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Functional Conversion and Dominance of γδ T Subset in Mouse Experimental Autoimmune Uveitis

Dongchun Liang,* Hong Nian,† Hui Shao,‡ Henry J. Kaplan,‡ and Deming Sun*

We have previously shown that activated γδ T cells have a much stronger proinflammatory effect in the development of experimental autoimmune uveitis than their nonactivated counterparts. Our present study explored γδ T cell subsets are functionally distinct in autoimmune pathogenesis and determined the pathogenic contribution of biased Vγ4$^+$ γδ T cell activation in this disease. By systematically comparing two major peripheral γδ T cell subsets, the Vγ1$^+$ and the Vγ4$^+$ cells, we found that the Vγ4$^+$ cells were readily activated in B6 mice during experimental autoimmune uveitis development, whereas Vγ1$^+$ cells remained nonactivated. Cytokines that were abundantly found in the serum of immunized mice activated Vγ4$^+$, but did not activate Vγ1$^+$, cells. The Vγ4$^+$ cells had a strong proinflammatory activity, whereas the Vγ1$^+$ cells remained nonactivated when tested immediately after isolation from immunized mice. However, when the Vγ1$^+$ cells were activated in vitro, they promoted inflammation. Our results demonstrated that activation is a major factor in switching the enhancing and inhibiting effects of both Vγ1$^+$ and Vγ4$^+$ γδ T cell subsets, and that γδ T cell subsets differ greatly in their activation requirements. Whether the enhancing or inhibiting function of γδ T cells is dominant is mainly determined by the proportion of the γδ T cells that are activated versus the proportion not activated. ***The Journal of Immunology*****, 2017, 198: 000–000.**

The γδ T cells play a major role in both innate and adaptive immunity (1, 2) and are the early infiltrating cells in inflammatory disorders such as autoimmune diseases (3–10). Studies have shown that γδ T cells can either enhance (9, 11–15) or inhibit (8, 15–20) an adaptive immune response. This functional diversity has been previously credited to γδ T cell subsets that express distinct TCRs (21–25). Later studies have also demonstrated that the enhancing and inhibiting activities of γδ T cells could be reversed if γδ T cells were pre-exposed to bacterial products (23, 25). Clinical approaches have been developed to use γδ T cells as a therapeutic modality (26–28). A better knowledge of how these cells exert their enhancing and inhibitory functions should improve their therapeutic use.

More than 90% of the γδ T cells in the peripheral lymphoid tissues of naive mouse are either Vγ1$^+$ or Vγ4$^+$ γδ T cells; among these, Vγ1$^+$ γδ T cells are the major components (29). This dominance shifts during disease. Thus, at the peak of the peripheral immune response in induced autoimmune diseases such as experimental autoimmune encephalomyelitis and autoimmune uveitis (EAU), the dominant Vγ1$^+$ γδ T cells are rapidly replaced by Vγ4$^+$ γδ T cells; meanwhile, the total γδ T cell number is increased by 5- to 10-fold (11, 30–33). We have previously shown that activated γδ T cells have a much stronger proinflammatory effect than their nonactivated counterparts (34–36). Studies on the mechanism of how γδ T cells become activated and whether manipulation of γδ activation could allow us to control disease progression should have implications for their therapeutic use.

Using a mouse EAU model that consistently demonstrated a dominant activation of Vγ4$^+$ γδ T cells in a preclinical stage (30), we investigated the mechanism leading to this biased γδ activation and the correlation of disease pathogenesis and the dominance shift. Our results showed that activation of macrophages/dendritic cells (DCs) in the immunized mice produces increased amounts of cytokines; these cytokines have a biased stimulatory effect on Vγ4$^+$ cells, but not on Vγ1$^+$ cells, leading to a preferential activation and expansion of the Vγ4$^+$ γδ subset.

Structural and functional comparison between Vγ1$^+$ and Vγ4$^+$ γδ T cell subsets isolated from EAU-prone B6 mice before or after immunization showed that the enhancing and inhibitory functions of both Vγ1$^+$ and Vγ4$^+$ T cells are determined by their activation status. The Vγ4$^+$ cells are dominant among the activated γδ T cells in immunized mice, and these cells possessed greatly increased proinflammatory activity. By contrast, the Vγ1$^+$ cells remained nonactivated in immunized mice and are functionally suppressive. However, the suppressive effect of Vγ1$^+$ cells could also be converted to an enhancing effect if these cells were rendered activated. The balance of enhancing or inhibiting function of γδ T cells is mainly determined by the proportion of the γδ T cells that are activated versus those that are not activated.

Our results demonstrated that activation is a major factor in switching the enhancing and inhibitory functions of both the Vγ1$^+$ and the Vγ4$^+$ γδ T subsets. The difference in activation requirements accounted for the selective dominance of a specific γδ T subset. The net functional balance between the enhancing and inhibiting effects of γδ T cells is mainly determined by the number of activated γδ T cells and the proportion of activated versus nonactivated γδ T cells.

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Abbreviations used in this article: BMDC, bone marrow DC; DC, dendritic cell; EAU, experimental autoimmune uveitis; IRBP, interphotoreceptor retinoid-binding protein.

Materials and Methods

Animals and reagents

Female C57BL/6 (B6) and TCR-$\delta^{-/-}$ mice on the B6 background, purchased from The Jackson Laboratory (Bar Harbor, ME), were housed and maintained in the animal facilities of the University of California, Los Angeles, and were used at 12–16 wk of age. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles (Protocol ARC#2014-029-03A). Recombinant murine IL-12, IL-23, and GM-CSF were purchased from R&D Systems (Minneapolis, MN). FITC-, PE-, or allophycocyanin-conjugated mouse mAbs against mouse αβ-TCR (clone H57-597), mouse γδ-TCR Vγ4 (UC3), mouse γδ-TCR Vγ1 (211), mouse γδ-TCR (clone GL3), mouse IL-17, mouse IFN-γ, mouse CD73, or mouse CD44 and isotype control Abs were all purchased from BioLegend (San Diego, CA).

Cell preparation

At day 13 postimmunization, $CD3^+$ T cells were purified from the spleen or draining lymph nodes of B6 or TCR-$\delta^{-/-}$ mice immunized with peptide interphotoreceptor retinoid-binding protein $(IRBP)_{1–20}$ (aa 1–20 of human IRBP; LifeTein, Hillsborough, NJ) by positive selection using a combination of FITC-conjugated anti-mouse CD3 Abs and anti-FITC Ab-coated Microbeads. Cells were then separated on an auto-MACS separator, according to the manufacturer's suggested protocol (Miltenyi Biotec, Auburn, CA).

The γδ T cells were isolated from $IRBP_{1–20}$-immunized mice at 13 d postimmunization, using a combination of FITC-conjugated anti–TCR-δ Abs and anti-FITC Ab-coated Microbeads, followed by separation using an auto-MACS. To test the effect of activation by either cytokines or DCs, freshly prepared γδ T cells from $IRBP_{1–20}$-immunized B6 mice were cultured in cytokine-free medium for 5 d to generate the resting state because γδ T cells freshly isolated from $IRBP_{1–20}$-immunized mice are activated. The cells were then incubated for 48 h with a combination of IL-1, IL-7, and IL-23 (10 ng/ml of each), as described previously (35), or for 48 h with bone marrow DCs (BMDCs) and anti-CD3 Abs.

EAU induction and evaluation

To induce EAU, B6 mice were injected s.c. at six spots at the tail base and on the flank with a total of 200 μl emulsion consisting of equal volumes of 150 μg peptide $IRBP_{1–20}$ in PBS and CFA (BD Biosciences, San Diego, CA), and i.p. with 200 ng pertussis toxin (Sigma-Aldrich, St. Louis, MO).

Assessment of Th17 responses

Responder TCR-$\alpha\beta^+$ T cells (3×10^6) prepared from $IRBP_{1–20}$-immunized TCR-$\delta^{-/-}$ mice were cocultured for 48 h with $IRBP_{1–20}$ (10 μg/ml) and irradiated spleen cells (2×10^6 per well) as APCs in a 12-well plate under Th17 polarized conditions (culture medium supplemented with 10 ng/ml IL-23), with or without the addition of γδ T cells. IL-17 levels in the culture medium were then measured using ELISA kits (R&D Systems), and the number of Ag-specific T cells expressing IL-17 was determined by intracellular staining, followed by FACS analysis, as described below (35, 37).

ELISA measurement of cytokine levels in serum and culture supernatants

ELISA was used to measure cytokine (IL-1, IL-7, IL-12, IL-23, and IL-17) levels in the serum on day 13 postimmunization and in the 48-h culture

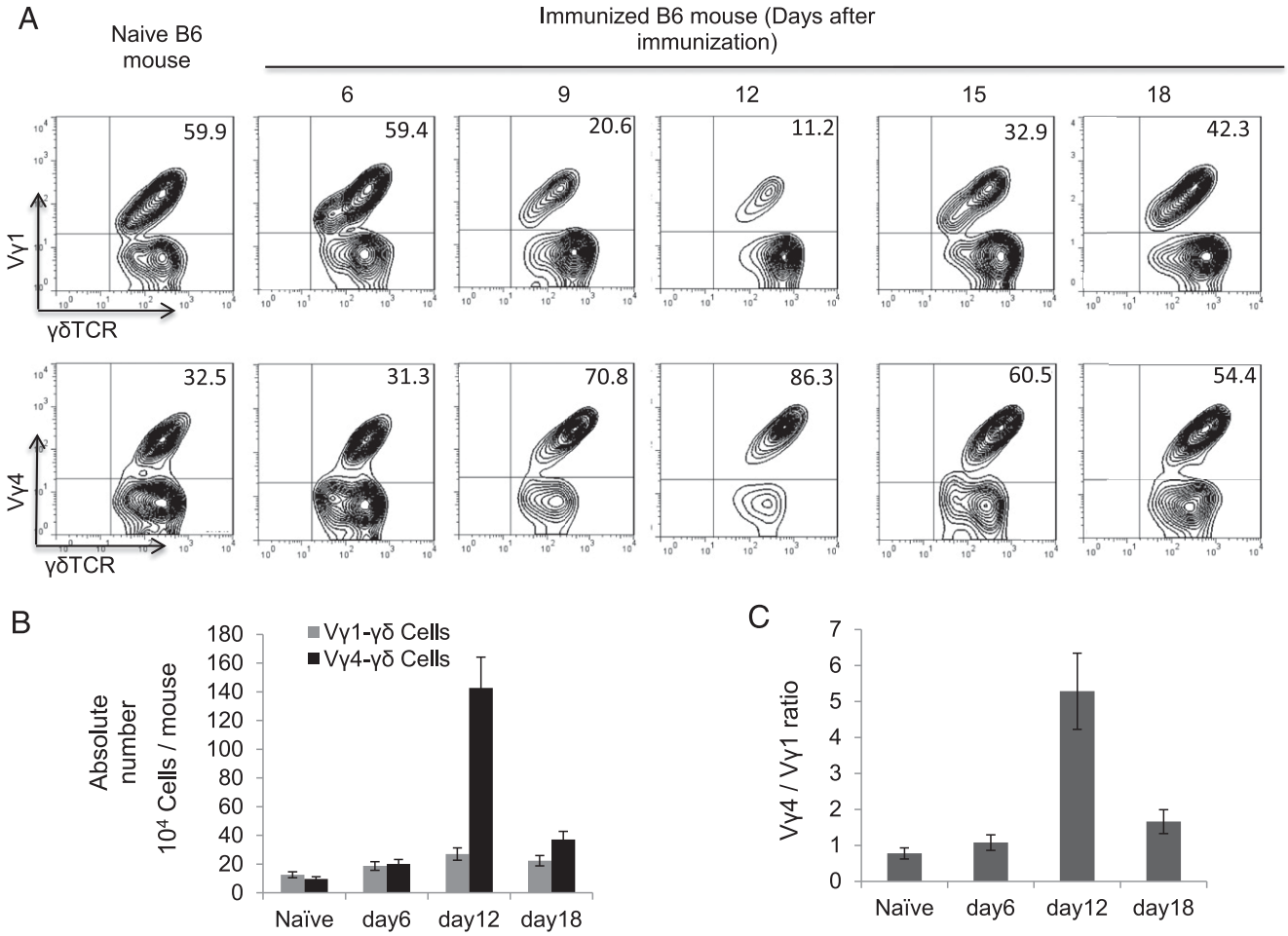

FIGURE 1. $V\gamma4^+$ γδ T cells gradually become the dominant γδ subset in the periphery of immunized B6 mice. (**A**) Relative number of $V\gamma1^+$ and $V\gamma4^+$ γδ T cells. Splenic T cells from $IRBP_{1–20}$-immunized B6 mice ($n = 6$) were double stained with anti-mouse Vγ1 (upper panels) or anti-mouse Vγ4 (lower panels) and anti-mouse γδ-TCR Abs, on the indicated days after immunization. *Pan* γδ T cells were gated for FACS analysis. (**B** and **C**) Absolute number of $V\gamma1^+$ and $V\gamma4^+$ γδ T cells. Total numbers of splenic $V\gamma1^+$ and $V\gamma4^+$ γδ T cells were evaluated from immunized B6 mice (B), and the ratio change between $V\gamma1^+$ and $V\gamma4^+$ γδ T cells is shown in (C). The results are from a single experiment ($n = 6$) and are representative of three independent studies.

supernatants of responder T cells isolated from immunized TCR-$\gamma^{-/-}$ mice in the absence or presence of Vγ1 or Vγ4 cells.

Immunofluorescence flow cytometry for surface and cytoplasmic Ags

In vivo primed T cells were stimulated with the immunizing Ag and APCs for 5 d; then the T cells were separated using Ficoll gradient centrifugation and stimulated in vitro for 4 h with 50 ng/ml PMA, 1 μg/ml ionomycin, and 1 μg/ml brefeldin A (all from Sigma-Aldrich). Aliquots of cells (2×10^5 cells) were then fixed, permeabilized overnight with Cytofix/Cytoperm buffer (eBioscience, San Diego, CA), and intracellularly stained with PE-conjugated anti-mouse IFN-γ Abs or FITC-labeled anti-mouse IL-17 Abs. Data collection and analysis were performed on a FACScalibur flow cytometer using CellQuest software.

Generation of BMDCs

BMDCs were generated by incubating bone marrow cells from B6 mice for 5 d in the presence of 10 ng/ml murine granulocyte rM-CSF (R&D Systems), as described previously (38). To test the stimulating effect of these cells on γδ T cells, the BMDCs were pretreated for 48 h with 100 ng/ml LPS (39).

CFSE assays

Purified αβ-TCR$^+$ T cells from $IRBP_{1–20}$-immunized TCR-$\delta^{-/-}$ mice were stained with CFSE (Sigma-Aldrich), as described previously (40). Briefly, the cells were washed and suspended at 50×10^6 cells/ml in serum-free RPMI 1640 medium; cells were then incubated at 37°C for 10 min with gentle shaking with a final concentration of 5 μM CFSE before being washed twice with, and suspended in, complete medium, stimulated with immunizing peptide in the presence of APCs, and analyzed by flow cytometry.

Measurement of adenosine receptor mRNA levels

Cytokine (IL-1, IL-7, and IL-23) receptor mRNA levels were determined by real-time PCR. Vγ1$^+$ and Vγ4$^+$ γδ T cells were purified from $IRBP_{1–20}$-immunized B6 mice by autoMACS separation. Total RNA was extracted from 2×10^5 cells using an RNA isolation kit (Invitrogen, Carlsbad, CA) and treated with DNase I (GE Healthcare, Piscataway, NJ); then 0.1 μg was reverse transcribed into cDNA using a Moloney murine leukemia virus reverse-transcription kit (Invitrogen) and tested in a Cyber Green real-time PCR assay. Levels of each cDNA were measured in triplicate, using GAPDH cDNA as reference. Each cDNA sample was amplified for the gene of interest. The concentration of the mRNA for the gene of interest was

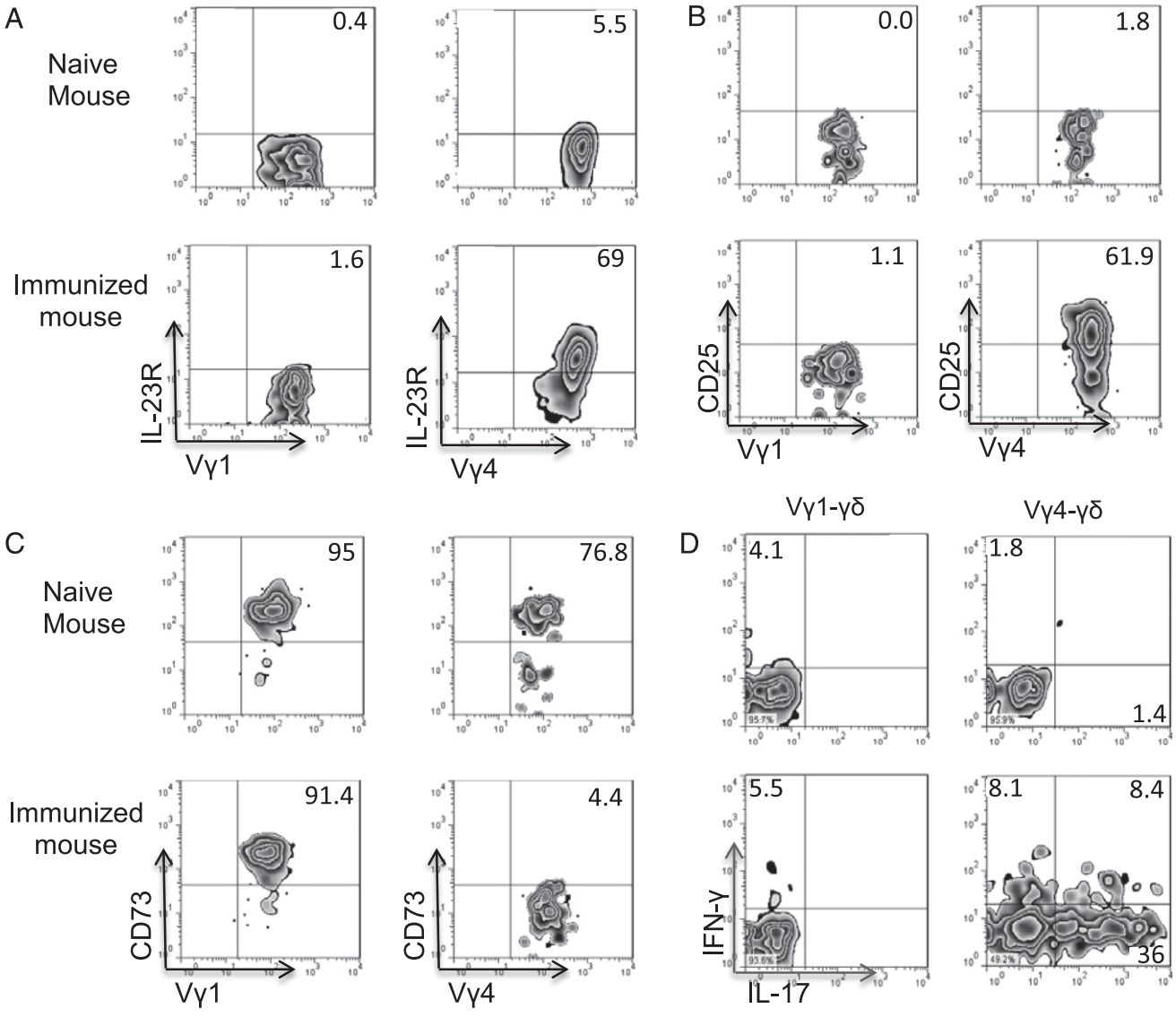

FIGURE 2. Vγ4$^+$ cells became activated, whereas the Vγ1$^+$ γδ T cells remained nonactivated in EAU-induced B6 mice. Splenic CD3$^+$ cells were enriched from a group ($n = 6$) of naive mice or from immunized mice 13 d postimmunization using MACS column. They were double stained with anti-mouse Vγ1/Vγ4 Abs and anti–IL-23R (**A**); anti-mouse CD25 Ab (**B**); or anti-CD73 Ab (**C**). Alternatively, there was intracellular staining of IFN-γ and IL-17 (**D**). Vγ1 and Vγ4 cells were selectively gated for FACS analysis.

determined using the comparative threshold cycle number and normalized to that of the internal GAPDH control. Results were shown as $2^{-\triangle Ct}$.

Statistical analyses

All experiments were repeated three to five times. Experimental groups typically consisted of six mice, and the figures show the data from a representative experiment. The statistical significance of differences between the values for different groups was examined using the Mann–Whitney *U* test.

Results

Vγ4$^+$ cells gradually become the dominant γδ cells in the spleen of immunized B6 mice

Kinetic examination of the intensity of induced autoimmune responses and the appearance of Vγ1$^+$ and Vγ4$^+$ T cells in the spleen of immunized B6 mice demonstrated that the maximal responses to the immunizing Ag were constantly detected ∼13 d after immunization, ∼1 wk before clinical symptoms were detected. The Vγ1$^+$ γδ T cells constitute approximately two-thirds of the total splenic γδ T cells in naive mice, and in immunized mice until 6 d postimmunization, whereas the Vγ4$^+$ γδ T cells constitute only one-third (Fig. 1A). The non-Vγ4 non-Vγ1 cells are very few. These proportional numbers shifted dramatically, starting 6–7 d postimmunization. By 12 d postimmunization, the Vγ1$^+$ γδ T cells had declined quickly to 11.2% of the total splenic γδ T cells, whereas the Vγ4$^+$ γδ T cells had increased to 86.5%, leading to a change in the ratio of Vγ1:Vγ4 from 7:3 to 1:8. Estimation of the absolute numbers of Vγ1 and Vγ4 cells showed, additionally, the absolute number of Vγ1$^+$ γδ T cells remained largely unchanged, whereas that of the Vγ4$^+$ γδ T cells increased 5- to 10-fold (Fig. 1B, 1C).

Vγ4$^+$ cells in immunized B6 mice are activated, whereas the Vγ1$^+$ γδ T cells remain nonactivated

We have assessed the activation status of Vγ1 and Vγ4 cells immediately after isolation from splenic T cells of naive or immunized mice. Fig. 2A shows that both Vγ1 and Vγ4 cells from naive mice were CD25^{low}CD44^{low}. In immunized mice, the Vγ4$^+$ cells became CD25^{high}CD44^{high}, but the Vγ1$^+$ T cells of immunized mice remained CD25^{low}CD44^{low}. As our previous study found activated γδ T cells expressed downregulated CD73 (41, 42), we examined whether Vγ1$^+$ and Vγ4$^+$ γδ T cells express different levels of CD73. The results showed that a majority of the Vγ4$^+$ cells from immunized mice were CD73^{low} or CD73$^-$; by contrast, the Vγ1$^+$ cells from immunized mice remained CD73^{high} (Fig. 2C). In addition, a significant portion of the Vγ4$^+$ T cells, but not of the Vγ1$^+$ T cells, expressed IL-17, in the absence of additional in vitro stimulation (Fig. 2D). We have previously reported that activated γδ T cells have a strong enhancing effect on Th17 responses (30, 34, 35, 42). We then determined whether Vγ1$^+$ γδ T cells isolated from immunized mice differed in their enhancing autoimmune response from the Vγ4$^+$ cells isolated from immunized mice. Responder αβ T cells isolated from immunized TCR-δ$^{-/-}$ mice were stimulated with the immunizing Ag and APCs, with an addition of Vγ1$^+$ or Vγ4$^+$ cells isolated from immunized mice. Five days after in vitro stimulation, the

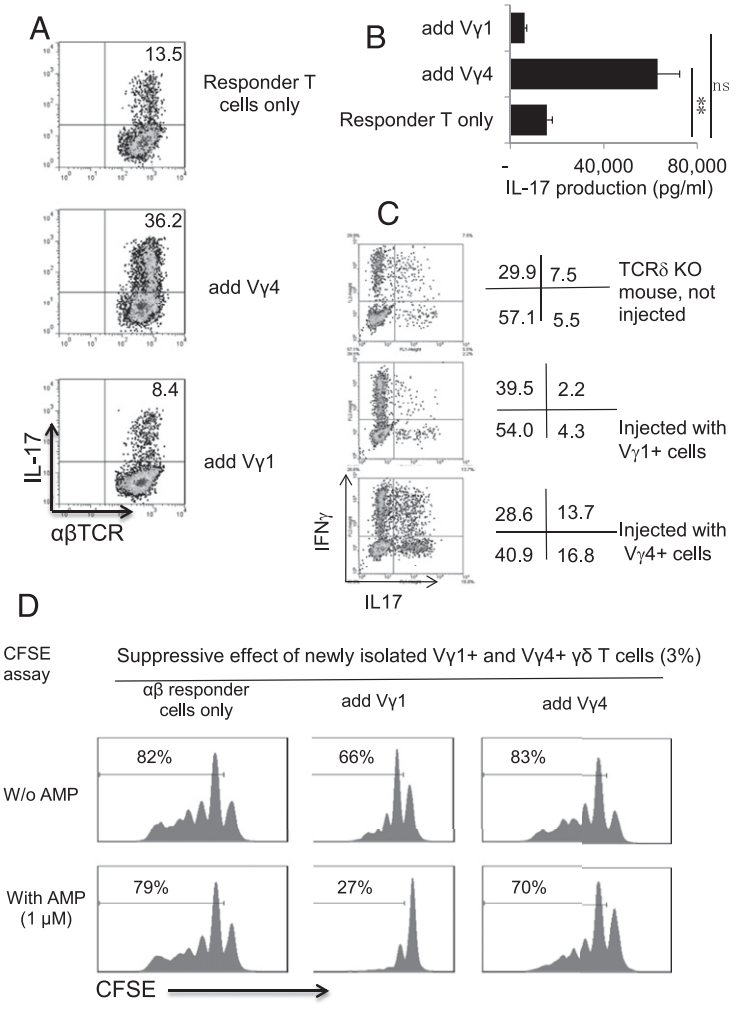

FIGURE 3. The Vγ4$^+$ cells, but not the Vγ1$^+$ cells isolated from immunized mice, had an enhancing effect on activation of autoreactive T cells. (**A**) Splenic αβ T cells were enriched from a group of immunized TCR-δ$^{-/-}$ mice 13 d postimmunization using MACS column. They were stimulated with the immunizing Ag and APCs for 5 d, in the presence of Vγ1 or Vγ4 T cells, before they were stained with anti–IL-17 and anti-mouse αβ Abs; and αβ T cells were gated for FACS analysis. Results are from one experiment representing five separate studies. (**B**) ELISA test of IL-17 production of the responder T cells activated in the presence of Vγ1 or Vγ4 T cells; groups ($n = 6$) of TCR-δ$^{-/-}$ mice with or without injection of Vγ1$^+$ and Vγ4$^+$ γδ T cells (1×10^6 per mouse) were immunized 1 d later with a pathogenic dose of IRBP$_{1-20}$/CFA, and the IL-17 production of the responder T cells was assessed 2 d after in vitro stimulation under Th17 polarized conditions, as described earlier. The numbers indicated are calculated from triplicated samples. Data are from a single experiment, representative of three independent experiments. $^{**}p < 0.01$; ns, not significant. (**C**) Injection of TCR-δ$^{-/-}$ mice with Vγ4$^+$, but not Vγ1$^+$, γδ T cells, before IRBP$_{1-20}$ immunization increases the generation of IL-17$^+$ IRBP-specific T cells. Groups ($n = 6$) of TCR-δ$^{-/-}$ mice with or without injection of Vγ4$^+$, but not Vγ1$^+$, γδ T cells (1×10^6 per mouse) were immunized 1 d later with a pathogenic dose of IRBP$_{1-20}$. The IFN-γ$^+$ and IL-17$^+$ T cells were assessed 5 d after in vitro stimulation. The αβ T cells were gated for FACS analysis. (**D**) Vγ1$^+$, but not Vγ4$^+$, cells newly isolated from immunized mice, showed greatly augmented suppressive effect in the presence of exogenous AMP. Responder T cells isolated from TCR-δ$^{-/-}$ mice were labeled with CFSE and stimulated with the immunizing IRBP$_{1-20}$ and APCs, in the absence or presence of added (2%) Vγ1$^+$ or Vγ4$^+$ cells, freshly prepared from immunized mice. The test was conducted in the absence of additions (left panels), in the presence of AMP (middle panels), and in the presence of AMP and adenosine 5′-(α,β-methylene)diphosphate, the CD73 inhibitor (right panels).

IL-17$^+$ cells among the responder T cells were assessed by intracellular staining with IL-17 (Fig. 3A). In addition, T cell supernatants were tested for IL-17 production by ELISA 48 h after stimulation (Fig. 3B). Our results showed that the addition of a small number (2%) of Vγ4$^+$ cells strongly enhanced the Th17 response, whereas the addition of Vγ1$^+$ cells was ineffective or slightly suppressive (Fig. 3A, 3B). In vivo test agreed with this prediction: TCR-δ$^{-/-}$ recipient mice injected with Vγ4$^+$, but not with Vγ1$^+$, γδ T cells showed significantly enhanced Th17 responses (Fig. 3C), as examined by intracellular staining of the responder T cells, or by assessing cytokine production in 48 cultured supernatants by ELISA (data not shown). In addition, when the suppressive effect was tested, the Vγ1$^+$ cells, but not Vγ4$^+$ cells, that were freshly isolated from immunized mice showed greater suppressive effect, particularly when in the presence of exogenously added AMP (Fig. 3D).

Cytokine exposure activates the Vγ4$^+$, but not the Vγ1$^+$, γδ T cells in vitro

To determine whether different activation requirements were due to the biased activation of Vγ4$^+$ γδ subset during autoimmune induction, we examined the serum cytokines that were increased in immunized mice and found IL-1, IL-23, and IL-7 increased significantly in the serum of immunized mice, as compared with that of naive mice (Fig. 4). When separated Vγ1$^+$ and Vγ4$^+$ γδ T cells were exposed to a combination of IL-1, IL-23, and IL-7, only the Vγ4$^+$, but not the Vγ1$^+$, γδ T cells were activated (Fig. 5A–C). The stimulated Vγ4$^+$ T cells produced significantly increased amounts of IL-17 after exposure to either cytokines or anti-CD3 Ab (Fig. 5D). The Vγ1$^+$ cells, in contrast, expressed increased levels of CD44 and CD25 only and downregulated CD73 after exposure to anti-CD3 Abs, but not to cytokines (Fig. 5A–C). To determine the mechanism by which cytokines biasedly activated Vγ4$^+$ γδ T cells, we have compared the cytokine receptor expression between Vγ1$^+$ and Vγ4$^+$ γδ T cells. The new RT-PCR results (Fig. 5E) showed that Vγ4$^+$, but not Vγ1$^+$ cells in immunized mice expressed increased amounts of IL-1R and IL-23R, even though expression of IL-7R was indistinguishable between the two cells.

Role of DC in biased Vγ4$^+$ T cell activation

Our previous studies showed that DCs have a stimulating effect on γδ T cells (36, 43, 44), if pre-exposed to TLR ligands such as LPS (39, 43). BMDCs were cultured from bone marrow cells of immunized mice in medium containing GM-CSF. To determine whether LPS-treated BMDCs stimulated Vγ1$^+$ and Vγ4$^+$ γδ subsets, CD3$^+$ responder T cells were isolated from the spleens of naive B6 mice, and the gated Vγ1$^+$ and Vγ4$^+$ γδ cells were assessed for expression of CD25 and CD44 by FACS analysis, 2 d after coculture with BMDCs. Our results showed that the Vγ4$^+$ cells expressed greatly increased amounts of CD25 and CD44, whereas the Vγ1$^+$ did not (Fig. 6B compares to Fig. 6A) after exposure to LPS-treated BMDCs. We have also examined whether direct cell–cell contact is mandatory for DCs to render a Vγ4$^+$ T cell activated. Vγ1$^+$ and Vγ4$^+$ cells were cultured in medium with supernatants of LPS-stimulated BMDCs (1:10 dilution) for 48 h, before assessment of γδ T cell activation molecules, CD44 and CD25 (42). The results showed that LPS-treated BMDC supernatant has a strong stimulating effect on Vγ4$^+$, but not on Vγ1$^+$ cells (Fig. 6C). Cytokine test (Fig. 6D) detected increased IL-17 production from Vγ4$^+$, but not from Vγ1$^+$ cells after incubation with BMDCs.

Vγ1$^+$ γδ T cells' enhancing activity is increased, but suppressive activity is decreased after exposure to anti-CD3 mAb

To determine whether activation can convert the enhancing and suppressive effect of both Vγ1$^+$ and Vγ4$^+$ subsets, Vγ1$^+$ and Vγ4$^+$ T cells were assessed for their enhancing and inhibiting effects before or after exposure to anti-mouse CD3 mAb (34, 42). The enhancing effect on Th17 response was assessed by testing the response of in vivo primed αβ T cells stimulated with the immunizing Ag and APCs under Th17 polarized conditions (culture medium containing IL-23) and in the absence or presence of added γδ T cells (Fig. 7A). The enhancing effect was significantly augmented when Vγ1$^+$ or Vγ4$^+$ cells were added after anti-CD3 stimulation. As expected, the enhancing effect was minimal when nonactivated cells were added to responder T cells.

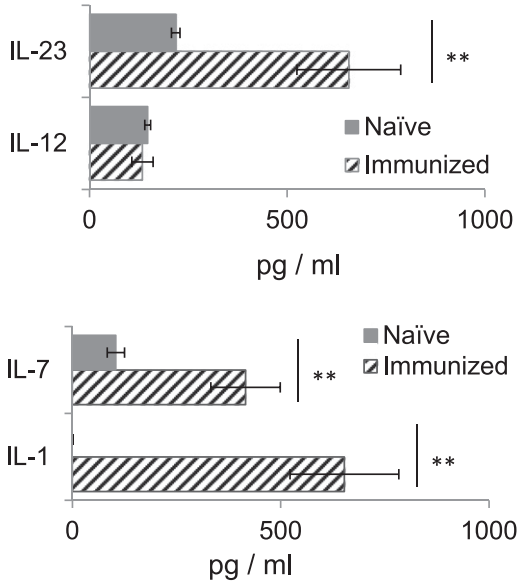

FIGURE 4. Serum cytokine detection of naive and immunized B6 mice. Blood samples collected from groups ($n = 6$) of naive and immunized mice (on day 13 postimmunization) were pooled and tested in triplicate by ELISA. The SEs were calculated from triplicated samples. Data are from a single experiment, representative of three independent experiments. The SEs were calculated from triplicated samples. Data are from a single experiment, representative of three independent experiments. $^{**}p < 0.01$.

We previously reported that the inhibitory effect of γδ T cells was better demonstrated if exogenous AMP was provided because nonactivated γδ T cell expresses higher amounts of CD73, which more effectively degrades AMP to adenosine (42, 45, 46). Activated γδ T cells, in contrast, expressed decreased levels of CD73, which weakened their ability to convert AMP to adenosine (42). Inhibition test used a CFSE assay, in which the responder αβ T cells were prelabeled with CFSE before stimulation with the immunizing peptide and APCs (Fig. 7B). Unlabeled Vγ1$^+$ and Vγ4$^+$ γδ T cells, with or without prior anti-CD3 mAb stimulation, were added to responder cells at a ratio of γδ:αβ = 1:20. The results, assessed 7 d after in vitro stimulation, showed that, in the presence of AMP, both Vγ1$^+$ and Vγ4$^+$ resting cells showed a suppressive effect, whereas both Vγ1$^+$ and Vγ4$^+$ activated cells showed a significantly diminished suppressive activity. Importantly, the suppressive effect was partially neutralized by the CD73 inhibitor, the adenosine 5′-(α,β-methylene)diphosphate (Fig. 7B) (47, 48).

Discussion

Although a regulatory effect of γδ T cells on adaptive immunity has been repeatedly observed (22, 26–28), knowledge of how these cells regulate remains very limited, and the mechanisms by which they enhance an immune response in some cases (49–51) but inhibit it in others (16–18, 52) remain largely obscure. A better understanding of the mechanisms by which γδ T cells regulate

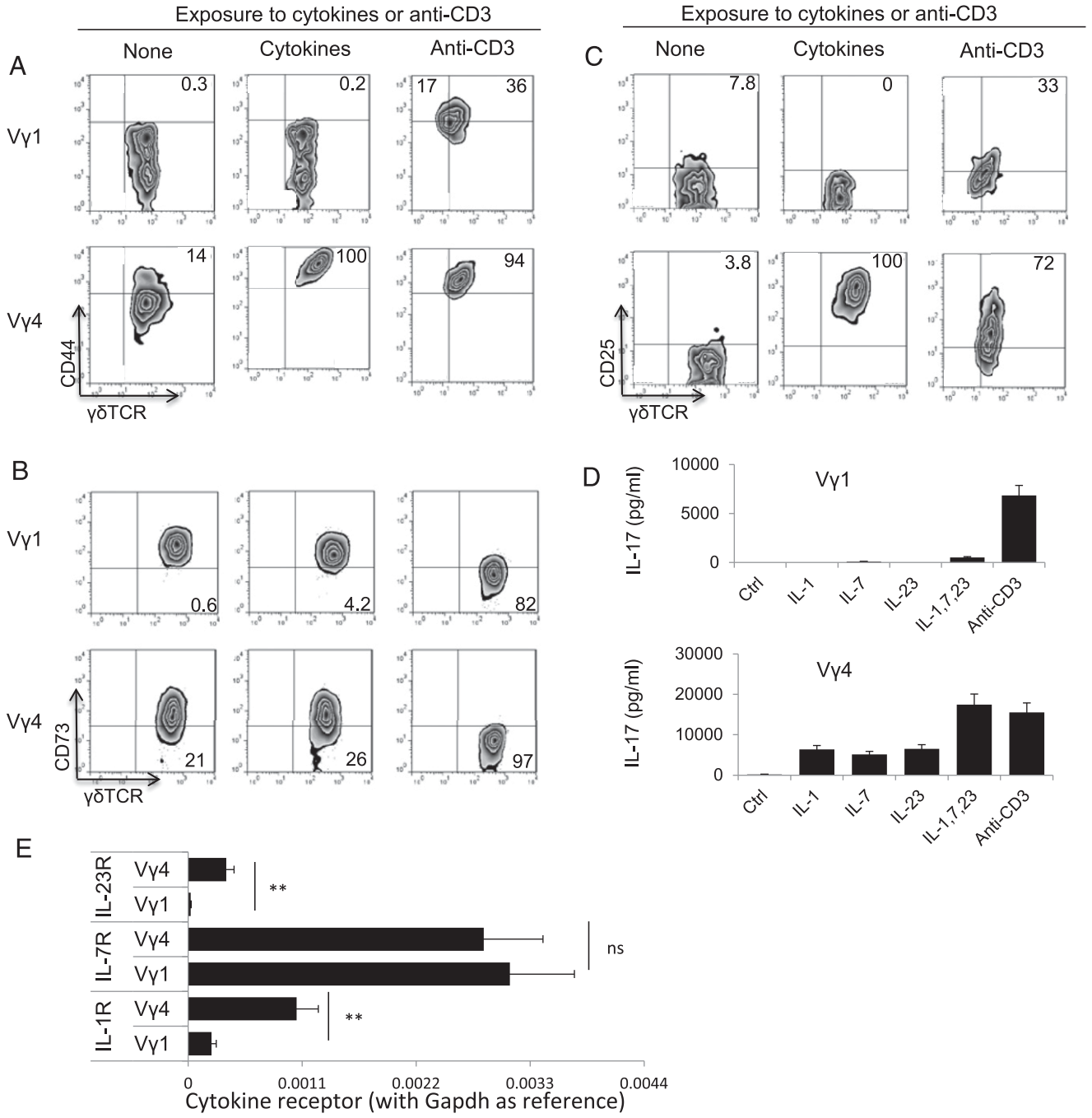

FIGURE 5. The cytokine combination (IL-1 + 7 + 23) activated Vγ4$^+$, but not Vγ1$^+$, γδ T cells. (**A**–**C**) Expression of CD44 (A), CD73 (B), and CD25 (C) by Vγ1$^+$ and Vγ4$^+$ cells was assessed before and after an exposure to cytokine or anti-CD3 Ab. Vγ1$^+$ and Vγ4$^+$ γδ subsets were separated from immunized B6 mice 13 d postimmunization. They were exposed for 48 h in culture to a combination of IL-1, IL-23, and IL-7 (10 ng/ml) or anti-CD3 Ab (1 μg/ml). Expression of CD44 (A), CD73 (B), and CD25 (C) of Vγ1$^+$ and Vγ4$^+$ cells before and after cytokine or Ab stimulation was compared, after staining with related Abs, followed by FACS analysis. A representative experiment of five separate repeats. (**D**) Cytokine production assay. Supernatants of cultured Vγ1$^+$ and Vγ4$^+$ cells were assessed in triplicate for IL-17 before and after an exposure to single or pooled cytokines of IL-1 + 7 + 23 (10 ng/ml), as indicated. (**E**) Real-time RT-PCR analysis of IL-1R, IL-7R, and IL-23R transcripts among total RNA isolated from Vγ1$^+$ and Vγ4$^+$ γδ T cells isolated from $IRBP_{1–20}$-immunized B6 mice. Vγ1$^+$ and Vγ4$^+$ cells were purified from splenocytes and drainage lymphocytes of immunized B6 mice by auto-MACs purification, using (PE)-conjugated anti-Vγ1 or anti-Vγ4 Abs and anti-PE Ab-conjugated magnetic beads. Quantitative PCR was performed with GAPDH as the internal reference. Results were represented as $2^{-\Delta Ct}$. $^{**}p < 0.01$; ns, not significant.

immune response should facilitate the development of γδ T cell–related therapeutic approaches. In a number of induced autoimmune diseases, including EAU (9, 11, 30, 53), a prevailing activation of Vγ4$^+$ cells was observed; however, the mechanism of such a biased γδ activation to disease pathogenesis remains unclear. The current study is aimed at determining the mechanism that causes the biased γδ T cell activation and determining the factors that convert the enhancing and inhibiting effect of γδ T cells. Previous studies have shown that the functional diversity of γδ T cell subsets is correlated to distinct TCRs they express (21, 22, 54, 55). Later studies also demonstrated that the enhancing and inhibiting activity of γδ T cells could be converted by a pre-exposure of these cells to bacterial products (23, 25). Furthermore, studies by our laboratory have demonstrated that activated γδ T cells possessed a greatly

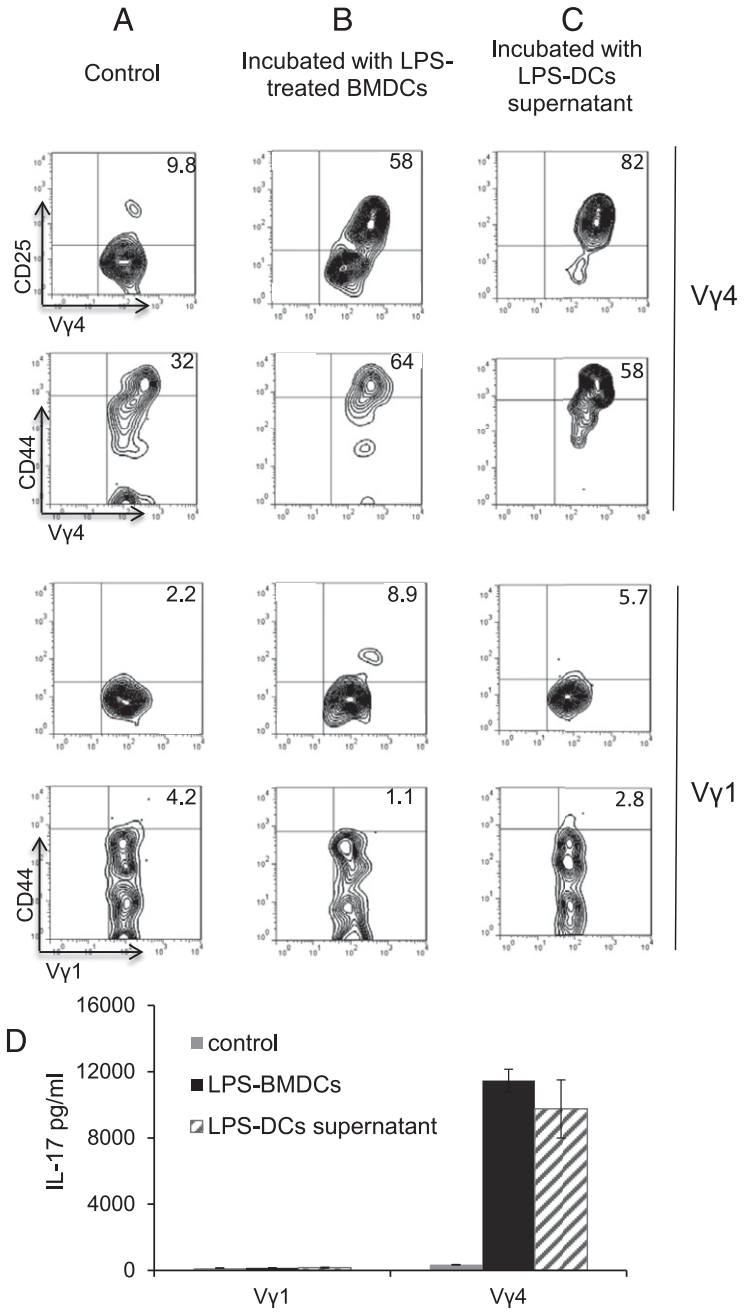

FIGURE 6. LPS-treated BMDCs activated $V\gamma4^+$, but not $V\gamma1^+$ γδ T cells. Resting $V\gamma1^+$ and $V\gamma4^+$ cells were coincubated with medium alone (**A**) or with LPS-treated (100 ng/ml) BMDCs (**B**) (ratio γδ:DC = 10:1) in six-well plates for 3 d. The treated $V\gamma1^+$ and $V\gamma4^+$ T cells were separated by Ficoll gradient centrifugation and stained with anti-CD44 and anti-CD25 Abs, followed by FACS analysis. (**C**) Separated $V\gamma1^+$ and $V\gamma4^+$ cells ($n = 6$) were incubated with LPS-treated (100 ng/ml) BMDC supernatants (1:10 diluted) for 3 d; the T cells were separated by Ficoll gradient centrifugation and stained with anti-CD44 and anti-CD25 Abs, respectively, followed by FACS analysis. (**D**) IL-17 in the supernatant of treated $V\gamma1^+$ and $V\gamma4^+$ cells was determined by ELISA ($n = 6$).

increased ability to enhance autoimmune response (34, 35, 43). Mice deficient of γδ T cells (TCR-$\delta^{-/-}$) demonstrated milder Th17 response when induced for EAU as compared with wild-type B6 mice (30, 34), and administration of γδ T cells to TCR-$\delta^{-/-}$ mice before immunization greatly enhanced the disease susceptibility associated with an augmented Th17 response (34, 35, 43). In vitro study also showed that the addition of a small number (2%) of γδ T cells to responder αβ T cells greatly enhanced their Th17 responses in vitro (30, 35, 41, 56).

Development of induced EAU in the EAU-prone B6 mouse is associated with an increased activation and dominance of $V\gamma4^+$ γδ T cells (30). The contribution of these biased γδ T cells to disease development remained unclear. In this study, we show that, in the preclinical phases of EAU, a vigorous activation and expansion of $V\gamma4^+$ cells are caused by cytokines produced by activated myeloid cells. The $V\gamma1^+$ γδ T cells are the poor responder cells of cytokines in this inflammatory environment. As a result, $V\gamma4^+$ cells are activated and become the dominant γδ subset. Our results demonstrated that this dominance shift of γδ subsets is due to the preferred activation requirements of the different γδ subsets. We conclude that the appearance of a larger number of activated γδ T cells, rather than the γδ subset expressing a specific TCR segment, or the $V\gamma4^+$ subset, is the major factor leading to disease progression. This assumption is supported by the evidence that $V\gamma1^+$ cells also gained enhancing activity after being activated.

We previously observed that administration of γδ-specific Ab removed γδ T cells more effectively in naive mice, whereas the γδ T cells of immunized mice were more resistant to the depletion, because the activated γδ T cells express decreased numbers of surface-expressed TCR that allowed them to escape removal by the injected Abs (31, 56). Injection of γδ-specific Ab may remove the non- or less activated γδ T cell population more easily than activated cells. The outcome of this treatment will weaken the suppressive effect, shifting the balance from suppression toward

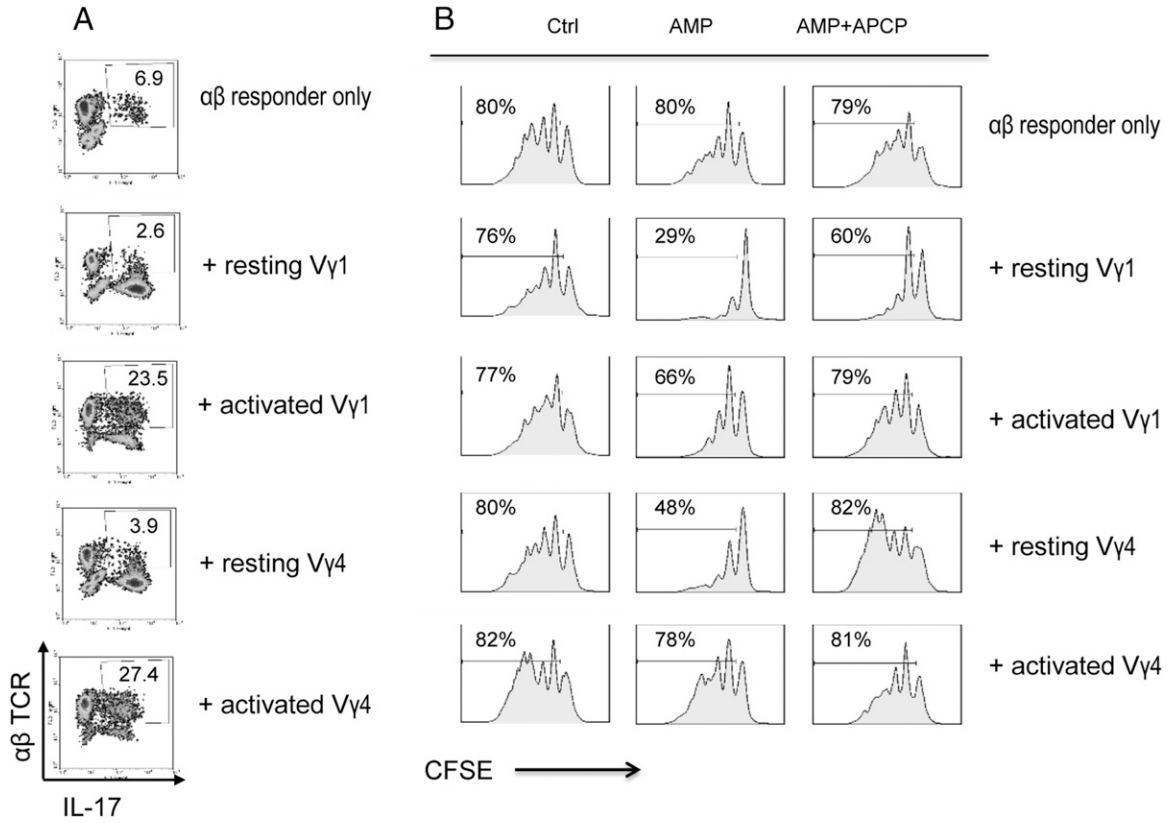

FIGURE 7. Both Vγ1$^+$ and Vγ4$^+$ γδ T cells possessed an enhancing effect on autoimmune response in their activated state and suppressive effect in their nonactivated state. (**A**) Enhancing activity of activated Vγ1$^+$ and Vγ4$^+$ γδ T cells. Responder T cells were isolated from TCR-δ$^{-/-}$ mice 13 d post-immunization. They were stimulated with the immunizing $IRBP_{1–20}$ and APCs, in the absence or presence of added (2%) Vγ1$^+$ and Vγ4$^+$ cells, with or without a prior exposure to anti-CD3 mAb. The activated T cells were then separated by Ficoll centrifugation and stained with anti–αβ-TCR and anti–IL-17 Abs, followed by FACS analysis. Results of gated αβ T cells were shown. A representative study of five separate experiments. (**B**) Nonactivated Vγ1$^+$ and Vγ4$^+$ γδ T cells are both suppressive. Responder T cells were isolated from TCR-δ$^{-/-}$ mice 13 d postimmunization. They were labeled with CFSE and stimulated with the immunizing $IRBP_{1–20}$ and APCs, in the absence or presence of added (2%) Vγ1$^+$ or Vγ4$^+$ cells. Activated Vγ1$^+$ or Vγ4$^+$ cells were prepared by exposure to anti-CD3 Ab. The test was conducted in the absence of additions (left panels), in the presence of AMP (middle panels), and in the presence of AMP and adenosine 5′-(α,β-methylene)diphosphate, the CD73 inhibitor (right panels). FACS analysis was conducted 7 d after in vitro stimulation. The demonstrated results were from a single experiment representative of five independent studies.

enhancement. Our results showing that the γδ T subsets differed in activation requirements suggested that both Vγ1$^+$ and Vγ4$^+$ γδ T cell subsets can either enhance or inhibit, depending on the disease-associated microenvironment that determines γδ T cell activation. Such a prediction is supported by observations that Vγ1$^+$ γδ T cells are dominantly activated in many different infectious disease models (57–62).

We found that cytokines (IL-1, IL-23, IL-7) that are stimulatory to Vγ4$^+$ γδ T cells were mainly produced by myeloid rather than by T cells (data not shown). Indeed, cytokines in LPS-stimulated BMDC supernatants showed a strong stimulatory effect, indicating that DC activation and release of cytokines play a major role in γδ activation in the early stage of induced autoimmune disease.

In studies clarifying the mechanism by which activated γδ T cells gain increased enhancing activity, we have made efforts to identify molecules that contribute to such a functional switch. We were able to show that activated γδ T cells express altered levels of IL-23R (35), giving γδ T cells a competitive ability to bind IL-23, which would abate subsequently initiated Th17 αβ T cell responses that require IL-23 (35). In recent studies (41, 42), we also demonstrated that activated γδ T cells express low levels of CD73 molecules, an ecto-enzyme that converts proinflammatory extracellular ATP to adenosine, which is suppressive for adaptive responses (63, 64). Expression of low levels of CD73 causes activated γδ T cells to convert less adenosine from extracellular ATP, which would predispose to stronger T cell responses. Such a hypothesis has been supported by the evidence that the suppressive effect of γδ T cells is readily amplified if exogenous AMP, a precursor molecule of adenosine, is provided; and the suppressive effect is abolished in the presence of a CD73 inhibitor (41, 42), indicating expression of different amounts of CD73 allows γδ T cells to modulate their regulatory effect. Results in the current study further confirmed this hypothesis by showing that the Vγ1$^+$ cells in EAU-induced mice retained the ability to express higher amounts of CD73; thus, the inhibiting effect prevailed in the Vγ1$^+$ cells. Nevertheless, the enhancing activity prevailed when the function of the entire γδ T cells is assessed, because the Vγ1$^+$ cells in immunized mice were greatly outnumbered by activated Vγ4$^+$ T cells. The fact that anti-CD3 mAb-activated Vγ1$^+$ γδ T cells showed an increased enhancing effect, but a decreased suppressive activity, appeared to support the notion that activation status is an important factor balancing the enhancing and suppressive effects in the two γδ T cell subsets.

In summary, our previous results demonstrated that activation switches the inhibitory and enhancing effect of γδ T cell (34, 35, 42, 43). In the current study, we further demonstrate that the activation-induced functional change is not restricted to Vγ4$^+$ cells, but also applies to Vγ1$^+$ cells. An abundance of activated Vγ4$^+$ γδ T cells shifts the enhancing and suppressing activities toward the former, leading to enhanced disease susceptibility.

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Disclosures

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References

1. Mak, T. W., and D. A. Ferrick. 1998. The gammadelta T-cell bridge: linking innate and acquired immunity. *Nat. Med.* 4: 764–765.
2. Chien, Y. H., C. Meyer, and M. Bonneville. 2014. γδ T cells: first line of defense and beyond. *Annu. Rev. Immunol.* 32: 121–155.
3. Selmaj, K., C. S. Raine, M. Farooq, W. T. Norton, and C. F. Brosnan. 1991. Cytokine cytotoxicity against oligodendrocytes: apoptosis induced by lymphotoxin. *J. Immunol.* 147: 1522–1529.
4. Wucherpfennig, K. W., J. Newcombe, H. Li, C. Keddy, M. L. Cuzner, and D. A. Hafler. 1992. γ δ T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc. Natl. Acad. Sci. USA* 89: 4588–4592.
5. Shimonkevitz, R., C. Colburn, J. A. Burnham, R. S. Murray, and B. L. Kotzin. 1993. Clonal expansions of activated γ/δ T cells in recent-onset multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 90: 923–927.
6. Peng, S. L., M. P. Madaio, D. P. M. Hughes, I. N. Crispe, M. J. Owen, L. Wen, A. C. Hayday, and J. Craft. 1996. Murine lupus in the absence of α β T cells. *J. Immunol.* 156: 4041–4049.
7. Bucht, A., K. Söderström, T. Hultman, M. Uhlén, E. Nilsson, R. Kiessling, and A. Grönberg. 1992. T cell receptor diversity and activation markers in the V δ 1 subset of rheumatoid synovial fluid and peripheral blood T lymphocytes. *Eur. J. Immunol.* 22: 567–574.
8. Peterman, G. M., C. Spencer, A. I. Sperling, and J. A. Bluestone. 1993. Role of γ δ T cells in murine collagen-induced arthritis. *J. Immunol.* 151: 6546–6558.
9. Sutton, C. E., S. J. Lalor, C. M. Sweeney, C. F. Brereton, E. C. Lavelle, and K. H. G. Mills. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31: 331–341.
10. Mukasa, A., M. Lahn, E. K. Pflum, W. Born, and R. L. O'Brien. 1997. Evidence that the same γ δ T cells respond during infection-induced and autoimmune inflammation. *J. Immunol.* 159: 5787–5794.
11. Roark, C. L., J. D. French, M. A. Taylor, A. M. Bendele, W. K. Born, and R. L. O'Brien. 2007. Exacerbation of collagen-induced arthritis by oligoclonal, IL-17-producing γ δ T cells. *J. Immunol.* 179: 5576–5583.
12. Moser, B., and M. Eberl. 2007. gammadelta T cells: novel initiators of adaptive immunity. *Immunol. Rev.* 215: 89–102.
13. Carding, S. R., and P. J. Egan. 2000. The importance of γ δ T cells in the resolution of pathogen-induced inflammatory immune responses. *Immunol. Rev.* 173: 98–108.
14. Nanno, M., Y. Kanari, T. Naito, N. Inoue, T. Hisamatsu, H. Chinen, K. Sugimoto, Y. Shimomura, H. Yamagishi, T. Shiohara, et al. 2008. Exacerbating role of gammadelta T cells in chronic colitis of T-cell receptor α mutant mice. *Gastroenterology* 134: 481–490.
15. Ponomarev, E. D., and B. N. Dittel. 2005. γ δ T cells regulate the extent and duration of inflammation in the central nervous system by a Fas ligand-dependent mechanism. *J. Immunol.* 174: 4678–4687.
16. Tsuchiya, T., S. Fukuda, H. Hamada, A. Nakamura, Y. Kohama, H. Ishikawa, K. Tsujikawa, and H. Yamamoto. 2003. Role of γ δ T cells in the inflammatory response of experimental colitis mice. *J. Immunol.* 171: 5507–5513.
17. Uezu, K., K. Kawakami, K. Miyagi, Y. Kinjo, T. Kinjo, H. Ishikawa, and A. Saito. 2004. Accumulation of gammadelta T cells in the lungs and their regulatory roles in Th1 response and host defense against pulmonary infection with *Cryptococcus neoformans*. *J. Immunol.* 172: 7629–7634.
18. D'Souza, C. D., A. M. Cooper, A. A. Frank, R. J. Mazzaccaro, B. R. Bloom, and I. M. Orme. 1997. An anti-inflammatory role for γ δ T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. *J. Immunol.* 158: 1217–1221.
19. Chen, Y., K. Chou, E. Fuchs, W. L. Havran, and R. Boismenu. 2002. Protection of the intestinal mucosa by intraepithelial γ δ T cells. *Proc. Natl. Acad. Sci. USA* 99: 14338–14343.
20. Inagaki-Ohara, K., T. Chinen, G. Matsuzaki, A. Sasaki, Y. Sakamoto, K. Hiromatsu, F. Nakamura-Uchiyama, Y. Nawa, and A. Yoshimura. 2004. Mucosal T cells bearing TCRgammadelta play a protective role in intestinal inflammation. *J. Immunol.* 173: 1390–1398.
21. Dalton, J. E., J. Pearson, P. Scott, and S. R. Carding. 2003. The interaction of γ δ T cells with activated macrophages is a property of the V γ 1 subset. *J. Immunol.* 171: 6488–6494.
22. Huber, S. A., D. Graveline, M. K. Newell, W. K. Born, and R. L. O'Brien. 2000. V γ 1+ T cells suppress and V γ 4+ T cells promote susceptibility to coxsackievirus B3-induced myocarditis in mice. *J. Immunol.* 165: 4174–4181.
23. Spencer, C. T., G. Abate, A. Blazevic, and D. F. Hoft. 2008. Only a subset of phosphoantigen-responsive γ9δ2 T cells mediate protective tuberculosis immunity. *J. Immunol.* 181: 4471–4484.
24. van der Heyde, H. C., M. M. Elloso, W. L. Chang, B. J. Pepper, J. Batchelder, and W. P. Weidanz. 1996. Expansion of the gammadelta T cell subset in vivo during bloodstage malaria in B cell-deficient mice. *J. Leukoc. Biol.* 60: 221–229.
25. Peng, G., H. Y. Wang, W. Peng, Y. Kiniwa, K. H. Seo, and R. F. Wang. 2007. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique Toll-like receptor signaling pathway. *Immunity* 27: 334–348.
26. Kabelitz, D., D. Wesch, and W. He. 2007. Perspectives of gammadelta T cells in tumor immunology. *Cancer Res.* 67: 5–8.
27. Girardi, M. 2006. Immunosurveillance and immunoregulation by gammadelta T cells. *J. Invest. Dermatol.* 126: 25–31.
28. Poccia, F., C. Agrati, F. Martini, G. Mejia, M. Wallace, and M. Malkovsky. 2005. Vgamma9Vdelta2 T cell-mediated non-cytolytic antiviral mechanisms and their potential for cell-based therapy. *Immunol. Lett.* 100: 14–20.
29. Hayday, A. C. 2000. [γ][δ] cells: a right time and a right place for a conserved third way of protection. *Annu. Rev. Immunol.* 18: 975–1026.
30. Cui, Y., H. Shao, C. Lan, H. Nian, R. L. O'Brien, W. K. Born, H. J. Kaplan, and D. Sun. 2009. Major role of γ δ T cells in the generation of IL-17$^+$ uveitogenic T cells. *J. Immunol.* 183: 560–567.
31. Cheng, L., Y. Cui, H. Shao, G. Han, L. Zhu, Y. Huang, R. L. O'Brien, W. K. Born, H. J. Kaplan, and D. Sun. 2008. Mouse gammadelta T cells are capable of expressing MHC class II molecules, and of functioning as antigen-presenting cells. *J. Neuroimmunol.* 203: 3–11.
32. Hahn, Y. S., C. Taube, N. Jin, K. Takeda, J. W. Park, J. M. Wands, M. K. Aydintug, C. L. Roark, M. Lahn, R. L. O'Brien, et al. 2003. V γ 4+ γ δ T cells regulate airway hyperreactivity to methacholine in ovalbumin-sensitized and challenged mice. *J. Immunol.* 171: 3170–3178.
33. Lahn, M., A. Kanehiro, K. Takeda, J. Terry, Y. S. Hahn, M. K. Aydintug, A. Konowal, K. Ikuta, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2002. MHC class I-dependent Vgamma4+ pulmonary T cells regulate α β T cell-independent airway responsiveness. *Proc. Natl. Acad. Sci. USA* 99: 8850–8855.
34. Nian, H., H. Shao, R. L. O'Brien, W. K. Born, H. J. Kaplan, and D. Sun. 2011. Activated gammadelta T cells promote the activation of uveitogenic T cells and exacerbate EAU development. *Invest. Ophthalmol. Vis. Sci.* 52: 5920–5927.
35. Liang, D., A. Zuo, H. Shao, W. K. Born, R. L. O'Brien, H. J. Kaplan, and D. Sun. 2013. IL-23 receptor expression on γδ T cells correlates with their enhancing or suppressive effects on autoreactive T cells in experimental autoimmune uveitis. *J. Immunol.* 191: 1118–1125.
36. Liang, D., A. Zuo, H. Shao, W. K. Born, R. L. O'Brien, H. J. Kaplan, and D. Sun. 2012. Role of CD25$^+$ dendritic cells in the generation of Th17 autoreactive T cells in autoimmune experimental uveitis. *J. Immunol.* 188: 5785–5791.
37. Peng, Y., G. Han, H. Shao, Y. Wang, H. J. Kaplan, and D. Sun. 2007. Characterization of IL-17+ interphotoreceptor retinoid-binding protein-specific T cells in experimental autoimmune uveitis. *Invest. Ophthalmol. Vis. Sci.* 48: 4153–4161.
38. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176: 1693–1702.
39. Chen, M., D. Liang, A. Zuo, H. Shao, H. J. Kaplan, and D. Sun. 2015. An A2B adenosine receptor agonist promotes Th17 autoimmune responses in experimental autoimmune uveitis (EAU) via dendritic cell activation. *PLoS One* 10: e0132348.
40. Lyons, A. B., and C. R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J. Immunol. Methods* 171: 131–137.
41. Liang, D., A. Zuo, H. Shao, M. Chen, H. J. Kaplan, and D. Sun. 2014. Roles of the adenosine receptor and CD73 in the regulatory effect of γδ T cells. *PLoS One* 9: e108932.
42. Liang, D., A. Zuo, R. Zhao, H. Shao, W. K. Born, R. L. O'Brien, H. J. Kaplan, and D. Sun. 2016. CD73 expressed on γδ T cells shapes their regulatory effect in experimental autoimmune uveitis. [Published erratum appears in 2016 *PLoS One* 11: e0164502.] *PLoS One* 11: e0150078.
43. Liang, D., A. Zuo, H. Shao, W. K. Born, R. L. O'Brien, H. J. Kaplan, and D. Sun. 2013. Retinoic acid inhibits CD25+ dendritic cell expansion and γδ T-cell activation in experimental autoimmune uveitis. *Invest. Ophthalmol. Vis. Sci.* 54: 3493–3503.
44. Liang, D., A. Zuo, H. Shao, M. Chen, H. J. Kaplan, and D. Sun. 2015. A2B adenosine receptor activation switches differentiation of bone marrow cells to a CD11c(+)Gr-1(+) dendritic cell subset that promotes the Th17 response. *Immun. Inflamm. Dis.* 3: 360–373.
45. Antonioli, L., P. Pacher, E. S. Vizi, and G. Haskó. 2013. CD39 and CD73 in immunity and inflammation. *Trends Mol. Med.* 19: 355–367.
46. Beavis, P. A., J. Stagg, P. K. Darcy, and M. J. Smyth. 2012. CD73: a potent suppressor of antitumor immune responses. *Trends Immunol.* 33: 231–237.
47. Forte, G., R. Sorrentino, A. Montinaro, A. Luciano, I. M. Adcock, P. Maiolino, C. Arra, C. Cicala, A. Pinto, and S. Morello. 2012. Inhibition of CD73 improves B cell-mediated anti-tumor immunity in a mouse model of melanoma. *J. Immunol.* 189: 2226–2233.
48. Romio, M., B. Reinbeck, S. Bongardt, S. Hüls, S. Burghoff, and J. Schrader. 2011. Extracellular purine metabolism and signaling of CD73-derived adenosine in murine Treg and Teff cells. *Am. J. Physiol. Cell Physiol.* 301: C530–C539.
49. Rajan, A. J., V. C. Asensio, I. L. Campbell, and C. F. Brosnan. 2000. Experimental autoimmune encephalomyelitis on the SJL mouse: effect of γ δ T cell depletion on chemokine and chemokine receptor expression in the central nervous system. *J. Immunol.* 164: 2120–2130.
50. Spahn, T. W., S. Issazadah, A. J. Salvin, and H. L. Weiner. 1999. Decreased severity of myelin oligodendrocyte glycoprotein peptide 33-35-induced experimental autoimmune encephalomyelitis in mice with a disrupted TCR δ chain gene. *Eur. J. Immunol.* 29: 4060–4071.
51. Tagawa, T., H. Nishimura, T. Yajima, H. Hara, K. Kishihara, G. Matsuzaki, I. Yoshino, Y. Maehara, and Y. Yoshikai. 2004. Vdelta1+ gammadelta T cells producing CC chemokines may bridge a gap between neutrophils and macrophages in innate immunity during *Escherichia coli* infection in mice. *J. Immunol.* 173: 5156–5164.
52. Born, W., C. Cady, J. Jones-Carson, A. Mukasa, M. Lahn, and R. O'Brien. 1999. Immunoregulatory functions of γ δ T cells. *Adv. Immunol.* 71: 77–144.

53. Blink, S. E., M. W. Caldis, G. E. Goings, C. T. Harp, B. Malissen, I. Prinz, D. Xu, and S. D. Miller. 2014. γδ T cell subsets play opposing roles in regulating experimental autoimmune encephalomyelitis. *Cell. Immunol.* 290: 39–51.
54. Hahn, Y. S., C. Taube, N. Jin, L. Sharp, J. M. Wands, M. K. Aydintug, M. Lahn, S. A. Huber, R. L. O'Brien, E. W. Gelfand, and W. K. Born. 2004. Different potentials of γ δ T cell subsets in regulating airway responsiveness: V γ 1+ cells, but not V γ 4+ cells, promote airway hyperreactivity, Th2 cytokines, and airway inflammation. *J. Immunol.* 172: 2894–2902.
55. O'Brien, R. L., X. Yin, S. A. Huber, K. Ikuta, and W. K. Born. 2000. Depletion of a γ δ T cell subset can increase host resistance to a bacterial infection. *J. Immunol.* 165: 6472–6479.
56. Nian, H., H. Shao, G. Zhang, W. K. Born, R. L. O'Brien, H. J. Kaplan, and D. Sun. 2010. Regulatory effect of gammadelta T cells on IL-17+ uveitogenic T cells. *Invest. Ophthalmol. Vis. Sci.* 51: 4661–4667.
57. Dieli, F., J. Ivanyi, P. Marsh, A. Williams, I. Naylor, G. Sireci, N. Caccamo, C. Di Sano, and A. Salerno. 2003. Characterization of lung γ δ T cells following intranasal infection with *Mycobacterium bovis* bacillus Calmette-Guérin. *J. Immunol.* 170: 463–469.
58. Belles, C., A. K. Kuhl, A. J. Donoghue, Y. Sano, R. L. O'Brien, W. Born, K. Bottomly, and S. R. Carding. 1996. Bias in the gamma delta T cell response to *Listeria monocytogenes*: V delta 6.3+ cells are a major component of the gamma delta T cell response to *Listeria monocytogenes*. *J. Immunol.* 156: 4280–4289.
59. Naiki, Y., H. Nishimura, S. Itohara, and Y. Yoshikai. 2000. gammadelta T cells may dichotomously modulate infection with avirulent *Salmonella choleraesuis* via IFN-γ and IL-13 in mice. *Cell. Immunol.* 202: 61–69.
60. Ninomiya, T., H. Takimoto, G. Matsuzaki, S. Hamano, H. Yoshida, Y. Yoshikai, G. Kimura, and K. Nomoto. 2000. Vgamma1+ gammadelta T cells play protective roles at an early phase of murine cytomegalovirus infection through production of interferon-γ. *Immunology* 99: 187–194.
61. Huber, S. A., A. Moraska, and M. Choate. 1992. T cells expressing the γ δ T-cell receptor potentiate coxsackievirus B3-induced myocarditis. *J. Virol.* 66: 6541–6546.
62. Hou, S., J. M. Katz, P. C. Doherty, and S. R. Carding. 1992. Extent of γ δ T cell involvement in the pneumonia caused by Sendai virus. *Cell. Immunol.* 143: 183–193.
63. Naganuma, M., E. B. Wiznerowicz, C. M. Lappas, J. Linden, M. T. Worthington, and P. B. Ernst. 2006. Cutting edge: critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. *J. Immunol.* 177: 2765–2769.
64. Zarek, P. E., C.-T. Huang, E. R. Lutz, J. Kowalski, M. R. Horton, J. Linden, C. G. Drake, and J. D. Powell. 2008. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood* 111: 251–259.