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Role of CD25⁺ Dendritic Cells in the Generation of Th17 Autoreactive T Cells in Autoimmune Experimental Uveitis

Dongchun Liang,* Aijun Zuo,* Hui Shao,[†] Willi K. Born,[‡] Rebecca L. O'Brien,[‡] Henry J. Kaplan,[†] and Deming Sun*

In the current study, we showed that in vivo administration of an anti-CD25 Ab (PC61) decreased the Th17 response in C57BL/6 mice immunized with the uveitogenic peptide interphotoreceptor retinoid-binding protein (1–20), while enhancing the autoreactive Th1 response. The depressed Th17 response was closely associated with decreased numbers of a splenic dendritic cell (DC) subset expressing CD11c⁺CD3[−]CD25⁺ and decreased expansion of $\gamma\delta$ T cells. We demonstrated that ablation of the CD25⁺ DC subset accounted for the decreased activation and the expansion of $\gamma\delta$ T cells, leading to decreased activation of IL-17⁺ interphotoreceptor retinoid-binding protein-specific T cells. Our results show that an enhanced Th17 response in an autoimmune disease is associated with the appearance of a DC subset expressing CD25 and that treatment of mice with anti-CD25 Ab causes functional alterations in a number of immune cell types, namely DCs and $\gamma\delta$ T cells, in addition to CD25⁺ $\alpha\beta$ TCR⁺ regulatory T cells. *The Journal of Immunology*, 2012, 188: 5785–5791.

Knowledge about factors that affect or regulate the activation of autoreactive T cells is required for therapeutics for autoimmune diseases. Over the past two decades, circumstantial evidence has been obtained that, in a number of experimental autoimmune diseases, including encephalomyelitis (1–4), arthritis (5, 6), and uveitis (7, 8), a major subset of pathogenic autoreactive T cells produces IFN- γ and IL-2 and belongs to the Th1 type of CD4 T cells. Recent studies identified a new and crucial autoreactive T cell subset that produces IL-17, but not IFN- γ or IL-4, designated Th17 cells (9–13). Studies showed that the requirements for activation of Th1 and Th17 autoreactive T cells differ (14–18) and that Th1 and Th17 autoreactive T cells may not be regulated by the same cells (19, 20). These observations prompted us to determine how Th17 autoreactive T cells differ from Th1 autoreactive T cells in their regulation by functionally counterreactive cells and molecules, whether different mechanisms leading to the activation of Th1 or Th17 pathogenic T cell subsets could be identified, and whether a single therapeutic strategy could control the pathogenic activity of both types of T cells.

Anti-CD25 mAb binds to the α -chain of IL-2R (21, 22). CD25 expression is not restricted to T cells (23), and it can easily be detected on human (24–26) and mouse (27–30) dendritic cells (DCs) and myeloid cells. To determine whether Th1 and Th17 pathogenic T cells are regulated by different immunoregulatory mechanisms, we examined whether decreasing the activity of

CD25⁺ regulatory T cells, a treatment found to result in enhanced function of Th1 autoreactive T cells (31–35), would have a similar or a different effect on Th17 autoreactive T cell responses. We found that mice treated with anti-CD25 mAb before immunization with human interphotoreceptor retinoid-binding protein (IRBP) peptide had a significantly decreased Th17 response but an enhanced Th1 response. Mechanistic studies on the effects on functionally different autoreactive T cell responses in anti-CD25 mAb-treated mice showed that a decrease in the numbers of CD25⁺ and Foxp3⁺ T cells was associated with decreased activation of $\gamma\delta$ TCR⁺ T cells. Moreover, the altered responses were also evident when in vivo-primed T cells from mice with or without prior Ab treatment were stimulated with the immunizing Ag in the presence of splenic APCs from mAb-treated mice, suggesting an involvement of functionally altered APCs in the treated mice. We also showed that ~10% of CD11c⁺CD3[−] DCs in spleens from immunized mice coexpressed CD25 and that purified CD25⁺ DCs were much more effective than were CD25[−] DCs in stimulating the activation of IL-17⁺ autoreactive T cells and $\gamma\delta$ T cells. Our data demonstrate that an enhanced Th17 response in an autoimmune disease is associated with the appearance of a DC subset expressing CD25 and that treatment of mice with anti-CD25 mAb causes functional alterations in multiple immune cell types, rather than only in CD4⁺CD25⁺ T cells.

Materials and Methods

Animals and reagents

Female C57BL/6 (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were housed and maintained in the animal facilities of the University of Southern California. Institutional approval was obtained, and institutional guidelines regarding animal experimentation were followed. Recombinant murine IL-23 and IL-12 were purchased from R&D Systems (Minneapolis, MN). FITC- or PE-conjugated Abs against mouse IFN- γ , IL-17, CD25, CD11c, $\alpha\beta$ TCR, and $\gamma\delta$ TCR were purchased from BioLegend (San Diego, CA); all other Abs were from BD Bioscience (La Jolla, CA).

Immunization procedure and in vitro stimulation of in vivo-primed T cells

B6 mice were immunized s.c. over six spots at the tail base and on the flank with 200 μ l emulsion containing 150 μ g the uveitogenic peptide IRBP_{1–20}

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Abbreviations used in this article: B6, C57BL/6; DC, dendritic cell; EAU, experimental autoimmune uveitis; IRBP, interphotoreceptor retinoid-binding protein; LDA, limiting dilution analysis.

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[aa 1–20 of human IRBP; Sigma, St. Louis, MO)] emulsified in CFA (Difco, Detroit, MI). Concurrently, 200 ng pertussis toxin (Sigma, St. Louis, MO) was injected i.p. At day 13 postimmunization, T cells were isolated from lymph node cells and spleen cells by passage through a nylon wool column. A total of 1×10^7 cells in 2 ml RPMI 1640 medium (Cellgro, Mediatech, Manassas, VA) containing 10% FCS was added to each well of a six-well plate (Costar) and stimulated for 48 h with 10 μ g/ml IRBP_{1–20} in the presence of 1×10^7 irradiated syngeneic spleen cells, as APCs, together with IL-12 (Th1 polarized) or IL-23 (Th17 polarized) (10 ng/ml). Activated T cell blasts were separated by Ficoll-gradient centrifugation and cultured for another 72 h in the same medium used for stimulation without the peptide.

In vivo administration of anti-CD25 Ab

The PC61 (anti-CD25) hybridoma was purchased from the American Type Culture Collection (Manassas, VA), and ascites was produced in SCID mice by Taconic (Hudson, NY). Ammonium sulfate-precipitated IgG from PC61 ascites was used for all injections. C57BL/6 mice were injected i.p. with three doses of 500 μ g PC61 on days –7, –4, and –1 before immunization on day 0. Flow cytometry was performed on a BD FACScan or FACS-Calibur, and the results were analyzed using CellQuest software (BD Immunocytometry Systems, San Jose, CA).

Cytokine assays

Enriched T cells (3×10^4 cells/well) from the draining lymph nodes and spleens were cultured at 37°C for 48 h in 96-well microtiter plates with irradiated syngeneic spleen APCs (1×10^5) in the presence of IRBP_{1–20}. A fraction of the culture supernatant was assayed for IL-17 using ELISA kits (R&D Systems).

Scoring of experimental autoimmune uveitis

The mice were examined three times a week for clinical signs of experimental autoimmune uveitis (EAU) by indirect fundoscopy. The pupils were dilated using 0.5% tropicamide and 1.25% phenylephrine hydrochloride ophthalmic solutions, and fundoscopic grading of disease was performed using the scoring system described previously (36). For histopathological evaluation, whole eyes were collected at the end of the experiment and immersed for 1 h in 4% glutaraldehyde in phosphate buffer (pH 7.4) and then transferred to 10% formaldehyde in phosphate buffer until processed. The fixed and dehydrated tissues were embedded in methacrylate, and 5- μ m sections were cut through the pupillary–optic nerve plane and stained with H&E. Presence or absence of disease was evaluated blindly by examining six sections cut at different levels for each eye. Disease was graded pathologically based on cellular infiltration and structural changes (37).

Intracellular staining and FACS analysis

For intracellular staining, T cells (2×10^5 in 100 μ l PBS) were incubated for 4 h with 50 ng/ml PMA, 1 μ g/ml ionomycin, and 1 μ g/ml brefeldin A (Sigma-Aldrich) and then were washed, fixed, permeabilized overnight with Cytofix/Cytoperm buffer (eBioscience), intracellularly stained with Abs against IFN- γ and IL-17, and analyzed on a FACSCalibur flow cytometer.

Limiting dilution analysis

B6 mice were immunized with IRBP_{1–20} emulsified in CFA, the spleen and draining lymph nodes were removed 13 d later, a single-cell suspension was prepared, and T cells were enriched by nylon wool adherence. They were then seeded in two sets in 96-well flat-bottom culture plates containing irradiated spleen cells (1×10^5 /well) under Th1- or Th17-polarizing conditions, with one set of plates containing an optimal dose of immunizing peptide (10 μ g/ml). Activation of IRBP_{1–20}-specific T cells was estimated by comparing the proliferation and IFN- γ and IL-17 production of graded numbers (3,000–200,000/well) of T cells in the presence and absence of IRBP_{1–20} under polarizing conditions; 24 replicate wells were used for each cell density. After 44 h of incubation, the plates were centrifuged, and 50 μ l supernatant was harvested for lymphokine assays; the rest of the cultures were pulsed with [³H]thymidine for 6 h, harvested, and counted in a beta counter. Positive microcultures were defined as those in which lymphokine activity or incorporated thymidine exceeded the mean activity in control cultures (no responder cells) by >3 SD (38–40). The frequency of responder T cells was obtained from the minimum estimates of precursor frequency calculated using a program developed to analyze the limiting dilution analysis (LDA) data acquired (41, 42). This program uses the Poisson distribution to calculate the frequency of responder T cells with 99% confidence limits.

Statistical analysis

Experiments were repeated at least twice, usually three or more times. Experimental groups were typically composed of four mice. The figures show data from a representative experiment. Differences between the values for different groups were examined using the two-tailed *t* test.

Results

Treatment of B6 mice with anti-mouse CD25 Ab (PC61) decreases the Th17 autoreactive T cell response

Previous studies showed that treatment of mice with an anti-mouse CD25 Ab enhances autoreactivity by abolishing the function of regulatory T cells (31–35). To determine whether Ab treatment affected both Th1 and Th17 autoreactive T cells, B6 mice were randomly divided into two groups. One group was injected with three doses of anti-CD25 Ab (PC61), and the other group was injected with an isotype-matched rat IgG on days –7, –4, and –1 before immunization with IRBP_{1–20} emulsified in CFA (see *Materials and Methods*) or remained untreated. In vivo-primed T cells, collected on day 13 postimmunization, were restimulated in vitro for 2 d with immunizing peptide and syngeneic APCs under either Th1- or Th17-polarizing conditions (culture medium containing IL-12 or IL-23, respectively), and the activated T cells were intracellularly stained with Abs specific for IFN- γ , IL-17, or $\alpha\beta$ TCR and analyzed by flow cytometry. As shown in Fig. 1A, the number of IL-17⁺ T cells among the responder T cells obtained from PC61-treated mice was significantly decreased by ~50%, contrasting with the number of IFN- γ ⁺ T cells, which increased ~2-fold compared with mice that were not treated with Ab or were treated with an irrelevant control Ab. ELISA tests gave results similar to those for intracellular staining, showing that T cells from PC61-treated mice produced increased amounts of IFN- γ but decreased amounts of IL-17 (Fig. 1B). To determine whether IRBP-specific T cells from PC61-treated mice had increased or decreased pathogenic activity, 2×10^6 Th1- or Th17-polarized activated T cells from each group were transferred to naive recipients, and the development of EAU in the recipient mice was followed by fundoscopy and pathological examination. Fig. 1C shows a set of representative pathologic photographs along with a summary plot to demonstrate that IL-17⁺ IRBP-specific T cells from PC61-treated mice showed significantly decreased uveitogenic activity, whereas the uveitogenic activity of IFN- γ ⁺ IRBP-specific T cells from the same mice was slightly enhanced. To determine whether the Ab treatment directly affected the in vivo priming of Th1 and Th17 autoreactive T cells, we performed LDAs to measure the frequencies of in vivo-primed Th1 and Th17 IRBP-specific T cells in PC61-treated and non-treated mice. As shown in Fig. 1D, the average frequency of IRBP-specific Th17 responder T cells decreased by up to 50% in PC61-treated mice (12 cells/100,000 responder T cells) compared with untreated mice (25 cells/100,000 responder T cells), whereas the frequency of IFN- γ ⁺ IRBP-specific T cells increased from ~3 to 5 cells/100,000 responder T cells.

Decreased activation of $\gamma\delta$ T cells in PC61-treated mice

To determine the mechanism by which in vivo administration of PC61 decreased the generation of IL-17⁺ autoreactive T cells, we examined cellular components in the spleen before (Fig. 2A–C) and after (Fig. 2D–F) in vitro activation. As shown in Fig. 2A, before immunization, ~5% of splenic T cells from immunized mice expressed CD25; most of this T cell population disappeared after Ab administration. In parallel, there was a significant decrease in the numbers of Foxp3⁺ cells (Fig. 2B) and $\gamma\delta$ T cells (Fig. 2C). After stimulation with the immunizing Ag and expan-

FIGURE 1. Injection of B6 mice with anti-mouse CD25 Ab (PC61) decreases the Th17 autoreactive T cell response. **(A)** Splenic T cells from IRBP_{1–20}/CFA-immunized mice, with or without prior PC61 treatment, were enriched by passage through nylon wool and stimulated for 48 h with an optimal dose of IRBP_{1–20} (10 μg/ml) under Th1- or Th17-polarized conditions. The activated T cells were separated by Ficoll-gradient centrifugation on day 3, cultured under the same polarized conditions for an additional 5 d, and intracellularly stained with PE-conjugated anti-IFN-γ Abs and FITC-conjugated anti-IL-17 Abs, followed by FACS analysis. **(B)** IFN-γ and IL-17 levels in the culture supernatants were measured by ELISA after the 48-h incubation with peptide. **(C)** IRBP-specific T cells (2×10^6 /mouse) from untreated and PC61-treated IRBP-immunized mice activated under Th1- or Th17-polarized conditions were adoptively transferred to syngeneic B6 mice. Pathologic examination was conducted 10 d after disease induction. A set of representative pathologic slides is shown (left panels) along with summarized *in vivo* results (right panel). H&E staining. Original magnification $\times 200$. **(D)** Responder T cell numbers were evaluated by LDA, as detailed in *Materials and Methods*. The results shown are representative of those from more than five experiments.

sion under Th17-polarized conditions for 8 d, the number of IL-17⁺ T cells decreased >6 -fold in PC61-treated mice compared with untreated mice (Fig. 2D). Because IL-17⁺ T cells contain both $\alpha\beta$ and $\gamma\delta$ T cells, we separately assessed the IL-17⁺ $\alpha\beta$ TCR⁺ and IL-17⁺ $\gamma\delta$ TCR⁺ T cells. We found that the number of IL-17⁺ $\alpha\beta$ TCR⁺ T cells decreased >5 -fold (Fig. 2E), and the number of IL-17⁺ $\gamma\delta$ TCR⁺ T cells declined even more dramatically (Fig. 2F).

Splenic APCs from PC61-treated mice show a decreased ability to stimulate IL-17⁺ autoreactive T cells *in vitro*

To determine whether the Ab treatment has an effect on immune cells other than CD25⁺ T cells, we performed criss-cross experiments, in which *in vivo*-primed T cells from PC61-treated or untreated mice were stimulated with immunizing Ag in the presence of splenic APCs from PC61-treated or untreated mice. When *in vivo*-primed T cells were stimulated for 5 d *in vitro* with the immunizing peptide in the presence of APCs from PC61-treated mice, the activated T cells contained significantly fewer IL-17⁺ cells, regardless of whether the T cells were from Ab-treated or nontreated mice, and T cells from PC61-treated mice showed a significantly decreased response compared with untreated mice (Fig. 3A). Consistent with the intracellular staining analysis, ELISA tests showed that the use of APCs from PC61-treated mice resulted in less activation of IL-17⁺ T cells (Fig. 3B)

but slight enhancement of the activation of IFN-γ⁺ IRBP-specific T cells (data not shown). These data show that splenic APCs from PC61-treated mice are functionally altered in their ability to stimulate Th17 and Th1 T cells.

CD25⁺CD11c⁺ cells are more effective than CD25[−]CD11c⁺ cells in stimulating activation of IL-17⁺ IRBP-specific T cells and $\gamma\delta$ T cells

To determine the mechanism underlying the functional change in splenic APCs from PC61-treated mice, we examined whether the injected Ab depleted a subset of APCs that express CD25. As shown in Fig. 4A, $\sim 10\%$ of the CD11c⁺ cells in the spleen of immunized mice expressed CD25, and this cell population disappeared in mice pretreated with PC61 (Fig. 4A, 4B). Phenotypic studies showed that the CD25⁺CD11c⁺ cells were CD3[−]CD161[−] (data not shown). To further determine whether CD25⁺CD11c⁺ cells were functionally distinct from their CD25[−] counterparts, we magnetically separated the CD25⁺ and CD25[−] fractions from immunized mice (Fig. 4C), stimulated the *in vivo*-primed T cells with the immunizing peptide in the presence of CD25⁺CD11c⁺ or CD25[−]CD11c⁺ cells as APCs for 5 d, stained the activated T cells with Abs, and performed flow cytometry. As shown, CD25⁺CD11c⁺ cells showed a significantly decreased ability to stimulate the activation of both IL-17⁺ IRBP-specific T cells (Fig. 4D) and IL-17⁺ $\gamma\delta$ T cells (Fig. 4E) compared with CD25[−]CD11c⁺ cells.

FIGURE 2. Phenotypic change of T cells in PC61-injected mice. B6 mice were randomly separated into two groups ($n = 8$) and immunized with IRBP₁₋₂₀/CFA, with or without prior treatment with PC61. After 13 d, splenic T cells were enriched and stimulated with the immunizing peptide. The phenotype of the T cells was analyzed either before (**A–C**) or after (**D–F**) in vitro stimulation. Effect of PC61 injection on CD25⁺CD3⁺ cells (**A**), Foxp3⁺ cells (**B**), and $\gamma\delta$ T cells (**E**). (**D–F**) After in vitro stimulation with the immunizing Ag and expansion under Th17-polarized conditions, the activated T cells were stained with the indicated Abs and analyzed by flow cytometry. The experiments were repeated more than five times, and the results of one representative experiment are shown.

Furthermore, responder T cells produced larger amounts of IL-17 when they were stimulated by CD25⁺CD11c⁺ DCs than when they were stimulated by CD25[−]CD11c⁺ DCs (Fig. 4F).

FIGURE 3. Criss-cross tests showing that dysfunction of splenic APCs accounts for the decreased generation of IL-17⁺ IRBP-specific T cells in PC61-treated mice. (**A**) Responder T cells from immunized PC61-treated or nontreated mice were stimulated in vitro for 48 h with IRBP₁₋₂₀ peptide in the presence of splenic APCs from PC61-treated or nontreated mice. The percentage of IL-17⁺ T cells was measured. (**B**) IL-17 levels in the culture supernatants after a 48-h incubation with peptide and APCs were assessed by ELISA.

Th17 response of PC61-treated mice is restored by addition of a small number of activated $\gamma\delta$ T cells to the responder T cells

To test the possibility that the decrease in the number of $\gamma\delta$ T cells contributed to the hyporesponsiveness of Th17 autoreactive T cells, we added a small number (2%) of $\gamma\delta$ T cells, preactivated by exposure to anti-CD3 Ab for 2 d, to the in vivo-primed T cells from PC61-treated mice before stimulating with the immunizing Ag and APCs under Th1- or Th17-polarized conditions. As shown in Fig. 5, Th17-polarized T cells from PC61-treated mice generated a significantly smaller number of IL-17⁺ T cells (Fig. 5B) than did those from untreated mice (Fig. 5A). However, the addition of 2% of activated $\gamma\delta$ T cells restored the intensity of the Th17 response, suggesting that $\gamma\delta$ T cells are responsible, at least in part, for the activation of Th17 autoreactive T cells in this system.

Discussion

Approximately 10% of peripheral CD4⁺ cells and <1% of CD8⁺ cells in normal unimmunized adult mice express the IL-2R α -chain (CD25) (32, 43). Studies showed that cells within the CD4⁺ T subset that constitutively express CD25 have immunosuppressive activity (31, 44, 45). This conclusion was supported by the demonstration that treatment of mice with Abs specific for mouse CD25 leads to an increased frequency and severity of autoimmune diseases (31–35) and that functional abnormalities in the CD25⁺ T cell population are closely associated with an increased development of autoimmune diseases (43, 46). Regardless of whether the administered anti-CD25 Ab eliminated (33, 47–49) or inactivated (50, 51) CD25⁺CD4⁺ T cells, the fact remains that the Ab caused altered function of T cells expressing CD4 and CD25. However, these previous studies did not examine whether the Ab caused dysregulated function of different autoreactive T cell subsets, such as Th1 and Th17 autoreactive T cells.

FIGURE 4. CD25⁺CD11c⁺ cells are more effective than are CD25[−]CD11c⁺ cells in stimulating the activation of IL-17⁺ IRBP-specific T cells and $\gamma\delta$ TCR⁺ T cells. (**A** and **B**) Splenic cells of immunized mice, with or without PC61 injection, were stained for expression of CD11c and/or CD25. (**C**) CD25⁺CD11c⁺ and CD25[−]CD11c⁺ DCs were separated using magnetic beads. (**D** and **E**) Splenic T cells isolated from immunized B6 mice were stimulated with the immunizing peptide IRBP_{1–20} in the presence of CD25[−]CD11c⁺ (left panels) or CD25⁺CD11c⁺ (right panel) DCs for 5 d. The activated T cells were stained for the expression of IL-17 (**D**) and IFN- γ (**E**). (**F**) ELISA assay. Culture supernatant of immunized splenic T cells was tested for IL-17 production after a 48-h stimulation with the immunizing peptide IRBP_{1–20} in the presence of either CD25[−]CD11c⁺ or CD25⁺CD11c⁺ DCs.

Regulatory T cells participate in the maintenance of peripheral tolerance and prevention of autoimmunity (31–35). One of the experimental tools used to assess the effect of regulatory T cells

in vivo is injection of mice with an anti-CD25 Ab. In this study, we found that mice treated with an anti-CD25 (PC61) Ab had decreased Th17 responses, which were associated with a de-

FIGURE 5. Restoration of the Th17 response by addition of activated $\gamma\delta$ T cells to functionally defective T cells. In vivo-primed T cells from IRBP_{1–20}/CFA-immunized mice with (**B**, **C**) or without (**A**) prior treatment with PC61 were stimulated for 5 d with immunizing peptide under Th1- or Th17-polarized conditions (**A**, **B**) or after addition of 2% of activated $\gamma\delta$ T cells [2×10^4 /well (**C**)]. Activated T cells were intracellularly stained for IFN- γ and IL-17 and analyzed by FACS for numbers of IL-17⁺ or IFN- γ ⁺ T cells. (**D**) IFN- γ and IL-17 levels in the culture supernatants at 48 h were assessed by ELISA.

creased activation/expansion of $\gamma\delta$ T cells. Furthermore, fewer CD25⁺ cells among splenic DCs (CD11c⁺CD3⁺CD25⁺ cells) probably accounted for the reduced Th17 response and the diminished activation of $\gamma\delta$ TCR⁺ T cells. Thus, treatment of mice with anti-CD25 Abs directly or indirectly caused functional alterations in a number of immune cells, namely DCs and $\gamma\delta$ T cells, in addition to CD25⁺ $\alpha\beta$ TCR⁺ regulatory T cells.

Anti-CD25 mAb binds to the α -chain of IL-2R (21, 22). Expression of CD25 is not restricted to T cells (23), and it can easily be detected on human (24–26) and mouse DCs (27–30) and myeloid cells. We were able to show that ~10% of CD11c⁺ cells in the spleens of immunized mice expressed CD25 and that purified CD3⁺CD11c⁺CD25⁺ cells had a greater ability than did CD11c⁺CD25[−] cells to stimulate the activation of IL-17⁺ uveitogenic T cells and $\gamma\delta$ T cells. It is likely that an increase in this DC subset enhances the Th17 response via pathways involving $\gamma\delta$ T cell activation, whereas removal of this DC subset diminishes Th17 responses.

We reported previously that $\gamma\delta$ T cells play a major role in the activation of Th17 autoreactive T cells (52–54). $\gamma\delta$ -deficient mice (TCR- $\delta^{-/-}$) have decreased activation of Th17 autoreactive T cells, and the transfer of a small number of $\gamma\delta$ T cells to TCR- $\delta^{-/-}$ mice restores the Th17 response (52–54). We showed that activation of $\gamma\delta$ T cells promoted the generation of the Th17 response in vitro and in vivo and, thus, promoted the development of EAU. Moreover, the enhancing and suppressing effect of $\gamma\delta$ T cells on EAU is convertible and dependent on their state of activation (52). In the current study, we found that the activation or expansion of $\gamma\delta$ T cells was significantly inhibited in PC61-treated mice and that the decreased numbers of the CD25⁺ DC subset accounted for the hypofunction of $\gamma\delta$ T cells. These observations support our previous finding that $\gamma\delta$ T cells play a major role in regulating Th17 autoreactive T cell responses in EAU (52–54).

The possibility that the decreased number of $\gamma\delta$ T cells in PC61-treated mice was due to the elimination of activated $\gamma\delta$ T cells expressing CD25 was not supported by our studies, because we failed to demonstrate any CD25⁺ $\gamma\delta$ T cells among freshly isolated T cells from either naive or immunized mice (data not shown). The mechanism by which activated $\gamma\delta$ T cells cause an increased autoreactive T cell response remains to be determined. It is likely that the recorded changes are accompanied by a series of reciprocal interactions between $\gamma\delta$ and $\alpha\beta$ T cells and between $\gamma\delta$ T cells and DCs. Clarification of these issues may help us to understand the mechanism by which Th17 responses are regulated and the mechanism by which $\gamma\delta$ T cells regulate the Th17 response. The observation that specifically activated DCs or DC subsets are important for $\gamma\delta$ T cell activation and function should help in efforts to manipulate the Th17 response by acting on $\gamma\delta$ T cell activation.

There is an unresolved question about whether previously identified regulatory T cells that suppress Th1 cells can also suppress Th17 cells. Indeed, there are suggestions that, in contrast to IFN- γ production, IL-17 production and/or Th17 cell development may not be effectively downregulated by previously identified regulatory T cells (19, 20). Thus, efforts aimed at identifying factors that regulate the generation and expansion of Th17 autoreactive T cells are important.

Disclosures

The authors have no financial conflicts of interest.

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