Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice

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Transgenic mice expressing the mouse interleukin 33 (IL-33) gene driven by a keratin 14 promoter were generated. The skin-selective expression of the IL-33 gene was enhanced, and intense immunofluorescence for IL-33 was evident in the nuclei of the epidermis. Spontaneous itchy dermatitis developed in those mice at 6–8 wk of age in specific pathogen-free conditions. In the lesional skin, the epidermis was thickened and the eosinophils were infiltrated with increased expression of the eosinophil peroxidase and major basic protein genes. Mast cells were also abundant there, and blood histamine and total IgE levels were high. Those phenotypes closely resemble the features of atopic dermatitis. In peripheral blood and lesional skin, IL-5, IL-13, regulated upon activation, normally T-expressed, and presumably secreted (RANTES)/CCL5, and Eotaxin 1/CCL11 were increased, whereas TNF-α, IFN-γ, and thymic stromal lymphopoietin (TSLP) were unaltered. Furthermore, the proportion of group 2 innate lymphoid cells (ILC2s), which produce IL-5, were significantly increased in the lesional skin, peripheral blood, and regional lymph nodes. The dermatitis with eosinophil infiltration was improved by the administration of an anti-IL-5 antibody. These results suggest that the expression of IL-33 in the skin activates an immune response involving ILC2 and that this process might play a crucial role in the pathogenesis of allergic inflammation that is characteristic of atopic dermatitis.

Atopy | natural helper cells | nuocytes

IL-33 is a member of the IL-1 family of cytokines and is a ligand for IL-1 receptor-like-1 or ST2 (1). In contrast with other members of that family, IL-1 and IL-18, which are activated in inflammasomes by caspase 1, IL-33 is produced in an active full-length form and is stored in the nucleus. In response to external insults or various types of cellular damage, IL-33 is released from cells and binds to ST2 on Th2 cells and on various types of innate immune cells including basophils, mast cells, eosinophils, and natural helper cells (2) or nuocytes (3), currently termed group 2 innate lymphoid cells (ILC2s) (4), which induces and activates those cells. IL-33 has been suggested to be involved in the pathogenesis of various allergic disorders such as asthma (5), allergic rhinitis (6), allergic conjunctivitis (7), and even rheumatoid arthritis (8) and inflammatory bowel diseases (9).

Atopic dermatitis (AD) is one of the major allergic disorders with a wide prevalence. Indeed, the morbidity of AD reaches 20–30% in developed countries (10). AD is characteristic of chronic dermatitis with severe, long-lasting itching, which often compromises the quality of life of patients and thereby influences social and economic activities. Clinically, eosinophils and mast cells accumulate in the lesional skin of patients with AD, often with an elevation of histamine and total IgE levels in peripheral blood (11). The association of AD with a polymorphism of the ST2 gene suggests that immune regulation via the IL-33-ST2 system may be pivotal as a genetic background of AD (12). Recently, the upregulation of IL-33 in the epidermis and the infiltration of ST2-positive cells in the dermis of the lesional skin of patients with AD have been reported (13, 14). In contrast, an increase in IL-33 has also been shown in the epidermis of patients with psoriasis, which is mediated mainly by Th1 and Th17 cells (14–16). However, it is still unclear how IL-33 contributes to those inflammatory conditions involving skin in association with ILC2s, which produce massive amounts of Th2 cytokines in response to IL-33 (4).

In this study, we established transgenic mice with a skin-specific overexpression of IL-33. Interestingly, spontaneous dermatitis developed with the activation of ILC2s in those mice under specific pathogen-free (SPF) conditions, and the characteristics of those mice closely resemble atopic dermatitis.

Results

Development of Spontaneous Itchy Dermatitis in Transgenic Mice with Skin-Specific Expression of IL-33. To elucidate the role of IL-33 in cutaneous inflammation, we developed transgenic mice with skin-specific expression of IL-33. A transgene driven by the human keratin 14 promoter was constructed (Fig. 1*A*) to generate transgenic mice expressing the full-coding sequence of mouse IL-33 cDNA. The integration of the transgene was confirmed in genomic DNA from12 of 122 pups born from foster mothers. The increased expression of the IL-33 gene was confirmed in ear RNA from 6 transgenic mice. Skin lesions spontaneously developed in 2 mice, and transgenic mouse lines were established from those 2 mice. Variations in phenotypes were minimal in the offspring from one mouse line termed hK14mIL33tg. Male heterozygous hK14mIL33tg mice were crossed with female wild-type C57BL/6 mice to generate littermates, which were used for experiments.

Immunofluorescence using an anti-IL-33 antibody was examined in the ears of the transgenic and wild-type mice. In the skin, IL-33 was localized in the nuclei of the epidermis, and the intensity of staining for IL-33 was higher in hK14mIL33tg mice than in wild-type mice (Fig. 1*B*). The expression of the IL-33 gene was compared in various organs of those mice, using quantitative real-time PCR. A marked induction of *Il33* expression was found in the skin of hK14mIL33tg mice compared with the other tissues of those mice (Fig. 1*C*). hK14mIL33tg mice were born and grew normally up to 5 wk, but all mice spontaneously developed

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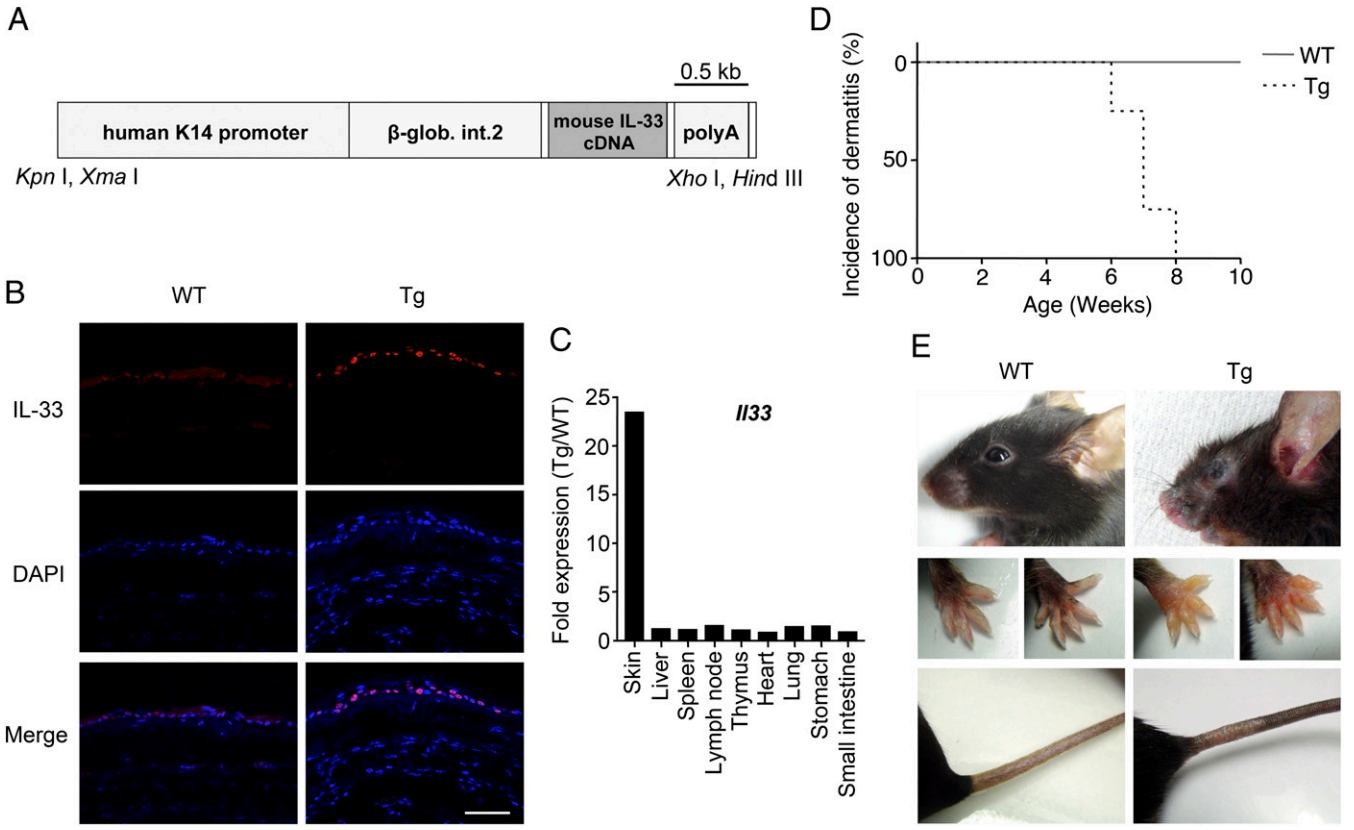

Fig. 1. Spontaneous development of AD-like skin lesions in hK14mIL33tg mice. (*A*) Schematic structure of the transgene DNA. An active full-length form of mouse IL-33 cDNA was placed downstream of the human keratin 14 promoter to drive the keratinocyte-specific expression of IL-33. The transgene construct also contained a rabbit β-globin intron sequence and a keratin 14 polyadenylation signal for the stable processing of transcripts. (*B*) Immunofluorescence of IL-33 in the epidermis of wild-type (WT) and hK14mIL33tg (Tg) ear skins. Intense staining of IL-33 was evident in nuclei of the epidermis in hK14mIL33tg mice. (Scale bar, 50 μm.) Data are representative of three independent experiments. (*C*) The skin-selective expression of the IL-33 gene in hK14mIL33tg mice. Total RNAs from various organs of mice were used as templates for quantitative real-time PCR. Each bar shows the expression of the IL-33 gene in hK14mIL33tg mice relative to wild-type mice. Data are representative of two independent experiments. (*D*) Incidence of spontaneous dermatitis that develops in hK14mIL33tg mice. Skin lesions spontaneously developed between 6 and 8 wk of age and remained thereafter in all hK14mIL33tg mice. Wild-type mice (WT; $n = 7$) and hK14mIL33tg mice (Tg; $n = 4$). $P < 0.001$ by the two-tailed log-rank test. (*E*) Cutaneous manifestations of hK14mIL33tg mice. Skin lesions, such as erythemas with exudations, erosions, crusts, scales, and/or hair loss, were found at the periorbital and perinasal regions of the face, ears, neck, hands, feet, and tail. Representative photographs taken from three 24–28-wk-old mice are shown. Similar skin lesions were observed in three independent experiments.

dermatitis between 6 and 8 wk under SPF conditions (Fig. 1*D*). The eruptions were erythemas with erosions, exudations, and scales and/or crusts on the face, mainly around the eyes and nose, ears, neck, hands, feet, and tail, which are easily subject to friction and damage (Fig. 1*E*). Periorbital skins from wild-type and hK14mIL33tg mice were used for histological and immunological analyses unless otherwise indicated. hK14mIL33tg mice showed severe scratching behavior, possibly resulting from itching of the eruptions (Movie S1).

Induction of Eosinophils in hK14mIL33tg Mice. The histology of the dermatitis was examined by H&E staining. In the lesional skin of hK14mIL33tg mice, the thickening of the epidermis and the infiltration of inflammatory cells including eosinophils were evident compared with the normal skin of wild-type mice (Fig. 2*A*). The proportion of $CCR3^+Siglec\text{-}F^+$ eosinophils in the skin and peripheral blood was examined by flow cytometry (Fig. 2 *B*-*E*). Eosinophils infiltrating the lesional skin were about 7.4 times more abundant than in normal wild-type mouse skin (Fig. 2*C*). Eosinophils in blood were also increased about 4.5 times more in hK14mIL33tg mice than in wild-type mice (Fig. 2 *D* and *E*). In contrast, the proportions of $Gr\text{-}1^+CD11b^+$ neutrophils, $DX5^+$ IgE^+ basophils, and $CD3^+$ or $B220^+$ lymphocytes were not different in peripheral blood between hK14mIL33tg and wild-type mice (Fig. 2*D*). According to the infiltration of eosinophils, the expression of the genes for eosinophil peroxidase (*Epx*) and major basic protein (*Prg2*) was significantly higher in the lesional skin of hK14mIL33tg mice than in the normal skin of wild-type mice (Fig. 2*F*).

Induction of Mast Cells, Histamine, IgE, and Itching in hK14mIL33tg Mice. Severe scratching behavior in mice is suggestive of the activation of mast cells. When the skin sections were examined by toluidine blue staining, mast cells were abundant in the lesional dermis of hK14mIL33tg mice (Fig. 3*A*), and degranulating mast cells were also observed. Flow cytometry of the lesional skin revealed that the proportion of $c\text{-}kit^+IgE^+$ mast cells is significantly increased compared with the normal skin of wild-type mice (Fig. 3 *B* and *C*). The concentration of plasma histamine was 1.46 μM in hK14mIL33tg mice (Fig. 3*D*), which was much higher than 0.18 μM in wild-type mice, possibly because of the activation of mast cells. Total IgE in serum was 386 ng/mL in hK14mIL33tg mice (Fig. 3*E*), which was more than 20 times higher than the 15 ng/mL seen in wild-type mice. Scratching behavior observed over the course of 20 min was significantly longer in hK14mIL33tg mice than in wild-type mice (Fig. 3*F*).

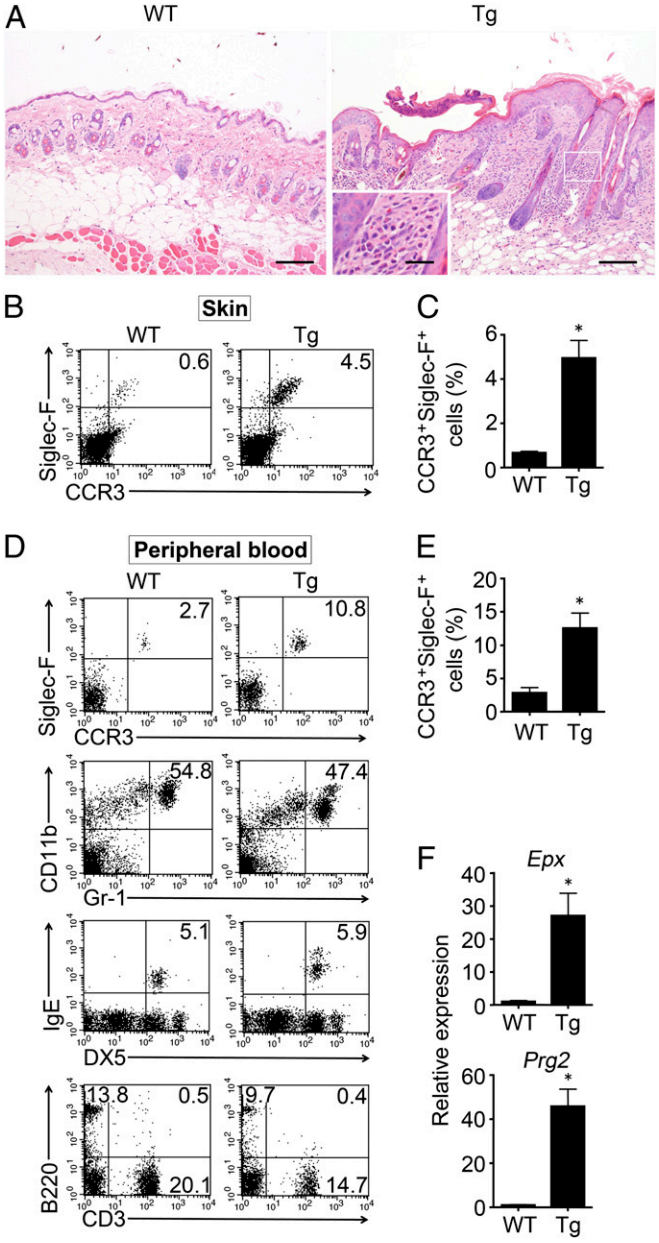

Fig. 2. Dermatitis with eosinophil infiltrates and eosinophilia in hK14mIL33tg mice. (*A*) H&E staining of wild-type (WT) and hK14mIL33tg (Tg) mouse skin. The infiltration of eosinophils was evident in the dermis of hK14mIL33tg lesional skin. Panels are representative of three mice and three independent experiments. (Scale bars, 200 μm; inset, 50 μm.) (*B* and *D*) Flow cytometry of $B220^{-}CD3^{-}CD45^{+}$ skin cells (*B*) or $CD45^{+}$ peripheral blood cells (*D*) from wild-type (WT) and hK14mIL33tg (Tg) mice. The numbers indicate the proportion of cells in each quadrant. Data are representative of four mice and two independent experiments. (*C* and *E*) The proportions of eosinophils determined by flow cytometry of $B220^{-}CD3^{-}CD45^{+}$ skin cells (*C*) or $CD45^{+}$ peripheral blood cells (*E*). Eosinophils were markedly increased in the lesional skin and in the peripheral blood of hK14mIL33tg mice. Data are expressed as means ± SEM ($n = 4$) $^{*}P < 0.05$ (Student *t* test). (*F*) The expression of genes for eosinophil peroxidase *(Epx)* and eosinophil granule major basic protein *(Prg2)* in the skins of wild-type (WT) and hK14mIL33tg (Tg) mice. *Epx* and *Prg* were significantly increased in the lesional skin of hK14mIL33tg mice. Data are expressed as means ± SEM ($n = 5$) $^{*}P < 0.05$ (Student *t* test).

Scratching time in hK14mIL33tg mice could be shortened by an i.p. administration of 2 mg diphenhydramine hydrochloride, an H_1-receptor antagonist (Fig. 3*G*).

Th2 Cytokines and Chemokines Induced in hK14mIL33tg Mice. Eosinophils are induced and activated by Th2 cytokines such as IL-5 and IL-13 and by chemokines such as regulated upon activation, normally T-expressed, and presumably secreted (RANTES)/CCL5 and Eotaxin 1/CCL11, which are ligands for CCR3 expressed in eosinophils (17). Therefore, cytokines and chemokines in the serum and skin were examined by protein array analysis (Fig. 4). The concentration of IL-5 was much higher in the serum and lesional skin from hK14mIL33tg mice than in the serum and skin from wild-type mice. RANTES/CCL5 and Eotaxin 1/CCL11 were also significantly increased in the lesional skin. These results strongly suggest that those cytokines and chemokines are important for cutaneous inflammation with eosinophil infiltration and eosinophilia in hK14mIL33tg mice. In contrast, Th1 cytokines such as TNF-α and IFN-γ were unaltered in hK14mIL33tg mice. IL-18, thymic stromal lymphopoietin (TSLP), and IL-25, epithelial cell-derived proinflammatory cytokines other than IL-33, were

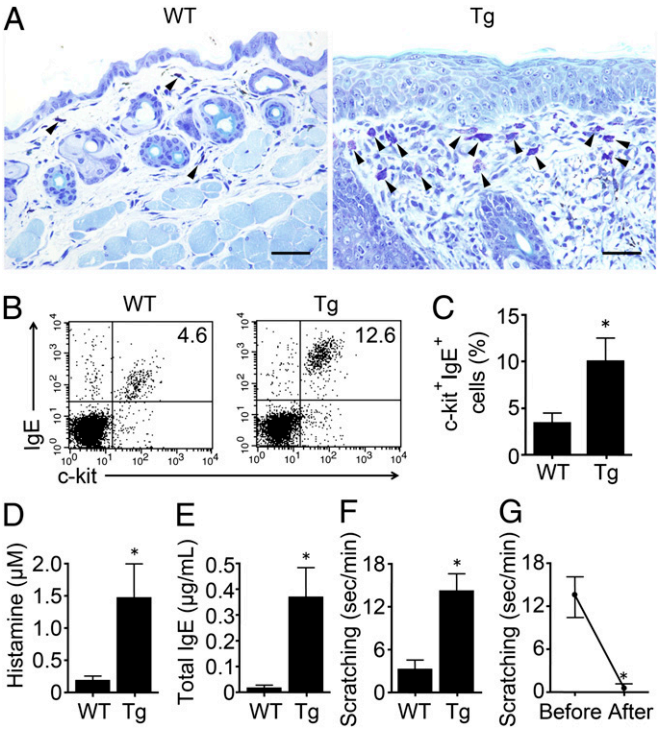

Fig. 3. Dermatitis with abundant mast cells, high levels of blood histamine and total IgE, and itching in hK14mIL33tg mice. (*A*) Toluidine blue staining of wild-type (WT) and hK14mIL33tg (Tg) skins. Toluidine blue-positive mast cells (arrowheads) were abundant in the lesional skin of hK14mIL33tg mice. Panels are representative of three mice and two independent experiments. (Scale bars, 50 μm.) (*B* and *C*) Flow cytometry of skin cells from wild-type (WT) and from hK14mIL33tg (Tg) mice. (*B*) Numbers indicate the proportion of $c\text{-}kit^{+}$ IgE^{+} mast cells. (*C*) The proportions of mast cells in flow cytometry of $B220^{-}CD3^{-}CD45^{+}$ skin cells. Mast cells were significantly increased in the lesional skin of hK14mIL33tg mice. Data are expressed as means ± SEM ($n = 4$) $^{*}P < 0.05$ (Student *t* test). (*D* and *E*) Plasma histamine (*D*) and serum total IgE levels (*E*) in wild-type (WT) and hK14mIL33tg (Tg) mice. Histamine and total IgE concentrations in the blood were significantly increased in hK14mIL33tg mice. Data are expressed as means ± SEM ($n = 4$) $^{*}P < 0.05$ (Student *t* test). (*F*) Itching in wild-type (WT) and hK14mIL33tg (Tg) mice. Itching was evaluated by total time of skin-scratching behavior during an observation period of 20 min. Data are expressed as means ± SEM (seconds per minute; $n = 3$). $^{*}P < 0.05$ (Student *t* test). The time for skin-scratching behavior was significantly elongated in hK14mIL33tg mice. (*G*) Itching of hK14mIL33tg mice before and after the administration of diphenhydramine hydrochloride. The H_1-receptor antagonist improved itching in hK14mIL33tg mice. Data are expressed as means ± SEM (seconds per minute) ($n = 3$). $^{*}P < 0.05$ (paired *t* test).

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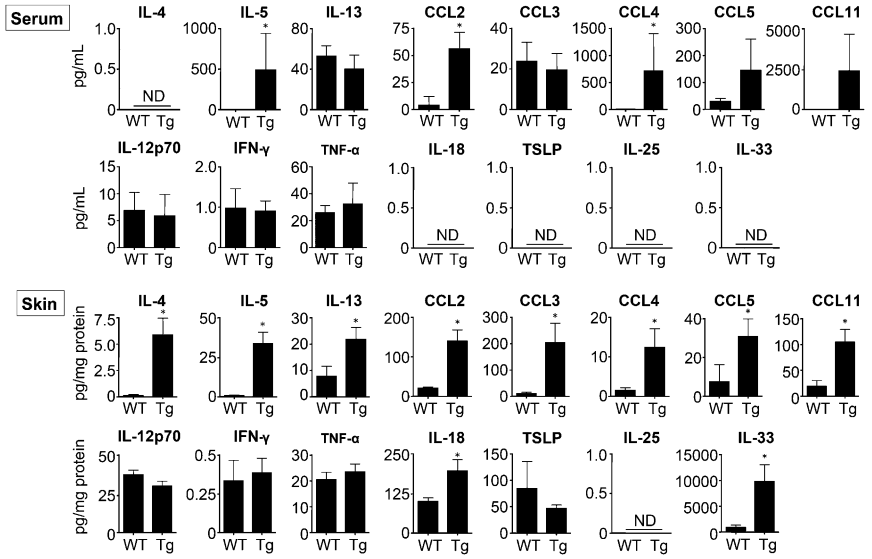

Fig. 4. Serum and cutaneous cytokine and chemokine profiles in wild-type and hK14mIL33tg mice. The concentrations of cytokines and chemokines in the serum and skin of wild-type (WT) and hK14mIL33tg (Tg) mice were measured as described in the *Materials and Methods*. IL-5, IL-13, RANTES/CCL5, and Eotaxin 1/CCL11 were increased, whereas TNF-α, IFN-γ, and TSLP were unaltered in both the serum and the lesional skin of hK14mIL33tg mice. The minimum detectable concentrations of IL-4, IL-33, IL-18, TSLP, and IL-25 are 2.1, 0.2, 31.8, 6.3, and 9.0 pg/mL, respectively. Data are expressed as means ± SEM ($n = 6$). *$P <$ 0.05 (Student *t* test). ND, not detected.

also examined. However, the increase in IL-18 in the lesional skin was small, and in contrast with other AD mouse models (18), serum levels of IL-18 were not different between wild-type and hK14mIL33tg mice. No significant increase in TSLP or IL-25 was observed in hK14mIL33tg mice.

Induction of ILC2s in hK14mIL33tg Mice. The marked increase of IL-5 and IL-13 in hK14mIL33tg mice prompted us to examine ILC2s because those cells massively produce those cytokines in response to IL-33 (4). Flow cytometry revealed that $Lin^{-}ST2^{+}$ $Sca\text{-}1^{+}$ ILC2s were increased in the skin lesions, peripheral blood, and regional lymph nodes from hK14mIL33tg mice in comparison with wild-type mice (Fig. 5*A*). Furthermore, lymph node cells from hK14mIL33tg mice were subjected to intracellular staining for IL-5 and IL-13 and were examined by flow cytometry to identify cells producing those cytokines. Cells expressing IL-5 and IL-13 matched the $Lin^{-}ST2^{+}$ $Sca\text{-}1^{+}$ ILC2s (Fig. 5 *B* and *C*). However, ILC2s producing IL-5 or IL-13 were not found in lymph node cells from wild-type mice. These results show that IL-33 contributes to the activation of ILC2s, which induce eosinophils through the production of IL-5 and IL-13.

The Effect of a Neutralizing IL-5 Antibody on Dermatitis with Eosinophil Infiltrates of hK14mIL33tg Mice. Whether the induction of eosinophils in hK14mIL33tg mice is dependent on IL-5 was examined using a neutralizing anti-IL-5 antibody. When 20 μg of that anti-IL-5 antibody was administered to hK14mIL33tg mice every 2 d for 2 wk, the peripheral blood eosinophil count was significantly decreased (Fig. 6 *A* and *B*). Moreover, the dermatitis with thickened epidermis became milder, and inflammatory infiltrates including eosinophils were improved by the treatment (Fig. 6*C*). The increased expression of the Prg2 gene was also reduced (Fig. 6*D*), whereas expression of the IL-33 gene was not altered by the neutralization of IL-5. Therefore, the induction of eosinophils in the lesional skin and the peripheral blood may be mediated via IL-5, increased in hK14mIL33tg mice.

Discussion

In this study, we demonstrated that the skin-specific expression of IL-33 causes AD-like cutaneous manifestations with the induction of eosinophils in transgenic mice under SPF conditions. The contribution of IL-33 to the pathogenesis of cutaneous inflammation had not been fully elucidated, although the intradermal injection of mouse recombinant IL-33 has been shown to elicit a scleroderma-like reaction with an increase in dermal collagen fibers (19) or a psoriasis-like dermatitis with a thickened epidermis (16). However, the phenotype of hK14mIL33tg mice was inconsistent with those pathologic changes. The itchy long-standing dermatitis with infiltrations of eosinophils and mast cells and high serum IgE levels in hK14mIL33tg mice are more compatible with AD.

Several animal models for AD have been established (20), but none of those were produced to look for the upregulation of IL-33 in the epidermis. In contrast, the expression pattern of IL-33 in the epidermis of hK14mIL33tg mice (Fig. 1*B*) closely resembles the expression pattern of IL-33 in AD (13). In hK14mIL33tg mice, the dermatitis develops in skin areas in which coat hairs are sparse and are vulnerable to external stimuli (Fig. 1*E*). This suggests that the Koebner phenomenon (in which eruptions are induced by stimuli to the skin) is reproduced in the transgenic mice.

The dermatitis that develops in hK14mIL33tg mice is accompanied by an abundant infiltration of eosinophils (Fig. 2). In contrast, transgenic mice expressing caspase 1 or an active form of IL-18 in the epidermis show neutrophil predominance in the skin (21). Eosinophil infiltrates are usually observed in AD (22), and therefore hK14mIL33tg transgenic mice may resemble AD in that respect. We have previously reported that IL-33 stimulates the production of IL-4 by eosinophils (7). As shown in Fig. 2, eosinophils infiltrating in the skin were more evident in hK14mIL33tg mice than in wild-type mice. Because group 2 ILCs hardly produce IL-4, we speculate that eosinophils in the skin might produce IL-4 in response to IL-33 in Kh14mIL33tg mice.

The induction of mast cells (Fig. 3 *A*–*C*) and the increases in blood histamine (Fig. 3*D*) and total IgE levels (Fig. 3*E*) observed in hK14mIL33tg mice have been documented in other AD model mice (20). Those findings in hK14mIL33tg mice are consistent with reports that IL-33 is involved in the increased numbers of mast cells in the skin (16) and the induction of serum IgE (1). However, the mechanism for increase in serum IgE is

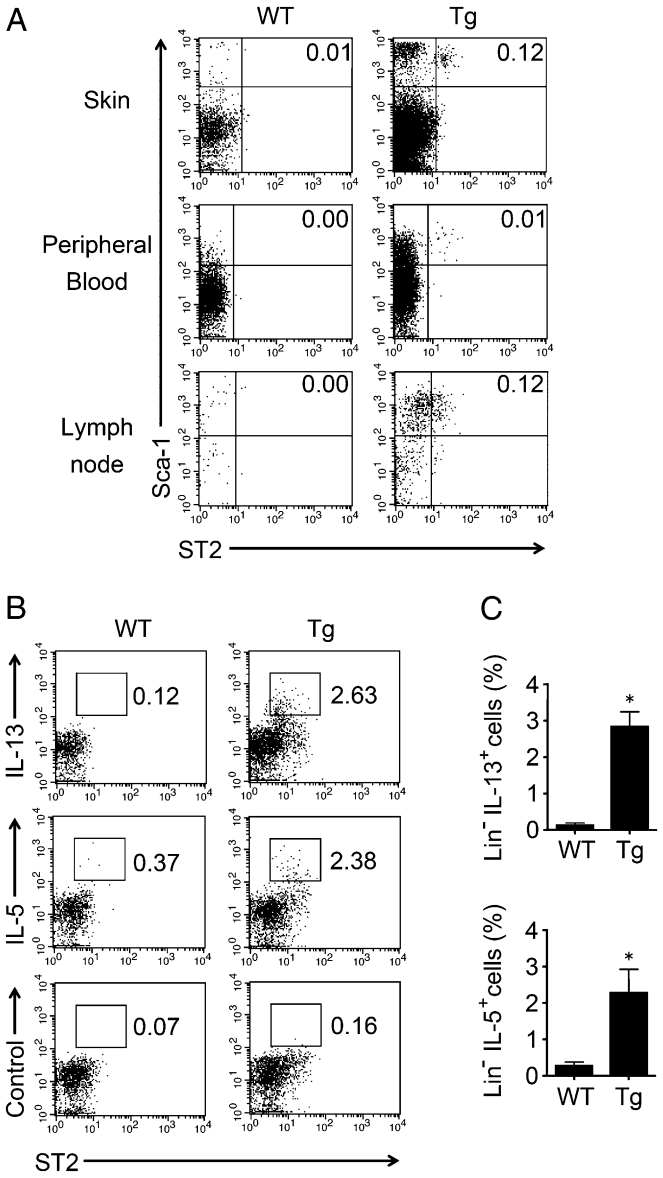

Fig. 5. Induction of Lin$^-$ST2$^+$ Sca-1$^+$ ILC2s in hK14mIL33tg mice. (*A*) Flow cytometry of cells from the skin, peripheral blood, and lymph nodes of wild-type (WT) of hK14mIL33tg (Tg) mice. Cells were gated on the Lin$^-$ fraction. The numbers indicate the proportion of Lin$^-$ST2$^+$Sca-1$^+$ ILC2s in flow cytometry of FSC^{low}SSC^{low} cells (lymphocyte gate). Skins were sampled from periorbital and perinasal regions and ears. Data are representative of three mice and two independent experiments. Lin$^-$ST2$^+$ ILC2s were induced in the lesional skin, peripheral blood, and lymph nodes of hK14mIL33tg (Tg) mice. Note that Sca-1$^+$ST2$^-$ cells are not lymphocytes but are CD45$^-$ skin cells. (*B*) Production of IL-5 and IL-13 in Lin$^-$ST2$^+$ cells from lymph nodes of wild-type (WT) and hK14mIL33tg (Tg) mice. Cells were gated on the Lin$^-$ fraction. The numbers indicate the proportion of ST2$^+$IL-5$^+$ or ST2$^+$IL-13$^+$ cells. Data are representative of three mice and three independent experiments. (*C*) The proportions of Lin$^-$ST2$^+$IL-5$^+$ or Lin$^-$ST2$^+$IL-13$^+$ cells in flow cytometry of Lin$^-$ cells from lymph nodes of wild-type (WT) and hK14mIL33tg (Tg) mice. Data are expressed as means ± SEM (n = 3) *P < 0.05 (Student t test). Lin$^-$ST2$^+$ ILC2s producing IL-5 and IL-13 were markedly induced in hK14mIL33tg mice.

unknown. We have found that IL-33 induces the activation of mast cells in vitro (6), but the phenotype of hK14mIL33tg mice suggests mast cells could also be highly activated in vivo by IL-33. Administration of an antihistamine diphenhydramine hydrochloride relieves the itching behavior of the mice (Fig. 3*G*), which implies that mast cells are responsible for the itching associated with dermatitis in hK14mIL33tg mice.

The infiltration of eosinophils in dermatitis may be a result of the increased levels of IL-5, IL-13, RANTES/CCL5, and Eotaxin 1/CCL11 in the serum or lesional skin of hK14mIL33tg mice (Fig. 4). Notably, the marked increase in IL-5 seems to be characteristic of the transgenic mice, because it was not found in wild-type mice. Serum levels of IL-33 are not increased in hK14mIL33tg mice or in patients with AD (23). IL-33 in the serum might remain low in skin allergies, although it is high in patients with allergic rhinitis (24).

Interestingly, we demonstrated that ILC2s are increased in the skin with dermatitis, in peripheral blood, and in lymph nodes (Fig. 5*A*), and furthermore, that ILC2s are IL-5-producing in hK14mIL33tg mice (Fig. 5*B*). A recent study has shown that a population of group 2 ILCs is enriched in the lesional skin of patients with AD (25). However, group 2 ILCs have been reported at least to be IL-33-independent, and it is unknown whether those ILC2s correspond to mouse ILC2s increased in hK14mL33tg mice. The present study provides evidence that ILC2s are involved in the pathophysiology of IL-33-induced cutaneous inflammation, although the induction of ILC2s in response to IL-33 has been shown in other pathogenic conditions, such as influenza virus (26), helminthic infection (3, 27), and chronic rhinosinusitis (28).

Because a neutralizing antibody for IL-5 abolishes eosinophils in vivo (Fig. 6*C*), the induction of eosinophils is possibly dependent on IL-5 in hK14mIL33tg mice. The expression of the IL-5 gene is enhanced (29), and eosinophil infiltrates have been observed in the lesional skin of patients with AD, regardless of blood eosinophilia (22). The efficacy of anti-IL-5 for treating AD

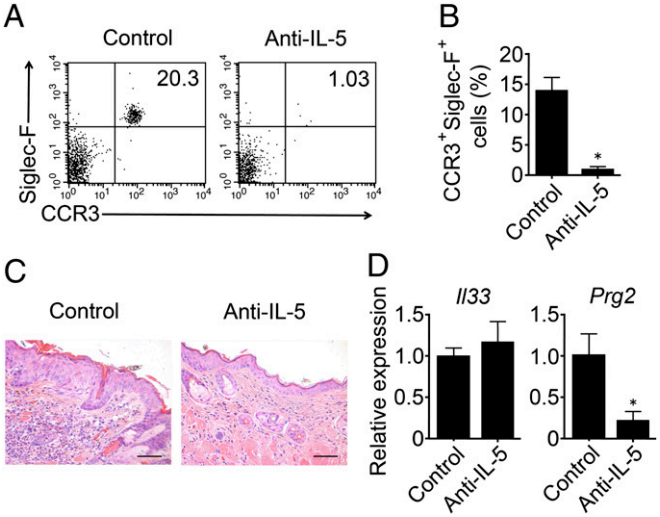

Fig. 6. IL-5-dependent induction of eosinophils in hK14mIL33tg mice. A neutralizing monoclonal anti-IL-5 antibody or a control IgG1 antibody (20 μg per mouse) was administered intraperitoneally to hK14mIL33tg mice every 2 d for 2 wk. (*A*) Flow cytometry of CCR3$^+$Siglec-F$^+$ eosinophils in peripheral blood cells of hK14mIL33tg mice. The numbers indicate the proportion of CCR3$^+$Siglec-F$^+$ eosinophils. Data are representative of four mice. (*B*) The proportions of eosinophils in flow cytometry of CD45$^+$ cells from peripheral blood cells. Data are expressed as means ± SEM (n = 4). *P < 0.05 (Student t test). Eosinophilia in hK14mIL33tg mice was significantly improved by the anti-IL-5 antibody. (*C*) H&E staining of the lesional skin of hK14mIL33tg mice. Data are representative of four mice. (Scale bars, 50 μm.) Dermatitis with eosinophil infiltrates was resolved by the anti-IL-5 antibody. (*D*) The expression of the IL-33 and Prg2 genes in the lesional skin of hK14mIL33tg mice. Data are expressed as means ± SEM (n = 4). *P < 0.05 (Student t test). The expression of *Il33* was unchanged, whereas that of *Prg2* was significantly suppressed by the anti-IL-5 antibody.

was expected, but a clinical trial of an anti-human IL-5 antibody (mepolizumab) resulted in only a modest improvement of the disease that was not considered successful (30). That result has been assumed to be a result of the low dose of mepolizumab used, which reduced blood eosinophil levels only to 70% (30). However, mepolizumab has been proven to be effective for severe asthma afterward (31), and this report revives interest in the biologic targeting of IL-5. Psoriasis is also an intractable skin disorder, but effective regimens using biologics have now been established to dramatically improve the quality of life of patients with psoriasis. In contrast, useful biologics have not been available for treating patients with AD (32), and further investigations are necessary to explore immunological processes involving IL-33 and ILC2s as novel targets for the therapy of AD.

In conclusion, we propose that IL-33 from epidermal keratinocytes induces ILC2s in the skin and lymph nodes and stimulates the production of IL-5 from those cells to induce AD-like dermatitis with eosinophil infiltrates. The phenotype of hK14mIL33tg mice strongly suggests that IL-33 is crucial for the development of AD. This transgenic mouse, in which long-lasting dermatitis with severe itching develops spontaneously, may be useful for studying the pathogenesis of AD and to test the therapeutic efficacy of drugs for AD. Further study using hK14mIL33tg mice will help understand the mechanisms of release, activation, and regulation of IL-33 in vivo, which might open the way to novel therapeutic drugs targeting innate immune responses involving IL-33.

Materials and Methods

Preparation of cell suspensions from mouse skin, used for flow cytometric analysis, was as described previously (33). In brief, skin sheets were incubated in 40 mL RPMI 1640 containing 10% (vol/vol) FCS, 0.2% collagenase D (Roche) and 0.01% DNase I (Roche) at 37 °C for 60 min. After vigorous pipetting, cells were isolated by gradient centrifugation on a Lymphosepar II (IBL). Cells from mouse skin, lymph nodes, and blood were stained with each antibody and were examined using a FACS Calibur (BD Biosciences). The classification of cells is as follows. $B220^{-}CD3^{-}CD45^{+}CCR3^{+}Siglec\text{-}F^{+}$ cells, eosinophils; $B220^{-}CD3^{-}CD45^{+}Gr\text{-}1^{+}CD11b^{+}$ cells, neutrophils; $B220^{-}CD3^{-}CD45^{+}DX5^{+}IgE^{+}$cells, basophils; $B220^{-}CD3^{-}CD45^{+}c\text{-}kit^{+}IgE^{+}$cells, mast cells; and lineage markers (CD3, CD4, CD8, CD19, Gr-1, IgE, NK1.1, Siglec-F)$^{-}Sca\text{-}1^{+}$ $ST2^{+}$ cells, ILC2s. Intracellular IL-5 or IL-13 staining was as described previously (27). In brief, cells from lymph nodes were incubated in culture medium for 6 h, and surface antigens were stained in the presence of monensin without phorbol 12-myristate 13-acetate/ionomycin. Cells were fixed with 4% (wt/vol) paraformaldehyde, permeabilized with 0.1% saponin buffer (PBS with 0.1% saponin, 1 mM Hepes, and 0.1% BSA) and then stained with anti-IL-5, anti-IL-13, or a control rat IgG1 antibody (BD Biosciences). Details of analyses are described in *SI Materials and Methods*.

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