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

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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;129:1611-20.)

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

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

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]

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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.

Available online March 3, 2012.

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0091-6749/$36.00

doi:10.1016/j.jaci.2011.12.976

which favors T_H1 differentiation.[40,41] We have previously shown that cognate interaction between CD8 T cells and DCs is able to 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 expressed as means ± SEM (*P* value range was indicated). Flow cytometric