

The effects of CD8⁺γδ T cells on late allergic airway responses and airway inflammation in rats

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Background: Gamma-delta (γδ) T cells regulate immune responses to foreign protein at mucosal surfaces. Whether they can modify allergen-induced early (EAR) and late airway responses (LAR) is unknown.

Objective: We have tested the hypothesis that the CD8⁺ sub-type of γδ T cells decreases allergen-induced LAR and airway eosinophilia in the rat.

Methods: Brown Norway rats were administered, intraperitoneally, 3.5×10^4 lymph node CD8⁺γδ T cells from naive or sensitized rats. The recipients were sensitized to ovalbumin (OVA) in Al(OH)₃ 3 days after cell transfer and challenged with aerosolized OVA 14 days later. Serum IgE was measured before allergen challenge. After challenge, lung resistance was monitored for 8 hours and then bronchoalveolar lavage (BAL) was analyzed for eosinophil major basic protein (MBP), IL-4, IL-5, IL-13, and IFN-γ messenger RNA-expressing cells.

Results: γδ T cells from naive donors significantly decreased LAR in OVA-challenged sensitized rats, whereas MBP⁺ eosinophils were decreased by both γδ T cells from naive and sensitized donors. EAR and serum IgE levels were unchanged. The expression of IL-4, IL-5, and IL-13 by BAL cells of γδ T cell recipients was attenuated compared with OVA-challenged controls. This was accompanied by an increase in the expression of IFN-γ.

Conclusions: Our results are consistent with a suppressive role of CD8⁺γδ T cells on allergic airway responses. However, only γδ T cells from naive donors inhibit LAR. (J Allergy Clin Immunol 2003;112:547-55.)

Key words: CD8⁺γδ T cells, TCR-γδ, late allergic responses, LAR, BN rat

Abbreviations used

BAL:	Bronchoalveolar lavage
BN:	Brown Norway (rats)
EAR:	Allergen-induced early airway responses
ip:	Intraperitoneal injection
LAR:	Allergen-induced late airway responses
MACS:	Magnetic cell sorting
MBP:	Eosinophil-derived major basic protein
ND group:	Animals that were transferred CD8 ⁺ γδ T cells from naive donors
OVA group:	Animals that were injected with PBS and challenged with OVA
R _L :	Lung resistance
Saline group:	Animals that were injected with PBS and challenged with saline
SD group:	Animals that were transferred CD8 ⁺ γδ T cells from OVA-sensitized donors
TCR:	T-cell receptor

T cells play a pivotal role in the immune system of mammals. Of the various T cell subsets, the γδ T-cell receptor (TCR)-bearing cells are the least well understood. In contrast to the αβ TCR⁺ cells, the γδ TCR⁺ cells might recognize some antigens directly.¹ However, they might also recognize peptides that bind to MHC molecules with the assistance of co-receptors.^{2,3} Conflicting observations have been reported about the potential role of γδ T cells in asthma. Airway responsiveness to methacholine is increased after inhalational challenge with ovalbumin (OVA) in naive γδ T-cell-deficient mice,⁴ whereas allergen-specific IgE and allergen-induced pulmonary IL-5 release, airway eosinophil, and T-cell infiltration are lower in sensitized γδ T-cell-deficient mice.⁵ The net effect of the γδ T cells is to augment allergen-induced airway bronchoconstrictive responses, because airway hyperresponsiveness is attenuated in antigen-challenged sensitized γδ T-cell-deficient mice.⁶

The surface marker of γδ T cells is mainly CD8⁺, whereas the CD4⁺CD8[−] cells and the even rarer CD4⁺ cells represent only a small fraction of the total γδ pool. The roles of the different γδ T-cell subsets have received little attention. The adoptive transfer of a small number of

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Supported by Canadian Institutes for Health Research Grant 10381.

Received for publication January 9, 2003; revised May 16, 2003; accepted for publication May 20, 2003.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1682

CD8⁺γδ T cells from OVA-tolerant mice suppresses IgE antibody production.⁷ This effect is mediated by IFN-γ secretion.⁷ In a rodent model of allergic asthma, antigen-primed CD8⁺ cells, not characterized as to their TCR (αβ and γδ), have a potent suppressive effect on allergen-induced late airway responses (LARs), T_H2 cytokine expression, and allergen-induced airway eosinophilia.⁸ We reasoned that the CD8⁺γδ T-cell subset might account for the observed suppression of allergen-induced airway responses in the rat. To address this question, we used the OVA-sensitized Brown Norway (BN) rat model of allergic asthma that shows late airway responses and a T_H2-type airway inflammation after OVA challenge.⁹ Adoptive transfer of γδ cells to recipient animals was used to test the effects of the CD8⁺ subset of these cells on sensitization and on airway responses to allergen challenge. We examined the effects of the transfer of γδ cells from both sensitized and naive donor animals on allergen-induced airway eosinophilia and LARs. We explored the mechanisms of the observed inhibitory effects of the γδ cells by assessing the effects on serum IgE, T_H1, and T_H2 cytokine-expressing cells in the bronchoalveolar lavage (BAL) fluid and eosinophil clearance by macrophages.

METHODS

Animals and sensitization

BN rats (males; aged 10–12 weeks; 190–240 g) were purchased from Harlan Sprague Dawley UK (Blackthorn, UK) and were maintained in conventional animal facilities. Experimental procedures were approved by an institutional animal care committee. The animals were sensitized by a single subcutaneous injection of 20 μg OVA (grade V, Sigma, St Louis, Mo) in 1.0 mL normal saline containing 4.28 mg aluminum hydroxide gel (Anachemia Chemicals, Montreal, Canada). Simultaneously, 0.5 mL of *Bordetella pertussis* vaccine containing 5×10^8 heat-killed bacilli were injected intraperitoneally (ip).

Study protocol

CD8⁺γδ T cells were harvested from the cervical lymph nodes of either naive donor rats or OVA-sensitized rats and purified by magnetic cell sorting (MACS) with negative selection columns (Miltenyi Biotec Inc, Auburn, Calif). Donor rats were sensitized 2 weeks before harvesting of the T cells. Recipient BN rats were given 3.5×10^4 CD8⁺γδ T cells ip on day 1, whereas control rats were given PBS ip. All animals were sensitized to OVA on day 3. Serum samples were taken for the measurement of OVA-specific IgE levels on day 17. BN rats that were given CD8⁺ γδ T cells from either naive donor rats (animals that were transferred CD8⁺γδ T cells from naive donors [ND group]; n = 9) or OVA-sensitized rats (animals that were transferred CD8⁺γδ T cells from OVA-sensitized donors [SD group]; n = 9) were challenged with aerosolized OVA (5% in saline). Control rats that were given PBS were challenged with either aerosolized OVA (OVA group; n = 12) or saline (saline group; n = 9). Lung resistance (R_L) was measured before aerosol challenge at 5, 10, 15, 20, and 30 minutes after aerosol challenge, and subsequently at 15-minute intervals for a total of 8 hours. On completion of the lung function measurements, BAL was performed.

To exclude the possibility that cells that were not removed during cell sorting and therefore contaminated the CD8⁺ γδ T cell population might have accounted for the observed effects of the cell transfers, we also tested the effects of the transfer of an equivalent num-

ber of these cells (3.5×10^3). We administered the contaminating cell population harvested from naive and sensitized donor animals to sensitized recipients (n = 4/group) and compared the allergic airway responses and airway inflammation with a control sensitized group (n = 7) following the previous protocol.

Antibodies

ED9 (MCA620R, mouse anti-rat CD172a mAb, IgG1), MRC OX-33 (MCA340G, mouse anti-rat CD45RA mAb, IgG1), and 10/78 (MCA1427, mouse anti-rat CD161, IgG1) were purchased from Serotec Inc (Raleigh, NC). W3/25 (CL003AP, mouse anti-rat CD4 mAb, IgG1) was purchased from Cedarlane Laboratories (Hornby, ON, Canada). R73 (22281D, mouse anti-rat αβ TCR mAb, IgG1) was purchased from BD Pharmingen (Mississauga, ON, Canada). Rat anti-mouse IgG1 microbeads were purchased from Miltenyi Biotec Inc., BMK13, a mouse anti-human major basic protein (MBP) antibody, was provided by Dr R. Moqbel (University of Alberta, Edmonton, Canada). For fluorescence-activated cell sorter analysis, OX-8 (MCA48C, mouse anti-rat CD8 mAb: RPE-Cy5) was purchased from Serotec Inc, V65 (22464D, mouse anti-rat γδ mAb: FITC) was purchased from BD Pharmingen.

Adoptive transfer of CD8⁺γδ T cells

T cells were harvested from the cervical lymph nodes of 3 rats and pooled before administration to 3 recipients. Purification of CD8⁺γδ T cells was done using magnetic cell sorting in 2 steps. First, CD8⁺ T cells were purified by incubating cells with primary antibodies (W3/25; anti-CD4, OX-33; anti-B cell, ED9; anti-myeloid cell, 10/78; anti-NK cell). Then, cells were incubated with MACS beads. Unlabeled cells that passed through the MACS column were mainly CD8⁺ T cells. Second, CD8⁺γδ T cells were purified by incubating the CD8⁺ cells with primary antibody against the αβ TCR (R73; anti-αβ). Then the cells were incubated with MACS beads. Unlabeled cells that passed through the MACS column were again collected. The purity of the cells was measured using flow cytometry (FACScan, Becton Dickinson, Mountain View, Calif). CD8⁺γδ T cells (3.5×10^4) were transferred to each recipient ip. To study the effects of the cells contaminating the enriched CD8⁺γδ T cell population, the second purification step was performed with a mixture of antibodies against both the αβ and γδ TCR.

Immunocytochemical evaluation of IL-4 and IFN-γ expression

Cytospin slides were prepared from CD8⁺γδ T-cell isolates before transfer to recipient animals. The slides were fixed in acetone-methanol, incubated with antibodies OX-81 (24051A, mouse anti-rat IL-4 mAb, BD Pharmingen), DB-1 (20441N, mouse anti-rat IFN-γ mAb, BD Pharmingen), or A112.2 (20351C, mouse IgG1 isotype control, BD Pharmingen) and stained using a streptavidin-biotin kit (DAB II, DAKO, Mississauga, Ontario, Canada). The numbers of immunoreactive cells were assessed by a blinded investigator.

OVA-specific IgE measurement by ELISA

OVA-specific IgE was measured by ELISA as previously described¹⁰ on serum samples obtained before OVA challenge.

Measurement of airway responses to OVA

On day 17, the BN rats were anesthetized by urethane (1.2 g/kg body weight) ip and instrumented as described previously.¹¹ R_L was calculated from airflow and esophageal pressure measurements using the technique of multiple linear regression to solve the equation of motion for the lung.

BAL

Twenty-five milliliters of sterile saline was instilled in 5-mL increments 8 hours after antigen challenge, and total BAL cell counts were determined using a hemacytometer. BAL cytospin slides were prepared using a Cytospin Model II (Shandon, Pittsburgh, Pa). The slides were Wright-Giemsa stained, and cellular differentials were assessed by a blinded investigator on a count of 300 cells.

Immunocytochemical evaluation of eosinophil-derived MBP expression

BAL cytospin slides were fixed in 4% paraformaldehyde for 30 minutes and were stained with mouse anti-human MBP mAb (BMK-13) using the alkaline phosphatase anti-alkaline phosphatase method.

Detection of apoptotic cells in BAL

To detect apoptotic cells in BAL, we used a fragmented DNA labeling technique (ApopTag peroxidase detection kit; Intergen, Purchase, NY). The BAL cytospin samples were pretreated with 20 μ g/mL proteinase K (Intergen) for 15 minutes at 37°C, and endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 5 minutes. This was followed by polymerization of digoxigenin-labeled uridine triphosphate on nicked DNA ends, application of anti-digoxigenin peroxidase conjugate, and development with 3,3'-diamino-benzidine chromogen substrate.

Cytokine messenger RNA (mRNA) detection by in situ hybridization

Cytospin slides were prepared using BAL fluid, and fixed by 4% paraformaldehyde for 30 minutes. Measurement of the expression of mRNA for IL-4, IL-5, IL-13, and IFN- γ was performed according to previously described methods.¹² Antisense and sense riboprobes were prepared from cDNA coding for rat IL-4, IL-5, IL-13, and IFN- γ mRNA. The probes for IL-4, IL-5, IL-13, and IFN- γ were gifts from Drs A. Neil Barclay (Oxford, UK), T. Blankenstein (Berlin, Germany), Fadi G. Lakkis (Atlanta, Ga), and P. H. van der Meide (Rijswijk, The Netherlands), respectively.

Statistical analysis

Unless otherwise indicated data are presented as mean $\pm$ SE. When data were not normally distributed, we have shown the median, 10th, 25th, 75th, and 90th percentiles. Statistical comparisons were performed using the Kruskal-Wallis and the Mann-Whitney *U* tests. *P* values less than .05 were considered significant.

RESULTS

CD8 $^{+}$ $\gamma\delta$ T-cell purification and transfer

The efficacy of immunomagnetic cell purification of CD8 $^{+}$ $\gamma\delta$ T cells harvested from the cervical lymph nodes was assessed by FACS analysis and demonstrated 88.8% $\pm$ 2.4% purity (*n* = 3). The survival ratios of CD8 $^{+}$ $\gamma\delta$ T cells (live cells/total cells) by Trypan blue exclusion, before their injection into the animals were 95.9% $\pm$ 2.2% (*n* = 3) and 95.8% $\pm$ 2.6% (*n* = 3) for cells harvested from naive and sensitized donors, respectively.

Cytokine expression by purified CD8 $^{+}$ $\gamma\delta$ T cells before transfer

Inhibitory immunomodulatory effects of $\gamma\delta$ T cells have been previously attributed to IFN- γ .¹³ For this rea-

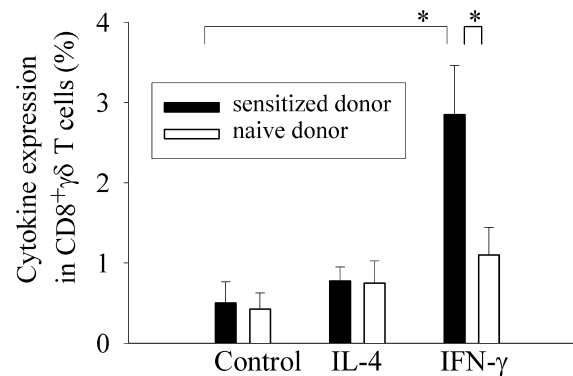


FIG 1. Cytokine expression in CD8 $^{+}$ $\gamma\delta$ T cells purified from cervical lymph nodes by immunohistochemical staining (**P* < .05).

son, we examined the expression of IFN- γ by purified CD8 $^{+}$ $\gamma\delta$ T cells before transfer. The number of cells that were IFN- γ positive was assessed by immunostaining and was higher in CD8 $^{+}$ $\gamma\delta$ T cells harvested from the sensitized donor group than the naive donor group (**P* < .05; Fig 1). There was no evidence of significant IL-4 expression by cells from either group.

Effect of CD8 $^{+}$ $\gamma\delta$ T cells on the BAL leukocytes

There were significant but highly selective anti-inflammatory effects of $\gamma\delta$ T cell transfers as evidenced from the changes in BAL fluid leukocytes. There was no significant difference in the total cell counts among the groups. However, ND and SD groups showed substantially reduced eosinophil numbers compared with the OVA group (*P* < .05; Table I). The sham-challenged saline group showed fewer neutrophils compared with that of the OVA group, ND, and SD groups, respectively (**P* < .05; ****P* < .005; Table I). There were no significant differences in lymphocytes and macrophages among groups.

To confirm the effects of the $\gamma\delta$ cell transfers on OVA-induced eosinophilia, we performed immunostaining for MBP on BAL fluid cytopins. MBP $^{+}$ cells with the morphologic characteristics of eosinophils are shown in Fig 2, A. The percentage of MBP $^{+}$ eosinophils in BAL fluid is shown in Fig 3, A. All of the ND, SD, and saline groups had significantly lower levels of eosinophils compared with the OVA group (**P* < .05, ****P* < .005).

MBP-immunoreactive macrophages in BAL fluid

We counted MBP immunoreactive cells with the morphology of macrophages as an index of the rate of ingestion of eosinophils and therefore clearance of these cells by alveolar macrophages (Fig 2, B). The percentage of MBP $^{+}$ macrophages in BAL fluid is shown in Fig 3, B. MBP $^{+}$ macrophages in the SD group were significantly higher than the saline group (**P* < .05). To correct the index of eosinophil clearance by macrophages for the absolute number of eosinophils in the BAL fluid, we calculated the ratio of MBP $^{+}$ macrophages to MBP $^{+}$

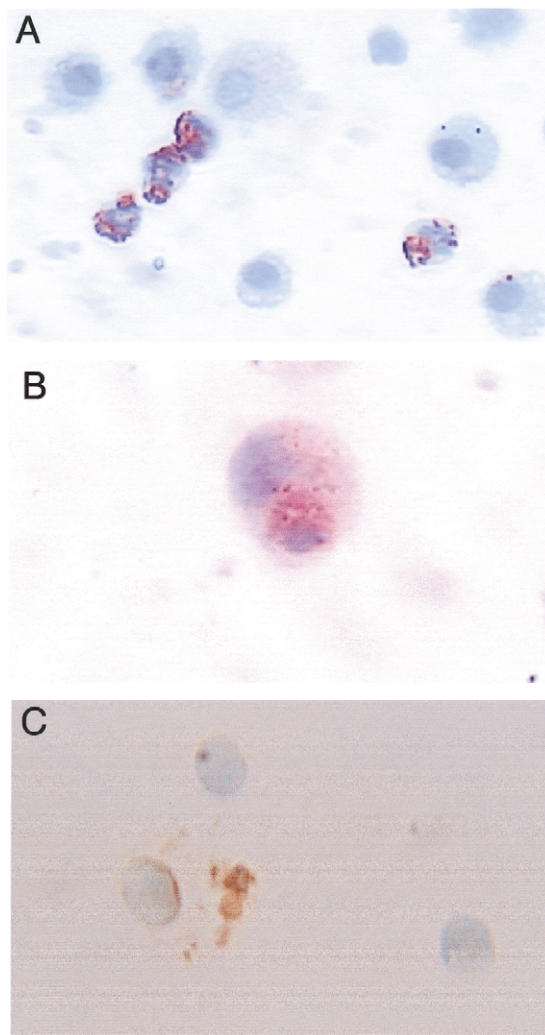


FIG 2. Representative photomicrographs of an eosinophil-derived major basic protein (MBP⁺), immunoreactive eosinophils (**A**), and an MBP⁺ macrophage that phagocytosed an MBP⁺ eosinophil (**B**) in bronchoalveolar lavage fluid (BALF) from the ovalbumin control group. **C**, A representative example of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling apoptosis immunoreactivity in BALF from animals that were transferred CD8⁺γδ T cells from naive donors. A macrophage contains phagocytosed apoptotic cells.

eosinophils for each group (Fig 3, C). The ratio for the 2 test groups (those receiving CD8⁺γδ T cells) was higher than that of the 2 control groups. In the 2 test groups, it is possible that either the activity of the macrophages increased or the survival time of eosinophils decreased. A representative example of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling apoptosis immunoreactivity in BAL fluid from the naive donor group is shown in Fig 2, C, confirming that, indeed, the macrophages are phagocytosing apoptotic eosinophils.

Cytokine mRNA-expressing cells in BAL fluid

To assess the contribution of alterations in T-cell cytokine expression to the changes in inflammation, we

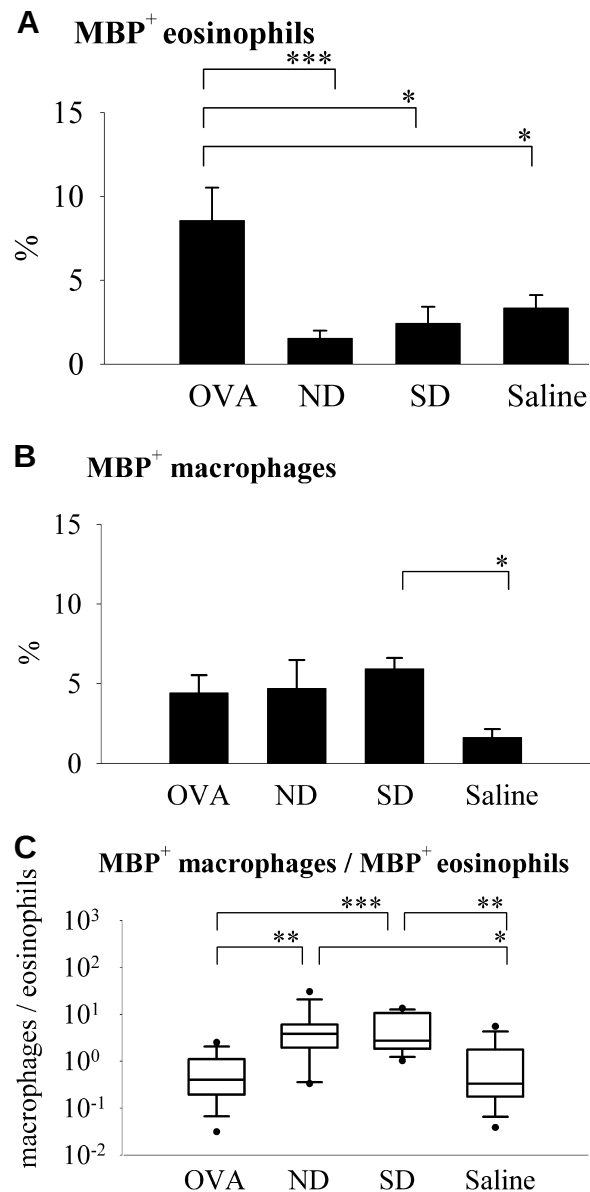


FIG 3. Eosinophil-derived major basic protein (MBP) immunoreactivity in bronchoalveolar lavage (BAL) cells. **A**, The percentage of MBP⁺ eosinophils in BAL fluid. **B**, The percentage of MBP⁺ macrophages in BAL fluid. Figures in indicate the means and SEMs. **C**, The ratio of MBP⁺ macrophages/MBP⁺ eosinophils was calculated. In (**C**) the median, 10th, 25th, 75th, and 90th percentiles are shown (* $P < .05$, ** $P < .01$, *** $P < .005$).

determined the number of BAL fluid cells expressing IL-4, IL-5, and IL-13 by use of in situ hybridization. The number of cells positive for IL-4 mRNA was significantly lower in both the ND and SD group than in the OVA group (* $P < .05$, *** $P < .005$; Fig 4, A). Also, the number of positive cells in the SD group was significantly lower than in the ND group (*** $P < .005$). The number of cells positive for IL-5 mRNA was significantly lower in both the ND and SD groups compared with the OVA control group (*** $P < .005$; Fig 4, B). As well, the num-

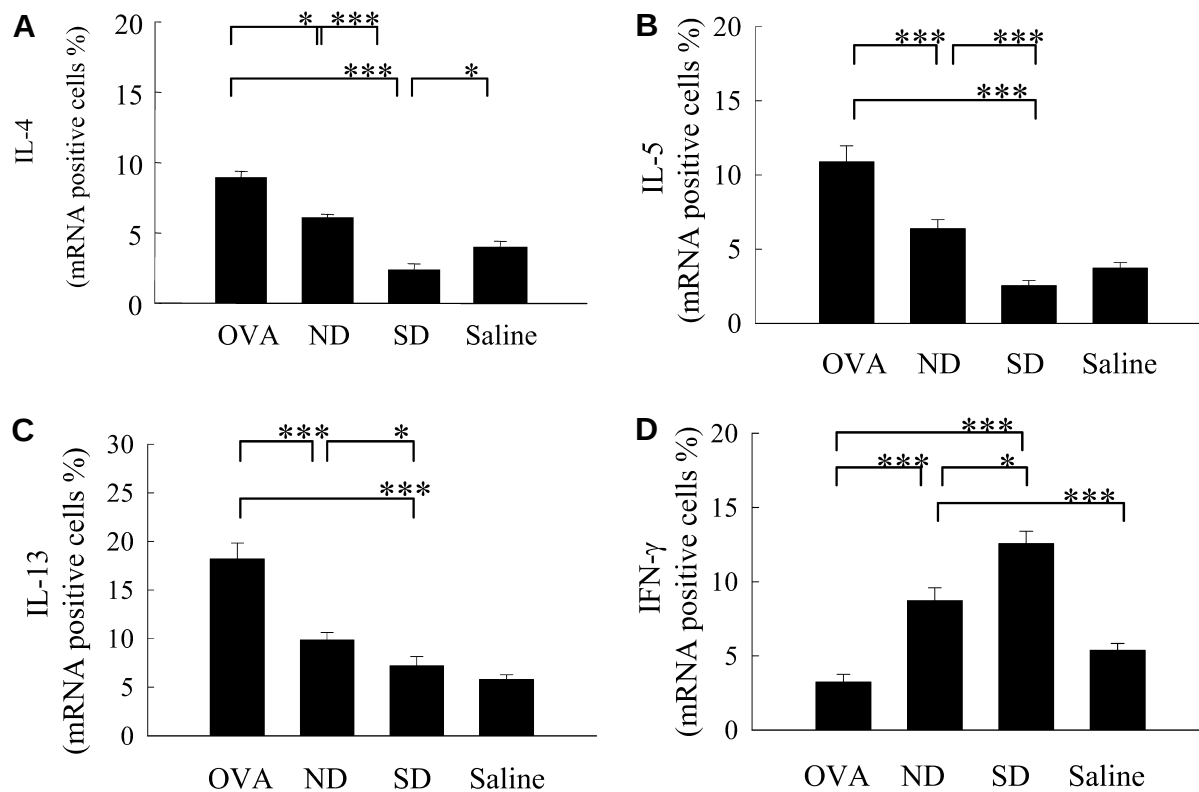


FIG 4. IL-4, IL-5, IL-13, and IFN- γ messenger RNA (mRNA)-positive cells detected in bronchoalveolar lavage (BAL) by in situ hybridization. **A**, The number of positive cells for IL-4 mRNA is shown. **B**, The number of positive cells for IL-5 mRNA is shown. **C**, The number of cells positive for IL-13 mRNA is shown. **D**, The number of cells positive for IFN- γ mRNA is shown ($*P < .05$, $***P < .005$).

ber of positive cells in the SD group was significantly lower than the ND group ($***P < .005$). The number of cells positive for IL-13 mRNA was significantly lower in the ND and SD groups than the OVA control group ($***P < .005$; Fig 4, C).

We examined the possibility that the $\gamma\delta$ T cells might have exerted their effects through IFN- γ . The number of cells positive for IFN- γ mRNA was assessed by in situ hybridization and was significantly higher in the ND and SD groups than both the OVA and saline groups ($***P < .005$; Fig 4, D), consistent with a switch in T-cell differentiation to a T_H1 phenotype by $\gamma\delta$ T cells. Again, the number of IFN- γ -positive cells in the SD group was significantly higher than that of the ND group ($*P < .05$).

OVA-specific IgE

To examine the effects of the $\gamma\delta$ cells on the response to sensitization with OVA, we measured OVA-specific IgE on sera obtained immediately before OVA challenge. Sera from naive donor rats were used as negative controls (naive group; $n = 4$). Sera of the OVA and saline groups were pooled, because both groups were sensitized in the same manner. The absorbance values for OVA-specific IgE in the groups of OVA + saline, ND, and SD were significantly higher than the naive group ($*P < .05$, $**P < .01$, $***P < .005$, Fig 5).

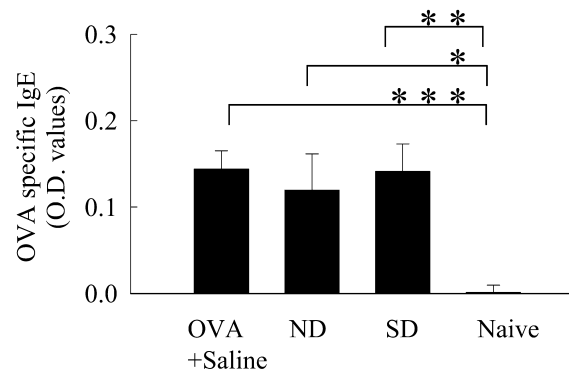


FIG 5. Serum ovalbumin (OVA)-specific IgE levels in sensitized control rats (OVA + saline), sensitized recipients of CD8 $^{+}$ $\gamma\delta$ T cells from naive donors (ND) and sensitized donors (SD), and naive rats (naive). Sera of control rats that were given PBS and were challenged with either aerosolized OVA (OVA group) or saline (saline group) were pooled and served as positive controls. Sera from naive rats were used as negative controls (naive group; $n = 4$) ($*P < .05$, $**P < .01$, $***P < .005$).

There were no significant differences among these 3 groups, indicating that $\gamma\delta$ T cell transfers did not alter the production of IgE.

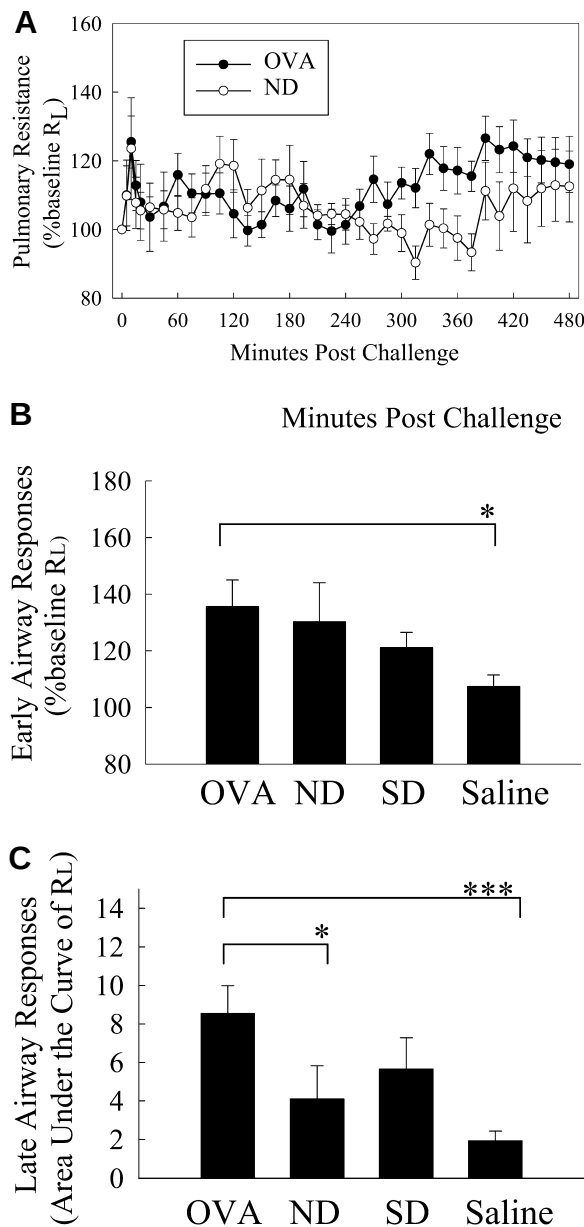


FIG 6. Measurement of airway responses to ovalbumin (OVA). **A**, Data for 2 groups illustrating the time course of lung resistance (R_L) after OVA challenge are shown. Symbols indicate the mean values for each group, and the vertical bars indicate the SEs. **B**, Allergen-induced early airway response were determined from the peak in the R_L within 1 hour after the challenge. **C**, The allergen-induced late airway responses were determined by calculation of the area under the curve of R_L against time from 3 to 8 hours after the challenge (* $P < .05$, *** $P < .005$).

Effect of CD8 $^{+}$ $\gamma\delta$ T cells on airway responses to OVA

Baseline lung function was not affected by cell transfers as indicated by the fact that R_L before antigen challenge was not significantly different among the 4 groups. The time course of R_L after OVA challenge is shown in Fig 6, A, for the ND and OVA groups. Allergen-induced

early airway responses (EARs) were 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 the saline-challenged PBS-injected animals (* $P < .05$; Fig 6, B). The EAR after $\gamma\delta$ T cell transfers was not different from that of the OVA group. The LAR was determined by 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 saline and ND groups were significantly lower than the OVA group (* $P < .05$, *** $P < .005$, Fig 6, C). There was also a nonsignificant trend ($P = .155$) for the LAR to be lower in the SD group.

To exclude the possibility that the small percentage of cells (12%, approximately) contaminating the CD8 $^{+}$ $\gamma\delta$ cell population might have affected the results of our experiments, we tested the effects of transfer of an equivalent number of the contaminating cells (3.5×10^3) on airway responses and BAL fluid eosinophilia. Neither the LAR nor the degree of BAL fluid eosinophilia was altered significantly by the transferred cells (Tables II and III).

DISCUSSION

In this study, we found that the administration of $\gamma\delta$ T cells to rats before sensitization resulted in a marked inhibition of the LAR and of allergic inflammation evoked by aerosol challenge. The mechanism of the inhibition was not dependent on IgE; serum levels of OVA-specific IgE were unaffected by the $\gamma\delta$ T cell transfers. T-cell cytokine profiles were biased toward a T_H1 profile, consistent with the observed reduction in BAL fluid eosinophilia. Macrophages in the BAL fluid of recipients of $\gamma\delta$ T cells transfers showed disproportionately high numbers of phagocytosed eosinophils, suggesting the possibility of an enhanced rate of phagocytosis as an additional mechanism for the reduction in eosinophilia.

We chose to study CD8 $^{+}$ $\gamma\delta$ T cells because we reasoned that this subset would be likely to express the prototypic T_H1 cytokine IFN- γ . The literature to date suggests that CD4 $^{+}$ $\gamma\delta$ T cells tend to secrete T_H2 -type cytokines¹⁴ and CD8 $^{+}$ $\gamma\delta$ T cells T_H1 -type cytokines.¹³ Only a small proportion of the cells we harvested from the cervical lymph nodes expressed IFN- γ by immunostaining, but the number of cells expressing this cytokine was greater when cells were harvested from sensitized animals than from naive animals. Rat CD8 $^{+}$ $\gamma\delta$ T cells that have been harvested from OVA-tolerant animals strongly express IFN- γ .¹³ IL-4 expression was not detectable by this technique. In contrast to the CD8 $^{+}$ $\gamma\delta$ T cells, there is strong association of murine CD4 $^{+}$ $\gamma\delta$ T cells to the T_H2 cytokine profile.¹⁵ Likewise, CD4 $^{+}$ $\gamma\delta$ T cells in the nasal mucosa of human subjects express T_H2 -type cytokines,¹⁴ including IL-4, and not IFN- γ .¹⁶ Although CD4 $^{+}$ $\gamma\delta$ T cells have been reported to be increased in the BAL of patients with asthma,¹⁷ the cytokine profile secreted by these cells does not seem to have been examined.

Eosinophilia and T_H2 cytokine expression were seen in BAL after OVA challenge of OVA-sensitized rats, sim-

TABLE I. Total cell count and cell profile in bronchoalveolar lavage cells*

Group	Total cells ($\times 10^6$)	Macrophages ($\times 10^6$)	Neutrophils ($\times 10^3$)	Eosinophils ($\times 10^3$)	Lymphocytes ($\times 10^3$)	Basophils ($\times 10^3$)
OVA† (n = 11)	3.056 $\pm$ 0.439	2.592 $\pm$ 0.364	164.0 $\pm$ 50.9‡	273.4 $\pm$ 92.5	38.0 $\pm$ 10.8	1.1 $\pm$ 1.8
ND§ (n = 9)	2.939 $\pm$ 0.213	2.718 $\pm$ 0.185	121.0 $\pm$ 24.2‡	55.7 $\pm$ 27.5¶	50.7 $\pm$ 21.6	0.7 $\pm$ 0.6
SD** (n = 9)	2.778 $\pm$ 0.290	2.570 $\pm$ 0.239	108.0 $\pm$ 30.3††	63.1 $\pm$ 42.9¶	35.3 $\pm$ 8.7	0.7 $\pm$ 0.7
Saline‡‡ (n = 9)	2.713 $\pm$ 463.1	2.522 $\pm$ 0.419	20.2 $\pm$ 4.56	144.2 $\pm$ 85.2	27.3 $\pm$ 8.4	0.0 $\pm$ 0.0

*Data are expressed as average $\pm$ SEM.
†OVA group, animals that were injected with PBS and challenged with ovalbumin.
‡P < .005 compared with saline group.
§ND group, animals that were transferred CD8 $^{+}$ γ δ T cells from naive donors.
¶P < .05 compared with OVA group.
**SD group, animals that were transferred CD8 $^{+}$ γ δ T cells from ovalbumin-sensitized donors.
††P < .05 compared with saline group.
‡‡Saline group, animals that were injected with PBS and challenged with saline.

TABLE II. Effect of transfer of the cells contaminating the enriched CD8 $^{+}$ γ δ T cells on baseline lung resistance, allergen-induced early airway responses, and allergen-induced late airway responses after ovalbumin challenge*

Group	Baseline† (R _L)	Early airway responses† (Peak R _L / Baseline R _L %)	Late airway responses,† (area under the curve of R _L)
C-OVA‡ (n = 7)	0.151 $\pm$ 0.01	131.0 $\pm$ 21.0	6.55 $\pm$ 1.68
C-ND§ (n = 4)	0.155 $\pm$ 0.01	117.2 $\pm$ 4.5	6.66 $\pm$ 1.51
C-SD¶ (n = 4)	0.151 $\pm$ 0.01	113.0 $\pm$ 3.6	6.49 $\pm$ 4.29

R_L, Lung resistance.
*Data are expressed as mean $\pm$ SEM.
†There were no significant differences among the 3 groups.
‡C-ND group, animals that were transferred CD8 $^{+}$ γ δ T cells from naive donors.
§C-SD group, animals that were transferred CD8 $^{+}$ γ δ T cells from ovalbumin-sensitized donors.
¶C-OVA group, animals that were injected with PBS and challenged with ovalbumin.

TABLE III. Effect of the transfer of cells contaminating the enriched CD8 $^{+}$ γ δ T cells on total cell count and cell differentials in bronchoalveolar lavage cells after ovalbumin challenge*

Group	Total cells† ($\times 10^6$)	Macrophages† ($\times 10^6$)	Neutrophils† ($\times 10^3$)	Eosinophils† ($\times 10^3$)	Lymphocytes† ($\times 10^3$)	Basophils† ($\times 10^3$)
C-OVA‡ (n = 7)	4.100 $\pm$ 0.640	3.489 $\pm$ 0.656	300.0 $\pm$ 63.5	190.8 $\pm$ 53.18	64.1 $\pm$ 12.6	0.0 $\pm$ 0.0
C-ND§ (n = 4)	5.325 $\pm$ 0.189	4.646 $\pm$ 0.271	295.0 $\pm$ 110.0	242.2 $\pm$ 73.7	131.3 $\pm$ 35.6	0.0 $\pm$ 0.0
C-SD¶ (n = 4)	4.775 $\pm$ 1.430	4.111 $\pm$ 1.252	297.0 $\pm$ 106.0	274.0 $\pm$ 144.6	92.9 $\pm$ 42.0	0.0 $\pm$ 0.0

*Data are expressed as mean $\pm$ SEM (n = 12).
†There were no significant differences among the 3 groups.
‡C-OVA group, animals that were injected with PBS and challenged with ovalbumin.
§ND group, animals that were transferred contaminated cells of CD8 $^{+}$ γ δ T cells from naive donors.
¶SD group, animals that were transferred contaminated cells of CD8 $^{+}$ γ δ T cells from ovalbumin-sensitized donors.

ilar to previous reports.⁹ There was marked suppression of eosinophilia by the transferred CD8 $^{+}$ γ δ T cells. Recipients of cells from sensitized donors had a more T_H1-like cytokine profile in the BAL than the recipients from the naive donor group; IFN- γ -expressing cells were more numerous, and IL-4 and IL-5-expressing cells significantly fewer. IFN- γ is capable of differentiating resident CD4 $^{+}$ T cell to the T_H1 phenotype¹⁸ and might amplify the T_H1 response within the airways.¹⁹ The reduction in eosinophilia is likely caused by the reduction in T_H2 cytokines through loss of chemotactic and survival signals, as well as changes in adhesion molecule expression.^{20,21} The minimum duration required for the transferred γ δ T cells to condition the airway response is not known. However, if the inhibitory actions are mediated

by IFN- γ secretion from γ δ T cells, such effects could be exerted quite rapidly. The inhalation of IFN- γ just before OVA challenge changes cytokine expression in BAL cells from T_H2 to T_H1 within several hours.⁹ The ratio of MBP $^{+}$ macrophages to MBP $^{+}$ eosinophils was higher in the 2 γ δ T-cell-recipient groups than in the 2 control groups. In the 2 test groups, it is possible that either the activation of the macrophages increased the rate of phagocytosis of eosinophils or, more likely, that the proportion of viable eosinophils decreased. The former mechanism could result from an action of resident T_H1 cells and/or CD8 T cells on the macrophages. Perhaps macrophages are activated by γ δ T cells, potentially by means of IFN- γ ; alveolar macrophages activated in this way have been shown to have an anti-inflammatory

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action.²² The latter mechanism could be caused by an effect of IFN- γ secreted from resident T_H1 cells and/or CD8 T cells so as to induce the apoptosis of eosinophils.²³ The smaller numbers of IL-5-expressing cells in $\gamma\delta$ T-cell recipients also favors an enhanced rate of apoptosis.²⁴

There were differences between the effects of $\gamma\delta$ T cells from naive and sensitized donors on cytokine profiles and the inhibition of both eosinophilia and LAR. Cells from the sensitized donors were more effective in causing a T_H1-type cytokine response in BAL cells of recipient animals, but the cells from the naive donors caused a similar degree of inhibition of eosinophilia. This observation suggests that $\gamma\delta$ T cells might have effects on the regulation of eosinophilia that are independent of IL-5. The higher ratio of MBP positive macrophages, presumably a reflection of the clearance of these cells from the bronchoalveolar compartment of the lung in recipients of cells from naive donors, is consistent with enhanced clearance as a possible alternate explanation for the findings. Contrary to the effects on cytokines, the $\gamma\delta$ T cells from sensitized donors did not cause a statistically significant inhibition of the LAR in the recipient animals. This fact indicates that the inhibition of the LAR to OVA by CD8⁺ $\gamma\delta$ T cells might not be determined solely by the effects on CD4⁺ $\alpha\beta$ T-cell-derived T_H2 cytokines and IFN- γ secretion.⁴ A similar experiment using a mixed CD8⁺ T-cell population showed that these cells suppressed eosinophilia by an IFN- γ -dependent mechanism, whereas the inhibition of the LAR by these cells was independent of IFN- γ .²⁵ The results of this study also point strongly to an independence of the LAR and eosinophilia.

To know whether the differences in efficacy of CD8⁺ $\gamma\delta$ T cells were reflected in differences in the profile of cytokines, T_H1 vs T_H2, synthesized by these cells before transfer, we examined the numbers of cells that were positive for immunoreactive IFN- γ and IL-4. We did not detect any evidence of IL-4 synthesis by this technique. Likewise the expression of IFN- γ was low in the cells from naive donors but was higher in cells harvested from sensitized donors. There was concurrence between the anti-inflammatory effects of the cells and their IFN- γ expression but not their effects on the LAR, suggesting that the mechanisms of inhibition of inflammation and the LAR might not be the same. However, one caveat is that the number of cells expressing cytokines before transfer might not reflect their behavior after transfer and migration to the airways. A further caveat is the possibility that levels of secretion of cytokines might not be well reflected in the numbers of cells expressing these cytokines measured by immunostaining and in situ hybridization.

Transferred $\gamma\delta$ T cells did not influence the level of serum OVA-specific IgE in our model. McMenamin et al¹³ reported that $\gamma\delta$ T cells that were harvested from tolerized BN rats suppressed the OVA-specific IgE in syngeneic animals. There are substantial differences between the 2 models, however. We sensitized our animals subcutaneously with a single injection, whereas McMenamin et al used 10 inhalational challenges during a 2-week period. Consistent with the lack of effect on

OVA-specific IgE, $\gamma\delta$ T cells also did not influence the EAR in recipients, which reaction is dependent on IgE-mast cell interactions.²⁶

The composition of the $\gamma\delta$ T cell populations differs considerably as a function of organ and species. CD8⁺ represents 90% of the $\gamma\delta$ T cells, whereas CD4⁺ and double negative are rare in the peripheral lymph nodes of the rat. The structure of the CD8 marker also varies; CD8 $\alpha\beta$ is found in secondary lymphoid organs and CD8 $\alpha\alpha$ in gut.²⁷ The main subtype of $\gamma\delta$ T cells in peripheral lymph nodes in the mouse is double negative and comprises about 60% of the total $\gamma\delta$ T cell population. CD8⁺ T cells are 32% and CD4⁺ T cells are 8% of the total,²⁸ somewhat greater numbers than in the rat. In the case of human blood $\gamma\delta$ T cells, the double negative cells predominate, whereas CD8⁺ T cells are 20% to 30%, and CD4⁺ T cells are rare.^{29,30}

Our results indicate that the CD8⁺ subset is immunomodulatory, protecting against exaggerated airway responses to allergic challenge. The work of Lahn and colleagues³¹ indicates that the V γ 4⁺ subset of CD8⁺ $\gamma\delta$ T cells in the lungs of mice also has a protective function against the effects of inhaled foreign protein. A deficiency of these cells permits an increase in airway hyperresponsiveness after repeated OVA exposure of naive mice. IFN- γ production by the V γ 4⁺ cells is critical for their protective effect. The pertinence of the murine and rodent model systems for human asthma is uncertain. $\gamma\delta$ T cells are increased in the BAL of patients with asthma, but the increases are largely attributable to the CD4⁺ $\gamma\delta$ T cells and to a lesser extent the double negative cells.¹⁷ This finding suggests that a relative deficiency of CD8⁺ $\gamma\delta$ T cells might be part of the immunopathology of asthma.

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