Original article

Antigen-specific effector CD8 T cells regulate allergic responses via IFN-γ and dendritic cell function

Yafang Tang, BSc,[a,b,c] Shou Ping Guan, BSc,[b,d] Benson Y. L. Chua, BSc,[a,b] Qian Zhou, BSc,[a,b] Adrian W. S. Ho, BSc,[a,b] Kenneth H. S. Wong, BSc,[a,b] Kok Loon Wong, PhD,[e] W. S. Fred Wong, PhD,[b,d] and David M. Kemeny, PhD, FRCPath[a,b,c] *Singapore*

Background: Previous studies have shown that CD8 T cells can both prevent and cause allergic responses. However, the underlying mechanisms remain to be elucidated.
Objective: We aim to investigate the potential of CD8 T cells with different IFN-γ expressions to modulate the elicitation of allergic inflammation following ovalbumin (OVA) challenge and investigate the underlying mechanisms.
Methods: To study the role of IFN-γ in the effect of CD8 T cells, effector CD8 T cells from CD8 OVA transgenic (OT-I) mice and IFN-$\gamma^{-/-}$OT-I mice were transferred to OVA-sensitized mice the day before 3 challenges with OVA. The effect on lung dendritic cells (DCs) exerted by CD8 T cells was studied with *ex vivo* culture of sorted DCs from treatment mice with CD4 T cells.
Results: Effector OT-I, but not IFN-$\gamma^{-/-}$OT-I CD8 T cells, attenuated eosinophilia and mucus secretion in the lungs of sensitized mice in an antigen-specific manner. Effector IFN-$\gamma^{-/-}$OT-I CD8 T cells displayed a Tc2-/Tc17-biased phenotype with weaker cytotoxicity and were able to both induce and exacerbate eosinophilia as well as neutrophilia. OT-I CD8 T cells increased the ability of lung CD11b$^+$CD103$^-$ DCs to both prime the differentiation of naive OVA-specific CD4 T cells toward a T_H1 phenotype and enhance IFN-γ production by antigen-experienced lung CD4 T cells.
Conclusion: Effector CD8 T cells attenuate pulmonary inflammation and alter the ability of DCs within the allergic lung to polarize T cells to a T_H1 phenotype during a T_H2 response. In the absence of IFN-γ, CD8 T cells assume a Tc2-/Tc17-biased phenotype and potentiate inflammation. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: *Asthma, OT-I, IFN-$\gamma^{-/-}$OT-I, CD8 T cells, effector, IFN-γ, eosinophils, CD11b$^+$CD103$^-$DC, T_H1*

T cells are central to the pathogenesis of asthma, and the cytokines they secrete determine both the initiation and progression of asthmatic disease.[1,2] Type II cytokines such as IL-4, IL-5, and IL-13 are key mediators of eosinophilia, IgE production, goblet cell hyperplasia, and airway hyperresponsiveness (AHR).[3-7] Immunoregulatory functions of T cells were first described in the inhibition of allergic sensitization to ovalbumin (OVA) represented by IgE.[8] These regulatory cells were later shown to be CD8 T cells,[9] and their inhibitory effect on IgE was further demonstrated by different groups.[10,11] Subsequently, CD8 T cells were also shown to attenuate T_H2 responses in the airways.[12-14] Recently, CD8 T cells were demonstrated to be important in the suppressive role of regulatory T cells in allergic lung disease.[15]

However, it became apparent that there are different subsets of CD8 T cells. This was discovered when several subsequent studies showed that in contrast to their suppressor activity, CD8 T cells could also contribute to allergic responses.[16-18] These contrasting findings were partially explained when CD8 T cells were shown to exist in 2 different subsets, namely, Tc1 and Tc2.[19-21] While the proinflammatory Tc2 subtype predominates in the lungs of asthmatic patients and is always associated with exacerbation of asthma,[22-27] Tc1 cells can superimpose a T_H1-biased inflammation over T_H2 response and are capable of suppressing T_H2 inflammation, especially if present prior to sensitization.[13,14] IFN-γ from CD8 T cells was shown to play an important role in the attenuation of allergic lung inflammation[28-31] though the underlying mechanisms remain unclear. Recently, a new subset of CD8 T cells, Tc17, was also described,[32] and IL-17 produced by these cells was shown to be proinflammatory in pulmonary pathology.[33] In addition, the effector and memory status of CD8 T cells may also be crucial in influencing their regulatory effects, with the effector phenotype exhibiting a strong bias toward the promotion of allergic responses.[34,35]

We have shown *in vitro* that CD8 T-cell interaction with dendritic cells (DCs) modulates their ability to prime CD4 T cells.[36] Two main subsets of DCs are described in the lung: CD11b$^+$CD103$^-$ DCs, which are efficient at MHC class II–restricted presentation to CD4 T cells, and CD11b$^-$CD103$^+$ DCs, which cross-present antigens via MHC I to CD8 T cells.[37,38] Both subsets of DCs are capable of producing IL-12p70,[37,39] which favors T_H1 differentiation.[40,41] We have previously shown that cognate interaction between CD8 T cells and DCs is able to

From [a]the Department of Microbiology, Yong Loo Lin School of Medicine, [b]the Immunology Programme, Centre for Life Sciences, [c]the NUS Graduate School for Integrative Sciences and Engineering (NGS), and [d]the Department of Pharmacology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore; and [e]the Singapore Immunology Network, Agency for Science, Technology and Research, Biopolis.

This work was supported by the Biomedical Research Council (grant no. BMRC 05/1/21/19/41). Y.T. was supported by an NGS scholarship. D.M.K. is supported by the National Research Foundation HUJ-CREATE programme on Cellular and Molecular Mechanisms of Inflammation.

Disclosure of potential conflict of interest: D. M. Kemeny is on the Johnson & Johnson Scientific Advisory Committee. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 7, 2011; revised October 27, 2011; accepted for publication December 22, 2011.

Corresponding author: David M. Kemeny, PhD, FRCPath, Immunology Programme, Centre for Life Sciences, National University of Singapore #03-05, 28 Medical Dr, Singapore 117456. E-mail: mickdm@nus.edu.sg.

0091-6749/$36.00

© 2012 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2011.12.976

Abbreviations used
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
DC: Dendritic cell
OVA: Ovalbumin

induce potent DC production of IL-12p70 *in vitro* in an IFN-γ–dependent manner.[36]

Harnessing the ability of CD8 T cells to induce immune deviation of an allergic T_H2 response represents a promising strategy for attenuating inflammation, but the role of CD8 T cells in allergic inflammation remains controversial. The contribution of CD8 T-cell–derived IFN-γ in T_H1 polarization during pulmonary T_H2 inflammation is similarly poorly understood. The present study sought to address the ability of CD8 T cells of a specific phenotype to regulate allergic responses focusing on the role of IFN-γ as well as pulmonary DCs in a mouse model of OVA-induced asthma. Antigen-specific effector CD8 T cells were used in our study and introduced to immunized mice before allergen challenge. The participation of lung parenchyma DCs in the regulatory role of CD8 T cells was assessed by using an *ex vivo* coculture with CD4 T cells. In order to better identify the role of IFN-γ in the modulating effect of CD8 T cells, we employed a cross-bred transgenic IFN-$\gamma^{-/-}$OT-I mice. Our results showed that Tc1-like antigen-specific effector CD8 T cells could attenuate eosinophil infiltration and mucus secretion. Pulmonary DCs, particularly $CD11b^+CD103^-$ DCs, from CD8 T-cell–transferred recipient mice were capable of biasing CD4 T cells toward T_H1. However, in the absence of IFN-γ, CD8 T cells became more Tc2-/Tc17-like when activated, which induced an asthma-like pathology in nonimmunized mice and caused exacerbation of inflammation in immunized and challenged mice.

METHODS

Animals

Age- and sex-matched C57BL/6 mice (age 8-10 weeks) were purchased from the animal breeding center of the National University of Singapore. OT-I (C57BL/6-Tg(TcraTcrb)1100Mjb/Crl) and OT-II (C57BL/6-Tg(TcraTcrb) 425Cbn/Crl) mice were purchased from Charles River Laboratories (Wilmington, Mass). OT-I CD8 T cells recognize OVA peptide $OVA_{257-264}$ (SIINFEKL) associated with $H\text{-}2K^b$, while OT-II CD4 T cells recognize OVA peptide $OVA_{323-339}$. IFN-$\gamma^{-/-}$ mice were purchased from Jackson Laboratory (Bar Harbor, Me). To generate IFN-$\gamma^{-/-}$OT-I mice, OT-I mice were cross-bred with IFN-$\gamma^{-/-}$ mice for 3 to 5 generations. All mice were maintained under pathogen-free conditions in the satellite animal housing unit of the Centre for Comparative Medicine. All experiments were conducted in accordance with institutional guidelines and were approved by the National University of Singapore Institutional Animal Care and Use Committee under protocol number 029/09.

Sensitization and airway challenges

Mice were injected intraperitoneally with 100 μg of OVA (grade V; Sigma-Aldrich, St Louis, Mo) emulsified in 0.95 mg of aluminum potassium sulfate (Sigma-Aldrich) in a total volume of 100 μL of PBS on days 0 and 14. On days 22 to 24, mice were challenged intranasally with 100 μg of OVA in 20 μL of PBS under isoflurane (Abbott Laboratories, North Chicago, Ill) anesthesia. Age- and sex-matched control mice were sensitized and challenged with PBS. Mice were assessed for airway function or sacrificed for sample collection on day 25.

Isolation, activation, and adoptive transfer of CD8 T cells

CD8 T cells from OT-I or IFN-$\gamma^{-/-}$OT-I mice were purified by positive selection with anti-CD8α-antibody–conjugated magnetic cell sorting beads (Miltenyi Biotec, Singapore). Purified cells were cultured with 10 ng/mL of phorbol 12-myristate 13-acetate (Sigma-Aldrich) and 400 ng/mL of ionomycin (Sigma-Aldrich) for 48 hours. Three million activated cells were intravenously transferred into immunized mice on day 21. Carboxyfluorescein-succinimidyl-ester staining[42] of the CD8 T cells was carried out for either naive cells (*in vitro* prolifereation) or activated cells before transfer (*in vivo* proliferation).

Bronchoalveolar lavage analysis

After cardiac puncture, the lungs were lavaged via a trachea tube twice with 0.7 mL of PBS. The bronchoalveolar lavage (BAL) fluid cells were stained with fluorochrome-labeled antibodies for flow cytometric analysis: Siglec-F-PE (BD Biosciences, Franklin Lakes, NJ), Ly-6G-APC (BD Biosciences), CD11c-PerCP/Cy5.5 (Biolegend, San Diego, Calif), and CD3-PB (Biolegend).

Ex vivo assay of lung parenchymal DCs

Lung tissues were excised after BAL and digested with Liberase CI (Roche, Switzerland). Single-cell suspension in 1.062 g/mL of Optiprep (Sigma-Aldrich) was subjected to density centrifugation. Enriched cells were stained with CD11c-AF647 (Biolegend), CD11b–PE (BD Biosciences), CD103-AF488 (Biolegend), and IAIE-PB (Biolegend). $CD11c^+IAIE^+CD11b^+CD103^-$ DCs were sorted by using MoFlo (Beckman Coulter, Brea, Calif). To study the differentiation capacity of DCs, these cells were cocultured with either freshly isolated OT-II CD4 T cells or lung CD4 T cells isolated from respective treatment mice for 4 days. Production of cytokines was measured by ELISA.

Lung histology

Lung tissues were obtained after perfusion with PBS and fixation with 0.5 mL of 4% paraformaldehyde (Sigma-Aldrich). Tissues were further fixed with 4% paraformaldehyde for 3 to 5 days and then dehydrated and embedded in paraffin. Sections (4 μm thick) were cut and stained with periodic acid-Schiff's reagent and hematoxylin (Sigma-Aldrich) to identify mucus secreting globlet cells.

Assessment of airway function

Airway responsiveness was measured as the change in airway resistance to increased concentration of nebulized methacholine (0.5-8.0 mg/mL) (Sigma-Aldrich). Mice were anesthetized, tracheostomized, and mechanically ventilized by using FinePointe Series RC Sites (Buxco Research System, Wilmington, NC) and airway resistance was recorded. Results are expressed as percentages of respective basal values in response to PBS.

Measurement of cytokines

Cytokine levels were measured with mouse DuoSet ELISA development kits (R&D Systems, Minneapolis, Minn) according to the manufacturer's instruction.

^{51}Cr Cytotoxic assay

EL4 Target cells were labeled with radioactive ^{51}Cr (PerkinElmer, Waltham, Mass) and pulsed with 10 μg/mL of SIINFEKL peptide (Anaspec, Fremont, Calif). CD8 effector cells were cultured with target cells at desired ratios for 6 hours before supernatants were harvested. Supernatants were loaded into a 96-well Luma plate and left to dry overnight. Radioactive counts were determined using Beckman Top Count (PerkinElmer). Degranulation assay measuring CD107a accumulation is elaborated in this article's Online Repository materials at www.jacionline.org.

Statistical analysis

Unpaired Student *t* test was used for comparison between 2 groups, and 1-way ANOVA was used for comparison within multiple groups. Data are

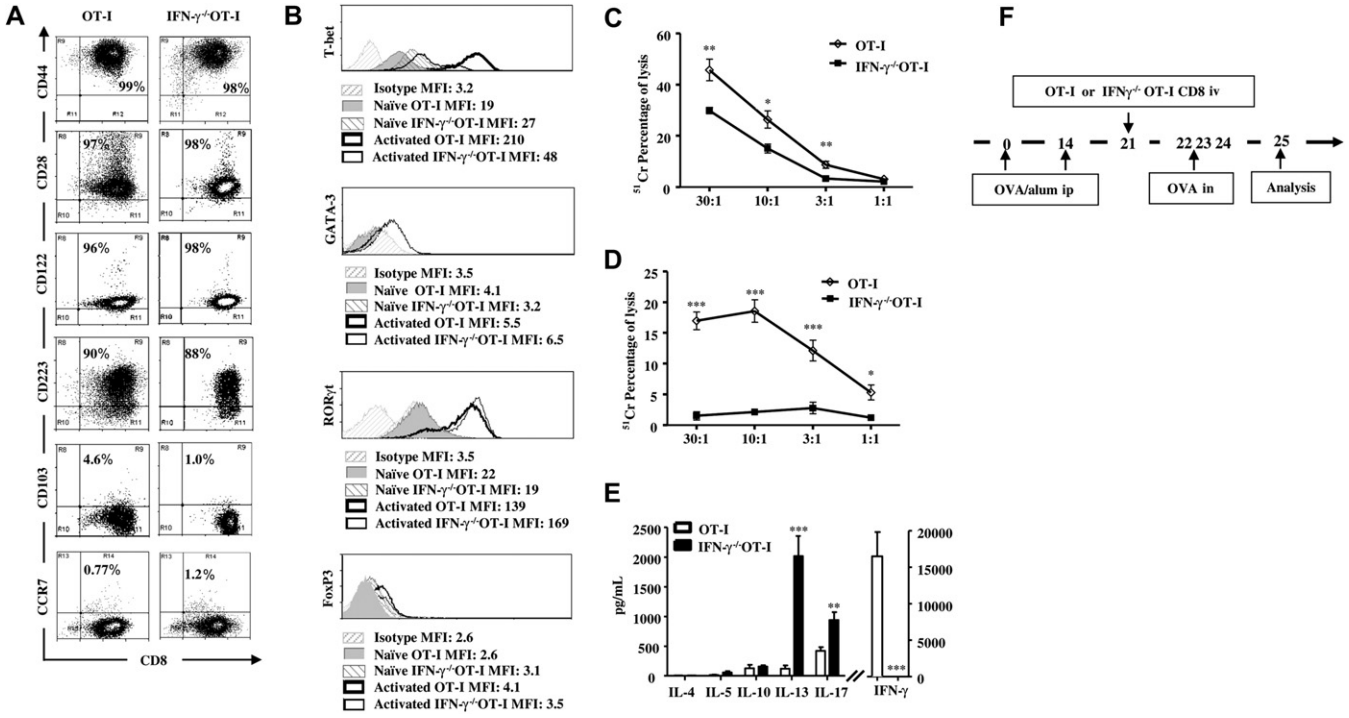

FIG 1. Characterization of effector OT-I and IFN-$\gamma^{-/-}$OT-I CD8 T cells. CD8 T cells from OT-I and IFN-$\gamma^{-/-}$OT-I mice were activated for 48 hours with phorbol 12-myristate 13-acetate and ionomycin. **A,** Flow cytometric analysis for surface marker expression. **B,** Transcription factor expression by intracellular staining. **C,** Cytotoxic profile of naive CD8 T cells determined by ^{51}Cr assay, expressed as percentage of specific lysis of target cells. **D,** Cytotoxic profile of activated CD8 T cells determined by ^{51}Cr assay, expressed as percentage of specific lysis of target cells. **E,** Cytokine production in the supernatants measured by ELISA. **F,** Sensitized mice were adoptively transferred with activated CD8 T cells from either OT-I or IFN-$\gamma^{-/-}$OT-I mice before 3 consecutive intranasal challenges. n = 3; *P < .05, **P < .01, ***P < .001. *in*, Intranasally; *ip*, intraperitoneally; *iv*, intravenously; *MFI*, mean fluorescence intensity.

expressed as means ± SEM (P value range was indicated). Flow cytometric profiles and histological pictures are representations of repeated experiments.

RESULTS

Phenotype of effector CD8 T cells is highly dependent on IFN-γ expression

Effector OT-I and IFN-$\gamma^{-/-}$OT-I CD8 T cells, generated by stimulation with phorbol 12-myristate 13-acetate and ionomycin for 48 hours, expressed high levels of CD44, CD28, and CD223, low to intermediate level of CD122, but not CCR7 or CD103 (Fig 1, *A*). These surface molecules indicated an activated effector phenotype. OT-I CD8 T cells exhibited a Tc1-like phenotype, expressing high levels of T-bet (11-fold increase) and IFN-γ production, although they also upregulated RORγt expression (6.3-fold increase) and produced some IL-17. In contrast, IFN-$\gamma^{-/-}$OT-I CD8 T cells exhibited a Tc2/Tc17 phenotype with the upregulation of RORγt expression (8.9-fold increase) and predominantly produced IL-13 and IL-17 (Fig 1, *B* and *E*). No obvious increase was observed for the expressions of GATA-3 or Foxp3 (Fig 1, *B*). Interestingly, ^{51}Cr release assay revealed that IFN-$\gamma^{-/-}$OT-I CD8 T cells were poorer killers both when naive and activated as compared with OT-I CD8 T cells (Fig 1, *C* and *D*). The difference was further confirmed by using CD107α degranulation assay (see Fig E1 in this article's Online Repository at www.jacionline.org). These data show that because of their lack of IFN-γ, effector IFN-$\gamma^{-/-}$OT-I CD8 T cells differ significantly in their phenotype from OT-I CD8 T cells.

Effector CD8 T cells are efficiently recruited into the lungs of sensitized mice

Transfer of 3×10^6 OT-I or IFN-$\gamma^{-/-}$OT-I CD8 T cells into OVA-sensitized mice was carried out prior to 3 daily OVA intranasal challenges (Fig 1, *F*). OVA-specific CD8 T cells in the lung parenchyma were detected by using H2K^b/SIINFEKL tetramer. Significant increase (4%) of tetramer-positive cells was detected in OVA-immunized and challenged mice (Fig 2, *A* and *B*), which increased 7-fold following the transfer of either effector OT-I or IFN-$\gamma^{-/-}$OT-I CD8 T cells (Fig 2, *A* and *B*). Both OT-I and IFN-$\gamma^{-/-}$OT-I CD8 T cells proliferated at a similar rate both *in vitro* (Fig 2, *C*) and *in vivo* (Fig 2, *D*). Together, these data demonstrate that both types of cells are efficiently recruited to the lung parenchyma and continue to proliferate, regardless of IFN-γ.

Effector OT-I CD8 T cells inhibit and IFN-$\gamma^{-/-}$OT-I CD8 T cells exacerbate allergic inflammation

The analysis of BAL cytokine levels revealed that OT-I CD8 T cells induced more IFN-γ production while IFN-$\gamma^{-/-}$OT-I CD8 T cells slightly enhanced eotaxin production (Fig 3). To evaluate the ability of CD8 T cells with different phenotypes and different effects on the BAL environment to modulate airway inflammation, BAL eosinophils (Siglec-F$^+$CD11c$^-$), neutrophils (Ly-6G$^+$CD11c$^-$), T lymphocytes (Siglec-F$^-$Ly-6G$^-$CD11c$^-$CD3$^+$), and alveolar macrophages (Siglec-F$^+$CD11c$^+$Ly-6G$^-$) were identified by flow cytometry. Transfer of effector OT-I CD8 T cells reduced

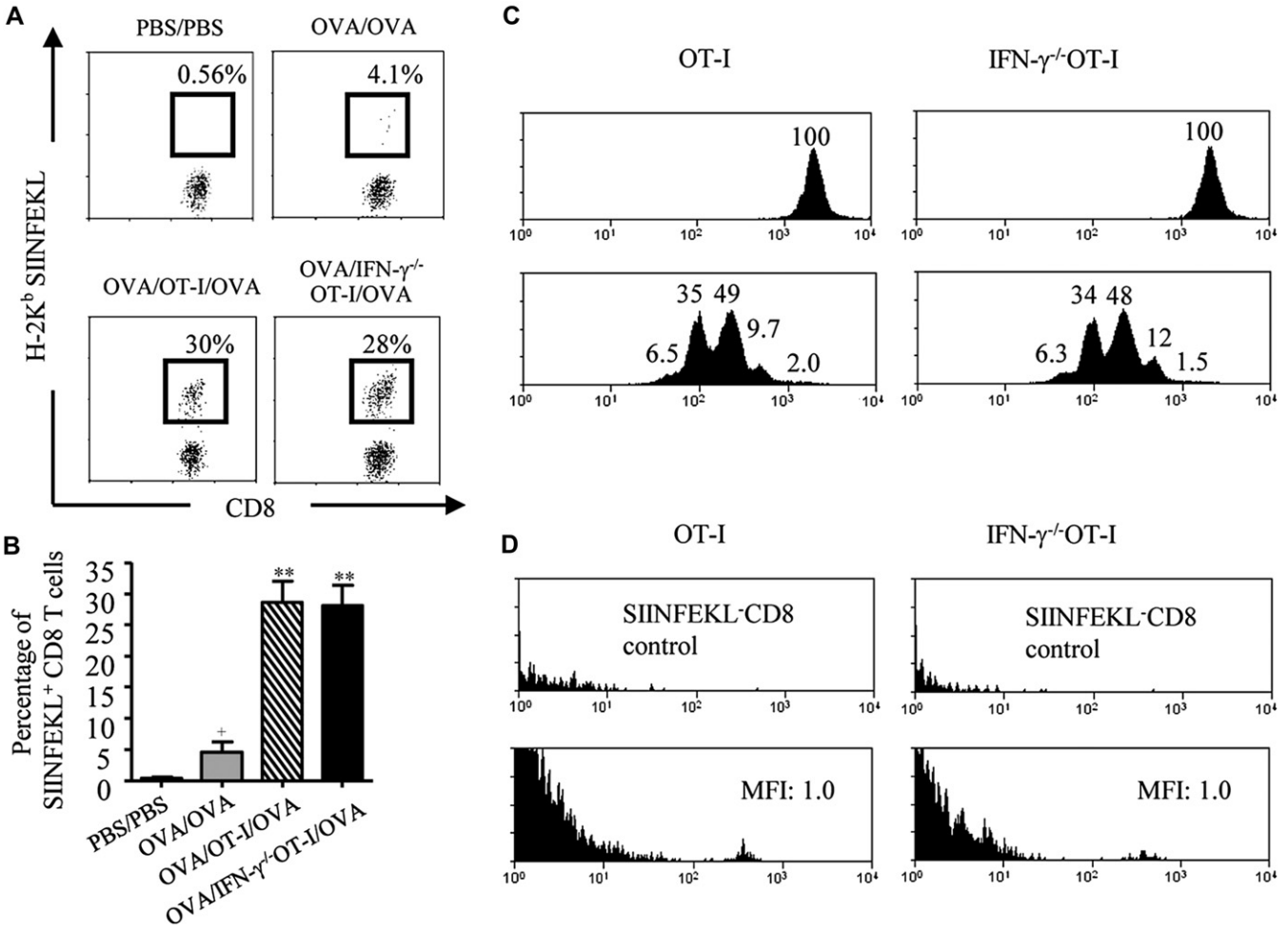

FIG 2. Recruitment of OVA-specific CD8 T cells in the lung parenchyma in response to CD8 T-cell transfer. 3 $\times 10^6$ effector OT-I or IFN-$\gamma^{-/-}$OT-I CD8 T cells were transferred to OVA-sensitized mice as described in Fig 1, *F*. **A,** OVA-specific CD8 T cells in the lung identified by fluorescent-labeled SIINFEKL tetramer. **B,** Quantitative analysis of OVA-specific CD8 T cells in the lung. **C,** *In vitro* proliferation of CD8 T cell after 64-hour phorbol 12-myristate 13-acetate and ionomycin activation. **D,** *In vivo* proliferation of CD8 T cells that were labeled prior to adoptive transfer. n = 10; $^{+}P < .05$ (compared with PBS/PBS mice) and $^{**}P < .01$ (compared with OVA/OVA mice). *MFI,* Mean fluorescence intensity.

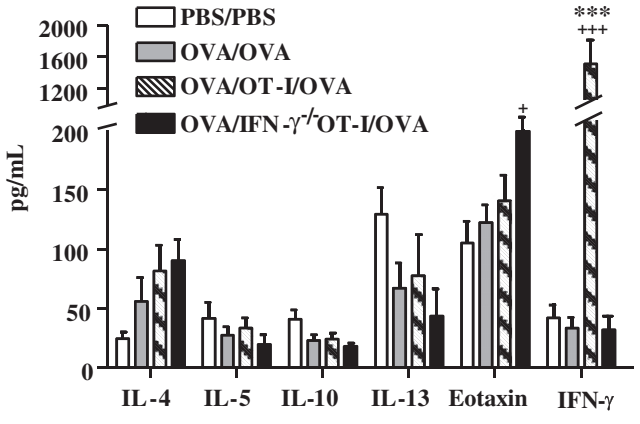

FIG 3. Effect on BAL cytokine production in response to CD8 T-cell transfer. Sensitized mice were adoptively transferred with CD8 T cells and challenged 3 times before sacrifice. Cytokine levels in the BAL fluid were measured by ELISA. n = 10; $^{+}P < .05$, $^{+++}P < .001$ (compared with PBS/PBS mice), $^{***}P < .001$ (compared with OVA/OVA mice).

eosinophilia (Fig 4, *A*, upper panel, and *B*) while mice that received IFN-$\gamma^{-/-}$OT-I CD8 T cells had higher numbers of eosinophils. Neutrophil infiltration was increased in mice that received CD8 T cells regardless of their ability to produce IFN-γ (Fig 4, *B*). Increased numbers of lymphocytes were most likely the result of the recruitment of transferred CD8 T cells. Collectively, these data demonstrate that the introduction of OT-I CD8 T cells into the allergic lung attenuates eosinophilia while potentiating neutrophilia and lymphocyte infiltration. In the absence of IFN-γ, IFN-$\gamma^{-/-}$OT-I CD8 T cells not only induce neutrophilia and lymphocyte infiltration but also exacerbate rather than attenuate eosinophilia.

Effector OT-I but not IFN-$\gamma^{-/-}$OT-I CD8 T cells attenuate goblet cell mucus production

Mucus production by goblet cells was assessed by periodic acid staining of lung sections. Mucus production was significantly inhibited in response to OT-I but not IFN-$\gamma^{-/-}$OT-I CD8 T-cell transfer (Fig 5, *A*). To assess the lung function of treated mice,

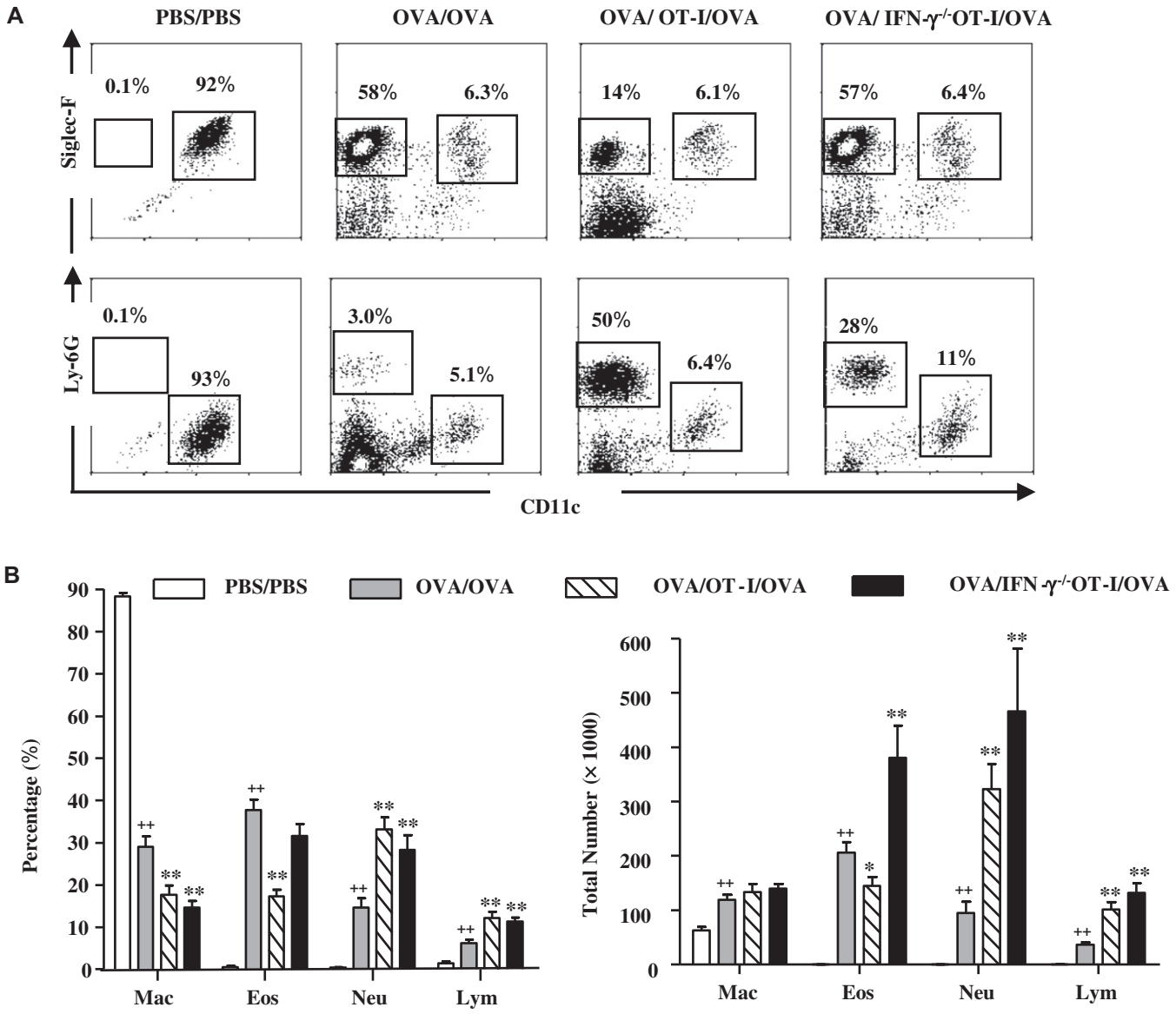

FIG 4. Altered cell infiltration in the airway in response to CD8 T-cell transfer. Sensitized mice were adoptively transferred with CD8 T cells and challenged 3 times before sacrifice. **A,** Representative fluorescence-activated cell sorting plots shown for the percentages of eosinophils *(upper panel)* and neutrophils *(lower panel)*. **B,** Quantitative analysis of BAL cells, both in percentage *(left)* and in total cell number *(right)*. n = 10; $^{++}P < .01$ (compared with PBS/PBS mice), $^{*}P < .05$, $^{**}P < .01$ (compared with OVA/OVA mice). *Eos,* Eosinophils; *Lym,* lymphocytes; *Mac,* macrophages; *Neu,* neutrophils.

AHR was measured at increasing doses of methacholine. No significant difference was found following the transfer of either OT-I or IFN-$\gamma^{-/-}$OT-I CD8 T cells compared with OVA-immunized and challenged mice (Fig 5, *B*). So, while airway inflammation and mucus secretion are inhibited by OT-I CD8 T cells, there is no evidence that these cells, transferred at the elicitation phase, could modify lung function.

Effector OT-I CD8 T cells condition DCs for T_H1 differentiation

Since the inhibition of eosinophilia by CD8 T cells was shown to be highly dependent on IFN-γ production and their Tc1 phenotype, we then looked into the underlying linking mechanisms. First, to determine whether the inhibition effect was dependent on antigen specificity, cell infiltration following the transfer of CD8 T cells from wild-type CD45.1^{+} mice and OT-I mice was compared. Without antigen specificity and potential cell-cell interaction, the transferred CD45.1^{+} CD8 T cells were less efficiently recruited to the lung (Fig 6, *A*) and failed to inhibit eosinophilia (Fig 6, *B*). This indicated that cognate interaction with OVA-specific CD8 T cells is important and led us to examine the effect of CD8 T-cell transfer on lung DCs. The numbers of both CD11b^{+}CD103^{-} and CD11b^{-}CD103^{+} DCs in the lung were increased in response to CD8 T-cell transfer and was independent of their ability to produce IFN-γ (Fig 7, *A*). CD11b^{+}CD103^{-} became the dominant DC subtype in the lungs once the mice were exposed to OVA (Fig 7, *A*). *Ex vivo* culture of

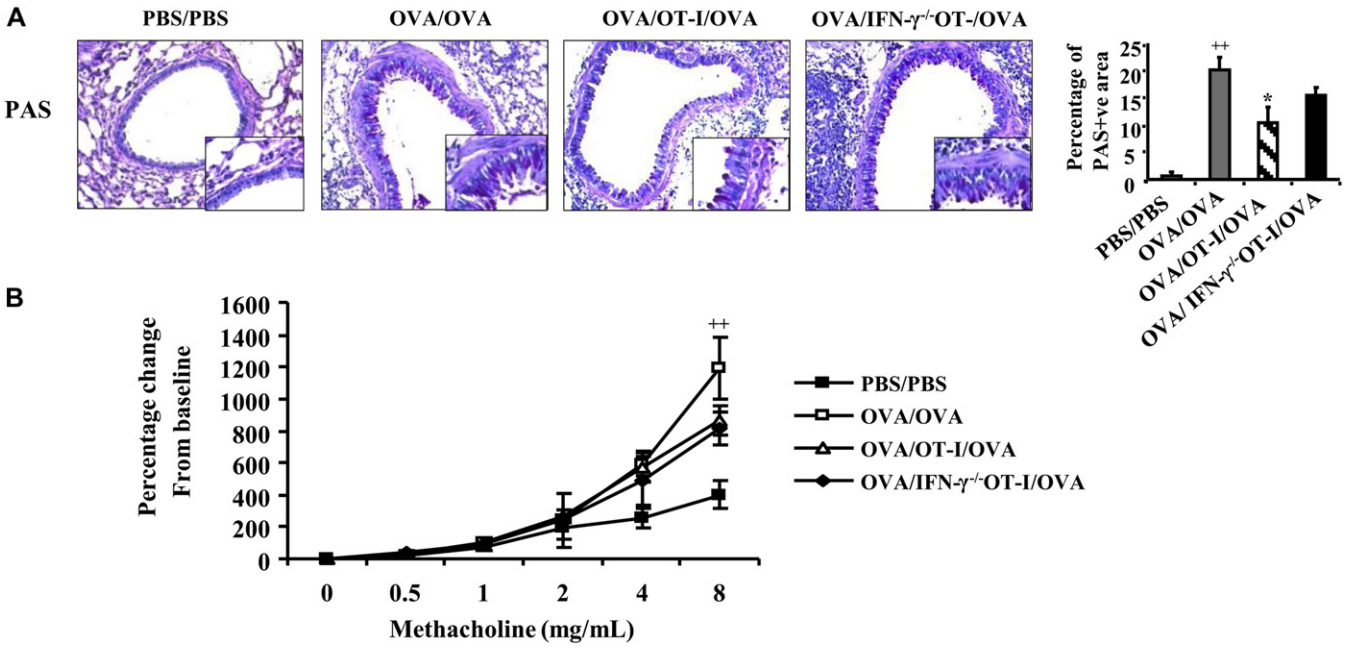

FIG 5. Effect on mucus production and lung function in response to CD8 T-cell transfer. Sensitized mice were adoptively transferred with CD8 T cells and challenged 3 times before sacrifice. **A,** Periodic acid staining (PAS) of lung tissue sections. n = 3. **B,** Airway resistance in response to increasing concentrations of nebulized methacholine (0.5-8.0 mg/mL). Results were expressed as percentages of respective basal values in response to PBS. n = 6; $^{++}P < .01$ (compared with control) and $*P < .05$ (compared with OVA/OVA mice).

these cells with OT-II CD4 T cells or antigen-experienced lung CD4 T cells polarized or potentiated the T cells for IFN-γ production, respectively, while those from mice that received IFN-$\gamma^{-/-}$OT-I CD8 failed to do so (Fig 7, *B* and *C*). $CD11b^{-}CD103^{+}$ DCs, on the other hand, had a weaker effect on IFN-γ production and no significant difference could be found among groups (Fig 7, *D* and *E*). Collectively, these data demonstrate that antigen-specific CD8 T cells and their IFN-γ production result in a functional change in DCs within the allergic lung, increasing their ability to skew toward a T_H1 environment.

Intrinsically altered effector IFN-$\gamma^{-/-}$OT-I CD8 T cells induce asthma-like pathology in nonimmunized mice

To determine whether the different regulatory effects we observed were dependent on IFN-γ alone or more on the intrinsic differences induced by the silencing of IFN-γ, we employed IFN-γ–neutralizing antibody. We found that blockage of IFN-γ attenuated the inhibitory effect of CD8 T cells but did not potentiate inflammation (see Fig E2 in this article's Online Repository at www.jacionline.org), indicating that the changed phenotype rather than the production of IFN-γ might be responsible for the promotion of inflammation by IFN-$\gamma^{-/-}$OT-I CD8 T cells.

To find out whether IFN-$\gamma^{-/-}$OT-I but not OT-T CD8 T cells could induce inflammation, effector CD8 T cells were transferred into unimmunized mice, followed by 3 intranasal challenges. Compared with OT-I CD8 T cells, IFN-$\gamma^{-/-}$OT-I CD8 T cells were recruited into the lung more efficiently (Fig 8, *A*) and induced both significant eosinophilia and neutrophilia whereas OT-I CD8 T cells did not cause significant infiltration (Fig 8, *B*). Neither neutralization of IFN-γ (see Fig E3, *A* and *B* in this article's Online Repository at www.jacionline.org) nor increasing the number of transferred cells (see Fig E3, *C* and *D*) could induce the infiltration of granulocytes in OT-I CD8 T-cell transferred mice, indicating that the proinflammatory nature of the effector IFN-$\gamma^{-/-}$OT-I CD8 T cells was an intrinsic property, which OT-I CD8 T cells do not possess.

DISCUSSION

The role of CD8 T cells in host defense against viruses and intracellular bacteria is well established,[43-46] but their function in allergic disease is less clear. In this study, we demonstrate that a robust Tc1-biased phenotype and IFN-γ production are essential for CD8 T-cell–mediated attenuation of eosinophilia and goblet cell hyperplasia. In contrast, effector CD8 T cells from IFN-$\gamma^{-/-}$OT-I mice, which display a Tc2/Tc17 phenotype and reduced cytotoxicity, fail to ameliorate these 2 hallmarks of allergic inflammation but rather potentiate eosinophil and neutrophil infiltration. The adoptive transfer of IFN-$\gamma^{-/-}$OT-I CD8 T cells in the absence of prior allergen sensitization induces a profound asthma-like inflammation in response to allergen challenge, further highlighting the importance of IFN-γ in CD8-mediated regulation of allergy. We further demonstrate that both types of effector CD8 T cells increase the number of $CD11b^{+}CD103^{-}$ lung DCs but only those from OT-I but not IFN-$\gamma^{-/-}$OT-I mice could induce IFN-γ production by CD4 T cells *ex vivo*, indicating that IFN-γ–producing CD8 T cells alter DC function, creating a more T_H1-permissive environment.

As intervention therapy for asthma is likely to take place after patients have become allergic, we introduced CD8 T cells into mice after sensitization but prior to allergen challenges. Consistent with previous reports, we observed that CD8 T cells were capable of attenuating pulmonary inflammation.[13,14,28-31] However, previous studies by Miyahara et al[34] and Taube et al[35]

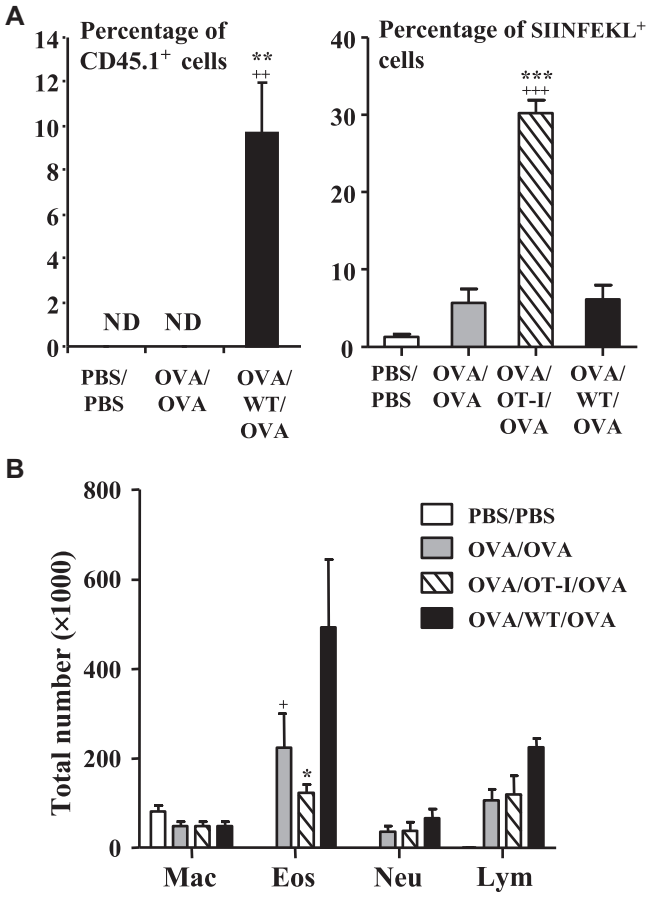

FIG 6. Role of non–antigen-specific CD8 T cells on allergic responses. Sensitized mice were adoptively transferred with CD8 T cells and challenged 3 times before sacrifice. **A,** Recruitment of non–antigen-specific CD8 T cells ($CD45.1^+CD45.2^-$ cells) into the lung. **B,** Quantitative analysis of cell infiltration in the BAL fluid. n = 3; $^+P < .05$, $^{++}P < .01$, $^{+++}P < 0.001$ (compared with PBS/PBS mice), $*P < .05$, $**P < .01$, $***P < .001$ (compared with OVA/OVA mice). *Eos,* eosophinils; *lym,* lymphocytes; *mac,* macrophages; *ND,* not detectable; *neu,* neutrophils; *WT,* wild type.

observed increased inflammation, attributing these changes to proinflammatory effector memory rather than central memory T cells. This conflict could partly be explained by the different approaches utilized. In these previous studies, endogenous CD8 T cells were depleted during the sensitization phase as opposed to our study in which CD8 T cells were transferred to mice in which an endogenous CD8 response was already established. Our study therefore focuses more on the ability of CD8 T cells to influence an ongoing T_H2 inflammation as well as the ability of these cells to alter T_H2 cell polarization.

To focus our study on the role of IFN-γ produced by CD8 T cells, we generated IFN-$\gamma^{-/-}$OT-I mice. Effector IFN-$\gamma^{-/-}$OT-I cells expressed similar surface markers to effector OT-I CD8 T cells, but exhibited reduced T-bet expression, increased production of IL-13 and IL-17, and weaker cytotoxic activity. Consistent with previous findings,[13,14,28-31] our data showed that the absence of IFN-γ ameliorated CD8 T-cell–mediated inhibition of eosinophilia and goblet cell hyperplasia. Indeed IFN-$\gamma^{-/-}$OT-I CD8 T cells greatly increased eosinophilia and neutrophilia, resulting in exaggerated T_H2 and T_H1 responses, unlike IFN-γ neutralization treatment for mice with OT-I CD8 T-cell transfer, which merely restored the level of inflammation. This profound increase in inflammation led us to examine the role of adoptively transferred IFN-$\gamma^{-/-}$OT-I CD8 T cells in nonsensitized hosts. Transfer of IFN-$\gamma^{-/-}$OT-I CD8 T cells into naive C57BL6 mice followed by OVA challenges resulted in an asthma-like pathology that included both eosinophilia and neutrophilia. The cytokine profile of effector IFN-$\gamma^{-/-}$OT-I CD8 T cells indicated that the induction of inflammation was probably dependent on IL-13 and IL-17. IL-13 has been shown to play an important role in stimulating eosinophil infiltration via various mechanisms: IL-5 and eotaxin expression[47]; promotion of eosinophil entry into the lung[48]; and upregulation of various chemotactic factors.[49] Indeed, we observed an elevation of eotaxin in BAL of OVA-sensitized mice that received IFN-$\gamma^{-/-}$OT-I CD8 T cells (Fig 3, *A*). Moreover, there is an emerging body of evidence that IL-17 contributes to eosinophil and neutrophil infiltration through the induction of a range of proinflammatory cytokines, chemokines, and adhesion molecules.[50-54] Our data are consistent with these findings, demonstrating that in the absence of IFN-γ, the increased production of IL-17 from IFN-$\gamma^{-/-}$OT-I CD8 T cells might have contributed to the promotion of cell infiltration, especially neutrophils. It is still unclear how reduced cytotoxicity could be involved in the regulation of inflammation.

While the reduction of cell infiltration and mucus production by CD8 T cells was evident, AHR did not seem to be affected. Some previous studies have shown that some features of asthma such as eosinophilia and airway remodeling were also shared by eosinophilic bronchitis while AHR was exclusive to asthma,[55-57] suggesting that the participation of different factors may contribute to different features of asthma.[58] Thus, it is possible that the inhibition of AHR might also require the coordination of other factors in concert with CD8 T cells. Moreover, although modulating inflammation was found to have an effect on AHR, different cell types were involved, including eosinophils, neutrophils, and mast cells.[59-61] In our study, neutrophils were increased in response to CD8 T-cells transfer, which may counter the inhibitory effect on AHR by reduced eosinophilia.

Although the involvement of DCs in asthma was previously described,[62,63] not much detail was revealed on the role of different subsets. Lung DCs can be broadly divided into $CD11b^-CD103^+$ and $CD11b^+CD103^-$ subsets, of which $CD11b^+CD103^-$ predominates in OVA-induced inflammation. $CD11b^+CD103^-$ DCs from effector OT-I, but not IFN-$\gamma^{-/-}$OT-I CD8 T cell, recipient mice are able to skew OT-II and lung parenchymal CD4 T cells for IFN-γ production, while $CD11b^-CD103^+$ DCs are much weaker inducers probably because of their less efficient antigen presentation via the MHC-II pathway.[38,64] These findings indicate that CD8 T-cell–derived IFN-γ is essential to alter DC cell function, favoring CD4 T-cell differentiation to the T_H1 phenotype even within a predominantly T_H2 lung and demonstrate a novel potential mechanism through which CD8 T cells attenuate eosinophilia.

In summary, we have shown that OVA-specific effector CD8 T cells are skewed toward Tc1, and when transferred to sensitized mice at the elicitation phase of an allergic lung response, are able to skew toward a type I response and inhibit type II inflammation via IFN-γ production and DC T_H1 priming in an antigen-specific manner. Although not AHR modifying, the ability of these Tc1-biased cells in modulating inflammation and mucus production suggests that they could be used in therapeutic approaches aiming to alleviate asthma symptoms. In the absence of IFN-γ, CD8 T cells become functionally biased toward Tc2/Tc17 upon activation, producing IL-13 and IL-17, exhibit weaker cytotoxicity, and exacerbate both type I and type II inflammation. This study

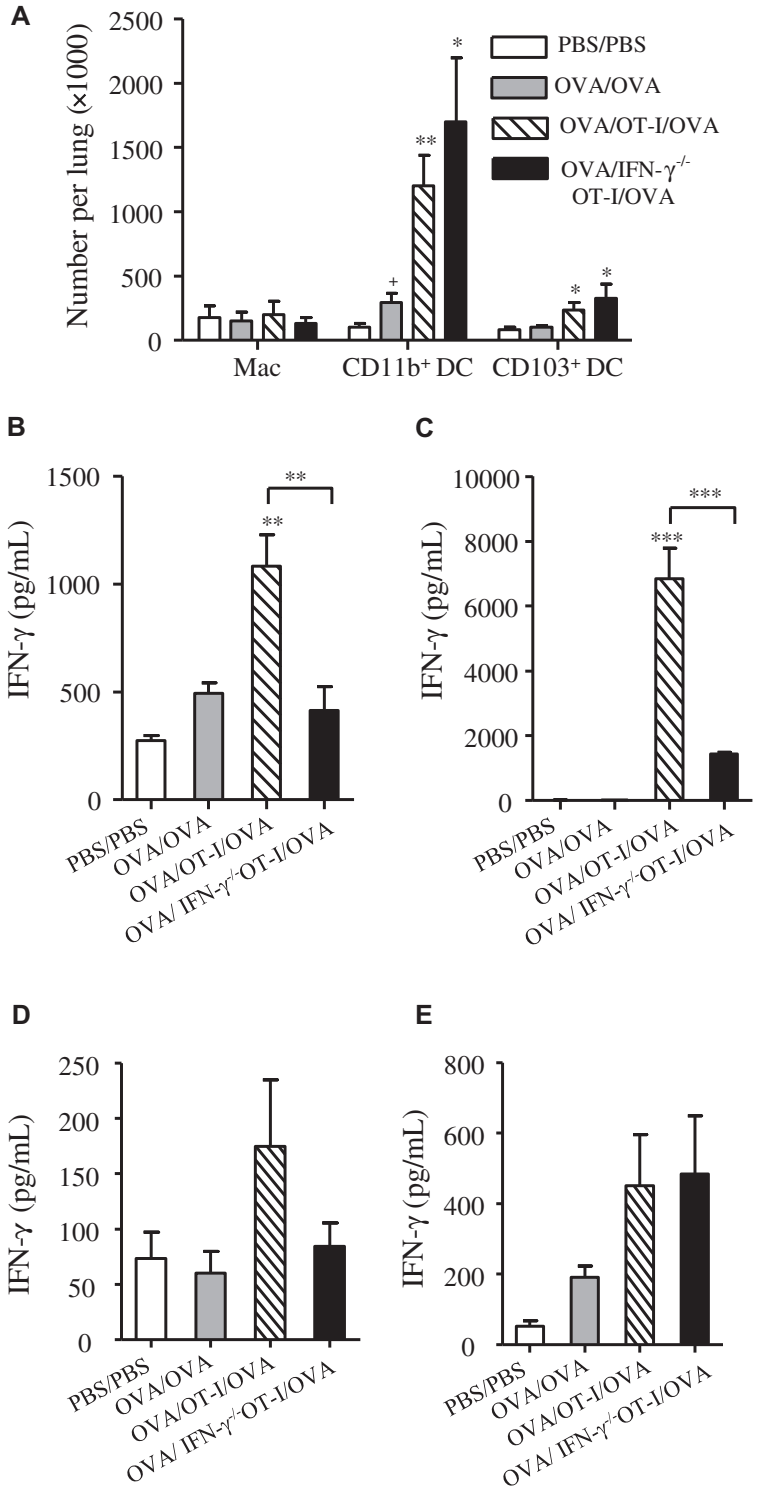

FIG 7. Effect of lung parenchymal DCs on CD4 T-cell priming. Sensitized mice were adoptively transferred with CD8 T cells and challenged 3 times before sacrifice. **A,** Numbers of different antigen-presenting cells in the lung parenchyma. **B,** Production of IFN-γ by naive OT-II CD4 T cells after coculture with sorted lung $CD11b^+CD103^-$ DCs from different treatment groups. **C,** Production of IFN-γ by lung parenchymal CD4 T cells isolated from respectively treated mice after coculture with sorted lung $CD11b^+CD103^-$ DCs correspondingly. **D,** Production of IFN-γ by naive OT-II CD4 T cells after coculture with sorted lung $CD11b^-CD103^+$ DCs from different treatment groups. **E,** Production of IFN-γ by lung parenchymal CD4 T cells isolated from respectively treated mice after coculture with sorted lung $CD11b^-CD103^+$ DCs correspondingly. n = 3; ^+P <.05, $*P$ < .05, $**P$ < .01, $***P$ < .001 (compared with OVA/OVA mice). *Mac,* Macrophages.

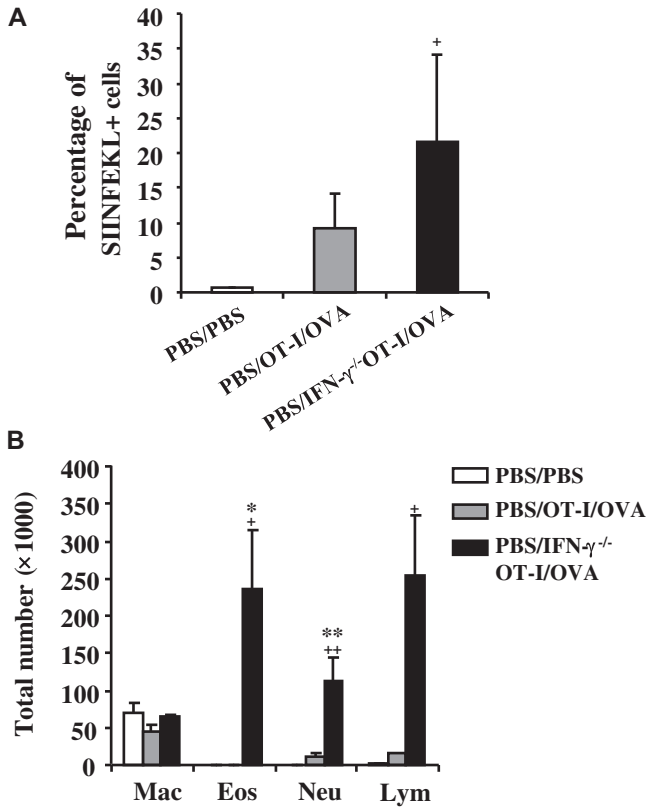

FIG 8. Infiltration of cells into the airway following adoptive transfer of CD8 T cells in nonimmunized mice. Naive mice were adoptively transferred with activated CD8 T cells followed by 3 intranasal challenges. Mice were sacrificed 1 day after the last challenge. **A,** Percentage of OVA-specific CD8 T cells in the lung, stained with SIINFEKL tetramer. **B,** Quantitative analysis of cell infiltration in the BAL fluid. n = 3; $^{+}P < .05$, $^{++}P < .01$ (compared with PBS control) and $*P < .05$, $**P < .01$ (compared with PBS/OT-I/OVA mice). *Eos,* Eosophinils; *lym*, lymphocytes; *mac,* macrophages; *neu,* neutrophils.

also demonstrates the utility of IFN-$\gamma^{-/-}$OT-I mice as a useful tool to elucidate the role of IFN-γ and Tc2/Tc17 phenotype in the study of CD8 T cells in asthma models.

We thank Lew Fei Chuin and Dr Paul Edward Hutchinson for assistance with cell sorting, Too Chien Tei for antibody purification, and Dr Paul A. MacAry for suggestions on the manuscript.

Key messages

- **Effector CD8 T cells are able to modulate allergic responses at the effector phase.**
- **CD8-primed CD11b^{+}CD103^{-} DCs are capable of skewing the differentiation of CD4 T cells toward a T_H1 phenotype.**
- **IFN-$\gamma^{-/-}$OT-I CD8 T cells have decreased cytotoxic capacity, display a Tc2-/Tc17-biased phenotype, and induce as well as exacerbate eosinophilic and neutrophilic infiltration.**
- **IFN-$\gamma^{-/-}$OT-I mice serve as a useful tool to study the involvement of IFN-γ in the regulatory role of antigen-specific CD8 T cells.**

REFERENCES

1. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. Annu Rev Immunol 2004;22:789-815.
2. Busse W, Coffman R, Gelfand E, Kay A, Rosenwasser L. Mechanisms of persistent airway inflammation in asthma: a role for T cells and T-cell products. Am J Respir Crit Care Med 1995;152:388-93.
3. Lewis C, Aronow B, Hutton J, Santeliz J, Dienger K, Herman N, et al. Unique and overlapping gene expression patterns driven by IL-4 and IL-13 in the mouse lung. J Allergy Clin Immunol 2009;123:795-804.e8.
4. McLane MP, Haczku A, van de Rijn M, Weiss C, Ferrante V, MacDonald D, et al. Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. Am J Respir Cell Mol Biol 1998;19:713-20.
5. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258-61.
6. Webb DC, McKenzie AN, Koskinen AM, Yang M, Mattes J, Foster PS. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. J Immunol 2000;165:108-13.
7. Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. J Immunol 2010;184:1663-74.
8. Tada T, Okumura K, Taniguchi M. Regulation of homocytotropic antibody formation in the rat, VII: carrier functions in the anti-hapten homocytotropic antibody response. J Immunol 1972;108:1535-41.
9. Okumura K, Takemori T, Tokuhisa T, Tada T. Specific enrichment of the suppressor T cell bearing I-J determinants: parallel functional and serological characterizations. J Exp Med 1977;146:1234-45.
10. Diaz-Sanchez D, Lee TH, Kemeny DM. Ricin enhances IgE responses by inhibiting a subpopulation of early-activated IgE regulatory CD8+ T cells. Immunology 1993;78:226-36.
11. Sedgwick JD, Holt PG. Suppression of IgE responses in inbred rats by repeated respiratory tract exposure to antigen: responder phenotype influences isotype specificity of induced tolerance. Eur J Immunol 1984;14:893-7.
12. Renz H, Lack G, Saloga J, Schwinzer R, Bradley K, Loader J, et al. Inhibition of IgE production and normalization of airways responsiveness by sensitized CD8 T cells in a mouse model of allergen-induced sensitization. J Immunol 1994;152:351-60.
13. Ishimitsu R, Nishimura H, Yajima T, Watase T, Kawauchi H, Yoshikai Y. Overexpression of IL-15 in vivo enhances Tc1 response, which inhibits allergic inflammation in a murine model of asthma. J Immunol 2001;166:1991-2001.
14. Wells J, Cowled C, Giorgini A, Kemeny D, Noble A. Regulation of allergic airway inflammation by class I-restricted allergen presentation and CD8 T-cell infiltration. J Allergy Clin Immunol 2007;119:226-34.
15. Joetham A, Okamoto M, Takeda K, Schedel M, Ohnishi H, Dakhama A, et al. CD8 regulates T regulatory cell production of IL-6 and maintains their suppressive phenotype in allergic lung disease. J Immunol 2011;186:113-20.
16. Hamelmann E, Oshiba A, Paluh J, Bradley K, Loader J, Potter T, et al. Requirement for CD8+ T cells in the development of airway hyperresponsiveness in a marine model of airway sensitization. J Exp Med 1996;183:1719-29.
17. Miyahara N, Takeda K, Kodama T, Joetham A, Taube C, Park J, et al. Contribution of antigen-primed CD8+ T cells to the development of airway hyperresponsiveness and inflammation is associated with IL-13. J Immunol 2004;172:2549-58.
18. Holmes BJ, Diaz-Sanchez D, Lawrence RA, Bell EB, Maizels RM, Kemeny DM. The contrasting effects of CD8+ T cells on primary, established and *Nippostrongylus brasiliensis*-induced IgE responses. Immunology 1996;88:252-60.
19. Seder R, Boulay J, Finkelman F, Barbier S, Ben-Sasson S, Le Gros G, et al. CD8+ T cells can be primed in vitro to produce IL-4. J Immunol 1992;148:1652-6.
20. Croft M, Carter L, Swain S, Dutton R. Generation of polarized antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. J Exp Med 1994;180:1715-28.
21. Noble A, Macary P, Kemeny D. IFN-gamma and IL-4 regulate the growth and differentiation of CD8+ T cells into subpopulations with distinct cytokine profiles. J Immunol 1995;155:2928-37.
22. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. J Immunol 1997;158:3539-44.
23. Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma. Am J Respir Crit Care Med 2005;171:224-30.
24. Meissner N, Kussebi F, Jung T, Ratti H, Baumgarten C, Werfel T, et al. A subset of CD8+ T cells from allergic patients produce IL-4 and stimulate IgE production in vitro. Clin Exp Allergy 1997;27:1402-11.

25. Stock P, Kallinich T, Akbari O, Quarcoo D, Gerhold K, Wahn U, et al. CD8(+) T cells regulate immune responses in a murine model of allergen-induced sensitization and airway inflammation. Eur J Immunol 2004;34:1817-27.
26. Schaller M, Lundy S, Huffnagle G, Lukacs N. CD8+ T cell contributions to allergen induced pulmonary inflammation and airway hyperreactivity. Eur J Immunol 2005;35:2061-70.
27. Sawicka E, Noble A, Walker C, Kemeny D. Tc2 cells respond to soluble antigen in the respiratory tract and induce lung eosinophilia and bronchial hyperresponsiveness. Eur J Immunol 2004;34:2599-608.
28. Suzuki M, Taha R, Ihaku D, Hamid Q, Martin J. CD8+ T cells modulate late allergic airway responses in Brown Norway rats. J Immunol 1999;163:5574-81.
29. Suzuki M, Maghni K, Molet S, Shimbara A, Hamid Q, Martin J. IFN-gamma secretion by CD8T cells inhibits allergen-induced airway eosinophilia but not late airway responses. J Allergy Clin Immunol 2002;109:803-9.
30. Huang T, MacAry P, Kemeny D, Chung K. Effect of CD8+ T-cell depletion on bronchial hyper-responsiveness and inflammation in sensitized and allergen-exposed Brown-Norway rats. Immunology 1999;96:416-23.
31. Takeda K, Dow S, Miyahara N, Kodama T, Koya T, Taube C, et al. Vaccine-induced CD8+ T cell-dependent suppression of airway hyperresponsiveness and inflammation. J Immunol 2009;183:181-90.
32. Intlekofer AM, Banerjee A, Takemoto N, Gordon SM, Dejong CS, Shin H, et al. Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin. Science 2008;321:408-11.
33. Yen HR, Harris TJ, Wada S, Grosso JF, Getnet D, Goldberg MV, et al. Tc17 CD8 T cells: functional plasticity and subset diversity. J Immunol 2009;183:7161-8.
34. Miyahara N, Swanson B, Takeda K, Taube C, Miyahara S, Kodama T, et al. Effector CD8+ T cells mediate inflammation and airway hyper-responsiveness. Nat Med 2004;10:865-9.
35. Taube C, Miyahara N, Ott V, Swanson B, Takeda K, Loader J, et al. The leukotriene B4 receptor (BLT1) is required for effector CD8+ T cell-mediated, mast cell-dependent airway hyperresponsiveness. J Immunol 2006;176:3157-64.
36. Wong K, Lew F, MacAry P, Kemeny D. CD40L-expressing CD8 T cells prime CD8alpha(+) DC for IL-12p70 production. Eur J Immunol 2008;38:2251-62.
37. Sung S, Fu S, Rose CJ, Gaskin F, Ju S, Beaty S. A major lung CD103 (alphaE)-beta7 integrin-positive epithelial dendritic cell population expressing Langerin and tight junction proteins. J Immunol 2006;176:2161-72.
38. del Rio M, Rodriguez-Barbosa J, Kremmer E, Förster R. CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells. J Immunol 2007;178:6861-6.
39. D'Andrea A, Rengaraju M, Valiante N, Chehimi J, Kubin M, Aste M, et al. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. J Exp Med 1992;176:1387-98.
40. Hsieh C, Macatonia S, Tripp C, Wolf S, O'Garra A, Murphy K. Development of TH1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. Science 1993;260:547-9.
41. Manetti R, Parronchi P, Giudizi M, Piccinni M, Maggi E, Trinchieri G, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 1993;177:1199-204.
42. Aguilar-Pimentel JA, Alessandrini F, Huster KM, Jakob T, Schulz H, Behrendt H, et al. Specific CD8 T cells in IgE-mediated allergy correlate with allergen dose and allergic phenotype. Am J Respir Crit Care Med 2010;181:7-16.
43. Uzonna JE, Joyce KL, Scott P. Low dose Leishmania major promotes a transient T helper cell type 2 response that is down-regulated by interferon gamma-producing CD8+ T cells. J Exp Med 2004;199:1559-66.
44. Sud D, Bigbee C, Flynn JL, Kirschner DE. Contribution of CD8+ T cells to control of *Mycobacterium tuberculosis* infection. J Immunol 2006;176:4296-314.
45. Peterson KE, Stromnes I, Messer R, Hasenkrug K, Chesebro B. Novel role of CD8(+) T cells and major histocompatibility complex class I genes in the generation of protective CD4(+) Th1 responses during retrovirus infection in mice. J Virol 2002;76:7942-8.
46. Heidema J, Lukens MV, van Maren WW, van Dijk ME, Otten HG, van Vught AJ, et al. CD8+ T cell responses in bronchoalveolar lavage fluid and peripheral blood mononuclear cells of infants with severe primary respiratory syncytial virus infections. J Immunol 2007;179:8410-7.
47. Pope SM, Brandt EB, Mishra A, Hogan SP, Zimmermann N, Matthaei KI, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. J Allergy Clin Immunol 2001;108:594-601.
48. Castilow EM, Meyerholz DK, Varga SM. IL-13 is required for eosinophil entry into the lung during respiratory syncytial virus vaccine-enhanced disease. J Immunol 2008;180:2376-84.
49. Wills-Karp M. Interleukin-13 in asthma pathogenesis. Immunol Rev 2004;202:175-90.
50. Wong C, Ho C, Ko F, Chan C, Ho A, Hui D, et al. Pro-inflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin Exp Immunol 2001;125:177-83.
51. Kawaguchi M, Kokubu F, Fujita J, Huang S, Hizawa N. Role of interleukin-17F in asthma. Inflamm Allergy Drug Targets 2009;8:383-9.
52. Cheung P, Wong C, Lam C. Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. J Immunol 2008;180:5625-35.
53. Moseley T, Haudenschild D, Rose L, Reddi A. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 2003;14:155-74.
54. Dragon S, Rahman M, Yang J, Unruh H, Halayko A, Gounni A. IL-17 enhances IL-1beta-mediated CXCL-8 release from human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2007;292:L1023-9.
55. Brightling C, Symon F, Birring S, Bradding P, Pavord I, Wardlaw A. TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis. J Allergy Clin Immunol 2002;110:899-905.
56. Brightling C, Bradding P, Symon F, Holgate S, Wardlaw A, Pavord I. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699-705.
57. Gibson P, Dolovich J, Denburg J, Ramsdale E, Hargreave F. Chronic cough: eosinophilic bronchitis without asthma. Lancet 1989;1:1346-8.
58. Robinson D. The role of the T cell in asthma. J Allergy Clin Immunol 2010;126:1081-91.
59. Gibson PG, Saltos N, Borgas T. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. J Allergy Clin Immunol 2000;105:752-9.
60. Berend N, Salome CM, King GG. Mechanisms of airway hyperresponsiveness in asthma. Respirology 2008;13:624-31.
61. Busse WW. The relationship of airway hyperresponsiveness and airway inflammation: airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest 2010;138:4S-10S.
62. van Rijt L, Jung S, Kleinjan A, Vos N, Willart M, Duez C, et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. J Exp Med 2005;201:981-91.
63. Thomas M, Noble A, Sawicka E, Askenase P, Kemeny D. CD8 T cells inhibit IgE via dendritic cell IL-12 induction that promotes Th1 T cell counter-regulation. J Immunol 2002;168:216-23.
64. Desch AN, Randolph GJ, Murphy K, Gautier EL, Kedl RM, Lahoud MH, et al. CD103+ pulmonary dendritic cells preferentially acquire and present apoptotic cell-associated antigen. J Exp Med 2011;208:1789-97.

METHODS

CD107α assay

EL4 cells were pulsed with 10 μg/mL of SIINFEKL peptide (Anaspec) for 30 minutes and used as target cells. Freshly isolated CD8 effector cells were cultured with target cells at the desired ratio (3:1) in the presence of 5 μg/mL of brefeldin A (BD Biosciences), 5 μg/mL of monesin (BD Biosciences), and 10 μg/mL of CD107α conjugated to antigen-presenting cell (eBioscience) for 6 hours. Cells were then harvested and stained with surface markers as per normal procedure. The accumulation of CD107α on the surface of CD8 T cells was analyzed using flow cytometry.

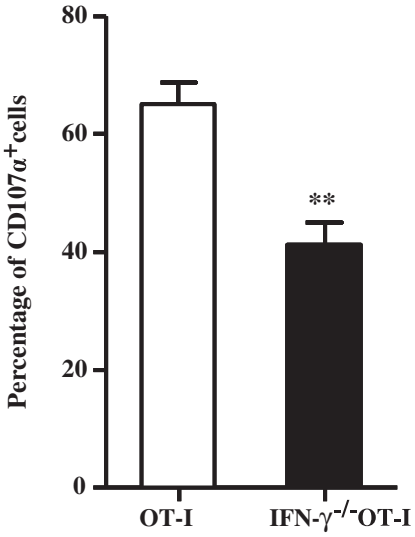

FIG E1. Degranulation of CD8 T cells detected by the accumulation of CD107α. Freshly isolated OT-I or IFN-$\gamma^{-/-}$OT-I CD8 T cells were cocultured with target cells EL4 murine lymphoblast cells for 6 hours in the presence of brefeldin A, monesin, and CD107α. Result was expressed as the percentage of CD107α^{+} CD8 T cells. n = 4; $^{**}P < .01$.

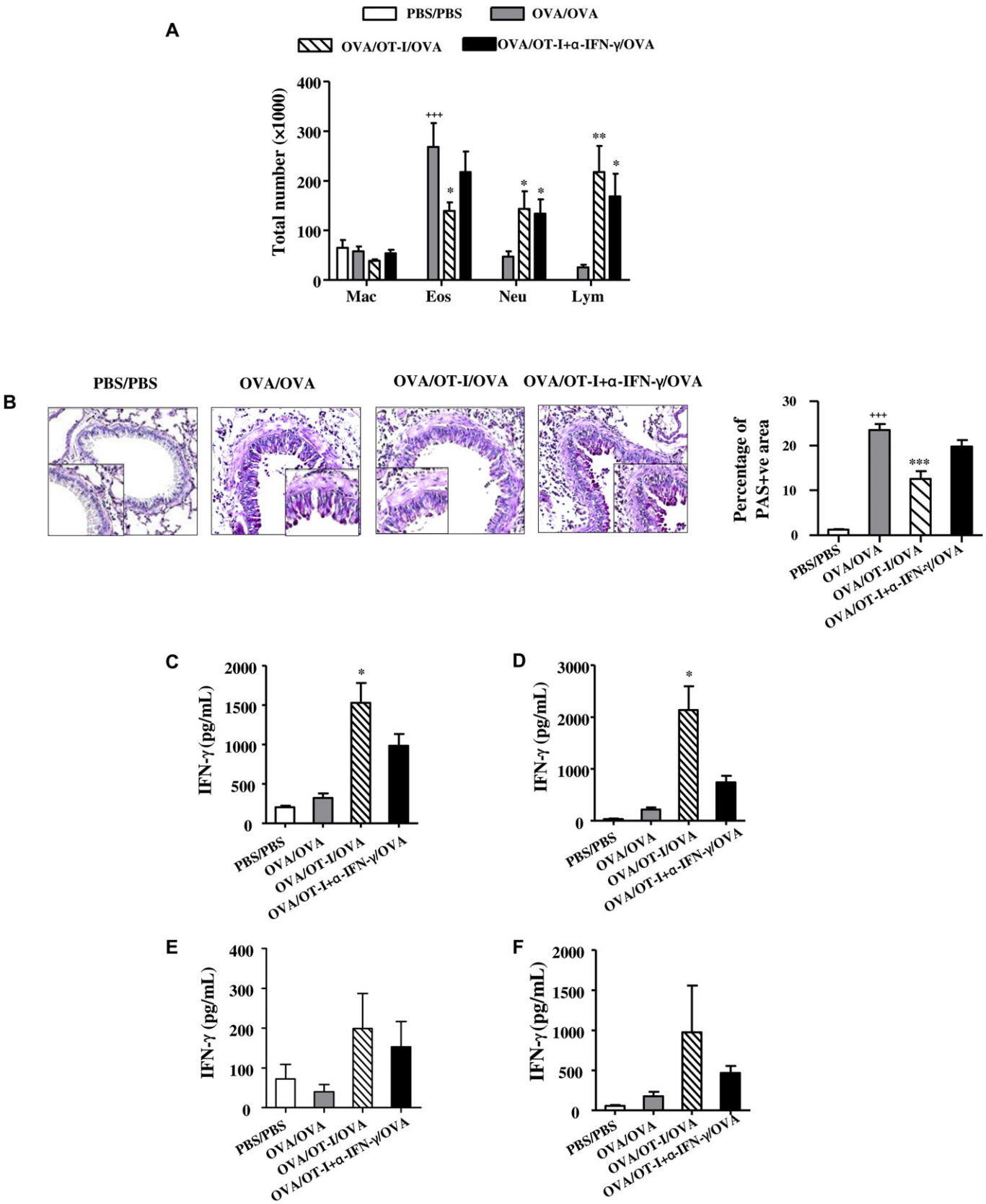

FIG E2. Effect of IFN-γ neutralization on CD8 T-cell–induced suppression of T_H2 responses. Sensitized mice were treated with α-IFN-γ antibody via intratracheal transfer before the adoptive transfer of activated OT-I CD8 T cells. Mice were then challenged with OVA for 3 consecutive days. **A,** Quantitative analysis of cell infiltration in the BAL fluid. **B,** Periodic acid staining *(PAS)* of lung tissue sections and statistical analysis.

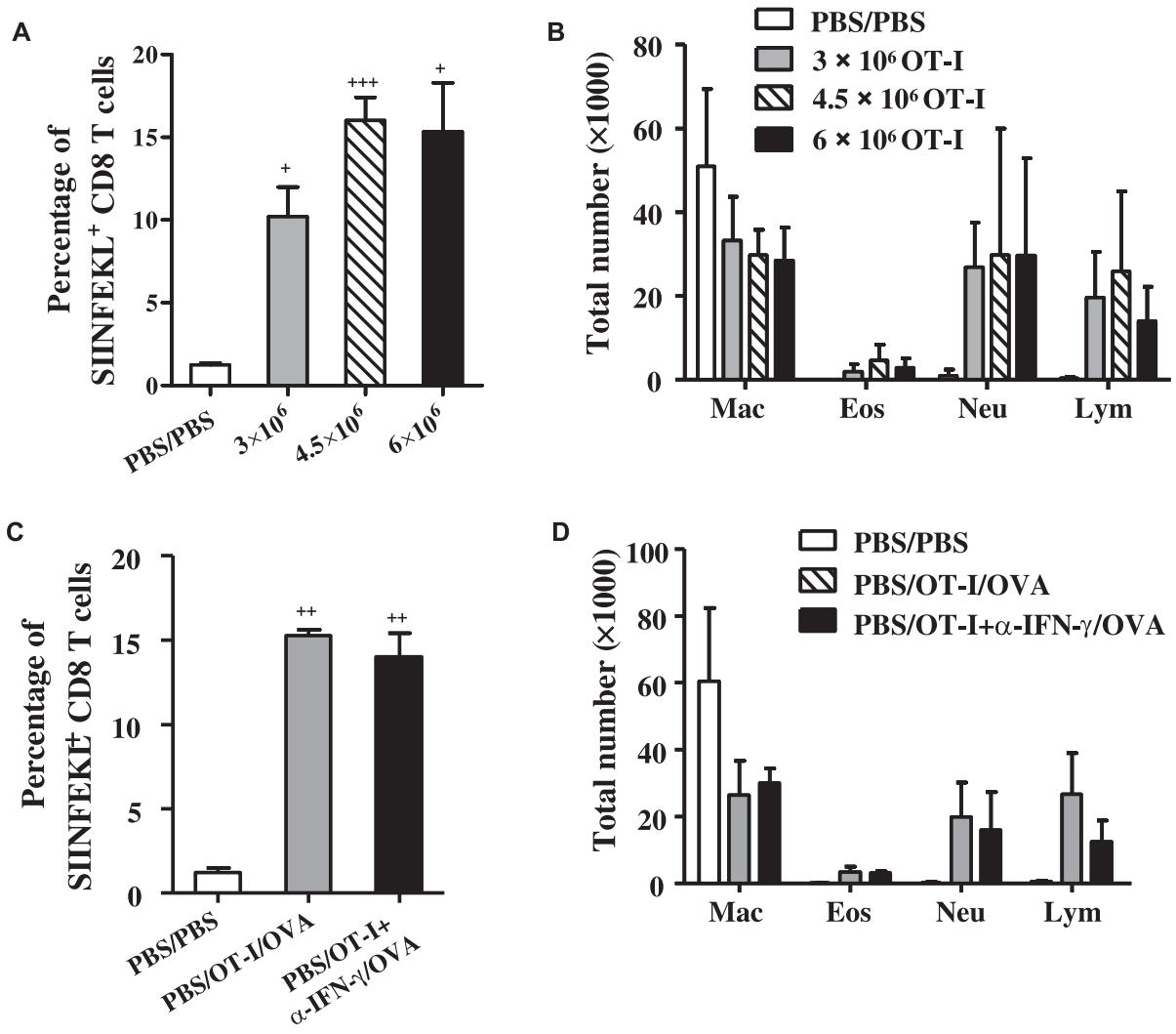

FIG E3. Effect of IFN-γ neutralization and number of CD8 T cells on cell infiltration into the airway following OT-I CD8 T-cell transfer and challenges in nonimmunized mice. Naive mice were adoptively transferred with different numbers of activated OT-I CD8 T cells followed by 3 intranasal challenges. Mice were sacrificed 1 day after the last challenge. **A,** Percentage of OVA-specific CD8 T cells in the lung, stained with SIINFEKL tetramer. **B,** Quantitative analysis of cell infiltration in the BAL fluid. For IFN-γ neutralization, α-IFN-γ antibody was given to mice right before the transfer of 3×10^6 CD8 T cells. **C,** Percentage of OVA-specific CD8 T cells in the lung, stained with SIINFEKL tetramer. **D,** Quantitative analysis of cell infiltration in the BAL fluid. n = 3; $^{+}P < .05$, $^{++}P < .01$, $^{+++}P < .001$ (compared with PBS/PBS mice). *Eos*, Eosophinils; *lym*, lymphocytes; *mac*, macrophages; *neu*, neutrophils.

C, Production of IFN-γ by naive OT-II CD4 T cells after coculture with sorted lung $CD11b^{+}CD103^{-}$ DCs from different treatment groups. **D,** Production of IFN-γ by lung parenchymal CD4 T cells isolated from respectively treated mice after coculture with sorted lung $CD11b^{+}CD103^{-}$ DCs correspondingly. **E,** Production of IFN-γ by naive OT-II CD4 T cells after coculture with sorted lung $CD11b^{-}CD103^{+}$ DCs from different treatment groups. **F,** Production of IFN-γ by lung parenchymal CD4 T cells isolated from respectively treated mice after coculture with sorted lung $CD11b^{-}CD103^{+}$ DCs correspondingly. $^{+++}P < .001$ (compared with PBS/PBS mice). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ (compared with OVA/OVA mice). *Eos*, Eosophinils; *lym*, lymphocytes; *mac*, macrophages; *neu*, neutrophils.