

An obligate role for T-cell receptor $\alpha\beta^+$ T cells but not T-cell receptor $\gamma\delta^+$ T cells, B cells, or CD40/CD40L interactions in a mouse model of atopic dermatitis

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Background: We recently described a murine model of atopic dermatitis (AD) elicited by epicutaneous sensitization with ovalbumin (OVA). The skin lesions in these mice were characterized by a dermal infiltrate consisting of eosinophils and T cells and by increased expression of the T_H2 cytokines IL-4 and IL-5. Epicutaneous sensitization induces a rise in the levels of serum total IgE and OVA-specific antibodies, further indicating that it elicits a predominantly T_H2 response.

Objective: This study was undertaken to assess the roles of T cells, B cells, and CD40L-CD40 interactions in AD.

Methods: Mice with targeted gene deletions were sensitized with OVA. Histologic and immunohistochemical examinations, as well as measurements of IL-4 mRNA, were performed on OVA-sensitized skin. Total and antigen-specific serum IgE levels were determined.

Results: RAG2^{-/-} mice, which lack both T and B cells, did not exhibit cellular infiltration, induction of dermal IL-4 mRNA, or elevation of serum IgE after OVA sensitization; all of these features were present in B-cell-deficient IgH^{-/-} mice. T-cell receptor α^- mice did not display cellular infiltration, IL-4 mRNA expression, or increased IgE levels after OVA sensitization, but these responses were elicited in T-cell receptor δ^- mice after sensitization. Absence of CD40 had no effect on these responses.

Conclusion: These results suggest that $\alpha\beta$ T cells, but not $\gamma\delta$ T cells, B cells, or CD40L-CD40 interactions, are critical for skin inflammation and the T_H2 response in AD. (J Allergy Clin Immunol 2001;107:359-66.)

Key words: Atopic dermatitis, $\alpha\beta$ T cells, $\gamma\delta$ T cells, B cells, CD40L, CD40

Abbreviations used

AD: Atopic dermatitis
EC: Epicutaneous
HPF: High-power field
OVA: Ovalbumin
TCR: T-cell receptor
WT: Wild type

Atopic dermatitis (AD) is a chronic relapsing inflammatory disease of the skin often associated with elevated levels of total and antigen-specific IgE.¹ T cells and eosinophils are thought to play a major role in the pathogenesis of AD. Skin lesions in AD contain a mononuclear cell infiltrate, predominantly in the dermis, consisting of activated memory CD4⁺ T cells and macrophages.¹ The occasional presence of eosinophils in the dermis and the common finding of eosinophil-derived major basic protein and eosinophil cationic protein, which are associated with eosinophil degeneration, are indicative of eosinophil involvement in AD.^{2,3} There is a significant increase in the number of cells expressing the T_H2 cytokines IL-4, IL-5, and IL-13 in the acute skin lesions of AD.^{4,5} Cells expressing the T_H1 cytokine IFN- γ are also found in affected skin of chronic AD.⁶

We recently described a murine model of AD elicited by repeated epicutaneous (EC) sensitization with ovalbumin (OVA).⁷ The skin lesions exhibited significant dermal and epidermal thickening, a cellular infiltrate consisting of eosinophils and T cells, and consistently elevated levels of IL-4 and IL-5 mRNA in 4 strains tested to date, Balb/C, C57BL/6, 129Sv, and WBB6F1.^{7,8} IFN- γ mRNA is variable, being elevated, unchanged, or decreased in the different strains tested.^{7,8} These findings, together with a rise in total serum IgE level and the induction of antigen-specific IgE antibodies,⁷ strongly indicate that EC sensitization with antigen elicits a predominantly T_H2 response. Studies with mice deficient in cytokines have demonstrated that IL-4 and IL-5 are critical for eosinophil infiltration of the skin in our model, whereas IL-5 and IFN- γ may play a role in skin hypertrophy.⁸

T cells differentiate along 2 major pathways to generate T-cell receptor (TCR) $\alpha\beta$ -bearing cells and TCR $\gamma\delta$ -bearing cells.^{9,10} Both cell types reside in normal mouse skin^{11,12} and both can secrete a wide array of cytokines.¹³

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B cells may also play a role in AD either through secreted Igs, including IgE, or as cells that focus antigen for presentation to T cells. Finally, interaction between CD40L expressed on activated T cells and CD40 expressed on antigen-presenting cells, including macrophages, B cells, and endothelial cells, is thought to play an important role in local immune responses and in tissue inflammation.^{14,15}

We used knockout mice to investigate the role of $\alpha\beta$ versus $\gamma\delta$ T cells, B cells, and CD40L-CD40 interactions in our T_H2 -dominated model of allergic dermatitis. The results indicate that $\alpha\beta$ T cells, but not $\gamma\delta$ T cells, B cells, or CD40/CD40L interactions, are critical for the eosinophilic cutaneous inflammation and the T_H2 responses induced by EC sensitization with allergen.

METHODS

Mouse sensitization

$TCR\alpha^{-/-}$, $TCR\delta^{-/-}$, $IgH^{-/-}$, and F2 (129Sv $\times$ C57BL/6) mice were obtained from Jackson Laboratories (Bar Harbor, Me). C57BL/6 mice were purchased from Taconic Laboratories (Germantown, NY). $RAG2^{-/-}$ mice were donated by Dr Fred Alt (Boston, Mass).¹⁶ The $CD40^{-/-}$ mice were generated in our laboratory as previously described.¹⁷ The $IgH^{-/-}$, $TCR\alpha^{-/-}$, and $TCR\delta^{-/-}$ mice are on C57BL/6 background. The $RAG2^{-/-}$ and $CD40^{-/-}$ mice are on 129Sv $\times$ C57BL/6 background. All mice were kept in a pathogen-controlled environment. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of Children's Hospital, Boston.

Epicutaneous sensitization of 4- to 6-week-old mice was performed as previously described.⁷ In brief, mice were shaved with an electric razor. The shaved area was then tape stripped 3 times with transparent bio-occlusive dressing (Johnson and Johnson Medical Inc, Arlington, Tex) to remove loose hair and to introduce standardized skin injury. One hundred micrograms of OVA (Grade V; Sigma Chemical Co, St Louis, Mo) in 100 μ L of normal saline solution or placebo (100 μ L of normal saline solution) was placed on a 1 $\times$ 1-cm patch of sterile gauze. The gauze was secured to the skin with the same transparent bio-occlusive dressing. The patches were placed for a 1-week period and then removed. Two weeks later, an identical patch was reapplied to the same skin site. Each mouse had a total of three 1-week exposures to the patch separated by 2-week intervals.

Antibodies

Rat and hamster mAbs were used for immunohistochemical analysis and were directed against mouse CD3e (145-2C11), CD4 (H129.19), CD8a (53-6.7), and CD45 (30F11.1). mAbs used in OVA-specific ELISA were biotin-conjugated rat antibodies to mouse IgE (clone R35-92). IgE mAb clone R35-72 and clone R35-92 were used for detection of total IgE. All antibodies were purchased from PharMingen (San Diego, Calif).

ELISA

Sera were obtained 1 day after the completion of epicutaneous sensitization. The standard PharMingen protocol for sandwich ELISA was used to quantify the total amount of IgE in serum, with clone R35-72 used for coating and biotin-conjugated clone R35-92 used for detection of IgE as previously described.⁷ IgE OVA-specific antibodies were measured as previously described.⁷ Results are expressed as ELISA titers relative to an internal isotype standard run in each assay.⁷

Histologic analysis

Specimens were obtained from patched areas on the skin 24 hours after the patch from the third sensitization was removed. Multiple 4-mm sections of formalin-fixed skin were stained with standard hematoxylin-eosin. Individual inflammatory cell types were counted in the dermis in 10 to 20 high-power fields (HPFs) at a magnification of 1000 $\times$ by an individual (A.L.W.) blinded to the identity of the sections and were expressed as cells per HPF. Sections from 4 to 8 mice were counted for each strain. The mouse-to-mouse variability in infiltrating cells was small. In each experiment, statistical significance of differences in data were established with the Student *t* test. *P* values < .05 were considered significant.

Immunohistologic analysis

Skin sections were embedded in Tissue-Tek O.C.T. compound (Miles Inc, Elkhart, Ind) on dry ice. Four-micrometer sections were stained by an avidin-biotin complex method as described previously.⁷ Stained cells were counted in 10 to 20 HPFs at a magnification of $\times$ 400.

RNA preparation, cDNA synthesis, and PCR amplification of reverse transcribed cDNA

Skin biopsy specimens obtained 24 hours after completion of sensitization were homogenized in Trizol (Gibco-BRL, Grand Island, NY) with a Polytron RT-3000, and RNA was prepared according to the standard protocol.

cDNA was synthesized from 10 μ g of total RNA in 50- μ L of reaction mixture with Superscript II (Gibco-BRL) for 90 minutes at 37°C. The primers used to amplify cDNA for β_2 -microglobulin and IL-4 were as previously described.⁷ To quantify mRNA, we coamplified a fixed amount of reverse-transcribed cellular mRNA in the presence of serial dilutions of a multispecific internal plasmid control, pMUS, which contained nucleotide sequences of multiple cytokines.⁷ The results were expressed as a ratio of cytokine cDNA to cDNA of the constitutively expressed β_2 -microglobulin gene.

RESULTS

T cells, but not B cells, are required for infiltration of OVA-sensitized skin sites with mononuclear cells and eosinophils and for IL-4 expression in sensitized skin

We have previously shown that EC sensitization with OVA induces a cellular infiltrate in the dermis containing T cells and eosinophils, significant thickening of the dermis and epidermis, as well as a vigorous humoral response evidenced by the production of antigen-specific IgE.⁷ To assess the roles of cellular and humoral immunity in the cutaneous response to allergen, we studied $RAG2^{-/-}$ mice, which lack B and T cells.¹⁶ $RAG2^{-/-}$ mice failed to display dermal infiltration by eosinophils or mononuclear cells after OVA sensitization (Fig 1 and Fig 2, A and B). Furthermore, there was no increase in the level of IL-4 mRNA expression in OVA-sensitized skin sites of $RAG2^{-/-}$ mice (Fig 2, C). In contrast and as previously reported,⁸ EC sensitization with OVA elicited a dermal inflammatory response consisting of eosinophils and mononuclear cells and caused increased IL-4 mRNA expression in sensitized skin sites of wild-type (WT) 129Sv $\times$ C57BL/6 controls (Figs 1 and 2). As expected, no serum IgE was detected in either saline- or OVA-

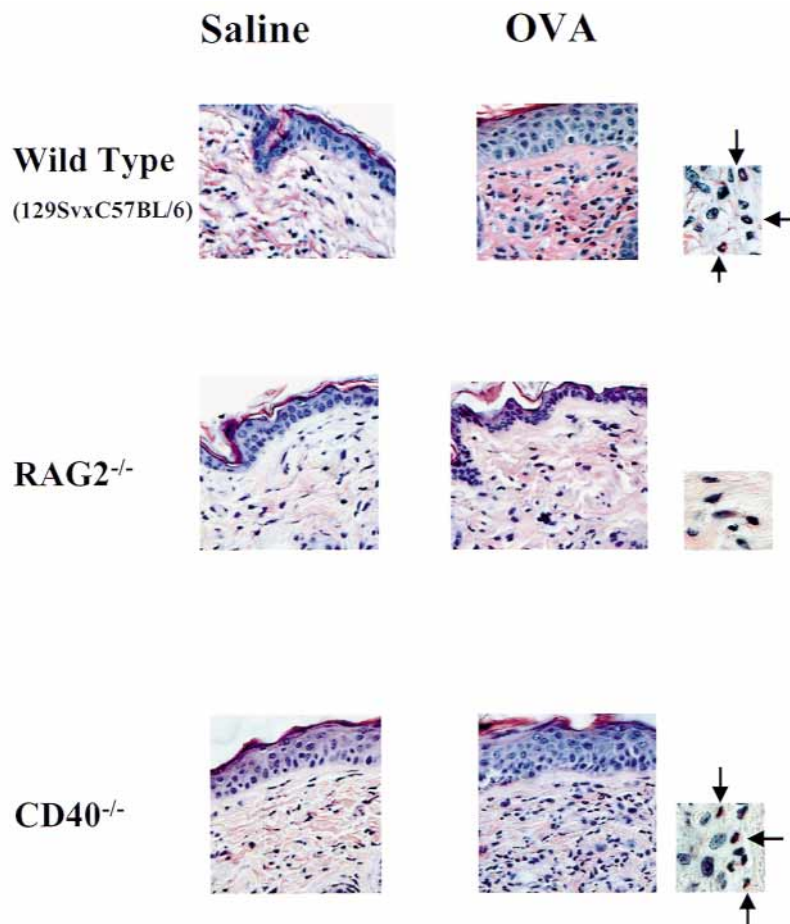


FIG 1. Histologic features of saline-sensitized and OVA-sensitized skin sites in $RAG2^{-/-}$ and $CD40^{-/-}$ mice compared with their WT (129Sv $\times$ C57BL/6) controls. Skin sections were stained with standard hematoxylin-eosin. Magnification for *large panels*: 400 $\times$; *small panels*: 600 $\times$. Arrows indicate eosinophils. $RAG2^{-/-}$ mice do not exhibit an increase in dermal eosinophils and total cellular infiltrate after OVA sensitization, as is seen in WT mice. $CD40^{-/-}$ mice exhibit an increase in dermal eosinophils and total cellular infiltrate after OVA sensitization comparable to that observed in WT mice.

sensitized $RAG2^{-/-}$ mice, whereas as previously reported,⁸ there was a rise in the total serum IgE level and development of OVA-specific IgE antibodies in WT controls (data not shown).

$IgH^{-/-}$ mice lack mature B cells but have a normal complement of T cells.¹⁸ After OVA sensitization $IgH^{-/-}$ mice developed a dermal infiltrate of eosinophils and mononuclear cells equivalent to those observed in WT C57BL/6 controls (Fig 3 and Fig 2, A and B). In addition, the numbers of CD45⁺, CD3⁺, CD4⁺, and CD8⁺ cells in the sensitized skin of $IgH^{-/-}$ mice were equivalent to those in identically sensitized WT controls (data not shown). Moreover, IL-4 mRNA was induced in the skin of OVA-sensitized $IgH^{-/-}$ mice to the same degree as observed in WT controls (Fig 2, C). There was no detectable serum Ig in either saline- or OVA-sensitized $IgH^{-/-}$ mice, whereas as previously reported,⁸ there was a rise in the total serum IgE level and development of OVA-specific IgE antibodies in WT C57BL/6 controls (data not shown).

Taken together, these results suggest that T but not B lymphocytes are essential for the development of skin inflammation and for increased cutaneous expression of IL-4 in our model of allergic dermatitis.

$\alpha\beta$ T cells, but not $\gamma\delta$ T cells, are required for infiltration of OVA-sensitized skin with eosinophils and mononuclear cells and for IL-4 expression in sensitized skin

T cells differentiate along 2 major pathways to generate TCR $\alpha\beta$ -bearing cells and TCR $\gamma\delta$ -bearing cells; both cell types reside in normal mouse skin.¹⁰ Given our observation that T cells are essential for the development of skin inflammation after allergen exposure, we set out to determine the relative contribution of $\alpha\beta$ T and $\gamma\delta$ T cells to the skin lesion induced by EC sensitization with OVA. To this purpose, mice deficient in either TCR $\alpha\beta$ or TCR $\gamma\delta$ cells were used in our model.

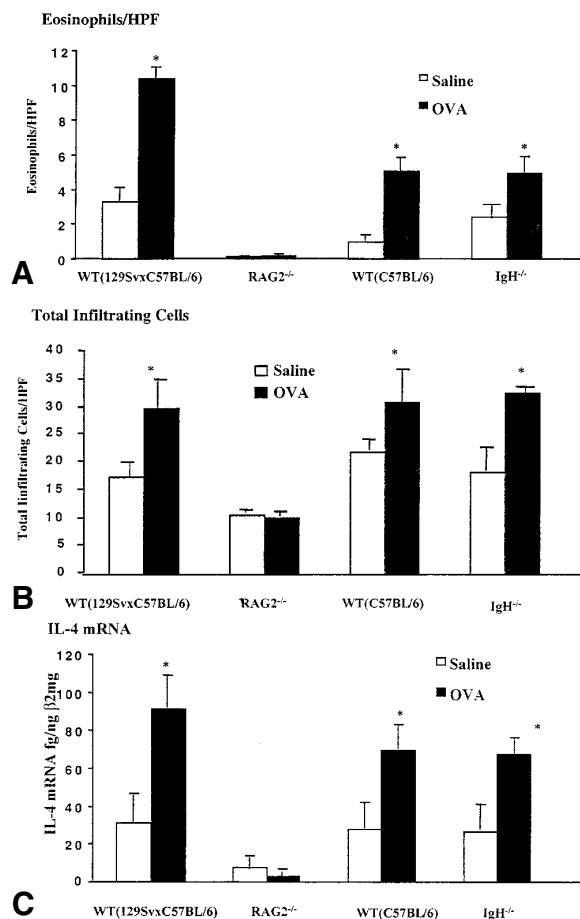


FIG 2. Histologic features and IL-4 mRNA expression in saline and OVA-sensitized skin sites from RAG2^{-/-}, IgH^{-/-}, and WT control mice. **A**, Eosinophils/HPF at 1000× magnification. **B**, Infiltrating mononuclear cells/HPF at 1000× magnification. Mean values from 10 to 20 HPFs per animal are shown (n = 4-8 animals per strain). **C**, IL-4 mRNA levels as a ratio of IL-4 cDNA to cDNA of the constitutively expressed β₂-microglobulin. Columns and bars represent mean values ± SE (n = 4-8 animals per strain). *P < .05.

Saline-sensitized skin of TCRα^{-/-} mice had significantly higher numbers of eosinophils and mononuclear cells than that of WT controls ($P = .00168$ for eosinophils, $P = .044$ for mononuclear cells). However, OVA-sensitized skin sites from TCRα^{-/-} mice failed to exhibit an increase in dermal infiltration with eosinophils or mononuclear cells after OVA sensitization (Fig 3 and Fig 4, A and B). In addition, there was no detectable increase of IL-4 mRNA in OVA-sensitized skin sites of TCRα^{-/-} mice (Fig 4, C). In contrast to WT controls, TCRα^{-/-} mice failed to increase their total serum IgE levels and failed to develop OVA-specific IgE antibodies after OVA sensitization (Fig 5).

TCRδ^{-/-} mice exhibited dermal infiltration with eosinophils and mononuclear cells after OVA sensitiza-

tion similar to that observed in WT controls (Fig 3 and Fig 4, A and B). In addition, the numbers of CD45⁺, CD3⁺, CD4⁺, and CD8⁺ cells in the skin of TCRδ^{-/-} mice showed a rise after sensitization as seen in WT controls (data not shown). Furthermore, IL-4 mRNA expression was induced in OVA-sensitized skin sites of TCRδ^{-/-} mice to an extent similar to that of WT controls (Fig 4, C). TCRδ^{-/-} mice responded to EC sensitization with a rise in serum IgE level and development of OVA-specific IgE antibody titers similar to those observed in WT controls (Fig 5).

The above results indicate that αβ T cells, but not γδ T cells, are essential for the development of skin inflammation, increased cutaneous IL-4 expression, and production of antigen-specific IgE levels.

CD40L-CD40 interactions are not necessary for infiltration of OVA-sensitized skin sites with eosinophils and mononuclear cells or for IL-4 expression in sensitized skin

Interaction between CD40L expressed on activated T cells and CD40 expressed on endothelial cells, epithelial cells, tissue macrophages, and other resident cells is thought to play an important role in tissue inflammation.¹⁴ To assess the role of CD40L-CD40 interactions in our model of allergic dermatitis, we studied mice deficient in CD40.

OVA-sensitized skin sites from CD40^{-/-} mice exhibited significant infiltration with eosinophils and mononuclear cells, which was comparable to that of WT controls (Fig 1 and Fig 6, A and B). Furthermore, there was significantly increased expression of IL-4 mRNA in OVA-sensitized skin sites of CD40^{-/-} mice to an extent similar to that of WT (Fig 6, C). As expected, total serum IgE and antigen-specific IgE remained undetectable in CD40^{-/-} mice after OVA sensitization (data not shown).

The above results suggest that CD40L-CD40 interactions are not essential for the development of skin inflammation and increased cutaneous IL-4 expression after epicutaneous sensitization with protein antigen.

DISCUSSION

This study demonstrates an obligate role for αβ T cells, but not γδ T cells, B cells, or CD40/40L interaction for eosinophilic skin inflammation, increased cutaneous IL-4 expression, and IgE antibody response after EC sensitization with antigen in a murine model of AD.

Immunocompetent WT mice sensitized epicutaneously with the antigen OVA develop skin lesions exhibiting significant infiltration of the dermis by mononuclear cells and eosinophils, as well as consistently increased expression of the T_H2 cytokines IL-4 and IL-5. Thus the hallmark of our model of allergic skin inflammation is the development of a T_H2 response. We used knockout mice to investigate the role of T cells, B cells, and CD40L-CD40 interactions in our T_H2-dominated model of allergic dermatitis. Because IL-5 is required for

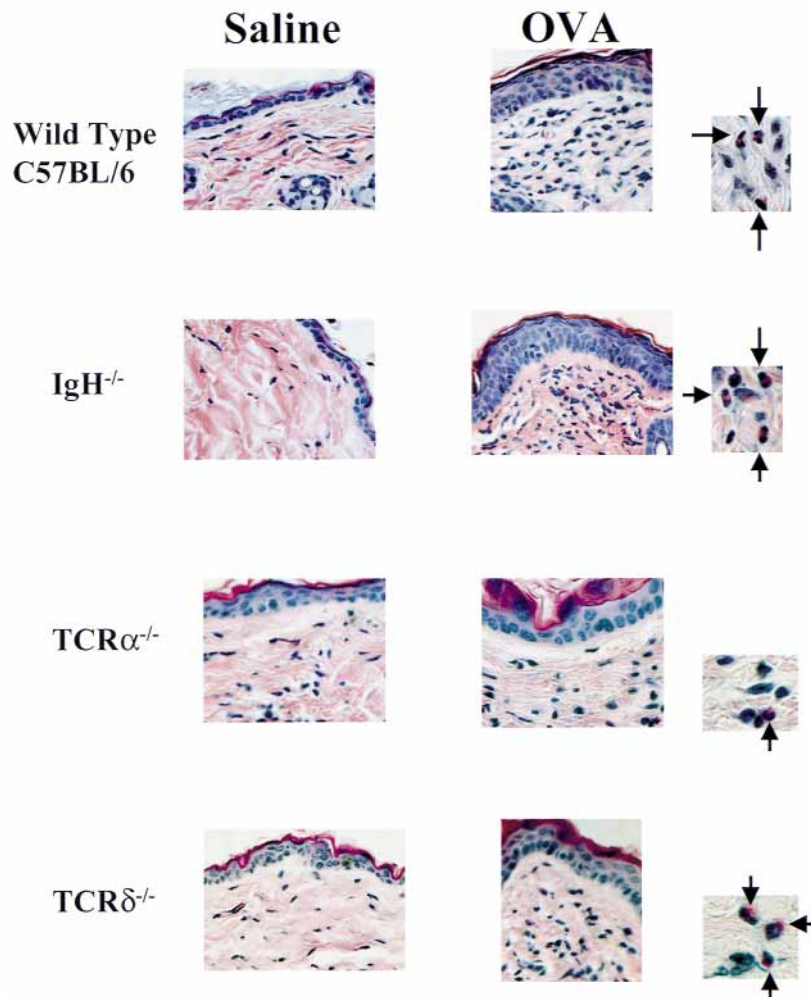


FIG 3. Histologic features of saline-sensitized and OVA-sensitized skin sites in $IgH^{-/-}$, $TCR\alpha^{-/-}$, and $TCR\delta^{-/-}$ mice compared with their WT (C57BL/6) controls. Skin sections were stained with standard hematoxylin-eosin. Magnification for *large panels*: 400 $\times$; *small panels*: 600 $\times$. Arrows indicate eosinophils. $IgH^{-/-}$ mice and $TCR\delta^{-/-}$ mice exhibit an increase in dermal eosinophils and total cellular infiltrate after OVA sensitization comparable to that observed in WT mice. $TCR\alpha^{-/-}$ mice do not exhibit an increase in dermal eosinophils and total cellular infiltrate after OVA sensitization, as is seen in WT mice.

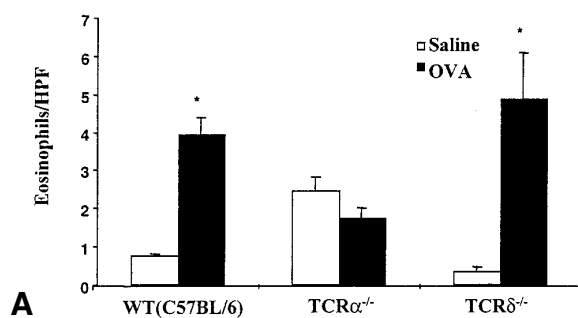
eosinophilia¹⁹ and our previous studies have shown IL-5 to be required for the skin eosinophilia in our model,⁸ we used skin eosinophilia as an indirect measure of IL-5 expression and limited our measurement of T_H2 cytokine mRNA expression in the skin to IL-4. In contrast to the consistently elevated expression of IL-4 and IL-5, expression of the T_H1 cytokine IFN- γ in the skin in our model was variable and depended on the strain used.^{7,8} We did, however, measure the levels of IFN- γ mRNA in the skin and found them to be similar in $IgH^{-/-}$, $TCR\delta^{-/-}$, and $CD40^{-/-}$ mice and their genetically identical WT controls and to be absent in $RAG2^{-/-}$ and $TCR\alpha^{-/-}$ mice (data not shown).

$RAG2^{-/-}$ mice, which lack both T and B cells, did not exhibit an inflammatory dermal infiltrate or increased IL-4 mRNA expression after EC sensitization with OVA. The detection of IL-4 mRNA in the saline-sensitized skin of

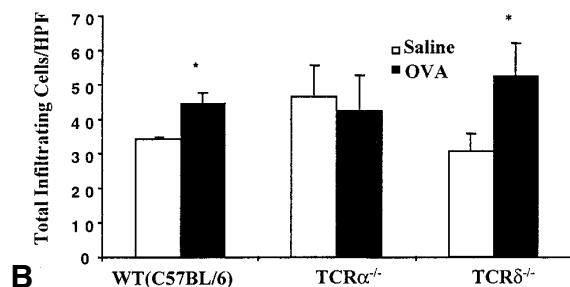
$RAG2^{-/-}$ mice suggests a source of IL-4 other than T cells. A good candidate is the mast cell, which is capable of expressing IL-4. This is supported by our recent observation that IL-4 mRNA is detectable in the undisturbed skin of WT controls but not in that of mast cell-deficient W/W^v mice (Alenius et al, unpublished observations, 2000).

In contrast to $RAG2^{-/-}$ mice, $IgH^{-/-}$ mice, which lack mature B cells and are incapable of producing antibodies but have a normal complement of T cells, developed eosinophilic skin lesions and increased in IL-4 mRNA expression equivalent to those observed in WT mice. This is consistent with observations in models of allergic airway inflammation, where normal eosinophilic inflammatory responses have been observed in the lungs of B-cell-deficient mice after sensitization and challenge with OVA.²⁰ Taken together, our observations in $RAG2^{-/-}$ and $IgH^{-/-}$ mice indicate that T cells, but not B cells, are

Eosinophils/HPF



Total Infiltrating Cells



IL-4

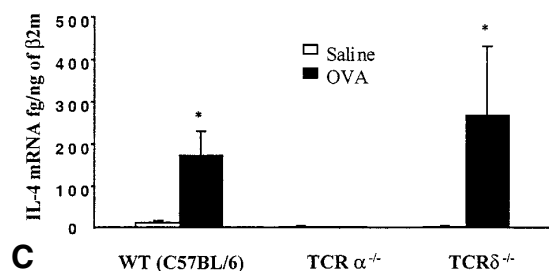
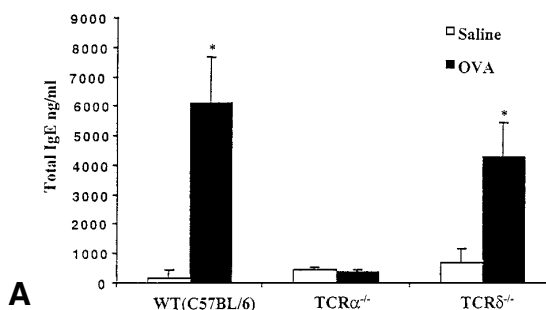


FIG 4. Histologic features and IL-4 mRNA expression in saline- and OVA-sensitized skin sites from TCR $\alpha^{-/-}$ and TCR $\delta^{-/-}$ mice compared with WT controls. Sections are stained with hematoxylin-eosin. **A**, Eosinophils/HPF at $\times 1000$ magnification. **B**, Infiltrating mononuclear cells/HPF at $\times 1000$ magnification. **C**, IL-4 mRNA levels. Columns and bars represent mean values $\pm$ SE ($n = 4-8$ animals per strain). * $P < .05$.

required for the development of cutaneous allergic inflammation.

Because both $\alpha\beta$ and $\gamma\delta$ T cells normally reside in mouse skin,^{11,12} we studied mice deficient in the individual T-cell populations to determine their role in the development of cutaneous inflammation in our model. Our data indicate that $\alpha\beta$ T cells, but not $\gamma\delta$ T cells, are critical for the development of skin lesions in our model. TCR $\alpha^{-/-}$ mice did not exhibit an increase in the inflammatory cellular infiltrate in the dermis or an increase in IL-4 mRNA expression as observed in WT mice. TCR $\alpha^{-/-}$ also failed to raise antigen-specific antibodies after epicutaneous exposure to OVA. In contrast, TCR $\delta^{-/-}$ mice exhibited

Total IgE



Anti-OVA IgE

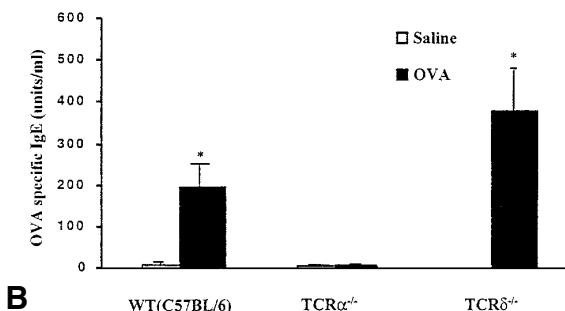


FIG 5. Serum IgE levels (**A**) and serum IgE anti-OVA (**B**) in TCR $\alpha^{-/-}$ and TCR $\delta^{-/-}$ and WT controls. Columns and bars represent mean values $\pm$ SE ($n = 4-8$ animals per strain). * $P < .05$.

dermal infiltrate, exhibited increased IL-4 mRNA levels, and mounted antigen-specific antibody responses equivalent to those observed in WT mice.

TCR $\alpha^{-/-}$ mice are known to have a unique population of CD4⁺ T cells that express the TCR β chain in the absence of the TCR α chain.²¹ This population responds to superantigens but fails to respond to conventional antigens such as OVA.²² Our results indicate that this population is not sufficient to produce a dermal inflammatory response to OVA, thereby suggesting that a full complement of functional TCR $\alpha\beta$ T cells is necessary to develop the skin lesions in our model. The presence of increased numbers of mononuclear cells and eosinophils in the saline-sensitized skin of TCR $\alpha^{-/-}$ mice may reflect increased susceptibility of the TCR $\alpha^{-/-}$ mice to developing inflammatory reactions in the skin.

$\gamma\delta$ T cells have been shown to produce T_H1- and T_H2-type cytokines,²³ and it has been reported that $\gamma\delta$ T cells are necessary for the development of allergic airway inflammation in a model in which mice are sensitized intraperitoneally and then challenged intranasally with OVA.²⁴ $\gamma\delta$ T cells have also been implicated in negative regulation of airway hyperresponsiveness.²⁵ In the origi-

nal description of our model, we reported significantly lower levels of antigen-specific IgE in mice sensitized to OVA by intraperitoneal injection compared with mice sensitized by epicutaneous exposure,⁷ suggesting a fundamental difference in the inflammatory response elicited by the 2 routes of sensitization. The fact that $\gamma\delta$ T cells are not necessary in our model of EC sensitization, but have been found to be necessary in a system of intraperitoneal sensitization,²⁴ suggests differences in the response to the intraperitoneal versus the EC route of sensitization.

Recent studies regarding the role of CD40 and CD40L in mouse asthma models have yielded discrepant results. Hogan et al,²⁶ using CD40^{-/-} mice, found no difference in bronchoalveolar lavage eosinophilia and airway hyperresponsiveness in knockout mice compared with WT controls. In contrast, Lei et al,²⁷ using CD40L^{-/-} mice sensitized to OVA through intraperitoneal injection, found decreased inflammatory cellular infiltrate in both bronchoalveolar lavage and histologic sections of the lungs of knockout versus WT control mice.²⁷ Our own studies show that neither CD40 nor CD40L is necessary for pulmonary eosinophilia in response to inhaled sensitization to *Aspergillus fumigatus* via the intranasal route; however, CD40L but not CD40 was found to be necessary for induction of bronchial hyperreactivity.²⁸

Because of these discrepant reports in the literature, we studied CD40^{-/-} mice in our model of AD. CD40-CD40L interactions are necessary for isotype switching to IgE as well as for the activation of inflammatory cells that include monocytes, dendritic cells, and endothelial cells.¹⁴ Our previous studies have shown that IgE^{-/-} mice develop the same degree of skin inflammation as WT mice.⁸ We therefore did not expect the absence of IgE by itself in CD40^{-/-} mice to result in a diminished cutaneous inflammatory response in these mice. Our findings indicate that CD40-CD40L interactions are not necessary for the development of eosinophilic dermal inflammation or increased IL-4 mRNA expression in our model of EC. This suggests that CD40 ligation on endothelial and inflammatory cells is not essential for the development of an allergic skin response. The difference in the requirement of CD40-CD40L interactions between different routes of sensitization (eg, intraperitoneal vs inhalational vs EC) suggests that there may be important differences in allergic sensitization through different routes that are yet to be elucidated.

This study provides new insights into the pathogenesis of AD and may therefore suggest future avenues for therapeutic investigation. We have found that neither B cells nor CD40-CD40L interactions are necessary for the development of skin lesions in our model. Because B cells are required for Ig production and CD40-CD40L interactions are necessary for Ig isotype switching, our current results suggest that IgE is not necessary for the development of skin lesions in our model, which are characterized by dermal cellular infiltrate and induction of IL-4 mRNA. This complements our previous findings that IgE^{-/-} mice develop skin lesions identical to those seen in their WT controls after OVA sensitization.⁸ Taken

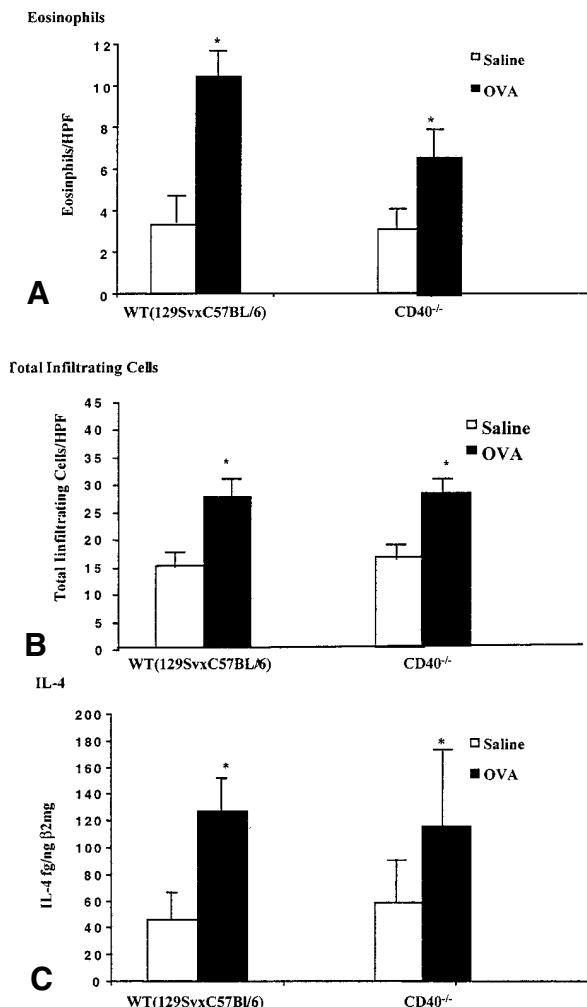


FIG 6. Histologic features and IL-4 mRNA levels of OVA-sensitized skin sites from CD40^{-/-} mice and WT controls. **A**, Eosinophils/HPF at $\times 1000$ magnification. **B**, Infiltrating mononuclear cells/HPF at $\times 1000$ magnification. **C**, IL-4 mRNA levels. Columns and bars represent mean values $\pm$ SE ($n = 4-8$ animals per strain). * $P < .05$.

together, these investigations indicate that allergen-induced cellular infiltration and IL-4 production in skin lesions arise by IgE-independent mechanisms and suggest that anti-IgE therapies may not prove to be effective in modulating these aspects of AD. On the other hand, our current study established that TCR $\alpha\beta$ T-cells are necessary for the development of skin lesions in our model. This suggests that therapies that target T cells, such as topical tacrolimus, may prove to be efficacious in the treatment of AD.

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