

Histamine receptor H1 signaling on dendritic cells plays a key role in the IFN- γ /IL-17 balance in T cell-mediated skin inflammation

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Background: The diverse effects of histamine on immune regulation are a result of the differential expression and regulation of 4 histamine receptors. Many of the immediate allergic and inflammatory actions of histamine are mediated via the type 1 receptor (H1R).

Objectives: We hypothesized that H1R was involved in the fine-tuning of the initiation of T cell-mediated skin pathology—that is, dermatitis.

Methods: The impact of the H1R invalidation on the development of skin inflammation was analyzed in a mouse model of atopic dermatitis.

Results: We show that *H1r*^{-/-} mice developed reduced allergen-specific skin lesions. Lack of H1R expression on dendritic cells (DCs) led to diminished IL-12, upregulated IL-23, and IL-6 production upon allergen stimulation. H1R engagement on dendritic cells was necessary for DC activation and subsequent priming of effector IFN- γ ⁺CD8⁺ T cells. We demonstrate here that H1R blockade on DCs promotes generation of noneffector IL-17⁺CD8⁺ T cells that are unable to initiate the skin inflammation.

Conclusion: Our data identify that histamine signaling through the H1R on DCs is an important early event conditioning the quality of the skin effector immune response. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Atopic dermatitis, CD8⁺ T cell, histamine, H1R, dendritic cell

Abbreviations used

AD:	Atopic dermatitis
BMDC:	Bone marrow-derived dendritic cell
CFSE:	Carboxy-fluorescein diacetate succinimidyl ester
DC:	Dendritic cell
DLN:	Draining lymph node
DNFB:	2,4-Dinitrofluorobenzene
DTH:	Delayed-type hypersensitivity
H1R-H4R:	Histamine type 1-4
ICOS-L:	Inducible T-cell costimulator ligand
LN:	Lymph node
PMA:	Phorbol 12-myristate 13-acetate
SFC:	Spot-forming cell
Tc:	T cytotoxic
WT:	Wild type

Histamine is well known as a chemical mediator that play important roles in allergic inflammatory and immune reactions.^{1,2} Histamine mediates its effects through 4 receptor types (H1R-H4R), each characterized by a specific pattern of cellular expression and function.³ H1R and H2R have a ubiquitous expression on both lymphoid and nonlymphoid cells.⁴ H3R and H4R are mainly expressed in the brain and on hematopoietic cells, respectively.⁵ Many of the allergic and inflammatory actions of histamine are mediated via H1R. H1R is a G α q/11 receptor that contains 7 membrane-spanning domains and an extracellular NH2 terminal glycosylated domain. Activation of cellular process after histamine binding to H1R occurs via phospholipase C-mediated calcium mobilization,⁶ protein kinase C activation, and nuclear factor- κ B-mediated signaling pathways.⁷

Histamine, originally considered as a mediator of acute inflammatory and immediate hypersensitivity responses, has also been demonstrated to affect chronic inflammation and regulate several events in the immune response.⁸⁻¹⁰ Recently, it has been described that H1R is involved in airway inflammation and hyperactivity, including the findings that *H1r*^{-/-} mice developed reduced allergen-induced lung inflammation in a mouse model of asthma.¹¹⁻¹³ Therefore, we hypothesized that H1R deficiency might be crucial in allergen-induced inflammation in the skin. Many skin disorders, such as atopic dermatitis (AD), contact dermatitis, and psoriasis, are T cell-mediated diseases. AD is a chronic inflammatory skin disease associated with cutaneous hyperactivity to environmental antigens that are innocuous to healthy nonatopic individuals. In this respect, the house dust

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mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are major allergens in AD pathophysiology.¹⁴ Contact dermatitis is a dendritic cell (DC)–dependent, T cell–mediated cell immune response elicited by epicutaneous sensitization with haptens, which become immunogenic after binding to discrete amino acid residues of proteins or peptides. We have previously shown that CD8⁺ T cells producing IFN- γ are playing a pivotal role in initiating skin lesions in both mouse models of *D farinae*–induced AD and in 2,4-dinitrofluorobenzene (DNFB)–induced contact dermatitis.^{15–17} We report here that *Hlr*^{−/−} mice developed reduced allergen-specific skin lesions. This reduction was associated with an immune deviation of the elicited CD8⁺ T-cell population from a T cytotoxic (Tc) 1 to a Tc17 response in draining lymph nodes. Upon allergen maturation, *Hlr*^{−/−} DCs expressed low MHC class I and II, low CD86, and high inducible T-cell costimulator ligand (ICOS-L) molecules compared with their wild-type counterparts. *Hlr*^{−/−} DCs produced reduced quantities of IL-12 and upregulated the production of IL-23 and IL-6 on allergen stimulation and subsequently primed preferentially IL-17 rather than IFN- γ –producing CD8⁺ T cells *in vitro*. These data demonstrate the essential role of H1R triggering in the fine-tuning of the DC function in respect to the outcome of the allergic skin inflammation. Our observations collectively identify H1R as an attractive target for controlling the initiation of the skin immune response.

METHODS

Animals

Female C57BL/6 wild-type (WT) mice were purchased from Charles River Breeding Laboratories (L'Arbresle, France). *Hlr*^{−/−} mice were kindly provided by T. Watanabe.¹⁸ *Hlr*^{+/+}, *Hlr*^{−/−} littermate mice, F2 generation, obtained from F1x F1 crossbreeding of *Hlr*^{−/−} x C57BL/6. C57BL/6 mice with a mutation in the CD3 cluster (CD3 ϵ ^{−/−}) were provided by M. Malissen (Centre d'Immunologie, Marseille-Lumigny, Marseille, France). All mice were used between 2 and 3 months of age. Groups consisted of 10 mice each. All experimental procedures were in accordance with the Comité régional d'éthique pour l'expérimentation animale guidelines on animal welfare.

Mouse model of *D farinae* eczema

Ear mouse eczema was induced as previously described.¹⁵ Alternatively, mouse AD was induced by injecting bone marrow dendritic cells (BMDCs) uploaded with *D farinae* antigen. Mice were challenged 7 days later, and the ear edema was measured 48 hours after the challenge.

DC generation and maturation

Dendritic cells were generated *in vitro* from bone marrow progenitors from C57BL/6 or *Hlr*^{−/−} mice with GM-CSF as previously described.¹⁹ Immature DCs were collected at day 6 of culture. DCs were further matured with *D farinae* for 1 hour or overnight culture. Alternatively, DC were treated in the same time as the antigen with anti-H1R inhibitor tripelennamine hydrochloride (Sigma, St Quentin Fallavier, France) at a concentration of 10 μ mol/L. DCs were used for *in vivo* immunization of *in vitro* cell culture. For the DC migration assay, BMDCs were stained with a solution of 5 μ mol/L carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes) according to the manufacturer and exposed to *D farinae* before subcutaneous injection in WT recipients. At 24 hours postinjection, the draining lymph nodes (DLNs) were recovered and stained for flow cytometry analysis. For flow cytometry analysis, DC were stained with the following antimouse antibodies: MHC class I -anti-H-2K^b, MHC class II anti-IA/IE, CD86, CD11c, and ICOS-L (BD Pharmingen). IL-5, IL-4, IL-13, and IL-10 were titrated by using a Multiplex cytokine assay (BioRad) according to the manufacturer's instructions. Cytokine production (IL-12p40, IL-23, IL-6, IL-10) was evaluated by ELISA

in culture supernatants according to the manufacturer's instructions (R&D Systems DuoSet).

T-cell isolation and cell culture

CD8⁺ T cells were purified by using anti-CD8 mAb-coated microbeads and positive selection columns (Miltenyi Biotec). Purity was routinely >95%. IFN- γ and IL-17 production was titrated by ELISA by using the R&D Systems DuoSet kit following the manufacturer's protocol. The ELISPOT assay (IFN- γ , R46A2; BD Pharmingen; and IL-17, SEL421; R&D) was performed as previously described.¹⁵ For IL-17 and IFN- γ (BD Pharmingen) intracellular staining, isolated CD8⁺ T cells restimulated with antigen-presenting cells loaded with *D farinae* were stimulated for 4 hours with phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) and ionomycin (500 ng/mL), and cytokine secretion was blocked with Golgi STOP (BD Pharmingen) for the last 2 hours of culture. The release of cytotoxic granules was assessed by flow cytometry by the staining of CD107a (1D4B; BD Pharmingen) during restimulation with PMA and ionomycin for 4 hours. For cytotoxicity assay, irradiated splenocytes were labeled with 0.5 μ mol/L or 5 μ M CFSE. Those labeled with 0.5 mmol/L were further pulsed with *D farinae* antigen. Control and pulsed target cells were mixed at a ratio of 1:1 and were incubated with different ratio of CD8⁺ T cells (issued from vehicle-treated WT mice, WT, or *Hlr*^{−/−} BMDC-immunized mice). After 24 hours, the cells were analyzed by flow cytometry, and the percentage of specific lysis was calculated as follows:

$$(1 - \% \text{ targets} / \% \text{ control cells}) \times 100.$$

Adoptive transfer

Axillary and inguinal lymph nodes (LNs) from allergen-treated mice (C57BL/6 and *Hlr*^{−/−} mice) were harvested at day 21 after sensitization and used to prepare single LN-cell suspensions and purified CD8⁺ T cells. A total of 1×10^6 cells (WT of *Hlr*^{−/−})/200 μ L saline were intravenously transferred through the orbital vein into naive syngenic CD3 ϵ ^{−/−} mice. Recipient mice were challenged after cell transfer, and ear swelling was measured the following days.

Histology

Ear were fixed in a 3% formalin solution for 24 hours and processed through a routine 15-hour cycle to paraffin embedding. The 4-mm sections were cut by using a microtome and mounted onto Superfrost Plus slides (Fisher Scientific, Illkirch, France). Sections were dried overnight at 37°C. The slides were dewaxed in Ottix bath and stained with hematoxylin and eosin.

Statistical analysis

Three or 5 mice per group were used in each experiment. Statistical analyses were performed by using the paired 2-tailed Student *t* test. *P* < .05 was used as the level of significance.

RESULTS

H1R modulates T cell–driven skin inflammation

Hlr^{−/−} or *Hlr*^{+/+} mice were sensitized by epicutaneous application of a solution of *D farinae* once a week for 4 weeks as previously described.¹⁵ Forty-eight hours after the last allergen application (day 23), *Hlr*^{+/+} developed a typical AD skin inflammation response as manifested by increased ear swelling (Fig 1, A). No ear edema was recorded either in *Hlr*^{−/−} or in *Hlr*^{+/+} mice before the fourth application of the allergen, excluding the possibility of a different kinetic of inflammation in H1R-deficient mice (see this article's Fig E1 in the Online Repository at www.jacionline.org). Histopathologic analysis of the ear skin in *Hlr*^{+/+} mice revealed characteristics of AD lesions

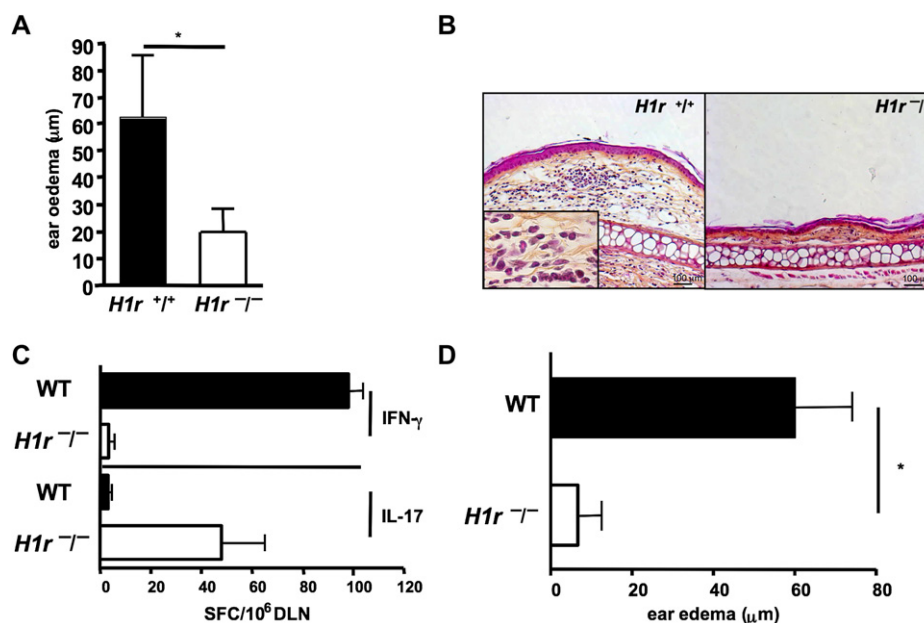


FIG 1. Atopic dermatitis is reduced in *H1r*^{-/-} mice. **A**, *D farinae*-induced skin inflammation was analyzed in *H1r*^{-/-} and *H1r*^{+/+} mice painted onto the ear with a solution of *D farinae* once a week for 4 weeks. Results are expressed as the mean ear swelling at 48 hours after the last application (day 23). Results are representative of 3 independent experiments using 5 mice per group. **B**, Histologic analysis of ear sections (hematoxylin and eosin staining) from *D farinae*-treated WT and *H1r*^{-/-} mice at day 23. Original magnification $\times 100$, inset $\times 1000$. **C**, *D farinae*-specific IFN- γ and IL-17-producing cells were determined in total LN cells. Results are expressed as the number of SFCs per 10⁶ LN. Representative of 3 independent experiments. **D**, CD8⁺ T cells were purified from the draining lymph nodes of WT or *H1r*^{-/-} mice at day 21. The cells were intravenously transferred into naive T cell-deficient *CD3 ϵ* ^{-/-} recipients that were challenged with *D farinae* the same day. The results are expressed as ear swelling at day 3 after challenge. Representative of 3 independent experiments. **P* < .05.

including infiltration of mononuclear cells and eosinophils (Fig 1, B). In contrast, AD was drastically reduced in *H1r*^{-/-} mice measured by ear edema and illustrated by histologic staining on tissue sections (Fig 1, A and B). Notably, we obtained similar results in 2 other models of cellular immunity in mice, the DNFB model of contact dermatitis and the delayed-type hypersensitivity (DTH) reaction to BSA (see this article's Fig E2 in the Online Repository at www.jacionline.org), which suggest that *H1r*^{-/-} mice develop reduced DTH reactions in general.

Next, we analyzed the allergen-specific immune response generated in the DLNs of the sensitization site at day 21. First we analyzed the cellular composition of the T-cell compartment in *H1r*^{+/+} compared with *H1r*^{-/-} mice. The CD4⁺/CD8⁺ T-cell ratio was similar in naive (data not shown) and sensitized *H1r*^{+/+} and *H1r*^{-/-} mice (see this article's Fig E3 in the Online Repository at www.jacionline.org). Whole DLNs cells from *H1r*^{+/+} and *H1r*^{-/-} were *D farinae*-restimulated *in vitro*. The frequency of *D farinae*-specific IFN- γ spot-forming cells (SFCs) in DLNs was dramatically reduced in *H1r*^{-/-} mice compared with control littermates (Fig 1, C). In contrast, the frequency of *D farinae*-specific IL-17 SFCs was significantly increased in *H1r*^{-/-} mice (Fig 1, C). The frequencies of IFN- γ and IL-17 SFCs in isolated CD8⁺ T cells were similar to IFN- γ and IL-17 SFCs in whole DLNs, suggesting that IL-17 was essentially produced by CD8⁺ T cells (see this article's Fig E4 in the Online Repository at www.jacionline.org). Finally, we evaluated the capacity of the *in vivo* primed CD8⁺ T cells to transfer skin inflammation in naive T cell-deficient *CD3 ϵ* ^{-/-} recipients. We performed adoptive transfer of CD8⁺ T cells purified from draining LNs of *D*

farinae-sensitized WT or *H1r*^{-/-} mice. Cohorts that received CD8⁺ T cells from WT *D farinae*-sensitized mice developed skin inflammation peaking at day 3 after challenge (Fig 1, D). In contrast, no skin inflammation was observed in *CD3 ϵ* ^{-/-} adoptively transferred with CD8⁺ T cells from *H1r*^{-/-} *D farinae*-sensitized mice. Altogether, these results indicate that reduced allergen-specific skin inflammation in H1R-deficient mice is associated with diminished IFN- γ and increased IL-17 CD8⁺ T-cell responses.

H1R signaling on DCs is required for AD development

We further addressed the question on which cell type the H1R signaling was mandatory for the skin inflammation development. To assess directly the impact of the H1R deficiency on the function of allergen presenting cells, we replaced the epicutaneous sensitization by a subcutaneous injection of BMDCs loaded with *D farinae* followed by a *D farinae* challenge onto the ear 7 days later. WT mice sensitized by injection of *D farinae* WT BMDCs (WT $\rightarrow$ WT) developed 48 hours after ear challenge a typical AD response, in contrast with *H1r*^{-/-} $\rightarrow$ WT mice. Similar results were obtained by using CD11c⁺ spleen DCs from either WT or *H1r*^{-/-} mice (data not shown). Furthermore, we analyzed the allergen-specific immune response generated in the DLNs at day 7 after immunization. *H1r*^{-/-} $\rightarrow$ WT mice produced reduced numbers of *D farinae*-specific IFN- γ SFCs in the DLNs compared with WT $\rightarrow$ WT (Fig 2, C). In contrast, the number of *D farinae*-specific IL-17 SFCs was increased in

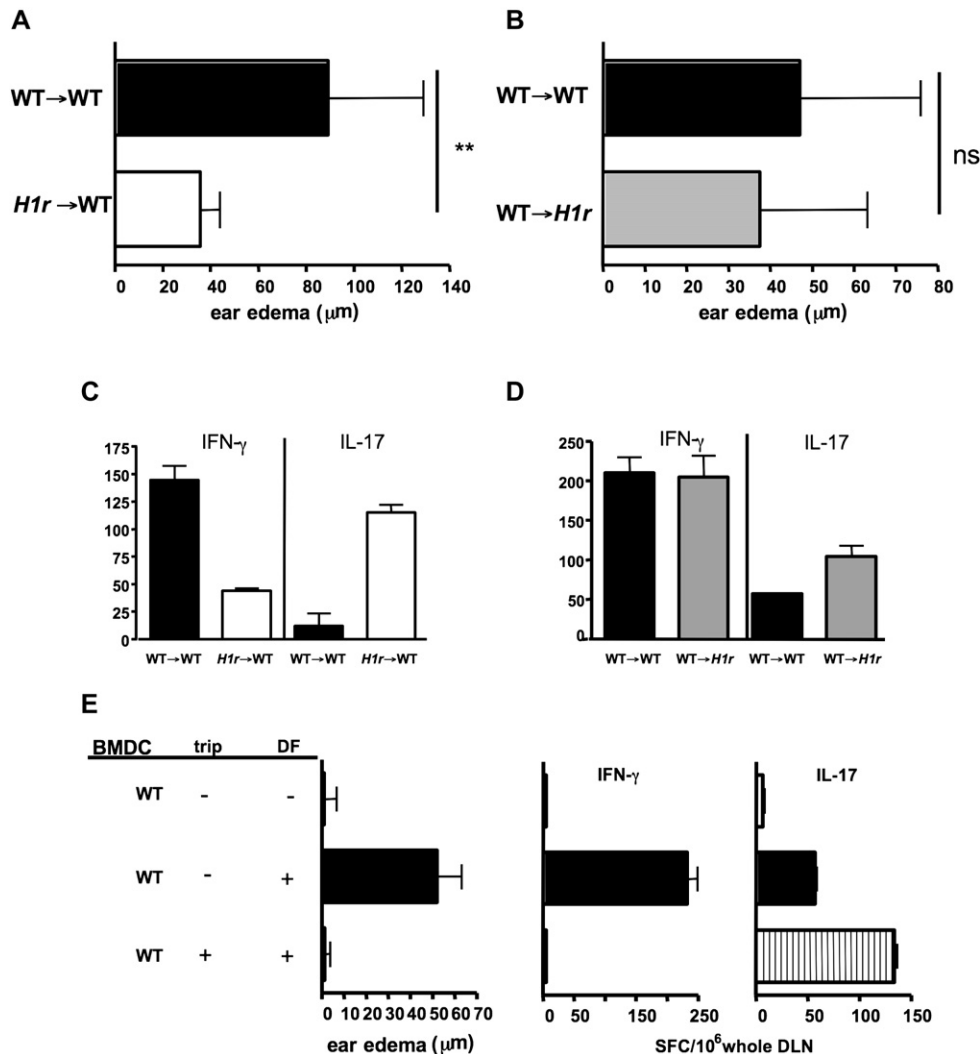


FIG 2. H1R deficiency on DCs is responsible for the reduced skin inflammation. **A**, WT C57BL/6 mice were sensitized by a subcutaneous injection of 3×10^6 *D. farinae* (DF) BMDCs derived from either WT mice or *H1r*^{-/-} mice. Seven days later, the mice are challenged on the ear with a solution of DF. The ear edema was measured at 48 hours after the challenge. Results are representative of 3 independent experiments using 5 mice per group. **B**, *H1r*^{-/-} or WT mice were sensitized by a subcutaneous injection of WT DF BMDCs. Results are representative of 3 independent experiments using 5 mice per group. **C** and **D**, DF-specific IFN- γ and IL-17 cells were determined by ELISPOT assay in LN at day 7 in mice immunized as described in the text. Results are expressed as the number of SFCs per 10^6 LN cells. Results are representative of 3 independent experiments using 3 mice per group. **E**, Ear swelling measurement at 24 hours after challenged in WT mice sensitized with WT DF BMDCs, tripelennamine (*trip*)-treated WT DF BMDCs, or no antigen WT BMDCs. Results are representative of 3 independent experiments using 5 mice per group. DF-specific IL-17 and IFN- γ -producing cells were determined by ELISPOT assay in recovered DLN at day 7. ** $P < .005$. ns, Not significant.

H1r^{-/-}→WT compared with WT→WT immunized mice. In addition, the quantity of IFN- γ was reduced, and the quantity of IL-17 was increased (see this article's Fig E5 in the Online Repository at www.jacionline.org). To exclude that H1R expression on T cells was essential for the AD response, *H1r*^{-/-} mice were immunized with *D. farinae* WT BMDCs (WT→*H1r*^{-/-}). There was no statistical difference between WT→WT and WT→*H1r*^{-/-}-immunized mice *in vivo* (Fig 2, B) or *in vitro* (Fig 2, D). These findings were confirmed by using a specific pharmacologic inhibitor of H1R, tripelennamine.⁹ We showed that BMDC treatment with H1-antihistamine before *D. farinae* exposure led to reduced ear edema and IFN- γ SFCs and increased IL-17 SFCs, similar to *H1r*^{-/-} BMDC use (Fig 2, E). Collectively these

findings reveal that H1R deficiency or blockade on DCs leads to impaired generation of antigen-specific skin inflammation.

H1r^{-/-} DCs display a tolerogenic phenotype on allergen stimulation

Because the expression of H1R on DCs seemed to play a key role in the development of skin inflammation, we have next analyzed the phenotype of BMDCs issued from *H1r*^{-/-} mice and control WT mice on *D. farinae* stimulation. Similar concentrations of histamine were detected in both WT and *H1r*^{-/-} BMDC cultures (see this article's Fig E6 in the Online Repository at www.jacionline.org). We show that *in vitro*-generated BMDCs from WT or *H1r*^{-/-} defined as CD11c⁺ cells had similar

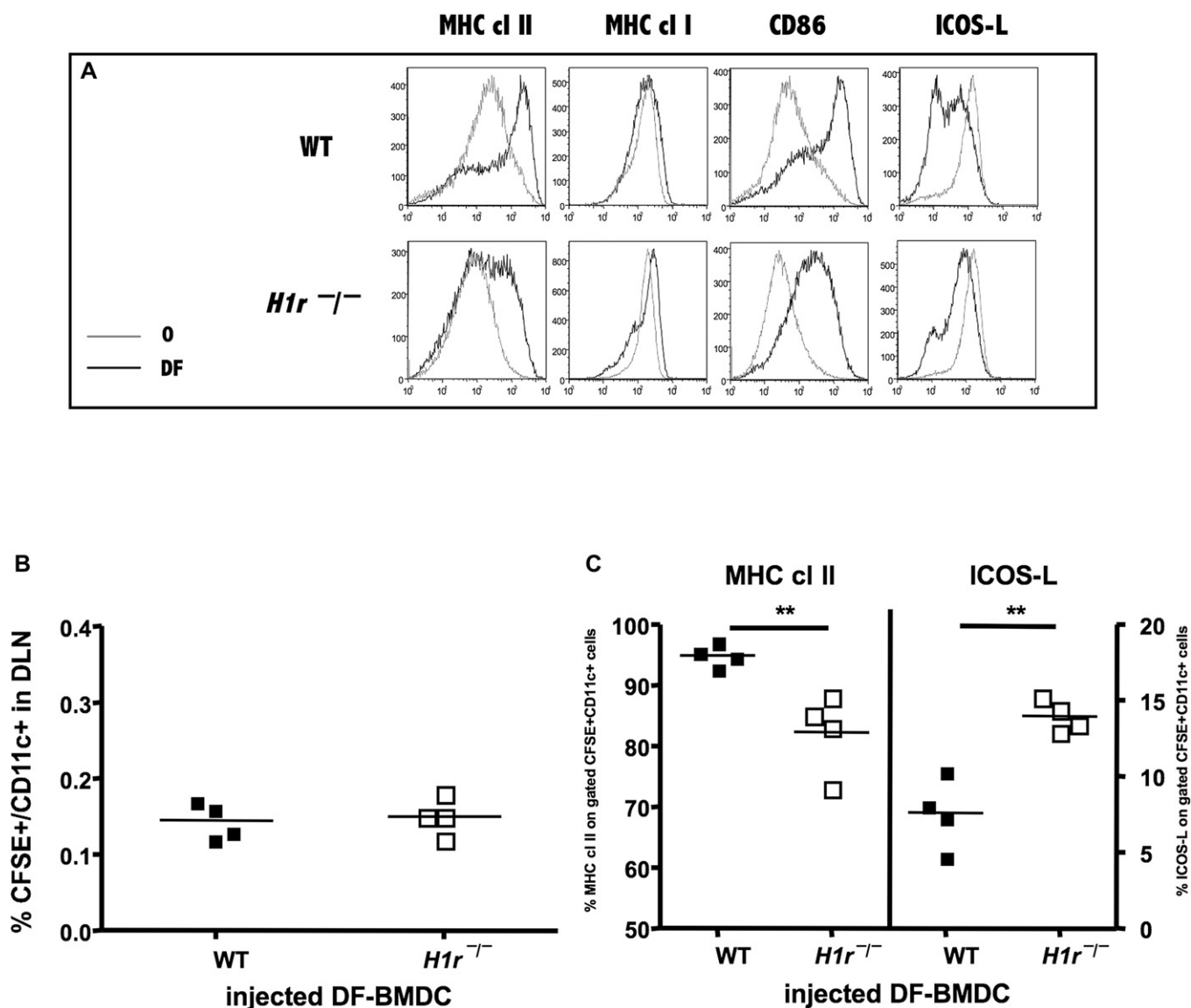


FIG 3. Impact of H1R deficiency on the phenotype of DCs on allergen stimulation. **A**, WT or *H1r*^{-/-} BMDCs were generated *in vitro* with GM-CSF for 6 days. *D farinae* (DF) was added for the last 16 hours of culture. The phenotype of BMDCs in the presence/absence of DF was analyzed by flow cytometry staining with CD11c, MHC class I and II, CD86, ICOS-L-specific antibodies. Histogram overlays represent surface staining gated on CD11c⁺ cells (WT in upper row, *H1r*^{-/-} in lower row). Representative of 3 independent experiments. **B** and **C**, WT of *H1r*-deficient BMDCs were stained with CFSE and exposed to DF before subcutaneous injection in WT recipients. At 24 hours postinjection, the DLNs were recovered and stained for CD11c, MHC class (cl) II, and ICOS-L. **B**, The graph represents the percentage of CFSE-labeled DF BMDCs (WT or *H1r*^{-/-}) that have migrated to the draining lymph node 24 hours postinjection. **C**, Phenotype of CFSE-labeled injected BMDCs (WT or *H1r*^{-/-}) upon migration in the DLN at 24 hours (% of MHC class II and ICOS on gated CFSE⁺CD11c⁺). Each symbol represents an individual mouse. ***P* < .005.

expression of costimulatory molecules at steady state—that is, in the absence of the allergen (Fig 3, A and B). *D farinae* induced up-regulation of MHC II, MHC I, and CD86. This upregulation was reduced in *H1r*^{-/-} mice compared with WT mice. ICOS-L, which is a negative costimulatory molecule, was highly expressed in the absence of the antigen. Upon *D farinae* uptake, ICOS-L was less downregulated in *H1r*^{-/-} BMDCs compared with WT BMDCs. Similar results were obtained by using *D pteronyssinus* antigen as well as Toll-like receptor 4 ligand LPS (see this article's

Fig E7, A and B, in the Online Repository at www.jacionline.org). These findings were confirmed by using anti-H1R, tripe-lennamine (Fig E7, C).

Furthermore, to confirm the phenotypical differences *in vivo*, WT of *H1r*^{-/-} BMDCs were stained with CFSE and exposed to *D farinae* before subcutaneous injection in WT recipients and further assessed at 24 hours postinjection in the DLNs. As shown in Fig 3, B, equivalent percentages of CFSE⁺CD11c⁺ WT or *H1r*^{-/-} BMDCs migrated to the DLN on transfer. In contrast,

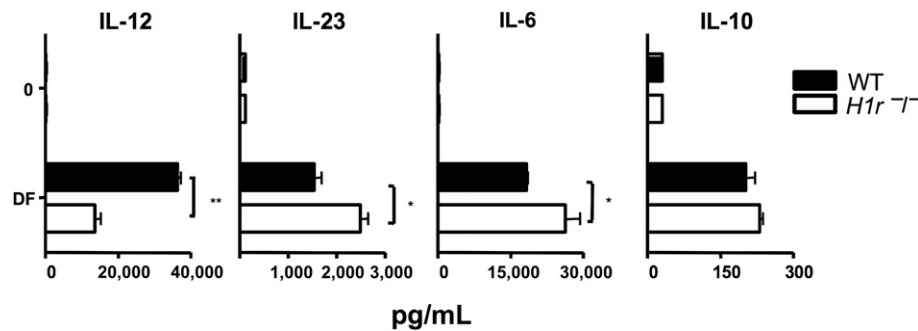


FIG 4. *H1r*^{-/-} DCs downregulate IL-12 and upregulate IL-23 and IL-6 production on allergen stimulation. WT or *H1r*^{-/-} BMDCs were generated *in vitro* for 6 days and either left unstimulated (0) or further stimulated with *D farinae* (DF) overnight. Supernatants were collected, and IL-12, IL-23, IL-6, and IL-10 production was measured by ELISA. Results are representative of 3 independent experiments. **P* < .05, ***P* < .005.

CFSE⁺CD11c⁺ WT BMDCs displayed significantly higher expression of MHC class II than their *H1r*-deficient counterparts. Conversely, ICOS-L was significantly more expressed on *H1r*^{-/-} than on WT BMDCs (Fig 3, C). Altogether, these results show that H1R signaling modulates DC phenotype upon allergen stimulation.

H1r^{-/-} DCs produce increased IL-23 on allergen stimulation

The results obtained so far indicated a CD8⁺ T-cell cytokine production switch from IFN- γ to IL-17 in *H1r*^{-/-} mice as well as in WT mice immunized with H1R-deficient DCs. Induction of IL-12 after allergen exposure on DCs is considered to be crucial for efficient generation of Tc1 cells. Moreover, induction of IL-23 on DCs is considered to be pivotal for IL-17 production. Therefore, we analyzed the production of IL-12p40 and IL-23 by BMDCs on overnight allergen stimulation. As shown in Fig 4, WT or *H1r*^{-/-} BMDCs produced no IL-12p40 in the absence of allergen. On allergen stimulation, WT BMDCs produced large quantities of IL-12p40 compared with *H1r*^{-/-} BMDCs. In contrast, *H1r*^{-/-} BMDCs produced more IL-23 and IL-6 than their WT counterparts. These findings were confirmed by using anti-H1R, tripeleppamine (see this article's Fig E8, A, in the Online Repository at www.jacionline.org). Similar results were obtained by using Toll-like receptor 4 ligand LPS (Fig E8, B). These results collectively indicate that on allergen stimulation, *H1r*^{-/-} BMDCs have a distinct pattern of cytokine secretion from WT BMDCs that may be responsible for the generation of IL-17-producing CD8⁺ T cells.

H1r^{-/-} DCs prime preferentially IL-17⁺CD8⁺ T cells *in vitro*

To establish whether the lack of H1R signaling on DCs was sufficient to generate IL-17-producing CD8⁺ T cells, we performed *in vitro* allergen-specific T-cell activation. Naive CD8⁺ T cells were stimulated with either WT or *H1r*^{-/-} BMDCs loaded with *D farinae* or with no antigen (0) for 6 days, and the proliferation was evaluated by ³H-thymidine uptake. Similar concentrations of histamine were detected in both WT and *H1r*^{-/-} BMDC cultures (data not shown). As shown in Fig 5, A, CD8⁺ T-cell proliferation was comparable in cultures conducted with WT or *H1r*^{-/-} *D farinae* BMDCs. Furthermore, WT *D farinae* BMDCs induced a significant increase in the production of IFN- γ compared with *H1r*^{-/-} *D farinae* BMDCs. In contrast, *H1r*^{-/-} *D farinae* BMDC

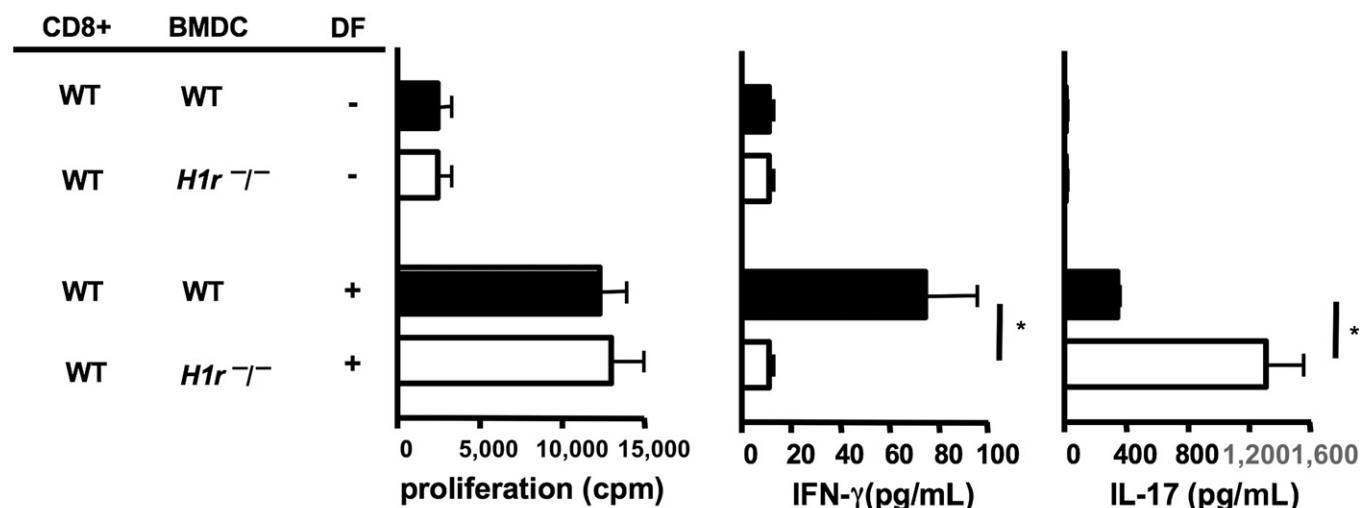
stimulation induced significantly more IL-17 than the WT counterparts. No production of IL-4 or IL-5 and no differential production of IL-13 and IL-10 was observed in both WT and *H1r*^{-/-} BMDC conditions (see this article's Fig E9 in the Online Repository at www.jacionline.org). Naive CD4⁺ T cells stimulated with either WT or *H1r*^{-/-} BMDCs loaded with *D farinae* or with no antigen and CD8⁺ T cells showed similar cytokine profiles (see this article's Fig E10 in the Online Repository at www.jacionline.org).

To determine whether the IL-17 and IFN- γ -producing CD8⁺ T cells were distinct CD8⁺ T-cell populations, the cells were recovered and restimulated with PMA and ionomycin for 4 hours and stained for CD8 and intracellular IFN- γ and IL-17. Stimulation with BMDCs in the absence of *D farinae* led to few IFN- γ ⁺CD8⁺ T cells and no IL-17⁺CD8⁺ T cells. After WT *D farinae* BMDC stimulation, a high percentage of CD8⁺ T cells produced IFN- γ , and a small percentage of CD8⁺ T cells produced IL-17 (Fig 5, B). On the contrary, after *H1r*^{-/-} *D farinae* BMDC stimulation, the percentage of CD8⁺ T cells producing IFN- γ was reduced, and conversely the production of IL-17 was upregulated. Collectively, these data reveal that *H1r*^{-/-} and WT BMDCs have the same capacities to prime naive CD8⁺ T cells (CD8⁺ T cells proliferated equivalently) but with a distinct phenotype (Tc17 rather than Tc1).

IL-17⁺CD8⁺ T cells are less cytotoxic than IFN- γ ⁺CD8⁺ T cells

Our data indicated that distinct populations of CD8⁺ T cells were producing IFN- γ and IL-17. To characterize further the phenotype of IL-17⁺CD8⁺ and IFN- γ ⁺CD8⁺ T cells, we stained for activation molecules CD25 and ICOS. We showed that IFN- γ ⁺CD8⁺ T cells expressed CD25 and that IL-17⁺CD8⁺ T cells coexpressed CD25 and ICOS (Fig 6). The expression of ICOS by IL-17⁺CD8⁺ was correlated with the high expression of ICOS-L on *D farinae* *H1r*^{-/-} BMDCs (Fig 3, A). To establish whether those distinct phenotypes were associated with different cytotoxicity capacities, we measured the degranulation of CD8⁺ T cells by the assessment of cell surface expression of CD107a as well as the intracellular staining with anti-granzyme B on PMA and ionomycin stimulation. As shown in Fig 6, IFN- γ ⁺CD8⁺ cells express approximately 6% (5.9% $\pm$ 0.3) of CD107a, in contrast with IL-17⁺CD8⁺ T cells (0.50% $\pm$ 0.1) on activation. Similarly, IFN- γ ⁺CD8⁺ T cells express high levels of granzyme B, in contrast with IL-17⁺CD8⁺ T cells. We next compared the cytotoxic activity of *in vivo*-primed CD8⁺ T cells. CD8⁺ T cells

A



B

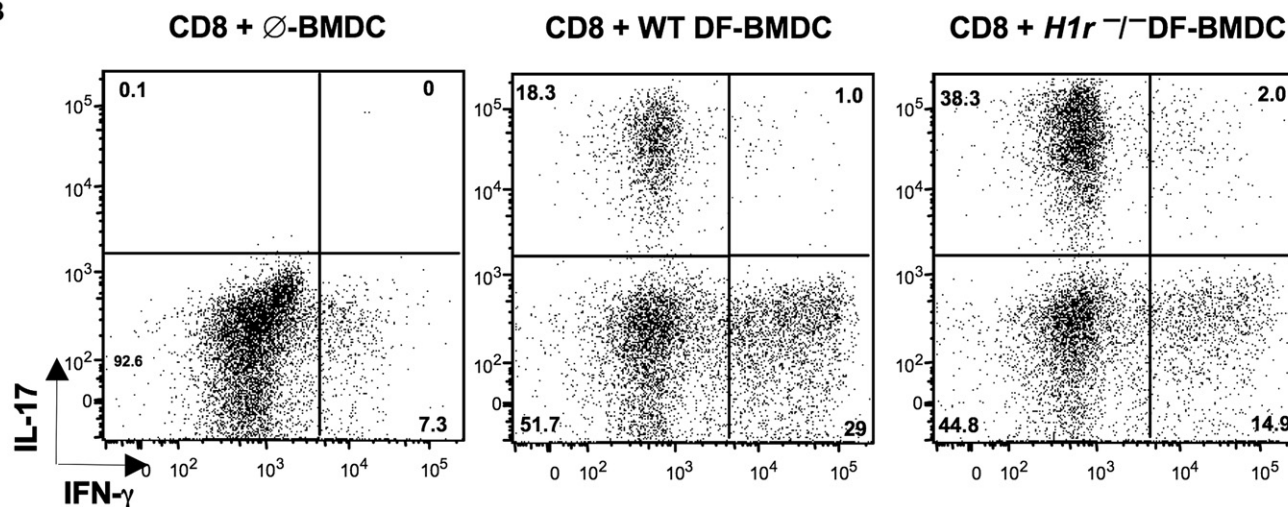


FIG 5. *H1r*^{-/-} DCs prime preferentially IL-17-producing CD8⁺ T cells. WT or *H1r*^{-/-} BMDCs pulsed with or without *D. farinae* (DF) were cocultured with naive WT CD8⁺ T cells for 5 days, and the proliferation was measured by the thymidine uptake. The production of IFN-γ and IL-17 was measured in the supernatants by ELISA. **A**, Intracellular staining of IL-17 and IFN-γ on gated blastic CD8⁺ T cells after stimulation with PMA and ionomycin. **B**, The percentages of CD8⁺IFN-γ⁺ and CD8⁺IL-17⁺ are indicated. Representative of 3 independent experiments. **P* < .05. *cpm*, Counts per minute,

from DLNs of *D. farinae* WT or *H1r*^{-/-} BMDC-sensitized mice were restimulated *ex vivo* for 48 hours with *D. farinae* BMDCs (WT or *H1r*^{-/-}) and then cultured for 24 hours with CFSE-labeled targets. As shown in Fig 6, B, WT *D. farinae*-primed CD8⁺ T cells have a high cytotoxic activity compared with their *H1r*^{-/-} *D. farinae*-primed CD8⁺ T cell counterparts. Altogether, these data suggest that H1R-deficient BMDCs prime CD8⁺ T cells with reduced cytotoxic capacities that are unable to initiate skin inflammation.

DISCUSSION

The local release of histamine during allergic reactions has long been recognized as an important step in immediate hypersensitivity reactions to allergen. Here, we show an important role of

histamine signaling through the H1R in the development of an antigen-specific skin inflammation. The first important finding was that *H1r*^{-/-} mice displayed reduced AD. It was essential to establish whether this was caused by the deficiency of H1R in the T-cell effector compartment (CD8⁺ T cells) or in antigen-presenting cells. Several reports showed that H1R deficiency directly affects the cytokine response in T cells.^{9,13,20,21} Jutel et al⁹ showed that *H1r*^{-/-} mice displayed lower IFN-γ-producing T cells after anti-CD3 and anti-CD28 stimulation compared with WT mice. *H1r*^{-/-} mice exhibited a significant delay in the onset of experimental autoimmune encephalomyelitis and a reduced severity of the clinical signs compared with WT mice. This phenotype was associated with a significantly lower production of IFN-γ by CD4⁺ T cells because of an impaired activation of p38 mitogen-activated protein kinase by T cell-receptor

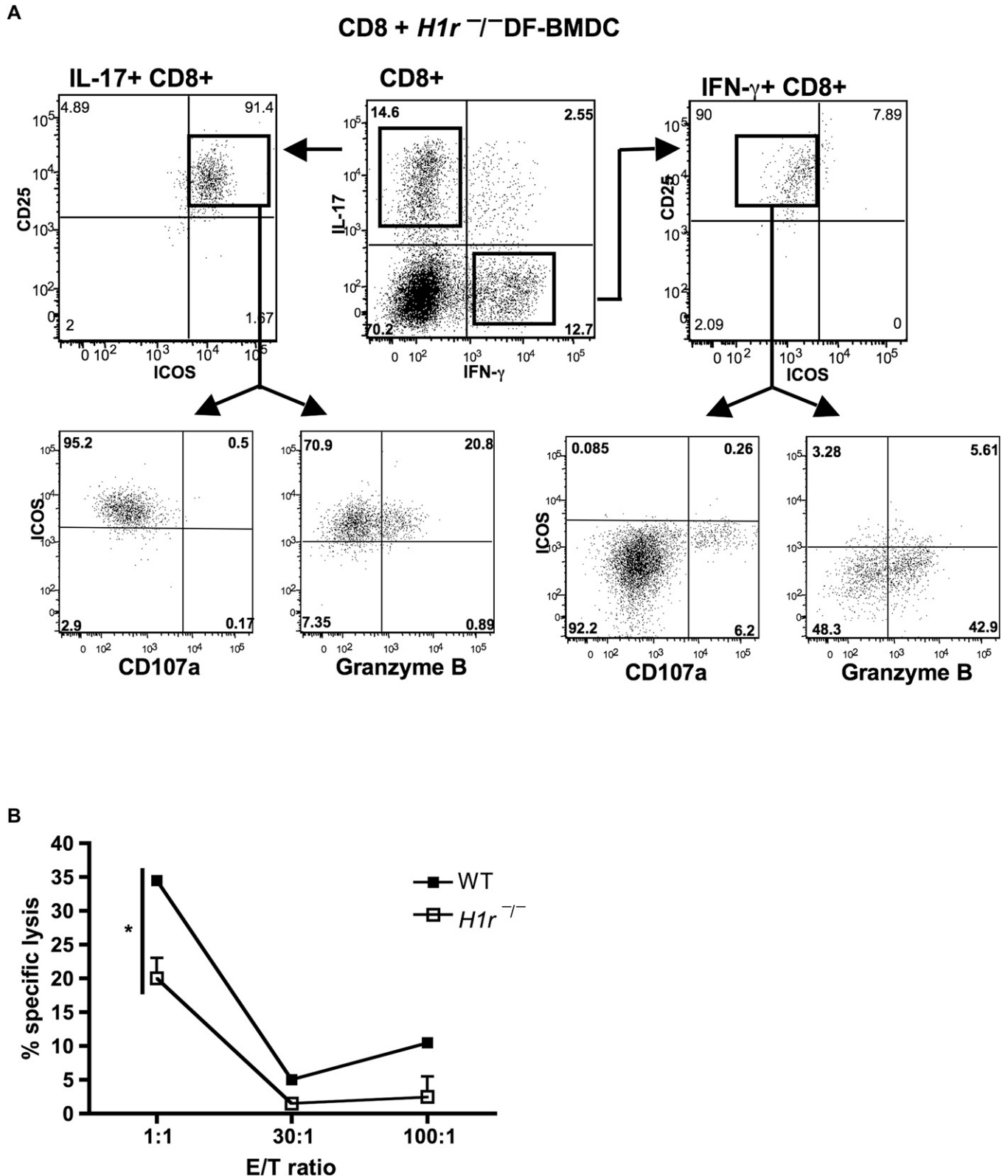


FIG 6. IL-17⁺CD8⁺ T cells are less cytotoxic than IFN-γ⁺CD8⁺ T cells. **A**, *H1r*^{-/-} BMDCs pulsed with *D. fariniae* (DF) were cocultured with naive CD8⁺ T cells for 5 days. The phenotype of CD8⁺IL-17⁺ or CD8⁺IFN-γ⁺ T cells stained with anti-CD25 and anti-ICOS is indicated. Staining for granzyme B and CD107a in CD8⁺IL-17⁺ and CD8⁺IFN-γ⁺ T cells is indicated. Representative of 3 independent experiments. **B**, Irradiated splenocytes were labeled with 0.5 μmol/L or 5 μmol CFSE. Those labeled with 0.5 μmol/L were further pulsed with DF antigen. Control and pulsed target cells were mixed at a ratio of 1:1 and were incubated with different ratio of CD8⁺ T cells (issued from vehicle-treated WT mice, WT or *H1r*^{-/-} BMDC-immunized mice). After 24 hours, the cells were analyzed by flow cytometry, and the percentage of specific lysis was calculated as (1 – % targets/% control cells) × 100. Representative of 3 independent experiments. **P* < .05. E/T, Effector/target ratio.

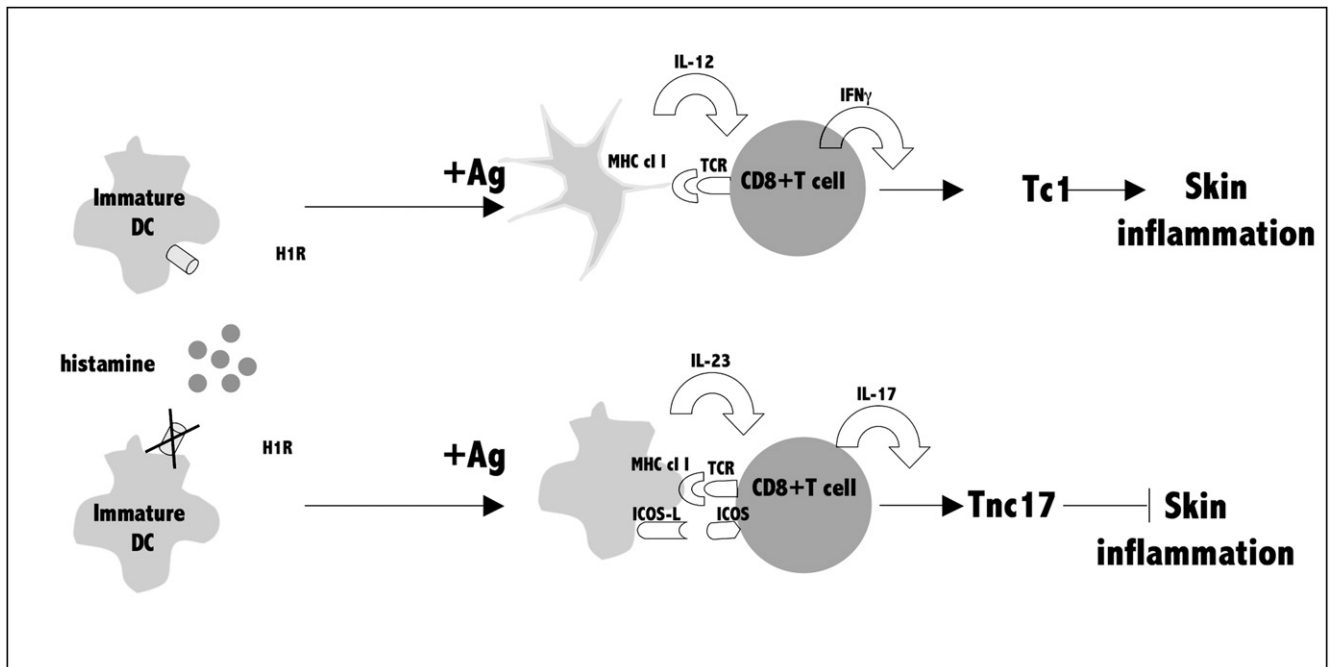


FIG 7. Role of H1R in the development of antigen (Ag)-specific skin inflammation. This hypothetical model proposes that in the sensitization phase of atopic dermatitis, immature DCs uptake the Ag, mature, and produce IL-12. Cognate interaction with naive CD8⁺ T cells leads to the generation of cytotoxic Tc1 cells that initiate the development of AD. In contrast, in the absence of H1R signaling, immature DCs mature less on Ag uptake and preferentially produce IL-23. In this case, cognate interaction with naive CD8⁺ T cells leads to the generation of noncytotoxic Tnc17 cells that block the development of skin inflammation. *cl*, Class.

ligation.²⁰ In a mouse model of asthma, H1R regulated T-cell migration and was essential for the recruitment of passively transferred antigen-specific CD4⁺ and CD8⁺ T cells to the site of antigen exposure and for the subsequent generation of inflammatory responses.¹³ We show for the first time that the antigen-specific skin inflammation is significantly reduced in *H1r*^{-/-} mice and associated with low frequencies of *D farinae*-specific IFN- γ CD8⁺ T cells because of the impaired expression of H1R on DCs. This assertion is substantiated by 2 lines of evidence: (1) skin inflammation induced by injection of *D farinae* *H1r*^{-/-} BMDCs was similarly decreased in topically sensitized *H1r*^{-/-} mice, and (2) injection of allergen-charged WT BMDCs in *H1r*^{-/-} recipients led neither to reduction of skin inflammation nor to the reduction of frequency of specific IFN- γ CD8⁺ T cells. Previous reports did not take into account the contribution of antigen-presenting cells to cognate activation of T cells because the experiments were done with isolated naive T cells stimulated *in vitro* with anti-CD3 antibodies⁹ or on *in vivo*-primed T cells in *H1r*^{-/-} mice, in which both antigen-presenting cells and T cells are deficient for H1R.²⁰ Our findings show that H1R signaling plays a key role in DC instruction for T-cell priming upstream of the T-cell activation.

Upon *D farinae* stimulation, H1R-deficient BMDCs displayed a modified phenotype compared with WT counterparts. *D farinae* and *D pteronyssinus* are major allergens in AD pathophysiology.¹⁴ *D farinae* has enzymatic activity and is capable of affecting the DC phenotype.^{22,23} Histamine has been shown to modulate the DC phenotype. Endogenous histamine is actively synthesized during cytokine-induced DC differentiation, which acts in an autocrine and paracrine fashion and modifies DC markers.²⁴ Studies on human *in vitro*-derived DCs showed that H1R acts as a positive

stimulant that increases antigen-presentation capacity, proinflammatory cytokine production, and T_H1-priming activity.^{25,26} Our results show that on allergen stimulation, *H1r*^{-/-} BMDCs expressed lower levels of MHC class I, MHC class II, and CD86 and higher levels of ICOS-L than their WT counterparts. This “less mature” phenotype was associated with reduced production of IL-12p40. More importantly, we show that H1R deficiency was an active phenomenon responsible for DC cytokine production switch from IL-12 to IL-23. IL-23 cytokine is secreted by professional antigen-presenting cells during inflammatory responses to pathogenic as well as nonpathogenic stimuli.²⁷ The IL-23–IL-17 system has been reported to be important in the pathogenesis of several inflammatory disorders like psoriasis and inflammatory bowel disease.^{28–30} Consistent with the IL-23 production, *H1r*^{-/-} BMDCs primed IL-17–producing instead of IFN- γ –producing CD8⁺ T cells. Unlike T_H17, Tc17 has received only marginal attention. However, it has been documented that the basic factors that determined the development of mouse CD8⁺ T cells in the presence of TGF- β and IL-6 result in the expression of retinoic acid–related orphan receptor γ t and development of Tc17.^{31–33} Originally it was described that CD8⁺ T cells secreting IL-17 played a pathogenic role in a variety of diseases. Tajima et al³⁴ demonstrated that IL-6 drove spontaneous expansion of Tc17 cells, which induced colitis in a mouse model. In a contact hypersensitivity model, allergen sensitization induced the development of CD8⁺ T-cell subpopulations that produce IFN- γ and IL-17 and are both required to initiate the response.^{35,36} In another study, the majority of CD8⁺ and CD4⁺ T cells derived from lesional psoriatic skin expressed IL-17A, suggesting that skin-infiltrating IL-17A T cells contribute to disease pathogenesis.³⁷ However, recent reports showed that IL-17 may play a

protective role. O'Connor et al³⁸ showed that in addition to IL-10 and TGF- β , IL-17A is crucial in directly inhibiting the T_H1 subset and suppressing gut inflammation in the CD45RB^{hi} transfer model of colitis. T cells deficient in IL-17A induced an accelerated disease and correlated with a higher expression of IFN- γ .³⁸ Our results show that IL-17⁺CD8⁺ T cells are less cytotoxic and therefore unable to initiate the development of AD. Our findings are in accordance with 2 previous reports demonstrating that CD8⁺ T cells differentiated in the presence of TGF- β and IL-6 into IL-17-producing cells and that those cells were not cytotoxic (Tnc17) in mixed lymphocyte culture.^{33,39} Perforin and granzyme B are cytotoxic molecules produced by activated CD8⁺ T cells, which are strongly expressed in AD lesional skin.⁴⁰ Previous studies have shown that cytotoxicity is mandatory for the development of antigen-specific skin inflammation.⁴¹ Therefore, the reduced cytotoxicity of IL-17⁺CD8⁺ T cells accounted for the diminished AD in *H1r*^{-/-} and *H1r*^{-/-} BMDC-immunized mice.

Which factors define the pathogenic role of IL-17? Ciric et al⁴² showed that IL-17⁺CD8⁺ T cells are pathogenic or nonpathogenic depending on the cytokine environment and that IL-23 plays an important role in driving pathogenic IL-17-producing CD8⁺ T cells. In contrast, our data demonstrate that IL-23 production by *H1r*^{-/-} BMDCs was not associated with the induction of pathogenic IL-17-producing cells. One explanation might be the dose of IL-23 used *in vitro* to generate pathogenic IL-17⁺CD8⁺ T cells, which was 5 times higher⁴² than the production that we observed in our cultures.

We show that IL-17-secreting CD8⁺ T cells were distinct from IFN- γ -producing CD8⁺ T cells and express ICOS. These data are consistent with high expression of ICOS-L on *H1r*^{-/-} BMDCs and therefore might be involved in the generation of IL-17-producing CD8⁺ T cells. Furthermore, these results are in accordance with a previous report showing that ICOS signaling is required for the generation of IL-17-producing cells. T cells deficient in ICOS (CD4⁺ T cells issued from *Icos*^{-/-} mice) produced less IL-17 and IL-4 and more IFN- γ than their WT counterparts.⁴³

Our data suggest a model to explain how H1R modulates antigen-specific skin inflammation. As depicted in Fig 7, immature DCs capture the antigen, mature, and produce IL-12. Cognate interaction with naive CD8⁺ T cells leads to primed cytotoxic Tc1 cells, which initiate the development of antigen-specific skin inflammation. On the contrary, immature DCs in the absence of H1R signaling mature less and produce IL-23. Cognate interaction with naive CD8⁺ T cells leads to primed noncytotoxic Tnc17 cells, which block the development of skin inflammation.

In conclusion, the current study demonstrates that H1R is an essential receptor involved in the efficient generation of allergic skin inflammation. Therefore, pharmacologic antagonists of H1R that are commonly used for the treatment of immediate allergic reactions might be useful in preventing chronic allergic conditions like AD. A previous study showed that treatment with the H1R antagonist cetirizine in young children with AD over a period of 18 months was reported to delay or prevent the onset of asthma in some cases.⁴⁴ Such treatment, attractive given the availability of antihistamines, might be useful in preventing eczema in young children with a high risk for developing AD.

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Clinical implications: H1R signaling on DCs conditions the outcome of the skin effector immune response.

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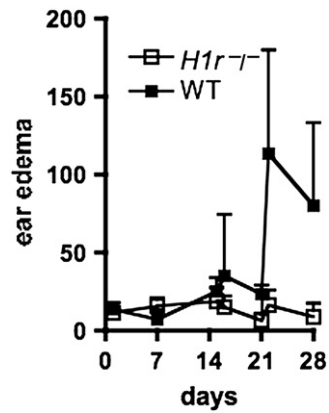


FIG E1. Kinetic of AD response in WT and *H1r*^{-/-}. *D. farinae*-induced skin inflammation was analyzed in *H1r*^{-/-} and *H1r*^{+/+} mice painted onto the ear with a solution of *D. farinae* once a week for 4 weeks. Results are representative of 3 independent experiments using 5 mice per group.

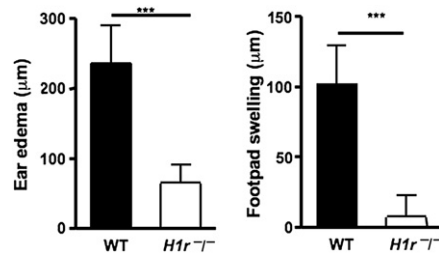


FIG E2. DNFB-CHS and BSA-DTH are reduced in *H1r*^{-/-} mice. Contact hypersensitivity to DNFB and DTH to BSA were performed as previously described.⁴¹ The ear edema and the foodpad swelling are expressed in micrometers. Representative of 3 independent experiments. ****P* < .0001.

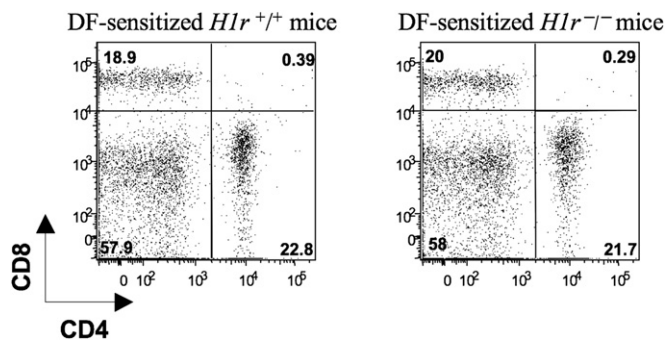


FIG E3. Analysis of the T-cell compartment in *H1r*^{-/-} mice. Flow cytometry analysis of auricular LN cells of sensitized mice. Cells were recovered from LNs and stained with anti-CD4 and anti-CD8. Dot plot analysis shows the percentage of CD8⁺ and CD4⁺ T cells in *H1r*^{+/+} and *H1r*^{-/-} mice. Representative of 3 independent experiments. *DF*, *D. farinae*.

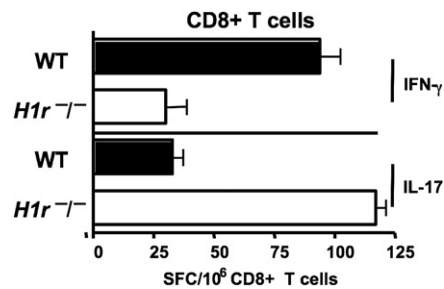


FIG E4. IL-17 is mainly produced by CD8⁺ T cells in the AD model. *D. farinae*-specific IFN- γ and IL-17-producing cells were determined in isolated CD8⁺ T cells recovered at day 21 from H1r^{-/-} or H1r^{+/+} *D. farinae*-sensitized mice on *D. farinae* restimulation *in vitro* in the presence of irradiated splenocytes as APC. Results are expressed as the number of SFCs per 10⁶ CD8⁺ T cells. Representative of 3 independent experiments.

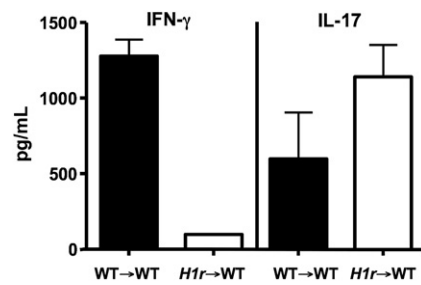


FIG E5. Production of IFN- γ and IL-17 in sensitized animals. IFN- γ and IL-17 production in LNs at day 7 in WT mice sensitized by *D farinae* WT of *H1r*^{-/-} BMDCs. *D farinae*-specific IFN- γ and IL-17 production was evaluated by ELISA on *D farinae* restimulation *in vitro* of whole DLN cells. Representative of 4 independent experiments.

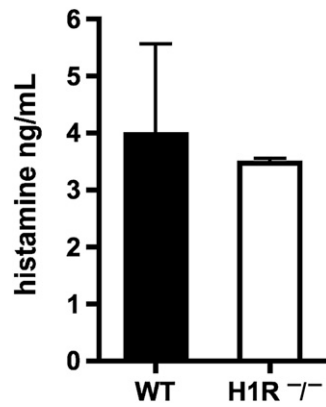


FIG E6. Production of histamine in BMDC (WT or *H1r*^{-/-}) cultures. Histamine was determined using ELISA (Neogen Corp, Lansing, Mich). Representative of 3 independent experiments.

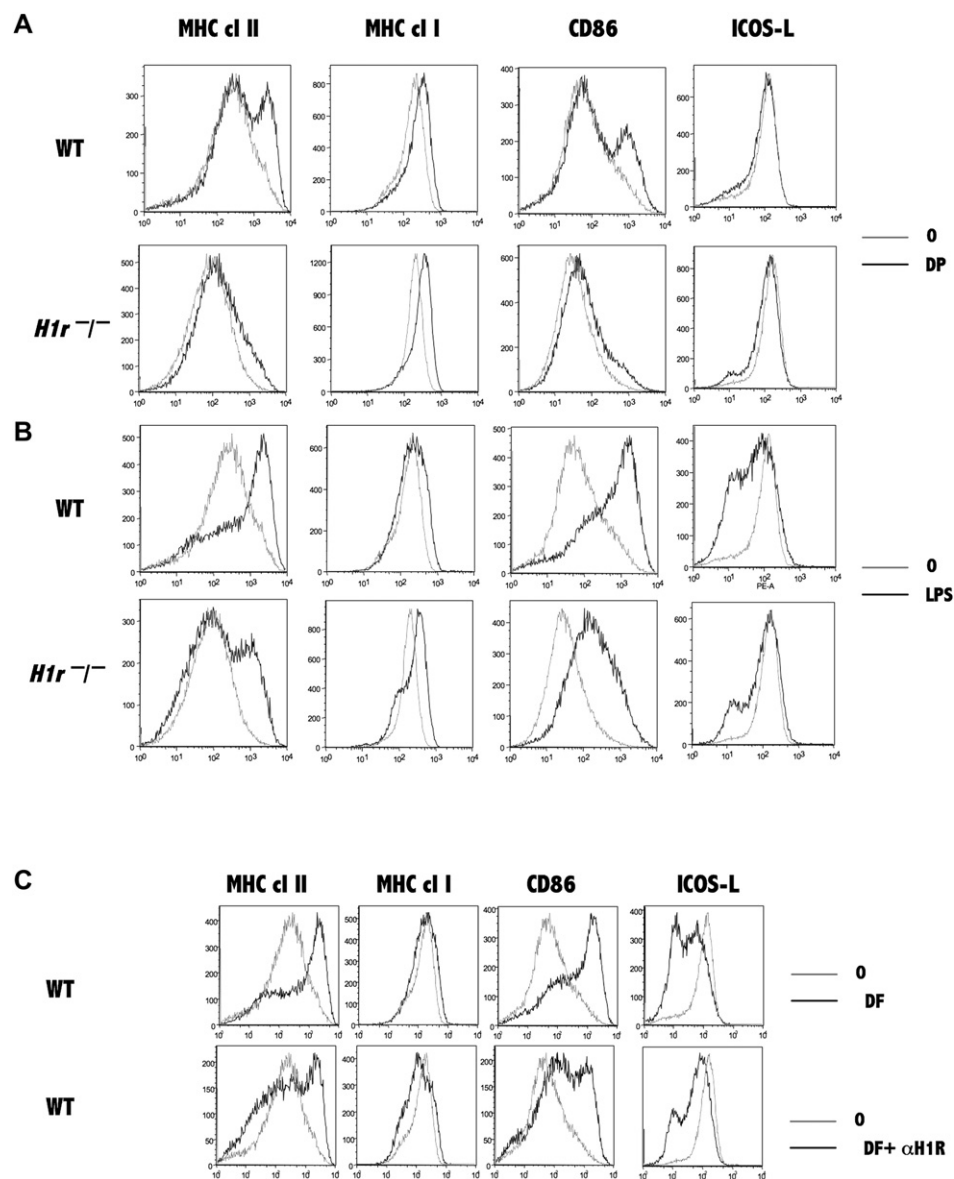


FIG E7. Impact of H1R deficiency or blockade on the phenotype of DCs. Histogram overlays represent surface staining of MHC class (cl) II, MHC class I, CD86, and ICOS-L in BMDCs gated on CD11c⁺ cells after *D pteronyssinus* stimulation (**A**), LPS (**B**). Tripelennamine anti-H1R was used (**C**). Representative of 3 independent experiments. *DF*, *D farinae*.

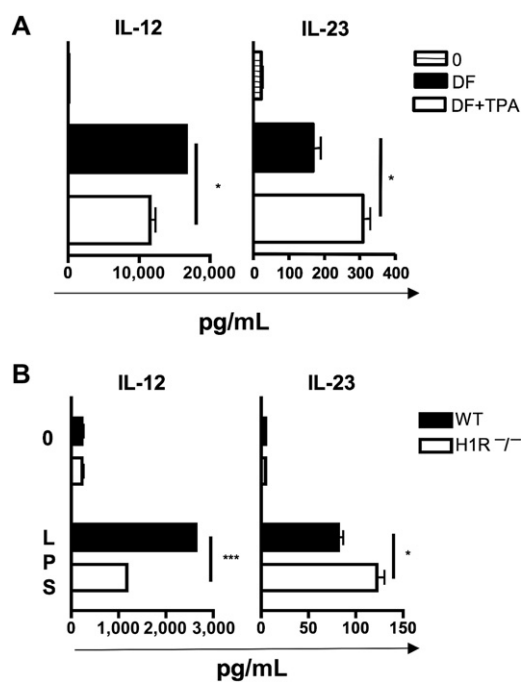


FIG E8. Modulation of the cytokine secretion of DCs by H1R. **A**, WT *D. farinae* (DF) BMDCs were treated or not with tripelennamine (TPA) before antigen uptake. The IL-12 and IL-23 production was measured by ELISA. **B**, WT or H1R^{-/-} BMDCs were matured with LPS, and the production of IL-12 and IL-23 was measured. Representative of 3 independent experiments. * $P < .05$, *** $P < .0001$.

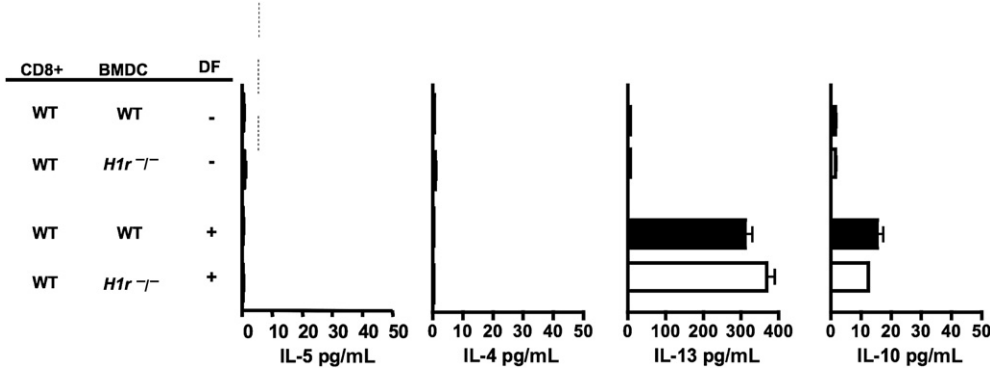


FIG E9. Cytokine profile of *in vitro*-primed CD8⁺ T cells. WT or H1r^{-/-} BMDCs pulsed or not with *D. farinae* (DF) were cocultured with naïve WT CD8⁺ T cells for 5 days. IL-4, IL-5, IL-13, and IL-10 production was measured in the supernatants by ELISA. Representative of 3 independent experiments.

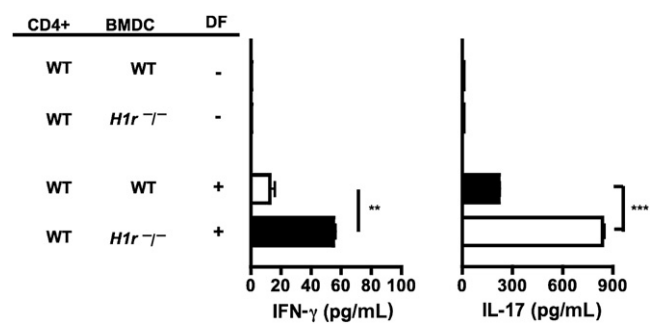


FIG E10. Cytokine profile of *in vitro*-primed CD4⁺ T cells. WT or H1r^{-/-} BMDCs pulsed or not with *D farinae* (DF) were cocultured with naive WT CD4⁺ T cells for 5 days. IFN-γ and IL-17 were measured in the supernatants by ELISA. Representative of 3 independent experiments. ***P* < .005, ****P* < .0001.