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CCR5-Dependent Regulatory T Cell Migration Mediates Fungal Survival and Severe Immunosuppression

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Paracoccidioidomycosis, a debilitating pulmonary mycosis, is caused by the dimorphic fungus Paracoccidioides brasiliensis. The infection results in the formation of granulomas containing viable yeast cells that are the fungal sources for disease reactivation. Because CD4⁺CD25⁺ regulatory T cells (Tregs) are in the lesions of patients with paracoccidioidomycosis, the migration of Treg cells is dependent on the axis chemokine-chemokine receptors, and CCR5 ligands are produced in P. brasiliensis-induced lesions, we investigated the role of CCR5 in the control of the infection. The results showed that CCR5⁻/⁻ mice are more efficient in controlling fungal growth and dissemination and exhibited smaller granulomas than wild-type (WT) mice. In the absence of CCR5, the percentage of CD4⁺CD25⁺ T cells expressing Foxp3, glucocorticoid-induced TNFR (GITR), CD103, CD45low, and CTLA-4 in the granulomas was significantly decreased. Interestingly, P. brasiliensis infection resulted in an absence of T cell proliferation in response to Con A in WT but not CCR5⁻/⁻ mice that was abrogated by anti-CTLA-4 mAb and anti-GITR mAb. Moreover, the adoptive transfer of CD4⁺CD25⁺ but not CD4⁺CD25⁻ T cells from infected WT to infected CCR5⁻/⁻ mice resulted in a significant increase in fungal load. Overall, CCR5 is a key receptor for the migration of Treg cells to the site of P. brasiliensis infection, leading to down-modulation of effector immune response and the long-term presence of the fungus in the granulomas. Thus, a tight control of Treg cell migration to the granulomatous lesions could be an important mechanism for avoiding exacerbation and reactivation of the disease. The Journal of Immunology, 2008, 180: 3049–3056.

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ligands. Therefore, we investigated the role of CCR5 in the control of \textit{P. brasiliensis} infection and sought to demonstrate the kinetics of CD4^+ CD25^+ T cell migration to the periphery and its role in the control of immune response and granuloma formation during this infection. We found that CCR5-deficient mice have an impaired migration of CD4^+ CD25^+ regulatory T cells to the pulmonary lesions, which leads to an effective local immune response culminating in the formation of more compact granulomas and control of the disseminated disease.

Materials and Methods

\textbf{Mice}

Male C57BL/6 wild-type (WT; CCR5^{+/+}) and CCR5-deficient (CCR5^{−/−}) mice, 6 to 8 wk old at the beginning of the experiments, were used. The mice were bred and maintained in microisolator cages in the animal housing facility of Department of Immunology, University of São Paulo, Ribeirão Preto, Brazil. Mice with targeted disruption of chromosome 9 of the CCR5 gene were obtained from The Jackson Laboratory. All animal experiments were performed in accordance with protocols approved by the School of Medicine of Ribeirão Preto Institutional Animal Care and Use Committee (Ribeirão Preto, Brazil).

\textbf{Fungus and experimental infection}

Yeast cells of a \textit{P. brasiliensis} strain (Ph18 and 339) were cultured at 35°C in Fava-Netto’s medium for 7 to 14 days, harvested, and washed three times in PBS (pH 7.2), and viability was determined as previously described (18). The animals were anesthetized by i.p. injection of 100 \mu l of PBS with 2.5% of tribromoethanol and infected via the trachea with \(1 \times 10^7\) viable yeast cells of Pb18 suspended in 100 \mu l of PBS. Some mice were injected i.p. with 0.5 mg of anti-CD25 (mAb PC61) on days 7, −4, and −1, anti-glucocorticoid-induced TNFR (GITR) (mAb DTA) on day −1, or control Ab (rat IgG Ab) on days −7, −4, and −1.

\textbf{Assay for organ CFU}

To determine the growth and dissemination of \textit{P. brasiliensis} in lungs, livers, and spleens, the amounts of CFU recovered from the tissues were analyzed at 7, 15, 30, 60, and 90 days after infection. The organs were aseptically removed, weighed, and homogenized in sterile PBS using tissue grinders (IKA-Werke) and 100 \mu l of the samples were diluted in 900 \mu l of PBS. Aliquots of 100 \mu l of each sample were dispensed into petri dishes containing brain heart infusion agar (Difco Laboratories) supplemented with 4% normal horse serum and 5% Pb339 broth yeast culture filtrate containing brain heart infusion agar (Difco Laboratories) supplemented with 4% normal horse serum and 5% Pb339 broth yeast culture filtrate (containing brain heart infusion agar (Difco Laboratories)).

\textbf{Isolation of leukocytes from granulomas}

The lungs were harvested 30 days later, and the presence and localization of CCR5 (A) and CCL4 (B) (top two panels) were determined by immunohistochemistry. C (bottom panel) shows representative immunostaining pattern in lungs of C57BL/6 infected mice using a secondary Ab alone, either CCR5 or CCL4. Diaminobenzidine (DAB) was used as peroxidase substrate to generate a brown-staining signal, and the slices were counterstained with hematoxylin. Bar (lower right corner of each panel), 100 \mu m.

\textbf{Flow cytometry analysis}

The cell surface expression of CD3, CD4, CD8, CCR5, CD45Rb, CD103, CTLA-4 (CD152), GITR, and Foxp3 were assessed by flow cytometry. Leukocytes isolated from the lungs or granulomas of infected or normal mice were washed in cold PBS and incubated for 30 min at 4°C with 0.5 \mu g of anti-CD16/CD32 mAb (PC (B) followed by the addition of 0.5 \mu g per 10^6 cells of the Abs PerCP, FITC, or PE (all from BD Pharmingen). To detect intracellular expression of Foxp3, the cells were fixed with Cytofix and Cytoperm solution (Sigma-Aldrich) for 20 min at 4°C and then stained with PE-labeled anti-Foxp3 (all from BD Pharmingen) for 30 min at 4°C in the dark. Subsequently, cells were washed twice and resuspended in 300 \mu l of PBS-BSA (Sigma-Aldrich). Multivariate data analysis was performed in the FACSCalibur flow cytometer using the CellQuest software (BD Biosciences) by gating the live cells on a side scatter vs forward scatter dot plot or on the population of CD4^+ CD25^+ or CD4^+ CD25^- T cells to assess the regulatory cell phenotype.

\textbf{CFSE proliferation assay}

Total cells isolated from the spleens of uninfected or infected mice at the day 15 were suspended at a concentration of 10^6 cells/ml in PBS. CFSE

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Footnote:

1 Abbreviations used in this paper: WT, wild type; Treg, regulatory T cell; GITR, glucocorticoid-induced TNF receptor; p.i., postinfection.
FIGURE 2. Absence of CCR5 leads to the formation of compact granulomas after *P. brasiliensis* infection. Lung sections from WT and CCR5−/− mice, infected with *P. brasiliensis*, were fixed in formalin, paraffin embedded, stained with H&E, and analyzed by light microscopy. The percentage of lymphocytes expressing CD45RBlow was determined. CD45RBlow lymphocytes were gated and analyzed for coexpression of CCR5, CD25, CD103, CTLA-4, GITR, and Foxp3.

FIGURE 3. Decreased migration of CD4+ CD25+ T cell to *P. brasiliensis*-induced granulomas in CCR5−/− mice. Leukocytes were isolated from pulmonary granulomas of *P. brasiliensis*-infected WT and CCR5−/− mice and analyzed by flow cytometry. The percentage of lymphocytes expressing CD19, CD3+CD4+, and CD25+CD8- cells (A) and CD4+CD25+ T cells (B) was determined. CD4+CD25+ (C) or CD4+CD25− (D) T lymphocytes were gated and analyzed for coexpression of CCR5, CD45RBlow, CD103, CTLA-4, GITR, and Foxp3. The data represent the mean ± SEM of the results from three mice per group and are representative of two independent experiments. *, *p < 0.05 compared with WT mice.

FIGURE 3. Decreased migration of CD4+ CD25+ T cell to *P. brasiliensis*-induced granulomas in CCR5−/− mice. Leukocytes were isolated from pulmonary granulomas of *P. brasiliensis*-infected WT and CCR5−/− mice and analyzed by flow cytometry. The percentage of lymphocytes expressing CD19, CD3+CD4+, and CD25+CD8- cells (A) and CD4+CD25+ T cells (B) was determined. CD4+CD25+ (C) or CD4+CD25− (D) T lymphocytes were gated and analyzed for coexpression of CCR5, CD45RBlow, CD103, CTLA-4, GITR, and Foxp3. The data represent the mean ± SEM of the results from three mice per group and are representative of two independent experiments. *, *p < 0.05 compared with WT mice.

(Molecular Probes) was added at a final concentration of 1.25 μM. The solution was mixed well and incubated at room temperature for 5 min. An equal volume of serum was used to quench the reaction, after which, the cells were washed with PBS with 1% serum. Cells (1 × 10⁶) were then cultured in a 48-well plate with 500 μl of complete culture medium and stimulated or not with Con A (2 μM) or surface *P. brasiliensis* Ag (2 μg/ml) in the presence of the Abs anti-GITR (mAb DTA), anti-CTLA-4 (mAb 4F10), or isotype control anti-rat for 3 days at 37°C and 5% CO₂. Viable lymphocytes were gated based on forward/side scatter and the number of proliferating cells was determined using CellQuest software (BD Biosciences) and FlowJo software (Tree Star).

**Adoptive cell transfer experiments**

Leukocytes were obtained from the spleens of infected WT mice at day 15 after infection. CD4+CD25+ and CD4+CD25− T cells were isolated using magnetic beads (Miltenyi Biotech) according to the manufacturer’s protocol and labeled with CFSE as previously described. Briefly, leukocytes were stained with 5 μmol/L CFSE (5 min at 37°C in the dark). Staining was stopped by adding complete culture medium and the cells were centrifuged (5 min at 490 × g) and suspended in the same medium. Next, 1 × 10⁶ CD4+CD25+ and CD4+CD25− cells were i.v. injected into infected CCR5−/− mice (day 15 after infection). WT and CCR5−/− infected mice were used as control. After 5 days, the CFU were determined as previously described. Frozen lung sections from CCR5−/− were stained with 4’6-diamidino-2-phenilidole dihydrochloride (DAPI, blue fluorescence; Molecular Probes) and analyzed using a confocal microscope (Leica SP2). The images were processed using the Leica LCS program to characterize the presence of CFSE+ cells.

**Statistical analysis**

The results are expressed as the mean ± SEM and statistical analysis was performed using ANOVA comparing multiple groups or followed by the parametric Tukey-Kramer test for two group comparison (GraphPad software). *p < 0.05 was considered to indicate statistical significance.

**Results**

*P. brasiliensis*-induced granuloma formation in CCR5−/− mice

We previously showed the mRNA to CCR5 and their ligands in the spleen of *P. brasiliensis*-infected mice (7). In the current study, the presence of CCR5 and its ligand CCL4 in the lungs of WT mice was determined using immunohistochemistry. Our results revealed that 15 (data not shown) and 30 days postinfection (p.i.) a high number of CCR5+ cells and CCL4 were localized predominantly in the granulomatous lesions (Fig. 1). Positive stain was also observed for CCL3 and CCL5. Furthermore, the importance of CCR5 in the resistance of infection was evaluated in lung sections from...
P. brasiliensis-infected WT and CCR5−/− mice at several time points p.i. We noted the exudation of histiocytes, polymorphonuclear neutrophils, and mononuclear leukocytes on the day 7 p.i. in both WT and CCR5−/− mice. At this time, the extension of the inflammatory infiltrate in CCR5−/− mice was apparently higher when compared with that in WT mice (Fig. 2, A and B). At day 15 p.i. the inflammatory infiltrate was organized in both WT and CCR5−/− mice, with well defined granulomas containing yeast cells (Fig. 2, C and D). At day 30 p.i. the lung sections from both strains of mice exhibited histiocytes, neutrophils, epithelioid cells, and a few multinuclear giant cells surrounded by a rim composed of a large numbers of lymphocytes and some fibroblasts (Fig. 2, E and F). However, differing from WT mice, in the absence of CCR5 we observed more compact granulomas and preservation of the adjacent tissue (Fig. 2, E and F). The granuloma area, achieved by morphometry, was significantly reduced (p < 0.05) in infected CCR5−/− mice compared with WT mice (Fig. 2G).

CCR5−/− mice show competent cellular immune response and decreased migration of CD4+CD25+ T cell to the granulomas after P. brasiliensis-infection

To assess whether CCR5−/− mice present different cell migration to the lesions, we determined the frequency of leukocytes (CD19, CD3CD8, CD3CD4, and CD4CD25) from pulmonary granulomas at day 15 p.i. The results demonstrated that T cell recruitment was not inhibited in the absence of CCR5. However, the percentage of B cells was significantly decreased (p < 0.05). In the absence of this receptor the percentage of T cells (CD3+CD8+ and CD3+CD4+) was similar in both mouse strains (Fig. 3A). Interestingly, a significantly decreased (p < 0.05) level of CD4+CD25+ T cells was present in the lesions of CCR5−/− mice compared with WT mice (Fig. 3B), suggesting that regulatory T cells could be a modulatory component involved in the immune response in these P. brasiliensis-induced lesions. In fact, as shown in Fig. 4, there are apparently similar amounts of CD4+ cells in the granulomas of WT and CCR5−/− infected mice, while the number of Foxp3+ cells is higher in WT mice. Because CD25 is also expressed in conventional CD4+ T cells, the cells were gated for the populations of CD4+CD25+ and CD4+CD25− T lymphocytes and analyzed for coexpression of CD45Rblow, CD103, CTLA-4, GITR, and Foxp3. The results showed a high percentage of CD4+CD25+ T cells coexpressing all of these markers in WT and CCR5−/− mice except for the expression of CCR5, which was virtually absent in CCR5−/− mice (Fig. 3C). Differently, significantly lower expression of CD45Rblow, CD103, CTLA-4, GITR, and Foxp3 were found in CD4+CD25− T cells (Fig. 3D). These

FIGURE 4. Absence of CCR5 results in reduced migration of Foxp3-positive cells to the lungs of mice infected with P. brasiliensis. WT (A and C) and CCR5−/− (B and D) mice were infected with P. brasiliensis, the lungs were harvested 15 days later, and the presence of CD4+ (A and B) and Foxp3+ (C and D) cells were analyzed by immunohistochemistry. Diaminobenzidine (DAB) was used as the peroxidase substrate to generate a brown-staining signal and the slices were counterstained with hematoxylin. Bar (lower right corner of each panel), 100 μm.

FIGURE 5. CCR5−/− mice control P. brasiliensis growth and dissemination. CFU in the lungs, liver, and spleen of WT and CCR5−/− mice were determined on days 7, 15, 30, 60, and 90 p.i. The data represent the mean ± SEM of the results of three animals/group and are representative of two independent experiments. *, p < 0.05 compared with WT infected mice.

FIGURE 6. T cell proliferation is inhibited in WT and normal in CCR5−/− mice during the P. brasiliensis infection. Spleen cells from WT and CCR5−/− mice infected or not with P. brasiliensis were labeled with CFSE and cultured with or without Con A for 3 days. The cells were harvested and assessed for CFSE staining using cytometry (A). The number of proliferating cells is demonstrated in B. The data represent the mean ± SEM of the results of three animals/group and are representative of two independent experiments. *, p < 0.05 compared with WT infected mice.
data show that the CD4⁺CD25⁺ T cells found in granulomas express a classical phenotype of regulatory T cells. Furthermore, a decreased percentage of CD4⁺CD25⁺ cells recovered from CCR5⁻/⁻ mice coexpressed Foxp3 compared with WT mice (p < 0.05; Fig. 3D). These data strongly suggest that CCR5 is critical for the accumulation of regulatory T cells (Treg) in the granulomas of mice infected with P. brasiliensis. Thus, we speculate that a lower number of CCR5-infected mice effectively control the growth and dissemination of P. brasiliensis.

**CCR5⁻/⁻ infected mice effectively control the growth and dissemination of P. brasiliensis**

To directly assess the role of CCR5 in the resistance to P. brasiliensis infection, we determined the number of viable fungi (CFU) recovered from lungs, liver, and spleen from infected WT and CCR5⁻/⁻ mice. All infected mice survived for at least 100 days p.i., being that the CCR5⁻/⁻ mice were more efficient in controlling the growth of yeast cells and avoiding its dissemination to spleen and liver when compared with WT mice (Fig. 5). Except on day 7 p.i., the amount of yeast cells recovered from all organs studied was significantly (p < 0.05) lower in CCR5⁻/⁻ when compared with WT mice. Both strains of mice controlled the disease and presented a reduced number of CFU by day 90 p.i. (Fig. 5). Overall, these data indicate that in the absence of CCR5, the control of the disease is more efficient and suggest that some CCR5⁺ cells contribute to the long-term persistence of fungus in the lesions.

**GITR⁺ and CTLA-4⁺ cells modulate the T cell proliferative response in the infection with P. brasiliensis**

The lymphoproliferative response is a classical parameter that characterizes the immunological status during the infection with P. brasiliensis. To address the suppressor mechanisms observed during the disease, we performed a proliferation assay with spleen cells from WT and CCR5⁻/⁻ mice on day 15 p.i. The cells were stained with CFSE and cultured with or without Con A (2 ng/ml). As predicted, the lymphocytes obtained from WT infected mice showed decreased proliferation compared with WT uninfected mice (Fig. 6, A and B). Interestingly, the proliferation of lymphocytes obtained from CCR5⁻/⁻ infected mice was similar to that observed in cells from CCR5⁻/⁻ uninfected mice in the presence of Con A. Moreover, the T cell proliferation of CCR5⁻/⁻ infected mice was higher than that observed with cells from P. brasiliensis-infected WT mice (Fig. 6), showing an absence of systemic suppression in infected-CCR5⁻/⁻ mice. Because GITR and CTLA-4 are constitutively expressed in Treg cells, the blockage of CTLA-4 and the activation of GITR by interaction with the Abs may enhance the T cell proliferation by attenuating Treg function. To determine the mechanisms involved in the immunosuppression of infected WT mice, we cultured spleen cells with anti-CTLA-4 or anti-GITR Abs. Similar results were obtained in three animals per group and the experiment was performed twice with similar results.*

* p < 0.05 compared with WT mice treated with control Ab.
CD4⁺ CD25⁺ T cells play a crucial role in the control of P. brasiliensis growth

To determine the role of CCR5⁻/⁻ in the control of P. brasiliensis infection in mice, we adoptively transferred WT infected CD4⁺ CD25⁺ and CD4⁺ CD25⁻ T cells, stained with CFSE and obtained from spleen, to CCR5⁻/⁻ mice on day 15 p.i. Five days later, lung sections were stained with 4′,6-diamidino-2-phenylindole dihydrochloride and the cell migration to the lung was assessed using confocal microscopy. The results revealed that the transferred CFSE⁺ cells migrated to the lungs of CCR5⁻/⁻ (Fig. 8, A and B). Moreover, the CFU recovered from the lungs showed a significant increase (p < 0.05) of viable yeast cells in the lungs of CCR5⁻/⁻ mice that received CD4⁺ CD25⁺ cells compared with the CCR5⁻/⁻ only infected mice. In contrast, the transference of CD4⁺ CD25⁻ resulted in a significantly decreased (p < 0.05) CFU compared with that in CCR5⁻/⁻ mice (Fig. 8C). We confirmed the role of Treg cells in the maintenance of fungus survival through the treatment of WT mice with 500 μg of anti-CD25 mAb on days −7, −4, and −1, anti-GITR mAb on day −1, or rat-Ig on days −7, −4, and −1. The anti-CD25 and anti-GITR Ab treatment resulted in a significant decrease of CFU recovered from lungs on day 15 p.i. Overall, the data demonstrate that the recruitment of CD4⁺ CD25⁺ T cells into granulomas is triggered by CCR5 and favor fungal growth in the lesion.

Discussion

In this study we describe the involvement of CCR5 in the modulation of granuloma formation, the control of immune response, and, consequently, the maintenance of the fungal infection in mice. First, we observed CCR5⁺ cells and its ligands in mature granulomas induced by P. brasiliensis infection. CCR5 is expressed in resident or migrating cells after stimulation with IL-12, IFN-γ, and TNF-α (19–21), which are produced after infection with yeast forms of P. brasiliensis (2, 3, 5, 22, 23). These proinflammatory cytokines are involved in the leukocyte influx toward target tissues through the induction of chemokine generation (7, 24), leading to an environment that favors the formation of granulomas and limiting fungal proliferation and dissemination. Interestingly, the control of infection was more efficient in CCR5-deficient mice, resulting in the formation of compact and organized granulomas. These results suggest that the presence of CCR5⁺ cells inhibited the migration of effector immune response against P. brasiliensis in WT mice. Because CD4⁺ CD25⁺ regulatory T cells express CCR5 on their surface and migrate to the peripheral inflamed tissues through interaction with CCR5 ligands (15, 25–27), we sought the involvement of these cells in the granuloma formation induced during P. brasiliensis infection. We observed significantly less CD4⁺ CD25⁺ T lymphocytes in the pulmonary lesions of infected CCR5⁻/⁻ mice compared with WT mice. Moreover, the CD4⁺ CD25⁺ T lymphocytes found into the granulomas coexpressed CD45RBlow, CTLA-4, CD103, GITR, and Foxp3, which are expressed on Treg (28–30), suggesting that these cells could be involved in the control of the granuloma formation, fungal growth and dissemination, contributing to maintenance of the chronic disease. Indeed, Treg cells are known to regulate the effector immune response, limiting tissue damage and promoting pathogen survival (25, 31, 32). In the current study our data show that, in addition to the lower number of potentially suppressive T cells at the site of infection in CCR5⁻/⁻ mice, those animals are able to control the fungal load, which is enough to impair collateral tissue damage.

According to our results, CCR5 regulates critical aspects of the P. brasiliensis-induced disease. The migration of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ T lymphocytes into the lesions occurred in a CCR5-independent way, because the absence of this receptor did not affect the final number of these cells into lesions. However, the migration of the subpopulation of CD4⁺ CD25⁺ T cells was CCR5 dependent. In accordance, CCR5 is expressed in at least 70% of CD4⁺ CD25⁺ T cells but not in CD4⁺ CD25⁻ T cells, indicating that this receptor can be critical to the suppression of effector immune response against P. brasiliensis. Similarly, its been shown that CCR5 directs the homing of CD4⁺ CD25⁺ T cells to Leishmania major-infected dermal sites, where they suppress CD4⁺ T cells responses (25).

Although Treg cells promote a tight control of the magnitude of the immune response, a detrimental consequence of their presence at the sites of P. brasiliensis-infection could be the maintenance of latent focus. In fact, P. brasiliensis-infected patients present active disease for longer than a decade after the contact with endemic areas (1). Moreover, patients with paracoccidioidomycosis present T cell unresponsiveness to P. brasiliensis Ags (3, 10, 33), and the mechanisms proposed to explain this are associated with the suppressor activity of Treg cells, such as high expression of CTLA-4 (14) and increased TGF-β and IL-10 production (1, 9, 10). In this study we show that the T cell unresponsiveness of spleen cells from infected WT mice was completely abrogated by the additions of Abs against CTLA-4 or GITR to the cultures. Moreover, the pretreatment of WT mice with anti-CD25 or anti-GITR Ab resulted in a significant decrease in the amount of fungus in their lungs, indicating the prominent role of functional Treg cells in the control of the fungus growth. Similarly, the blockade of CTLA-4 in leukocytes from humans infected with P. brasiliensis results in the inhibition of T cell unresponsiveness and increased IFN-γ production (10). Because GITR and CTLA-4 are constitutively expressed in Treg cells at higher levels than in other T cells (29, 34), the blockade of CTLA-4 and activation of GITR by interaction with the Abs may enhance T cell proliferation by attenuating Treg, damping the suppression. Interestingly, the infection with P. brasiliensis does not inhibit T cell proliferation in CCR5⁻/⁻ mice, most likely because, as demonstrated, CCR5⁺ Treg cells are highly more suppressive than CCR5⁻ Treg cells (26). Thus, besides the participation of CCR5 in the migration of Treg cells to the granulomas, our data indicate that CCR5 deficiency results in a drastic reduction of the systemic suppression. We observed that the number of CD4⁺ CD25⁺ T cells expressing Foxp3 was significantly decreased in P. brasiliensis-induced granulomas of CCR5⁻/⁻ mice. Because Foxp3 plays an essential role in the differentiation and suppressor activity of Treg (35–39), including in the induction of genes to IL-2, CTLA-4, and GITR (36), fewer Foxp3⁺ cells in CCR5⁻/⁻ mice certainly results in the observed decreased suppression. However, how CCR5 affects the function of Treg cells remains to be determined.

Another important question is whether or not Treg cells can be differentiated inside the inflammatory environment. Our results indicate that this possibility is plausible. As shown in Fig. 4, only a small percentage of CD4⁺ CD25⁺ T cells in the lesions from WT and CCR5⁻/⁻ mice coexpressed CD45RBlow, CTLA-4, CD103, and GITR, but ~42% of these cells from WT mice also coexpressed Foxp3. Because Foxp3 can bind to the promoters of CD25, CTLA-4, and GITR genes, inducing their transcription (40–43),
these data indicate that Treg cells are being generated in the granulomas and may contribute to the control of the effector response at the site of infection. In fact, the differentiation of conventional CD4+ T cells in induced Treg cells occurs in the presence of TGF-β and IL-10 (44), both cytokines that are highly produced in the site of infection with *P. brasiliensis* (2, 9). Moreover, the CD4+ T cells expressing Foxp3 acquire Treg cell function (39). Overall, nearly 50% of CD4+ T cells in the granulomas (7% of CD4+CD25+ and 42% of CD4+CD25− T cells) are potentially suppressor T cells, resulting in the marked immunosuppression. Indeed, these results demonstrated that a straight equilibrium of the immune response is induced in the granulomas to avoid excessive pulmonary damage.

Previous studies have shown that Tregs migrate to the site of infection and favor pathogen persistence (45). In this study we showed that the migration of CD4+CD25+ T cells to the granulomas is CCR5 dependent and that it inhibits effector memory response, facilitating disease reactivation. In fact, the adoptive transfer of CD4+CD25+ T cells to infected WT mice also resulted in increased fungal load in the granulomas (data not shown), once again suggesting that these cells may acquire the suppressive phenotype dependent on the inflammatory environment (44). In vitro, CD4+CD25+ T cells render naïve CD4+CD25− T cells anergic and suppressive (46). If induced Treg cells can really be generated in vivo in the granulomas, the understanding of this mechanism may provide a way to avoid it, leading in the control of disease. Overall, the results presented in this issue high light some aspects of the maintenance of chronicity during the infection with *P. brasiliensis*. CD4+CD25+ T cells expressing CCR5 and with potent suppressor activity migrated to the granulomas in response to chemokine ligands produced in the lesions. These cells have an important role avoiding exacerbation of immune response and tissue damage. However, our results clearly demonstrated that a lesser number of Treg cells favor higher control of fungal growth and dissemination. Indeed, the recruitment of Treg cells to the lesions may be an efficient mechanism by which the yeast cells escape the effector immune response and persist in the host tissue. Because natural (31) or induced (32) Treg cells can control several parameters of the immune response, such as decreased production of proinflammatory cytokines and inhibition of the T and B lymphocyte proliferation, the development of drugs that blocks CCR5 may constitute potent immunotherapeutic strategies to control this and possibly other chronic diseases.

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Disclosures

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References


