Mast Cells Are Required for Full Expression of Allergen/SEB-Induced Skin Inflammation

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Atopic dermatitis (AD) 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 AD skin and allergen/staphylococcal enterotoxin B–induced mouse skin lesions, particularly in the 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 T helper type 2 (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, this clinically relevant model provides mechanistic insights into the pathogenic mechanism of human AD.

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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 type 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 IFN-γ-producing Th1 cells (Tsicopoulos *et al.*, 1994; Thepen *et al.*, 1996; Werfel *et al.*, 1996). Thus, as Irvine *et al.* (2011) have stated, AD was considered for many years to be primarily immunologically driven disease with secondary barrier defect (the so-called inside–outside hypothesis). In contrast, some investigators had hypothesized that the primary defect is in the skin barrier (the outside–inside hypothesis). 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 in *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

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Abbreviations: AD, atopic dermatitis; B6, C57BL/6; DC, dendritic cell; Der f, Dermatophagoides farinae extract; EC, epicutaneous; K, keratin; OVA, ovalbumin; SEB, Staphylococcal enterotoxin B; Th2, T helper type 2; TNF-α, tumor necrosis factor-α; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor; TWEAK, TNF-like weak inducer of apoptosis; WT, wild type

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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 (Irvine *et al.*, 2011; De Benedetto *et al.*, 2012). 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 past 15 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 (Woodward *et al.*, 2001; Alenius *et al.*, 2002). 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 >90% of AD patients are colonized with *Staphylococcus aureus* as compared with ~5% of healthy subjects. *S. aureus* infection is thought to be critical in the pathogenesis and/or worsening of skin lesions (Strange *et al.*, 1996; Jappe, 2000). Moreover, there is a strong association of human AD with house dust mite allergens (Kimura *et al.*, 1998; Scalabrin *et al.*, 1999; Fuiano and Incorvaia, 2012). 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, whereas $r=0.976$ 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 (Supplementary Figure S1A online). These data imply that the same pathogenic mechanisms may underlie the development of AD-like skin lesions in both strains of mice. In contrast, comparison between different tissues gave lower values, e.g., $r=0.855$ between normal skin and normal spleen of B6 mice, and $r=0.857$ 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 National Center for Biotechnology Information (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 Supplementary Figure S1B online). 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*, and *Lce1m*)), skin barrier function (e.g., several kallikrein-related peptidases and serine protease inhibitors (*Serpinb3d*, *3a*, and *13a*)), immune responses (e.g., several cytokines, chemokines, and their receptors, *S100A8*, and *S100A9*), lipid/energy metabolism (e.g., *Slc27a2* and *Pck1*), and extracellular matrix/adhesion (e.g., several matrix metalloproteinases, their inhibitor *Timp4*, and *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 online). 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 transepidermal water loss (Supplementary Figure S2 online). Expression of select genes among the top similarity contributors was confirmed by quantitative real-time reverse-transcriptase–PCR (Supplementary Figure S1C online).

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,

Table 1. Similarity analysis of human and mouse microarray data

			B6 AD-like lesional/B6 normal skin			NC/Nga AD-like lesional/NC/Nga normal skin		
GEO accession	Orthologs common in the lists	Expression fold change between	Score	*P*-value	Genes	Score	*P*-value	Genes
GSE6012	10,873	AD lesional/normal skin	1,198.9	<0.001	94	1,327.5	<0.001	131
GSE5667	14,325	AD lesional/normal skin	972.3	<0.001	75	976.0	<0.001	97
		AD lesional/nonlesional skin	1,029.0	<0.001	61	976.6	<0.001	81
		AD nonlesional/normal skin	277.8	0.845	69	366.0	0.502	65
GSE32924	14,878	AD lesional/normal skin	768.2	<0.001	56	553.7	0.014	75
		AD lesional/nonlesional skin	1,283.8	<0.001	126	1,015.4	<0.001	143
		AD nonlesional/normal skin	552.4	0.022	29	485.2	0.068	41
GSE16161	10,873	AD lesional/normal skin	994.1	<0.001	97	753.1	0.004	116
GSE27887	14,878	AD lesional/nonlesional skin	1,156.9	<0.001	117	910.6	<0.001	134
GSE26952	14,889	AD nonlesional/normal epidermis	888.8	<0.001	46	849.5	<0.001	50
		PS nonlesional/normal epidermis	577.9	0.006	29	484.5	0.069	34

Abbreviations: AD, atopic dermatitis; GEO, Gene Expression Omnibus; PS, psoriasis.
Similarity of expression changes in human AD or PS and mouse induction models were compared using OrderedList algorithm as described in the Materials and Methods. Human data series obtained from National Center for Biotechnology Information (NCBI) GEO are shown by GEO accession. As each data set 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 gave a high similarity score. The similarity scores were compared with the random distribution of similarity scores (Supplementary Figure S1B online), 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 Supplementary Table S1 online.

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 cell–deficient *TCRβ*$^{-/-}$ and T cell/B cell–deficient *Rag1*$^{-/-}$ mice. For comparison, B cell–deficient *μMT* and WT mice were also tested. Both *TCRβ*$^{-/-}$ and *Rag1*$^{-/-}$ mice exhibited substantially lower clinical scores than WT mice (Figure 1a). In contrast, the clinical scores of *μMT* mice were similar to those of WT mice. These observations were reflected in the thicknesses of skin (Figure 1b–d). Although the epidermis was thickened in all AD-induced mice, the dermis in *TCRβ*$^{-/-}$ or *Rag1*$^{-/-}$ mice was not thickened after Der f/SEB treatment (Figure 1c). The clinical scores correlated with the thicknesses of epidermis and dermis (Figure 1d). In comparison with 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 (Supplementary Figure S3 online). Consistent with the perturbation of epidermal homeostasis, there was an increase in K6 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 ($3,076 \pm 839\,ng\,ml^{-1}$, $n=7$) were high in eczematous WT mice. In contrast, without T-cell help, IgE levels ($700 \pm 279\,ng\,ml^{-1}$, $n=6$) were lower in *TCRβ*$^{-/-}$ mice. As expected from the lack of antibody-producing B cells, *μMT* and *Rag1*$^{-/-}$ mice did not have detectable levels of serum IgE ($<15.6\,ng\,ml^{-1}$, 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 (Figure 2a). However, no correlation was found between the numbers of eosinophils in skin lesions and clinical scores observed in these mice (Figure 2b). Furthermore, the clinical scores in Der f/SEB-treated eosinophil-deficient *PHIL* or *ΔdblGATA* mice were not different from those in WT control (Figure 2c and d), indicating that eosinophils are dispensable for allergen-induced skin inflammation. In contrast, the numbers of mast cells correlated positively with clinical scores (Supplementary Figure S4 online). 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 (Figure 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 (Figure 3b and c). To further confirm the role of mast cells, *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice were engrafted with bone marrow–derived mast cells generated from WT mice. These mice exhibited clinical scores similar to WT mice (Figure 3a). The numbers of engrafted mast cells were at near-normal levels ($1,131 \pm 98\,mm^{-2}$ in engrafted mice vs. $1,770 \pm 49\,mm^{-2}$ in WT mice). In the absence of mast cells,

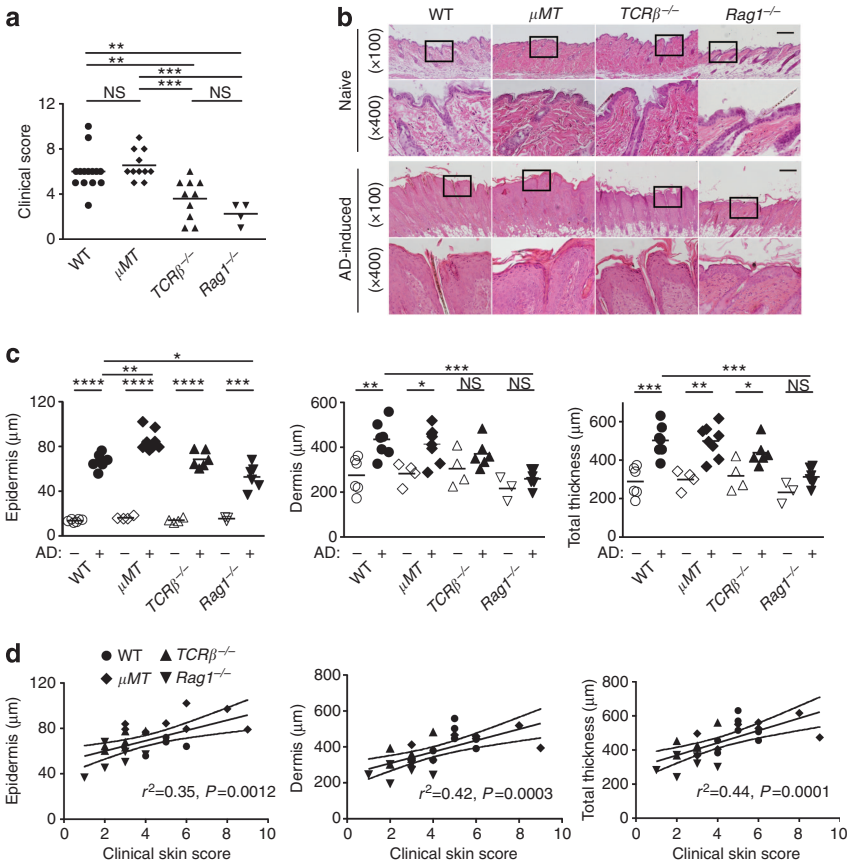

Figure 1. T cells, but not B cells, are indispensable for maximal skin inflammation. Dermatitis induction by epicutaneous applications of *Dermatophagoides farinae* extract (Der f) and Staphylococcal enterotoxin B (SEB) was performed as described in the Materials and Methods. Each symbol represents one mouse. (**a**) Clinical skin scores. (**b**) Hematoxylin and eosin (H&E) staining of naive (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. *$P<0.05$, **$P<0.01$, ***$P<0.001$, and ****$P<0.0001$. AD, atopic dermatitis; NS, not significant; WT, wild type.

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 (Figure 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*$^{fl/fl}$ (Lilla *et al.*, 2011). These mice also exhibited significantly blunted skin inflammation (Supplementary Figure S5 online). Interestingly, eosinophil infiltration was increased in mast cell–deficient mice (Figure 3e), whereas neutrophils were decreased (Figure 3f). Moreover, the numbers of neutrophils were significantly correlated with the clinical scores (Supplementary Figure S6 online). 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 *μMT* mice (Figure 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% of AD patients (Leung and Bieber, 2003), and anti-IgE therapy is efficacious in treating severe AD patients (Vigo *et al.*, 2006; Amrol, 2010). Therefore, we tested whether the IgE/FcεRI axis is involved in skin inflammation. As shown in Figure 4a, the clinical scores were significantly lower in $Fc\varepsilon RI\alpha^{-/-}$ mice than in WT mice. Although hematoxylin and eosin staining showed that epidermal/dermal thicknesses were not altered compared with WT mice (Figure 4b and c), immunofluorescence microscopy analysis indicated that the increase in differentiating cell populations is slightly higher in AD-induced $Fc\varepsilon RI\alpha^{-/-}$ mice compared with AD-induced WT counterparts (Figure 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 $Fc\varepsilon RI\alpha^{-/-}$ mice (Figure 4e). One of the major cytokines acutely secreted from FcεRI-activated mast cells is tumor necrosis factor-α (TNF-α), which is important for late-phase allergic reactions and neutrophil accumulation (Wershil *et al.*, 1991). However, mice lacking TNF-α failed to show a reduction in clinical scores (Figure 4f),

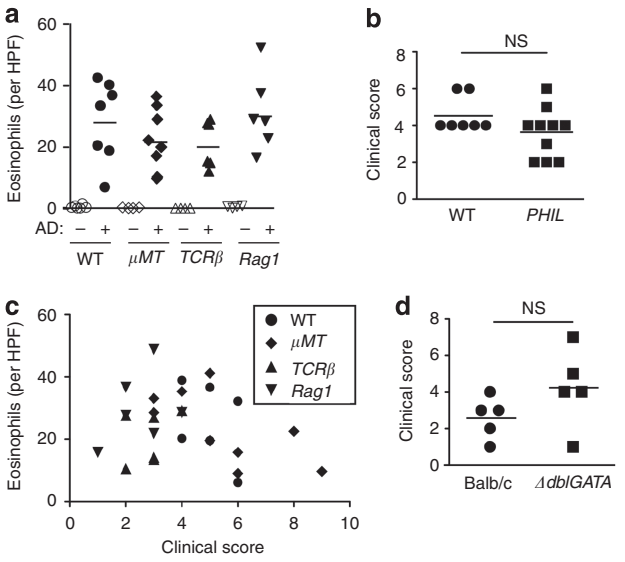

Figure 2. Eosinophils are dispensable for allergen-induced skin inflammation. (**a**, **b**) Eosinophils stained with Congo red were enumerated in the skin sections derived from experiments shown in Figure 1. Each symbol represents one mouse. There was no significant correlation between eosinophil numbers and clinical scores. (**c**, **d**) Dermatitis induction by epicutaneous applications of *Dermatophagoides farinae* extract and Staphylococcal enterotoxin B (SEB) was performed on eosinophil-deficient (**c**) *PHIL* and (**d**) *ΔdblGATA* mice. Clinical scores are shown. AD, atopic dermatitis; HPF, high power field; NS, not significant; WT, wild type.

suggesting that 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

Thymic stromal lymphopoietin (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 Figure 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 nonspecific. 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 TSLP receptor (TSLPR) exhibited substantial reduction in clinical scores (Figure 5b). Although the thicknesses of epidermis and dermis were not significantly different between WT and *TSLPR*$^{-/-}$ mice (Figure 5c and d), the numbers of neutrophils and eosinophils, but not mast cells, were drastically reduced in *TSLPR*$^{-/-}$ mice (Figure 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 (Figure 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 ($10.1 \pm 3.8\,\mu g\,ml^{-1}$, $n=4$ vs. WT $4.4 \pm 1.1\,\mu g\,ml^{-1}$, $n=7$). 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 DCs, and these cells migrate and mature to present allergens to naive helper T cells in lymph nodes; activated and differentiated Th2 cells migrate back to skin sites reexposed 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 tissue-damaging 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

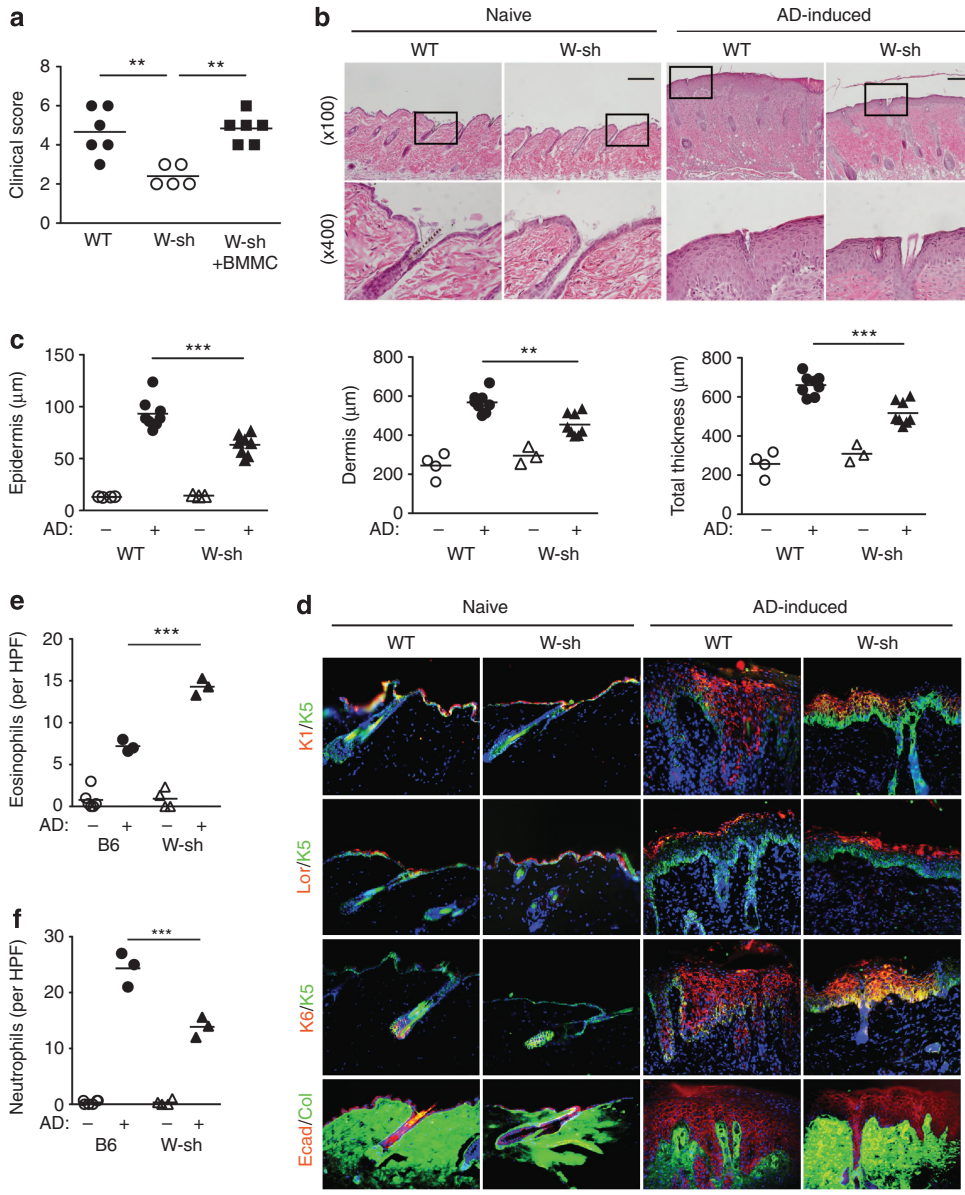

Figure 3. Mast cells are indispensable for maximal skin inflammation. (**a**) Mast cell–deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice exhibited lower clinical scores than wild-type (WT) mice. The scores similar to WT mice were restored by engraftment of bone marrow–derived mast cells (BMMCs; W-sh + BMMC). (**b**) Hematoxylin and eosin (H&E) staining of naive 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 naive and lesional skin tissues. Numbers of (**e**) eosinophils and (**f**) neutrophils before and after atopic dermatitis (AD) induction. $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$. Col, collagen; Ecad, E-cadherin; HPF, high power field; K, keratin; Lor, loricrin.

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 (Woodward *et al.*, 2001; Alenius *et al.*, 2002). In 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). Although reduced natural killer cell activity was shown in our model and it led to severe erosive skin lesions upon vaccinia virus infection (Kawakami *et al.*, 2009b), the natural killer 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.

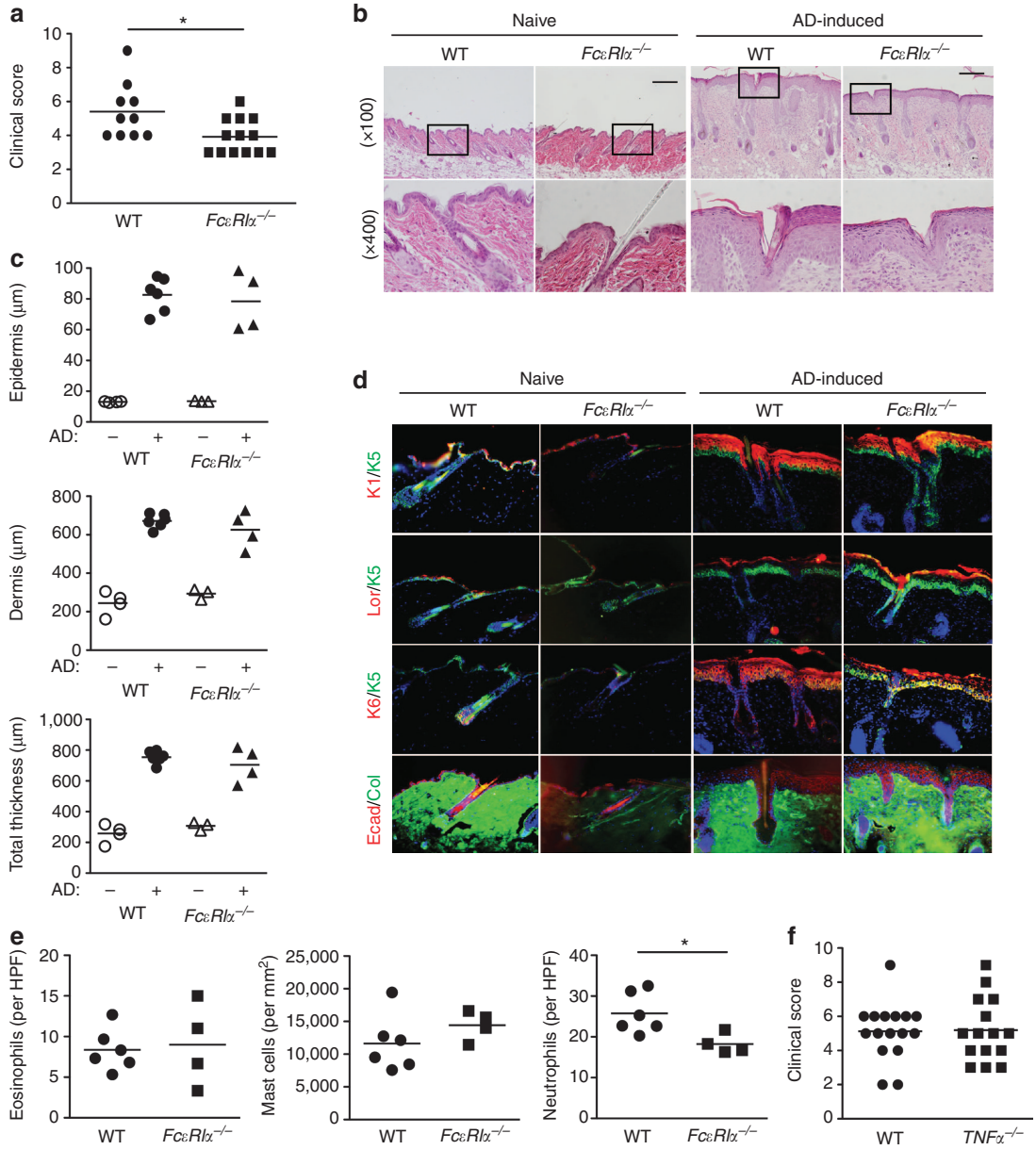

Figure 4. FcεRI, but not tumor necrosis factor-α (TNF-α), is required for maximal skin inflammation. *Dermatophagoides farinae* extract/Staphylococcal enterotoxin B (Der f/SEB) induction experiments were performed on (**a**–**e**) *FcεRIα*$^{-/-}$ and (**f**) *TNF-α*$^{-/-}$ mice. (**a**, **f**) Clinical skin scores. (**b**–**d**) Thicknesses of epidermis, dermis, and total skin layers, and (**e**) inflammatory cell infiltration for these mice are also shown. (**b**) Hematoxylin and eosin (H&E) staining and (**d**) immunofluorescence microscopy were performed on naive and lesional skin tissues in *FcεRIα*$^{-/-}$ mice. *$P<0.05$. AD, atopic dermatitis; Col, collagen; Ecad, E-cadherin; HPF, high power field; K, keratin; Lor, loricrin; WT, wild type.

Several studies have 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 c-Jun N-terminal kinase–dependent pathway (Kim *et al.*, 2011); and 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. As anti-TNF-α therapy is effective in treating psoriasis (Kircik and Del Rosso, 2009; Langley *et al.*, 2010), but not AD (Pua and Barnetson, 2006; Belloni *et al.*, 2008), 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

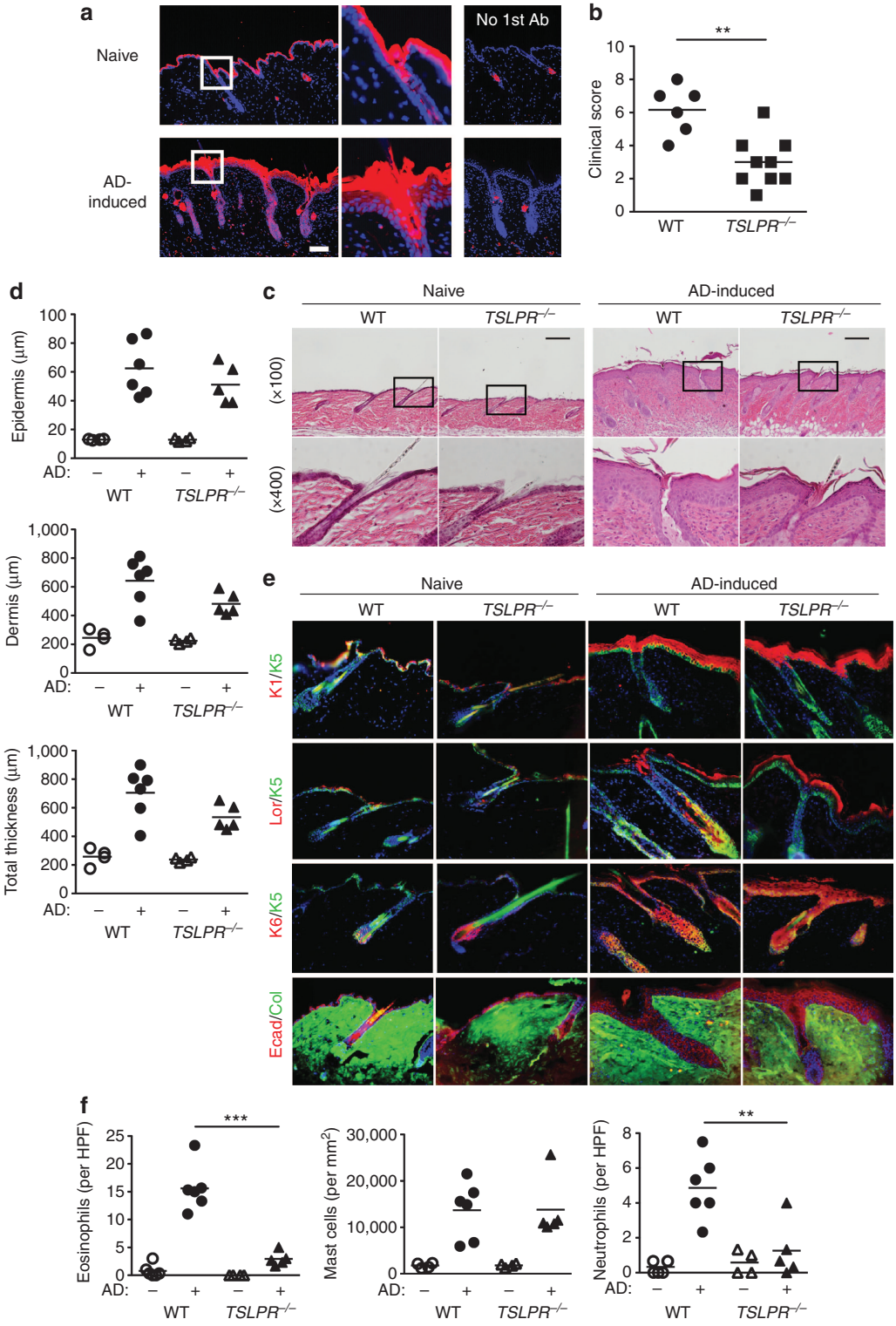

Figure 5. The T helper type 2 (Th2)–promoting cytokine thymic stromal lymphopoietin (TSLP) contributes to skin inflammation. (**a**) Expression of TSLP (red) before (upper) and after (lower) dermatitis induction with *Dermatophagoides farinae* extract/Staphylococcal enterotoxin B (Der f/SEB) in wild-type (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**) Hematoxylin and eosin (H&E) staining and (**f**) immunofluorescence microscopy were performed on naive and lesional skin tissues in *TSLPR*$^{-/-}$ mice. $^{**}P<0.01$ and $^{***}P<0.001$ by Student's *t*-test. AD, atopic dermatitis; Col, collagen; Ecad, E-cadherin; HPF, high power field; K, keratin; Lor, loricrin.

naive T cells to produce Th2 cytokines (IL-4, IL-5, and 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 keratinocyte-specific 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, as mast cells stimulated via FcεRI produce TSLP (Soumelis *et al.*, 2002; Okayama *et al.*, 2009) 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 (Yokohama, Japan) (www.crj.co.jp). *μMT* (Kitamura *et al.*, 1991), *TCRβ*$^{-/-}$ (Mombaerts *et al.*, 1991), *Rag1*$^{-/-}$ (Mombaerts *et al.*, 1992), *Kit*$^{W\text{-}sh/W\text{-}sh}$ (Grimbaldeston *et al.*, 2005), *Cpa3-Cre;Mcl-1*$^{fl/fl}$ (Lilla *et al.*, 2011), *PHIL* (Lee *et al.*, 2004), *ΔdblGATA* (Yu *et al.*, 2002), *FcεRIα*$^{-/-}$ (Dombrowicz *et al.*, 1993), *TNF-α*$^{-/-}$ (Pasparakis *et al.*, 1996), *TSLPR*$^{-/-}$ (Al-Shami *et al.*, 2004), and *GM-CSF*$^{-/-}$ (Stanley *et al.*, 1994) mice have been previously described. Briefly, solutions of 500 ng of SEB (Sigma-Aldrich, St Louis, MO) and 10 μg of Der f extract (100 μg ml^{-1}, 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. After 3 days, 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 1-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; and 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 to 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, and Supplementary Table and Figures, can be found in the Supplementary Data online.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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