FoxP3\(^+\) Regulatory T Cells Restrain Splenic Extramedullary Myelopoiesis via Suppression of Hemopoietic Cytokine-Producing T Cells

Jee H. Lee, Chuanwu Wang and Chang H. Kim

*J Immunol* 2009; 183:6377-6386; doi: 10.4049/jimmunol.0901268

http://www.jimmunol.org/content/183/10/6377
FoxP3⁺ Regulatory T Cells Restrain Splenic Extramedullary Myelopoiesis via Suppression of Hemopoietic Cytokine-Producing T Cells¹

Jee H. Lee, Chuanwu Wang, and Chang H. Kim²

Extramedullary myelopoiesis occurs in peripheral organs such as spleen and produces many types of myeloid cells with diverse functions in response to inflammation and infection. It is increased during immune responses and chronic inflammation and is a significant factor in regulating inflammatory diseases and immunity. Increased myeloid cells are found in FoxP3-deficient mice but the mechanism has been unclear. We investigated the mechanism by which FoxP3⁺ regulatory T cells regulate the extramedullary myelopoiesis. We found that Ab or genetic depletion of FoxP3⁺ regulatory T cells greatly increased the number of the myeloid progenitors in spleen during immune responses. Consistently, the splenic myelopoiesis was effectively suppressed by increased numbers of natural or induced FoxP3⁺ regulatory T cells. We demonstrated that myelopoiesis is positively regulated by splenic CD4⁺ T cells that produce myelopoietic cytokines (GM-CSF and IL-3), and these effector CD4⁺ T cells are induced from naive CD4⁺ T cells in response to antigenic stimulation. FoxP3⁺ regulatory T cells were able to effectively suppress the differentiation of naive T cells into myelopoietic cytokine-producing T cells. This suppression was found to be dependent on cell contact but independent of TGFβ. Unlike splenic myelopoiesis, marrow myelopoiesis is not significantly affected by FoxP3⁺ regulatory T cells. We conclude that FoxP3⁺ T cells can negatively regulate splenic extramedullary myelopoiesis by suppressing the naive T cell differentiation into myelopoietic cytokine-producing CD4⁺ T cells. Our results provide new insights into regulation of extramedullary myelopoiesis. *The Journal of Immunology, 2009, 183: 6377–6386.*

B one marrow is the primary site of hemopoiesis in adult mammals (1). During embryo development before formation of functional bone marrow, however, yolk sac, para-aortic splanchnopleural mesoderm, fetal liver, and spleen serve as hemopoietic sites for survival of the fetus (2). Spleen is a site of extramedullary hemopoiesis also in adult mammals (3, 4). Extramedullary hemopoiesis, and more specifically, extramedullary myelopoiesis (EM),¹ is important for production of sufficient numbers of leukocytes such as phagocytes and APCs during immune responses, but excessive EM is often seen in autoimmunity and systemic inflammation. The important role of splenic EM in development of immunity is well evidenced by the marked reduction in phagocytosis and clearance of extracellular pathogens in asplenic patients (5, 6). Excessive splenic EM is a feature of many autoimmune diseases and chronic infection in humans and animals (7–9). Moreover, a heterogeneous group of myeloid cells called myeloid-derived suppressor cells with immunoregulatory functions are increased in peripheral organs following infection and cancer formation and are likely to be a product of EM (10).

Myelopoiesis is both positively and negatively regulated by a number of cell types and cytokines in the body. For example, IL-3, stem cell factor, G-CSF, GM-CSF, and IL-6 are important promyelocytic cytokines (11–14). In contrast, some inflammatory cytokines and many chemokines negatively regulate myelopoiesis (15). Although it is still unclear what cell types regulate extramedullary hemopoiesis, there is evidence that T cells have the potential to regulate the process (16). This is probably because T cells can produce GM-CSF, IL-3, and other hemopoietic cytokines upon activation. In line with this, it has been reported that reduced myelopoiesis occurs in T cell-deficient mice (17–21).

Forkhead box P3 (FoxP3-positive regulatory T cells (Treg; Ref. 2) constitute a major subset of T cells with immunosuppressive functions (22–24). Tregs can suppress various cell types such as T cells, B cells, dendritic cells, macrophages, and NK cells to achieve immune tolerance (25–31). Deficiency of these T cells due to congenital mutations in the FoxP3 gene or other genes important for induction or expansion of Tregs leads to autoimmune diseases in multiple organs (32–34). The important role of Tregs in regulation of myelopoiesis is well evidenced by the greatly increased numbers of Mac1 (CD11b/CD18)⁺ cells including neutrophils, monocytes, and eosinophils in various tissues (33, 35–37). In this study, we investigated the roles of hemopoietic cytokine-producing T cells and Tregs in the regulation of EM. We found that Tregs negatively regulate the splenic myelopoiesis but have a minimal effect on marrow myelopoiesis. Tregs regulate myelopoiesis through suppression of T cells that produce myelopoietic cytokines through a cell contact-dependent but TGFβ-independent manner.
FoxP3+ T cells regulate extramedullary myelopoiesis

Materials and Methods

Mice

BALB/c mice were purchased from Harlan. FoxP3-deficient scurfy mice and dominant negative (dn) TGFβRII mice were purchased from The Jackson Laboratory. DO11.10 rag2−/− mice were purchased from Taconic. mOVA × DO11.10 rag2−/− transgenic mice were maintained at Purdue University. GM-CSF-deficient mice have been described previously (38). Mice were housed at Purdue University and used according to approved protocols and institutional guidelines. The FoxP3-deficient scurfy mice were used at 3 wk of age, and other mice were used between 6 and 8 wk of age.

Cell isolation and in vitro studies

CD4+CD25+ T cells were isolated with a magnetic sorting method (Miltenyi Biotec) by isolation of splenic CD4+ T cells and then by positive selection of CD25+ cells (purity >90% in FoxP3 expression). The CD25 T cell fraction of CD4+ T cells was used as target T cells in many experiments. Naive CD4+ T cells were further isolated by depleting the T cells expressing CD44, CD69, and CD25. For preparation of the culture supernatant of T cells, the two populations were cultured together or separately in the presence of staphylococcal enterotoxin B (SEB; 2 μg/ml) for 3 days in RPMI medium supplemented with 10% FBS. ELISA assays of GM-CSF and IL-3 were performed on the culture supernatant. Neutralizing anti-GM-CSF, anti-IL-3 (clone MP2-8F5), and anti-IL-6 (clone MP1-22E9) Abs were added to the culture at 0.1 μg/ml (BioLegend) when indicated. The culture supernatant was assayed also for its activity in supporting myelopoiesis with the hematopoietic colony-forming assay as described below with splenic myeloid progenitor cells. Transwell membranes (Corning Life Sciences) with 0.4-μm diameter pores were used to physically separate the CD4+ CD25+ target cells from the spleen cells during co-culture in some experiments. For Treg suppression assays, 1 × 10⁴ CD4+CD25+ T cells or naive CD4+ T cells were cocultured with Tregs at various ratios for 6 days in the presence of SEB (2 μg/ml).

Methylcellulose CFU-GM assay

The myeloid colony-forming assay was performed as described before (39). Marrow cells and spleen cells were plated in 3-cm plates containing methylcellulose medium supplemented with cytokines or conditioned media. Marrow cells were plated at 5 × 10⁴ cells/plate, and spleen cells were plated at 5 × 10⁴ cells/plate in 1.0% methylcellulose culture medium containing 30% v/v FBS (HyClone). Recombinant human erythropoietin (1 U/ml; Amgen Biologics), 50 μM 2-ME, 10 ng/ml recombinant murine stem cell factor (Peprotech), 5% v/v PWM mouse spleen cell-conditioned medium or T cell-conditioned medium, and 0.1 mM hemin (Fluka) were added. Colonies of CFU-GM were scored after 8 days of incubation in a humidified environment at 5% CO₂.

In vivo studies

For depletion studies, BALB/c mice were injected twice with 400 μg of anti-CD25 (PC61) at days 1 and 3. The mice were injected i.v. with 50 μg of SEB (Sigma-Aldrich) at day 5 and sacrificed at day 7, 10, or 20. As the result of the depletion of CD25+ cells, the frequency of FoxP3+ T cells was decreased from 10–12% to 3–4% of CD4+ T cells in the spleen at day 10 (5 days after the SEB immunization). FoxP3-deficient scurfy mice and wild-type mice were sacrificed at 3 wk of age. Bone marrow and spleen cells were harvested and assayed to determine the number of CFU-GM. Spleen tissues were examined for immunohistochemical detection of FoxP3 and CD11b+ GR−1− myeloopoietic patches as described below by immunohistochemistry.

For adoptive transfer of primary Tregs into scurfy mice, CD4+CD25+ T cells (1 × 10⁷) were transferred into 1- to 2-day-old newborn pups. The mice were sacrificed 3 wk later, and the marrow and spleen were examined for CFU-GM by the methylcellulose CFU-GM assay described above, and for lineage (CD3/CD19/CD11b−) negative c-Kit+ progenitor cells, GM-CSF+ T cells, IL-3− T cells, FoxP3+ T cells, and non-FoxP3− T cells by flow cytometry.

DO11.10 rag2−/−/mice (thymus-derived FoxP3+ (FoxP3+)+ cell-deficient) and mOVA × DO11.10 rag2−/− transgenic mice (FoxP3+ cell-excessive) were immunized i.v. with SEB (50 μg/mouse), and the mice were sacrificed 5 days later. The marrow and spleen tissues were examined for CFU-GM by the colony assay and for FoxP3+ and FoxP3− CD4+ T cells by flow cytometry.

Immunohistochemistry

Spleens were harvested and frozen in the Tissue-Tek freezing medium (Sakura Finetek). Tissue sections (6 μm) were fixed in cold acetone for 10 min and rehydrated in 1× PBS for surface staining. Sections were blocked with 10% rat serum for 10 min and stained with fluorescent anti-mouse GR-1 (clone RB6-8C5), anti-mouse CD11b (clone M1/70), and anti-mouse CD4 (clone GK1.5) for 40 min at 4°C. For nuclear FoxP3 staining, sections were stained with fluorescent anti-mouse FoxP3 (clone FJK-16s) for 40 min at 4°C according to the to the manufacturer’s protocol (eBioscience). The slides were analyzed with a Leica microscope equipped with epifluorescence.

Flow cytometry

Fresh bone marrow cells and spleen cells, harvested from BALB/c mice or transgenic mice, were stained with Abs to CD4 (clone RM4-5), CD11b, and GR-1 for 30 min on ice. The cells were further stained with anti-FoxP3 (clone FJK-16s) according to the manufacturer’s protocol (eBioscience). For intracellular staining of GM-CSF and IL-3, cells were stained for surface Ags and activated for 4 h in RPMI 1640 (10% FBS) with PMA (50 ng/ml) and ionomycin (1 μM) in the presence of monensin (2 μM). Cells were fixed, permeabilized, and stained with anti-GM-CSF (clone MP1-22E9), anti-IL-3 (clone MP2-8F8), and anti-IFN-γ (clone XMG1.2) Abs. The CD11b− Gr−/−/mört cells were identified with Abs to mouse CD11b (clone M1/70), GR-1 (clone RB6-8C5), c-Kit (clone 2B8), L-selectin (CD62L; ME-14), and CD69 (H1.2F3). The Abs were purchased from BD Biosciences or BioLegend. The stained cells were analyzed with a FACSCanto II (BD Biosciences).

Statistical analyses

Averages with SEM are shown in most of the figures. Student’s paired and unpaired t tests were used to determine the significance of the differences between two groups of data. p values ≤0.05 were considered significant.

Results

FoxP3-deficient mice display extramedullary, but normal marrow, myelopoiesis

Scurfy mice have a nonfunctional mutation in the FoxP3 gene and, thus, are completely deficient with FoxP3+ T cells (32, 33). These mice have hematological abnormalities with increased CD11b+ cells in blood and lymphoid tissues with concomitantly increased production of GM-CSF (36, 37, 40). We found sites of myelopoiesis characterized by patches of CD11b+ GR−1− cells in the spleen of the FoxP3-deficient mice but not in wild-type mice (Fig. 1A). Also increased was the number of myeloid progenitors (CFU-GM) in the spleen of the FoxP3-deficient mice compared with wild-type mice (Fig. 1B). In contrast, the numbers of CFU-GM in the bone marrow were not significantly increased in the FoxP3-deficient mice. Because effector T cells are increased in the FoxP3-deficient mice, we examined also the frequencies of GM-CSF+ and IL-3−/CD4+ T cells in the spleen and marrow. The spleen T cells and marrow T cells were very different in expression of GM-CSF and IL-3 (Fig. 1C). Only some spleen, but not marrow, CD4+ T cells of the FoxP3-deficient mice were able to produce the two myeloopoietic cytokines. These results show a positive correlation between EM and GM-CSF+ and IL-3−/CD4+ T cells; and a negative correlation between EM and FoxP3+ T cells and the clear differences between spleen and marrow in myeloopoietic activity and T cell cytokine phenotype.

Depletion of Tregs increased the super Ag-induced splenic myelopoiesis

The excessive EM in the FoxP3-deficient mice may be due to the Treg deficiency and excessive development of T cells that produce
myelopoietic cytokines, but it is also likely that this is an indirect result of the chronic autoimmune disease in these mice. To clarify this, we established a mouse model of EM, induced with a superantigen staphylococcal enterotoxin B (SEB; Fig. 2A), which polyclonally activates CD4^{+} T cells by binding both to TCR of the T cells and MHC class II molecules of APCs (41). We found that SEB was effective in inducing EM in the spleen (Fig. 2A). Single injection (i.v.) of SEB increased the number of the myeloid progenitors (CFU-GM) in the spleen. This EM was transient following the injection of SEB, peaking at day 5. The bone marrow myelopoiesis did not change significantly upon the SEB challenge, even more suppressive, suggesting the roles of the T cell-derived hemopoietic cytokines in supporting myelopoiesis. We tested also the T cells isolated from GM-CSF-deficient mice. We observed the lower activity of GM-CSF and IL-3 compared with GM-CSF^{+/-} T cells in supporting myelopoiesis, which was further suppressed when IL-3 was blocked at the same time with a neutralizing Ab (Fig. 3B). These results demonstrate that T cell activation in combination with Treg deficiency can cause excessive EM.

Myelopoietic activities of T cell-derived GM-CSF and IL-3

To determine the role of GM-CSF and IL-3 produced from T cells in regulation of myelopoiesis, we prepared conditioned media from the culture of CD4^{+}CD25^{+} T cells and examined their activity in supporting myelopoiesis in a semisolid methylcellulose assay. The T cell-conditioned medium was effective in supporting the splenic myeloid progenitors (Fig. 3A). Only the conditioned medium with SEB-activated T cells but not with resting T cells without Ag stimulation was effective in supporting the myelopoiesis of the splenic progenitors. Blocking of GM-CSF or IL-3 with neutralizing Abs decreased the myelopoietic activity of the conditioned medium (Fig. 3A). Blocking of both GM-CSF and IL-3 was even more suppressive, suggesting the roles of the T cell-derived hemopoietic cytokines in supporting myelopoiesis. We tested also the T cells isolated from GM-CSF-deficient mice. We observed the lower activity of GM-CSF^{+/-} T cells compared with GM-CSF^{+/-} T cells in supporting myelopoiesis, which was further suppressed when IL-3 was blocked at the same time with a neutralizing Ab (Fig. 3B). These results demonstrate that the T cell-derived myelopoietic cytokines can positively regulate the myelopoiesis.

Tregs directly suppress the T cells that produce myelopoietic cytokines

It is a question of interest how Tregs can regulate the EM. We hypothesized that Tregs would suppress the T cells that produce myelopoietic cytokines to suppress EM. To test the hypothesis, we cultured the two T cell populations (Tregs and CD4^{+}CD25^{−} effector T cells) separately or together in the presence or absence of

---

**FIGURE 1.** Increased myelopoietic activities and hemopoietic cytokine-producing cells in the spleen but not in the bone marrow of FoxP3^{−/−} mice. A, Immunohistochemistry detection of the cells expressing GR-1 and CD11b in the spleen of FoxP3^{−/−} and wild-type mice. B, Total number of CFU-GMs in the spleen and marrow (number per 2.8 × 10^{8} marrow cells). C, Frequencies of GM-CSF^{+}CD4^{+} T cells and IL-3^{+}CD4^{+} T cells in spleen and marrow. Combined data of three independent experiments (averages and SEM; three to five mice) are shown. FoxP3^{−/−} mice were sacrificed at ∼3 wk of age. The T cells were stimulated with PMA and ionomycin for 4 h to detect the cytokine-producing T cells. *, Significant differences between the wild-type (WT) and FoxP3^{−/−} (KO) mice (p < 0.05).
SEB and examined the production of GM-CSF/IL-3 and myelo-
poietic activity of the culture supernatant (Fig. 4). Tregs sup-
pressed the production of GM-CSF and IL-3 by the CD4
CD25
T cells in a dose-dependent manner (Fig. 4
A). It was apparent that
Tregs decreased the myelopoiesis-supporting activity of the
CD4
CD25
effector T cells in a dose-dependent manner (Fig.
4
B). Unlike the CD4
CD25
T cells, Tregs, themselves, were not
good producers of the myelopoietic cytokines.

Tregs suppress the differentiation of naive T cells into
myelopoietic cytokine-producing T cells

Naive T cells do not produce GM-CSF and IL-3. Naive T cells
have to differentiate into effector T cells to gain the ability to pro-
duce the cytokines. Perhaps, Tregs would suppress the differenti-
ation of naive T cells into hemopoietic cytokine-producing effector
T cells. To determine the possibility, naive T cells, depleted of
Tregs and memory/effector T cells, were isolated from spleen cells
and cultured with irradiated splenocytes as APCs in the presence
of SEB. This condition was conducive for generation of GM-
CSF
T cells from the naive T cells (Fig. 5
A). In the absence
of SEB, no GM-CSF
T cells were made, suggesting the impor-
tance of the Ag signal in this process. Tregs were effective in
suppressing the differentiation of naive T cells into GM-CSF
T cells (Fig. 5
A). Tregs were effective also in suppressing the pro-
duction of myelopoietic factors by the T cells differentiated from
the naive T cells (Fig. 5
B).

FIGURE 2. Depletion of Tregs increased a superantigen-induced myelopoiesis in BALB/c mice. A, BALB/c mice were depleted (deplet and dep) of
CD4
CD25
cells with an anti-CD25 Ab (injected twice with a 2-day interval). Two days after the final injection of the Ab, SEB was injected i.v. into
mouse tail veins. The mice were sacrificed at days 2, 5, and 15, and the change in CFU-GM numbers in bone marrow (number per 2.8 \times 10^8 marrow cells)
and spleen was determined by a myelopoietic colony-forming assay (n = 5 per group). p values for the comparisons: 1*, 0.0001 between CD25-depleted/
SEB and SEB groups; 2*, 0.029 between CD25-depleted/SEB and control groups; 3*, 0.053 between SEB and control groups. B, Increased CD11b
GR-1
and CD11b
GR-1
myeloid cells in the spleen of Treg-depleted mice (n = 4). CD3
CD19 splenic cells are shown. Total numbers of CD11b
GR-1
and CD11b
GR-1
myeloid cells in the spleen are also shown. C, Increased CD11b
GR-1
myelopoietic sites in the spleen of Treg-depleted
mice. Frozen tissue sections were stained with fluorescent Abs in C. Bars, SEM. The control mice were injected with control rat IgG. **, Significant
differences (p < 0.05).
The roles of cell contact and TGFβ signaling in suppression of GM-CSF+ T cells

Cell contact is typically required for the suppression of target cells by Tregs, and TGFβ is implicated in the process (42, 43). We investigated whether cell contact and TGFβ are important also for the suppression of myelopoietic cytokine-producing T cells by Tregs. We used transwell membranes to separate the target cells in the lower chamber from the Tregs in the upper chamber. This separation was effective in abolition of the suppressive effect of Tregs on GM-CSF+ T cells (Fig. 6A).

To determine the role of TGFβ signaling in the cell contact-dependent suppression of myelopoietic cytokine-producing T cells, we used the CD4+CD25+ T cells isolated from the dnTGFβRII mice, which are defective in transmitting TGFβ signaling (44). Tregs were able to suppress the CD4+CD25+ T cells isolated from both wild-type and dnTGFβRII mice in production...
of GM-CSF (Fig. 6B), suggesting that the TGFβ signaling is not required for the process. These results demonstrate that Tregs need cell contact but not the TGFβ signaling in suppression of the GM-CSF+ T cells.

**Increased numbers of FoxP3+ T cells limit the numbers of myelopoietic cytokines and EM in the spleen**

To confirm the positive function of Tregs in limiting splenic myelopoiesis, we transferred Tregs isolated from secondary lymphoid tissues of wild-type mice into 1- or 2-day-old scurfy mice. As the result, the frequency of FoxP3+ T cells in the spleen was greatly increased in the scrufy mice with concomitant decreases in activated (CD69+) memory or effector (CD62L-) CD4+ T cells at 3 wk of age (Fig. 7A). The levels of CD4+ T cells expressing GM-CSF or IL-3 were nearly normalized (i.e., decreased) following the Treg transfer (Fig. 7B). Furthermore, the Treg transfer decreased the numbers of lineage-negative c-Kit+ progenitor cells and CFU-GM progenitor cells (Fig. 7, C and D).

The FoxP3+ T cells that are made in the periphery upon immunization are called induced FoxP3+ T cells (iFoxP3+ cells), whereas the FoxP3+ T cells that are made in the thymus are called thymus-generated FoxP3+ T cells (tFoxP3+ cells). We examined whether iFoxP3+ cells can suppress the splenic myelopoiesis induced upon antigenic stimulation. We used DO11.10 rag2−/− mice, which lack tFoxP3+ cells (Ref. 45 and Fig. 8A). We transferred iFoxP3+ cells, generated in vitro from DO11.10 rag2−/−...
CD4+ T cells with TGFβ1, into DO11.10 rag2−/− mice and immunized the mice with SEB. For comparison, we transferred activated conventional non-Treg CD4+ T cells prepared without TGFβ1. The Treg transfer increased the frequency of FoxP3+ cells from 0.1 to ~2.5% in the spleen at day 5 postimmunization (Fig. 8B). As the consequence, the numbers of lineage− c-Kit+ cells

FIGURE 7. Tregs suppress the extramedullary myelopoiesis in the spleen of FoxP3−/− scurfy mice. A, Frequencies of FoxP3+/CD4+ T cells and FoxP3−/CD4+CD69+CD62L− in the spleen of ~3-wk-old FoxP3−/− scurfy mice transferred with Tregs at day 1 or 2 following birth compared with age-matched wild-type (WT) mice and control scurfy mice. B, Frequencies of GM-CSF+CD4+ T cells and IL-3+CD4+ T cells in the spleen. C, Frequencies of lineage (CD3/CD19/CD11b)-negative c-Kit+ progenitor cells in the spleen. D, Frequencies of CFU-GM myeloid progenitor cells in the spleen. Averages and SEM of the data obtained from four to six mice are shown. * and **, Significant differences from wild-type mice (*, p < 0.05) or FoxP3−/− mice (**, p < 0.05).

FIGURE 8. Induced Tregs suppress the EM in the spleen of DO11.10 rag2−/− mice. A, iTreg were prepared by activating DO11.10 rag2−/− CD4+ T cells with TGFβ1 for 6 days in vitro. Conventional CD4+ T cells (Conv.) were prepared without using TGFβ1. B, Frequencies of FoxP3+ T cells among splenic CD4+ T cells in the DO11.10 rag2−/− mice transferred with iTreg or conventional CD4+ T cells. Frequencies of lineage (CD3/CD19/CD11b)-negative c-Kit+ progenitor cells in the spleen (C), CFU-GM (D), CD11b+GR-1dim cells (E), and CD11b+GR-1bright cells (F) in the spleen of DO11.10 rag2−/− mice transferred with iTreg or conventional CD4+ T cells. Averages and SEM of the data obtained from four to five mice are shown. * and **, Significant differences from the SEB group (*, p < 0.05) or the SEB/conventional T cell group (**, p < 0.05).
Discussion
Regulation of splenic EM in response to antigenic stimulation or inflammation has been largely unknown. Through the studies, we found that the splenic EM can be positively regulated by effector T cells that produce GM-CSF and IL-3 and that FoxP3$^+$ T cells suppress the T cells that produce GM-CSF and IL-3 and negatively regulate splenic EM but do not significantly affect marrow myelopoiesis.

The T cells that produce hemopoietic cytokines such as GM-CSF and IL-3 are thought to be important for production of necessary phagocytes and APCs during immune responses. However, excessive activities of these T cells and myelopoiesis would be harmful for the host because they could induce hyperimmune responses and inflammation. This view is supported by the fact that overexpression of GM-CSF induces chronic inflammation and autoimmune reactions (46, 47). In contrast, GM-CSF-deficient mice have no problem in basic myelopoiesis but are impaired in mounting immunity against bacterial pathogens (48–51). Appropriate regulation of myelopoietic cytokine-producing cells would be important to achieve both immunity and immune tolerance. Therefore, there is the need to negatively regulate EM. We found that Tregs provide the necessary negative signal to down-regulate EM induced upon antigenic stimulation or in systemic inflammation. Tregs themselves are not a good source of the myelopoietic cytokines. Rather, Tregs effectively suppress the production of the myelopoietic cytokines by the effector T cells. These findings are well supported by the increased numbers of GM-CSF$^+$ and IL-3$^+$ T cells and the high myelopoietic activity in the spleen of the FoxP3-deficient mice. Through the SEB-EM model, we found that the splenic EM occurs in the splenic red pulp areas surrounding white pulp areas following an immunological challenge. Depletion of FoxP3$^+$ T cells greatly expanded the size and number of the CD11b$^+$ GR-1$^+$ myelopoietic patches in spleen. On the other hand, transfer of Tregs decreased the myelopoietic activity in the spleen. These results demonstrate that Tregs play a negative role in the process.

In suppression of EM, FoxP3$^+$ T cells may suppress the effector T cells in the white pulp areas or they may suppress the production of hemopoietic cytokines in the red pulp areas. We found almost...
no FoxP3+ T cells within the CD11b+GR-1+ myelopoietic patches in the red pulp areas during immune responses. Instead, almost all FoxP3+ T cells were found in the white pulp areas and, more specifically, in the T cell area (Fig. 2C). This information supports the view that FoxP3+ Tregs function in white pulp where naïve T cells undergo activation and differentiation into effector T cells that produce myelopoietic cytokines. In this regard, our results demonstrated that Tregs are effective in blocking the differentiation of naïve T cells into GM-CSF+ effector T cells (Fig. 5).

FoxP3+ Tregs, although they regulate the myelopoiesis in the spleen, do not affect that in the bone marrow. This is somewhat unexpected because the bone marrow is highly enriched with FoxP3+ T cells (which constitute ~40% of the marrow CD4+ T cells; Refs. 45 and 52). We have two explanations for this. First, the frequency of effector CD4+ T cells in the bone marrow (~0.1% of total marrow cells vs ~15% of total spleen cells) is too low to affect the marrow hemopoiesis at significant levels. Second, the T cells in the bone marrow are intrinsically different from those of spleen in that they are unable to produce GM-CSF and IL-3. Thus, suppression of marrow effector T cells would not have any significant impact on production of the myelopoietic cytokines. The CD11b+GR-1+ myeloid cells with certain immune regulatory functions are receiving a lot of attention these days. Many groups noted the emergence of these myeloid-derived suppressor cells in spleen and other organs in infection and cancer (10, 53). The myeloid cells that are induced in the absence of Tregs in our study could include these myeloid cells. However, one should note that the CD11b+GR-1bright/mid myeloid cells induced in the spleen are highly heterogeneous and should not consider all of these cells as myeloid-derived suppressor cells. Indeed, it has been reported that CD11b+GR-1+ myeloid cells induced in the spleen of tumour-free animals often lack the regulatory function (54). The myeloid-derived suppressor cells themselves are highly heterogeneous and are considered normal constituents of myelopoiesis (10).

Overall, our results demonstrate that Tregs function to restrain the myelopoietic cytokine-producing T cells and, therefore, play an active role in prevention of extramedullary myelopoiesis induced upon antigen stimulation in the spleen. EM can occur in diverse forms and tissues in addition to the spleen in diseased conditions such as chronic inflammation and cancer. It remains to be determined whether Tregs have a similar function in regulation of other types of EM occurring in nonlymphoid tissues.

Acknowledgments

We thank S. Kang for helpful input (Purdue University).

Disclosures

The authors have no financial conflict of interest.

References


