

CD8⁺ αβ T cells can mediate late airway responses and airway eosinophilia in rats

Susumu Isogai, MD,^a Rame Taha, MD,^a Meiyo Tamaoka, MD,^a Yasuyuki Yoshizawa, MD,^b Outayba Hamid, MD, PhD,^a and James G. Martin, MD^a Montreal, Quebec, Canada, and Tokyo, Japan

Background: The function of CD8⁺ T-cell subsets in mediating late allergic responses is incompletely understood.

Objective: We sought to test the hypothesis that CD8⁺ αβ T cells are proinflammatory in the airways *in vivo* by using a well-characterized animal model and the technique of adoptive transfer

Methods: Brown Norway rats were administered CD8⁺ αβ T cells (10⁶) intraperitoneally purified from lymph node cells of either naive or ovalbumin (OVA)-sensitized rats and were challenged with aerosolized OVA 2 days later. Control rats were sensitized to 100 μg of OVA in Al(OH)₃ subcutaneously or sham sensitized to saline and were OVA challenged 2 weeks later.

Results: The OVA-sensitized and OVA-challenged group and the recipients of OVA-primed CD8⁺ αβ T cells had significant late airway responses calculated from lung resistance measured for an 8-hour period after challenge compared with the naive CD8⁺ αβ T cell-transferred group and the sham-sensitized control group. The number of eosinophils in bronchoalveolar lavage fluid increased in the OVA-sensitized group and the OVA-primed CD8⁺ αβ T-cell recipients compared with numbers in the naive CD8⁺ αβ T-cell recipients and the sham-sensitized control group. IL-4 and IL-5 cytokine mRNA expression in bronchoalveolar lavage fluid increased in the OVA-sensitized group and the OVA-primed CD8⁺ αβ T-cell recipients compared with that in the sham-sensitized group.

Conclusion: We conclude that antigen-primed CD8⁺ αβ T cells might have a proinflammatory role in allergen-driven airway responses in the rat. (*J Allergy Clin Immunol* 2004; 114:1345-52.)

Key words: Lung, allergy, inflammation, T lymphocytes, cytokines

CD4⁺ T cells are preferentially activated after inhalation of exogenous foreign protein as a result of antigen processing and presentation by dendritic cells in an MHC class II-restricted manner.¹ However, MHC class I-dependent activation of CD8⁺ T cells may also take place²⁻⁴ through cross-presentation.⁵ CD8⁺ T cells in the

Abbreviations used

AHR:	Airway hyperresponsiveness
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

airway have been associated with asthma severity⁶ and asthma-induced death.⁷ In rodents CD8⁺ T cells may have potent suppressive effects on the airway responses to allergen challenge.⁸⁻¹⁰ Somewhat contradictory evidence has been obtained on murine models, showing that CD8⁺ T cells are essential for allergen and virally induced airway hyperresponsiveness (AHR).^{11,12}

CD8⁺ T cells play an important role in preventing T_H2-driven immune responses through the synthesis and release of IFN-γ.^{4,13,14} However, CD8⁺ T cells that are positive for transgenic Vα2 and Vβ8.1 chains express higher IL-5 levels,¹⁵ indicating a possible dichotomy in the characteristics of certain subsets of CD8⁺ T cells. Although T cells bearing the αβ heterodimeric T-cell receptor (TCR) and the γδ TCR develop from common T-cell progenitors,¹⁶ the expression patterns of CD4 and CD8, their distributions, the sizes of the cell populations, and their reactivities differ and may be independently regulated.¹⁷ Both γδ and αβ T cells recognize the complexes of peptide antigen and MHC molecules with the assistance of the coreceptors CD4 and CD8.^{18,19} However, some γδ T cells recognize the peptide, pathogen, or damaged cell directly without class I- or class II-associated antigen presentation, and in this respect the TCRs of these cells are more like immunoglobulins than the classic αβ TCR.^{20,21}

The role of CD8⁺ T cells in airway inflammation and allergic airway response in the rodent has been explored by means of nonselective CD8⁺ T-cell depletion.^{8,10} Adoptive transfer techniques have also been applied, sometimes without attention to the TCR, and therefore the observed effects reflect the actions of mixed populations of CD8⁺ T cells.⁹ CD8⁺ γδ T cells from animals rendered tolerant to antigen are known to synthesize IFN-γ and inhibit IgE synthesis.²² CD8⁺ γδ T cells transferred

From ^aMeakins Christie Laboratories, Department of Medicine, McGill University, Montreal, and ^bthe Department of Geriatric and Pulmonary Medicine, Tokyo Medical and Dental University, Tokyo.

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Reprint requests: James G. Martin, MD, Meakins Christie Laboratories, Department of Medicine, McGill University, 3626 St Urbain, Montreal, H2X 2P2 Quebec, Canada. E-mail: james.martin@mcgill.ca.

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into sensitized rats before allergen challenge potentially inhibit the late airway response (LAR) and eosinophilia and cause a T-cell bias away from the T_H2 cytokine profile.²³ The presence of $\gamma\delta$ T cells in a mixed population of $CD8^+$ T cells might have profound effects on the function of the $\alpha\beta$ T cells that are present. Whether purified $CD8^+$ $\alpha\beta$ cells have proinflammatory or anti-inflammatory effects in the airways of allergen-sensitized and allergen-exposed rats is uncertain and prompted the current study. We used a well-characterized adoptive transfer technique in the Brown Norway (BN) rat model of asthma⁹ to study the effects of ovalbumin (OVA) challenge on the airway responses of rats that were administered primed or naive $CD8^+$ $\alpha\beta$ T cells. To better understand the differences in the effects of mixed $CD8^+$ T cells ($\alpha\beta$ and $\gamma\delta$) and purified $CD8^+$ $\alpha\beta$ T cells on airway responses *in vivo*, we examined also the expression of IL-4 and IFN- γ by $CD8^+$ T cells when cultured *ex vivo* as a pure population of $CD8^+$ $\alpha\beta$ cells or as a mixed population of $CD8^+$ $\alpha\beta$ and $\gamma\delta$ cells

METHODS

Animals and sensitization

Inbred male BN rats (10 weeks of age and 190–240 g; Harlan Sprague Dawley, Blackthorn, United Kingdom) were studied. Experimental procedures were approved by an institutional animal care committee. The animals were sensitized with a single subcutaneous injection of 100 μ g of OVA (grade V; Sigma, St Louis, Mo), with 4.28 mg of aluminum hydroxide gel (Anachemia Chemicals, Montreal, Quebec, Canada) as adjuvant. Simultaneously, 0.5 mL of *Bordetella pertussis* vaccine (Dr T. Issekutz, Dalhousie University, Halifax, Nova Scotia, Canada) containing 5×10^8 heat-killed bacilli was injected intraperitoneally.

Study protocol

$CD8^+$ $\alpha\beta$ T cells were purified from the cervical lymph nodes of either naive donor rats or rats sensitized to OVA 2 weeks previously. Recipient BN rats were given 1.0×10^6 $CD8^+$ $\alpha\beta$ T cells intraperitoneally on day 13, whereas control rats were given PBS. All animals were sensitized to either OVA or saline on day 1. BN rats that were given $CD8^+$ $\alpha\beta$ T cells from either naive donor rats (ND group, $n = 12$) or OVA-sensitized rats (SD group, $n = 12$) were challenged with aerosolized OVA (5% in saline). Control rats that were sensitized with either OVA (OVA group, $n = 12$) or saline (saline group, $n = 12$) were given PBS and challenged with aerosolized OVA. Because $\alpha\beta$ T cells could not be purified to 100%, we studied the effects of cells that were left after a purification procedure that included a step that removed the $CD8^+$ $\alpha\beta$ T cells. We administered 2×10^5 of these cells to a group of rats ($n = 5$) that was compared with a control group administered PBS intraperitoneally ($n = 5$).

Lung resistance (R_L) was measured before aerosol challenge and for 8 hours after aerosol challenge. On completion of the lung function measurements, bronchoalveolar lavage (BAL) was performed.

Antibodies

Cedarlane Laboratories (Hornby, Ontario, Canada): ED9 (IgG1 mouse anti-rat Myeloid Differentiation Antigen), OX33 (IgG1 mouse anti-rat B cell), OX8 (IgG1 mouse anti-rat CD8), ED9 (MCA620R, mouse anti-rat CD172a, IgG1), MRC OX-33 (MCA340G, mouse

anti-rat CD45RA mAb, IgG1), and 10/78 (MCA142.7, mouse anti-rat CD161, IgG1) were purchased from Serotec Inc (Raleigh, NC). W3/25 (CL003AP, mouse anti-rat CD4 mAb, IgG1) and OX8 (mouse anti-rat CD8, IgG1) were purchased from Cedarlane Laboratories. V65 (22461D, mouse anti-rat $\gamma\delta$ TCR, IgG1) was purchased from BD Pharmingen (Mississauga, Ontario, Canada). Rat anti-mouse IgG1 microbeads were purchased from Miltenyi Biotec Inc (Auburn, Calif). BMK13, a mouse anti-human major basic protein [MBP] antibody, was provided by Dr R. Moqbel (University of Alberta, Edmonton, Canada).

Adoptive transfer of $CD8^+$ $\alpha\beta$ T cells

T cells were harvested from the cervical lymph nodes by mincing and passing the tissues through a nylon mesh. $CD8^+$ $\alpha\beta$ T cells were purified by means of magnetic cell sorting (MACS; Miltenyi Biotec). Cells were incubated with primary antibodies (W3/25; anti-CD4, OX-33; anti-B cell, ED9; anti-myeloid cell, 10/78; anti-natural killer cell, V65; and anti- $\gamma\delta$ TCR) for 30 minutes and with MACS beads for 20 minutes at 4°C. Unlabelled cells that passed through the MACS column were collected. The purity of the cells was evaluated by means of flow cytometry (FACSscan; Becton Dickinson, Mountain View, Canada). $CD8^+$ $\alpha\beta$ T cells (1.0×10^6) were transferred to each recipient intraperitoneally. Cells (2.0×10^5) that remained after the above purification was performed but in which an anti-CD8 antibody, OX-8, was included were also administered intraperitoneally.

IL-4 and IFN- γ mRNA expression by $CD8^+$ $\alpha\beta$ T cells

Cytospin slides of $CD8^+$ $\alpha\beta$ T cells were fixed with 4% paraformaldehyde. The cells positive for mRNA for IL-4 and IFN- γ were identified by means of *in situ* hybridization.²⁴ Antisense and sense riboprobes were prepared from cDNA coding for rat IL-4 and IFN- γ mRNA (gifts from Drs A. Neil Barclay [Oxford, United Kingdom] and P. H. van der Meide [Rijswijk, The Netherlands], respectively). 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 counterstained with hematoxylin. Sense probes and RNase pretreatment of sections before antisense probe application were performed to confirm probe specificity.

Measurement of airway responses to OVA

Two days after T-cell transfers, the BN rats were anesthetized with intraperitoneal urethane (1.2 g/kg) and orotracheally intubated and instrumented for the measurement of pulmonary mechanics with a commercial device (Quadra-t; SCIREQ, Montreal, Quebec, Canada).²⁵

BAL

Sterile saline was instilled 5 times in 5-mL increments 8 hours after antigen challenge, and total BAL cell counts were determined with a hemacytometer. BAL cytospin slides (Cytospin model II; Shandon, Pittsburgh, Pa) were stained with Wright-Giemsa, and the cellular differential was assessed on 300 cells. For immunocytochemistry, slides were fixed in acetone-methanol, air-dried, and stored at -20°C until further use.

Immunocytochemical evaluation of eosinophil-derived MBP expression

Immunocytochemistry was performed by using a modified alkaline phosphatase–antialkaline phosphatase method (Dako Diagnostics Canada Inc, Mississauga, Ontario, Canada).²⁶ Slides were

incubated with the mouse anti-human MBP mAb overnight at 4°C. After washing in Tris-buffered saline, the secondary rabbit anti-mouse antibodies (1:60 dilution; Becton Dickinson, Mississauga, Ontario, Canada) were applied, and the slides were incubated with alkaline phosphatase–antialkaline phosphatase mouse antibodies (1:60 dilution, Becton Dickinson). The reaction was visualized by dissolving the Fast Red TR (Sigma Chemicals Co, St Louis, Mo) chromogen in alkaline phosphatase substrate. The slides were counterstained with hematoxylin. Negative controls were prepared, replacing the primary antibody with nonspecific mouse immunoglobulin.

Cytokine mRNA-expressing cells in BAL fluid: Detection by means of *in situ* hybridization

Cytospin slides were fixed with 4% paraformaldehyde. The technique of *in situ* hybridization was performed as described above for the determination of the expression of mRNA for IL-4, IL-5, and IFN- γ by a blinded observer. The probes for IL-5 were provided by Dr T. Blankenstein (Berlin, Germany).

IL-4 and IFN- γ production by CD8 $^{+}$ T cells or CD8 $^{+}$ $\alpha\beta$ T cells

Sensitized or naive CD8 $^{+}$ T cells were obtained from pooled cervical lymph nodes of 3 donors as described above by using antibodies (W3/25; anti-CD4, OX-33; anti-B cell, ED9; anti-myeloid cell, 10/78; anti-natural killer cell). CD8 $^{+}$ T cells (0.5×10^6) were placed in culture. The remaining CD8 $^{+}$ T cells were further purified to CD8 $^{+}$ $\alpha\beta$ T cells as above with V65, anti- $\gamma\delta$ antibody. CD8 $^{+}$ $\alpha\beta$ T cells (0.5×10^6) were cultured in 24-well plates with 0.5 mL of Dulbecco modified Eagle medium (12100-61; Invitrogen Corp, Burlington, Ontario, Canada), 10% heat-inactivated FBS (12483-020, Invitrogen), 5 μ L of antibiotics (10378-016, Invitrogen), 5 μ L of MEM nonessential amino acids solution (11140-050, Invitrogen), and 50 μ M of 2-mercaptoethanol (034461-100; Fisher Scientific, FairLawn, NJ) in a 37°C incubator for 18 hours. For cell stimulation, 500 ng/mL ionomycin (Bioscience Inc, Darmstadt, Germany) and 10 ng/mL phorbol 12-myristate 13-acetate (P-8139, Sigma) were added. Each sample was obtained by pooling cells from 3 animals, and 3 to 5 samples were prepared for each group. IL-4 and IFN- γ expression in supernatants of cultured cells were measured with ELISA kits (OptEIA ELISA sets, BD Pharmingen). Optical density was read with an Ultra Microplate Reader (Bio-tek Instrument Inc, Winooski, Vt).

Statistical analysis

Data are presented as means $\pm$ SE. Statistical comparisons were performed by using 1-way ANOVA, followed by the Fisher least-significant-difference test. Data that were not normally distributed were analyzed by using the Kruskal-Wallis and the Mann-Whitney U tests. *P* values of less than .05 were considered significant.

RESULTS

Purified and OVA-primed CD8 $^{+}$ $\alpha\beta$ T cells show a type II cytokine pattern before transfer

The purity of cervical lymph node CD8 $^{+}$ $\alpha\beta$ T cells by means of FACS analysis was $80.8\% \pm 2.51\%$ ($n = 3$). Contamination by CD4 $^{+}$ T cells, B cells, natural killer cells, or myeloid cells was less than 1%, and CD8 $^{+}$ $\gamma\delta$ T cells were less than 0.1%. The contaminating cells were CD45 $^{+}$ but were not further characterized.

We examined the expression of IL-4 and IFN- γ by purified CD8 $^{+}$ $\alpha\beta$ T cells before transfer. The number of cells that were IL-4 $^{+}$ as determined by means of *in situ* hybridization was higher in CD8 $^{+}$ $\alpha\beta$ T cells harvested from the sensitized donor group (4.6 ± 0.77) than from the naive donor group (1.4 ± 0.42 , $P < .05$). There was no evidence of a significant difference in IFN- γ expression by cells from either group (2.4 ± 1.22 and 3.9 ± 0.74 , sensitized and naive, respectively; $P =$ not significant).

CD8 $^{+}$ $\alpha\beta$ T cells can adoptively transfer late airway response but not early airway response

Baseline lung function was not affected by cell transfers: R_L before antigen challenge was not significantly different among the 4 groups. OVA-sensitized, PBS-injected, OVA-challenged animals (OVA group) showed small increases in R_L within 15 minutes after allergen challenge and more substantial increases in R_L 6 hours later compared with sham-sensitized, PBS-injected, OVA-challenged animals (saline group; Fig 1, A). Sham-sensitized recipients of CD8 $^{+}$ $\alpha\beta$ T cells from sensitized donors that were OVA challenged (SD group) showed significant increases in R_L from 2 hours onward after allergen challenge compared with sham-sensitized recipients of CD8 $^{+}$ $\alpha\beta$ T cells from naive donors that were OVA challenged (ND group; Fig 1, B).

Early airway response (EAR) was determined from the peak in the R_L within 1 hour after the challenge. The OVA group exhibited a significant increase in the peak EAR compared with both the saline and ND groups ($P < .05$; Fig 2, 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. The LARs of both the OVA and SD groups were significantly higher compared with those of the saline and ND groups ($P < .05$ and $P < .01$; Fig 2, B).

The contaminating cells that remained after cell purifications that included an anti-CD8 antibody were tested for their effects on R_L after OVA challenge. There was no evidence of either EAR or LAR in these animals. The LAR was 3.5 ± 1.55 cm H $_2$ O/mL/s $\times$ min in the cell-transferred group and 2.4 ± 0.79 cm H $_2$ O/mL/s $\times$ min in the PBS-injected rats ($P = .61$).

Eosinophils accumulate in the BAL fluid of CD8 $^{+}$ $\alpha\beta$ T cell-transferred animals after OVA challenge

There was no significant difference in the total cell counts among the groups (Table I). The number of lymphocytes was higher in the SD group than in the saline group ($P < .05$). The OVA group had significantly higher granulocyte counts than both the ND and saline groups ($P < .001$). The SD group also had significantly higher granulocyte counts than the saline group ($P < .01$).

The OVA group had significantly greater BAL eosinophilia (MBP $^{+}$) compared with both the saline group ($P < .005$) and the ND group ($P < .001$, Fig 3). Values in the SD

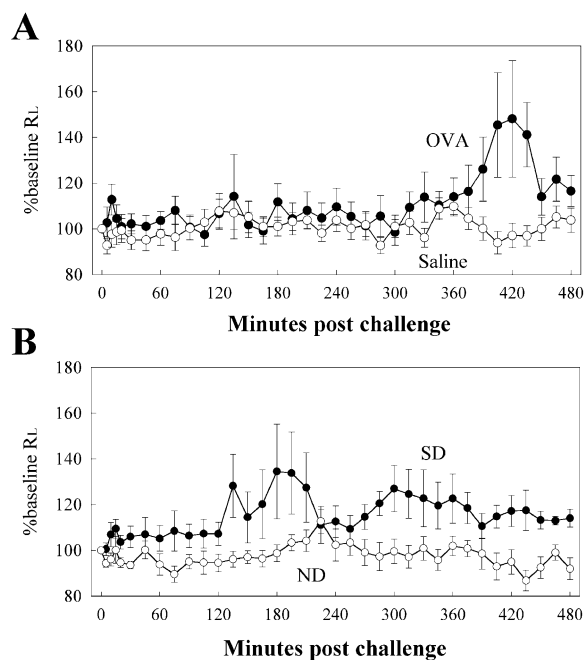


FIG 1. Time courses of changes in R_L after OVA challenge. OVA-sensitized, PBS-injected, OVA-challenged animals (OVA group, $n = 12$) are compared with saline-sensitized, PBS-injected, OVA-challenged animals (saline group, $n = 12$; **A**). Saline-sensitized, $CD8^+ \alpha\beta$ T cells of sensitized donor-transferred, OVA-challenged animals (SD group, $n = 12$) are compared with saline-sensitized, $CD8^+ \alpha\beta$ T cells of naive donor-transferred, OVA-challenged animals (ND group, $n = 12$; **B**).

group were higher than in both the saline group ($P < .005$) and the ND group ($P < .005$). The cell counts (absolute and differential) in the BAL fluid of the rats given the cells contaminating the $CD8^+ \alpha\beta$ population did not differ from the PBS-treated control group (Table II).

$CD8^+ \alpha\beta$ T cell-transferred animals show a type II cytokine pattern in the BAL fluid cells

The number of cells positive for IL-4 mRNA was higher in both the OVA and the SD groups compared with that in both the saline ($P < .05$, Fig 4) and the ND ($P < .05$) groups. Likewise, the number of IL-5 mRNA-positive cells was higher in both the OVA and SD groups compared with in both the saline ($P < .005$ and $P < .05$, respectively) and ND ($P < .05$) groups. The number of cells positive for IFN- γ mRNA was significantly lower in the OVA group compared with the saline control groups ($P < .05$) but was not significantly different among the SD, saline, and ND groups.

The cytokine expression pattern differed between mixed $CD8^+$ T cells ($\alpha\beta$ and $\gamma\delta$) and $CD8^+ \alpha\beta$ T-cell culture

$CD8^+$ T cells (mixed 10:1 $\alpha\beta$ and $\gamma\delta$) and $CD8^+ \alpha\beta$ T cells were cultured with and without (Fig 5) phorbol 12-myristate 13-acetate and ionomycin, and IL-4 and

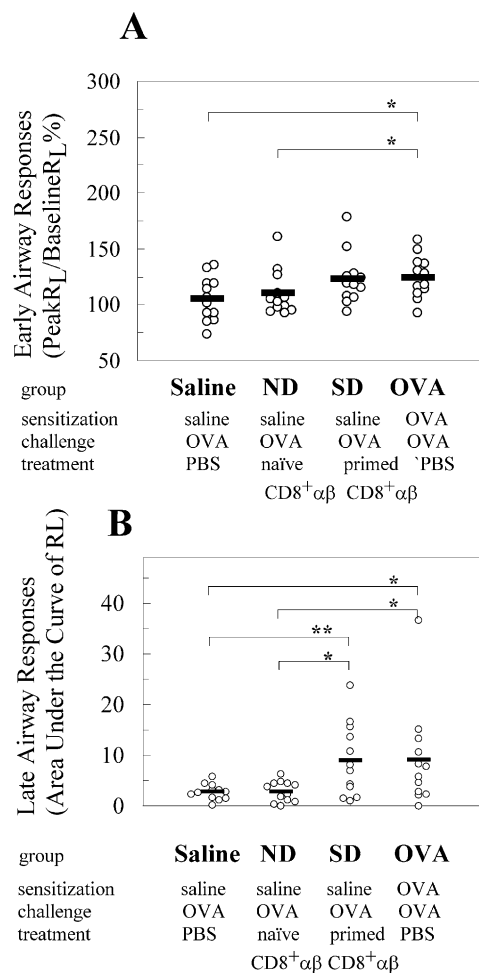


FIG 2. The EAR in the OVA-sensitized control group (OVA group), the saline-sensitized control group (saline group), the naive $CD8^+ \alpha\beta$ T cell-transferred group (ND group), and the primed $CD8^+ \alpha\beta$ T cell-transferred group (SD group) are shown in **A**. The LARs in the OVA group, SD group, saline group, and ND group are shown in **B**. * $P < .05$, ** $P < .01$.

IFN- γ levels were measured by means of ELISA. All data were within the range of detection.

Stimulated primed $CD8^+ \alpha\beta$ T cells expressed significantly more IL-4 than stimulated naive $CD8^+ \alpha\beta$ T cells ($P < .05$; Fig 5, A), whereas mixed $CD8^+$ T cells did not show a significant difference between the 2 groups. Stimulated primed $CD8^+$ T cells expressed more IFN- γ than did stimulated naive $CD8^+$ T cells ($P = .05$; Fig 5, B), whereas $CD8^+ \alpha\beta$ T cells did not show a significant difference between the 2 groups. Primed $CD8^+ \alpha\beta$ T cells produced significantly less IFN- γ than did primed and mixed $CD8^+$ T cells ($P < .05$).

DISCUSSION

The LAR after allergen exposure of sensitized subjects is prolonged and often more severe than the early response and is accompanied by a more intense inflammatory

TABLE I. Total cell count and cell profile in BAL cells*

Group	Total cells ($\times 10^6$)	Macrophages ($\times 10^6$)	Lymphocytes ($\times 10^6$)	Granulocytes ($\times 10^6$)
Saline	3.844 $\pm$ 0.304	3.599 $\pm$ 0.287	0.039 $\pm$ 0.007	0.206 $\pm$ 0.061
ND	4.771 $\pm$ 0.404	4.309 $\pm$ 0.375	0.102 $\pm$ 0.024	0.360 $\pm$ 0.075
SD	4.008 $\pm$ 0.628	3.248 $\pm$ 0.574	0.106 $\pm$ 0.038†	0.654 $\pm$ 0.158‡
OVA	4.338 $\pm$ 0.336	3.283 $\pm$ 0.327	0.094 $\pm$ 0.021	0.960 $\pm$ 0.121§

*Data are expressed as average $\pm$ SEM (n = 12).

†P < .05 compared with the saline group.

‡P < .01 compared with the saline group.

§P < .001 compared with the saline group.

||P < .001 compared with the ND group.

response than is seen in subjects with isolated EARs. The LAR is often followed by a period of enhanced AHR to inhaled agonists.²⁷ In the current experiment OVA-primed CD8⁺ $\alpha\beta$ T cells, which expressed IL-4, the prototypical type II cytokine, were administered to naive recipients 2 days before challenge and were found to adoptively transfer the LAR. The results were similar to those obtained previously with CD4⁺ T cells.²⁸ There was an associated eosinophilia in BAL fluid and increased numbers of IL-4 and IL-5 mRNA-expressing cells. These proinflammatory effects were not transferred by naive cells. These data indicate that primed CD8⁺ $\alpha\beta$ T cells might participate in airway responses to inhalational challenge with exogenous antigen and have net proinflammatory effects.

Adoptive transfer techniques have been used to advantage in elucidating the role of the T cell in the allergen-induced LAR²⁵ and AHR in BN rats.²⁸ Primed CD4⁺ T-cell transfers and subsequent exposure of the recipient animal with allergen evoke LAR, AHR, and airway inflammation in naive recipient animals.^{25,28} A leak in the processing and intracellular trafficking of exogenous antigen results in MHC class I-associated presentation to CD8⁺ T cells.^{2-5,29} Indeed, our previous studies have shown that CD8⁺ T cells from antigen-primed animals inhibit the LAR.⁹ However, in the current report CD8⁺ $\alpha\beta$ T cells showed a behavior similar to that of CD4⁺ T cells in transferring the LAR. Neither CD4⁺ nor CD8⁺ T cells cause the transfer of the typical EAR that is observed in an actively sensitized animal.⁹ This finding is not unexpected because there is no OVA-specific IgE in these animals to trigger an EAR.²⁵ The definition of the EAR, albeit an arbitrary one, is based on the usual time course (<1 hour) of the changes in pulmonary resistance after challenge of sensitized rats. However, in the current experiments there was a change in resistance as early as 2 hours after OVA challenge of CD8⁺ $\alpha\beta$ T cell-transferred animals. We have observed a similar early-phase reaction in adoptive transfer experiments with primed CD4⁺ T cells.²⁵ In a CD8⁺ T cell-dependent model of cutaneous delayed-type hypersensitivity that is a T_H1-type T cell-dependent response to cutaneous sensitization, there is also an early-phase reaction with a similar time course that is both complement and IgM dependent.³⁰

The transfer of allergic sensitivity by CD4⁺ T cells has been associated with eosinophilic airway inflammation

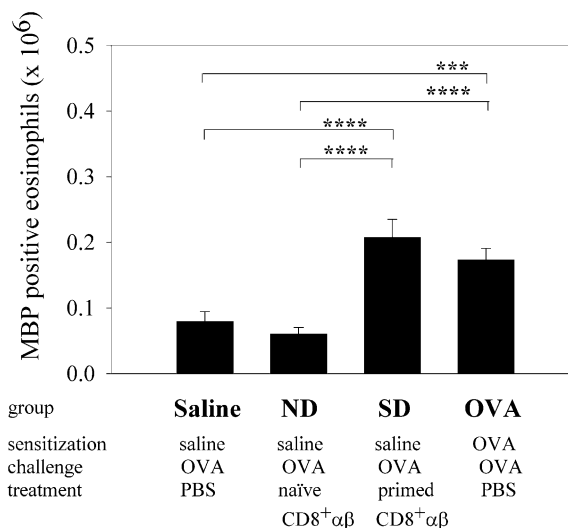


FIG 3. Eosinophil-derived MBP expression in BAL cells of the primed CD8⁺ $\alpha\beta$ T cell-transferred group (n = 12), the OVA-sensitized positive control group (OVA group, n = 12), the saline-sensitized control group (saline group, n = 12), and the naive CD8⁺ $\alpha\beta$ T cell-transferred group (ND group, n = 12). ***P < .005 and ****P < .001.

and T_H2 cytokine expression.³¹ The important role for IL-4 synthesis by transferred CD4⁺ T cells in evoking the LAR has been shown.³² In the current experiment there was also an increase in IL-4-expressing cells in the BAL fluid of the recipients of primed CD8⁺ $\alpha\beta$ T cells that might also account for the LAR. IL-5 was also expressed to a greater extent in animals with the LAR³³ and is likely important for the eosinophilic inflammatory response.¹⁰ It is somewhat controversial as to whether IL-5 can account for other manifestations of allergic sensitization and challenge, such as the LAR and AHR.³⁴ The production of IL-5 by murine CD8⁺ $\alpha\beta$ T cells has been reported previously¹⁵ and has led to the suggestion of a distinct T_C2 subset of CD8⁺ T cells mediating a similar inflammatory response to that of the T_H2 cells. Changes in the T_H1 cytokine IFN- γ might also be important in altering the inflammatory response. The number of cells expressing this cytokine decreased as IL-4-expressing cells increased. A defect in T_H1 inflammation has been identified in the BN rat, and CD8⁺ T cells appear to play a more important role than CD4⁺ T cells in this defect.³⁵

TABLE II. Total cell count and cell profiles in BAL fluid of OVA-challenged rats administered contaminating cells or PBS

Group	Total Cells ($\times 10^6$)	Macrophages ($\times 10^6$)	Lymphocytes ($\times 10^6$)	Neutrophils ($\times 10^6$)	Eosinophils ($\times 10^6$)
Cells	2.512 $\pm$ 0.750	2.042 $\pm$ 0.630	0.167 $\pm$ 0.074	0.258 $\pm$ 0.067	0.046 $\pm$ 0.021
PBS	1.736 $\pm$ 0.226	1.317 $\pm$ 0.171	0.120 $\pm$ 0.025	0.234 $\pm$ 0.050	0.065 $\pm$ 0.025

Data are expressed as averages $\pm$ SEM (n = 5).

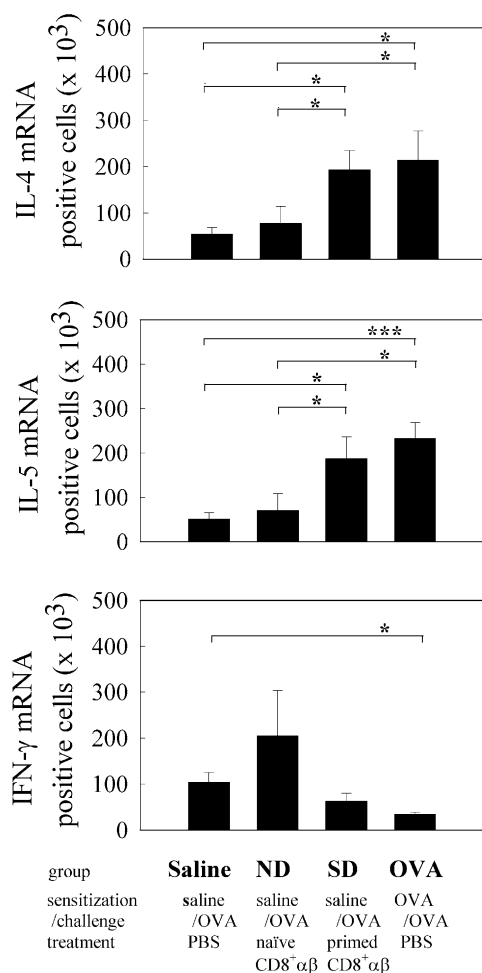


FIG 4. IL-4 (upper panel), IL-5 (middle panel), and IFN- γ (lower panel) mRNA-positive cells in BAL fluid in primed CD8 $^{+}$ $\alpha\beta$ T cell-transferred recipients (SD group, n = 6), the OVA-sensitized control group (OVA group, n = 6), the saline-sensitized control group (saline group, n = 6), and the naive CD8 $^{+}$ $\alpha\beta$ T cell-transferred group (ND group, n = 6). *P < .05.

Because an antigen-specific proinflammatory effect of CD8 $^{+}$ $\alpha\beta$ T cells was demonstrable in the BN rat, we concluded that the net inhibitory effect of mixed CD8 $^{+}$ T cells in prior experiments was likely attributable to CD8 $^{+}$ $\gamma\delta$ T cells that were transferred concurrently. Indeed, CD8 $^{+}$ $\gamma\delta$ T cells in the same animal model are markedly antiallergic. The inhibitory effect was caused by CD8 $^{+}$ $\gamma\delta$ T cells harvested from both naive and sensitized animals.²³ This is consistent with the observations that these cells can respond to antigen without the need for prior

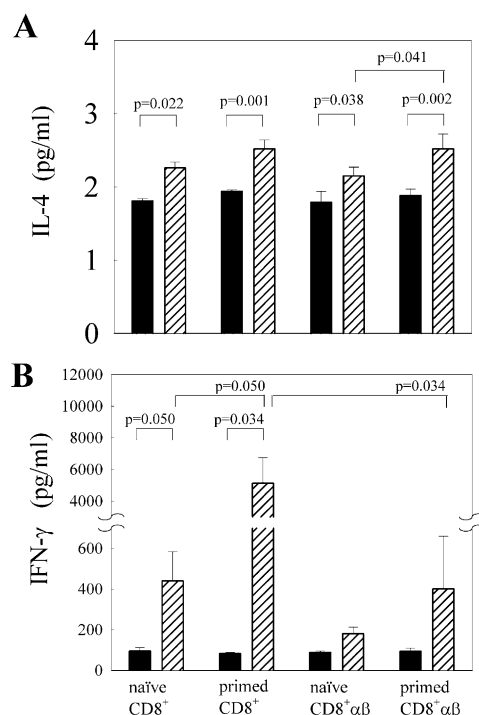


FIG 5. The cytokine expression by CD8 $^{+}$ T cells (mixed $\alpha\beta$ and $\gamma\delta$) and CD8 $^{+}$ $\alpha\beta$ T cells *in vitro*. CD8 $^{+}$ T cells or CD8 $^{+}$ $\alpha\beta$ T cells (0.5×10^6) were cultured with (hatched bars) and without (filled bars) phorbol 12-myristate 13-acetate and ionomycin (n = 3–5).

systemic sensitization.³⁶ However, the inhibitory effect of CD8 $^{+}$ T cells composed of a mixture of both CD8 $^{+}$ $\alpha\beta$ T cells and CD8 $^{+}$ $\gamma\delta$ T cells⁹ required prior sensitization for these cells to inhibit allergic airway responses. These observations suggest interactions of the 2 CD8 $^{+}$ T-cell subsets *in vivo*. The same number of CD8 $^{+}$ T cells (mixed $\alpha\beta$ and $\gamma\delta$) and CD8 $^{+}$ $\alpha\beta$ T cells was cultured and the expression of cytokines was measured to further explore this question. Stimulated and primed CD8 $^{+}$ $\alpha\beta$ T cells expressed significantly higher levels of IL-4 than those of stimulated naive CD8 $^{+}$ $\alpha\beta$ T cells. Total CD8 $^{+}$ T cells did not show any significant difference in IL-4 expression. Although IL-4 mRNA expression in CD8 $^{+}$ $\alpha\beta$ T cells was clearly detected by means of *in situ* hybridization, the IL-4 protein level produced by CD8 $^{+}$ $\alpha\beta$ T cells in culture was low, which is consistent with previous reports.^{37,38} Nevertheless, IL-4 expressed by CD4 $^{+}$ T cells is essential for airway inflammation and airway responsiveness in this strain.³² Primed CD8 $^{+}$ $\alpha\beta$ T cells expressed significantly less IFN- γ than did the primed and mixed CD8 $^{+}$ T cells. Because the ratio of $\alpha\beta$ to $\gamma\delta$ of the mixed CD8 $^{+}$ T cells

was 10:1, either the CD8⁺ γδ T cells accounted for the additional IFN-γ or the CD8⁺ γδ T cells promoted the biasing of the CD8⁺ αβ T cells in the culture toward a type I differentiation.

Recently, the influence of endotoxin on allergic airway responses has received considerable attention. Low-dose LPS has been reported to facilitate the development of a T_H2 response to sensitization with OVA, whereas a high dose is inhibitory in its effects.³⁸ Perhaps of more direct relevance to our study is the finding that the contamination of commercial OVA by LPS is sufficient to attenuate allergen-induced airway responses.³⁹ It is therefore possible that if we had used LPS-free OVA, we might have observed even larger effects of the challenge.

The results of the study show that antigen-primed CD8⁺ αβ T cells can transfer allergen sensitivity to naive rats and can mediate the development of late responses after allergen challenge. In this regard their function is similar to that of CD4⁺ T cells in the BN rat. The study does not address the issue of the relative contributions of MHC class I- and class II-restricted antigen presentation to the generation of allergic airway responses and inflammation after allergen challenge of actively sensitized animals. However, it is possible that the phenotype of the allergen-responsive CD8⁺ T cells might have a major role in determining whether airway exposure to allergen is associated with T_H2-type inflammation.

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