

The Influence of IgE-Enhancing and IgE-Suppressive $\gamma\delta$ T Cells Changes with Exposure to Inhaled Ovalbumin¹

Yafei Huang,*[†] Niyun Jin,*[†] Christina L. Roark,*[†] M. Kemal Aydinoglu,*[†] J. M. Wands,*[†] Hua Huang,*[‡] Rebecca L. O'Brien,*[†] and Willi K. Born^{2*†}

It has been reported that the IgE response to allergens is influenced by $\gamma\delta$ T cells. Intrigued by a study showing that airway challenge of mice with OVA induces in the spleen the development of $\gamma\delta$ T cells that suppress the primary IgE response to i.p.-injected OVA-alum, we investigated the $\gamma\delta$ T cells involved. We found that the induced IgE suppressors are contained within the V γ 4⁺ subset of $\gamma\delta$ T cells of the spleen, that they express V δ 5 and CD8, and that they depend on IFN- γ for their function. However, we also found that normal nonchallenged mice harbor IgE-enhancing $\gamma\delta$ T cells, which are contained within the larger V γ 1⁺ subset of the spleen. In cell transfer experiments, airway challenge of the donors was required to induce the IgE suppressors among the V γ 4⁺ cells. Moreover, this challenge simultaneously turned off the IgE enhancers among the V γ 1⁺ cells. Thus, airway allergen challenge differentially affects two distinct subsets of $\gamma\delta$ T cells with nonoverlapping functional potentials, and the outcome is IgE suppression. *The Journal of Immunology*, 2009, 183: 0000–0000.

Antibodies of the IgE class are prominent in the host response to parasitic infections and in allergic responses to many nonpathogenic Ags (1). The interest in IgE has heightened as ever-increasing proportions of the world's population suffer from allergies (2). In healthy mammals, IgE Abs acquired via the gastrointestinal tract by the newborn may serve as a first line of defense (3). IgE is synthesized and functions in the normal adult largely in the mucosal tissues where the IgE concentrations are high, whereas concentrations of IgE in the circulation remain low by comparison with other Igs (1). Mechanisms responsible for this biased anatomical distribution include the distribution and longevity of cells that express the receptors for IgE, as most of the IgE in the tissues is cell bound and thus protected from degradation, and local IgE synthesis, which is favored by the Th2 environment of the mucosal tissues that maintains local IgE levels (1).

IgE Abs are also induced during vaccination. Aluminum adjuvants (alum), currently the most widely used adjuvants in human and animal vaccines, stimulate the innate system and can favor Th2-biased reactivity (4). Immunization of previously untreated laboratory animals with soluble inert protein Ags using alum typically elicits Th2-type responses, accompanied by the development of IgE Abs. However, the outcome of this type of immunization also depends on prior exposure. Mucosal exposure to the same Ag may result in nonresponsiveness and the failure of the immunization to elicit Th2 reactivity (5). For example, repeated airway

challenge of rodents with OVA without adjuvant leads to nonresponsiveness to a subsequent i.p. injection of OVA-alum, which otherwise would induce Th2 reactivity and a strong OVA-specific IgE response (6). How airway exposure alters the outcome of OVA-alum immunization is not yet fully understood (7).

Several groups have provided evidence that $\gamma\delta$ T cells can modulate the OVA-alum-induced IgE response. Investigating the development of tolerance to inhaled OVA, McMenamin et al. (8) found that $\gamma\delta$ T cells from tolerized mice efficiently and selectively suppressed primary OVA-alum-induced IgE responses. In apparent contrast, others reported that $\gamma\delta$ T cells are required for the development of IgE responses to OVA and other Ags (9, 10). The underlying mechanisms responsible for these apparently opposing observations remained unresolved.

That $\gamma\delta$ T cells can exert both Th1-like and Th2-like effects on the immune responses to pathogens has been recognized (11), and later studies revealed the surprising circumstance that these different and sometimes opposed functional effects on the host responses segregate with TCR-V γ -definable subsets of $\gamma\delta$ T cells, such as V γ 4⁺ and V γ 1⁺ $\gamma\delta$ T cells (12, 13). Specific functional contributions of these and other TCR-defined subsets suggested that the $\gamma\delta$ TCR determines not only ligand specificity of $\gamma\delta$ T cells but also their functional potential (14). Consistent with this concept, a recent study showed that the ability of $\gamma\delta$ T cells with specificity for the T22 molecule to express IL-17 and IFN- γ depends on TCR-ligand interactions during their development (15).

Given the divergent observations regarding the role of $\gamma\delta$ T cells in the IgE response, we were interested in determining whether different TCR-V γ -definable subsets of $\gamma\delta$ T cells also exert opposed effects on IgE production. In the current study, we took advantage of the observation that normal mice immunized with a single i.p. injection of OVA-alum make a primary IgE response to OVA. The results of these experiments indicate that V γ 1⁺ $\gamma\delta$ T cells are able to enhance the primary IgE response induced by OVA-alum, whereas V γ 4⁺ cells in contrast are able to suppress it. Moreover, we found that in addition to their different functional potentials, the overall effect of the IgE-modulating $\gamma\delta$ T cells critically depends on the exposure history of the animal. Whereas the

*Integrated Department of Immunology, National Jewish Health, Denver, CO 80206; [†]University of Colorado, Denver, CO 80206; and [‡]Department of Medicine, National Jewish Health, Denver, CO 80206

Received for publication December 9, 2008. Accepted for publication May 6, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by National Institutes of Health Grants AI40611 and HL65410 (to W.K.B.) and AI44920 and AI063400 (to R.L.O.).

² Address correspondence and reprint requests to Dr. Willi K. Born, Integrated Department of Immunology, National Jewish Health, 1400 Jackson Street, GB K409, Denver, CO 80206. E-mail address: bornw@njc.org

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/\$2.00

IgE-enhancing $\gamma\delta$ T cells lose this ability upon airway allergen-exposure, the IgE-suppressive $\gamma\delta$ T cells gain theirs under the same circumstance.

Materials and Methods

Animals

Female C57BL/6 mice and several mutant strains of the same genetic background (B6.TCR- $\beta^{-/-}$, B6.TCR- $\delta^{-/-}$, B6.TCR- $\beta^{-/-}\delta^{-/-}$) were obtained from The Jackson Laboratory. TCR-V $\gamma 4^{-/-}$ 6 $^{-/-}$ mice deficient in V $\gamma 4^{+}$ and V $\gamma 6^{+}$ T cells were a gift from Dr. K. Ikuta (Kyoto University, Kyoto, Japan). They were backcrossed to the C57BL/6 genetic background and used after 11 backcross generations. B6.TCR- $\beta^{-/-}$ IFN- $\gamma^{-/-}$ mice were generated by crossing the single mutants and breeding double mutants identified in the F2 generation. B6.TCR-V $\gamma 1$ -transgenic mice were a gift from Dr. Pablo Pereira (Institut Pasteur, Paris, France). All mice were 8–12 wk old at the time of the experiments. Mice were maintained on an OVA-free diet and were cared for at National Jewish Health (Denver, CO), following guidelines for immune deficient animals. All experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee.

Ag exposure and immunization

The animals were exposed to 1% OVA (w/v) (5 $\times$ crystalline; EMD Biosciences) in saline aerosol inhalation for 30 min daily, 5 days/wk for up to 2 wk, and subsequently once per wk (designated 10N in this paper), following a method described by others (8). To induce OVA-specific IgE, mice were immunized by i.p. injection of 10 μ g of OVA in aluminum hydroxide (AlumImject; Pierce Scientific) (8).

Treatment with Abs against the TCR

Hamster pan anti-TCR-C δ mAbs (clone GL3), anti-V $\gamma 4$ mAb (clone UC3), and anti-V $\gamma 1$ mAb (clone 2.11) were purified from hybridoma culture supernatants using a protein G-Sepharose affinity column (Amersham Pharmacia Biotech). T cells were targeted by injection of 200 μ g of hamster anti-TCR- δ , anti-V $\gamma 4$ or anti-V $\gamma 1$ mAbs into the tail veins of mice 4 days before the i.p. immunization with OVA. The effect of these treatments on the targeted T cells was monitored as previously described (13). This approach, which is based on staining with non-cross-blocking anti-TCR mAbs, allows an assessment of the effect of treatment on TCR expression, but it does not assess the fate of the targeted T cells. The antibody treatments transiently reduce TCR expression by >90%. Sham Ab treatments were performed with nonspecific hamster IgG (The Jackson Laboratory). Throughout this article, we use the nomenclature for murine TCR-V γ genes introduced by Heilig and Tonegawa (16).

T cell purification from spleen and lung

Total spleens and lungs were harvested from naive or 10N mice at the time of the experiments. Lungs were dissected into small pieces and exposed to an enzymatic digestion mixture containing 0.125% dispase II (Roche), 0.2% collagenase II (Sigma-Aldrich), and 0.2% collagenase IV (Sigma-Aldrich) for 90 min at 37°C. After enzymatic digestion, a single-cell suspension was prepared by pushing the lung tissue fragments through a 70- μ m-diameter nylon mesh (BD Falcon). A suspension of splenocytes was prepared by mechanical dispersion. Cell suspensions were treated with Gey's RBC lysis solution and passed through nylon wool columns to obtain T lymphocyte-enriched cell preparations containing >75% T cells as previously described (13). Total cell counts were determined using a Coulter counter.

Adoptive transfer of $\gamma\delta$ T cells

Splenic nylon wool-nonadherent (NAD) cells from naive and 10N mice (B6.TCR- $\beta^{-/-}$, B6.TCR- $\beta^{-/-}$ IFN- $\gamma^{-/-}$) were incubated with biotinylated anti-V $\gamma 4$ mAb (clone UC3) or anti-V $\gamma 1$ mAb (clone 2.11) for 15 min at 4°C, washed, and incubated with streptavidin-conjugated magnetic beads (Streptavidin Microbeads; Miltenyi Biotec) for 15 min at 4°C and passed through magnetic columns to purify V $\gamma 4^{+}$ or V $\gamma 1^{+}$ cells as previously described in detail (17). This produced a cell population containing >90% V $\gamma 4^{+}$ or V $\gamma 1^{+}$ viable cells as determined by two-color staining with anti-TCR- δ and anti-V $\gamma 4$ or anti-V $\gamma 1$ mAbs. These splenic V $\gamma 4^{+}$ or V $\gamma 1^{+}$ cells were washed in PBS and resuspended to a concentration of 1.5×10^5 cells/ml PBS, and 3×10^4 cells/mouse were injected in 200 μ l of PBS via the tail vein into B6.TCR- $\delta^{-/-}$ mice directly before the OVA immunization.

In some experiments, subpopulations of V $\gamma 4^{+}$ or V $\gamma 1^{+}$ cells were purified using the MoFlo cell sorter. NAD cells were incubated with FITC-

conjugated anti-V $\gamma 4$ mAb (clone UC3) or FITC-conjugated anti-V $\gamma 1$ mAb (clone 2.11) and PE-conjugated anti-CD8 α (clone 53-6.7; BD Pharmingen) or biotinylated anti-V $\delta 5$ (clone F45-152), followed by PE-conjugated streptavidin (20 min at 4°C), and then washed. Cells were next sorted based on their expression of V γ chain and V $\delta 5$ or CD8 α using a MoFlo cell sorter (Dako Cytomation). Purified cells were washed in PBS and resuspended to a concentration of 1.5×10^5 cells/ml of PBS, and 3×10^4 cells/mouse injected in 200 μ l of PBS via the tail vein into B6.TCR- $\delta^{-/-}$ mice directly before the OVA immunization. In general, the cell sorter-purified cells were less effective than those prepared by magnetic bead selection and therefore have only been compared with each other.

Flow cytometric analysis

For flow cytometric analyses, NAD cells (2×10^5 /well) in 96-well plates (Falcon; BD Biosciences) were stained for TCR expression using a PE-labeled pan C δ Ab (clone GL3) and FITC-conjugated anti-V $\gamma 1$ (clone 2.11), or anti-V $\gamma 4$ (clone UC3) Ab followed by biotinylated anti-V δ mAbs: anti-V $\delta 4$ (clone GL-2), anti-V $\delta 5$ (clone F45-152), anti-V $\delta 6.3$ (clone 17-C), anti-V $\delta 6\lambda 12$ (clone F4.22) and anti V $\delta 8$ (clone B20.1), plus streptavidin-allophycocyanin. All samples were analyzed on a FACScan flow cytometer (BD Biosciences) counting a minimum of 25,000 events per gated region, and the data were processed using FlowJo 6.4.1 software (Tree Star).

Determination of serum IgE levels

Sera were harvested on day 14 after the i.p. immunization with OVA-alum. For OVA-specific serum IgE determinations, plates were coated overnight at 4°C with 2 μ g/ml rat anti-mouse IgE Ab (clone R35-72; BD Biosciences). The serum samples were then added, and biotin-labeled OVA subsequently added to the wells. Before biotinylation, OVA was first dialyzed at 4°C overnight against 0.1 M borate buffer (pH 8.4). Biotinylated OVA was then prepared by reacting 1 ml of OVA in PBS (1 mg/ml) with 150 μ l of *N*-hydroxysuccinimidobiotin in DMSO (1 mg/ml) for 4 h at room temperature, followed by overnight dialysis against PBS at 4°C. The bound OVA-biotin was detected with streptavidin-conjugated HRP (BD Biosciences) followed by 100 μ l/well of TMB substrate solution. OVA-specific IgE levels in the samples were compared with an internal standard obtained from pooled sera of hyperimmunized BALB/c mice, which was arbitrarily assigned to equal 1000 ELISA U. Total IgE levels were measured by sandwich ELISA using rat anti-mouse IgE at 2 μ g/ml (clone R35-72; BD Biosciences) as a capture Ab followed by biotinylated rat anti-mouse IgE H chain mAb (clone R35-118; BD Biosciences) at 2 μ g/ml, and detected as described above. All samples were read using a VERSA-max tunable microplate reader and processed using a SoftMax Pro 4.7.1 software.

Statistical analysis

Data are presented as means $\pm$ SEM. The unpaired *t* test was used for two group comparisons, and ANOVA was used for analysis of differences in three or more groups. Statistically significant levels are indicated as follows: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001; NS, not significant.

Results

Altered IgE response in $\gamma\delta$ T cell genetically modified mice and in normal mice treated with Abs against the $\gamma\delta$ TCR

To test the concept that $\gamma\delta$ T cells are capable of modulating the IgE response, we initially examined genetically modified mice, including mice lacking all T cells (B6.TCR- $\beta^{-/-}\delta^{-/-}$), all $\gamma\delta$ T cells (B6.TCR- $\delta^{-/-}$), V $\gamma 4^{+}$ and V $\gamma 6^{+}$ $\gamma\delta$ T cells only (B6.TCR-V $\gamma 4^{-/-}$ 6 $^{-/-}$), and mice expressing a rearranged V $\gamma 1$ J $\gamma 4$ C $\gamma 4$ transgene (B6.TCR-V $\gamma 1$ Tg), plus wild-type controls (C57BL/6). We also examined mice lacking all $\alpha\beta$ T cells (B6.TCR- $\beta^{-/-}$). Total serum IgE levels were measured by ELISA, in adult age- and sex-matched untreated mice, and in mice injected i.p. with OVA-alum, 14 days after the injection (Fig. 1a). Without treatment, basal serum IgE levels were low in comparison with wild-type controls in mice lacking all $\gamma\delta$ T cells (B6.TCR- $\delta^{-/-}$ vs C57BL/6, *p* < 0.01), but high in mice deficient in V $\gamma 4^{+}$ and V $\gamma 6^{+}$ $\gamma\delta$ T cells (B6.TCR-V $\gamma 4^{-/-}$ 6 $^{-/-}$ vs C57BL/6, *p* < 0.0001) and in mice expressing the V $\gamma 1$ J $\gamma 4$ C $\gamma 4$ transgene (B6.TCR-V $\gamma 1$ transgenic vs C57BL/6, *p* < 0.001). Mice lacking $\alpha\beta$ T cells did not express IgE at significant levels. At day 14 after OVA-alum injection, all mice except those lacking $\alpha\beta$ T cells exhibited substantially increased

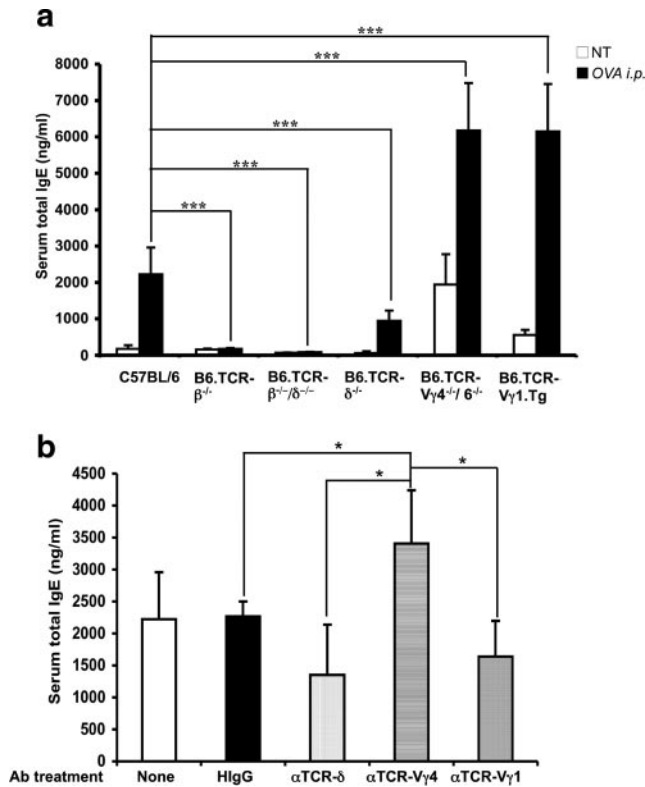


FIGURE 1. Altered IgE response in $\gamma\delta$ T cell-modified mice. *a*, Background serum IgE levels and primary OVA-alum-induced IgE responses in genetically altered mouse strains. Total serum IgE concentrations in C57BL/6, B6.TCR- $\beta^{-/-}$, B6.TCR- $\beta^{-/-}\delta^{-/-}$, B6.TCR- $\delta^{-/-}$, B6.TCR-V $\gamma 4^{-/-}6^{-/-}$, and B6.TCR-V $\gamma 1$ Tg mice was measured using sandwich ELISA 14 days after i.p. injection of OVA-alum and in nontreated controls. Results for each group are expressed as the mean $\pm$ SEM ($n = 18, 4, 4, 20, 15, 6$, in the order shown). NT, Nontreated; ***, Significant difference ($p < 0.001$) compared with OVA-challenged C57BL/6 mice. Significant differences in basal IgE levels between the mouse strains shown in *a* are listed in the text. *b*, Effect of treatment with anti-TCR mAbs on the primary OVA-alum-induced IgE response in C57BL/6 mice. Normal C57BL/6 mice were injected i.v. with nonspecific hamster IgG (HIgG), anti-TCR- δ , anti-TCR-V $\gamma 4$, and anti-TCR-V $\gamma 1$ mAbs on day -4, and total serum IgE concentrations were determined on day 14 after i.p. injection of OVA-alum. Mice that received the OVA-alum injection but no Ab treatment were included as controls. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). *, Significant difference compared with untreated or hamster IgG-treated controls ($p < 0.05$).

serum IgE levels, and the relative increases in IgE resembled the pattern of the untreated mice.

In a follow-up experiment with wild-type C57BL/6 mice only, we again examined serum IgE levels on day 14 after the i.p. OVA-alum injection, comparing groups that had been treated with Abs against TCR- δ , TCR-V $\gamma 4$, and TCR-V $\gamma 1$ or with nonspecific Ig, 4 days before the OVA-alum sensitization (Fig. 1*b*). We have shown previously that the i.v.-injected anti-TCR mAbs inactivate the targeted T cells (13). Although the treatments with anti-TCR- δ and TCR-V $\gamma 1$ mAbs did not significantly decrease serum IgE levels by comparison with the controls, the treatment with anti-TCR-V $\gamma 4$ mAb significantly increased IgE levels, and there were significant differences between the different Ab-treated groups (Fig. 1*b*), consistent with the findings in the genetically modified mice (Fig. 1*a*). Taken together, the data shown in Fig. 1 suggested that the overall effect of $\gamma\delta$ T cells in untreated mice, and in mice sensitized with OVA-alum, is to increase the primary IgE response but

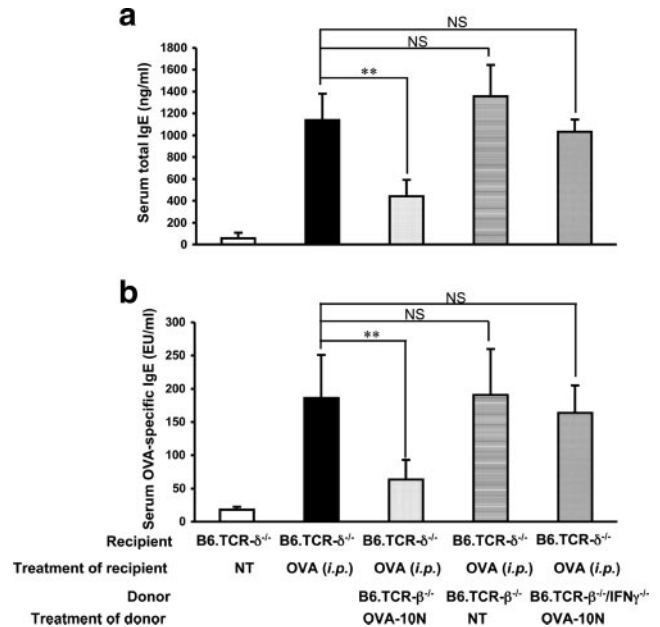


FIGURE 2. Primary OVA-alum-induced IgE response in B6.TCR- $\delta^{-/-}$ mice is inhibited following adoptive transfer of V $\gamma 4^{+}$ cells from airway OVA-challenged B6.TCR- $\beta^{-/-}$ mice. Purified V $\gamma 4^{+}$ cells (3×10^4 /inoculum) from naive or 10N-treated B6.TCR- $\beta^{-/-}$ mice, or from 10N-treated B6.TCR- $\beta^{-/-}$ IFN- $\gamma^{-/-}$ mice, were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients, just before i.p. OVA-alum injection. Serum total IgE (*a*) and OVA-specific IgE (*b*) levels were measured 14 days after the OVA-alum treatment. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. **, $p < 0.01$; NS, not significant; NT, not treated; EU, ELISA units.

also suggested that different V γ -defined subsets are capable of modulating the IgE response in opposite directions: whereas V $\gamma 1^{+}$ cells might enhance it, V $\gamma 4^{+}$ cells might suppress it.

Adoptively transferred V $\gamma 4^{+}$ cells from OVA-challenged but not untreated mice, inhibit the primary anti-OVA IgE response in TCR- $\delta^{-/-}$ mice

To further investigate the effect of V $\gamma 4^{+}$ $\gamma\delta$ T cells on the IgE response, we positively selected V $\gamma 4^{+}$ cells from the spleen of untreated B6.TCR- $\beta^{-/-}$ mice (using magnetic beads), and transferred 3×10^4 of the purified cells to B6.TCR- $\delta^{-/-}$ mice by i.v. injection, directly before the i.p. OVA-alum sensitization. We examined both serum total IgE (Fig. 2*a*) and OVA-specific IgE in the cell transfer recipients (Fig. 2*b*). V $\gamma 4^{+}$ cells derived from the untreated donors did not significantly alter serum IgE levels in the OVA-alum-sensitized recipients. However, others have reported that mice exposed to aerosolized OVA by inhalation on at least 10 consecutive days (10N-treated mice) lose their ability to mount an IgE response upon i.p. OVA-alum injection, and give rise to suppressive $\gamma\delta$ T cells (8). We therefore prepared V $\gamma 4^{+}$ cells from the spleen of 10N-treated B6.TCR- $\beta^{-/-}$ mice. Unlike those from the untreated donors, these cells markedly suppressed both total IgE and OVA-specific IgE levels in the serum of the B6.TCR- $\delta^{-/-}$ cell transfer recipients (Fig. 2, *a* and *b*). These data confirmed that IgE-suppressive $\gamma\delta$ T cells are induced by airway allergen challenge (8) and showed that this occurs independently of $\alpha\beta$ T cells. In an earlier study, we made essentially the same observations with airway hyperresponsiveness (AHR)³-suppressive $\gamma\delta$ T cells and found that V $\gamma 4^{+}$ $\gamma\delta$ T cells derived from

³ Abbreviations used in this paper: AHR, airway hyperresponsiveness; DC, dendritic cell.

Table I. Sizes of $\gamma\delta$ T cell subpopulations and their relative percentage in spleen and lung of naive and 10N-treated B6.TCR- $\beta^{-/-}$ mice^a

Group	V γ 4 δ 4	V γ 4 δ 5	V γ 4 δ 6.3	V γ 4 δ 6 λ 12	V γ 4 δ 8	V γ 1 δ 4	V γ 1 δ 5	V γ 1 δ 6.3	V γ 1 δ 6 λ 12	V γ 1 δ 8
Naive spleen	143 $\pm$ 23 (11.5 $\pm$ 0.6)	296 $\pm$ 69 (24 $\pm$ 1.5)	20 $\pm$ 8 (1.6 $\pm$ 0.5)	21 $\pm$ 5.6 (1.7 $\pm$ 0.4)	15 $\pm$ 4.5 (1.2 $\pm$ 0.4)	163 $\pm$ 45 (7.4 $\pm$ 0.1)	568 $\pm$ 141 (25.9 $\pm$ 0.5)	114 $\pm$ 50 (5.2 $\pm$ 1.7)	199 $\pm$ 15 (9.4 $\pm$ 1.7)	23 $\pm$ 7.5 (1.1 $\pm$ 0.4)
10N spleen	128 $\pm$ 33 (14 $\pm$ 0.6)	285 $\pm$ 81 (30.7 $\pm$ 3.7)*	14 $\pm$ 4 (1.6 $\pm$ 0.6)	14 $\pm$ 5.3 (1.6 $\pm$ 0.8)	21 $\pm$ 13 (1.4 $\pm$ 0.6)	115 $\pm$ 67 (8.7 $\pm$ 2.1)	319 $\pm$ 89 (25.8 $\pm$ 3.0)	84 $\pm$ 11 (7.0 $\pm$ 1.4)	101 $\pm$ 22 (8.3 $\pm$ 1.0)	14 $\pm$ 5.8 (1.1 $\pm$ 0.1)
Naive lung	14 (11.4)	34 (26.9)	1.8 (1.4)	2.7 (2.1)	5.2 (4.1)	3.3 (1.9)	43 (24.1)	10 (5.7)	8.5 (4.9)	2.5 (0.9)
10N lung	21 (11.5)	68 (37.1)	4.8 (2.7)	1.4 (0.8)	9.7 (5.4)	5.7 (3.2)	53 (35.6)	11.4 (8.6)	7.8 (5.0)	2.9 (1.1)

^a Nylon wool nonadherent (NAD) cells derived from spleen and lung were counted. The percentages of individual $\gamma\delta$ T cell subpopulations in NAD cells were determined cytofluorimetrically. Absolute cell numbers ($\times 1000$) were calculated as percentage $\times$ NAD cell numbers. Numbers in parentheses, percentages of $\gamma\delta$ T cell subpopulations in total $\gamma\delta$ T cells. Four mice were used in each group, and data for spleen cells were expressed as means $\pm$ SEM. Lung cells were pooled, and the average numbers are shown. Significant differences between naive and challenged mice are indicated. *, $p < 0.05$.

wild-type C57BL/6 mice and B6.TCR- $\beta^{-/-}$ mice are functionally equivalent (17). Finally, on the basis of the data of others suggesting that IgE-suppressive $\gamma\delta$ T cells depend on IFN- γ (8), we tested whether V γ 4⁺ cells from the spleens of 10N-treated B6.TCR- $\beta^{-/-}$ –IFN- $\gamma^{-/-}$ mice had suppressive activity. In clear contrast to their wild-type counterparts, these cells failed to suppress the IgE response (Fig. 2). These data confirmed that IFN- γ -dependent $\gamma\delta$ T cells can become potent inhibitors of the IgE response (8) and identified V γ 4⁺ cells as the source population of the suppressors.

IgE-suppressive V γ 4⁺ cells express V γ 4V δ 5-TCRs and CD8

To further investigate the properties of IgE-suppressive V γ 4⁺ $\gamma\delta$ T cells, we examined the V δ usage of V γ 4⁺ and V γ 1⁺ cells in

spleen and lung of untreated and 10N-treated B6.TCR- $\beta^{-/-}$ mice (Table I). After 10N treatment, the overall numbers and relative frequency of V γ 4⁺ cells remained stable or slightly decreased in spleen and increased in lung, whereas V γ 1⁺ cells more generally decreased in spleen and less prominently increased in lung. Certain V γ 4⁺ subpopulations (V γ 4V δ 5⁺ cells and V γ 4V δ 8⁺ cells) showed relative and absolute or at least relative increases in lung and spleen suggesting that these cells are selected in the 10N-treated mice and hence that they might be enriched in IgE suppressors.

Of the two subpopulations of V γ 4⁺ cells, V γ 4V δ 5⁺ cells are more accessible because they are present in larger numbers in both organs. Therefore, to test the idea that IgE suppressors among V γ 4⁺ cells are enriched during the 10N treatment, we purified V γ 4⁺V δ 5⁺ and V γ 4⁺V δ 5[−] cells by FACS sorting of splenocytes

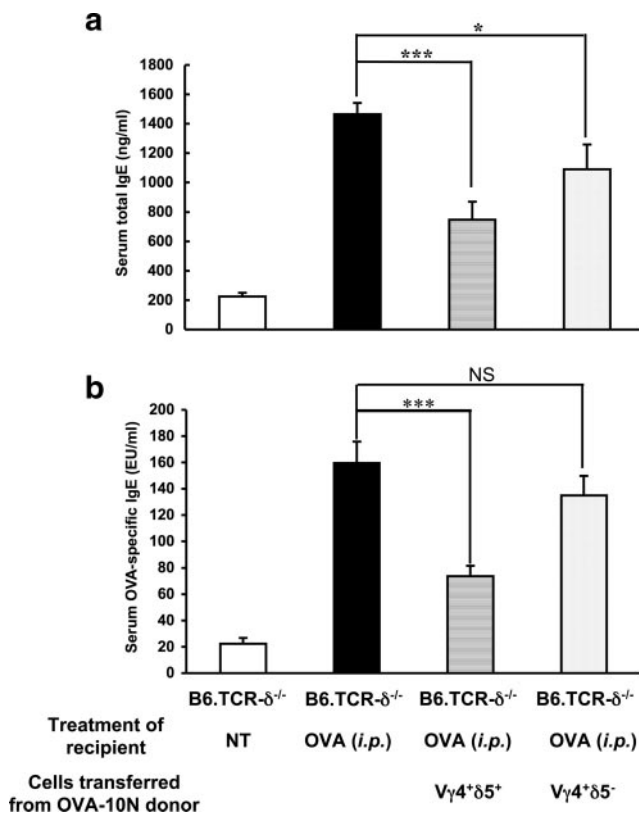


FIGURE 3. IgE-suppressive V γ 4⁺ $\gamma\delta$ T cells coexpress V δ 5. V δ 5⁺ and V δ 5[−] V γ 4 cells were purified from the spleen of 10N-treated B6.TCR- $\beta^{-/-}$ mice by FACS sorting. Purified V γ 4⁺V δ 5⁺ or V γ 4⁺V δ 5[−] cells (3×10^4 /inoculum) were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients just before i.p. injection of OVA-alum. Serum total IgE (a) and OVA-specific IgE (b) levels were measured 14 days after the OVA-alum injection. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. ***, $p < 0.001$; NS, not significant; NT, not treated; EU, ELISA units.

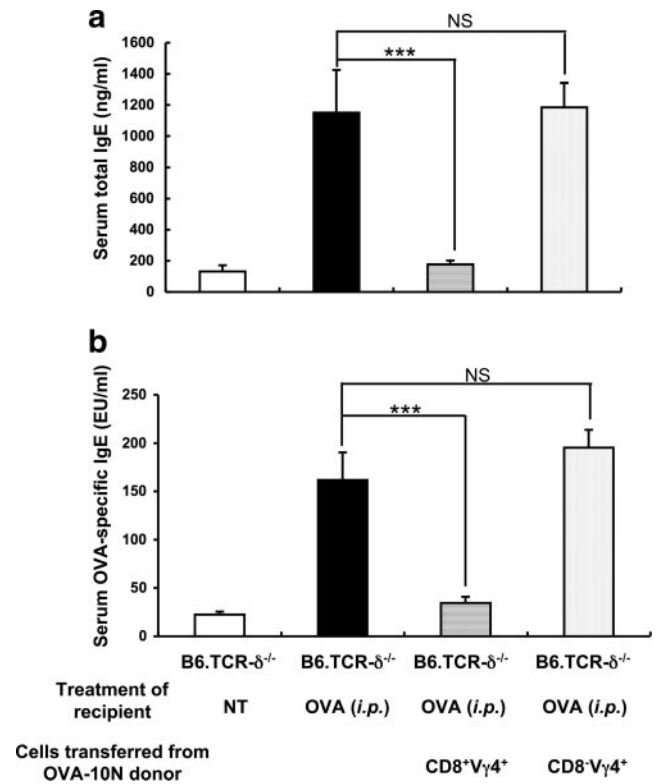


FIGURE 4. IgE-suppressive V γ 4⁺ $\gamma\delta$ T cells express CD8. CD8 α^+ and CD8 α^- V γ 4 cells were purified from the spleen of 10N-treated B6.TCR- $\beta^{-/-}$ mice by FACS sorting. Three $\times 10^4$ cells of each type were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients just before i.p. injection of OVA-alum. Serum total IgE levels were measured 14 days after the OVA-alum injection. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. ***, $p < 0.001$. NS, not significant; NT, not treated; EU, ELISA units.

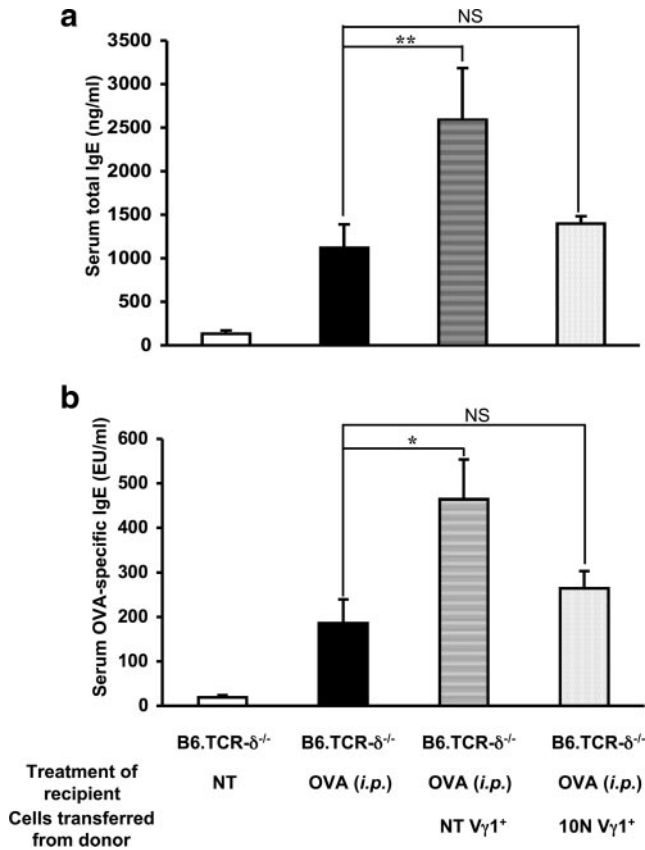


FIGURE 5. Adoptively transferred $V\gamma 1^{+}$ $\gamma\delta$ T cells from nontreated but not airway OVA-challenged B6.TCR- $\beta^{-/-}$ mice enhance the primary OVA-alum-induced IgE response in B6.TCR- $\delta^{-/-}$ mice. Purified $V\gamma 1^{+}$ cells (3×10^4 /inoculum) from nontreated (NT) or 10N-treated B6.TCR- $\beta^{-/-}$ mice were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients just before i.p. OVA-alum injection. Serum total IgE (a) and OVA-specific IgE (b) levels were measured 14 days after the OVA-alum injection. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. *, $p < 0.05$; **, $p < 0.01$. NS, not significant; NT, not treated; EU, ELISA units.

from 10N-treated B6.TCR- $\beta^{-/-}$ mice. Cells of either type (3×10^4 /inoculum) were then adoptively transferred to B6.TCR- $\delta^{-/-}$ recipients by i.v. injection directly before i.p. OVA-alum sensitization (Fig. 3). Overall, the FACS-sorted cells were less effective than the cells enriched by magnetic bead selection. However, the FACS-selected $V\gamma 4^{+}V\delta 5^{+}$ cells significantly decreased both serum total IgE (Fig. 3a) and OVA-specific IgE (Fig. 3b), whereas $V\gamma 4^{+}V\delta 5^{-}$ cells had a lesser effect, and only serum total IgE was significantly reduced. These data confirm that $V\gamma 4^{+}V\delta 5^{+}$ $\gamma\delta$ T cells can suppress IgE. Our experiment leaves open the possibility that other $V\gamma 4^{+}$ cells, e.g., $V\gamma 4^{+}V\delta 8^{+}$ $\gamma\delta$ T cells, also might function as IgE suppressors.

It has been proposed that IgE-suppressive $\gamma\delta$ T cells express CD8 (8); in addition, $V\gamma 4^{+}$ $\gamma\delta$ T cells express CD8 more frequently than other $\gamma\delta$ T cells (18). To examine the possibility that the $V\gamma 4^{+}$ IgE suppressors coexpress CD8, both $V\gamma 4^{+}CD8\alpha^{+}$ and $V\gamma 4^{+}CD8\alpha^{-}$ cells were purified by FACS sorting of splenocytes from 10N-treated B6.TCR- $\beta^{-/-}$ mice, and subsequently tested in the cell transfer assay (Fig. 4). Whereas the $V\gamma 4^{+}CD8\alpha^{+}$ cells completely suppressed the OVA-specific IgE response, $V\gamma 4^{+}CD8\alpha^{-}$ cells had no effect whatsoever. Taken together, these data suggest that a subset of $\gamma\delta$ T cells expressing $V\gamma 4V\delta 5$ TCRs and CD8 contains most if not all IgE suppressors in our model.

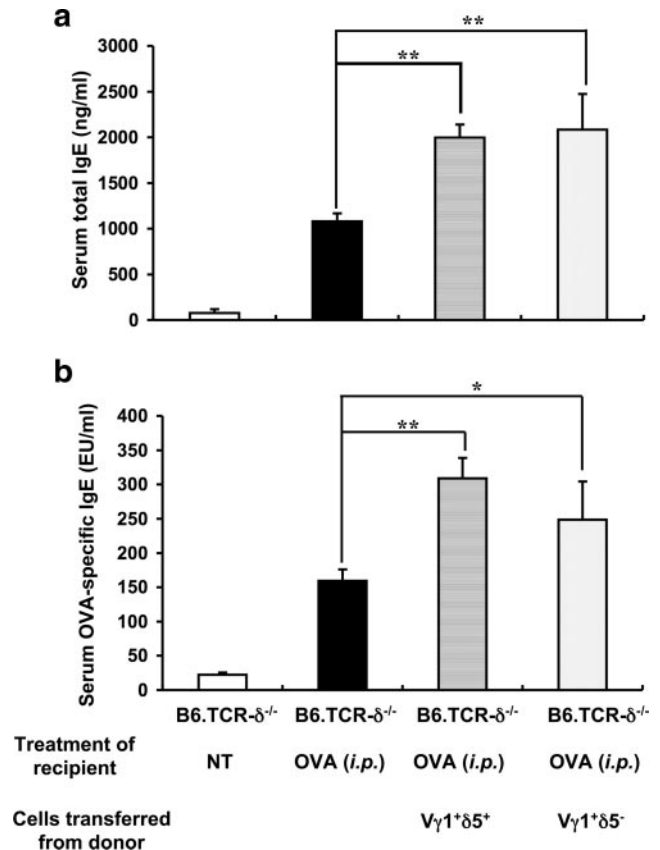


FIGURE 6. Co-expression of $V\delta 5$ is not required for $V\gamma 1^{+}$ $\gamma\delta$ T cells from nontreated mice to enhance the primary OVA-alum-induced IgE response. Purified $V\gamma 1^{+}V\delta 5^{+}$ or $V\gamma 1^{+}V\delta 5^{-}$ cells (3×10^4 /inoculum) from nontreated (NT) B6.TCR- $\beta^{-/-}$ mice were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients directly before Ag challenge. Serum total IgE (a) and OVA-specific IgE (b) levels were measured 14 days after the OVA-alum injection. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. *, $p < 0.05$; **, $p < 0.01$. EU, ELISA units.

Adoptively transferred $V\gamma 1^{+}$ cells from untreated but not OVA-challenged mice enhance the primary anti-OVA IgE response in TCR- $\delta^{-/-}$ mice

To further investigate the effect of $V\gamma 1^{+}$ $\gamma\delta$ T cells on the IgE response, we positively selected $V\gamma 1^{+}$ cells from the spleens of untreated B6.TCR- $\beta^{-/-}$ mice, and transferred 3×10^4 purified cells to B6.TCR- $\delta^{-/-}$ mice, shortly before the i.p. OVA-alum sensitization. $V\gamma 1^{+}$ cells derived from untreated donors significantly increased serum total IgE (Fig. 5a) and OVA-specific IgE levels (Fig. 5b) in the OVA-alum-sensitized recipients, in marked contrast to the $V\gamma 4^{+}$ cells, which required OVA challenge of the donor mice to become functional IgE suppressors (Fig. 2). However, $V\gamma 1^{+}$ cells from 10N-treated B6.TCR- $\beta^{-/-}$ mice no longer had any significant effect on the IgE level (Fig. 5). This is reminiscent of our earlier observation that $V\gamma 1^{+}$ cells from untreated mice enhance AHR (19), and it suggests that $V\gamma 1^{+}$ $\gamma\delta$ T cells in untreated mice already exert a Th2-like influence which enables them to promote both AHR and the IgE responses.

We had also found that AHR-enhancing $V\gamma 1^{+}$ $\gamma\delta$ T cells express $V\delta 5$ (19). To test whether the IgE enhancers also are $V\gamma 1V\delta 5^{+}$ cells, we purified both $V\gamma 1^{+}V\delta 5^{+}$ and $V\gamma 1^{+}V\delta 5^{-}$ cells by FACS sorting of splenocytes from nontreated B6.TCR- $\beta^{-/-}$ mice, and adoptively transferred either type to B6.TCR- $\delta^{-/-}$ recipients by i.v. injection shortly before i.p. OVA-alum sensitization (Fig. 6). However, both

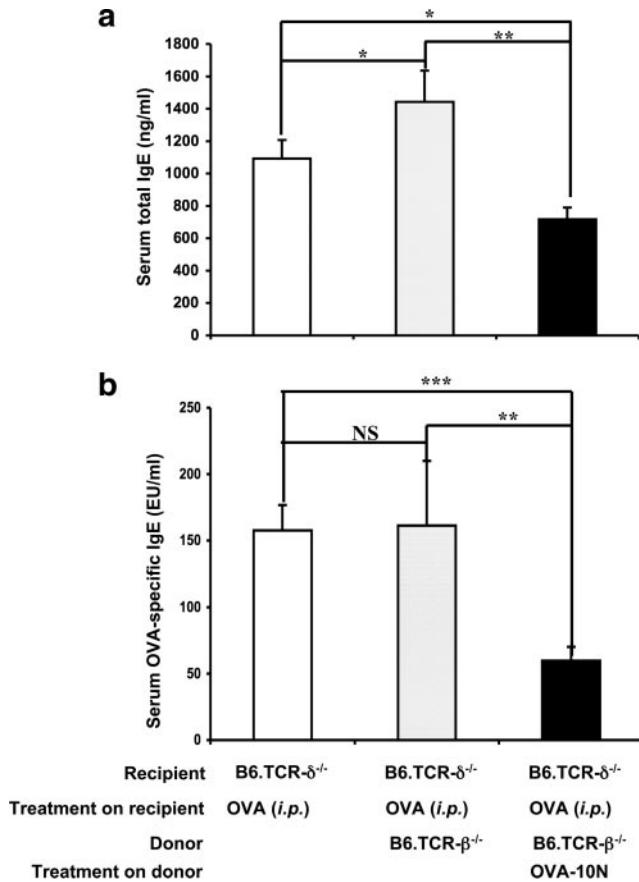


FIGURE 7. Net effect of total $\gamma\delta$ T cells on the primary OVA-alum-induced IgE response. Purified $\gamma\delta$ T cells (3×10^4 /inoculum) from non-treated (NT) or OVA-challenged (10N) B6.TCR- $\beta^{-/-}$ mice were adoptively transferred i.v. to B6.TCR- $\delta^{-/-}$ recipients directly before Ag challenge. Serum total IgE (a) and OVA-specific IgE (b) levels were measured 14 days after the OVA-alum injection. Results for each group are expressed as the mean $\pm$ SEM ($n = 4$ in each group). Significant differences between mice that received cells and those that received no cells are indicated. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$. EU, ELISA units.

$V\gamma 1^+V\delta 5^+$ and $V\gamma 1^+V\delta 5^-$ cells significantly increased serum total IgE and OVA-specific IgE in the B6.TCR- $\delta^{-/-}$ cell transfer recipients, indicating that the IgE enhancers among $V\gamma 1^+$ $\gamma\delta$ T cells, unlike the AHR enhancers, do not have to coexpress $V\delta 5$.

Net effect of nonseparated $\gamma\delta$ T cells on the primary anti-OVA IgE response in TCR- $\delta^{-/-}$ mice

Because our findings with transferred purified cells representing the $\gamma\delta$ T cell subsets suggested that the net effect of all $\gamma\delta$ T cells might be a switch from IgE enhancement to IgE suppression, we tested this idea by transferring total nonseparated $\gamma\delta$ T cells derived from donors that either remained untreated or were OVA challenged (10N; Fig. 7). Because the total number of transferred cells (3×10^4 i.v.) remained the same, in this experiment fewer cells from either $\gamma\delta$ T cell subset were transferred by comparison with the purified cells representing the individual subsets. As predicted, the nonseparated $\gamma\delta$ T cells from challenged donors suppressed the primary IgE response. However, nonseparated $\gamma\delta$ T cells from untreated donors did not significantly enhance the IgE response (see *Discussion*).

Discussion

In this study, we confirmed the earlier observation of others that $\gamma\delta$ T cells exert a regulatory influence on the IgE response (8–10, 20).

We extended these findings to show that there are IgE-suppressors and IgE enhancers among IgE-regulatory $\gamma\delta$ T cells, which belong to different subsets of $\gamma\delta$ T cells and are distinguished by their TCR expression. Furthermore, we demonstrate that although the suppressors require induction by airway exposure to allergen, the enhancers do not, and finally that airway allergen challenge, previously reported to induce $\gamma\delta$ T cell-dependent suppression of the IgE response (8, 20), actually has a dual effect. It induces the IgE suppressors and inactivates the IgE enhancers, thereby mediating $\gamma\delta$ T cell-dependent IgE suppression in the challenged mice.

These observations raise several new questions. If $\gamma\delta$ T cells indeed regulate the IgE response, when are they likely to play a role and how far-reaching might their influence be? In this study, we investigated the effect of $\gamma\delta$ T cells on serum IgE levels after a single i.p. injection of OVA-alum. After transfer of nonseparated $\gamma\delta$ T cells derived from untreated or challenged donors, the change in the primary IgE response was roughly 2-fold, and after transfer of purified $\gamma\delta$ T cells representing the regulatory subsets, the range of changes in total and OVA-specific IgE levels increased to ~ 10 -fold. Considering that IgE levels in circulation tend to be small (1), and that only 3×10^4 $\gamma\delta$ T cells were transferred, the regulatory potential of $\gamma\delta$ T cells may be substantial. We chose to investigate serum IgE and to transfer small numbers of splenic $\gamma\delta$ T cells mainly for experimental convenience. Conceivably, the regulatory effect of $\gamma\delta$ T cells on IgE in the mucosal tissues, where $\gamma\delta$ T cells are more concentrated (21) and where IgE Abs are present at much higher levels (2), might be greater. Our data also reveal differences in background serum IgE levels of $\gamma\delta$ T cell genetically altered mice, and here the range of differences reaches ~ 100 -fold. It seems clear, therefore, that $\gamma\delta$ T cells profoundly influence the IgE response and that their influence is not limited to the special case of immunization with OVA-alum.

What might be the significance of the correlation between TCR expression and IgE-regulatory function? We found that the effect of $V\gamma 4^+$ cells on the IgE response ranged from none to suppression, whereas that of $V\gamma 1^+$ cells ranged from enhancement to none. These nonoverlapping functional effects are reminiscent of other studies on which the two types of $\gamma\delta$ T cells also had non-overlapping and opposed functional effects (12). In particular, $V\gamma 1^+$ cells also enhanced AHR whereas $V\gamma 4^+$ cells suppressed it (13, 17, 19, 22). Our recent studies have shown that the $V\gamma 1^+$ $\gamma\delta$ T cell subset in normal untreated mice contains AHR-enhancing cells (19, 23) but the $V\gamma 4^+$ subset does not. However, the AHR and IgE-regulatory $\gamma\delta$ T cell populations do not appear to be identical. Whereas the AHR enhancers seem to be limited to those cells that express $V\gamma 1V\delta 5$ (19), IgE enhancers were also found among $V\gamma 1^+V\delta 5^-$ cells. Alternatively, most of the IgE suppressors appear to express $V\gamma 4V\delta 5$ whereas the AHR suppressors which also express $V\gamma 4$ might be less biased with regard to $V\delta$ expression (17). Moreover, we found that the IgE suppressors tend to express CD8, unlike the AHR suppressors which may be $CD8^+$ or $CD8^-$ (22). At least for those functional populations that depend on defined $V\gamma V\delta$ pairs (the AHR enhancers and the IgE suppressors), it seems likely that the TCR is somehow involved with their function, either because TCR-ligand interactions help determine cellular differentiation or because they define function itself through ligand specificity (14, 24). Consistent with the former possibility, a recent study showed that TCR-ligand interactions in the thymus determine the ability of peripheral $\gamma\delta$ T cells to express certain cytokines (15). However, possible ligands for the IgE-regulatory $\gamma\delta$ T cells remain unknown.

How can airway challenge mediate IgE suppression? Having examined both IgE suppressors and enhancers, it is clear that airway challenge does more than simply change the ratio of these

cells in favor of the suppressors but rather affects the two regulatory populations separately. The experiments shown in this paper do not formally rule out that $\gamma\delta$ T cells migrate from the challenged lung to the spleen. However, it would be difficult for pulmonary $\gamma\delta$ T cells to change the composition of splenic $\gamma\delta$ T cells substantially because the splenic population of $\gamma\delta$ T cells is much larger (25, 26). More likely perhaps, $\gamma\delta$ T cells in the spleen are influenced by other signal carriers from the lung, e.g., pulmonary dendritic cells (DC). Our preliminary studies with $\gamma\delta$ T cells exposed to transferred non-T cells from challenged mice are consistent with such a mechanism (Y. Huang, unpublished observations). Moreover, we found both $V\gamma 1^+$ and $V\gamma 4^+$ $\gamma\delta$ T cells in the spleen in close proximity to $CD8^+$ DC in the splenic periarteriolar lymphoid sheaths (22), a known destination of the peripheral signal carriers or shuttles. Furthermore, the splenic $\gamma\delta$ T cells required the presence of $CD8^+$ splenic DCs or their functional development in another model (22). Therefore, we envision that the splenic $\gamma\delta$ T cells might be compelled to change their function under the influence of $CD8^+$ splenic DC, which must have received signals from the challenged lung (27). Indeed, such a mechanism has already been described, where $CD8^+$ DC, which are known to remain in the lymphoid tissues (28), depend on shuttle cells to receive stimulatory signals and Ags from the peripheral tissues including the lung (29–32).

Despite the clear IgE-enhancing effect of purified $V\gamma 1^+$ cells derived from nonchallenged donors, total $\gamma\delta$ T cells derived from such donors enhanced IgE only weakly (detected in the assay for total serum IgE, but not for OVA-specific IgE). This simply might be due to the smaller number of transferred $V\gamma 1^+$ cells in this experiment (only approximately one-half of total splenic $\gamma\delta$ T cells are $V\gamma 1^+$) or it might be caused by interactions among the IgE-regulatory $\gamma\delta$ T cell subsets. In any case, the net effect of $\gamma\delta$ T cells in untreated mice therefore might be only slightly supportive of the primary IgE response, whereas their effect in challenged mice is clearly suppressive.

In sum, we found that airway challenge brings about coordinated and opposite functional changes of the IgE-enhancing and -suppressive $\gamma\delta$ T cells. This coordinated change of two antagonistic cell-types appears to represent an efficient mechanism in the regulation of the primary IgE response.

Acknowledgments

We acknowledge the advice and support of Dr. Katja Avikus and the expert help of Shirley Sobus and William Townsend with cell analysis and sorting.

Disclosures

The authors have no financial conflict of interest.

References

- Gould, H. J., B. J. Sutton, A. J. Beavil, R. L. Beavil, N. McCloskey, H. A. Coker, D. Fear, and L. Smurthwaite. 2003. The biology of IgE and the basis of allergic disease. *Annu. Rev. Immunol.* 21: 579–628.
- Gould, H. J., and B. J. Sutton. 2008. IgE in allergy and asthma today. *Nat. Rev. Immunol.* 8: 205–217.
- Thornton, C. A., J. A. Holloway, E. J. Popplewell, J. K. Shute, J. Boughton, and J. O. Warner. 2003. Fetal exposure to intact immunoglobulin E occurs via the gastrointestinal tract. *Clin. Exp. Allergy* 33: 306–311.
- Eisenbarth, S. C., O. R. Colegio, W. O'Connor Jr., F. S. Sutterwala, and R. A. Flavell. 2008. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 453: 1122–1127.
- Mowat, A. M. 1987. The regulation of immune responses to dietary protein antigens. *Immunol. Today* 8: 93–98.
- Sedgwick, J. D., and P. G. Holt. 1985. Down-regulation of immune responses to inhaled antigen: studies on the mechanism of induced suppression. *Immunology* 56: 635–642.
- Seymour, B. W. P., L. J. Gershwin, and R. L. Coffman. 1998. Aerosol-induced immunoglobulin (Ig)-E unresponsiveness to ovalbumin does not require $CD8^+$ or T cell receptor (TCR)- γ/δ^+ T cells or interferon (IFN)- γ in a murine model of allergen sensitization. *J. Exp. Med.* 187: 721–731.
- McMenamin, C., C. Pimm, M. McKersey, and P. G. Holt. 1994. Regulation of IgE responses to inhaled antigen in mice by antigen-specific $\gamma\delta$ T cells. *Science* 265: 1869–1871.
- Zuany-Amorim, C., C. Ruffie, S. Haile, B. B. Vargaftig, P. Pereira, and M. Pretolani. 1998. Requirement for $\gamma\delta$ T cells in allergic airway inflammation. *Science* 280: 1265–1267.
- Svensson, L., B. Lilliehook, R. Larsson, and A. Bucht. 2003. $\gamma\delta$ T cells contribute to the systemic immunoglobulin E response and local B-cell reactivity in allergic eosinophilic airway inflammation. *Immunology* 108: 98–108.
- Ferrick, D. A., M. D. Schrenzel, T. Mulvaney, B. Hsieh, W. G. Ferlin, and H. Lepper. 1995. Differential production of interferon- γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by $\gamma\delta$ T cells in vivo. *Nature* 373: 255–257.
- Huber, S. A., D. Graveline, M. K. Newell, W. K. Born, and R. L. O'Brien. 2000. $V\gamma 1^+$ T cells suppress and $V\gamma 4^+$ T cells promote susceptibility to coxsackievirus B3-induced myocarditis in mice. *J. Immunol.* 165: 4174–4181.
- Hahn, Y.-S., C. Taube, N. Jin, L. Sharp, J. M. Wands, M. Kemal Aydinutug, M. Lahn, S. A. Huber, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2004. Different potentials of $\gamma\delta$ T cell subsets in regulating airway responsiveness: $V\gamma 1^+$ cells, but not $V\gamma 4^+$ cells, promote airway hyperreactivity: Th2 cytokines, and airway inflammation. *J. Immunol.* 172: 2894–2902.
- O'Brien, R. L., C. L. Roark, N. Jin, M. K. Aydinutug, J. D. French, J. L. Chain, J. M. Wands, M. Johnston, and W. K. Born. 2007. $\gamma\delta$ T cell receptors: functional correlations. *Immunol. Rev.* 215: 77–88.
- Jensen, K. D. C., X. Su, S. Shin, L. Li, S. Youssef, S. Yamasaki, L. Steinman, T. Saito, R. M. Locksley, M. M. Davis, N. Baumgarth, and Y.-H. Chien. 2008. Thymic selection determines gamma/delta T cell effector fate: antigen-naïve cells make interleukin-17 and antigen-experienced cells make interferon γ . *Immunity* 29: 90–100.
- Heilig, J. S., and S. Tonegawa. 1986. Diversity of murine γ genes and expression in fetal and adult T lymphocytes. *Nature* 322: 836–840.
- Jin, N., C. Taube, L. Sharp, Y.-S. Hahn, X. Yin, J. M. Wands, C. L. Roark, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2005. Mismatched antigen prepares $\gamma\delta$ T cells for suppression of airway hyperresponsiveness. *J. Immunol.* 174: 2671–2679.
- Lahn, M., A. Kanehiro, K. Takeda, J. Terry, Y.-S. Hahn, M. K. Aydinutug, A. Konowal, K. Ikuta, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2002. MHC class I-dependent $V\gamma 4^+$ pulmonary T cells regulate $\alpha\beta$ T cell-independent airway responsiveness. *Proc. Natl. Acad. Sci. USA* 99: 8850–8855.
- Jin, N., N. Miyahara, C. L. Roark, J. D. French, M. K. Aydinutug, M. J. L., L. Gapin, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2007. Airway hyperresponsiveness through synergy of $\gamma\delta$ T cells and NKT cells. *J. Immunol.* 179: 2961–2968.
- McMenamin, C., M. McKersey, P. Kühnlein, T. Hünig, and P. G. Holt. 1995. $\gamma\delta$ T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. *J. Immunol.* 154: 4390–4394.
- Hayday, A., and R. Tigelaar. 2003. Immunoregulation in the tissues by $\gamma\delta$ T cells. *Nat. Rev. Immunol.* 3: 233–242.
- Cook, L., N. Miyahara, N. Jin, J. M. Wands, C. Taube, C. L. Roark, T. A. Potter, E. W. Gelfand, R. L. O'Brien, and W. K. Born. 2008. Evidence that $CD8^+$ dendritic cells enable the development of $\gamma\delta$ T cells that modulate airway hyperresponsiveness. *J. Immunol.* 181: 309–319.
- Jin, N., C. L. Roark, N. Miyahara, C. Taube, M. K. Aydinutug, J. M. Wands, Y. Huang, Y. S. Hahn, E. W. Gelfand, R. L. O'Brien, and W. K. Born. 2009. Allergic airway hyperresponsiveness-enhancing $\gamma\delta$ T cells develop in normal untreated mice and fail to produce IL-4/13, unlike Th2 and NKT cells. *J. Immunol.* 182: 2002–2010.
- O'Brien, R. L., M. Lahn, W. Born, and S. A. Huber. 2001. T cell receptor and function cosegregate in $\gamma\delta$ T cell subsets. *Chem. Immunol.* 79: 1–28.
- Hahn, Y.-S., C. Taube, N. Jin, K. Takeda, J.-W. Park, J. M. Wands, M. K. Aydinutug, C. L. Roark, M. Lahn, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2003. $V\gamma 4^+$ T cells regulate airway hyperreactivity to methacholine in ovalbumin-sensitized and challenged mice. *J. Immunol.* 171: 3170–3178.
- Wands, J. M., C. L. Roark, M. K. Aydinutug, N. Jin, Y.-S. Hahn, L. Cook, X. Yin, J. Dalporto, M. Lahn, D. M. Hyde, et al. 2005. Distribution and leukocyte contacts of $\gamma\delta$ T cells in the lung. *J. Leukocyte Biol.* 78: 1086–1096.
- Lambrecht, B. N., and H. Hamad. 2003. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* 3: 994–1003.
- Shortman, K., and S. H. Naik. 2007. Steady-state and inflammatory dendritic cell development. *Nat. Rev. Immunol.* 7: 19–30.
- Carbone, F. R., G. T. Belz, and W. R. Heath. 2004. Transfer of antigen between migrating and lymph node-resident DCs in peripheral T-cell tolerance and immunity. *Trends Immunol.* 25: 655–658.
- Allan, R. S., C. M. Smith, G. T. Belz, A. L. van Lint, L. M. Wakim, W. R. Heath, and F. R. Carbone. 2003. Epidermal viral immunity induced by $CD8\alpha^+$ dendritic cells but not by Langerhans cells. *Science* 301: 1925–1928.
- Mount, A. M., C. M. Smith, F. Kupresanin, K. Stoermer, W. R. Heath, and G. T. Belz. 2008. Multiple dendritic cell populations activate $CD4^+$ T cells after viral stimulation. *PLoS ONE* 3: 1–10.
- Belz, G. T., C. M. Smith, L. Kleinert, P. Reading, A. Brooks, K. Shortman, F. R. Carbone, and W. R. Heath. 2004. Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. *Proc. Natl. Acad. Sci. USA* 101: 8670–8675.