

Resident CD8⁺ T cells suppress CD4⁺ T cell-dependent late allergic airway responses

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Background: The role of CD8⁺ T cells in the immune response to airway challenge with an allergen is poorly understood.

Objective: The aim of this study was to test the hypothesis that resident naive CD8⁺ T cells modulate the magnitude of CD4⁺ T cell-dependent allergic airway responses.

Methods: Cervical lymph node CD4⁺ T cells (2×10^6) were harvested from ovalbumin (OVA)– or sham-sensitized rats and injected intraperitoneally into naive Brown Norway recipients. The recipients were treated with a CD8 α mAb (OX-8) to deplete the resident CD8⁺ T cells ($n = 12$) or mouse ascites ($n = 12$). Two days after adoptive transfer, the recipient animals were OVA challenged, lung resistance was measured for 8 hours, and bronchoalveolar lavage (BAL) was performed.

Results: After OVA challenge, primed CD4-transferred CD8-depleted rats had larger early airway responses and late airway responses compared with primed CD4-transferred CD8-nondepleted rats (early airway responses: $158.6\% \pm 19.2\%$ vs $115.7\% \pm 5.9\%$, $P < .05$; late airway responses: $8.5\% \pm 1.7\%$ vs $4.4\% \pm 0.9\%$, $P < .05$). BAL eosinophilia was also greater ($4.67\% \pm 0.45\%$ vs $2.34 \pm 0.26\%$, $P < .01$). The cells in BAL fluid expressing IL-4 mRNA were not significantly changed by CD8 depletion, but IL-5 mRNA⁺ cells were higher in number, and IFN- γ mRNA⁺ cells were fewer in the CD8-depleted group.

Conclusions: Resident CD8⁺ T cells downregulate the late allergic response and airway inflammation evoked by CD4⁺ T-cell transfers in Brown Norway rats. This downregulation does not require antigen priming. (*J Allergy Clin Immunol* 2005;115:521-6.)

Key words: CD8⁺ T cells, CD4⁺ T cells, T_H2 cytokines, eosinophils, late allergic responses, Brown Norway rat

T cells play an important role in allergen-induced late responses and allergic airway inflammation. The role of the CD4⁺ T-cell subset has been extensively studied in rodent¹⁻⁴ and murine^{5,6} models of asthma, whereas CD8⁺ cells have received less attention. Human studies suggest an association between the CD8⁺ T cell and allergic

Abbreviations used

BAL:	Bronchoalveolar lavage
BN:	Brown Norway
EAR:	Early airway response
LAR:	Late airway response
MACS:	Magnetic cell sorting
MBP:	Major basic protein
OVA:	Ovalbumin
R _L :	Lung resistance
TCR:	T-cell receptor

asthma. It has been previously postulated that CD8⁺ T cells normally limit the magnitude of the allergic response. Indeed, suppressor T-cell dysfunction has been invoked as a mechanism of allergic respiratory tract disease.⁷ The failure to develop a late airway response (LAR) after allergen challenge has been associated with an increase in CD8⁺ T cells in the bronchoalveolar lavage (BAL) fluid of asthmatic subjects.⁸ There is evidence for both proinflammatory and anti-inflammatory roles for the CD8⁺ T cells in the sensitized mouse.^{9,10} There is also convincing evidence that CD8⁺ T cells might serve to modulate the magnitude of the airway allergic response in the sensitized and challenged rat. Depletion of CD8⁺ T cells before challenge has been shown to augment IgE levels, LAR, T_H2 cytokine expression by BAL cells, and airway eosinophilia in the Sprague Dawley rat,^{11,12} whereas the adoptive transfer of CD8⁺ T cells suppresses the LAR and allergic inflammation in actively sensitized Brown Norway (BN) rats.¹³

The CD8⁺ T-cell subset responsible for the suppression of the LAR through administration of exogenous CD8⁺ T cells has not been characterized. However, the suppressive effect required that the cells be harvested from an actively sensitized donor,¹³ suggesting a role for CD8⁺ $\alpha\beta$ T-cell receptor (TCR)–bearing cells. However, the $\gamma\delta$ TCR–bearing CD8⁺ T cells have also the potential to inhibit airway responses to allergen challenge.^{14,15} These cells do not require a priori contact with antigen to have a protective effect. In the mouse a deficiency of the $V\gamma4^+$ CD8⁺ cells results in the development of airway hyperresponsiveness to methacholine challenge after 3 inhalational challenges with ovalbumin (OVA) without prior sensitization.¹⁴ Adoptive transfer experiments with CD8⁺ $\gamma\delta$ cells in the rat also have shown a marked anti-inflammatory effect of these cells and an inhibition of LAR.¹⁵ Thus we reasoned that resident CD8⁺ cells without prior antigen exposure

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have the potential to inhibit airway responses to allergen challenge. To address this question, we markedly reduced the number of resident CD8⁺ T cells in naive recipients by using a specific mAb, OX-8, and we examined the effects of the depletion of these cells on the LAR in an adoptive transfer model. We used adoptive transfer with CD4⁺ T cells from sensitized donor animals to permit passive sensitization of recipients and to avoid exposing resident T cells to the sensitizing antigen OVA.

METHODS

Animals, sensitization, and challenge

Eighteen inbred male BN rats (Harlan Olac, Bicester, United Kingdom; 160–220 g; 7–10 weeks old) were injected with 0.1 mg of OVA (Grade V; Sigma, St Louis, Mo) subcutaneously in a 1-mL 0.9% saline suspension of aluminum hydroxide (3.48 mg/mL). *Bordetella pertussis* (10⁹ heat-killed organisms/mL) was injected intraperitoneally as an adjuvant. Twenty-four naive BN rats received an OVA aerosol exposure and underwent a period of 8 hours of measurement of pulmonary mechanics.¹⁶

Study protocol

CD4⁺ T-cell donors were actively sensitized to OVA or were naive. Twelve days later, CD4⁺ T cells were isolated from the cervical lymph nodes and purified with magnetic cell sorting (MACS), and 2 × 10⁶ cells were injected intraperitoneally into naive recipient animals.¹ The primed CD4⁺ T cell-transferred animals were treated 24 hours previously with a single administration of 250 µg of mouse anti-rat CD8α monoclonal IgG antibody (OX-8) intraperitoneally to deplete the resident CD8⁺ T cells (n = 12) or 250 µL of IgG-containing mouse ascites fluid from BALB/c mice injected with SP2/0 myeloma cells (Cedarlane Labs, Hornby, Ontario, Canada) as a control (n = 12). The naive CD4⁺ T cell-transferred animals were also treated with mouse ascites as a control for the second group (n = 9). Two days after adoptive transfer, all recipient animals were OVA challenged, and lung resistance (R_L) was measured for 8 hours. BAL was performed at 8 hours.

Immunomagnetic purification of CD4⁺ T cells

Twelve days after OVA sensitization, mononuclear cells were harvested from the cervical lymph nodes of BN rats. For each purification experiment, pooled cells from 2 donors were used. The lymph nodes were minced and passed through a nylon mesh to remove cellular debris. The CD4⁺ cells were then isolated by means of negative selection with immuno-MACS.¹

In this protocol the cells were first incubated with 3 antibodies, OX-8 (anti-CD8α), ED-9 (anti-myeloid cell), and OX-33 (anti-B cell), for 30 minutes. The cells were then washed and incubated for 30 minutes with rat anti-mouse IgG1-labeled MACS beads. This cell-bead mixture was passed through a MACS column, and the CD4⁺ cell-containing eluate was collected. The purity of this cell population was tested by means of flow cytometry.

Immunofluorescent staining and FACS analysis

Flow cytometry was performed on cervical lymph node lymphocytes to quantify the effectiveness of OX-8 mAb in depleting CD8⁺ T cells and to measure the purity of MACS-isolated CD4⁺ T cells. The lymphocytes were incubated with the mAbs OX-8 (mouse anti-rat CD8α-CYC), 341 (mouse anti-rat CD8β-FITC), or both in 5% naive rat serum in PBS. The cells were fixed with 4% paraformaldehyde.

Ten thousand events were counted for each sample by using a FACScan flow cytometer (Becton Dickinson, Mississauga, Ontario, Canada). The MACS-isolated CD4⁺ T cells were incubated with OX-35 (mouse anti-rat CD4-CYC), fixed, and counted as previously described.² The cell population achieved 97% purity.

Measurement of airway responses to OVA challenge

Two days after the CD4⁺ T-cell transfer, rats were anesthetized with urethane (1.5 mg/g), with supplemental doses (10%) as needed, and intubated endotracheally. Pulmonary mechanics were assessed with a commercial device (Quadra-t; SCIREQ, Montreal, Quebec, Canada). R_L was calculated with commercial software to process the signals for airflow and esophageal pressure and linear regression analysis to solve the equation of motion of the lung.

Baseline R_L was measured over 15 to 20 minutes, and then each rat received a 5-minute inhalation challenge of 5% (wt/vol) OVA in 0.9% saline delivered with a Hudson nebulizer with an airflow of 8 L/min. R_L measurements were taken every 5 minutes for the first 30 minutes after challenge and thereafter every 15 minutes for 8 hours. Procedures were done by a person who was blinded to the experimental grouping of the animals.

BAL and cell counting

Twenty-five milliliters of sterile saline solution was instilled in 5-mL increments at the end of the 8-hour period of measurements of lung function, and total BAL cell counts were determined with a hemacytometer. BAL cytospin slides were prepared with 8 × 10⁴ cells per slide. The slides were Wright-Giemsa stained, and neutrophils, eosinophils, lymphocytes, and macrophages were identified from a count of 300 cells.

Immunocytochemistry for eosinophil-derived major basic protein

Immunocytochemistry was performed by using a modified alkaline phosphatase–antialkaline phosphatase (Dako Diagnostics Canada Inc, Mississauga, Ontario, Canada) method, as previously reported.¹⁵ Briefly, slides were incubated with the mouse anti-human major basic protein (MBP) mAb overnight at 4°C. The secondary rabbit anti-mouse antibody (1:60 dilution, Becton Dickinson) was applied, and the slides were incubated with alkaline phosphatase–antialkaline phosphatase mouse antibody (1:60 dilution, Becton Dickinson). The reaction was visualized by dissolving the Fast Red TR (Sigma Chemicals Co) chromogen in alkaline phosphatase substrate. The slides were counterstained with hematoxylin. Negative controls were prepared, replacing the primary antibody with non-specific mouse immunoglobulin.

Cytokine mRNA–expressing cells in BAL fluid and detection by means of *in situ* hybridization

Cytospin slides were fixed with 4% paraformaldehyde. The technique of *in situ* hybridization was performed for the determination of the expression of mRNA for IL-4, IL-5, and IFN-γ. Antisense and sense riboprobes were prepared from cDNA coding for rat IL-4 and IFN-γ mRNA. The probes for IL-4 and IFN-γ were gifts from Drs A. Neil Barclay (Oxford, United Kingdom) and P. H. van der Meide (Rijswijk, The Netherlands), respectively. The probes for IL-5 were provided by Dr T. Blankenstein (Berlin, Germany). Unhybridized single-stranded RNA was removed with RNase A. After dehydration, the slides were immersed in LM-1 hyperemulsion (Amersham Pharmacia Biotech, Oakville, Ontario, Canada) and exposed for 14 days. The autoradiographs were developed in Kodak D-19, fixed with

TABLE I. Results of flow cytometric analysis of immuno-fluorescent staining of CD8⁺ T cells from control mouse ascites fluid-treated versus OX-8 mAb-treated BN rats

	CD8 α ⁺ CD8 α ⁻	CD8 α ⁻ CD8 β ⁺	CD8 α ⁺ CD8 β ⁺
Control BN rats	2.59	0.00	2.66
CD8-depleted BN rats	0.51	0.08	0.02

These cells were examined 3 days after injection. Data are presented as the percentage of gated cells that have the cell surface marker indicated.

Rapid Fixer (Kodak, Rochester, NY), and counterstained with hematoxylin. Sense probes and RNase pretreatment of sections before antisense probe application were performed to confirm probe specificity.

Statistical analysis

Data were tested for normalcy. Means were compared for IL-4, IL-5, and IFN- γ by using an ANOVA with the post-hoc Fisher least-significance-difference test when data were normally distributed. A Kruskal-Wallis test was performed on EARs, LARs, and MBP-immunoreactive cells in BALF. When significant differences were established, comparisons between pairs of means were performed by using the Mann-Whitney *U* test on the basis of ranks. The data are presented as means $\pm$ 1 SEM.

RESULTS

CD8⁺ T-cell depletion *in vivo* by OX-8

Three days after injection of 250 μ g of OX-8 or the same amount of control ascites intraperitoneally, cells from cervical lymph nodes were harvested to quantify the effectiveness of OX-8 mAb in depleting CD8⁺ T cells. OX-8 markedly decreased the number of CD8 α ⁺ T cells from 2.59% to 0.51% and CD8 α β ⁺ T cells from 2.66% to 0.02%, corresponding to a depletion of these cell populations by 80.3% and 99.2%, respectively (Table I). A representative result is shown in Fig 1. Dosing studies with OX-8 revealed almost complete depletion at 24 hours, with 0.25, 0.5, and 1.0 mg administered intraperitoneally (data not shown).

Effect of CD8⁺ T-cell depletion on allergen-induced bronchoconstriction

Pulmonary resistance was measured for 8 hours after OVA challenge of primed CD4⁺ T cell-transferred BN rats that were CD8⁺ T cell depleted, and primed CD4⁺ T cell-transferred animals that were treated with ascites and served as control animals. Three other control groups were studied. A group of rats that received naive CD4⁺ T cells, a group that underwent CD8⁺ T-cell depletion but that was otherwise naive, and a group that received only PBS intraperitoneally were also challenged. The baseline *R_L* for each animal was determined from the mean of 7 to 9 *R_L* measurements taken over 20 minutes and was unaffected by cell transfers, cell depletion, or both, as indicated by the fact that *R_L* before antigen challenge was not significantly different among the 5 groups.

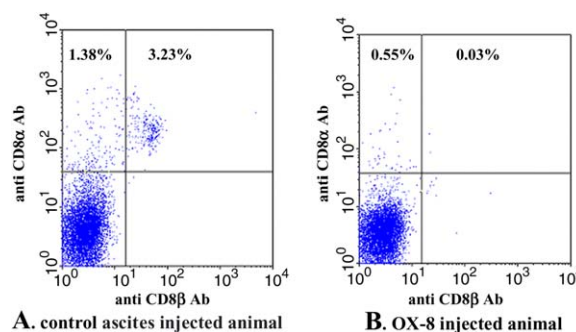


FIG 1. Flow cytometry on cervical lymph node cells harvested from a rat treated with mouse ascites (control; **A**) or OX-8 (mAb against the CD8 α chain; **B**). Expression of CD8 α and β chains was examined with OX-8-CYC and 341-FITC antibodies. The percentages of the cells in the various quadrants are expressed as a percentage of the total cells.

The changes in *R_L* after OVA challenge were measured for 8 hours, and the mean values of *R_L* at each 15-minute time point were calculated for both the OX-8-treated and control groups. The *R_L* increased sharply in the CD8-depleted group compared with control animals up to 75 minutes after OVA challenge (Fig 2). Thereafter, the CD8-depleted group maintained an increased mean *R_L* over that of control animals for the duration of the 8-hour experiment.

To quantify the effect of the depletion of the CD8⁺ T cells on the airway responses to OVA challenge, we compared the EARs and LARs in CD8-depleted versus control animals. The EAR was calculated from the highest *R_L* value as a percentage of baseline resistance during the first 60 minutes after OVA challenge. Fig 3, A, shows a significant increase in the mean EAR of control animals compared with OX-8-treated rats (115.7% $\pm$ 5.9% vs 158.6% $\pm$ 19.2%, *P* < .01). The mean LAR (Fig 3, B), calculated as the area under the *R_L* versus time curve between 3 and 8 hours after OVA challenge, was greater in OX-8-treated rats than in the CD8-nondepleted rats (8.5 $\pm$ 1.7 vs 4.4 $\pm$ 0.9 cm H₂O/mL/s.min, *P* < .05). The LAR in the CD8-nondepleted rats that received primed CD4⁺ T cells was in turn greater than that of the recipients of naive CD4⁺ T cells (1.2 $\pm$ 0.5 cm H₂O/L/s.min, *P* < .01).

Inflammatory cells in BAL fluid after CD8⁺ T-Cell depletion and OVA challenge

BAL fluid was collected after the 8-hour period of *R_L* measurements. There was no significant difference in the total numbers of cells among groups. The number of macrophages was slightly higher in the naive CD4⁺ T-cell recipients (3.53 $\pm$ 0.60 $\times$ 10⁶) than in the group that received antigen-primed CD4⁺ T cells and control ascites fluid (2.05 $\pm$ 0.16) and the group that received primed CD4⁺ T cells and OX-8 pretreatment (2.08 $\pm$ 0.28). Therefore there was no specific effect of OX-8 on the number of BAL fluid macrophages.

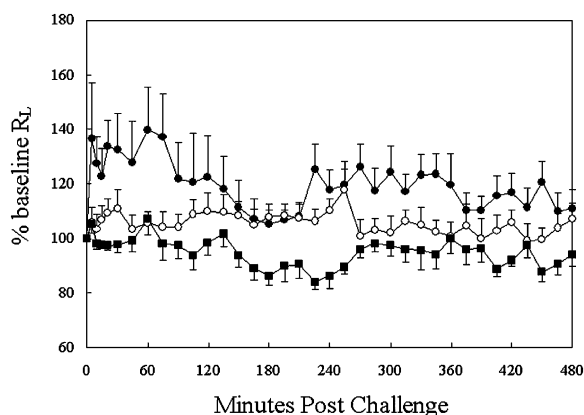


FIG 2. Time course of changes in R_L (percentage of baseline) after OVA challenge measured over 8 hours in control versus CD8⁺ T cell-depleted BN rats. All animals received 2×10^6 CD4⁺ T cells intraperitoneally (2 days before OVA challenge) isolated from cervical lymph nodes of OVA-sensitized donors. The CD8⁺ T cell-depleted animals ($n = 12$) received 0.250 mg of mouse anti-rat OX-8 mAb intraperitoneally 3 days before OVA challenge. The control animals ($n = 12$) were injected intraperitoneally with 250 μ L of mouse ascite fluid containing IgG. * $P < .05$.

There were substantial differences in eosinophil counts among groups. The results of immunostaining for eosinophil-derived MBP on BAL cytospin preparations are shown in Fig 4. The CD8-depleted/CD8-primed CD4 group had significantly greater BAL eosinophilia compared with the CD8-nondepleted/CD8-primed CD4 group ($P < .01$). Counts were also higher in the CD8-nondepleted/CD8-primed CD4 group than in the CD8-nondepleted/CD8-naïve CD4 group ($P < .01$).

T_H1 and T_H2 cytokine mRNA expression in BAL cells

The number of cells positive for IL-4 mRNA was higher in the CD8-nondepleted/primed CD4 group compared with that in the CD8-nondepleted/naïve CD4 group ($P < .001$). CD8 depletion did not further augment the IL-4⁺ cells (Fig 5). The number of IL-5 mRNA⁺ cells was higher in the CD8-depleted/primed CD4 group and the CD8-depleted/naïve CD4 group compared with that in the CD8-nondepleted/primed CD4 groups ($P < .005$). The number of IL-5 mRNA⁺ cells was higher in the CD8-nondepleted/primed CD4 group compared with that in the CD8-nondepleted/naïve CD4 group ($P < .05$, Fig 5). The number of cells positive for IFN- γ mRNA was significantly lower in the CD8-depleted/primed CD4 group compared with that in the CD8-nondepleted/primed CD4 group ($P < .005$) and in the CD8-nondepleted/primed CD4 group compared with that in the CD8-nondepleted/naïve CD4 group ($P < .001$, Fig 5).

DISCUSSION

Our results show that CD4⁺ T cells adoptively transfer antigen sensitivity to naïve recipients and that the

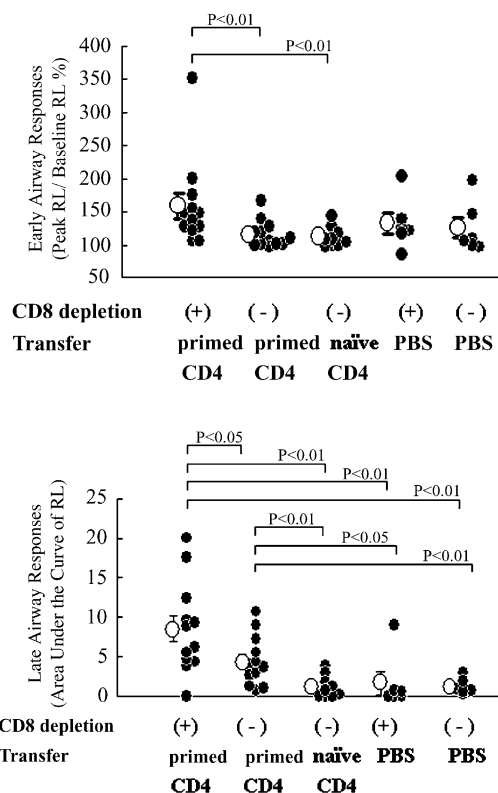


FIG 3. The EAR was determined from the peak in the R_L within 1 hour after challenge (A). The LAR was determined by means of calculation of the area under the curve of R_L against time from 3 to 8 hours after the challenge (B).

magnitude of the bronchoconstrictive responses and eosinophilic inflammation that develop after allergen challenge is affected by whether the recipient animal has been depleted of its resident CD8⁺ T cells by means of mAb treatment. There is an enhancement of the early and late responses after OVA challenge caused by CD8 depletion, and there is an accompanying increase in eosinophilia. The number of BAL cells expressing mRNA for the T_H1 cytokine IFN- γ was reduced by means of depletion of CD8⁺ cells. IL-5, but not IL-4, levels increased in these same animals.

To examine the effects of depletion of the resident population of CD8⁺ T cells on the late response, we used OX-8, a mouse anti-rat mAb directed against the CD8 α chain that leads to depletion of the CD8 cells. We have shown previously that CD8⁺ T cells were shown to be depleted by using antibodies that identified either the CD8 α chain or the CD8 β chain.¹⁷ The mechanism for this depletion might include both complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity. A single administration of 0.25 mg of OX-8 was shown by means of FACS analysis to effectively deplete CD8 $\alpha\beta$ and $\alpha\alpha$ T cells in the cervical lymph nodes. Published reports of OX-8 delivered intravenously at higher doses seemed to be less effective^{17,19} than the intraperitoneal route of administration. Alveolar macro-

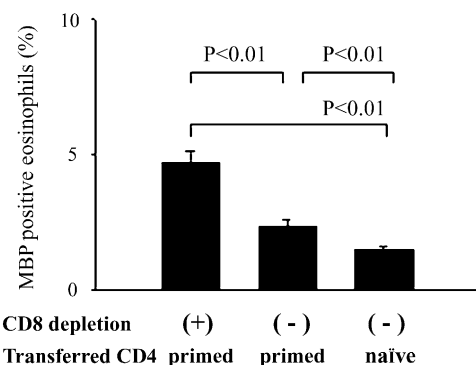


FIG 4. BAL fluid was taken from animals that underwent an OVA challenge followed by 8 hours of R_L measurements. The primed CD4-transferred CD8-depleted group, the primed CD4-transferred CD8-nondepleted group, and a naïve CD4-transferred CD8-non-depleted group were studied.

phages have been described to express CD8 α ¹⁸ and are therefore also a potential target for the antibody treatment. However, a count of macrophages in the BAL fluid indicated no difference between the CD4⁺ T cell-transferred groups with or without OX-8 treatment. This argues against the notion that modulation of the macrophage population accounted for our findings.

In the current experiment there is an alteration in the magnitude of the airway response to challenge by the CD8⁺ T-cell depletion. An adoptive transfer model of sensitization was used to evoke LAR and T_H2-type inflammation so that the role of naïve CD8⁺ T cells could be examined. It has been previously postulated that CD8⁺ T cells normally limit the magnitude of the allergic response in the airways.^{7,8} However, in these circumstances it is likely that antigen-specific CD8⁺ T cells were activated by means of antigen presentation through MHC class I pathways, whereas the results of the present study require alternative explanations. A model of CD4⁺ T cells and CD8⁺ T-cell interactions has been previously proposed that could potentially explain the current findings. When a naïve CD8⁺ T cell encounters antigen-binding MHC1 molecules on the surface of an antigen-presenting cell in the presence of an effector CD4⁺ T cell bound to the same antigen-presenting cell, the expression of costimulatory molecules, such as B7, can activate the CD8⁺ T cell.²⁰

The abovementioned mechanisms apply to CD8⁺ $\alpha\beta$ TCR-bearing T cells. However, there is evidence that the CD8⁺ $\alpha\beta$ TCR cells are proinflammatory in their effects in the airways, at least in the mouse.⁹ We have recently reported that the adoptive transfer of CD8⁺ $\alpha\beta$ TCR cells transfers allergic responsiveness to the rat, much as do the CD4⁺ T cells, causing late responses and T_H2 inflammation after allergen challenge.²¹ An alternative candidate for the observed inhibitory immune modulation is the CD8⁺ $\gamma\delta$ T-cell population. These cells might participate in the innate immune response, requiring no prior antigen exposure. McMenamin et al²² have shown that CD8⁺ $\gamma\delta$ T cells harvested from antigen-tolerized animals are potent

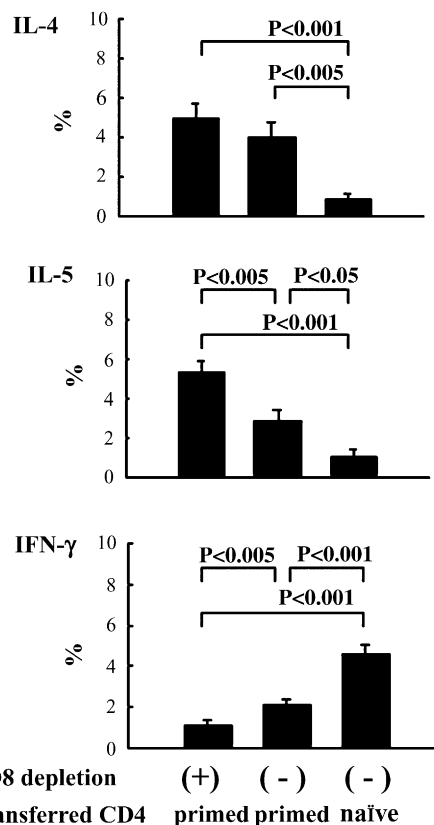


FIG 5. BAL fluid IL-4, IL-5, and IFN- γ mRNA-expressing cells (*in situ* hybridization) were determined in a CD4-transferred CD8-depleted group, a CD4-transferred CD8-nondepleted group, and a naïve CD4-transferred CD8-nondepleted group.

inhibitors of IgE synthesis.² We have previously reported a suppressive effect of CD8⁺ $\gamma\delta$ T cells on late responses and airway inflammation that is not dependent on the harvesting of cells from sensitized donors.¹⁵ Whether these cells have constitutive activity that is inherently anti-allergic or are activated by allergen challenge is not known.

Interestingly, there was a significant increase of pulmonary resistance within 1 hour of challenge seen in CD8⁺ T cell-depleted animals compared with pulmonary resistance in control animals. This EAR cannot be caused by IgE-mediated mast cell activation because there is no measurable serum OVA-specific IgE in the rats after CD4⁺ T-cell transfers.¹ The time course of the change in pulmonary resistance is reminiscent of the temporal changes in ear swelling in a murine model of cutaneous delayed-type hypersensitivity. In this model that is a T_H1-type T cell-dependent response to sensitization with a hapten, and there is an early-phase reaction that is complement dependent.²³ However, this early-phase reaction is specific IgM dependent,²³ and there is no opportunity for such antibody participation in our model because recipient animals are not exposed to antigen before the experiment. Interestingly, $\gamma\delta$ T cells also facilitate the cutaneous contact sensitivity that is mediated by $\alpha\beta$ T cells²⁴ but in the later stages of the delayed-type

hypersensitivity reaction. The nature of the early response in the current study is therefore quite unclear.

The LAR in BN rats has been shown to be a cell-mediated process involving the presence of antigen-primed CD4⁺ T cells.^{1,3} Whether the responses evoked by the transferred cells could be influenced by the unsensitized CD8⁺ cells in the recipient animals does not appear to have been addressed previously. The current study showed a clear influence of the resident CD8 cells on the LAR. One possible mechanism of this effect is that cytokines like IL-2 and IL-4 liberated from activated CD4⁺ T cells in turn activate CD8⁺ T cells. Once activated, the naive CD8⁺ T cell might express inhibitory cytokines, such as IFN- γ , that might inhibit allergic airway inflammation.^{9,13,25} Alternatively, direct effects by CD8 T cells, such as Fas ligand-Fas interaction or perforin-granzymes, might effect killing of either CD4⁺ T cells²⁶ or dendritic cells,²⁷ and if such effects are present *in vivo*, they would be expected to inhibit airway inflammation and airway responsiveness.

In summary, the results of the present study revealed an influence of the resident CD8⁺ cells on the CD4⁺ T cell-dependent late allergic airway responses and airway inflammation in the BN rat. The findings indicate that unprimed CD8⁺ T cells might serve an inhibitory function in limiting the magnitude of responses to inhaled foreign protein. The mechanism of action of the CD8⁺ cells is not known but might be mediated by IFN- γ through its reduction of T_H2 inflammation. Other mechanisms involving direct cell-to-cell interactions are also hypothetically possible but seem less likely to be plausible in the setting of the adoptive transfer model system used in the current experiments, given the small numbers of cells that are administered and the low probability of direct cell-to-cell encounters.

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