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Mast cells are required for full expression of allergen/SEB-induced skin inflammation

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Abstract

Atopic dermatitis is a chronic pruritic inflammatory skin disease. We recently described an animal model in which repeated epicutaneous applications of a house dust mite extract and staphylococcal enterotoxin B induced eczematous skin lesions. In this study we showed that global gene expression patterns are very similar between human atopic dermatitis skin and allergen/staphylococcal enterotoxin B-induced mouse skin lesions, particularly in expression of genes related to epidermal growth/differentiation, skin-barrier, lipid/energy metabolism, immune response, or extracellular matrix. In this model, mast cells and T cells, but not B cells or eosinophils, were shown to be required for the full expression of dermatitis, as revealed by reduced skin inflammation and reduced serum IgE levels in mice lacking mast cells or T cells (*TCRβ*$^{-/-}$ or *Rag1*$^{-/-}$). The clinical severity of dermatitis correlated with the numbers of mast cells, but not eosinophils. Consistent with the idea that Th2 cells play a predominant role in allergic diseases, the receptor for the Th2-promoting cytokine thymic stromal lymphopoietin and the high-affinity IgE receptor, FcεRI, were required to attain maximal clinical scores. Therefore,

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Conflict of Interest

The authors state no conflict of interest.

this clinically relevant model provides mechanistic insights into the pathogenic mechanism of human atopic dermatitis.

Keywords

atopic dermatitis; T cell; mast cell; house dust mite; staphylococcal enterotoxin B; FcεRI; TSLP; GM-CSF

Introduction

Atopic dermatitis (AD), or eczema, is a chronic or chronically relapsing, pruritic inflammatory skin disease. The etiology of this disease is incompletely understood, but it is multifactorial and the disease is manifested by complex interactions between genetic and environmental factors (Bieber, 2008; Boguniewicz and Leung, 2011). Pathological examination reveals hyperkeratosis, spongiosis, and parakeratosis in acute lesions and marked epidermal hyperplasia and perivascular accumulation of lymphocytes and mast cells in chronic lesions. Immunological abnormalities of AD are characterized by sensitization with various allergens (e.g., foods, aeroallergens, microbes, and autoallergens), high serum IgE levels, and skin lesions with apoptotic keratinocytes and infiltration with immune cells such as $CD4^+$ T cells, eosinophils, and mast cells. These T cells express IL-4, IL-5, and IL-13 (Grewe *et al*., 1998), and numerous studies suggest an association between AD development and T helper 2 (Th2) cell skewed immune responses. However, there are also data suggesting that AD development is independent of IgE, but correlates with an increase in interferon (IFN)-γ-producing Th1 cells (Thepen *et al*., 1996; Tsicopoulos *et al*., 1994; Werfel *et al*., 1996). Thus, as Irvine et al have stated, AD was considered for many years to be primarily immunologically driven disease with secondary barrier defect (the so-called insideoutside hypothesis). By contrast, some investigators had hypothesized that the primary defect is in the skin barrier (the outside-inside hypothesis) (Irvine *et al*., 2011). Various loss-of-function mutations in the *FLG* gene encoding filaggrin, a key protein for formation of the skin barrier, were recently found in a substantial proportion of AD patients (Palmer *et al*., 2006; Sandilands *et al*., 2007) and *flaky tail* mutant mice (Fallon *et al*., 2009). Furthermore, tight junction proteins claudin-1 and claudin-23 are reduced in AD patients. Silencing of claudin-1, whose expression is inversely correlated with Th2 biomarkers, in human keratinocytes diminishes tight junction function (De Benedetto *et al*, 2011). Thus, strong association of *FLG* mutations with AD and other studies have validated the outside-inside hypothesis (De Benedetto *et al*, 2012; Irvine *et al*, 2011). However, *FLG* mutations predispose subjects to allergen sensitization but these mutations are not sufficient for causing AD, as other genetic and environmental influences likely promote the Th2 immune response in susceptible individuals.

A number of mouse AD models have been developed over the last fifteen years, and have provided mechanistic insights into the pathogenesis of human AD (Gutermuth *et al*., 2004; Jin *et al*, 2009; Kawakami *et al*, 2009a). For example, a mouse model induced by epicutaneous (EC) sensitization with ovalbumin (OVA) mimicked skin lesions of human AD characterized by epidermal and dermal thickening, infiltration of $CD4^+$ T cells and

eosinophils, and local expression of mRNAs for IL-4, IL-5 and IL-13 (Spergel *et al*, 1998). Dermatitis in this model required αβ T cells, but not B cells or mast cells (Alenius *et al*, 2002; Woodward *et al*, 2001). Different roles of IL-4, IL-5, IL-10, IL-17, IFN-γ, chemokine receptors, complement components and complement receptors in this model were demonstrated using gene-manipulated mice (Jin *et al*, 2009). We also developed a highly reproducible mouse model that mimicked human AD, in which skin inflammation was induced by repeated treatments of *Dermatophagoides farinae* extract (Der f) and staphylococcal enterotoxin B (SEB) (Kawakami *et al*, 2007). Thus, AD patients often suffer from skin infections and more than 90% of AD patients are colonized with *Staphylococcus aureus*, as compared to about 5% of healthy subjects. *S. aureus* infection is thought to be critical in the pathogenesis and/or worsening of skin lesions (Jappe, 2000; Strange *et al*, 1996). Moreover, there is a strong association of human AD with house dust mite allergens (Fuiano and Incorvaia, 2012; Kimura *et al*, 1998; Scalabrin *et al*, 1999). In this study, we demonstrated the clinical relevance of this model to human AD by global gene expression analysis, and then investigated the cellular and molecular players involved in skin inflammation in this model. We focused particularly on the role of mast cells.

Results

Gene expression profiles in lesional skin of Der f/SEB-induced dermatitis are similar to those in human AD skin

Our previous study showed that AD-like skin lesions can be induced by epicutaneous applications of Der f and SEB in NC/Nga and C57BL/6 (B6) mice (Kawakami *et al*., 2007). Higher clinical scores were observed with dermatitis-prone NC/Nga mice than with B6 mice. Global gene expression analysis of skin RNAs showed r=0.956 (Spearman's correlation coefficient) between normal and eczematous skin in NC/Nga mice, while r=0.976 between normal and eczematous skin in B6 mice. Thus, the lower values of Spearman's correlation coefficient might reflect higher clinical scores in NC/Nga mice. Comparison between B6 and NC/Nga mice yielded r=0.962 when healthy skin was compared, and r=0.970 when eczematous skin was compared. Clustering analysis also showed higher similarity between eczematous B6 and eczematous NC/Nga mice than other comparisons (Fig. S1A). These data imply that the same pathogenic mechanisms may underlie the development of AD-like skin lesions in both strains of mice. By contrast, comparison between different tissues gave lower values, e.g., r=0.855 between normal skin and normal spleen of B6 mice; r=0.857 between eczematous skin and spleen of eczematous B6 mice.

To examine the clinical relevance of our Der f/SEB induction model, we compared skin gene expression data derived with B6 and NC/Nga mice with human AD skin data deposited in the NCBI Gene Expression Omnibus (GEO) database. The changes in expression of genes in human AD versus healthy skin from AD patients or healthy subjects were compared with those of orthologous genes in mouse eczematous versus healthy skin, using OrderedList algorithm (Lottaz *et al*., 2006; Yang *et al*., 2006). This analysis detected significant similarity in gene expression in the skin between human AD and mouse AD-like dermatitis (Table 1 and Fig. S1B). The top genes contributing to 95% of the similarity score

were similar when our B6 and NC/Nga results were compared with different human AD data, and were related to epidermal growth/differentiation (e.g., several keratin genes, cornified cell envelope-related genes [*Sprr1b*, *Sprr2k*, *Tgm3*, *Lce1m*]), skin barrier function (e.g., several kallikrein-related peptidases, serine protease inhibitors [*Serpinb3d*, *3a*, *13a*]), immune responses (e.g., several cytokines, chemokines, and their receptors, *S100A8*, *S100A9*), and lipid/energy metabolism (e.g., *Slc27a2*, *Pck1*), extracellular matrix/adhesion (e.g., several matrix metalloproteinases, their inhibitor *Timp4*, *Tnc*) Among these genes, *Il7r*, *Il21r*, *CD8a*, *Ltb*, *Ccl5*, *Cxcl9*, *Cxcl10*, *Dlg2*, *Zap70*, *Pik3r1*, and *Fos* are involved in the development and/or various aspects of functions of T cells, and *Fcer1a*, *Hck*, *Ccl2*, *Pik3r1*, and *Fos* are involved in the development and/or functions of mast cells (see more detail in Supplementary Description of Microarray Data and Table S1). Consistent with the altered expression of skin barrier-related genes, Der f/SEB-induced mice had impaired skin barrier, as revealed by high levels of TEWL (Fig. S2). Expression of select genes among the top similarity contributors was confirmed by RT-qPCR (Fig. S1C).

The above expression data, together with previous results showing high serum IgE levels in both the majority of AD patients and allergen-induced eczematous mice (Jin *et al*., 2009; Kawakami *et al*., 2009a), showed high similarity between human AD and Der f/SEB-induced skin inflammation, supporting the clinical relevance of our model. Thus, these results set the stage for detailed mechanistic investigations.

T cells, but not B cells, are required for maximal skin inflammation

CD4 T cells, particularly Th2 cells, play a predominant role in allergic diseases including AD (Boguniewicz and Leung, 2011; Novak and Leung, 2011). To begin to analyze the cellular requirement for allergen-induced dermatitis, we performed the Der f/SEB experiments on T celldeficient *TCR*$\beta^{-/-}$ and T cell/B cell-deficient *Rag1*$^{-/-}$ mice. For comparison, B cell-deficient μ*MT* and WT mice were also tested. Both *TCR*$\beta^{-/-}$ and *Rag1*$^{-/-}$ mice exhibited substantially lower clinical scores than WT mice (Fig. 1A). By contrast, the clinical scores of μ*MT* mice were similar to those of WT mice. These observations were reflected in the thicknesses of skin (Fig. 1B–D). While the epidermis was thickened in all AD-induced mice, the dermis in *TCR*$\beta^{-/-}$ or *Rag1*$^{-/-}$ mice was not thickened following Der f/SEB treatment (Fig. 1C). The clinical scores correlated with the thicknesses of epidermis and dermis (Fig. 1D). In comparison to the non-AD WT sample, the increased thickness of the epidermis in AD-induced samples could be attributed to expansion of differentiated layers, such as the stratum spinosum denoted by keratin 1 (K1) and the stratum granulosum marked by loricrin (Fig. S3). Consistent with the perturbation of epidermal homeostasis, there was an increase in keratin 6 expression. Despite a defect in tight junction formation, E-cadherin (which nucleates adherens junctions) expression appeared normal. Consistent with our previous data (Kawakami *et al*., 2007), serum IgE levels (3076 ± 839 ng/ml, n=7) were high in eczematous WT mice. By contrast, without T cell help, IgE levels (700 ± 279 ng/ml, n=6) were lower in *TCR*$\beta^{-/-}$ mice. As expected from the lack of antibodyproducing B cells, μ*MT* and *Rag1*$^{-/-}$ mice did not have detectable levels of serum IgE (<15.6 ng/ml, the detection limit, n=8 or 6), indicating that IgE is not essential for skin inflammation. However, this does not exclude the possibility that IgE might contribute to some aspects of

skin inflammation (see below). These results demonstrate that T cells, but not B cells, are required for the full expression of dermatitis in this model, similar to the EC OVA model.

Mast cells, but not eosinophils, are required for maximal skin inflammation

Histological analysis showed increased numbers of eosinophils in lesional skin in *TCRβ*$^{-/-}$, *Rag1*$^{-/-}$, *μMT*, and WT mice (Fig. 2A). However, no correlation was found between the numbers of eosinophils in skin lesions and clinical scores observed in these mice (Fig. 2B). Furthermore, the clinical scores in Der f/SEB-treated eosinophil-deficient *PHIL* or Δ*dblGATA* mice were not different from those in WT control (Fig. 2C–D), indicating that eosinophils are dispensable for allergen-induced skin inflammation. By contrast, the numbers of mast cells correlated positively with clinical scores (Fig. S4). Clinical scores were significantly lower in Der f/SEB-treated mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice than in the corresponding WT mice (Fig. 3A). Consistent with these observations, the thicknesses of the lesional epidermis and dermis were significantly reduced in *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (Fig. 3B–C). To further confirm the role of mast cells, *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice were engrafted with BMMCs generated from WT mice. These mice exhibited clinical scores similar to WT mice (Fig. 3A). The numbers of engrafted mast cells were at near-normal levels (1131 ± 98/mm^2 in engrafted mice versus 1770 ± 49 /mm^2 in WT mice). In the absence of mast cells, the decreased thickening of AD-induced skin was consistent with a lower expression of K1 in AD-induced *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice versus AD-induced WT mice (Fig. 3D). Concerned about the possible role of abnormalities other than the mast cell deficiency in *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice (Reber *et al*, 2012; Rodewald and Feyerabend, 2012), we performed Der f/SEB experiments using the recently engineered mast cell-deficient mouse strain *Cpa3-Cre;Mcl-1*$^{fl/f/}$ (Lilla *et al*, 2011). These mice also exhibited significantly blunted skin inflammation (Fig. S5). Interestingly, eosinophil infiltration was increased in mast cell-deficient mice (Fig. 3E), while neutrophils were decreased (Fig. 3F). Moreover, the numbers of neutrophils were significantly correlated with the clinical scores (Fig. S6). However, the role of neutrophils in our model remains to be determined. These results strongly indicate that mast cells are required for maximal skin inflammation.

FcεRI contributes to skin inflammation

High clinical scores in μ*MT mice* (Fig. 1A) do not necessarily indicate that immunoglobulins are not involved in AD pathogenesis, because there are both activating and inhibitory Fc receptors (Nimmerjahn and Ravetch, 2006) and IgE binding to FcεRI has positive effects on mast cell survival and activation (Asai *et al*, 2001; Kalesnikoff *et al*., 2001; Kitaura *et al*, 2003). Elevated IgE levels are found in up to 80 percent of AD patients (Leung and Bieber, 2003) and anti-IgE therapy is efficacious to treat severe AD patients (Amrol, 2010; Vigo *et al*., 2006). Therefore, we tested whether the IgE-FcεRI axis is involved in skin inflammation. As shown in Fig. 4A, the clinical scores were significantly lower in *FcεRIα*$^{-/-}$ mice than in WT mice. Although H&E staining showed that epidermal/dermal thicknesses were not altered compared with WT mice (Fig. 4B–C), immunofluorescence microscopy analysis indicated that the increase in differentiating cell populations is slightly higher in AD-induced *FcεRIα*$^{-/-}$ mice compared with AD-induced WT counterparts (Fig. 4D). The same trend of expression changes could be seen for K6 and E-cadherin. However, consistent with the lower clinical scores, lesional skin had less infiltration of neutrophils in

FcεRIα$^{-/-}$ mice (Fig. 4E). One of the major cytokines acutely secreted from FcεRI-activated mast cells is TNF-α, which is important for late-phase allergic reactions and neutrophil accumulation (Wershil *et al*, 1991). However, mice lacking TNF-a failed to show a reduction in clinical scores (Fig. 4F), suggesting that a factor(s) other than TNF-α may be critical for the mast cell contribution to skin inflammation in this model of AD.

TSLP contributes to skin inflammation

TSLP activates dendritic cells (DCs) and TSLP-activated DCs prime naive T cells to produce several cytokines such as IL-4, IL-5, IL-13 and TNF-α (Liu, 2006). TSLP is highly expressed by keratinocytes from AD patients (Soumelis *et al*., 2002), and transgenic mice overexpressing TSLP in keratinocytes develop AD-like eczematous lesions (Li *et al*., 2005; Yoo *et al*., 2005). As shown in Fig. 5A, TSLP protein was highly expressed by keratinocytes in lesional skin of B6 mice. Skin sections in which the primary antibody was omitted suggested that the fluorescence in hair follicles might be non-specific. TSLP mRNA levels were also increased in lesional skin (data not shown). Next, we tested whether TSLP is involved in skin inflammation in the Der f/SEB model. Importantly, mice lacking TSLPR exhibited substantial reduction in clinical scores (Fig. 5B). Although the thicknesses of epidermis and dermis were not significantly different between WT and *TSLPR*$^{-/-}$ mice (Fig. 5C–D), the numbers of neutrophils and eosinophils, but not mast cells, were drastically reduced in *TSLPR*$^{-/-}$ mice (Fig. 5E). Consistent with the histological analysis of AD-induced samples, expression of markers of epidermal differentiation was not significantly different between WT and *TSLPR*$^{-/-}$ mice (Fig. 5F). Interestingly, K6 expression was higher in AD-induced skin from *TSLPR*$^{-/-}$ mice versus WT mice. However, serum IgE levels were not lower in *TSLPR*$^{-/-}$ mice (10.1 ± 3.8 μg/ml, n=4 versus WT 4.4 ± 1.1 μg/ml, n=7). These results collectively indicate that the TSLP-TSLPR axis is critically involved in certain aspects of this AD model.

Discussion

This and previous (Kawakami *et al*, 2007) studies strongly support the clinical relevance of our Der f/SEB-induced AD model for the following reasons. First, eczematous mice thus induced exhibited similarity to human AD skin in gross and microscopic morphology and pruritus. Second, eczematous mice showed Th2 predominant skin inflammation and elevated serum IgE levels. Third, global gene expression in eczematous skin was similar to human AD skin, confirming altered epidermal differentiation (leading to impaired barrier function) and immune dysregulation in both human and mouse diseases. Fourth, consistent with the efficacy of anti-IgE therapy in treating severe AD patients (Belloni *et al*, 2008; Liu *et al*, 2011), the IgE-FcεRI axis was involved in Der f/SEB-induced dermatitis. Finally, the requirement of TSLPR for Der f/SEB-induced dermatitis was also consistent with Th2 inflammation in human AD.

To the best of our knowledge, this study represents a previously unreported comparison in gene expression at the genomic level between human AD and a mouse model of AD. The genes that contribute to similarity in human AD and our mouse model are related to epidermal growth and differentiation, skin barrier, lipid and energy metabolism, immune

response, and extracellular matrix. Many of these genes have been implicated in the pathophysiology of human AD (Barnes, 2010).

This study showed that mast cells and αβ T cells, but not B cells or eosinophils, are required for the full expression of AD-like skin lesions in B6 mice. This report also demonstrates the requirement for mast cells in an AD model (Kawakami *et al*, 2009a) by using a strict set of approaches including mast cell knock-in (Nakano *et al*., 1985). According to the widely accepted notion for AD development (Bieber, 2008; Boguniewicz and Leung, 2011), the impaired skin barrier function allows easy access of allergens to the inside of epidermis and dermis; allergens are taken up by Langerhans cells and/or dermal dendritic cells, and these cells migrate and mature to present allergens to naïve helper T cells in lymph nodes; activated and differentiated Th2 cells migrate back to skin sites re-exposed to allergens; these effector Th2 cells recruit eosinophils, mast cells, and other granulocytes to cause tissue damage. Our results support this scenario, particularly the roles of αβ T cells and mast cells. The dispensability of eosinophils shown in this study, as well as the dispensability of CCR3 (the chemokine receptor essential for eosinophil recruitment) in another AD model (Ma *et al*, 2002), probably indicates that tissuedamaging functions of eosinophils are redundant with those of other cells. Despite the apparent involvement of the IgE-FcεRI axis in certain features of Der f/SEB-induced dermatitis, including clinical score and numbers of neutrophils, B cells were not required for the full expression of the dermatitis. This could be interpreted as reflecting a balance between positive and negative regulatory functions of immunoglobulins in allergic inflammation. IgG receptors such as FcγRI, FcγRIIIA, and FcγRIV are activating receptors, whereas FcγRIIB is an inhibitory receptor (Nimmerjahn and Ravetch, 2006). FcγRIIB inhibits FcεRI-mediated activation as well (Kraft and Novak, 2006). The cellular requirements for dermatitis development in our model were not identical to those of the EC OVA model, as dermatitis in the latter model required αβ T cells, but not B or mast cells (Alenius *et al*, 2002; Woodward *et al*, 2001). By contrast, skin inflammation induced by EC sensitization with cedar pollen antigens was abolished in mast cell-deficient mice (Oiwa *et al*, 2008). The mast cell contribution to dermatitis development in that model and our model, but not in the EC OVA model, might be due to the use of complex allergens containing component(s) that trigger mast cell activation. Similar to our model, FcεRI was shown to be involved in dermatitis in an EC OVA sensitization model (Abboud *et al*., 2009). While reduced NK cell activity was shown in our model and it led to severe erosive skin lesions upon vaccinia virus infection (Kawakami *et al*., 2009b), NK cell activity seemed normal in the EC OVA model. Therefore, the two AD models might mimic different aspects of the AD phenotype. Alternatively, these different models reflect heterogeneity of human AD.

Several studies implicated TNF-α as an important factor in skin inflammation: TNF-α expression is high in AD and psoriatic lesional skin (Zimmermann *et al*., 2011); TNF-α and IFN-γ induce keratinocyte apoptosis (Konur *et al*., 2005); TNF-α inhibits barrier protein expression (filaggrin and loricrin) via a JNK-dependent pathway (Kim *et al*., 2011); TNF-α and TWEAK (TNF-like weak inducer of apoptosis) cooperate in the induction of apoptosis in primary keratinocytes and artificial skin equivalents. TWEAK upregulates TNF-α expression in keratinocytes. High TWEAK expression was observed in AD lesions, but not

in healthy skin or psoriatic lesions (Zimmermann *et al*., 2011). Although TNF-α could be produced by T cells and mast cells, the two cell types required for the full expression of dermatitis, TNF-α was not required for Der f/SEB-induced dermatitis. Since anti-TNF-α therapy is effective in treating psoriasis (Kircik and Del Rosso, 2009; Langley *et al*., 2010), but not AD (Belloni *et al*., 2008; Pua and Barnetson, 2006), this result also supports the relevance of our Der f/SEB-induced dermatitis as a model of human AD.

TSLP is considered a master regulator of allergic inflammation (Liu, 2006). TSLP activates DCs and TSLP-activated DCs prime naive T cells to produce Th2 cytokines (IL-4, IL-5, IL-13) and TNF-α. TSLPR is expressed on other immune cells as well, and TSLP is necessary and sufficient for allergic inflammation (Ziegler and Artis, 2010). Given that TSLP is highly expressed by keratinocytes from AD patients (Soumelis *et al*., 2002), and Der f/SEB-induced dermatitis and transgenic mice overexpressing TSLP in keratinocytes develop AD-like eczematous lesions (Li *et al*., 2005; Yoo *et al*., 2005), it was not surprising that TSLPR was required for Der f/SEB-induced dermatitis. Considering the requirement of T cells in maximal Der f/SEB-induced dermatitis and the dispensability of T cells for dermatitis in keratinocytespecific TSLP transgenic mice, it is tempting to speculate that T cells are required for the expression of TSLP in keratinocytes and they become dispensable after high-level expression of TSLP is attained. In this context, mast cells, which express TSLPR (Allakhverdi *et al*., 2007), might exert an effector role downstream of TSLP. Alternatively, mast cells, together with T cells, might also be required for TSLP production in keratinocytes, since mast cells stimulated via FcεRI produce TSLP (Okayama *et al*., 2009; Soumelis *et al*., 2002) and combinations of Th2 cytokines and inflammatory cytokines (IL-1α or TNF-α) can induce TSLP production in keratinocytes (Bogiatzi *et al*., 2007).

In summary, this study has strengthened the clinical relevance of Der f/SEB-induced model of AD. By establishing its cellular and molecular basis, this model should be a useful tool for further studying the pathogenesis of AD and developing novel therapeutic strategies to the treatment of human AD.

Materials and Methods

Der f/SEB-induced dermatitis

Dermatitis was induced in NC/Nga, C57BL/6 (B6) mice or mutant mice with a C57BL/6 genetic background as previously described (Kawakami *et al*, 2007). NC/Nga mice were purchased from Charles River Japan (www.crj.co.jp). µ*MT* (Kitamura *et al*, 1991), *TCR*$\beta^{-/-}$ (Mombaerts *et al*, 1991), *Rag1*$^{-/-}$ (Mombaerts *et al*., 1992), *Kit*$^{W\text{-}sh/W\text{-}sh}$ (Grimbaldeston *et al*., 2005), *Cpa3-Cre;Mcl-1*$^{f/fl}$ (Lilla *et al*., 2011), *PHIL* (Lee *et al*, 2004), Δ*dblGATA* (Yu *et al*, 2002), *FcεRI*$\alpha^{-/-}$ (Dombrowicz *et al*, 1993), *TNF*-$\alpha^{-/-}$ (Pasparakis *et al*, 1996), *TSLPR*$^{-/-}$ (Al-Shami *et al*, 2004), and *GM-CSF*$^{-/-}$ (Stanley *et al*, 1994) mice were previously described. Briefly, solutions of 500 ng of SEB (Sigma-Aldrich, St. Louis, MO) and 10 µg of Der f extract (100 µg/ml, Greer Laboratories, Lenoir, NC) were pipetted on a 1 cm × 1 cm square gauze pad placed on the shaved area. This portion of the back skin was occluded with a Tegaderm™ Transparent Dressing (3M HealthCare, St. Paul, MN) using bandages. Three days later, the dressings were replaced with a new one. After an additional 4 days had passed, the dressings were removed and the mice were kept without treatment for

the next week. The one-week Der f/SEB treatment was repeated once more. Clinical severity was scored by an investigator who did not know the identities of mice 2 days after removing the dressings in the last cycle. Clinical scores were based on the severity (0, no symptoms; 1, mild; 2, intermediate; 3, severe) of four possible symptoms (redness, bleeding, eruption, and scaling). Der f/SEB experiments were performed using 3–6 mice per group and cumulative data from 2–5 experiments are presented. Animal experiments were approved by the Animal Care and Use Committee of the La Jolla Institute for Allergy and Immunology. Other experimental procedures, together with detailed description of microarray data, supplementary Table and Figures, can be found in the Supplementary Data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AD	atopic dermatitis
B6	C57BL/6
BMMC	bone marrow-derived mast cell
CDSN	corneodesmosin
DC	dendritic cell
Der f	*Dermatophagoides farinae* extract
EC	epicutaneous
K	keratin
KLK	kallikrein
MMP	metalloproteinase
OVA	ovalbumin
SEB	staphylococcal enterotoxin B
Th2	T helper 2

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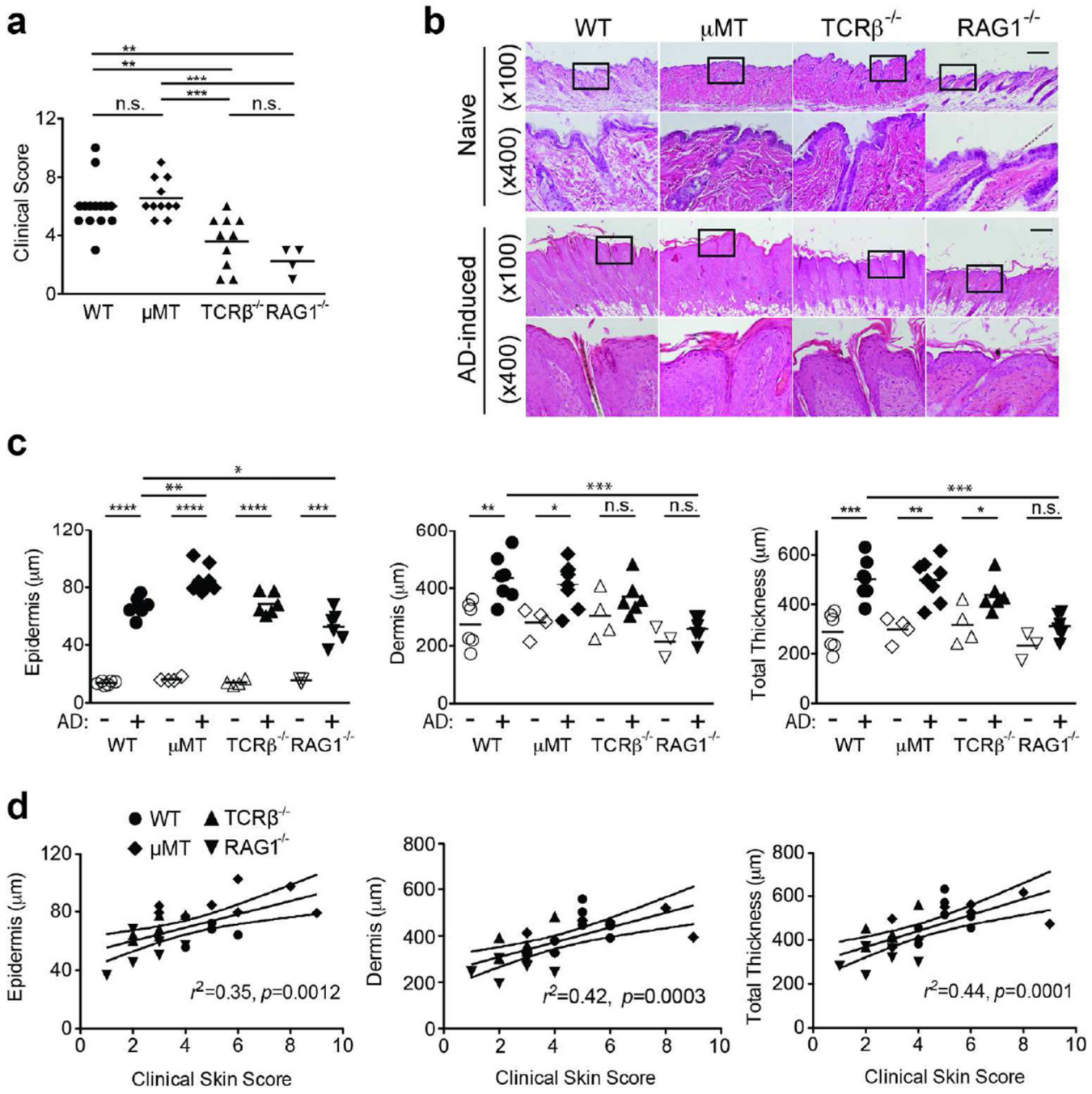

Figure 1. T cells, but not B cells, are indispensable for maximal skin inflammation

Dermatitis induction by epicutaneous applications of Der f and SEB was performed as described in the Materials and Methods. Each symbol represents one mouse. (A) Clinical skin scores. (B) H&E staining of naïve (upper rows) and lesional (lower rows) skin tissues. Bar, 200 μm. (C) Thicknesses of epidermis, dermis, and total skin (epidermis + dermis) layers, as measured in H&E-stained tissues. (D) Relationships between clinical scores and skin layer thicknesses. Linear regression lines are shown. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; n.s., not significant.

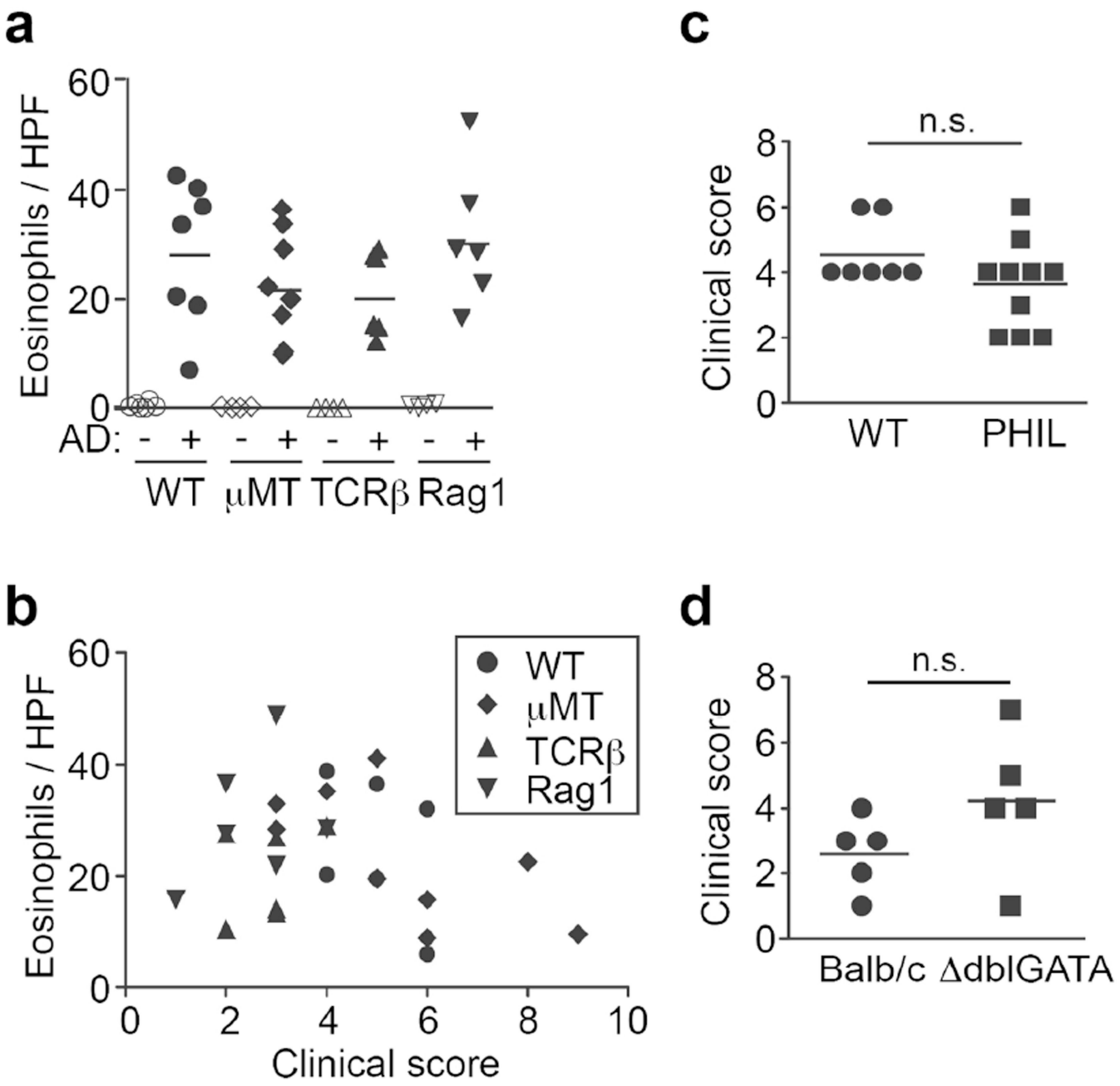

Figure 2. Eosinophils are dispensable for allergen-induced skin inflammation

(A,B) Eosinophils stained with Congo red were enumerated in the skin sections derived from experiments shown in Fig. 1. Each symbol represents one mouse. There was no significant correlation between eosinophil numbers and clinical scores. (C,D) Dermatitis induction by epicutaneous applications of Der f and SEB was performed on eosinophil-deficient *PHIL* (C) and Δ*dblGATA* (D) mice. Clinical scores are shown.

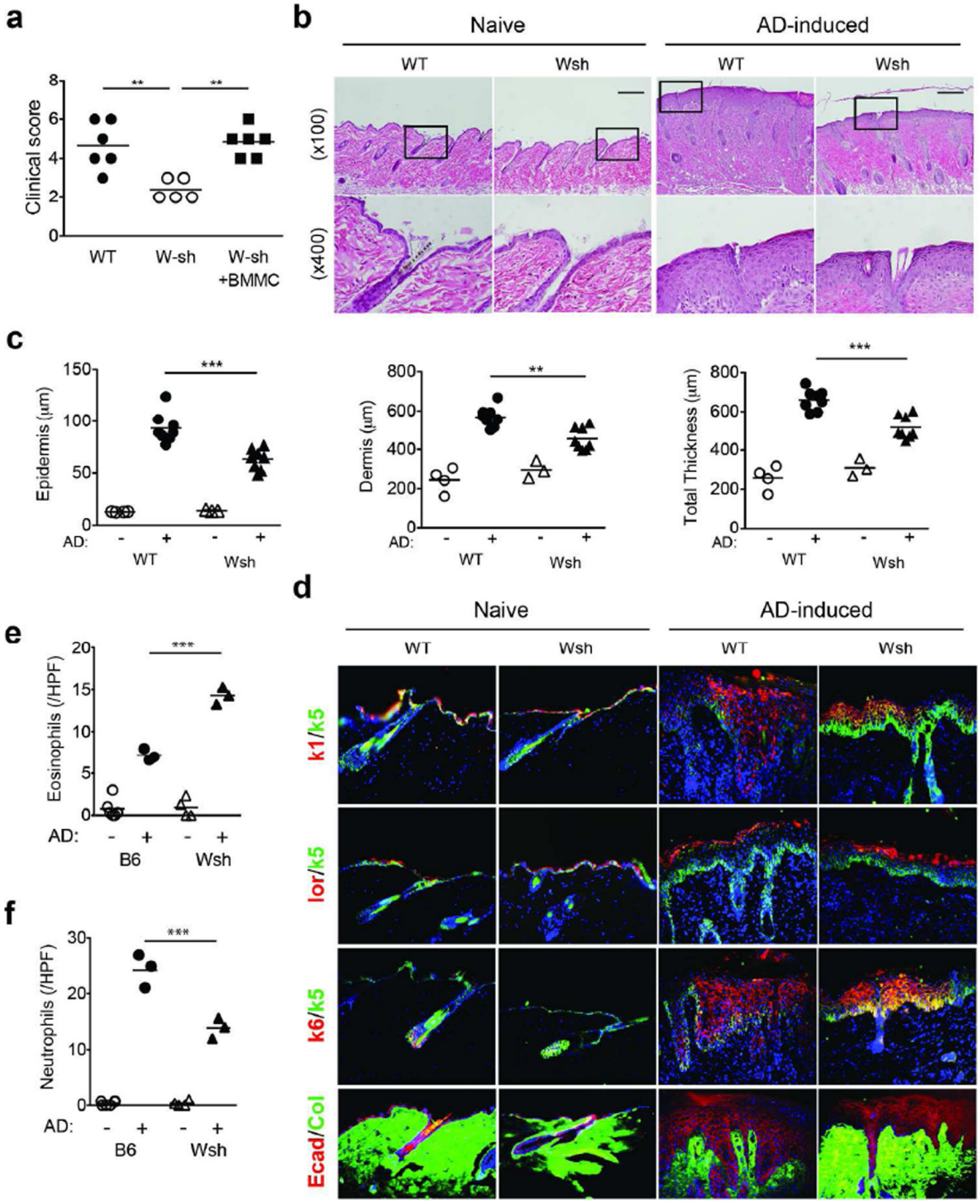

Figure 3. Mast cells are indispensable for maximal skin inflammation

(A) Mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice exhibited lower clinical scores than WT mice. The scores similar to WT mice were restored by engraftment of BMMC (W-sh + BMMC). (B) H&E staining of naïve and lesional skin tissues. Enlarged images of the areas indicated by rectangles are shown below. Bar, 200 μm. (C) Thicknesses of epidermis, dermis, and total skin layers. (D) Immunofluorescence microscopy was performed on naïve and lesional skin tissues. Numbers of eosinophil (E) and neutrophil (F) before and after AD induction. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; n.s., not significant.

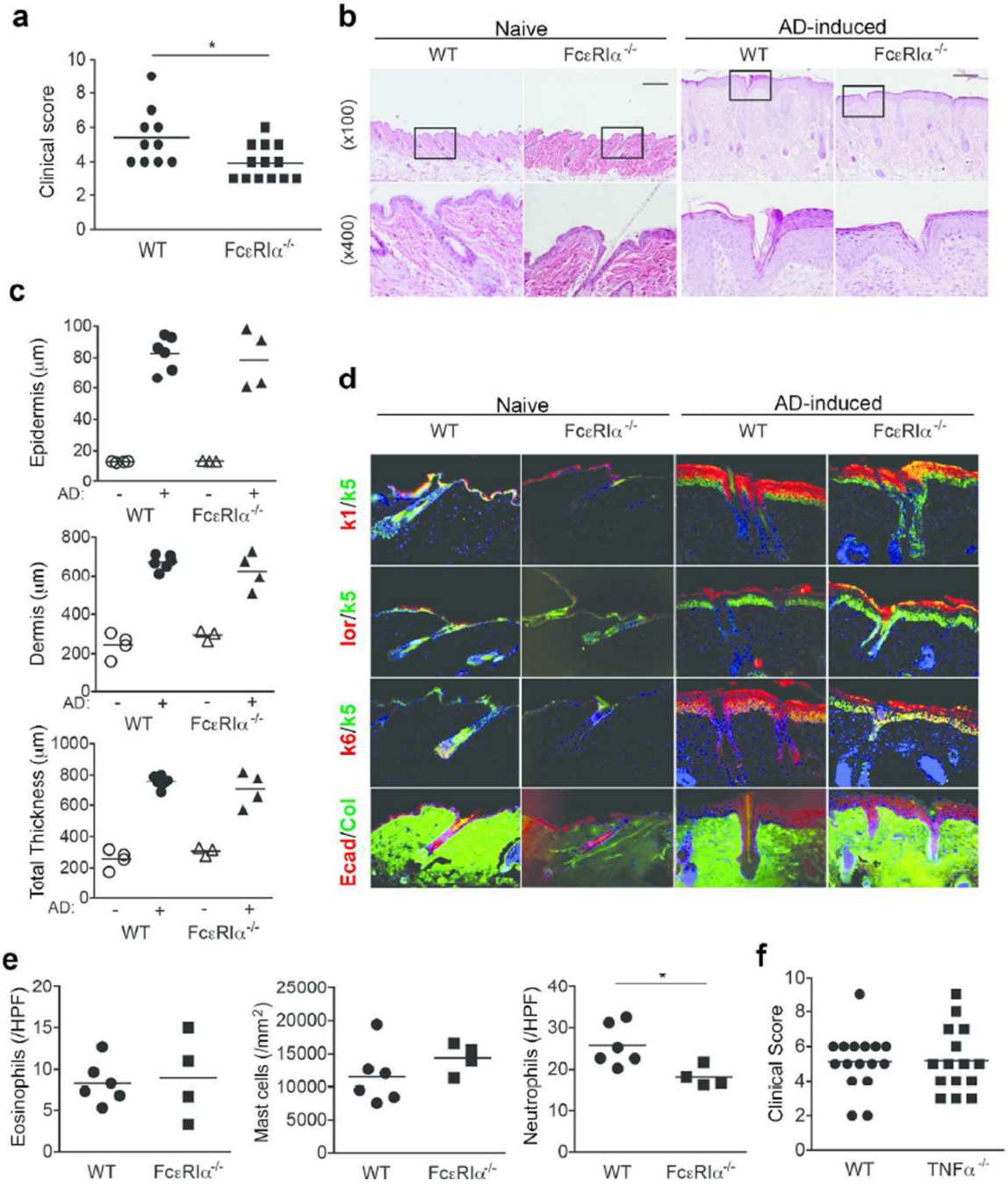

Figure 4. FcεRI, but not TNF-α, is required for maximal skin inflammation

Der f/SEB induction experiments were performed on *FcεRI*α$^{-/-}$ (A–E) and *TNF*-α$^{-/-}$ (F) mice. (A, F) Clinical skin scores. Thicknesses of epidermis, dermis, and total skin layers (B, C, D), and inflammatory cell infiltration (E) for these mice are also shown. (B) H&E staining and (D) immunofluorescence microscopy were performed on naïve and lesional skin tissues in *FcεRI*α$^{-/-}$ mice. *, $p<0.05$; n.s., not significant.

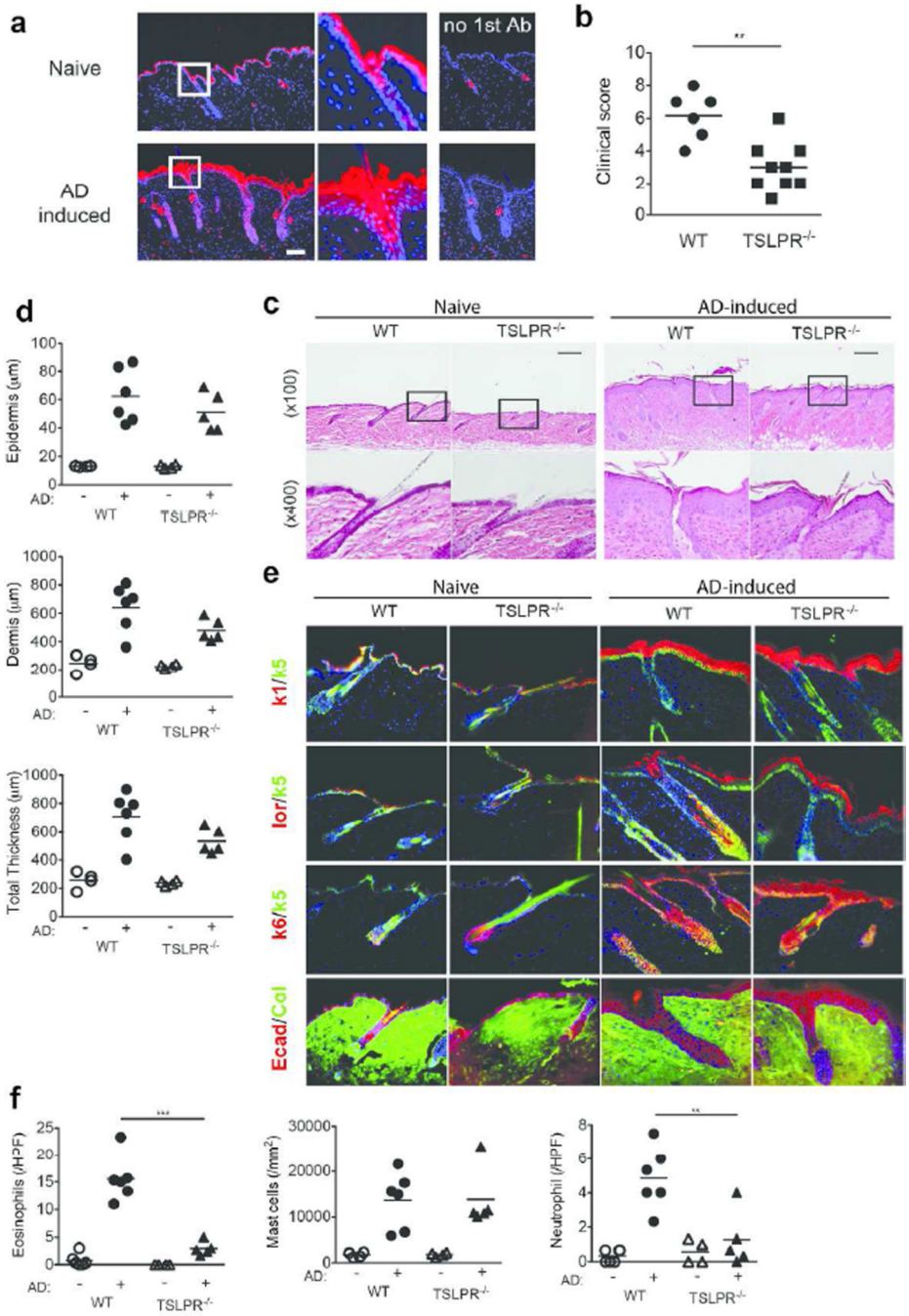

Figure 5. The Th2-promoting cytokine TSLP contributes to skin inflammation

(A) Expression of TSLP (red) before (Upper) and after (Lower) dermatitis induction with Der f/SEB in WT mice was revealed by immunofluorescence microscopy. Also shown are enlarged images of the areas indicated by rectangles as well as negative control without primary antibody. Bar, 100 μm. (B–F) Der f/SEB induction experiments were performed on *TSLPR*$^{-/-}$ mice. (B) Clinical skin scores, (C,D) thicknesses of epidermis, dermis, and total skin layers, and (E) inflammatory cell infiltration are shown. (C) H&E staining and (F)

immunofluorescence microscopy were performed on naïve and lesional skin tissues in *TSLPR*$^{-/-}$ mice. **, p<0.01; ***, p<0.001 by Student's *t* -test.

Table 1

Similarity analysis of human and mouse microarray data.

GEO accession	Orthologs common in the lists	Expression fold-change between	B6AD-like lesional / B6 Normal Skin			NC/NgaAD-like lesional / NC/Nga Normal Skin		
			Score	p-value	Genes	Score	p-value	Genes
GSE6012	10873	AD lesional / Normal Skin	1198.9	<0.001	94	1327.5	<0.001	131
GSE5667	14325	AD lesional / Normal Skin	972.3	<0.001	75	976.0	<0.001	97
		AD lesional / Non-lesional Skin	1029.0	<0.001	61	976.6	<0.001	81
		AD non-lesional / Normal Skin	277.8	0.845	69	366.0	0.502	65
GSE32924	14878	AD lesional / Normal Skin	768.2	<0.001	56	553.7	0.014	75
		AD lesional / Non-lesional Skin	1283.8	<0.001	126	1015.4	<0.001	143
		AD non-lesional / Normal Skin	552.4	0.022	29	485.2	0.068	41
GSE16161	10873	AD lesional / Normal Skin	994.1	<0.001	97	753.1	0.004	116
GSE27887	14878	AD lesional / Non-lesional Skin	1156.9	<0.001	117	910.6	<0.001	134
GSE26952	14889	AD non-lesional / Normal Epidermis	888.8	<0.001	46	849.5	<0.001	50
		PS non-lesional / Normal Epidermis	577.9	0.006	29	484.5	0.069	34

Similarity of expression changes in human AD or psoriasis (PS) and mouse induction models were compared using OrderedList algorithm as described in the Materials and Methods. Human data series obtained from NCBI Gene Expression Omnibus (GEO) are shown by GEO accession. Since each dataset uses a different microarray platform, first, orthologs were matched between human and mouse data according to NCBI HomoloGene Build 65. Numbers of the matched orthologs used in the comparisons are shown. OrderedList algorithm calculates similarity score according to the ranks of the genes listed in human and mouse data. We used expression fold changes for ranking. When the same genes were listed in the top (upregulated) or the bottom (downregulated) end of both human and mouse expression data, it gives a high similarity score. The similarity scores were compared with the random distribution of similarity scores (Fig. S1B), and *p*-values for the significance of similarity were obtained. Numbers of top genes that contributed to a total of 95% of the similarity score are also shown. A full gene list is available in Table S1.