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# Immune Sensitization in the Skin Is Enhanced by Antigen-Independent Effects of IgE

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## Summary

**Contact sensitivity responses require both effective immune sensitization following cutaneous exposure to chemical haptens and antigen-specific elicitation of inflammation upon subsequent hapten challenge. We report that antigen-independent effects of IgE antibodies can promote immune sensitization to haptens in the skin. Contact sensitivity was markedly impaired in IgE$^{-/-}$ mice but was restored by either transfer of sensitized cells from wild-type mice or administration of hapten-irrelevant IgE before sensitization. Moreover, IgE$^{-/-}$ mice exhibited impairment in the reduction of dendritic cell numbers in the epidermis after hapten exposure. Monomeric IgE has been reported to influence mast cell function. We observed diminished contact sensitivity in mice lacking FcεRI or mast cells, and mRNA for several mast cell-associated genes was reduced in IgE$^{-/-}$ versus wild-type skin after hapten exposure. We speculate that levels of IgE normally present in mice favor immune sensitization via antigen-independent but FcεRI-dependent effects on mast cells.**

## Introduction

The immunoglobulin IgE is involved in a variety of hypersensitivity responses. Upon interaction with polyvalent antigen, IgE bound to mast cells via FcεRI triggers immediate hypersensitivity reactions. We have shown that IgE, in the absence of antigen, regulates the expression of its receptor, FcεRI, both in vitro and in vivo (Yamaguchi et al., 1997, 1999). This has functional implications; upregulation of FcεRI on mast cells by IgE enhances serotonin release and cytokine production following subsequent challenge with antigen-specific IgE and relevant antigen (Yamaguchi et al., 1997).

Recently, two groups have reported that monomeric IgE can also function as a survival factor for mast cells (Asai et al., 2001; Kalesnikoff et al., 2001). Moreover, IgE antibodies, signaling via FcεRI, can enhance mast cell mediator and cytokine production in vitro, again, in the absence of exposure to known antigen (Kalesnikoff et al., 2001; Kitaura et al., 2003). In the present study, we sought to determine whether IgE, in the absence of specific antigen, could influence the expression of immune responses in vivo.

Contact sensitivity responses to chemical haptens have been widely used to study mechanisms of immune sensitization (Grabbe and Schwarz, 1998). The generation of contact sensitivity reactions requires both effective immune *sensitization* following initial antigen encounter at the skin and later, upon reexposure to the same antigen, the *elicitation* of an inflammatory response. Sensitization is mediated by the migration of haptenated dendritic cells from the skin to draining lymph nodes where they activate T cells (Macatonia et al., 1987). Sensitization is impaired in mice with defective migration of dendritic cells or T cell dysfunction (Cumberbatch et al., 1999; Forster et al., 1999; Wang et al., 1997).

Several investigators have suggested a role for IgE in the elicitation phase of contact sensitivity. Among mouse strains, the intensity of contact sensitivity responses correlates with the capacity to produce hapten-specific IgE (Nagai et al., 1999). Total IgE and IgE-positive cells increase after exposure of mice to contact sensitizers (Ban and Hettich, 2001). Askenase and colleagues, using passive transfer systems, found evidence for a role for antigen-specific IgE in contact sensitivity in enhancing the elicitation phase of the response (Graziano et al., 1983; Matsuda et al., 1995; Ptak et al., 1991).

In light of the recent evidence that IgE can exert antigen-independent effects on mast cell functions, we examined the requirements for IgE at both the immune sensitization phase (prior to the presence of specific antibodies) and the elicitation phase of contact sensitivity. We show that IgE, independent of exposure to specific antigen, can be required for optimal sensitization but not for elicitation in contact sensitivity. Mast cell-deficient mice and animals lacking FcεRI also have attenuated contact sensitivity in our model, and both IgE$^{-/-}$ and mast cell-deficient mice failed to display reductions in dendritic cell numbers in the epidermis following hapten exposure. The deficiency in sensitization in IgE$^{-/-}$ mice was associated with reduced cutaneous levels of mRNA for oxazolone-induced gene products that are mast cell derived and/or can influence dendritic cell migration and/or function after hapten exposure, such as TNFα, IL-1β, IL-6, MMCP-6, and MCP-1. Taken together, our findings suggest that levels of IgE normally present in mice can promote optimal sensitization in contact sensitivity by enhancing expression of mast cell-associated mediators that contribute to dendritic cell migration.

## Results

### IgE$^{-/-}$ Mice Have Diminished Contact Sensitivity Responses

IgE$^{-/-}$ and wild-type BALB/c mice were epicutaneously sensitized on the abdomen with hapten and challenged

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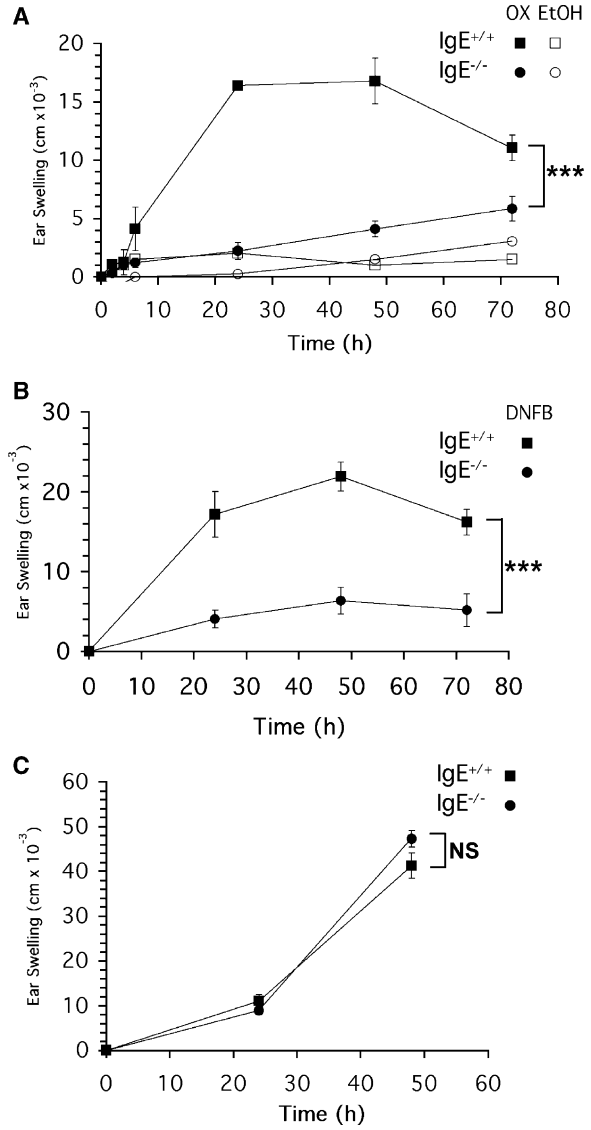


Figure 1. IgE$^{-/-}$ Mice Have Impaired Contact Sensitivity Responses to Chemical Haptens

Wild-type (filled squares) or IgE$^{-/-}$ mice (filled circles) were sensitized to oxazolone (A) or DNFB (B), challenged 5 days later, and ear thickness measured over time. Responses to oxazolone in unsensitized mice are indicated for wild-type (open squares) and IgE$^{-/-}$ mice (open circles). Responses to the irritant croton oil are shown in (C). Values represent the mean ± SEM (n = 5–10). ***$p < 0.001$ versus the corresponding values for IgE$^{+/+}$ mice; NS, no significant difference.

5 days later by application of the hapten to the ear. Sensitized wild-type animals displayed a robust increase in ear thickness to oxazolone challenge (Figure 1A) peaking at 24 hr (mean, 16.4 ± 0.34 cm$^{-3}$). In contrast, IgE$^{-/-}$ animals had minimal increases in thickness (mean, 2.2 ± 0.72 cm$^{-3}$ at 24 hr). Although modest swelling was evident at later time points (mean, 5.8 ± 1.06 cm$^{-3}$ at 72 hr), the thickness of hapten-challenged ears in sensitized IgE$^{-/-}$ mice was substantially less than that in the corresponding wild-type mice. This defect in contact sensitivity response to oxazolone was reproduced using the unrelated contact sensitizer DNFB (Figure 1B). The skin irritant croton oil, which does not require specific immune recognition to generate an inflammatory response, elicited normal ear swelling in IgE$^{-/-}$ mice (Figure 1C).

### The Defect in Contact Sensitivity Responses in IgE$^{-/-}$ Mice Resides at the Sensitization Phase

We employed cellular adoptive transfer and IgE-reconstitution approaches to evaluate the extent to which IgE antibodies participate in the sensitization or challenge phases. Transfer of splenocytes from sensitized wild-type animals conferred robust oxazolone-specific contact sensitivity to both naive wild-type and IgE$^{-/-}$ recipients (Figure 2A). In contrast, cells from oxazolone-exposed IgE$^{-/-}$ mice were unable to transfer oxazolone sensitivity into either wild-type or IgE$^{-/-}$ recipients. Thus, the impairment of contact sensitivity responses in IgE$^{-/-}$ animals is due to defective sensitization to the hapten. Elicitation of the response can proceed normally in the absence of endogenous IgE if sensitization has occurred in an environment with normal levels of IgE.

We reasoned that the impaired sensitization of IgE$^{-/-}$ mice to contact sensitizers must be directly related to their deficiency in IgE and that the defect ought to be overcome by administration of exogenous IgE. In a dose-dependent fashion, administration of IgE prior to sensitization enhanced the ability of IgE$^{-/-}$ animals to express contact sensitivity responses to oxazolone (Figure 2B). IgE doses of 10 μg or 100 μg were sufficient to facilitate swelling responses in IgE$^{-/-}$ mice that were statistically indistinguishable from those of wild-type animals, while 0.5 μg or 1.0 μg doses of IgE supported responses that were significantly greater than those of unreconstituted IgE$^{-/-}$ mice.

Correlation of serum IgE levels immediately after (i.e., within 2 min of) reconstitution (Figure 2C) with ear swelling responses (Figure 2B) revealed that transient induction of levels of IgE comparable to those observed in wild-type mice in our colony was sufficient for reconstitution of the responses to hapten (wild-type serum IgE levels were 787 ± 67 ng/mL). As expected, IgE was rapidly cleared following injection (see Figure 2C, inset); a 10 μg dose induced plasma levels of >1000 ng/ml at 2 min, which fell to less than 100 ng/ml within 6 hr and were undetectable after 24 hr. Exceeding normal levels of IgE by injection of 10–100 μg of IgE did not drive the ear swelling response beyond wild-type levels using the 2% sensitizing dose of this protocol. Giving the IgE i.v. immediately prior to challenge did not influence the defective contact sensitivity responses (data not shown).

Histological examination of contact sensitivity responses showed abundant inflammatory infiltrating cells within 6 hr of hapten challenge in wild-type animals (Figure 3). The cellular infiltrate consisted mainly of mononuclear cells, neutrophils, and some eosinophils and, by 24 hr, was associated with substantial dermal edema. Particularly at 24 hr after hapten challenge, occasional intraepithelial microabscesses containing neutrophils were also observed. In contrast, contact sensi-

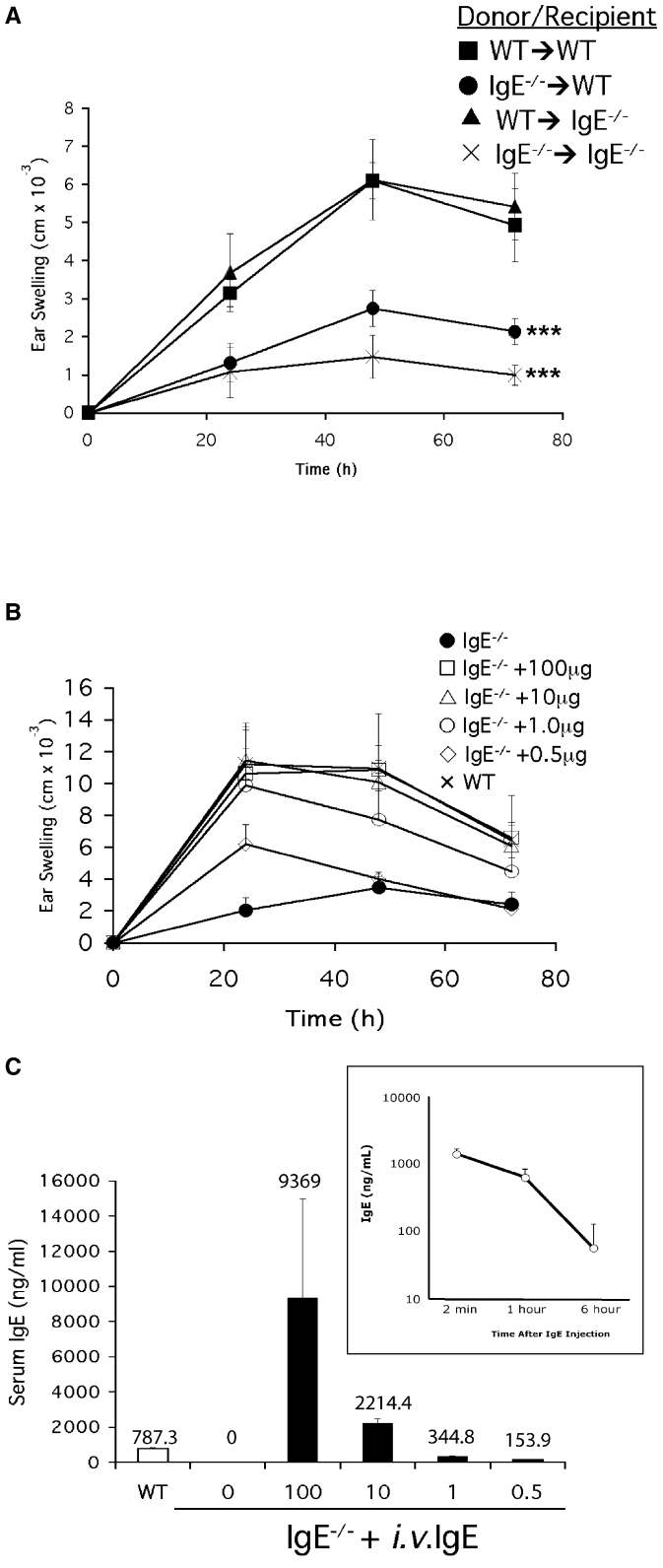


Figure 2. IgE Is Required for the Optimal Expression of the Sensitization Phase of Contact Sensitivity to Oxazolone

(A) Groups of five wild-type (squares) or IgE$^{-/-}$ mice (circles) were sensitized to oxazolone. Five days later splenocytes were transferred i.v. to naive recipients ($10^7$ cells/animal). Recipients were challenged with oxazolone and mean ear swelling measured ±SEM (n = 10–12). ***$p < 0.001$ versus the values for wild-type cells transferred into wild-type recipients. (B) IgE$^{-/-}$ mice received TNP-specific IgE (TIB142) or vehicle i.v. 24 hr before sensitization with oxazolone. Oxazolone challenge was performed 5 days later and mean ear swelling recorded ±SEM (n = 5–10). (C) Serum IgE levels 2 min after i.v. injection of various amounts of TIB142 IgE. Rate of clearance of a single IgE dose (10 μg) over 6 hr is shown in the inset.

tivity responses in the ears of IgE$^{-/-}$ mice contained few or no leukocytes at 6 hr and only a slight cellular infiltration, consisting predominantly of mononuclear cells and occasional eosinophils but no microabscesses. IgE$^{-/-}$ animals that received IgE (10 μg) prior to sensitization demonstrated cellular infiltration similar to

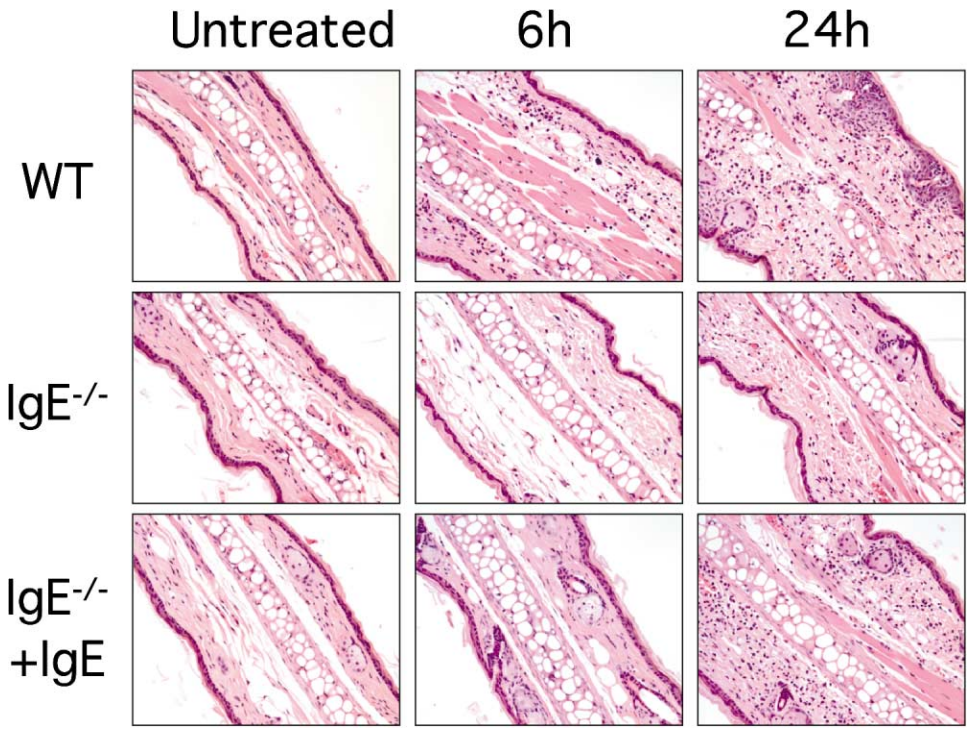


Figure 3. Histological Examination of Contact Sensitivity Responses

Hematoxylin- and eosin-stained ear sections following oxazolone sensitization and challenge (×400). Prior to sensitization, 10 μg IgE (TIB142) was given to one group of $IgE^{-/-}$ mice. Representative sections are shown from one of four individual animals that were examined per group.

that observed in wild-type animals, although no intraepithelial abscesses were observed.

### The Ability of IgE to Enhance the Sensitization Phase of Contact Sensitivity Is Independent of the Known Antigen Specificity of the IgE

Notably, reconstitution of the contact sensitivity response by exogenous IgE in $IgE^{-/-}$ mice did not require known antigenic specificity for the sensitizing hapten. TIB142, the monoclonal IgE antibody used, is directed against TNP, a chemical structurally unrelated to oxazolone. We determined that three other IgE monoclonal antibodies specific for TNP, DNP, or ovalbumin were also effective in reconstituting the ability to express contact sensitivity responses to oxazolone when given prior to sensitization, whereas a mouse anti-TNP IgG antibody had no detectable effect (Table 1). Our data suggest that the presence of IgE per se is permissive of sensitization to chemical haptens in a manner that is independent of the known antigen specificity of the antibody.

### Epidermal Dendritic Cell Numbers Are Not Diminished in the Skin of $IgE^{-/-}$ Mice after Hapten Application

Contact sensitizers are potent inducers of dendritic cell emigration from the skin epidermis, and mice with defective migration of dendritic cells exhibit diminished contact sensitivity responses (Cumberbatch et al., 1999; Shornick et al., 1996; Wang et al., 1997). After 18 hr exposure to oxazolone, wild-type mice showed reduced numbers of epidermal dendritic cells compared to values in vehicle-treated ears from matched animals (Figure 4B). This hapten-driven reduction of ~25% in the number of resident epidermal MHC $II^+$ cells (i.e., Langerhans cells) is comparable in magnitude to that previously reported by others using this assay (Cumberbatch et al., 2001). In $IgE^{-/-}$ animals, there was no significant difference between values for oxazolone versus vehicle-treated mice, but adoptive transfer of IgE to $IgE^{-/-}$ mice resulted in normalization of the effects of oxazolone on epidermal dendritic cell numbers.

### Both FcεRI and Mast Cells Are Required for Optimal Contact Sensitivity to Oxazolone

The observation that IgE can promote sensitization to haptens in this contact sensitivity model prompted us to evaluate whether this effect requires the presence of FcεRI and mast cells. In rodents, only the $\alpha\beta\gamma_2$ isoform of FcεRI is expressed, and this is exclusive to mast cells and basophils; although humans can express the $\alpha\gamma_2$ isoform of FcεRI in Langerhans cells and other lineages, that isoform can not be assembled in rodent cells (Dombrowicz et al., 1998; Kinet, 1999; Kawakami and Galli, 2002).

$FcR\gamma^{-/-}$ mice express neither FcεRI nor FcγRIII and have defects in mast cell-mediated responses to both IgE and IgG (Sylvestre and Ravetch, 1994; Takai et al., 1994). As a control, we examined mice lacking the α

Table 1. Several IgE Preparations of Unrelated Specificity Reconstitute Normal Sensitivity to Epicutaneously Applied Oxazolone in IgE$^{-/-}$ Mice

| Treatment | % of Wild-Type Response |
|---|---|
| No injection | 11.0 |
| IgG injection | |
| Anti-TNP (107.3) | 7.6 ± 2.3 NS |
| IgE injection | |
| Anti-TNP (TIB142) | 92.0 ± 8.1*** |
| Anti-TNP (C38-2) | 77.0 ± 5.8*** |
| Anti-DNP (SPE-7) | 90.6 ± 8.5*** |
| Anti-OVA (see Sawada et al., 1997) | 69.5 ± 12.3** |

Of the antibodies indicated, 10 μg of each was administered by tail vein injection to IgE$^{-/-}$ mice 24 hr prior to oxazolone sensitization and contact sensitivity elicited by application of oxazolone to the ear on day 5 (n = 3–7 per group). ***$p < 0.001$, **$p < 0.05$ versus values in IgE$^{-/-}$ mice that had not been injected with antibodies. NS, no significant difference ($p > 0.05$).

chain of FcγRIII. These mice lack FcγRIII function but retain FcεRI expression (Hazenbos et al., 1996). FcRγ$^{-/-}$ mice failed to show any significant ear swelling to oxazolone while FcγRIII$^{-/-}$ mice gave contact sensitivity responses to oxazolone that were similar (23.5 ± 5.01 cm$^{-3}$ at 24 hr) to those of wild-type animals (25.6 ± 2.50 cm$^{-3}$ at 24 hr) (Figure 5A). As expected, i.v. IgE could not reconstitute the defective contact sensitivity responses of the FcRγ$^{-/-}$-deficient animals. These observations show that interaction between IgE and FcεRI is required for IgE-mediated augmentation of cutaneous sensitization by chemical haptens.

WBB6F1/J-*Kit*$^{W}$/*Kit*$^{W-v}$ mice are profoundly deficient in mast cells and had a significantly impaired ear swelling response compared to wild-type controls (Figure 5B). Dendritic cells in mast cell-deficient animals also failed to leave the epidermis after oxazolone exposure (83.9 ± 5.0 cells/HPF after vehicle, 86.1 ± 4.7 after oxazolone in *WBBFI/J-Kit*$^{W}$/*Kit*$^{W-v}$ mice versus 81.9 ± 11.6 after vehicle, 54.4 ± 4.3 after oxazolone in littermate controls). We undertook passive cellular transfer to determine at which phase of contact sensitization mast cells might be acting. Figure 5C shows that wild-type splenocytes from sensitized animals conferred ear swelling responses to wild-type naive recipients challenged with hapten. By contrast, transfer of splenocytes from sensitized WBB6F1/J-*Kit*$^{W}$/*Kit*$^{W-v}$ mice into wild-type or WBB6F1/J-*Kit*$^{W}$/*Kit*$^{W-v}$ recipients resulted in little or no swelling response to hapten challenge. These findings are consistent with roles for mast cells in both the sensitization (IgE dependent) and elicitation phases (IgE independent) of contact sensitivity. There were no differences between wild-type and IgE$^{-/-}$ mice in numbers of mast cells per high-powered field in either abdominal

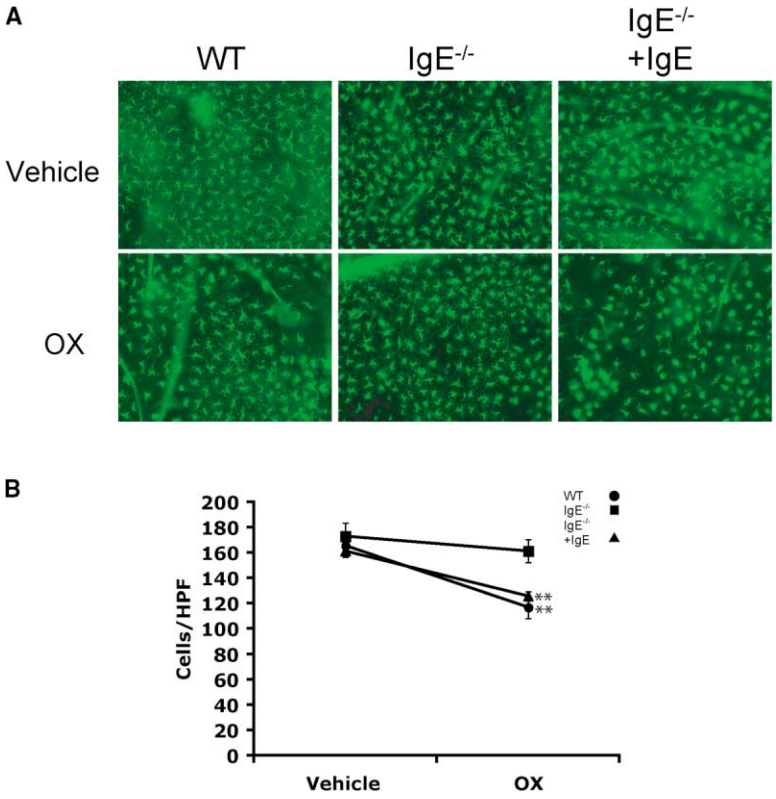


Figure 4. IgE$^{-/-}$ Mice Exhibit Impaired Reduction in Numbers of Epidermal Dendritic Cells in Response to Oxazolone

(A) Epidermal skin sheets were prepared from the ears of wild-type or IgE$^{-/-}$ mice 18 hr after application of 10 μl of 2% oxazolone or vehicle to each side. Some IgE$^{-/-}$ mice (IgE$^{-/-}$ + IgE) received 10 μg IgE (TIB142) i.v. 24 hr before hapten exposure. Dendritic cells were identified by staining with FITC anti-mouse I-A/I-E.

(B) The numbers of I-A/I-E$^{+}$ dendritic cells were counted in high-power fields of epidermal skin sheets. Four separate fields from each ear were counted and the results expressed as the mean ± SEM (n = 5). Values in wild-type (circles) and IgE$^{-/-}$ + IgE mice (triangles) were significantly different from those in IgE$^{-/-}$ (squares) by Student's t test (**$p < 0.05$).

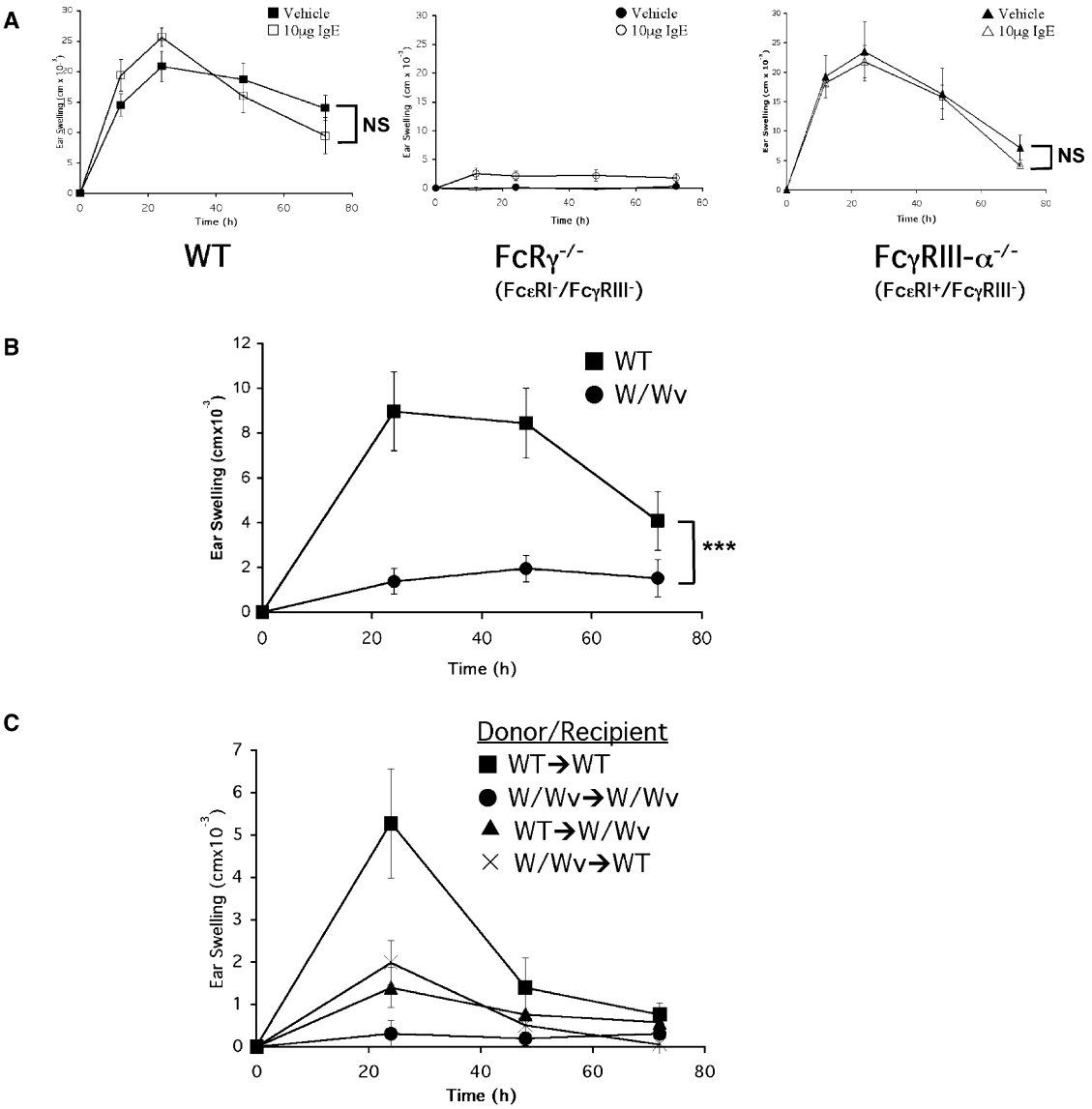


Figure 5. FcεRI and Mast Cells Are Required for Optimal Contact Sensitivity Responses

(A) Wild-type mice (squares), mice deficient in FcεRI and FcγRIII (FcRγ$^{-/-}$) (circles), or mice lacking in the α chain of FcγRIII (triangles) were sensitized and challenged with oxazolone. For each genotype, one group of animals received 10 μg of IgE (TIB142) i.v. 24 hr before sensitization (open symbols) and another group did not (filled symbols). Values represent the mean ear swelling ± SEM for each group (n = 4).

(B) Mast cell-deficient WBB6F1/J-*Kit*$^{W}$/*Kit*$^{W\text{-}v}$ mice (n = 9) (circles) or littermate WBB6F1/J-*Kit*$^{+/+}$ wild-type mice (n = 13) (squares) were sensitized to oxazolone, and ear swelling was measured after challenge, mean ± SEM. ***$p < 0.001$ by two-way ANOVA.

(C) Splenocytes from WBB6F1/J-*Kit*$^{W}$/*Kit*$^{W\text{-}v}$ or littermate WBB6F1/J-*Kit*$^{+/+}$ wild-type mice were prepared 5 days after sensitization exposure to oxazolone and transferred i.v. into naive recipients. Data shown represent mean ear swelling ± SEM (n = 4 to 5 per group).

skin (4.15 ± 0.3 in wild-type; 4.40 ± 0.4 in IgE$^{-/-}$) or ear skin (18.3 ± 1.5 in wild-type; 17.9 ± 0.2 in IgE$^{-/-}$). This indicates that the defective expression of contact sensitivity in the absence of IgE is not due to a deficiency in skin mast cell numbers.

### The Hapten-Induced Enhancement of mRNA for Several Inflammatory Mediators Is Diminished in the Skin of IgE$^{-/-}$ Mice

Kalesnikoff et al. recently showed that IgE, via FcεRI but independent of crosslinking of the IgE by exposure to known antigen, could influence cytokine production by mast cells in vitro (Kalesnikoff et al., 2001). We hypothesized that IgE antibodies might influence cytokine production in vivo. Real-time PCR was used to measure mRNAs encoding a panel of mediators thought to be mast cell associated (MMCP-6) or to contribute to the generation of contact sensitivity responses (IL-18) or both (IL-6, IL-1β, MCP-1, TNFα). Hapten exposure itself (for 1 hr), as previously observed by others, strongly induced the expression of several genes (Figure 6A), with greater than 100-fold induction of IL-6 over baseline

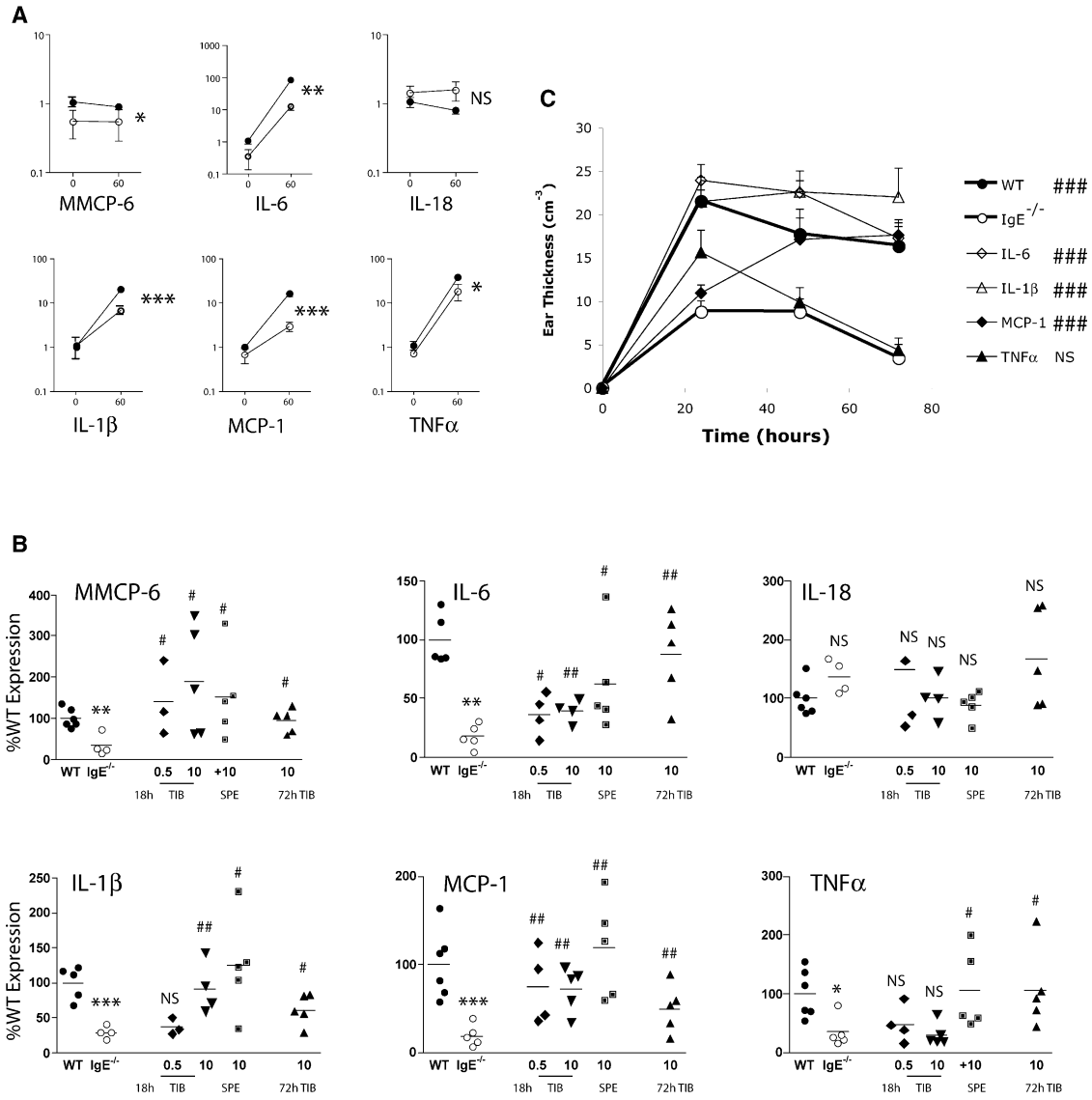


Figure 6. Defective Sensitization in the Absence of IgE Is Associated with Reduced Gene Expression

(A) The levels of an array of mRNAs in the abdominal skin of wild-type (filled circles) mice or IgE$^{-/-}$ (open circles) mice was determined by RT-PCR before (0) or 1 hr after oxazolone application. Relative expression of each product was determined by comparison to β actin. Each value is represented as fold induction relative to the mean wild-type expression in nonpainted skin (n = 5 per group).

(B) Cytokine transcripts were measured by RT-PCR 1 hr after oxazolone application in wild-type (wt) and IgE$^{-/-}$ mice and in IgE$^{-/-}$ mice 18 or 72 hr after reconstitution with i.v. injection of IgE antibodies TIB142 and SPE-7 (0.5 or 10 μg doses).

(C) Ear swelling thickness was recorded in wild-type or IgE$^{-/-}$ mice after oxazolone sensitization and challenge. Additional groups (n = 5) of IgE$^{-/-}$ mice received intradermal injections of recombinant mouse TNFα (100 ng), IL-6 (120 ng), MCP-1 (10 ng), IL-1 (50 ng), or vehicle immediately prior to sensitization. For time courses (A and C), p values were determined by ANOVA and for paired sets (B) by Student's t test. *$p < 0.05$, **$p < 0.005$, and ***$p < 0.001$ versus wt; #$p < 0.05$, ##$p < 0.005$, and ###$p < 0.001$ versus IgE$^{-/-}$. NS, not significant ($p > 0.05$).

levels and significant effects on IL-1β, MCP-1, and TNFα. Oxazolone painting did not alter mRNA levels for IL-18 or MMCP-6.

Oxazolone-induced mRNA levels of IL-1β, IL-6, MMCP-6, MCP-1, and TNFα but not IL-18 were all significantly lower in the skin of IgE$^{-/-}$ animals than in wild-type mice (Figure 6B). Restoration of IgE levels by i.v. administration of TIB142 IgE (0.5 or 10 μg) 18 hr before the mice were sacrificed resulted in dose-related increases in the levels of IL-1β and MMCP-6 and MCP-1 and IL-6 mRNAs. SPE-7 IgE, a clone with potent cytokine-inducing properties in mast cells in vitro (Kalesnikoff et al., 2001; Kitaura et al., 2003), enhanced oxazolone-induced transcription of the same group of cytokines and, in addition, enhanced TNFα transcription. TNFα induction was also evident in the skin of TIB142-treated animals when examined 72 hr after injection. As MMCP-6 is thought to be a mast cell-specific gene, these findings establish that ambient IgE levels can influence mast cell gene transcription in vivo. Intradermal injection of the IgE-regulated cytokines IL-6, MCP-1, or IL-1β immediately prior to oxazolone painting

corrected the sensitization defect in IgE$^{-/-}$ mice (Figure 6C). IgE$^{-/-}$ mice injected with TNF$\alpha$ showed a modest increase over vehicle at the 24 hr time point but the difference was not sustained or significant over the full 72 hr time course. Taken together, these findings suggest that the cytokine milieu of IgE$^{-/-}$ skin is not optimally supportive of immune sensitization to haptens. Furthermore, our data indicate that IgE, even in the absence of known specific antigen, can enhance hapten-induced cutaneous levels of a number of mRNAs for several mediators that are known to influence dendritic cell function and immune sensitization.

## Discussion

The role of *antigen-specific* IgE antibodies in mediating immediate hypersensitivity reactions has long been recognized. A number of studies have demonstrated that *antigen-independent* effects of IgE antibodies regulate several cellular functions, including IgE receptor expression, survival, and cytokine production (reviewed in Kawakami and Galli, 2002). However, it has not previously been established whether such antigen-independent effects of IgE influence the expression of active immune responses in vivo.

In this study, we have identified a function for IgE antibodies in regulating cutaneous immune sensitization to haptens (Figure 1). Using passive reconstitution with IgE, cellular adoptive transfer (Figures 2 and 3) and analysis of dendritic cell numbers in the epidermis (Figure 4), we have established that optimal immune sensitization to chemical haptens requires, at the time of antigen exposure, the presence of IgE. In our model, IgE clearly is not required at the elicitation phase of contact sensitivity, as shown by the normal ear swelling responses that are generated in IgE$^{-/-}$ mice that have been adoptively immunized with oxazolone-immune cells derived from wild-type mice. Our data strongly support the hypothesis that the effects of IgE during hapten sensitization involve Fc$\epsilon$RI, mast cells, and mast cell-derived products (Figures 5 and 6) and that IgE can contribute to optimal sensitization for contact sensitivity by influencing dendritic cell mobilization.

A role for hapten-specific IgE in the elicitation phase of contact sensitivity has previously been described by Askenase and colleagues, establishing that antigen-specific IgE also can facilitate the development of the response under certain conditions of testing (Graziano et al., 1983; Matsuda et al., 1995, 1997; Ptak et al., 1991). However, no prior studies examining the potential roles of IgE in contact sensitivity have evaluated its function during the initial immune sensitization. Our observations thus define a role for IgE antibodies during sensitization for contact sensitivity.

We initially were surprised by the lack of requirement for antigen specificity for the IgE antibodies conferring effective hapten sensitization. Taken together with our observations that Fc$\epsilon$RI and mast cells are also important for the induction of oxazolone responses under the conditions tested, these findings strongly suggest that IgE antibodies provide significant antigen-independent signals to mast cells residing in the skin. Our dose-response analysis (Figure 2) indicates that this antigen-independent effect of IgE can be conferred by IgE levels as low as those normally present in mice in a pathogen-free research colony, supporting the biological relevance of this phenomenon.

No further enhancement was achieved by transiently exceeding these levels at the single sensitizing dose of oxazolone (2%) used in these studies (see Figure 2). As the amplifying role of IgE in this response might be most pronounced at threshold levels of antigenic stimulation, we can not exclude the possibility that further enhancement of contact sensitivity responses would become evident at higher IgE levels under limiting conditions (lower oxazolone doses). Our findings provide strong evidence that at least "basal" IgE levels must be present for the maintenance of a cutaneous microenvironment that is permissive of efficient immune sensitization. However, our findings also leave open the possibility that in settings of lower antigen exposure, alternate routes of immunization or repeated sensitization, elevated IgE levels, such as those associated with parasite infestation or allergen exposure, might exert immunodultory effects that are even stronger than those of basal levels of IgE.

One concern raised in studies of "monomeric" IgE on mast cell functions in vitro has been the possibility of artifactual induction of responses by IgE aggregation under nonphysiologic conditions. Our data clearly demonstrate impaired contact sensitivity in IgE$^{-/-}$ mice but normal responses in wild-type mice, establishing an antigen-independent role for IgE in an in vivo system that is free of exogenous physical or chemical factors that could induce nonphysiologic aggregation. Furthermore, in our reconstitution studies we minimized the potential for multimerization or aggregation of IgE by ultracentrifuging all of the IgE preparations, prior to injection. Also, we tested the possibility that any ear swelling responses conferred by IgE injection might be artifactual "late phase" responses of mast cells acutely stimulated by IgE aggregates by measuring ear thickness after i.v. IgE injection: we found no evidence of any acute, IgE-induced swelling responses (data not shown).

The mechanism whereby IgE can activate mast cells in the absence of known antigen has not yet been elucidated (Kitaura et al., 2003). Engagement of Fc$\epsilon$RI by its ligand, monomeric IgE, may provide a signal in and of itself. Alternatively, receptor-bound IgE antibodies might have some tendency to associate in the absence of antigen, leading to Fc$\epsilon$RI aggregation and signaling. Finally, it has been suggested that molecules other than nominal antigen could interact with some IgE antibodies. James and colleagues observed that a single IgE antibody, SPE-7, can exist in two isomers, one which binds to DNP and another which binds to an unrelated protein antigen (James et al., 2003). SPE-7 has relatively potent mast cell simulating properties in culture (Kalesnikoff et al., 2001; Kitaura et al., 2003) and in our hands, SPE-7 was somewhat more effective than the other IgE antibodies studied in restoring both ear swelling responses and skin cytokine gene expression.

It is possible that such "antigenic promiscuity" may underlie the interesting effects of SPE-7 observed by us and others. However, attributing reconstitution of contact sensitivity responses in IgE$^{-/-}$ mice by each of the four different IgE monoclonal antibodies that have

hapten and protein specificities unrelated to each other and to oxazolone (Table 1) to such alternative antigen binding would require that they each have an alternative isoform capable of binding either oxazolone or an unrelated endogenous antigen. Although this seems unlikely given the lack of precedent among other immunoglobulin isotypes, it is possible that some low-affinity "alternative" antibody-antigen interactions may become experimentally apparent because of the intense biological amplification of the IgE/FcεRI/mast cell system.

Although previous analyses of cultured mouse mast cells have established a role for IgE antibodies in regulating mast cell survival (Asai et al., 2001; Kalesnikoff et al., 2001), our histological examinations revealed no differences in the number of mast cells in wild-type versus $IgE^{-/-}$ skin. It is possible that mast cell turnover is affected in $IgE^{-/-}$ mice but that this is obscured in our static analysis by compensatory increases in mast cell differentiation. Alternatively, since mast cell survival enhancement in culture was observed at levels of IgE (5–10 μg/ml) substantially greater than those in our wild-type mice (<1 μg/ml, see Figure 2), the survival signal may require high levels of IgE. It has been reported that the numbers of mast cells in gastrointestinal tissues of mice are significantly increased, in proportion to plasma IgE levels, by the adoptive transfer of IgE-producing but not IgG-producing hybridoma cells in vivo (Kitaura et al., 2003). In addition, we have found that $IgE^{-/-}$ mice infected with *Trichinella spiralis*, a parasite which typically elicits high IgE levels (>50 μg/ml) and splenic mastocytosis in wild-type mice have a marked blunting of this mast cell expansion, consistent with an IgE effect on mast cell homeostasis (Gurish et al., 2004).

Previous studies on the role of mast cells in contact sensitivity responses have given disparate results, leading to some confusion in the literature. Under some conditions, contact sensitivity responses appeared to be partially mast cell-*dependent* and under others they appeared to be mast cell-*independent*. We and others have reported *intact* contact sensitivity responses in mast cell-deficient animals (Thomas and Schrader, 1983; Galli and Hammel, 1984; Mekori and Galli, 1985; Ha et al., 1986; Mekori et al., 1987), while other groups have observed *diminished* responses (Askenase et al., 1983; Geba et al., 1996; Webb et al., 1998; Biedermann et al., 2000). Both groups contributing to this report have now independently confirmed that a significant defect in contact sensitivity to oxazolone can be detected in mast cell-deficient mice when ethanol is used as the vehicle and under the conditions of sensitization and challenge described in this report.

A wide range of experimental conditions have now been employed in studies of contact sensitivity in mast cell-deficient mice by several groups, working over a period of more than 20 years. While the exact reasons for the discrepancies reported in the different studies have yet to be determined, examination of the methods indicates that a number of variables might influence the outcome, including hapten chemical structure, hapten concentration (both at sensitization and challenge), vehicle, and genotype of mast cell-deficient mice. In addition, a variety of unreported variables, including mouse age and pathogen status of the colony, also could influence these results. It is further possible that the chemical stability of the haptens used (e.g., the rate of oxidation of oxazolone) varies among solvents, so that reported hapten concentrations using one solvent may not be strictly comparable to those reported when the hapten is used in another solvent. Moreover, given the known ability of mast cells to amplify other biological responses (reviewed in Kawakami and Galli, 2002), it is likely that the *potential* contributions of mast cells to immune responses may be most evident under limiting conditions of stimulation (i.e., less antigen, vehicle with less adjuvant or irritant activity, less traumatic method of application, etc.).

Interestingly, mast cells (unlike IgE antibodies) appear to be required for *both* optimal sensitization and optimal elicitation of contact sensitivity in this model, while IgE is required only at sensitization. This is consistent with an antigen-independent but IgE-dependent function of mast cells in providing signals (e.g., dendritic cell-activating cytokines) that support immune sensitization and a distinct, antigen-specific but IgE-independent mast cell function during elicitation, providing signals that promote tissue edema and granulocyte infiltration (Biedermann et al., 2000).

Although our studies show that IgE is *not* required at elicitation and that specific IgE antibodies are not detectable after the short 5 day sensitization period (data not shown), hapten-specific T cells are generated during this time (as confirmed by our adoptive transfer experiments). These T cells might produce factors, such as IL-4, that can induce mast cell activation (Boyce, 2003). Alternatively, antigen-specific mast cell activation at the time of elicitation of contact sensitivity reactions could be mediated by the effects of humoral factors other than IgE, including IgG1, immunoglobulin light chains, and B-1 cell-derived IgM (Paliwal et al., 2002; Redegeld et al., 2002; Tsuji et al., 2002).

Hapten exposure results in the induction of a number of cytokine mRNAs in skin, and we demonstrated that hapten-induced transcription of genes encoding several mast cell-associated mediators was significantly lower in $IgE^{-/-}$ animals than wild-type mice (Figure 6B). The reduced levels of mRNA for the mast cell-specific tryptase MMCP-6 indicates a defect in mast cell gene expression (Reynolds et al., 1991).

Administration of IgE (either TIB142 or SPE-7) to naive $IgE^{-/-}$ mice prior to oxazolone exposure led to dose-related increases in the expression of IL-1β, IL-6, MMCP-6, and MCP-1 at both 18 and 72 hr after IgE injection. TNFα levels rose more slowly following TIB142 IgE injection, becoming evident 72 hr later, but were increased even at the 18 hr time point following injection of SPE-7. We hypothesize that this IgE-reversible reduction in expression of a constellation of mediators in the skin of $IgE^{-/-}$ mice could contribute to the impaired migration of hapten-exposed dendritic cells and thereby affect the generation of a hapten-specific response.

IL-1β, IL-6, MCP-1, and TNFα have all been shown to be involved in the expression of contact sensitivity reactions, and TNFα is a direct stimulus for dendritic cell activation (Becke et al., 2001; Hope et al., 2000; Shornick et al., 1996; Yamazaki et al., 1998). MCP-1 (CCL2), which binds to the CCR2 chemokine receptor, is a known chemoattractant for Langerhans cells and is also important for their recruitment to skin and differenti-

ation from blood-borne progenitors (Merad et al., 2002; Yamaguchi et al., 1997). Transgenic overexpression of MCP-1 results in enhanced contact sensitivity (Mizumoto et al., 2001; Nakamura et al., 1995).

Mast cells are known producers of TNFα, IL-6, IL-1, and MCP-1 and have been shown to regulate MCP-1 expression by other dermal cell types (Eglite et al., 2003; Gordon, 2000; Gordon and Galli, 1990). We hypothesize that, in the absence of IgE, the mast cell production of these mediators and/or their induction in other cell types is attenuated, resulting in suboptimal signals driving migration of dendritic cells from the epidermis. Injection of IL-6, IL-1, MCP1, or TNFα immediately prior to sensitization partially or fully restored contact sensitivity (Figure 6C). Of course, only limited conclusions can be drawn from this experiment, as such injections might induce some local trauma as well as supraphysiologic cytokine levels. Since several cytokines each individually restored immune sensitization in $IgE^{-/-}$ mice, this experiment did not identify a direct link between IgE deficiency and the absence of one particular cytokine. Nevertheless, these results show that provision of local signals promoting immune sensitization can correct the cutaneous immune defect of $IgE^{-/-}$ mice.

IL-18, which is produced by dendritic cells and keratinocytes in skin (Stoll et al., 1998), was slightly increased in $IgE^{-/-}$ skin, perhaps reflecting relatively higher numbers of IL-18-producing dendritic cells. It has been established that IL-18 is important during the sensitization phase of contact sensitivity (Wang et al., 2002). Our findings do not exclude a critical role for IL-18 but instead suggest that the alterations in contact sensitivity we observed in $IgE^{-/-}$ and $W/W^v$ mice are not directly linked to any modulation of IL-18 production.

In conclusion, we have defined a previously unknown in vivo function of IgE, namely, facilitating the sensitization phase of contact sensitivity responses. Remarkably, although IgE mediates this effect via FcεRI and mast cells, it does so in a manner that does not require exposure to antigens for which the IgE has known specificity. We provide evidence that one mechanism whereby IgE can support optimal immune sensitization reflects the of the expression of cutaneous genes that can facilitate optimal mobilization of antigen-exposed dendritic cells from the epidermis.

## Experimental Procedures

### Reagents

4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) and 2,4-dinitrofluorobenzene (DNFB) (Sigma Chemical, St. Louis, MO) were dissolved in 100% ethanol. Croton oil was (Sigma Chemical, St. Louis, MO) was diluted to 1% in olive oil.

### Animals

$IgE^{-/-}$ mice (Oettgen et al., 1994) were bred onto a BALB/c background (ten generations). $Fc\gamma RIII\ \alpha$ chain $(CD16)^{-/-}$ (C57BL/6J strain) mice were provided by J.S. Verbeek (University Hospital, Utrecht, The Netherlands). WBB6F1/J-$Kit^W/Kit^{W\text{-}v}$ mice and the congenic wild-type WBB6F1/J-$Kit^{+/+}$ mice were from the Jackson laboratory (Bar Harbor, ME). $FcR\gamma^{-/-}$ mice or wild-type controls on the C57BL/6J background were purchased from the Jackson laboratory (Bar Harbor, ME). Wild-type BALB/c mice were from Taconic Farms (Germantown, NY). Mice were housed in a specific pathogen-free environment and were 6 to 12 weeks old. All experiments were carried out in accordance with Children's Hospital, Beth Israel Deaconess Medical Center, or Stanford University IACUC policies and procedures.

### Sensitization and Elicitation of Contact Hypersensitivity

Mice were sensitized by application of 100 μl of 2% oxazolone or 0.5% DNFB in 100% ethanol to the shaved abdomen. On day 5, 10 μl 1% oxazolone or 0.2% DNFB in 100% ethanol was applied to both sides of the ear and ear thickness measured serially over 72 hr using an engineer's micrometer (Mitotuoyo, Tokyo, Japan).

For IgE reconstitution, TIB142 IgE (anti-TNP) hollow-fiber culture supernatant or affinity-purified SPE-7 IgE (anti-DNP, Sigma Chemical, St. Louis, MO), affinity-purified C38-2 IgE (anti-TNP, Pharmingen, San Diego, CA), or culture supernatant containing OVA-specific IgE secreted by the hybridoma TOε, gift of Dr. M. Kiniwa (Sawada et al., 1997), was injected via tail vein immediately prior to sensitization or to hapten challenge.

### Nonspecific Irritant Responses

Of 1% croton oil in olive oil, 10 μl was applied to the anterior and posterior ear surfaces.

### Cellular Passive Transfer

Splenocytes were prepared 5 days postsensitization and $1 \times 10^7$ cells were injected i.v. in 100 μl into recipients that were then immediately challenged with oxazolone. There was no detectable IgE in $IgE^{-/-}$ recipients of wild-type cells after 5 days.

### Dendritic Cell Migration Analysis

Epidermal ear sheets were prepared as described (Cumberbatch et al., 2001) and stained with FITC anti-mouse I-A/I-E (BD Pharmingen, San Diego, CA). Sheets from each mouse were studied by fluorescent microscopy. Four individual fields from each ear were enumerated, and the mean numbers of dendritic cells ± SEM (I-A/I-E positive, dendritic morphology) were calculated.

### Serum IgE Levels

Serum IgE levels were measured using a sandwich ELISA with paired antibodies from BD Pharmingen (San Diego, CA).

### Histology

Ear sections (5 μm) were stained with hematoxylin and eosin. For mast cell quantification, skin samples were fixed in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% $CaCl_2$ in 0.1 M sodium cacodylate buffer [pH 7.3]), paraffin embedded, cut into 3 μM sections, and stained with Giemsa.

### Real-Time Quantitative RT-PCR

Total RNA was extracted from 10–30 mg mouse skin tissue (RNeasy fibrous tissue mini kit, Qiagen, Valencia, CA). cDNA (superscript II RNase H-reverse transcriptase kit, Invitrogen, Carlsbad, CA) was analyzed by PCR on an ABI Prism 7700 (Applied Biosystems, Foster City, CA). Each 20 μl reaction consisted of 1 μL cDNA, 0.5 U AmpliTaq gold enzyme (Applied Biosystems), 1× PCR gold buffer, 1× ROX dye (Ambion, Austin, TX), 400 μM dNTP, 4 mM $MgCl_2$, 1:40000 Sybr green dye (Molecular Probes, Eugene, OR), and 200 nM of each forward (F) and reverse (R) primer. For IL-6-labeled Taqman probe, 2× Taqman universal PCR master mix was used as directed (Applied Biosystems).

Primers were: TNFα, F 5′-cccacgtcgtagcaaacc-3′, R 5′-gcagccttgtcccttgaa-3′; MCP-1, F 5′-ctcagccagatgcagttaacg-3′, R 5′-ttgggatcatcttgctggtg-3′; MMCP-6, F 5′-cctctgaagcaagtgaaggttcc-3′, R 5′-gaatggctggcaggagtctctc-3′; IL-18, R 5′-ggttcactggcactttgat-3′, F 5′-acaactttggccgacttcac-3′; IL-1β, F 5′-caggcaggcagtatcactca-3′, R 5′-aggtgctcatgtcctcatcc-3′; β actin 135, F 5′-gctacagcttcaccaccacag-3′, R 5′-gctcatagctcttctccaggg-3′; β actin 152, R 5′-tgtggtgccagatcttctc-3′, F 5′-taggcaccagggtgtgatg-3′. For IL-6, primers and FAM-labeled probe were from Applied Biosystems.

Conditions for TNFα, MCP-1, IL-6, and β actin 135 were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min, immediately followed by melt curve analysis. Conditions for IL-18, IL-1β, MMCP-6, and β actin 152 were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 30 s. The relative gene expression among samples was determined

(Pfaffl, 2001). Efficiencies of each primer set used in the fold induction equation were obtained over five log dilutions of mouse skin cDNA at the appropriate PCR cycling temperatures for the primers to allow accurate analysis for each gene.

### Statistical Analysis

Differences in values for various experimental groups were examined for significance using the two-tailed Student's t test or by analysis of variance by two-tailed ANOVA using GraphPad Prism® software. All data are shown as the mean response ± SEM.

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