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dinitrofluorobenzene. IL-13 suppressed IL-12 production by mouse skin-derived DCs *in vitro* and *in vivo*. Scratching upregulated *IL13* expression in human skin, and IL-13 suppressed the capacity of LPS-stimulated human skin DCs to express IL-12 and promote IFN- γ secretion by CD4⁺ T cells. Conclusion: Release of IL-13 by cutaneous MCs in response to mechanical skin injury inhibits the T_H1 cell response to cutaneous antigen exposure in AD. (J Allergy Clin Immunol 2021;147:2305-15.)

Key words: Atopic dermatitis, IL-13, dendritic cells, T_H1 cells

Atopic dermatitis (AD) is characterized by a skin barrier defect, a T_H2 cell–dominated immune response to environmental allergens and foods, allergic skin inflammation with dermal infiltration by T_H2 cells and eosinophils, and susceptibility to cutaneous viral infection (eg, eczema herpeticum, eczema vaccinatum, and molluscum contagiosum).^{1,2} Mechanical injury inflicted by scratching dry itchy skin aggravates the skin barrier defect in AD. Normal skin is impermeable to cutaneously encountered antigens. Defective skin barrier function allows antigen entry, which is essential to drive an immune response. Mutations in genes coding for proteins involved in epidermal barrier function, such as *FLG*, *DSG1*, *SPINK5*, and *CDSN*, are associated with AD.³ The signals leading to a T_H2 cell–dominated immune response to cutaneously encountered antigens, development of allergic skin inflammation, and subsequent susceptibility to viral skin infections in AD are not well understood.

Dendritic cells (DCs) are abundant in the skin and in other body interfaces, and they are critical for the adaptive immune response to antigen. The composition and migration of skin DCs are heterogeneous.⁴ At steady state, the skin harbors conventional DCs and monocyte-derived DCs in the dermis and Langerhans cells in the epidermis; in addition, plasmacytoid DCs populate inflamed skin.⁴ Following its entry through a disrupted skin barrier, antigen is taken up and processed by conventional DCs in the dermis, which rapidly migrate to draining lymph nodes (dLNs), where they drive the expansion of recirculating naive antigen-specific CD4⁺ T cells and their differentiation into cytokine-producing cells.⁴⁻⁶ The migration of conventional DCs is later followed by the migration of Langerhans cells.⁷ DCs integrate the cues present in their microenvironment to polarize the T-cell response. We and others have previously shown that thymic stromal lymphopoietin released following mechanical skin injury acts on skin DCs to promote their ability to polarize T cells toward a T_H2 cell phenotype.⁸⁻¹¹ In addition, we have shown that mechanical skin injury inhibits the ability of skin DCs emigrants to drive T_H1 cell polarization.⁸ The molecules that mediate this inhibition remain unknown.

IL-13 is a type 2 cytokine produced by T_H2 cells, natural killer T cells, mast cells (MCs), basophils, and type 2 innate lymphoid cells (ILC2s).¹² IL-13 is expressed in AD skin lesions and plays an important role in pathogenesis of the disease.¹³ IL-13 upregulates the expression of T_H2 cell–attracting chemokines by stromal cells, impairs epidermal integrity, and promotes epidermal hyperplasia.¹⁴⁻¹⁶ There is strong evidence that IL-13 acts in the effector phase of the immune response to dampen T_H1 cell activation. In a mouse model of asthma DCs from mediastinal lymph nodes, *IL13*^{−/−} mice exhibit enhanced capacity to promote IFN- γ production in memory allergen-specific

Abbreviations used

AD:	Atopic dermatitis
DC:	Dendritic cell
BMMC:	Bone marrow–derived mast cell
CHS:	Contact hypersensitivity
dLN:	Draining lymph node
DNFB:	1-Fluoro-2,4-dinitrobenzene
ILC2:	Type 2 innate lymphoid cell
MC:	Mast cell
OVA:	Ovalbumin
rIL-13:	Recombinant IL-13
WT:	Wild-type

CD4⁺ T cells.¹⁷ Furthermore, IL-13 inhibits IFN- γ production and is protective in experimental models of autoimmune disease and graft rejection.¹⁸⁻²¹

The role for IL-13 in the initiation of the immune response to cutaneously encountered antigen has not been explored. We demonstrate that mechanical injury to mouse skin promotes IL-33–mediated upregulation of *IL13* expression in skin MCs, and that MC-derived IL-13 selectively inhibits the capacity of skin-derived DCs to polarize naive mouse CD4⁺ T cells to a T_H1 cell phenotype. Furthermore, we demonstrate that scratching induces *IL13* upregulation in human skin, and that IL-13 suppresses the ability of human skin DCs to promote IFN- γ secretion by CD4⁺ T cells.

METHODS

Mice

C57BL/6J wild-type (WT), BALB/c WT, and DO11.10 TCR transgenic mice were purchased from Charles River Laboratory (Wilmington, Mass). *Rag2*^{−/−} mice on a BALB/c background were purchased from Taconic (Germantown, NY). *Rag2*^{−/−}*gc*^{−/−} mice on a B6 background and *IL4/13*^{fllox/fllox} on a BALB/c background were purchased from the Jackson Laboratory (Bar Harbor, Me). *IL13*^{−/−}, *IL1rl1*^{−/−}, and *Kit*^{W-sh/W-sh} mice on a BALB/c background were previously described.²²⁻²⁴ *Mcpt5-cre* mice on a BALB/c background were crossed with *IL13*^{−/−} mice and then crossed with *IL4/13*^{fllox/fllox} mice. All of the mice were kept in a pathogen-free environment and fed an ovalbumin (OVA)-free diet. All procedures performed on the mice were in accordance with guidelines of the Animal Care and Use Committee of the Boston Children's Hospital.

Human subjects

After informed consent had been obtained, the inner side of the forearm of 3 healthy allergy-free adult subjects was scratched 30 times with a no. 11 sterile blade with care not to draw blood. Six hours later a 4-mm punch biopsy specimen was obtained from the scratched site and another biopsy specimen was obtained from a skin site on the contralateral forearm. The samples were kept in RNAlater (ThermoFisher Scientific, Waltham, Mass) and stored at −80°C. All procedures performed were in accordance with the guidelines of the institutional review board of the Boston Children's Hospital.

Preparation of skin cell suspensions from human skin and isolation of skin DCs

Human surgical discards of abdominal skin were obtained from the Human Skin Disease Resource Core at Brigham and Women's Hospital. To obtain the cells, all of the fat was removed by using a scalpel and the skin was chopped into small pieces and digested for 2 hours at 37°C with vigorous shaking in

complete RPMI medium containing Liberase LT (0.2 mg/mL; Roche, Indianapolis, Ind) and DNase I (0.2 mg/mL; Sigma, St Louis, Mo). Digested tissue was mechanically disrupted by using a plunger, filtered, centrifuged, and resuspended. Skin DCs were sorted by using a CD1c (BDCA-1)⁺ DC Isolation Kit from Miltenyi Biotec (Waltham, Mass), according to the manufacturer's instructions.

Functional analysis of human skin DCs

A total of 1×10^4 skin DCs were stimulated overnight with LPS (100 ng/mL) or medium in the absence or presence of recombinant IL-13 (rIL-13) (50 ng/mL). The next day the cells were harvested, kept in lysis buffer, and stored at -80°C for mRNA analysis. For the coculture experiments, stimulated skin DCs were extensively washed 3 times to remove LPS and rIL-13 and cocultured with 1×10^5 CD4⁺ T cells isolated from the blood of healthy donors by using a CD4⁺ T-Cell Isolation Kit from Miltenyi. The cells were cultured for 5 days in complete medium in the presence of T-Cell Activation/Expansion Kit beads (Miltenyi).

Tape stripping of mouse skin

The back skin of anesthetized mice was shaved and subjected to tape stripping 6 times with a film dressing (Tegaderm; 3M, Chelmsford, Mass). Mouse skin samples were collected at different time points, kept in RNAlater, and stored at -80°C .

RNA extraction and RT-PCR

RNA was extracted from human and mouse skin by using a Total RNA Isolation Kit (ThermoFisher Scientific). cDNA was prepared by using an iScript cDNA Synthesis Kit (Biorad, Irvine, Calif). Quantitative real-time PCR was done with the Taqman gene expression assay, universal PCR master mix, and ABI Prism 7300 Sequence Detection system (ThermoFisher Scientific). Fold induction was calculated by using the $2^{-\Delta\Delta C_t}$ method with normalization to the internal control β -2 microglobulin. An arbitrary unit of 1 was assigned to the mean value of unmanipulated skin samples.

Preparation of skin cell suspensions from mouse skin

Ears from vehicle or 1-fluoro-2,4-dinitrobenzene (DNFB)-treated mice were split in half, finely chopped by using scissors, and digested for 90 minutes in media containing Liberase TL (0.2 mg/mL; Roche) and DNase II (Sigma), with continuous shaking at 37°C . The digested skin homogenates were passed through a 70- μm cell strainer, washed and resuspended in PBS, and used for flow cytometry.

Isolation and functional analysis of mouse skin dLN DCs

For the *in vivo* DC priming experiment, OVA (1 mg in 100 μL of saline) was epicutaneously applied to the shaved, tape-stripped skin of the mice; 24 hours after sensitization, DCs were isolated from axillary lymph nodes by using CD11c beads from Miltenyi after enzymatic digestion with 1 mg/mL of collagenase IV. Naive CD4⁺ T cells were purified from the spleens of DO11.10 mice by using the Naive CD4⁺ T Cell Isolation Kit II from Miltenyi after mechanical dissociation. For proliferation studies, 2.5×10^4 CD11c⁺ cells were cocultured 1:1 with CellTrace Violet-stained naive DO11.10 CD4⁺ T cells without addition of exogenous OVA protein for 3 days and then analyzed by flow cytometry. For polarization studies, 1×10^5 CD11c⁺ cells were cocultured 1:1 with naive DO11.10 CD4⁺ T cells without addition of exogenous OVA protein for 5 days. Cell-free supernatant was used to detect mouse IL-5, IL-13, and IFN- γ by using ELISA Ready-SET Go kits (ThermoFisher Scientific).

IL-33 release by epidermis and dermis following tape stripping

Ears were excised from unmanipulated mice or immediately after tape stripping. The ears were split into dorsal and ventral halves, and the skin was floated on 4 mg/mL of dispase solution (Roche) for 30 minutes at 37°C . Epidermis and dermis were separated and cultured for 3 hours in complete RPMI medium. IL-33 in the supernatant was measured by using a Quantikine ELISA Kit (R&D, Minneapolis, Minn).

BMMC reconstitution of MC-deficient mice

Bone marrow cells from both the femurs and tibias of BALB/c WT or BALB/c *Il1rl1*^{-/-} mice were cultured for 4 weeks in complete RPMI medium supplemented with IL-3 (10 ng/mL) and stem cell factor (20 ng/mL) (Peprotech, Rocky Hill, NJ). Next, 1.5×10^6 bone marrow-derived MCs (BMMCs) were injected intradermally into 6- to 8-week-old female *Kit*^{W^{sh}/W^{sh} mice. To allow BMMC engraftment, the mice were given a 4-week rest before the tape stripping treatment.}

Flow cytometry

Cells were preincubated with Fc γ R-specific blocking mAb (2.4G2) and washed before staining with the following mAbs: PE-anti-CD124 (mIL4R-M1), FITC-anti-MHCII (M5/114.15.2), APC-anti-CD80 (16-10A1), PerCP-eFluor-710-anti-CD40 (1C10), PE-Cy7-anti-CD86 (GL1), Biotin-anti-CD11c (N418), AlexaFluor700-anti-MHC II (M5/114.15.2), and BV605-anti-CD11b (M1/70). Ebioscience Fixable Viability Dye eFluor 506 was used to exclude dead cells. For cytokine staining, cells were stimulated with ionomycin (0.5 $\mu\text{g}/\text{mL}$; Sigma), phorbol 12,13-dibutyrate (1 $\mu\text{g}/\text{mL}$; Sigma), brefeldin A (ThermoFisher Scientific), and monensin (ThermoFisher Scientific) in complete RPMI medium for 3 hours before surface staining. Subsequently, the cells were fixed and permeabilized (BD Biosciences Cytofix/Cytoperm, Franklin Lakes, NJ) and stained in permeabilization solution with antibodies against IFN- γ , IL-13, and IL-17A from eBioscience. The cells were analyzed on an LSRFortessa flow cytometer (BD Biosciences), and the data were analyzed with FlowJo software.

DNFB-induced contact hypersensitivity

The mice were sensitized with 50 μL of 0.5% DNFB (Sigma) in acetone-olive oil (4:1) on their shaved abdomens; the mice were then challenged 5 days later with 25 μL of 0.5% DNFB in acetone-olive oil (4:1) on the right ear and 25 μL of acetone-olive oil (4:1) (vehicle) on the left ear. Ear thickness was measured with a micrometer before challenge and 24 hours thereafter.

Statistical analysis

Statistical significance was determined by the 2-tailed Student *t* test. A *P* value less than .05 was considered statistically significant.

RESULTS

Keratinocyte-derived IL-33 drives IL-13 production by MCs following skin mechanical injury

Tape stripping increased *Il13* mRNA levels in mouse skin as early as 1 hour, with a return to baseline by 12 hours (Fig 1, A). To determine the source of IL-13 released following skin mechanical injury, we examined *Rag2*^{-/-} mice (which lack mature T and B cells), *Rag2*^{-/-}/*gc*^{-/-} mice (which lack ILCs in addition to mature T and B cells), and *Kit*^{W^{sh}/W^{sh} mice (which lack MCs) 3 hours after tape stripping. Upregulation of *Il13* in the skin was preserved in *Rag2*^{-/-} mice (Fig 1, B) and *Rag2*^{-/-}/*gc*^{-/-} mice (Fig 1, C) but abolished in *Kit*^{W^{sh}/W^{sh} mice (Fig 1, D), suggesting that MCs are the major source for IL-13 in mechanically injured skin. *Kit*^{W^{sh}/W^{sh} mice have abnormalities in cell lineages other than}}}

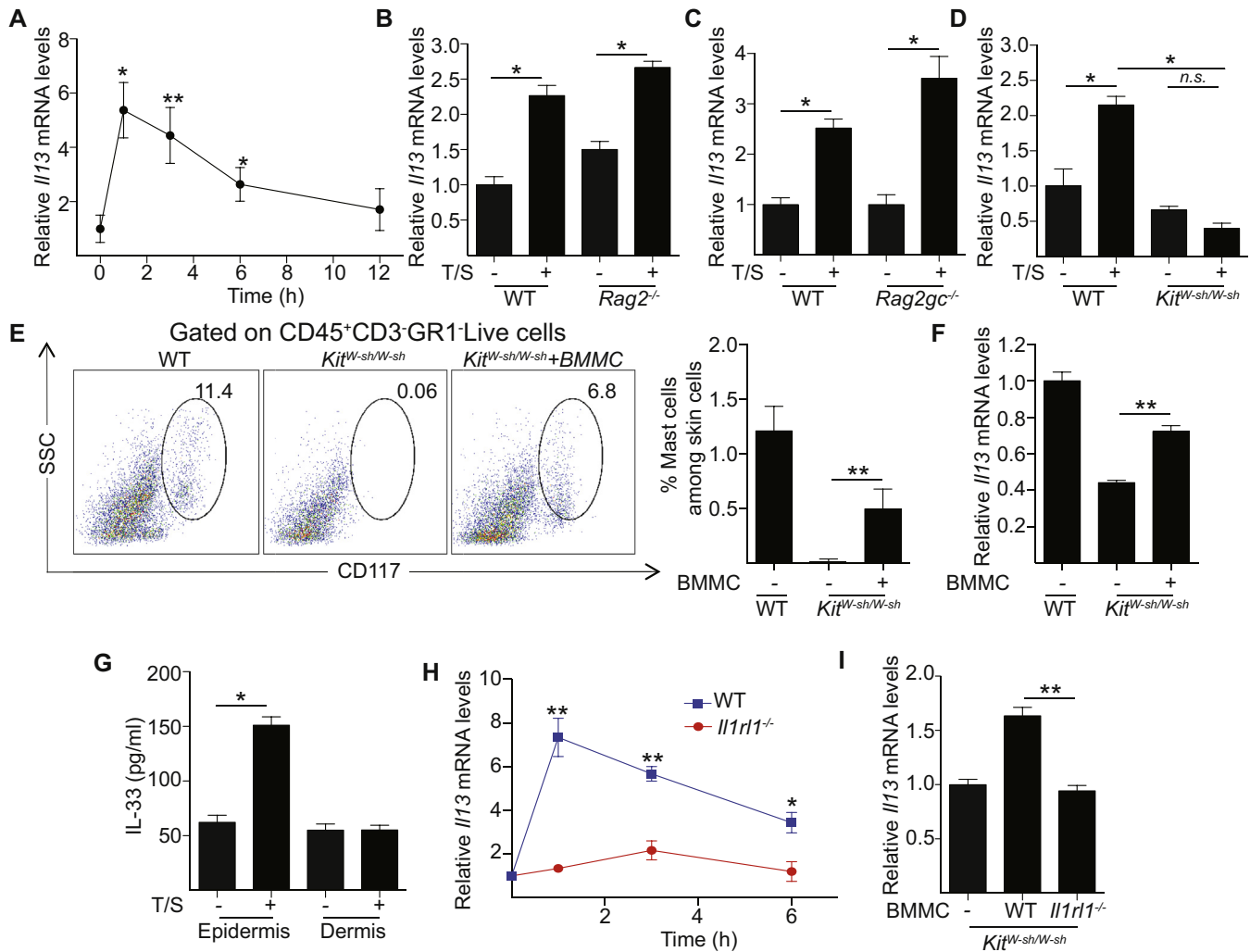


FIG 1. Keratinocyte-derived IL-33 drives IL-13 production by cutaneous MCs following mechanical skin injury. **A**, Time course analysis of cutaneous *Il13* mRNA levels after tape stripping (T/S). **B–D**, Relative *Il13* mRNA expression in unmanipulated and tape-stripped skin of *Rag2^{-/-}* mice (**B**), *Rag2gc^{-/-}* mice (**C**), and *Kit^{W-sh/W-sh}* mice (**D**) and WT controls. **E** and **F**, Representative flow cytometry plots (left) and quantitation (right) of CD45⁺CD3⁺G1⁺CD117⁺ MCs (**E**) and relative cutaneous *Il13* mRNA expression in the skin of WT mice and *Kit^{W-sh/W-sh}* and *Kit^{W-sh/W-sh}* mice that has been reconstituted with WT BMMCs. (**F**). **G**, IL-33 secretion by epidermal and dermal sheets from unmanipulated and tape-stripped ears of WT mice. **H**, Relative *Il13* mRNA expression in unmanipulated and tape-stripped skin of *Il1rl1^{-/-}* mice and WT controls at different time points. **I**, Relative cutaneous *Il13* mRNA expression in nonreconstituted skin of *Kit^{W-sh/W-sh}* mice and skin of *Kit^{W-sh/W-sh}* mice reconstituted with WT and *Il1rl1^{-/-}* BMMCs. Results in (**A**) to (**I**) are representative of 2 independent experiments with 4 or 5 mice/group. Columns and bars represent means $\pm$ SEMs. *P < .05; **P < .005. SSC, Side scatter.

MCs.²⁵ To ascertain the role of MCs in IL-13 induction after tape stripping, we reconstituted the skin of *Kit^{W-sh/W-sh}* mice with WT mouse BMMCs. Four weeks after intradermal administration of WT BMMCs, the reconstituted skin was subjected to tape stripping. Flow cytometry revealed absence of MCs in nonreconstituted skin of *Kit^{W-sh/W-sh}* mice. In contrast, MCs were present in BMMC-reconstituted skin of *Kit^{W-sh/W-sh}* mice, but their percentages among skin cells was about half that in WT controls (Fig 1, E). Reconstitution of the skin of *Kit^{W-sh/W-sh}* mice with WT BMMCs partially restored the increase in *Il13* mRNA 3 hours after tape stripping (Fig 1, F). Together, these results indicate that MCs are the major source of *Il13* induced in the skin by mechanical injury.

IL-33 promotes the production of IL-13 by MCs,²⁶ and its level increases in the skin following mechanical injury.^{27,28} Epidermal sheets, but not dermal sheets, prepared immediately after tape stripping the skin of WT mice demonstrated increased IL-33 secretion (Fig 1, G). We used IL-33 receptor-deficient *Il1rl1^{-/-}* mice to examine the role of IL-33 in *Il13* induction by mechanical skin injury. Induction of *Il13* mRNA following tape stripping was abolished in *Il1rl1^{-/-}* mice at all of the time points analyzed (Fig 1, H). To investigate whether IL-33 targets skin MCs directly to promote their production of IL-13 following mechanical injury, we reconstituted the skin of *Kit^{W-sh/W-sh}* mice with BMMCs derived from *Il1rl1^{-/-}* mice or WT controls. Tape stripping failed to increase *Il13* expression in the skin of *Kit^{W-sh/W-sh}* mice reconstituted

with *Il1rl1*^{-/-} BMMCs (Fig 1, I), but as a previously shown in Fig 1, F, it increased *Il13* expression in skin of *Kit*^{Wsh/Wsh} mice reconstituted with WT BMMCs. These results demonstrate that keratinocyte-derived IL-33 acts on skin MCs to promote IL-13 production following mechanical injury.

MC-derived IL-13 inhibits the capacity of skin-derived DCs to promote T_H1 cell polarization of naive CD4⁺ T cells to cutaneously introduced antigens

We previously reported that mechanical injury skews skin-derived DCs to promote T_H2 cell polarization and suppress T_H1 cell polarization.⁸ To determine the role of IL-13 in this process we made use of *Il13*^{-/-} mice. *Il13*^{-/-} mice and WT controls were subjected to epicutaneous sensitization with OVA; 24 hours later skin dLNs were examined by flow cytometry for CD11c⁺MHCII^{high} DCs, which represent recent skin emigrants.²⁹ The numbers of skin DC emigrants in dLNs, as well as expression of the activation markers CD40, CD80, CD86, and PDL2 by these cells, were comparable in *Il13*^{-/-} mice and WT controls (Fig 2, A and B and see Fig E1, A in the Online Repository at www.jacionline.org). Moreover, coinjection of rIL-13 with OVA did not alter either the accumulation of CD11c⁺MHCII^{high} DCs in the dLNs or the expression of activation markers by these cells (see Fig E1, B and C). These results indicate that IL-13 has no effect on the migration or activation of skin-derived DCs.

We next investigated the role of IL-13 in modulating the ability of skin-derived DCs to cause the proliferation and polarization of antigen-specific naive CD4⁺ T cells. *Il13*^{-/-} mice and WT controls were epicutaneously sensitized with OVA. Twenty-four hours later, DCs were purified from the skin dLNs and cocultured with splenic naive CD4⁺ T cells from DO11.10 transgenic mice, which carry a T-cell receptor specific for OVA. No exogenous OVA was added. DCs from the skin dLNs of *Il13*^{-/-} mice and WT controls induced comparable proliferation and secretion of IL-5, IL-13, and IL-17A by naive CD4⁺ T cells (Fig 2 C and D and see Fig E1, D). However, compared with DCs from the skin dLNs of WT controls, DCs from the skin dLNs of *Il13*^{-/-} mice caused increased production of IFN-γ by naive CD4⁺ T cells, as demonstrated by IFN-γ secretion into the supernatants (Fig 2, E) as well as by intracellular staining of the T cells for IFN-γ (Fig 2, F). In contrast, DCs from dLNs of skin coinjected with rIL-13 and OVA caused significantly less IFN-γ production by naive CD4⁺ T cells than did DCs from dLNs of skin intradermally injected with OVA alone (see Fig E1, E). DCs from skin dLNs of mice epicutaneously sensitized with saline failed to induce proliferation or cytokine secretion by naive CD4⁺ T cells in the absence of addition of OVA to the cultures (data not shown). These data suggest that IL-13 inhibits the capacity of antigen-bearing skin-derived DCs to promote T_H1 cell polarization.

To investigate whether IL-13 affects antigen presentation by DCs obtained from skin DLNs at later time points after cutaneous antigen exposure, we isolated CD11c⁺ cells from DLNs 72 hours after epicutaneous sensitization with OVA and analyzed their ability to promote polarization of naive CD4⁺ T cells. Neither T-cell proliferation nor secretion of IL-13, IL-17A, and IFN-γ were induced (data not shown). These results suggest that CD11c⁺ cells that migrate to DLNs late after cutaneous

sensitization do not support the activation of antigen-specific naive CD4⁺ T cells.

We next investigated whether IL-13 directly modulates the capacity of DCs from skin dLNs to promote T_H1 cell polarization. To this purpose, we used *Il4ra*^{-/-} mice, which lack the IL-4Rα chain common to the IL-4 and IL-13 receptors. DCs from the skin dLNs of unmanipulated WT and *Il4ra*^{-/-} mice were purified and cocultured with DO11-10 T cells and OVA with or without addition of rIL-13. In the absence of IL-13, DCs from WT and *Il4ra*^{-/-} mice caused comparable secretion of IFN-γ by naive CD4⁺ T cells (Fig 2, G). The addition of IL-13 significantly reduced IFN-γ secretion by naive CD4⁺ T cells cocultured with DCs from WT mice, but it had no effect on IFN-γ secretion by naive T cells cocultured with IL-4Rα-deficient DCs (Fig 2, G). To demonstrate that IL-13 released by MCs inhibits the capacity of skin-derived DCs to induce T_H1 cell polarization, we examined *Mcpt5-Cre*^{Tg/0}*Il4*^{fllox/+}*Il13*^{fllox/-} mice, which lack IL-13 selectively in MCs. Compared with DCs from dLNs of OVA-sensitized skin from *Il4*^{fllox/+}*Il13*^{fllox/-} control mice, DCs from the dLNs of OVA-sensitized skin from *Mcpt5-Cre*^{Tg/0}*Il4*^{fllox/+}*Il13*^{fllox/-} mice caused significantly more IFN-γ secretion but comparable IL-13 and IL-17A secretion by naive DO11.10 T cells (Fig 2, H and Fig E1, F). These results suggest that IL-13 released by MCs in mechanically injured skin acts directly on skin DCs to inhibit their capacity to drive T_H1 cell polarization.

MC-derived IL-13 controls IL-12 expression in skin-derived DCs

The cytokine IL-12 is produced by DCs and plays a critical role in DC-mediated T_H1 cell polarization.³⁰ IL-12 (p70) is a heterodimer of the p35 chain encoded by *Il12a* and the p40 chain encoded by *Il12b*. To investigate whether IL-13 regulates IL-12 production by DCs, we examined the effect of IL-13 on IL-12 secretion by LPS-stimulated DCs from the skin dLNs of WT mice. LPS stimulation increased IL-12p70 secretion by skin dLN DCs in a dose-dependent manner (Fig 3, A). Notably, rIL-13 decreased the secretion of IL-12p70 by skin dLN DCs (Fig 3, A). rIL-13 had no detectable effect on LPS induction of *Il12a* (IL-12p35) mRNA; however, it significantly inhibited LPS induction of *Il12b* (IL-12p40) (Fig 3, B). These *in vitro* results suggest that IL-13 inhibits LPS-driven production of IL-12p70 by DCs by downregulating *Il12b* (IL-12p40) expression.

To investigate whether IL-13 regulates IL-12 expression in skin-derived DCs *in vivo*, we measured *Il12a* and *Il12b* mRNA levels in DCs sorted from skin dLNs of WT and *Il13*^{-/-} mice 24 hours after epicutaneous sensitization with OVA. *Il12b* (IL-12p40) expression but not *Il12a* (IL-12p35) expression was significantly higher in DCs purified from the dLNs of OVA-sensitized skin of *Il13*^{-/-} mice than in the WT controls (Fig 3, C). No difference in the expression of *Il23a* (IL-23p19) or *Il6* was observed between DCs from the two mouse strains; however, *Tnf* expression was significantly higher in DCs of *Il13*^{-/-} mice than in the WT controls (see Fig E2, A in the Online Repository at www.jacionline.org). DCs purified from the dLNs of OVA-sensitized skin from *Mcpt5-Cre*^{Tg/0}*Il4*^{fllox/+}*Il13*^{fllox/-} mice exhibited significantly higher expression of *Il12b* and *Tnf*, but not *Il12a*, *Il23a*, or *Il6*, compared with DCs from their *Il4*^{fllox/+}*Il13*^{fllox/-} littermates (Fig 3, D and see Fig E2, B). These results indicate that IL-13 produced by skin MCs in response to mechanical injury suppresses IL-12 production in skin-derived DCs.

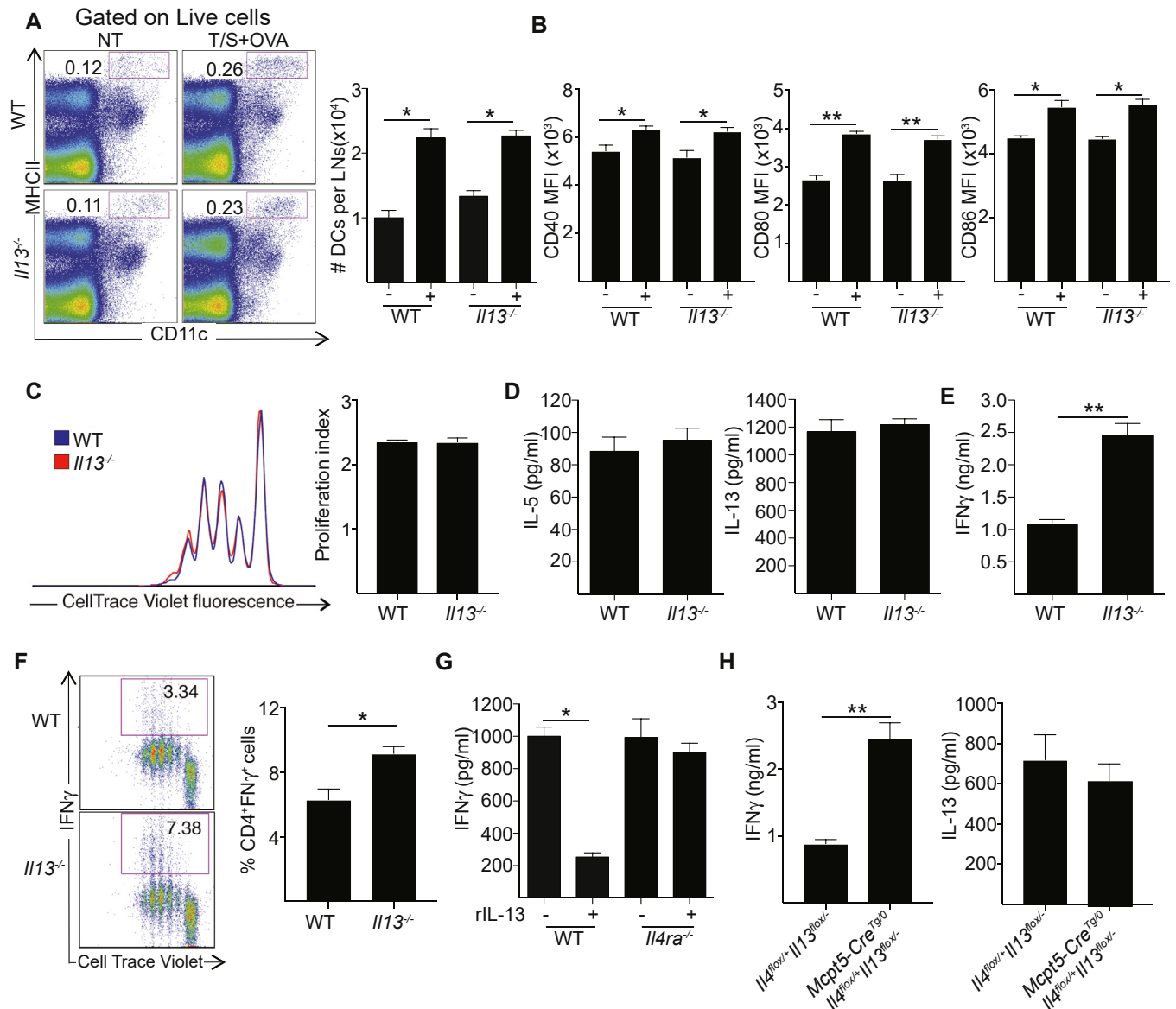


FIG 2. MC-derived IL-13 inhibits the capacity of antigen-capturing skin-derived DCs to promote T_H1 cell polarization of naive CD4⁺ T cells. **A** and **B**, Representative flow cytometry plots (*left*) and quantitation (*right*) of CD11c⁺MHCII^{high} skin-derived DCs (**A**) and mean fluorescence intensity (MFI) of CD40, CD80, and CD86 expression by skin-derived DCs (**B**) in dLNs of unmanipulated and OVA-sensitized skin of *Il13*^{-/-} mice and WT control mice. **C-F**, Representative flow cytometry histogram of proliferation (*left*) and proliferation index (*right*) (**C**), IL-5 and IL-13 secretion (**D**), IFN-γ secretion (**E**), and representative (*left*) and quantitative (*right*) analysis of intracellular IFN-γ expression (**F**) by naive constrained tibial vibration-loaded CD4⁺ T cells from DO11.10 mice cocultured with DCs from dLNs of OVA-sensitized skin of *Il13*^{-/-} mice and WT controls. **G**, IFN-γ production by naive CD4⁺ T cells from DO11.10 mice cocultured with DCs from dLNs of unmanipulated *Il4ra*^{-/-} mice and WT controls in the absence or presence of rIL-13. **H**, IL-13 and IFN-γ secretion by naive CD4⁺ T cells from DO11.10 mice cocultured with DCs from dLNs of OVA-sensitized skin of *Mcpt5-Cre*^{Tg}*Il4*^{fllox/+} mice and *Il4*^{fllox/+} control mice. Results in (**A**) to (**H**) are representative of 2 independent experiments with 4 or 5 mice/group. Columns and bars represent means ± SEMs. *P < .05; **P < .005. LN, Lymph node.

MC-derived IL-13 controls T_H1 cell differentiation during CHS induced by DNFB

We next investigated whether MC-derived IL-13 regulates T_H1 cell differentiation in a T_H1 cell-dominated cutaneous immune response. Contact hypersensitivity (CHS) to DNFB is mediated predominantly by IFN-γ produced by CD4⁺ T_H1 cells and

CD8⁺ T cells.³¹⁻³⁵ Compared with vehicle-treated ears, DNFB-painted ears from nonsensitized WT mice showed upregulation of *Il33* and *Il13* (see Fig E3, A and B in the Online Repository at www.jacionline.org). Moreover, *Il13* upregulation induced by DNFB painting was abolished in *Il1rl1*^{-/-} mice and significantly reduced in *Mcpt5-Cre*^{Tg}*Il4*^{fllox/+}*Il13*^{fllox/-} mice (see Fig E3, C

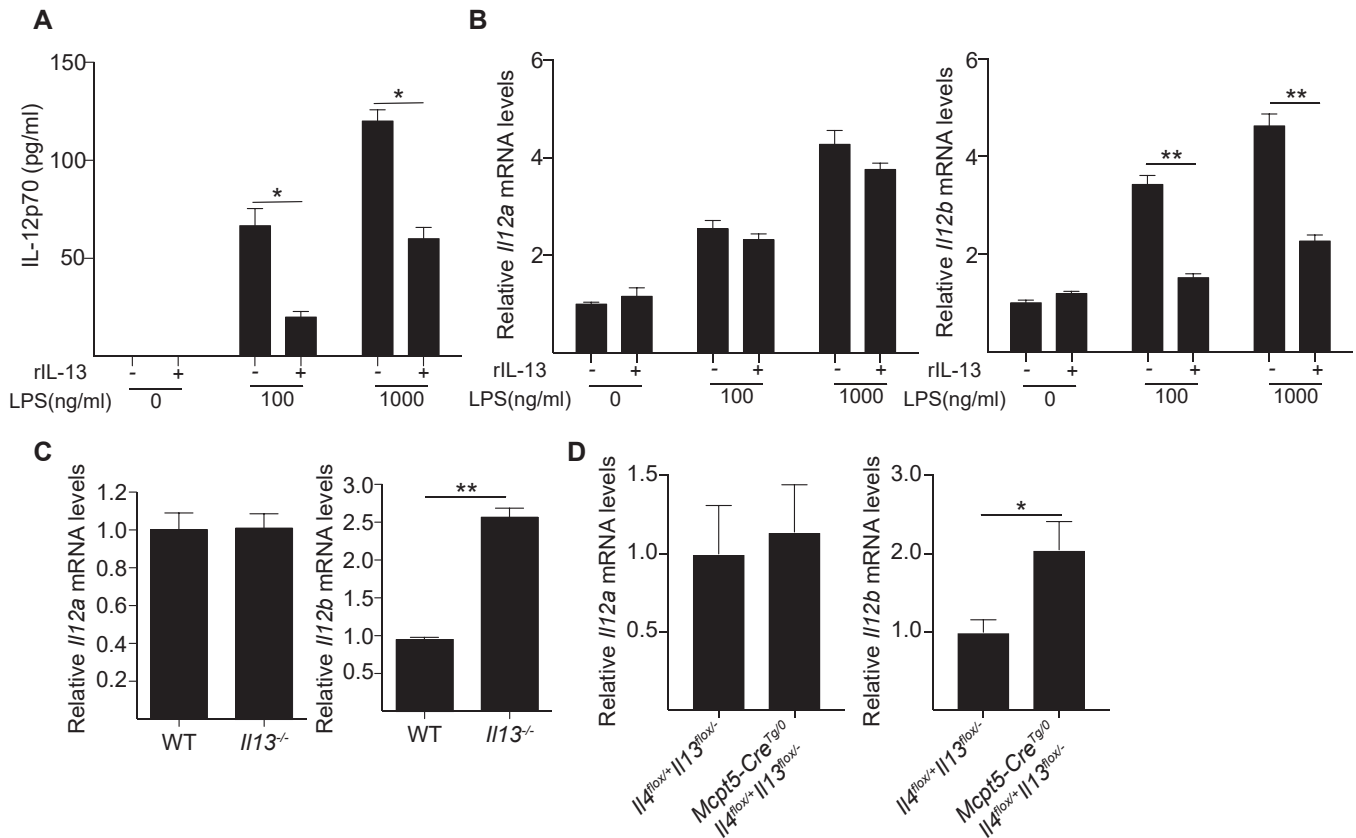


FIG 3. MC-derived IL-13 inhibits IL-12 expression in skin-derived DCs. **A** and **B**, IL-12p70 secretion (**A**) and relative *IL12a* and *IL12b* mRNA expression (**B**) in DCs stimulated with LPS in the absence or presence of added rIL-13. **C** and **D**, Relative *IL12a* and *IL12b* mRNA levels in sorted CD11c⁺ DCs from dLNs of OVA-sensitized skin of *IL13*^{-/-} mice and WT controls (**C**) and *Mcpt5-Cre*^{Tg/0} *IL4*^{fllox/+} *IL13*^{fllox/-} mice and *IL4*^{fllox/+} *IL13*^{fllox/-} control mice (**D**). Results in (**A**) and (**D**) are representative of 2 independent experiments with 4 or 5 mice/group. Columns and bars represent means $\pm$ SEMs. **P* < .05; ***P* < .005.

and E3, *D*). These results indicate that MCs are an important source of IL-33-dependent IL-13 production in DNFB-treated skin. To investigate whether IL-13 regulates CHS to DNFB, *IL13*^{-/-} mice and WT controls were sensitized by painting DNFB on their abdomens. Five days later, they were challenged by application of hapten or vehicle control to the ears. The ears were examined 24 hours after challenge for thickness and intracellular cytokine expression by CD4⁺ T cells. Challenge of WT mice with DNFB, but not with vehicle, caused a robust increase in ear thickness 24 hours later, as well as an increase in CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells, as determined by intracellular flow cytometry analysis (Fig 4, *A* and *B* [right panel]). The increase in ear thickness following DNFB challenge was significantly greater in *IL13*^{-/-} mice than in WT controls (Fig 4, *A*). The DNFB-challenged ears also showed a significantly higher percentage of CD3⁺CD4⁺IFN- γ ⁺ cells in *IL13*^{-/-} mice than in the WT controls (Fig 4, *B*). The percentages of CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells in vehicle-challenged ears were comparable among the 2 strains (Fig 4, *B* [right panel]).

We next investigated whether intradermal rIL-13 injection during the sensitization phase decreases ear swelling and T_H1 cell polarization following hapten challenge. WT mice were intradermally injected in the abdominal skin with rIL-13 or saline 30 minutes before DNFB sensitization. There was a significant

decrease in swelling and in the accumulation of CD3⁺CD4⁺IFN- γ ⁺ cells, but not CD3⁺CD4⁺IL-17A⁺ cells, in DNFB-challenged ears from mice intradermally injected before sensitization with rIL13 than in ears from saline-injected controls (Fig 4, *C* and *D* and data not shown).

To investigate whether MC-derived IL-13 is important for the inhibition of T_H1 cell-dominated CHS, we examined the CHS response of *Mcpt5-Cre*^{Tg/0} *IL4*^{fllox/+} *IL13*^{fllox/-} mice to DNFB. Ear swelling in response to DNFB challenge and the accumulation of CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells were significantly greater in *Mcpt5-Cre*^{Tg/0} *IL4*^{fllox/+} *IL13*^{fllox/-} mice than in *IL4*^{fllox/+} *IL13*^{fllox/-} control mice (Fig 4, *E* and *F*). The accumulation of CD3⁺CD4⁺IL-13⁺ cells in DNFB-challenged ears was comparable between both strains (Fig 4, *F* and data not shown). These data suggest that IL-13 derived from skin MCs inhibits the T_H1 cell response to hapten and thereby decreases the magnitude of ear swelling response in CHS.

IL-13 suppresses the ability of human skin DCs to promote T_H1 cell differentiation

We investigated whether suppression of T_H1 cell polarization by IL-13 produced in the skin is applicable to humans. We examined whether scratching induces *IL13* expression in human skin

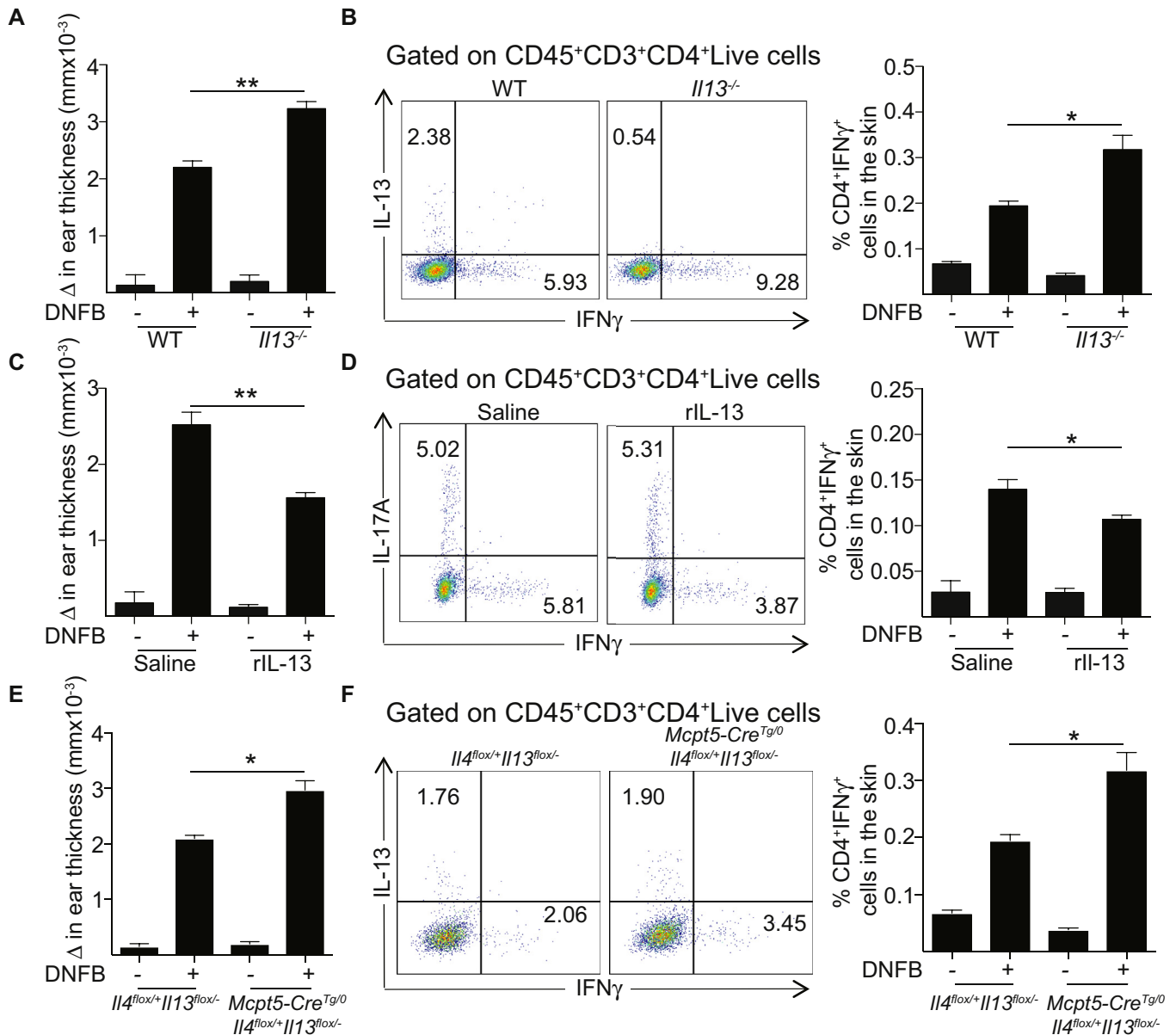


FIG 4. MC-derived IL-13 inhibits ear swelling and T_H1 cell differentiation in the CHS response to DNFB. **A.** Change in ear thickness on day 1 after hapten (+) or vehicle (-) challenge of DNFB-sensitized *Il13*^{-/-} mice and WT controls. **B.** Representative flow cytometry analysis (left) and quantitation (right) of the percentage of CD45⁺CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells among skin cells on day 1 after hapten challenge in ear skin of DNFB-sensitized *Il13*^{-/-} mice and WT controls. **C and D.** Change in ear thickness (**C**) and representative flow cytometry analysis (left) and quantitation (right) of CD45⁺CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells among skin cells in ear skin (**D**) on day 3 after hapten or vehicle challenge of DNFB-sensitized WT mice treated locally with rIL-13 or saline during sensitization. **E and F.** Change in ear thickness (**E**) and representative flow cytometry analysis (left) and quantitation (right) of CD45⁺CD3⁺CD4⁺IFN- γ ⁺ T_H1 cells among skin cells in ear skin (**F**) on day 1 after hapten or vehicle challenge of DNFB-sensitized *Mcpt5-Cre*^{Tg/0} *Il4*^{flox/+} *Il13*^{flox/-} mice and *Il4*^{flox/+} *Il13*^{flox/+} control mice. Results in (**A**) to (**F**) are representative of 2 independent experiments with 4 or 5 mice/group. Columns and bars represent means $\pm$ SEMs. **P* < .05; ***P* < .005.

and evaluated the effect of IL-13 on the ability of human CD1c⁺ skin DCs, which include Langerhans cells and conventional DCs,⁴ to promote T_H1 cell cytokine production *in vitro*. *IL13* mRNA level in human skin was significantly upregulated 6 hours after scratching (Fig 5, A). Human dermal CD1c⁺ DCs were found to express on their surface both chains of the IL-13 receptor (IL-4R α and IL-13R α), as indicated by flow cytometry using

anti-human IL-4R α and IL-13R α antibodies (Fig 5, B). Pretreatment of CD1c⁺ DCs isolated from normal human skin with IL-13 significantly suppressed LPS induction of *IL12B* (IL12p40), but not *IL12A* (IL12p35) (Fig 5, C). Importantly, CD4⁺ T cells stimulated with anti-CD3 plus anti-CD28 secreted significantly less IFN- γ following coculture with human skin DCs pretreated with LPS plus IL-13 than following coculture with DCs pretreated

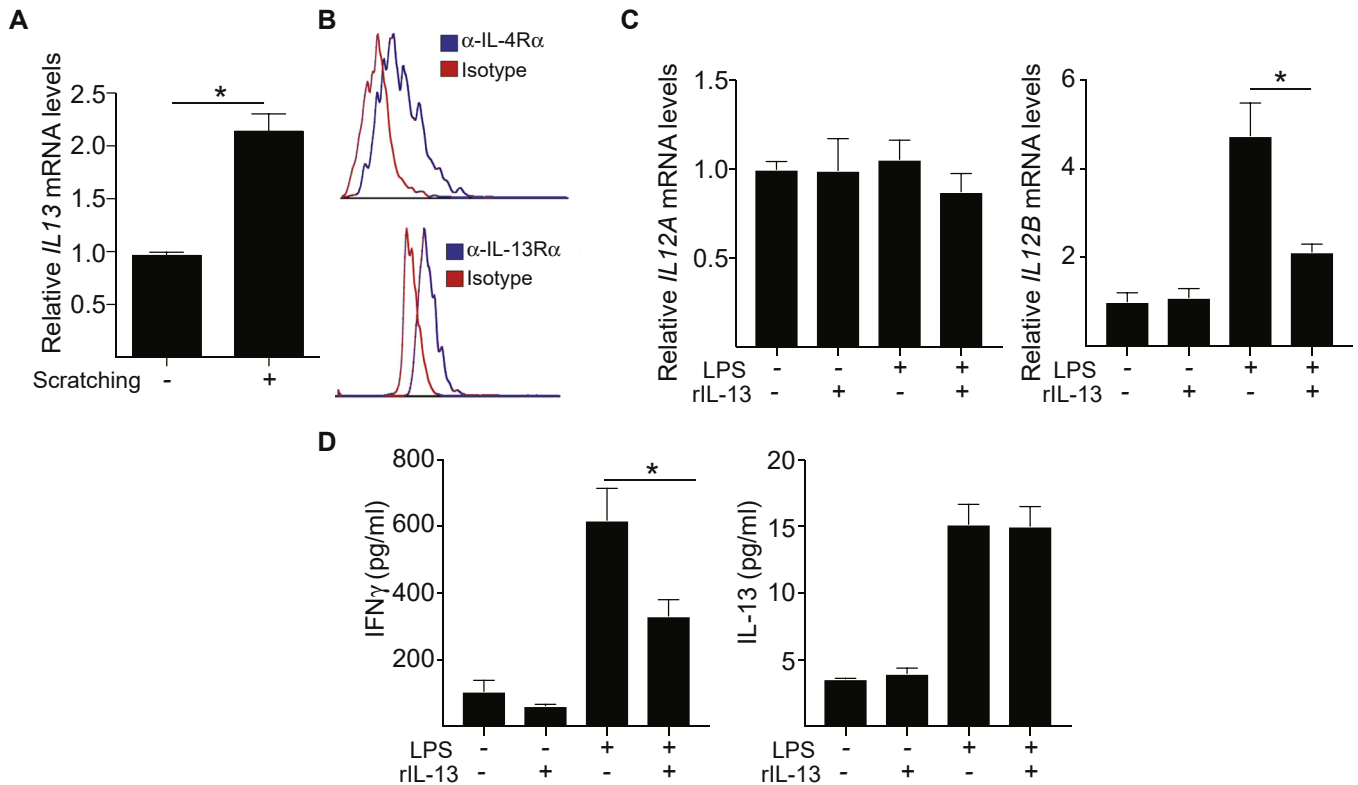


FIG 5. Scratching upregulates *IL13* expression in human skin and IL-13 suppresses the ability of LPS-stimulated human skin DCs to promote IFN- γ secretion by human CD4⁺ T cells. **A.** Relative *IL13* mRNA expression in unmanipulated and scratched skin (6 hours after scratching) of healthy subjects. **B.** Flow cytometry analysis of IL-4R α and IL-13R α expression by human skin CD1c⁺ DCs from surgical discard specimens. **C.** Relative *IL12A* and *IL12B* mRNA expression in human skin CD1c⁺ DCs induced by LPS in the absence or presence of rIL-13. **D.** IFN- γ and IL-13 secretion by CD4⁺ T cells from healthy donors cocultured with skin CD1c⁺ DCs from heterologous healthy donors who were untreated or treated with LPS in the absence or presence of added rIL-13. Results in **(A)** to **(D)** are representative of 1 experiment with 3 to 5 donors/group. Columns and bars represent means $\pm$ SEMs. * $P < .05$.

with LPS alone (Fig 5, D [left panel]). This effect was specific to IFN- γ , because IL-13 secretion was comparable in the 2 conditions (Fig 5, D [right panel]). These results strongly suggest that cutaneous IL-13 induced by scratching impairs the capacity of human skin DCs to promote IFN- γ secretion by CD4⁺ T cells.

DISCUSSION

We have demonstrated that mechanical skin injury drives IL-33-dependent production of IL-13 by cutaneous MCs, which suppresses the capacity of skin DCs to elicit a T_H1 cell response to cutaneously encountered antigens. This may contribute to the preferential induction of a T_H2 cell immune response to these antigens in patients with AD and to the susceptibility of these patients to cutaneous viral infections.

Mechanical skin injury inflicted by tape stripping upregulated *Il13* expression in cutaneous MCs. This upregulation was dependent on the alarmin IL-33 that was released by epidermal cells in response to tape stripping, as evidenced by the failure of tape stripping to upregulate *Il13* expression in the skin of MC-deficient *Kit^{Wsh/Wsh}* mice. This failure was reversed by reconstituting the skin of these mice with WT BMDCs but not with BMDCs that lack IL-33R expression. We found no role for ILC2s in *Il13* upregulation in mechanically injured skin, as there

was normal upregulation of *Il13* in the tape-stripped skin of *Rag2^{-/-}gc^{-/-}* mice, which lack ILCs. This is in contrast to the major role that ILC2s play in the induction of *Il13* expression in the lungs following intranasal rIL-33 treatment and in the IL-33-dependent *Il13* upregulation in the lungs following inhalation of papain, HDM, or *Alternaria*.^{36,37} Thus, IL-33 promotes innate IL-13 production by different tissue-resident innate cells in a tissue-specific and context-dependent manner.

MCs play an important role in initiation of the immune response in the skin, as MC-derived molecules (eg, TNF- α) promote the migration of skin DCs into dLNs.^{38,39} Our results show that MC-derived IL-13 is not required for DC migration from skin to dLNs or for induction of T_H2 cell polarization by these cells. Notably, MC-derived IL-13 inhibits the capacity of skin-derived DCs to promote T_H1 cell polarization of naive CD4⁺ T cells. This was evidenced in loss of function experiments in which skin-derived DCs from *Il13^{-/-}* mice and mice with conditional deletion of *Il13* in MCs promoted T_H1 cell polarization, as well as in gain-of-function experiments in which skin-derived DCs from mice intradermally injected with rIL-13 were impaired in driving T_H1 cell differentiation. Thus, MC-derived IL-13 inhibits the ability of skin DCs to drive a T_H1 cell response to cutaneously encountered antigen.

IL-13 likely acted directly on skin DCs *in vivo* to inhibit their ability to drive T_H1 cell polarization. The addition of IL-13

in vitro to skin-derived DCs from WT mice, but not from *Il4ra*^{-/-} mice, suppressed their capacity to drive T_H1 cell polarization. Importantly, IL-13 suppressed the production of the T_H1 cell-polarizing cytokine IL-12 by skin DCs. This was associated with inhibition of expression of *Il12b* encoding for the IL-12p40 chain. This result is consistent with the observation that rIL-13 inhibits the IL-12p40 and IL-12p70 production by human PMBCs stimulated with LPS.⁴⁰ Importantly, in a gain-of-function experiment we showed that expression of *Il12b* (IL-12p40), but not *Il12a* (IL-12p35), was significantly higher in DCs purified from the dLNs of OVA-sensitized skin of *Il13*^{-/-} mice and *Mcpt5-Cre*^{Tg/0}*Il4*^{fllox/+}*Il13*^{fllox/-} mice. PD-L2 inhibits the production of IL-12p70 in mouse bone marrow-derived DCs induced by house dust antigen.⁴¹ Our results indicate that PD-L2 expression by skin-derived DCs was unaffected by IL-13 deficiency. These results suggest that the inhibition of IL-12 expression in skin-derived DCs by MC-derived IL-13 is independent of PD-L2.

We used a T_H1 cell-dominated model of skin inflammation, namely, CHS to the hapten DNFB, to verify the role of MC-derived IL-13 in suppressing the T_H1 cell response to cutaneously encountered antigens. In this model, *Il33* and *Il13* mRNA are up-regulated in the sensitization phase. Importantly, IL-33 receptor was required for DNFB-induced IL-13, and MCs were an important source of this cytokine, suggesting that cutaneous sensitization with hapten and antigen sensitization use a common mechanism for the induction of IL-13 expression by skin MCs. Consistent with previous results, we showed that CHS response to DNFB, as measured by ear swelling and the accumulation of T_H1 cells in hapten-challenged ear skin, is exaggerated in *Il13*^{-/-} mice.⁴² In contrast, this response was diminished by intradermal injection of rIL-13 during hapten sensitization. Importantly, both ear swelling and accumulation of T_H1 cells in hapten-challenged ear skin were exaggerated in mice with selective deficiency of IL-13 in MCs, thereby establishing a role of MC-derived IL-13 in the T_H1 cell response to cutaneous hapten exposure.

Our findings in mice regarding the role played by MC-derived IL-13 in inhibiting the induction of a T_H1 cell response to cutaneously encountered antigens are likely applicable to humans. Scratching upregulated *IL13* expression in human skin; in addition, rIL-13 inhibited *IL12B* expression by human skin CD1c⁺ DCs and impaired their capacity to promote IFN- γ production by CD4⁺ T cells. IFN- γ plays an important role in controlling viral infections, including those caused by vaccinia and herpes simplex.⁴³⁻⁴⁵ Eczema herpeticum has been associated with *IFNG* and *IFNGR1* single-nucleotide polymorphisms. Moreover, patients with AD who experience this complication show defective production and response to IFN- γ .^{41,46}

Taken together, our results strongly suggest that MC-derived IL-13 suppresses the T_H1 cell response to cutaneously encountered antigens in AD. Local IL-13 blockade may be useful in correcting the T_H2 cell-to-T_H1 cell imbalance in AD and in promoting a protective T_H1 cell immune response to cutaneous viral infections that affect patients with AD.

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Clinical implications: Local IL-13 blockade may be useful in correcting the T_H2 cell-to-T_H1 cell imbalance in lesional AD skin and promoting a protective T_H1 cell immune response to cutaneous viral infections that affect patients with AD.

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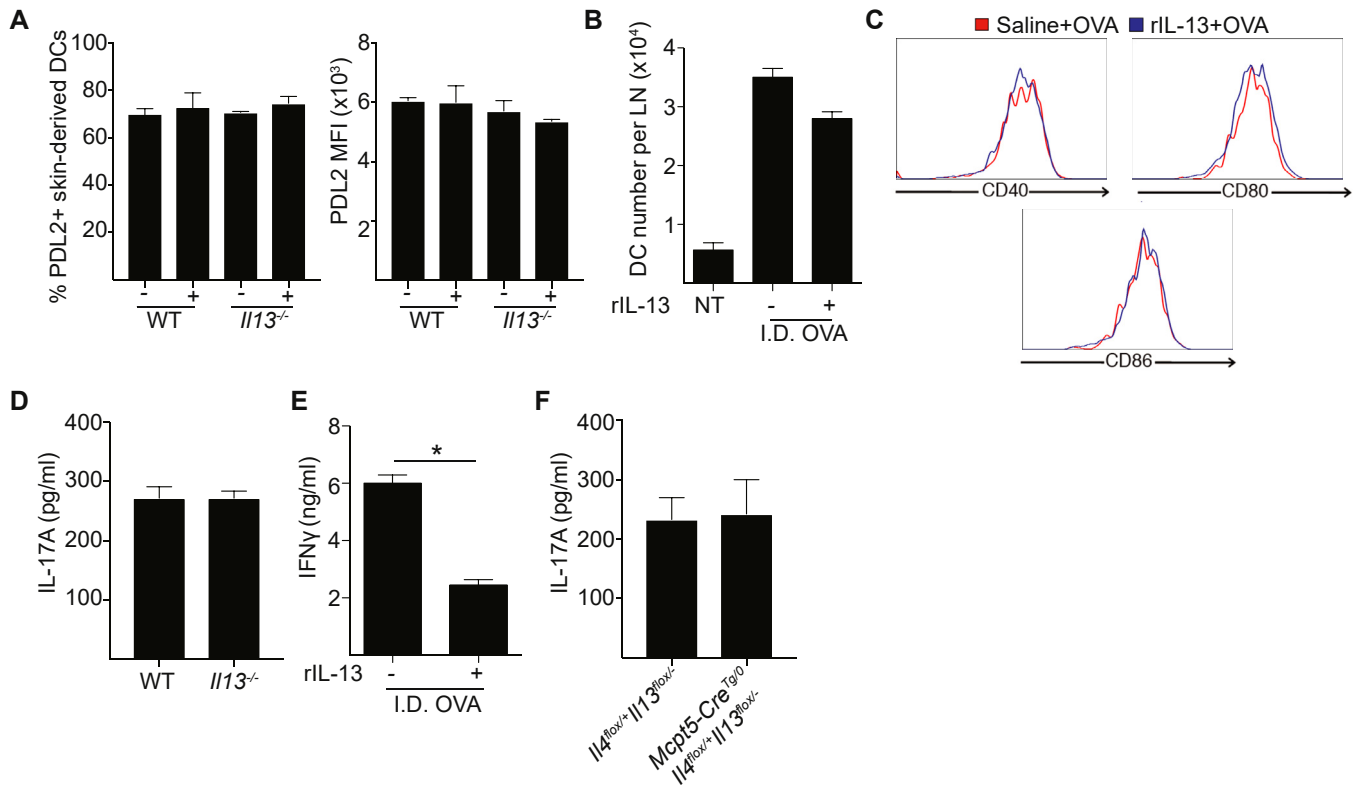


FIG E1. MC-derived IL-13 inhibits the capacity of skin-derived DCs to promote T_H1 cell polarization of naive CD4⁺ T cells. **A**, Percentages of PDL2⁺ (left) and PDL2 mean fluorescence intensity (MFI) (right) in skin-derived DCs from dLNs of unmanipulated and OVA-sensitized skin of *Il13*^{-/-} mice and WT controls. **B** and **C**, Quantitation of skin-derived CD11c⁺MHCII^{high} DCs (**A**) and expression of CD40, CD80, and CD86 (**B**) by these cells in dLNs of WT mouse unmanipulated (NT) skin or skin intradermally (I.D.) injected with OVA and rIL-13 or saline. **D**, IL-17A secretion by naive CD4⁺ T cells from DO11.10 mice cocultured with skin-derived DCs sorted from dLNs of OVA-sensitized skin of *Il13*^{-/-} mice and WT controls. **E**, IFN-γ secretion by naive CD4⁺ T cells from DO11.10 mice induced by sorted skin-derived DCs from dLNs of WT mice NT skin or skin I.D. injected with OVA and rIL-13 or saline. **F**, IL-17A secretion by naive CD4⁺ T cells cocultured with skin-derived DCs sorted from dLNs of OVA-sensitized skin of *Mcpt5-Cre*^{Tg/0}*Il4*^{lox/+}*Il13*^{lox/-} and *Il4*^{lox/-} + *Il13*^{lox/-} controls. Results in (**A**) to (**F**) are representative of 2 independent experiments with 5 mice/group. **P* < .05. LN, Lymph node.

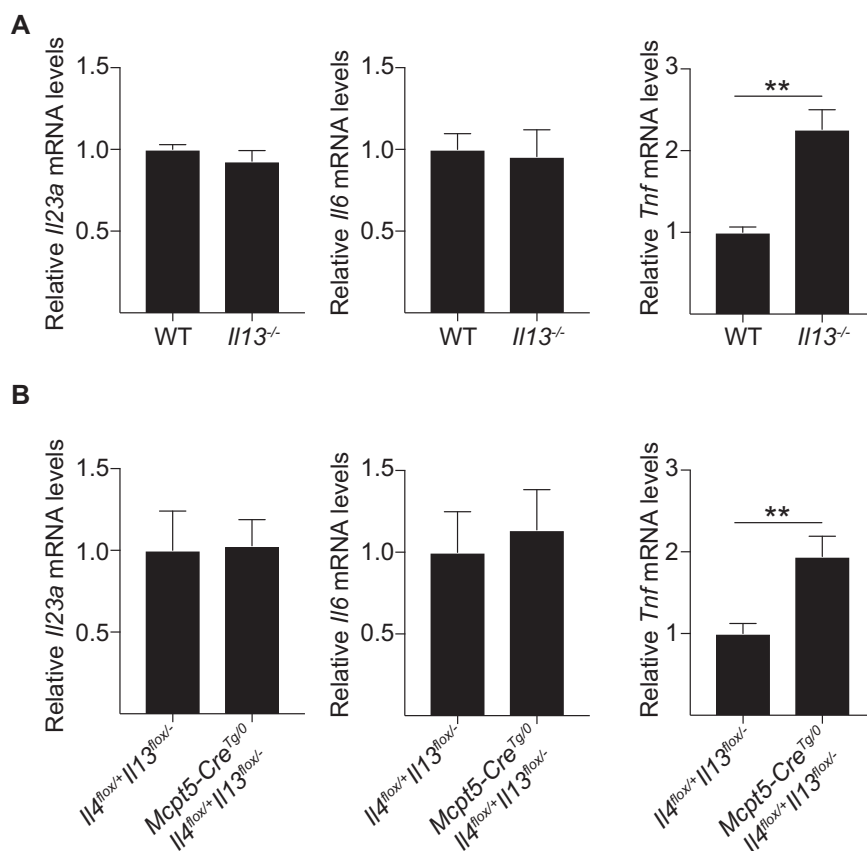


FIG E2. MC-derived IL-13 suppresses *Tnf* expression but not *IL23* or *IL6* expression in skin-derived DCs. **A** and **B**, Relative *IL23a*, *IL6*, and *Tnf* mRNA levels in skin-derived DCs sorted from DLNs of OVA-sensitized skin of *IL13*^{-/-} mice (**A**), *Mcpt5-Cre*^{Tg/0} *IL4*^{flox/+} *IL13*^{flox/-} (**B**), and controls. Results in (**A**) and (**B**) are representative of 2 independent experiments with 3 to 5 mice/group. Columns and bars represent means $\pm$ SEMs. ***P* < .005.

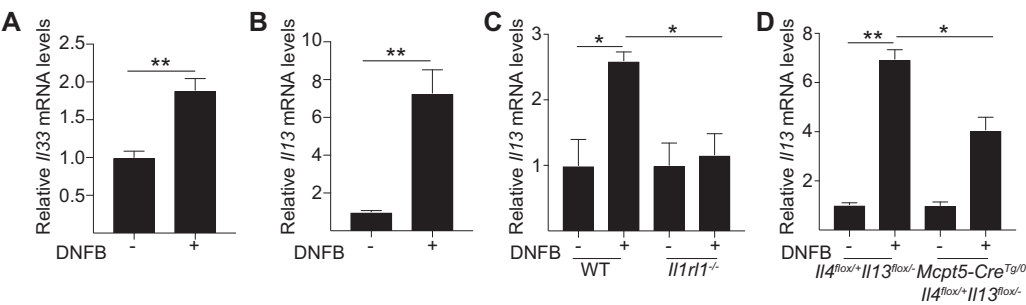


FIG E3. DNFB treatment promotes IL-33–dependent IL-13 production in skin MCs. **A** and **B**, Relative *Il33* (**A**) and *Il13* (**B**) mRNA levels in vehicle or DNFB-treated ears from WT mice. **C**, Relative *Il13* mRNA levels in vehicle or DNFB-treated ears from *Il1rl1*^{-/-} mice and WT controls. **D**, Relative *Il13* mRNA levels in vehicle or DNFB-treated ears from *Mcpt5-Cre*^{Tg/0}*Il4*^{fllox/+}*Il13*^{fllox/-} mice and *Il4*^{fllox/+}*Il13*^{fllox/-} controls. Results in (**A**) to (**D**) are representative of 2 independent experiments with 3 to 5 mice/group. Columns and bars represent means ± SEMs. ***P* < .005; **P* < .05.