for flow cytometry; J.K. helped with some experiments; and S.N., J.K. and M.T. assisted with interpretation of the findings and the writing of the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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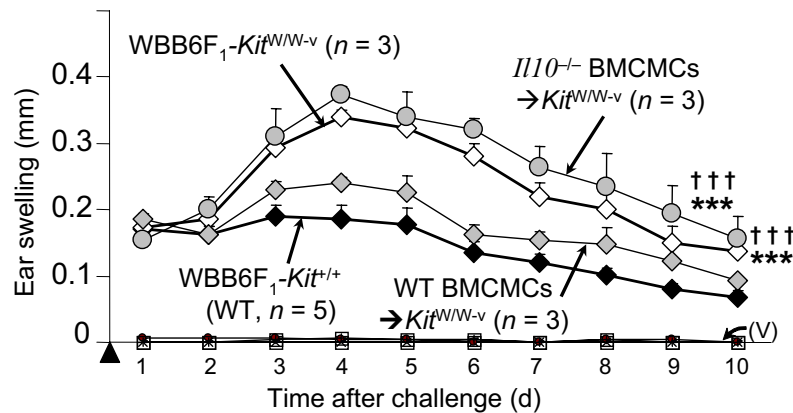
SUPPLEMENTARY ONLINE MATERIAL:

Supplementary Figures 1-14 & legends

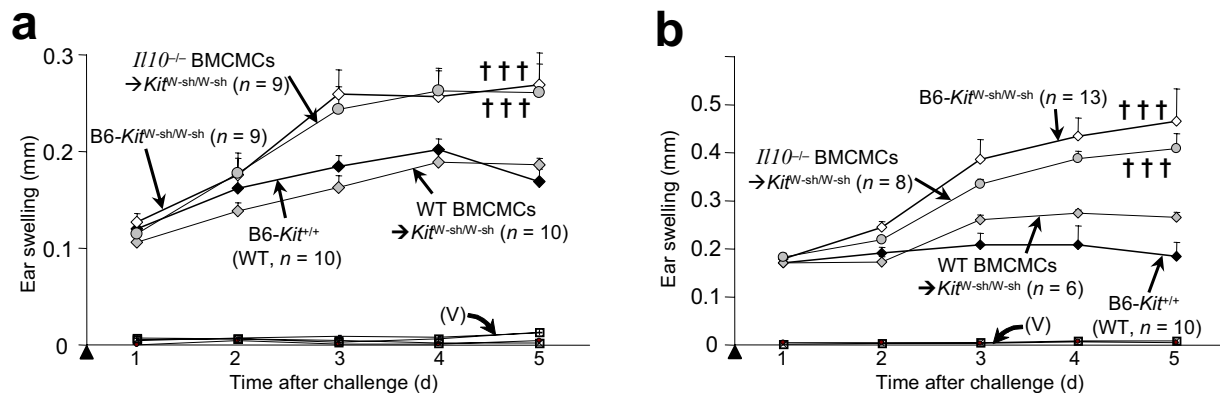
Supplementary Table 1

Supplementary Methods

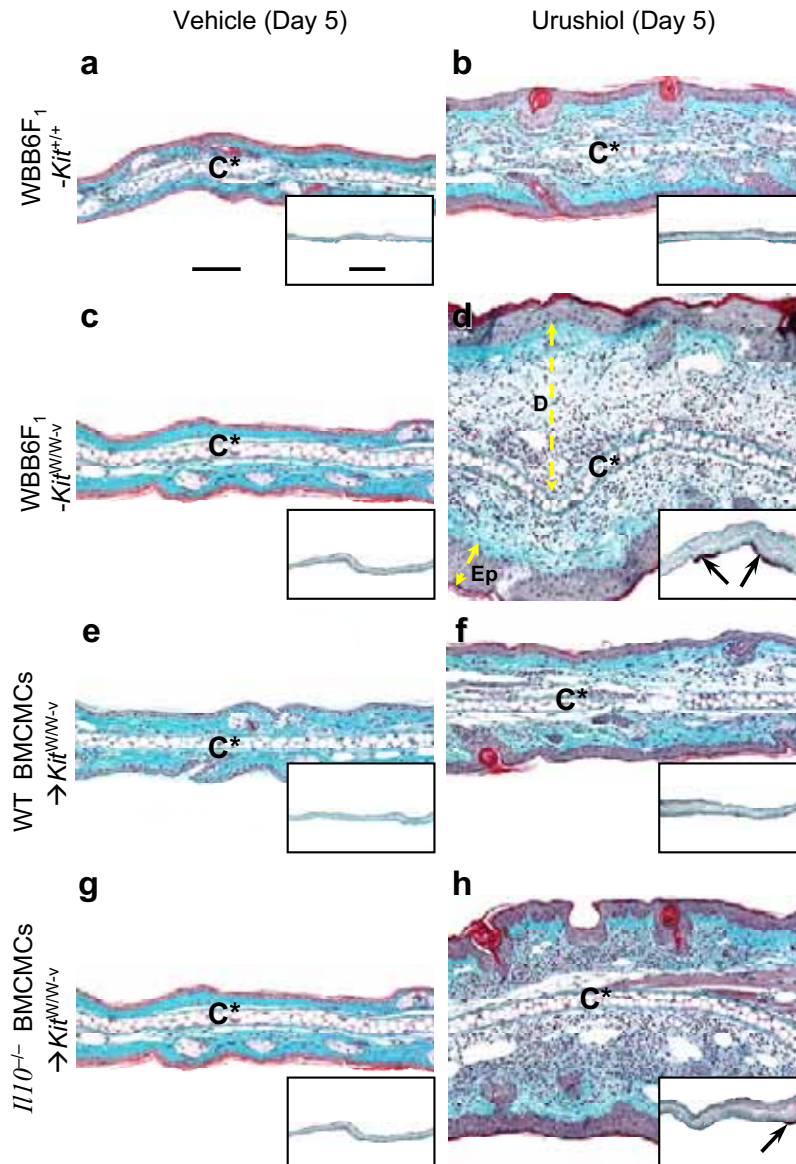
Supplementary Data



Supplementary Figure 1. Mast cell-derived IL-10 contributes significantly to the mast cell's ability to limit, and hasten the resolution of, the ear swelling associated with CHS responses to DNFB. CHS responses to DNFB and responses to vehicle (V) were examined for 10 d after DNFB challenge in mast cell-deficient *Kit*^{W/W-v} mice, the corresponding wild-type (*Kit*^{+/+}) mice, and in WBB6F₁-*Kit*^{W/W-v} mice that had been engrafted with wild-type (WT) BMCMCs (WT BMCMC → *Kit*^{W/W-v} mice) or *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC → *Kit*^{W/W-v} mice). *n* = 3-5 mice/group; the results shown are from a subgroup of the mice whose reactions over the first 5 d after DNFB challenge are shown in **Fig. 1d**. ****P* < 0.0001 versus corresponding DNFB-challenged wild-type (*Kit*^{+/+}) mice; †††*P* < 0.0001 versus corresponding DNFB-challenged WT BMCMCs → *Kit*^{W/W-v} mice. Arrowhead: DNFB or vehicle (v) challenge 5d after sensitization.



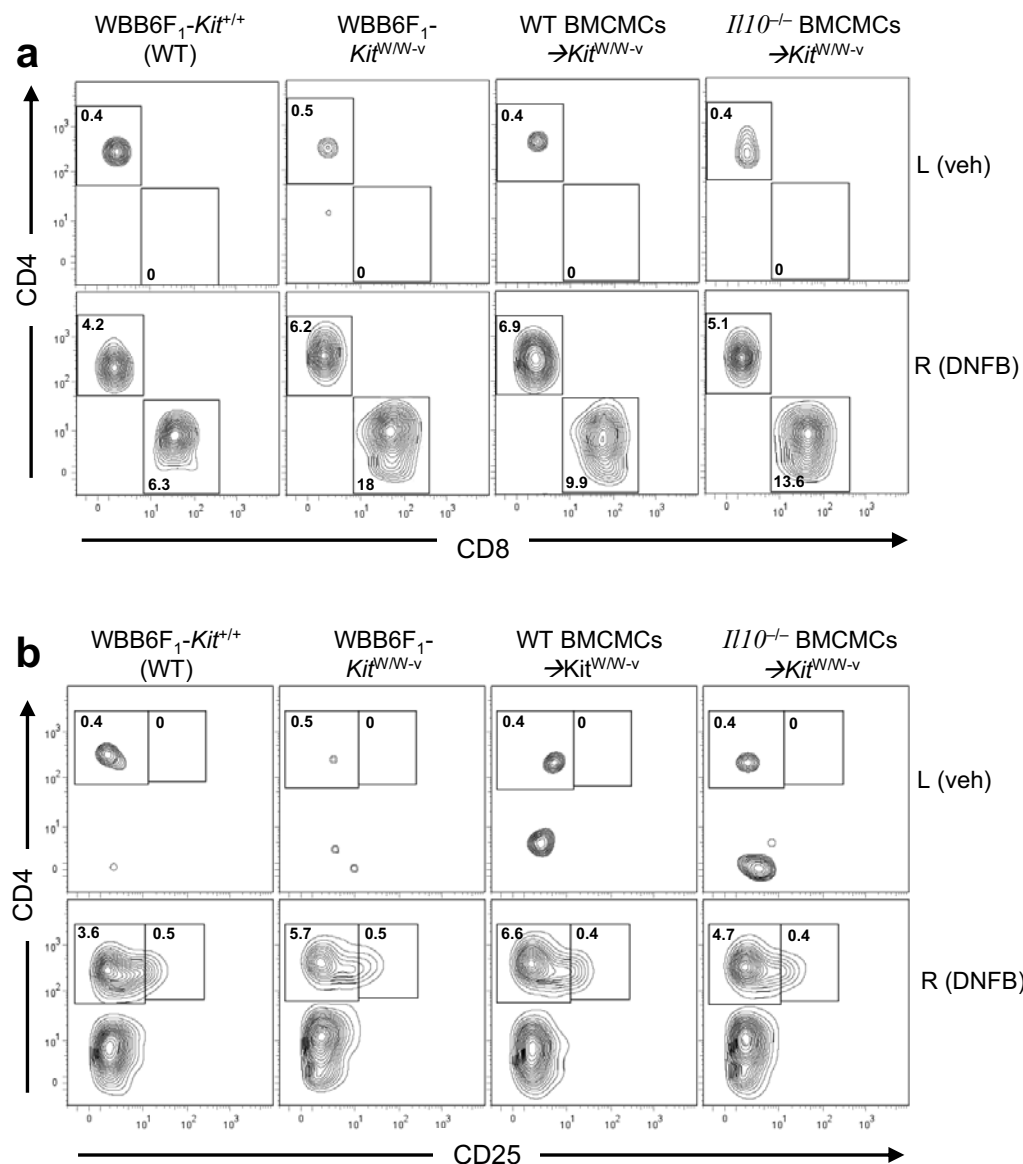
Supplementary Figure 2. Mast cell-derived IL-10 can contribute to the ability of mast cells to limit the tissue swelling associated with CHS responses to urushiol (a) or DNFB (b) in BMCMC-engrafted *Kit*^{W-sh/W-sh} mice. CHS responses and responses to vehicle (V) were examined in mast cell-deficient C57BL/6-*Kit*^{W-sh/W-sh} (B6-*Kit*^{W-sh/W-sh}) mice, the corresponding congenic wild-type (B6-*Kit*^{+/+}) mice, and in B6-*Kit*^{W-sh/W-sh} mice that had been engrafted with wild-type (WT) BMCMCs (WT BMCMC → *Kit*^{W-sh/W-sh} mice) or *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC → *Kit*^{W-sh/W-sh} mice); n = 3-7 mice/group per experiment. Results shown represent the findings in the 2 experiments we performed, each of which gave similar results. Numbers of mast cells in ears of BMCMCs → *Kit*^{W-sh/W-sh} mice at the conclusion of the CHS assays were similar to the numbers quantified in BMCMCs → *Kit*^{W/W-v} mice (see **Supplementary Data 1** online). †††P < 0.0001 versus the corresponding wild-type mice or WT BMCMCs → *Kit*^{W-sh/W-sh} mice. Arrowhead: urushiol, DNFB or vehicle (V) challenge 5 d after sensitization.



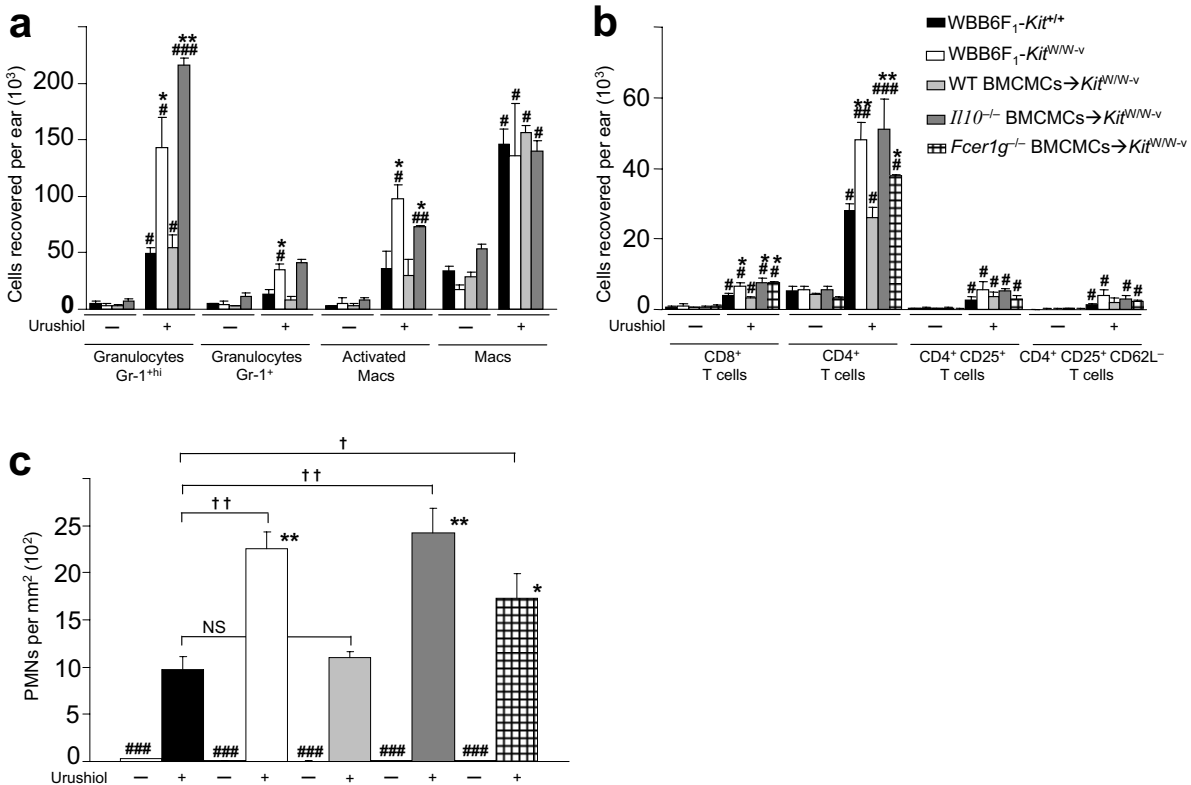
Supplementary Figure 3. Mast cells limit the pathology associated with CHS to urushiol.

Cross-sections of ears (stained with Masson's Trichrome) from WBB6F₁-*Kit*^{+/+} (wild-type) mice (**a,b**), *Kit*^{W/W-v} mice (**c,d**), or *Kit*^{W/W-v} mice engrafted 8 weeks before the experiment with WT BMCMCs (WT BMCMC→*Kit*^{W/W-v} mice) (**e,f**) or *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice) (**g,h**) were obtained 5 d after challenge with vehicle (100% acetone) only (**a,c,e,g**) or 5 mg/ml of urushiol (**b,d,f,h**). Focal full thickness necrosis of the epidermis and/or ulceration occurred in association with CHS responses to urushiol at 5 d after challenge in 8/10 of the mast cell-deficient *Kit*^{W/W-v} mice and in 8/8 *Il10*^{-/-} BMCMCs→*Kit*^{W/W-v} mice but in none of the 10 wild-type mice or 7 WT BMCMCs→*Kit*^{W/W-v} mice; **P* < 0.05 by Chi-square test for all comparisons between rates of epidermal necrosis and ulceration in wild-type (*Kit*^{+/+}) mice or WT BMCMC-engrafted *Kit*^{W/W-v} mice and the

corresponding mast cell-deficient *Kit*^{W/W^{-v}} mice or *Il10*^{-/-} BMCMC-engrafted *Kit*^{W/W^{-v}} mice. Similar findings were observed in association with CHS responses to urushiol in 5 of 7 *Kit*^{W-sh/W-sh} mice and in 16 of 17 *Kit*^{W-sh/W-sh} mice in response to DNFB. By contrast, epidermal necrosis and ulceration occurred in none of the corresponding congenic wild-type mice or WT BMCMC-engrafted *Kit*^{W-sh/W-sh} mice (10 or 8 for urushiol and 19 or 16 for DNFB, respectively); **P* < 0.05 by Chi-square test for all comparisons between rates of epidermal ulceration in wild-type or WT BMCMC-engrafted *Kit*^{W-sh/W-sh} mice and the corresponding mast cell-deficient *Kit*^{W-sh/W-sh} mice. C*: cartilage; double-headed arrows show thickness of dermis (D) or epidermis (Ep); arrow in insets: ulcers with adherent exudate (red). Scale bar in **a** = 100 μm & in inset in **a** = 1000 μm. Photomicrographs are representative of the findings observed in each of the 3 experiments we performed (*n* = 3-7 mice/group per experiment).



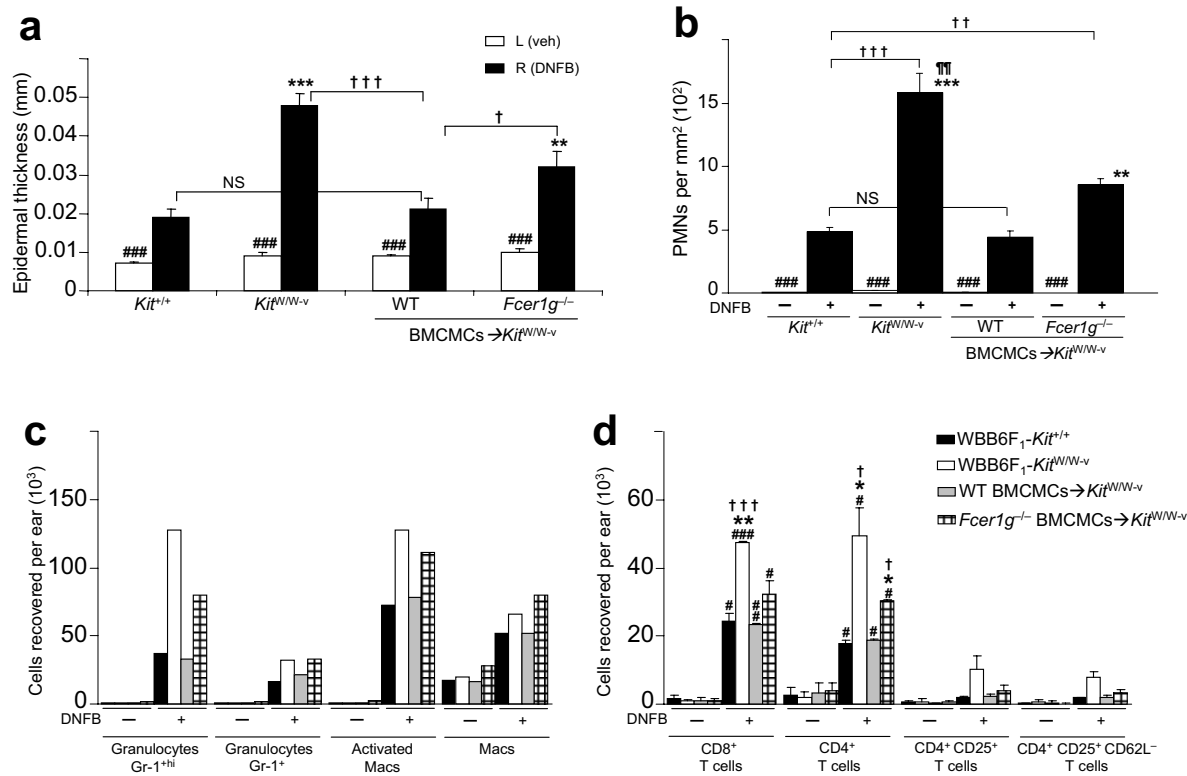
Supplementary Figure 4. Representative FACS plots of ear skin-derived T cells in CHS responses 5 d after challenge with DNFB. Assessment of CD4⁺ and CD8⁺ T cell populations (**a**) and CD4⁺ CD25⁺ T cells (**b**) in DNFB- or vehicle- (veh) challenged ear skin of WBB6F₁-*Kit*^{+/+} (wild-type) mice, mast cell-deficient *Kit*^{W/W-v} mice, and WBB6F₁-*Kit*^{W/W-v} mice that had been engrafted with wild-type (WT) BMCMCs (WT BMCMC $\rightarrow$ *Kit*^{W/W-v} mice) or *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC $\rightarrow$ *Kit*^{W/W-v} mice). For T cell populations, CD3⁺ T cells were first gated and then CD8⁺ and/or CD4⁺ T cells selected. FACS plots are representative of those observed in 4 different experiments in which cells were pooled from the ears of 4-5 mice/group in each experiment. The number associated with each selected cell population is the percentage that population represents of all the viable cells recovered.



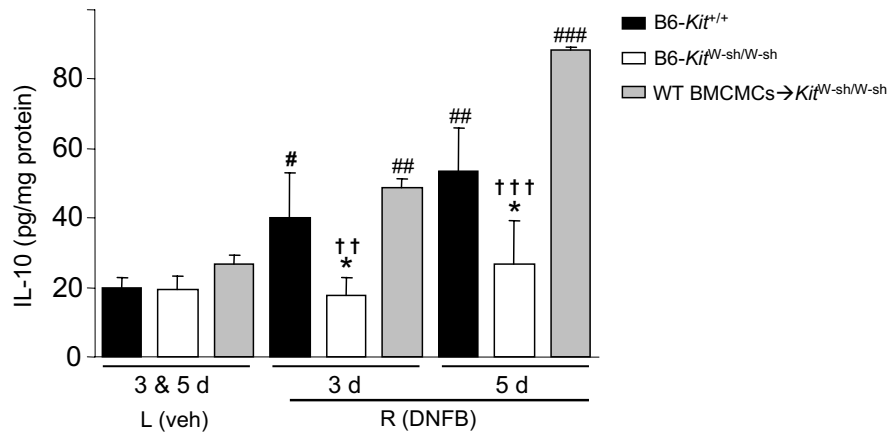
Supplementary Figure 5. Mast cell-derived IL-10 and mast cell FcR γ can contribute to the ability of mast cells to limit the numbers of leukocytes at sites of CHS to urushiol.

Assessment of granulocytes, activated macrophages (Activated Macs: F4/80^{hi} Gr-1⁺) (**a**), and T cells (**b**) in ear skin of wild-type mice, WBB6F₁-Kit^{W/W-v} mice or Kit^{W/W-v} mice engrafted with wild type (WT) BMCMCs (WT BMCMC→Kit^{W/W-v} mice), *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC→Kit^{W/W-v} mice) or *Fcεr1g*^{-/-} BMCMCs (*Fcεr1g*^{-/-} BMCMC→Kit^{W/W-v} mice) 5 d after challenge with 5 mg/ml urushiol (+) or vehicle (-). Data obtained from FACS analyses of leukocyte populations. (**a**) Granulocyte and macrophage data are from one experiment, in which we analyzed these cells in individual ears ($n = 2$ mice/group). (**b**) T cell data are from the 3 experiments which we performed, each of which gave similar results (in which cells were pooled from 4-5 mice/group in each experiment). The total number of viable cells (including leukocytes and other cell types) recovered per ear from the L (vehicle-treated) or R (urushiol-treated) ears were for (**a**), in which cells derived from individual ears of wild-type mice, WBB6F₁-Kit^{W/W-v} mice or Kit^{W/W-v} mice engrafted with WT or *Il10*^{-/-} BMCMCs were analyzed ($n = 2$ ears analyzed in each group of mice): 1.8 ± 0.3 , 1.6 ± 0.6 , 1.2 ± 0.1 or $2.5 \pm 0.7 \times 10^5$ cells, respectively, for the L ears, and 4.6 ± 0.7 , 8.8 ± 0.2 , 1.8 ± 0.4 or $6.2 \pm 0.3 \times 10^5$ cells, respectively, for the R ears, and for (**b**), in which the cells analyzed were pooled from 4-5 ears per group of wild-type mice, WBB6F₁-Kit^{W/W-v} mice or Kit^{W/W-v} mice engrafted with WT, *Il10*^{-/-} or *Fcεr1g*^{-/-} BMCMCs ($n =$ total number of ears analyzed in each group of mice): 1.4 ± 0.5 ($n = 15$), 1.3 ± 0.3 ($n = 15$), 1.3 ± 0.3 ($n = 13$), 1.2 ± 0.4 ($n = 12$) or 1.0 ± 0.1 ($n = 10$) $\times 10^5$ cells, respectively, for the L ears, and 4.4 ± 1.7 ($n = 15$), 6.6 ± 1.0 ($n = 15$), 3.2 ± 0.9 ($n = 13$), 5.9 ± 1.8 ($n = 12$) or $5.0 \pm 0.2 \times 10^5$ ($n = 10$) cells, respectively, for the R ears. Numbers of granulocytes (PMNs) per mm² detected in H&E-

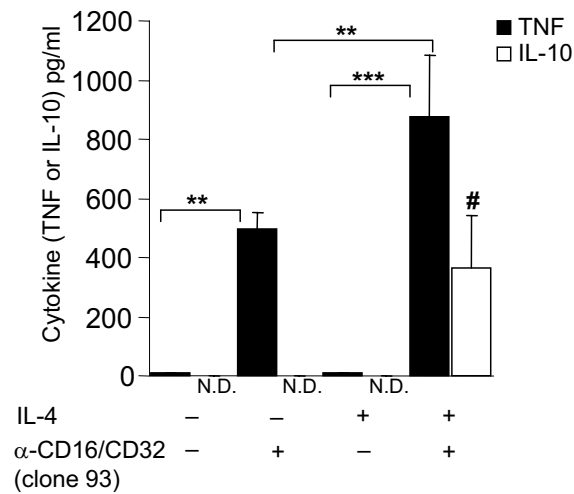
stained ear pinnae (**c**). For each group, we quantified 4-6 ears from the 2-3 experiments analyzed, each of which gave similar results. (**a,b**) $*P < 0.01$ or $**P < 0.001$ *versus* the corresponding wild-type mice or WT BMCMC $\rightarrow$ *Kit*^{W/W-v} mice; (**c**) $\dagger P < 0.01$ or $\dagger\dagger P < 0.001$ *versus* the corresponding wild-type mice; $*P < 0.01$ or $**P < 0.001$ *versus* the corresponding WT BMCMC $\rightarrow$ *Kit*^{W/W-v} mice; (**a-c**) $\#P < 0.05$, $\#\#P < 0.01$ or $\#\#\#P < 0.001$ for L (vehicle-treated) *versus* R (hapten-treated) ears in the same group of mice; N.S., not significant ($P > 0.05$).



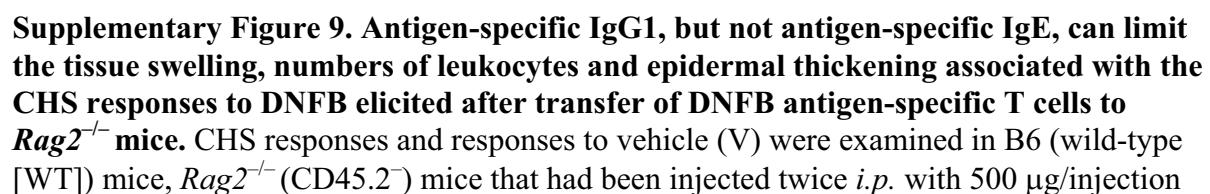
Supplementary Figure 6. Mast cell-FcR γ can contribute to the ability of mast cells to limit the epidermal thickening and the numbers of leukocytes at sites of CHS to DNFB. At 5 d after challenge, mast cell-deficient *Kit*^{W/W-v} mice, the corresponding congenic wild-type (*Kit*^{+/+}) mice or *Kit*^{W/W-v} mice engrafted with wild type (WT) BMCMCs (WT BMCMC→*Kit*^{W/W-v} mice) or *Fcer1g*^{-/-} BMCMCs (*Fcer1g*^{-/-} BMCMC→*Kit*^{W/W-v} mice) were assessed for epidermal thickness (*n* = 3 mice/group from one experiment) (a), numbers of granulocytes (PMNs) per mm² detected in H&E-stained ear pinnae (for each group we quantified PMNs in 6 ears from the 2 experiments analyzed, each of which gave similar results) (b), granulocytes, activated macrophages (Activated Macs: F4/80^{hi} Gr-1⁺) (c), and T cell populations (d). (c,d) Summarize the results of FACS analysis where the total number of recovered cells per ear (including leukocytes and other cell types) in the L (vehicle [-]) or R (DNFB-challenged [+]) ears of WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, and WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT or *Fcer1g*^{-/-} BMCMCs (*n* = total number of ears analyzed in each group of mice) were for (c), in which the cells derived from individual ears in a single experiment were pooled before FACS analysis: 1.0 (*n* = 5), 1.1 (*n* = 4), 1.0 (*n* = 4) or 1.7 (*n* = 4) × 10⁵ cells, respectively, for the L ears, and 1.6 (*n* = 5), 6.0 (*n* = 4), 3.1 (*n* = 4) or 4.2 (*n* = 4) × 10⁵ cells, respectively, for the R ears, and for (d), in which the cells derived from individual ears in the two experiments performed (using 4-5 mice/group), which gave similar results, each were pooled separately for FACS analysis: 1.0 ± 0.11 (*n* = 10), 1.1 ± 0.1 (*n* = 9), 0.9 ± 0.01 (*n* = 9) or 1.4 ± 0.4 (*n* = 9) × 10⁵ cells, respectively, for the L ears, and 1.4 ± 0.2 (*n* = 10), 5.0 ± 1.4 (*n* = 9), 2.3 ± 1.1 (*n* = 9) or 3.8 ± 0.6 (*n* = 9) × 10⁵ cells, respectively, for the R ears. **P* < 0.01, ***P* < 0.001, or ****P* < 0.0001 versus corresponding wild-type mice; †*P* < 0.01, ††*P* < 0.001, or †††*P* < 0.0001 versus group indicated by the bracket (a,b) or versus corresponding WT BMCMCs→*Kit*^{W/W-v} mice (d); ¶¶*P* < 0.001 versus corresponding *Fcer1g*^{-/-} BMCMCs→*Kit*^{W/W-v} mice; for R (hapten-treated) versus L (vehicle-treated) ears in the same group of mice: #*P* < 0.01, ###*P* < 0.001, or ####*P* < 0.0001; N.S., not significant (*P* > 0.05).



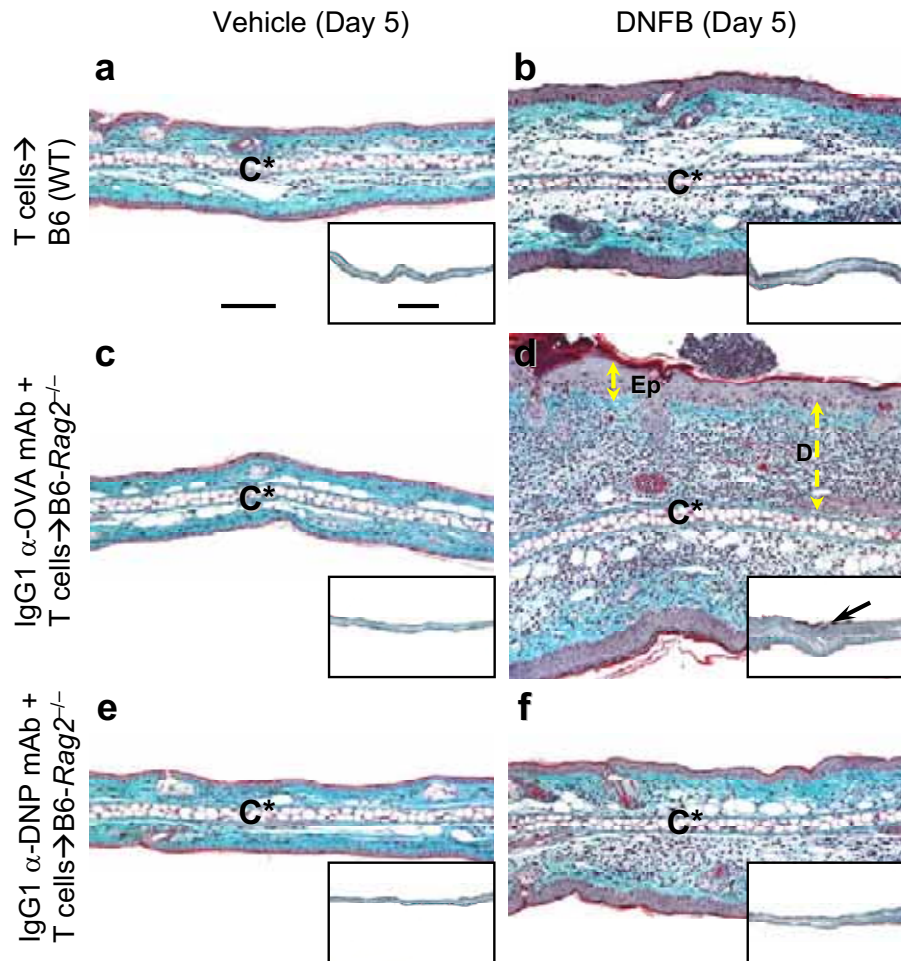
Supplementary Figure 7. Mast cells contribute to higher amounts of IL-10 in skin at sites of CHS to DNFB. Concentrations of IL-10 (pg/mg protein) in ears at 3 or 5 d after challenge with 0.2% DNFB or vehicle (veh) in mast cell-deficient B6-Kit^{W-sh/W-sh} mice, the corresponding congenic wild-type (B6-Kit^{+/+}) mice or B6-Kit^{W-sh/W-sh} mice engrafted with wild type (WT) BMCMCs (WT BMCMCs→Kit^{W-sh/W-sh} mice); $n = 3$ mice/group, from 1 experiment. * $P < 0.01$ versus the corresponding wild-type mice; †† $P < 0.001$ or ††† $P < 0.0001$ versus WT BMCMC→Kit^{W-sh/W-sh} mice; # $P < 0.01$, ## $P < 0.001$ or ### $P < 0.0001$ for L (vehicle-treated) versus R (hapten-treated) ears in the same group of mice.



Supplementary Figure 8. IL-4 can enhance FcRγ-mediated TNF and IL-10 production by BMCMCs *in vitro*. C57BL/6 (wild-type) BMCMCs were cultured (5×10^6 cells/ml) in IL-3 (5 ng/ml) $\pm$ IL-4 (20 ng/ml) for 4 d, incubated with 10 μ g/ml anti-CD16/CD32 (clone 93) mAb for 10 min at 4°C, and then incubated with 2.5 μ g/ml goat F(ab'2) α -rat IgG for 24 h at 37°C. TNF and IL-10 protein levels in the supernatants were measured by ELISA. The results shown represent findings from the 3 experiments we performed, in which each treatment group was done in triplicate. ** $P < 0.001$ or *** $P < 0.0001$ for the indicated TNF comparisons; # $P < 0.001$ versus any other IL-10 value; N.D. = IL-10 was not detected.

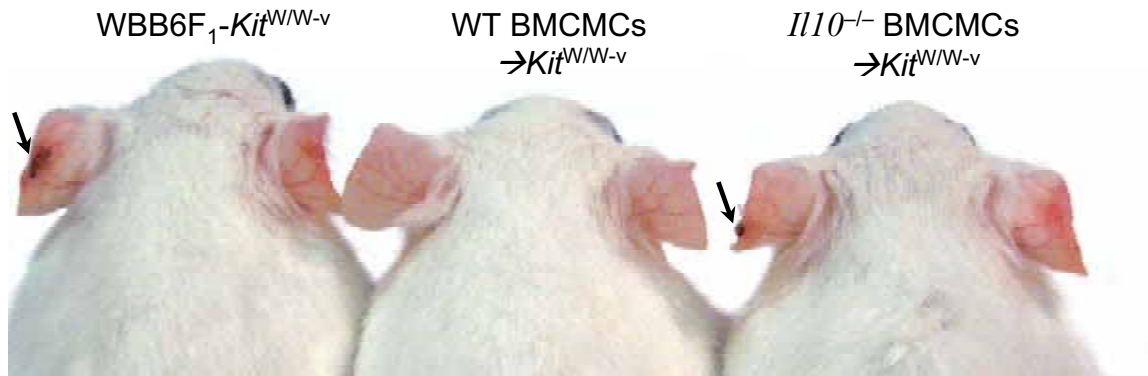


of IgG1 anti-OVA mAb (the control mAb), 280 ng/injection of IgE anti-DNP, or 500 µg/injection of IgG1 anti-DNP (at the time of T cell transfer and again 3 d after DNFB challenge) (a). T cell transfer was administered *i.v.* retro-orbitally with 1×10^7 T cells (obtained from the skin draining lymph nodes [LNs] of donor CD45.2⁺ B6 mice 5 d after sensitization with 0.5% DNFB). CD3⁺ T cells were isolated from lymph nodes *via* Streptavidin Particles Plus-DM and a BD IMagnet for cell separation to a purity of >97% CD3⁺ T cells; $n = 3-5$ mice/group per experiment. Results shown represent the findings in the 4 experiments we performed, each of which gave similar results, except for *Rag2*^{-/-} mice + IgE anti-DNP mAb, in which only one experiment was performed. † $P < 0.0001$ versus the corresponding wild-type mice or *Rag2*^{-/-} mice + IgG1 anti-DNP mAb. Arrowhead: DNFB or vehicle challenge. Arrows: IgG1 anti-DNP mAb or IgG1 anti-OVA mAb administration. Numbers of mast cells per mm of cartilage in ear pinnae (L ears; vehicle and R ears; DNFB) at 5 d after challenge (b). Mean ± s.d. in L ears: 23 ± 2 , 31 ± 2 , 29 ± 2 or 29 ± 4 , and in R ears: 24 ± 1 , 25 ± 0.2 , 29 ± 3 or 32 ± 2 for B6 mice or *Rag2*^{-/-} mice that had received IgG1 anti-OVA mAb, IgG1 anti-DNP mAb or IgE anti-DNP mAb, respectively; $n = 3-4$ mice/group, from 1 experiment. N.S., not significant ($P > 0.05$). Epidermal thickness at 5 d after challenge ($n = 10-13$ mice/group, where 3-4 mice were assessed from the 3 experiments we analyzed, each of which gave similar results) (c). Numbers of PMNs per mm² in H&E-stained ear pinnae at sites of CHS [+ DNFB] or control vehicle-treated [- DNFB] sites (for each group, we quantified 3-9 ears from 3 experiments, each of which gave similar results) (d). (e,f) Summarize data obtained from FACS analysis of infiltrating host-derived Gr-1⁺ granulocytes and activated macrophages (Activated Macs: F4/80^{hi} Gr-1^{hi}) (e), and infiltrating CD45.2⁺ donor-derived CD8⁺ and CD4⁺ T cells (f), in ear skin 5 d after challenge with 0.2% DNFB [+] or vehicle [-]. (e) Granulocyte and macrophage data are from one experiment, in which we analyzed these cells in individual ears ($n = 3$ mice/group). (f) T cell data are from 3 experiments (in which cells were pooled from 3-5 mice/group in each experiment), each of which gave similar results, except for the 3 mice treated with IgE anti-DNP mAb, which were analyzed in a single experiment). The total number of viable cells per ear (including leukocytes and other cell types) recovered from the L (vehicle-treated) or R (DNFB-treated) ears of B6 CD45.2⁻ (wild-type) mice, or of *Rag2*^{-/-} mice that had received IgG1 anti-OVA mAb, IgG1 anti-DNP mAb or IgE anti-DNP mAb ($n =$ total number of ears analyzed in each group of mice) were, for (e) ($n = 3$ ears per group): 1.1 ± 0.2 , 1.2 ± 0.1 , 1.2 ± 0.1 or $1.2 \pm 0.2 \times 10^5$ cells, respectively, for the L ears, and 5.7 ± 2.4 , 12.4 ± 1.9 , 5.0 ± 1.2 or $11.2 \pm 2.0 \times 10^5$ cells, respectively, for the R ears, and for (f): 1.9 ± 0.5 ($n = 14$), 1.7 ± 0.7 ($n = 12$), 1.6 ± 0.5 ($n = 13$) or 1.2 ± 0.2 ($n = 3$) $\times 10^5$ cells, respectively, for the L ears, and 3.9 ± 1.8 ($n = 14$), 8.5 ± 1.6 ($n = 12$), 4.4 ± 0.8 ($n = 13$) or 11.2 ± 2.0 ($n = 3$) $\times 10^5$ cells, respectively, for the R ears. (a) † $P < 0.0001$ versus B6 mice or *Rag2*^{-/-} mice that had received IgG1 anti-DNP; (b-f) * $P < 0.01$, ** $P < 0.001$ or *** $P < 0.0001$ versus B6 mice; † $P < 0.01$, †† $P < 0.001$ or ††† $P < 0.0001$ versus corresponding *Rag2*^{-/-} mice that had received IgG1 anti-DNP (c,e,f) or versus group indicated by the bracket (d); # $P < 0.05$, ## $P < 0.01$, or ### $P < 0.001$ for L (vehicle-treated) versus R (haptens-treated) ears in the same group of mice. N.S., not significant ($P > 0.05$).

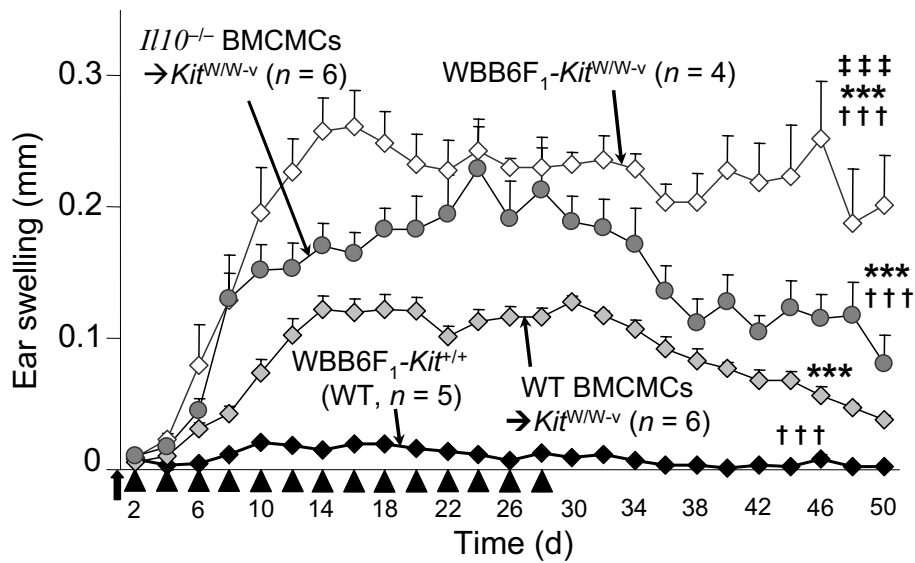


Supplementary Figure 10. Antigen-specific IgG1 can limit the pathology associated with CHS to DNFB after transfer of DNFB antigen-specific T cells into *Rag2*^{-/-} mice. Cross-sections of ears (stained with Masson's Trichrome) from B6 (WT) mice (**a,b**), *Rag2*^{-/-} (CD45.2⁻) mice that had been injected twice *i.p.* with 500 µg/injection of IgG1 anti-OVA mAb (α-OVA mAb, the control mAb) (**c,d**), or *Rag2*^{-/-} (CD45.2⁻) mice that had been injected twice *i.p.* with 500 µg/injection of IgG1 anti-DNP mAb (α-DNP mAb) (**e,f**), were obtained 5 d after transfer of DNFB Ag-specific T cells and challenge with vehicle (100% acetone) only (**a,c,e**) or 0.2% DNFB (**b,d,f**). C*: cartilage; double-headed arrows show thickness of dermis (D) or epidermis (Ep); arrows in insets: ulcers with adherent exudate (red). Scale bar in **a** = 100 µm and in inset in **a** = 1000 µm. Photomicrographs are representative of the findings observed in each of the 4 different experiments we performed (*n* = 3-5 mice/group per experiment).

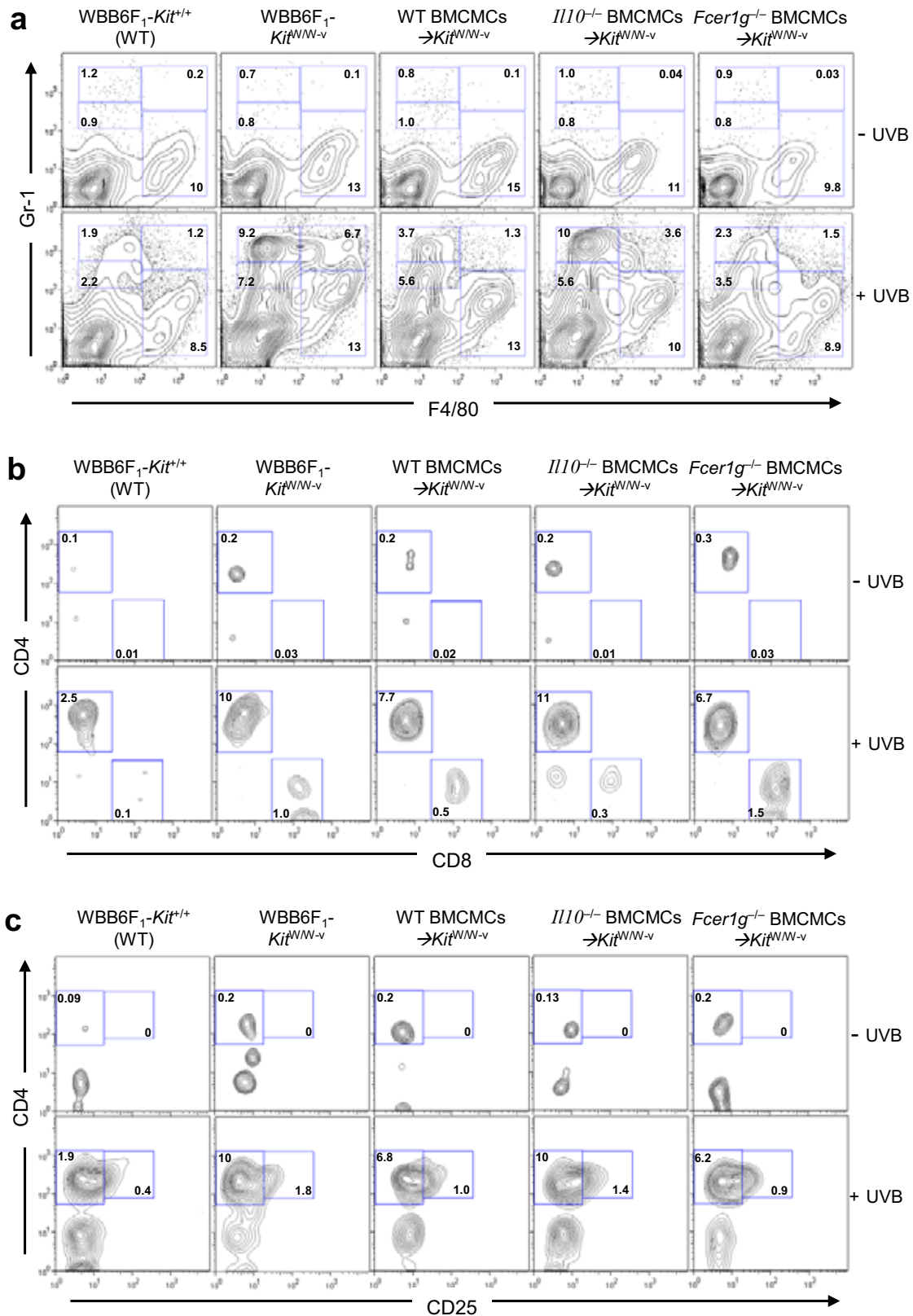
Mice exposed to chronic UVB irradiation of both ears



Supplementary Figure 11. Mast cells and mast cell-derived IL-10 can limit the skin erythema and ulceration induced by chronic low-dose UVB irradiation. Ears of mast cell-deficient WBB6F₁-*Kit*^{W/W-v} mice, *Kit*^{W/W-v} mice engrafted with wild type BMCMCs (WT BMCMCs → *Kit*^{W/W-v} mice) or *Kit*^{W/W-v} mice engrafted with *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMCs → *Kit*^{W/W-v} mice), after 15 exposures to 2 kJ/m² UVB. Arrows indicate focal ulceration or scabbing.

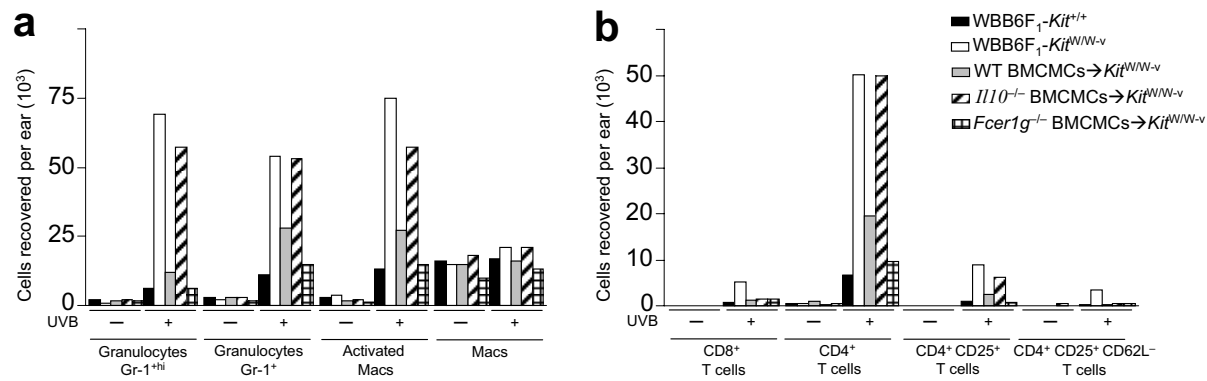


Supplementary Figure 12. Mast cells are required for optimal resolution of tissue swelling after chronic low-dose UVB irradiation. Ear swelling examined for up to 22 d after the last of 15 exposures of 2 kJ/m² UVB irradiation in WBB6F₁-Kit^{+/+} (wild-type [WT]) mice, mast cell-deficient Kit^{W/W-v} mice, or Kit^{W/W-v} mice engrafted with WT BMCMCs (WT BMCMCs → Kit^{W/W-v} mice) or Il10^{-/-} BMCMCs (Il10^{-/-} BMCMCs → Kit^{W/W-v} mice); n = 4-6 mice/group. The first exposure (arrow) was on day 0, immediately after we measured baseline ear thickness. Subsequent exposures are shown as arrowheads. For clarity, measurements in mice not treated with UVB radiation are not shown. The results shown are from a subgroup of the mice whose reactions over the time of UVB exposure are shown in **Fig. 6**. ***P < 0.0001 versus corresponding UVB-irradiated wild-type mice; †††P < 0.0001 versus corresponding UVB-irradiated WT BMCMCs → Kit^{W/W-v} mice; †††P < 0.0001 versus corresponding UVB-irradiated Il10^{-/-} BMCMCs → Kit^{W/W-v} mice.



Supplementary Figure 13. Representative FACS plots of ear skin-derived granulocytes, macrophages and T cells after chronic low-dose UVB irradiation. Assessment of

granulocytes, activated macrophages (Activated Macs: F4/80^{hi} Gr-1⁺) (**a**), CD4⁺ and CD8⁺ T cell populations (**b**), and CD4⁺ CD25⁺ T cells (**c**) in ear skin after the last of 15 exposures to 2 kJ/m² UVB irradiation (+ UVB) or at control sites not treated with UVB irradiation (– UVB) in WBB6F₁-*Kit*^{+/+} (wild-type [WT]) mice and mast cell-deficient *Kit*^{W/W-v} mice, and in WBB6F₁-*Kit*^{W/W-v} mice that had been engrafted with WT BMCMCs (WT BMCMC→*Kit*^{W/W-v} mice), *Il10*^{-/-} BMCMCs (*Il10*^{-/-} BMCMC→*Kit*^{W/W-v} mice) or *Fcer1g*^{-/-} BMCMCs (*Fcer1g*^{-/-} BMCMC→*Kit*^{W/W-v} mice). FACS plots are representative of those observed for cells derived from ears pooled from 4-5 mice/group in one experiment (**a**) or for cells in the 5 experiments analyzed, each of which gave similar results, in which cells were pooled from the ears of 4-5 mice/group in each experiment (**b,c**). For T cell populations, CD3⁺ T cells were first gated and then CD8⁺ and/or CD4⁺ T cells selected (**b,c**). The number associated with each selected cell population is the percentage that population represents of all the viable cells recovered (**a-c**).



Supplementary Figure 14. Mast cells and mast cell-derived IL-10, but not mast cell FcR γ , can limit the numbers of leukocytes at skin sites exposed to chronic low-dose UVB irradiation. FACS analysis of activated granulocytes (Gr-1^{hi}) and activated macrophages (Activated Macs: F4/80^{hi} Gr-1^{hi}) (a), as well as CD4⁺ and CD4⁺ CD25⁺ T cells (b), after the last of 15 exposures to 2 kJ/m² UVB irradiation in ear skin of WBB6F₁-Kit^{+/+} (wild-type) mice and mast cell-deficient Kit^{W/W-v} mice, and in WBB6F₁-Kit^{W/W-v} mice that had been engrafted with wild-type (WT) BMCMCs (WT BMCMC→Kit^{W/W-v} mice), Il10^{-/-} BMCMCs (Il10^{-/-} BMCMC→Kit^{W/W-v} mice) or Fcer1g^{-/-} BMCMCs (Fcer1g^{-/-} BMCMC→Kit^{W/W-v} mice). (a,b) Data were obtained from FACS analysis of leukocyte populations in ears pooled from 4-5 mice/group in one experiment (in which both ears of individual mice in the UVB irradiation [+] or control [-] groups received the same treatment), that gave tissue swelling results very similar to those in Fig. 5a. The total number of viable cells per ear (including leukocytes and other cell types) in the control (no UVB treatment) ears or UVB-irradiated ears of WBB6F₁-Kit^{+/+} mice, WBB6F₁-Kit^{W/W-v} mice, and WBB6F₁-Kit^{W/W-v} mice engrafted with WT, Il10^{-/-} or Fcer1g^{-/-} BMCMCs (n = total number of ears analyzed in each group of mice) were 1.9 (n = 8), 0.9 (n = 8), 0.5 (n = 8), 0.5 (n = 8) or 1.0 (n = 8) $\times 10^5$ cells, respectively, for the control (no UVB treatment) ears, and 1.8 (n = 10), 4.9 (n = 8), 2.6 (n = 8), 4.3 (n = 8) or 2.0 (n = 8) $\times 10^5$ cells, respectively, for the UVB-irradiated ears.

Supplementary Table 1. Concentrations of DNP-specific and total IgE and IgG1 in the serum 5 d after 0.2% DNFB challenge.

Mouse genotype	DNP-specific Ig		Total Ig	
	IgE (ng/ml)	IgG1 (μg/ml)	IgE (μg/ml)	IgG1 (μg/ml)
WBB6F ₁ - <i>Kit</i> ^{+/+}	61 ± 18	444 ± 56	5.5 ± 1.2	863 ± 119
WBB6F ₁ - <i>Kit</i> ^{W/W-v}	73 ± 16	317 ± 72	6.2 ± 0.7	885 ± 150
WT BMCMCs→ <i>Kit</i> ^{W/W-v}	52 ± 10	367 ± 55	5.7 ± 0.6	1161 ± 152
<i>Il10</i> ^{-/-} BMCMCs→ <i>Kit</i> ^{W/W-v}	79 ± 18	303 ± 65	6.4 ± 0.3	1014 ± 167
<i>Fcer1g</i> ^{-/-} BMCMCs→ <i>Kit</i> ^{W/W-v}	75 ± 16	347 ± 132	4.3 ± 0.5	1127 ± 192

Mean serum concentrations (mean ± s.e.m.) of DNP-specific and total IgE and IgG1 antibodies were measured by ELISA (*n* = 9-15 mice/group). The data represent findings from the 2-3 different experiments analyzed, each of which gave similar results. No statistically significant differences (by unpaired Student's *t* test, 2-tailed) were found for concentrations of DNP-specific or total IgE or IgG1 antibodies among the different groups of mice.

Supplementary Methods

Passive transfer of CHS responses to DNFB. C57BL/6-*Rag2*^{-/-} (B6-*Rag2*^{-/-}) mice lack mature T and B cells and consequently are unable to produce IgE or IgG1 antibodies. In our model of adoptive transfer of T cells into B6-*Rag2*^{-/-} mice, T cells for transfer were purified from skin draining lymph nodes (inguinal, axillary and submaxillary) of CD45.2⁺ donor C57BL/6 mice sensitized on the abdomen (30 μ l) and ears (10 μ l/ear) with 0.5% DNFB in 100% acetone (vehicle) 5 d before sacrifice.

Briefly, a single-cell suspension of lymph nodes was prepared, and red blood cells were lysed in RBC lysing buffer (Sigma). For CD3⁺ T cell purification, lymph node cells were incubated with biotinylated anti-mouse B220 (RA3-6B2), Gr-1 (RB6-8C5), CD11b (M1/70), CD11c (N418), CD49b (DX5), $\gamma\delta$ TCR (UC7-13D5), Ter119 (Ter119), and c-Kit/CD117 (2B8) antibodies for 20 min at 4°C. All Abs were obtained from eBioscience. The cells were then washed and incubated with 10 μ l Streptavidin Particles Plus-DM (BD Biosciences) per 1.0×10^7 cells for 20 min at 4°C, washed again, and placed in contact with a BD IMagnet for cell separation and negative selection of the >97% CD3⁺ T cell fraction. Purified CD3⁺ T cells (1.0×10^7 cells) were transferred *i.v.* into CD45.1⁺ B6-*Rag2*^{-/-} or CD45.1⁺ B6-WT recipient mice and 30 min later 0.2% DNFB in 100% acetone was applied to the right ear of each mouse.

B6-*Rag2*^{-/-} mice were injected *i.p.* twice (once at the time of T cell transfer and again 3 d after DNFB challenge) with 250 μ l of either: (1) 2 mg/ml IgG1 anti-DNP monoclonal antibody (mAb) (500 μ g/injection) (purified from the supernatant of mouse U7.6 hybridoma [ref. 36 of manuscript] and administered in PBS), (2) IgG1 anti-OVA mAb (500 μ g/injection), as a 1:14 dilution in PBS from 30 mg/ml of ascites of *Rag1*-deficient mice (The Jackson Laboratory) injected with the 3B2E6 hybridoma cell line, or (3) IgE anti-DNP mAb (280 ng/injection), 71 μ l injected *i.p.* of a 1:1000 dilution in PBS from 3.9 mg/ml stock concentration (purified from ascites induced by the hybridoma H1-DNP- ϵ 26 (ref. 35 of manuscript). Amounts of IgG1 anti-DNP mAb or IgE anti-DNP mAb injected into the B6-*Rag2*^{-/-} mice were based upon concentrations of antigen-specific IgG1 and IgE measured in the serum of mast cell-deficient *Kit*^{W/W-v} mice 5 d after 0.2% DNFB challenge (Supplementary Table 1 online).

Measurement of IL-10 protein in ear skin lysates. Ear skin lysates were prepared by sonication of finely chopped ear tissue in 250 μ l Tper EDTA-free lysis buffer (Pierce) containing protease inhibitors (Roche). Samples were frozen at -80 °C overnight then thawed and centrifuged at 16000 $\times$ g for 20 min at 4 °C and the supernatants were collected. IL-10 protein concentrations in the supernatants were measured by ELISA (eBioscience), according to the manufacturer's instructions (lower limit of detection = 30 pg/ml) and data obtained from each group were expressed as mean + s.e.m. pg/mg protein. Total protein concentrations in the supernatants were measured by a Bio-Rad D_c protein assay, according to the manufacturer's instructions (Bio-Rad Laboratories).

Measurement of DNP-specific and total IgE and IgG1 antibodies. Sera were obtained 5 d after 0.2% DNFB challenge in mice that had been sensitized with 0.5% DNFB. Total IgE and IgG1 titers were measured by ELISA. Briefly, ELISA plates were coated with 2 μ g/ml anti-mouse IgE antibody (Ab) (R35-72) or anti-mouse IgG1 Ab (A85-3) at 4 °C overnight. Sera

samples or standard Abs (IgE [IgE-3] or IgG1 [MOPC-31C]) were incubated in the coated capture Ab wells for 2 h at 25 °C, followed by incubation with 2 µg/ml biotinylated anti-mouse IgE (R35-118) or anti-mouse IgG1 (A85-1) Ab for 1 h at 25 °C, then incubation with streptavidin HRP for 1 h at 25 °C, and IgE or IgG1 titers were detected with 3,3',5,5'-tetramethylbenzidine (TMB) solution (Sigma). After adding 1N HCl stop solution, Ab titers were measured at 450 nm in a plate reader. All Abs were obtained from BD Pharmingen. For measurement of Ag-specific IgE or IgG1 titers, the aforementioned protocol was followed but the ELISA plates were coated with 10 µg/ml 2,4-dinitrophenol-human serum albumin (DNP-HSA; 30-40 DNP conjugated to each molecule of HSA, Sigma). The standard Abs used were IgE α -DNP mAb generated from ascites induced by the hybridoma H1-DNP- ϵ 26 (ref. 35 in the article), which produces IgE mAb to DNP, and the IgG1 α -DNP mAb generated from the hybridoma U7.6 (ref. 36 in the article), as described above.

Flow cytometric analysis of leukocytes in ear skin. For flow cytometry, left (L) vehicle- and right (R) hapten-treated ears, or UVB-irradiated or control (no UVB treatment) ears, were pooled from 3–5 mice per group per experiment for analysis of T cell populations. For assessment of granulocytes and activated macrophages (Gr-1⁺ F4/80⁺), individual ears of each mouse in each treatment group were analyzed in a given experiment. Ear skin was split parallel with the cartilage into two halves, cut into fine pieces and incubated in RPMI plus 0.5 mg/ml of Liberase CI (Roche) for 1 h at 37 °C. A suspension of single cells was obtained by using a 70 µm nylon cell strainer to separate undigested tissue. For FACS analysis of leukocyte populations, suspensions of single cells were incubated with anti-mouse CD16/CD32 mAb (eBioscience) on ice for 15 min. After FcR blocking, leukocytes were incubated on ice for 40 min with the following cell surface markers: anti-mouse CD3e (145-2C11, 2 µg/ml), CD4 (RM4-5, 2 µg/ml); CD8 β (CT-CD8 β , 1 µg/ml); CD25 (PC81, 5 µg/ml), CD62L (MEL-14, 2 µg/ml), F4/80 (BM8, 1 µg/ml), Gr-1 (Ly-6C RB6-8C5, 0.5 µg/ml), CD45.1 (A20, 2 µg/ml), CD45.2 (104, 2 µg/ml). All Abs were obtained from eBioscience with the exception of the Gr-1 mAb, that was obtained from BD Biosciences. The expression of cell surface markers was analyzed on a FACSCaliber (BD Biosciences) and using FlowJo Software (version 5.7.2). Gates for subpopulations of cells were based on isotype control antibody staining (Rat IgG2b, Rat IgG2a), as well as single color stain of the cells to determine compensation and non-specific fluorescence. 7-aminoactinomycin D (7-AAD) was used to detect dead cells. Only cells negative for 7-AAD were used for analysis. To calculate the number of live cells of a particular type recovered per ear (determined by gating on 7-aminoactinomycin D-negative cells), the following calculation was applied for each population quantified: live cells recovered per ear in a particular subset of leukocytes = (percentage gated of the total cell population in that group) x ([total number of cells recovered from the L or R ears (pooled) of 3 or 5 mice per group] ÷ [3 or 5], respectively).

Supplementary Data

1. Figure 1. Numbers of mast cells (mean $\pm$ s.d.) per mm of ear cartilage (R ears) at the end of the CHS assay: (a) 53 ± 8 , 0.4 ± 0.4 , 23 ± 4 or 0 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, B6-*Kit*^{+/+} mice or B6-*Kit*^{W-sh/W-sh} mice, respectively; (b) 70 ± 7 , 0.2 ± 0.2 , 47 ± 0.7 or 0.1 ± 0.04 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, B6-*Kit*^{+/+} mice, or B6-*Kit*^{W-sh/W-sh} mice, respectively; (c) 40 ± 8 , 0, 19 ± 3 or 20 ± 5 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, or *Kit*^{W/W-v} mice engrafted with WT BMCMCs or *Il10*^{-/-} BMCMCs, respectively; (d) 40 ± 9 , 0.2 ± 0.2 , 14 ± 4 or 16 ± 4 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, or WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT BMCMCs or *Il10*^{-/-} BMCMCs, respectively.

2. Figure 3a to c. As noted in Methods, total viable cells recovered per ear = (percentage gated) $\times$ [(in (b), the total number of cells recovered from individual L or R ears) or [in (c), total number of cells recovered from the pooled L or R ears of 4-5 mice per group] $\div$ 4 or 5 (as in c) or $\div$ 1 (as in b)). The total number of viable cells (including leukocytes and other cell types) recovered per ear from the L (vehicle) or R (DNFB-challenged) ears of WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, or WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT or *Il10*^{-/-} BMCMCs, were, for (b), in which cells derived from individual ears were analyzed (n = total number of ears analyzed in each group of mice): $0.6 + 0.7$ ($n = 2$), $1.2 + 0.7$ ($n = 2$), $1.1 + 0.1$ ($n = 2$) or $1.3 + 0.7$ ($n = 2$) $\times 10^5$ cells, respectively, for the L ears, and $4.4 + 0.2$ ($n = 2$), $9.1 + 0.3$ ($n = 2$), $1.9 + 0.1$ ($n = 2$) or $7.5 + 2.0$ ($n = 2$) $\times 10^5$ cells, respectively, for the R ears, and, for (c), in which cells were pooled from 4-5 ears per group (n = total number of ears analyzed in each group of mice): $1.0 + 0.3$ ($n = 20$), $1.2 + 0.2$ ($n = 17$), $1.0 + 0.1$ ($n = 17$) or $1.3 + 0.2$ ($n = 17$) $\times 10^5$ cells, respectively, for the L ears, and $1.7 + 0.6$ ($n = 20$), $5.7 + 1.2$ ($n = 17$), $2.0 + 0.5$ ($n = 17$), or $4.4 + 0.6$ ($n = 17$) $\times 10^5$ cells, respectively, for the R ears. The results shown for each group of mice in (b) are from 1 experiment and in (c) are from the 4 experiments that we performed using each group of mice, each of which gave similar results.

3. Figure 4. Numbers of mast cells per mm of ear cartilage (R ears) expressed as mean $\pm$ s.d. at the end of the CHS assay: (a) 46 ± 8 , 0, 20 ± 5 or 18 ± 6 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, or WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT BMCMCs or *Fcer1g*^{-/-} BMCMCs, respectively; (b) 42 ± 10 , 0, 16 ± 6 or 14 ± 5 for WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice, or WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT BMCMCs or *Fcer1g*^{-/-} BMCMCs, respectively.

4. Figure 5f. The total number of recovered cells per ear (including leukocytes and other cell types) in the control (no UVB treatment) ears or UVB-irradiated ears of WBB6F₁-*Kit*^{+/+} mice, WBB6F₁-*Kit*^{W/W-v} mice or WBB6F₁-*Kit*^{W/W-v} mice engrafted with WT, *Il10*^{-/-} or *Fcer1g*^{-/-} BMCMCs were $1.3 + 0.5$ ($n = 10$), $0.9 + 0.1$ ($n = 8$), $0.5 + 0.1$ ($n = 8$), $0.5 + 0.1$ ($n = 8$) or $1.0 + 0.1$ ($n = 8$) $\times 10^5$ cells, respectively, for the control (no UVB treatment) ears, and $1.6 + 0.4$ ($n = 35$), $3.9 + 1.1$ ($n = 24$), $2.1 + 0.3$ ($n = 30$), $3.3 + 0.8$ ($n = 20$) or $1.9 + 0.2$ ($n = 24$) $\times 10^5$ cells, respectively, for the R (UVB-irradiated) ears. The results shown are from the 3-5 experiments we performed employing the various groups of mice, each of which gave similar results; n = the total number of ears we analyzed for that group of mice.