MAJOR ARTICLE

$CD4^+$ Regulatory T Cells in a Cynomolgus Macaque Model of *Mycobacterium tuberculosis* Infection

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Background. *Mycobacterium tuberculosis* infection in humans results in either latent infection or active tuberculosis. We sought to determine whether a higher frequency of regulatory T (T_{reg}) cells predispose an individual toward active disease or whether T_{reg} cells develop in response to active disease.

Methods. In cynomolgus macaques infected with a low dose of *M. tuberculosis*, ~50% develop primary tuberculosis, and ~50% become latently infected. Forty-one animals were monitored for 6–8 months to assess the correlation of the frequency of $Foxp3^+$ cells in peripheral blood and airways with the outcome of infection.

Results. In all animals, the frequency of T_{reg} cells ($CD4^+Foxp3^+$) in peripheral blood rapidly decreased and simultaneously increased in the airways. Latently infected monkeys had a significantly higher frequency of T_{reg} cells in peripheral blood before infection and during early infection, compared with monkeys that developed active disease. Monkeys with active disease experienced increased frequencies of T_{reg} cells among peripheral blood mononuclear cells as they developed disease.

Conclusions. Our data suggest that increased frequencies of T_{reg} cells in active disease occur in response to increased inflammation rather than act as a causative factor in progression to active disease.

Tuberculosis is the leading cause of death by a single infectious agent [1]. Although 5%–10% of infected persons progress to primary tuberculosis, the majority control the infection, are asymptomatic, and are considered to be latently infected [2]. Factors contributing to these outcomes of infection are not well understood. The extended chemotherapy, increase in drug-resistant strains [3], and lack of an effective vaccine [4] make identification of factors that affect the outcome of infection imperative.

Once infected, the host mounts a robust type 1 T helper response and forms a granuloma, which can function to contain the bacilli. Presumably, the immune response must be controlled to limit damage to surrounding tissue. There is likely a fine balance in each granuloma among effector, inflammatory, and regulatory mechanisms. Patients with active tuberculosis produce more anti-inflammatory cytokines, such as interleukin 10 [5, 6] and transforming growth factor β [6, 7], than do those with latent tuberculosis, but whether aberrant immune modulation contributes to the development of active disease is unknown. It has been suggested that the $CD4^+$ T regulatory (T_{reg}) cell is a possible factor in the promotion of active tuberculosis.

$CD4^+$ T cells expressing CD25 are potent inhibitors of autoimmunity [8]; the transcription factor Foxp3 is a defining characteristic of these T_{reg} cells [9]. T_{reg} cells inhibit inflammatory responses and proliferation by producing cytokines, cell-to-cell contact, and inhibiting interleukin 2 [10]. Natural T_{reg} cells are thought to be self-reactive and prevent autoimmunity [11]. T_{reg} cells can also potentiate the persistence of certain pathogens [12–16].

Recent data suggest that T_{reg} cells play a role in the persistence of *M. tuberculosis*. Removal of T_{reg} cells in mice resulted in decreased bacterial burdens in lungs [17, 18], indicating that T_{reg} cells may down-regulate

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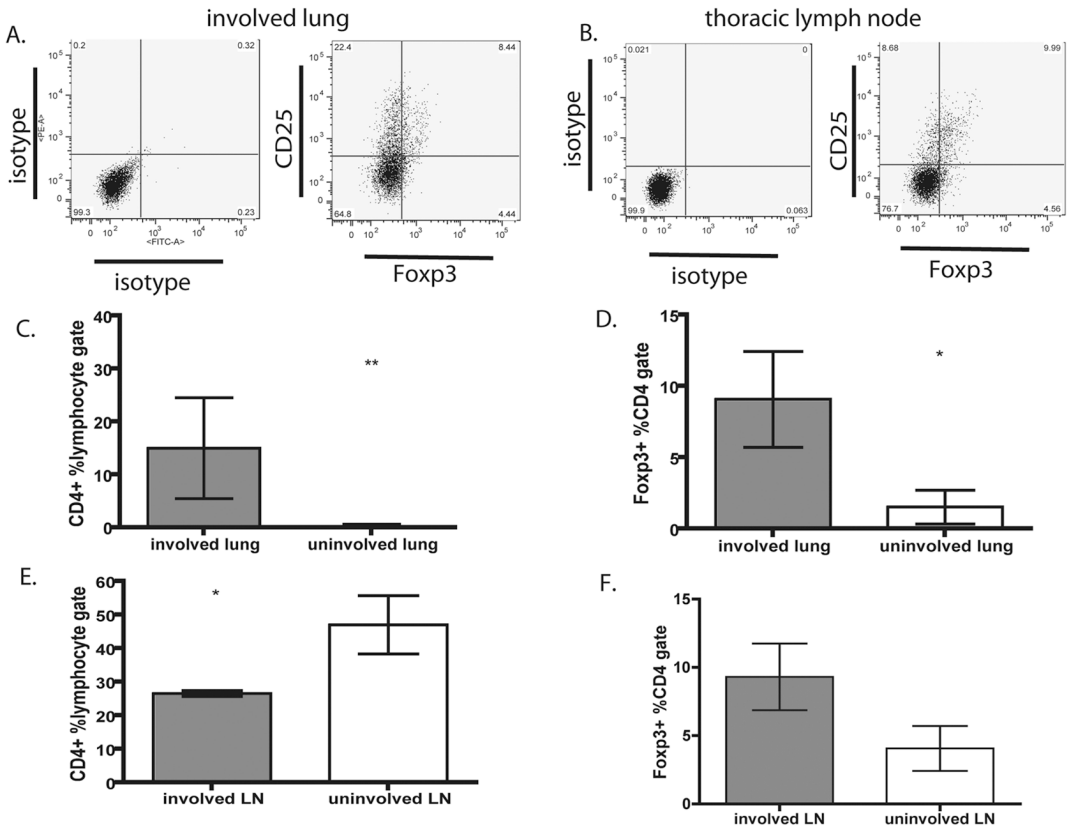

Figure 1. Enrichment of regulatory T (T_{reg}) cells in involved lung tissue and lymph nodes. The frequency of T_{reg} cells ($CD3^+CD4^+Foxp3^+$) was determined by gating on live cells, lymphocytes, and $CD3^+CD4^+$ cells in involved (*Mycobacterium tuberculosis*–positive) and uninvolved (*M. tuberculosis*–negative) lungs and thoracic lymph nodes at necropsy. Representative dot plots of involved lungs (*A*) and lymph nodes (*B*) from an infected monkey are shown. The frequency of $CD4^+$ cells (*C* and *E*) and T_{reg} cells within the $CD4^+$ gate (*D* and *F*) in lungs or lymph nodes were compared within involved and uninvolved tissue. LN, lymph node. $^*P < .05$; $^{**}P < .01$.

M. tuberculosis–specific immune responses. Peripheral blood mononuclear cells (PBMCs) from patients with active tuberculosis had increased frequencies of T_{reg} cells and decreased production of interferon γ (IFN-γ) in response to certain *M. tuberculosis* antigens, compared with PBMCs from patients with latent tuberculosis [19–21]. In vitro depletion of $CD25^+$ cells in patients with active tuberculosis increased *M. tuberculosis*–specific IFN-γ production, suggesting that T_{reg} cells suppress specific responses [19–21]. These studies were unable to differentiate between increased frequencies of T_{reg} cells contributing to the development of active tuberculosis and those occurring in response to inflammation in active disease. Studies of humans with tuberculosis are complicated by difficulties in defining the time of infection, extent of disease, mycobacterial strain, and size of inoculum, which may contribute to the quality of immune responses and disease outcome.

To address whether an increased frequency of T_{reg} cells affects the development of active disease or occurs in response to inflammation caused by active disease, we used a nonhuman primate (NHP) model of *M. tuberculosis* infection. This is the only established model that accurately mimics human latent infection [2]. When cynomolgus macaques are infected with a low dose by means of a bronchoscope, ~50% of animals exhibit no signs of disease despite being tuberculin skin test positive and are considered to be latently infected by 6 months; the rest develop primary tuberculosis [2]. These clinical classifications have been validated by pathological analysis and bacterial numbers at necropsy [22]. Using this model, we assessed the correlation between T_{reg} cells and outcome of infection as well as the dynamics of T_{reg} cells in the periphery and airways.

METHODS

Experimental animals. Cynomolgus macaques (*Macaca fascicularis*; Alpha-Genesis, Covance, and Valley Biosystems) were ≥4 years of age, 3.5–10 kg, housed in a biosafety level 3 facility

[23], and free of tuberculosis or other infections. The University of Pittsburgh School of Medicine Institutional Animal Care and Use Committee approved all procedures and protocols.

Infection of NHPs. Monkeys were infected with ~25 colony-forming units of *M. tuberculosis* strain Erdman by bronchoscopic instillation, as described elsewhere [22, 23]. Colony-forming units were determined in the inoculum by plating on 7H10 agar (Difco Laboratories). Infection of monkeys was confirmed by tuberculin skin test and on the basis of lymphocytic proliferation and IFN-γ production in response to mycobacterial antigens. Infection outcome was independent of age, sex, and weight [22].

PBMC isolation. Blood was collected by percutaneous venipuncture [23]. PBMCs were isolated by means of Percoll gradient (Amersham Bioscience).

Bronchoalveolar lavage cells. Cells were sampled from airways by bronchoalveolar lavage (BAL) [23].

Necropsy of animals. Before necropsy, animals were sedated and then euthanized by means of sodium pentobarbital (Schering-Plough Animal Health), as described elsewhere [23]. A veterinary pathologist conducted all necropsies; tissues and samples were obtained in a sterile fashion [22].

Isolation of cells from necropsy tissue. At necropsy, granulomatous and nongranulomatous lung and lymph node samples were excised [22]. Cell suspensions were obtained by homogenizing tissues in phosphate-buffered saline, using a MediMixer device (BD Biosciences). An aliquot of each suspension was plated for enumeration of *M. tuberculosis* colonies.

Depletion of CD25$^+$ cells from PBMCs. PBMCs were stained with phycoerythrin (PE)–conjugated anti-CD25 (Clone M-A251; BD Pharmingen). CD25$^+$ cells were isolated using anti-PE beads (Miltenyi).

Lymphocyte proliferation assay. For depletion experiments, PBMCs from before and 6 weeks after infection were suspended in AIM V medium (Invitrogen) at 200,000 cells/well in 200 μL. Cells were stimulated with phytohemagglutinin (PHA) (5 μg/mL), culture filtrate protein (CFP) (10 μg/mL; National Institute of Allergy and Infectious Diseases, National Institutes of Health, contract HHSN266200400091C), or medium in triplicate wells for 60 h at 37°C in 5% CO_2; for the final 18 h, [^{3}H]thymidine (1 μCi/well; Amersham) was added. Cells were harvested onto filters, and radioactive incorporation was measured. Data were reported as a stimulation index, defined as the fold increase in counts per minute over unstimulated control.

Flow cytometry. PBMCs, BAL cells, and cells from tissue were surface stained for CD3 (clone SP34; BD Pharmingen), CD4 (clone SK3; BD Biosciences), and CD25 (clone MA251; BD Biosciences) [23]; for CD39 (clone eBioA1; eBioscience), glucocorticoid-induced tumor necrosis factor–related protein (GITR) (clone eBioAITR; eBioscience), and intracellular cytotoxic T lymphocyte antigen 4 (CTLA-4) (clone BNI3; BD Bioscience); and then for Foxp3 (150D [BioLegend], PCH101, or 236A/E7 [eBioscience]) by means of the eBioscience Foxp3 staining kit. Data were collected using a FACSAria cell sorter (BD Biosciences) and analyzed using FlowJo software (version 8.6.3; TreeStar).

Immunofluorescence tissue staining. Antigen retrieval on formalin-fixed deparaffinized tissue sections was performed with High pH Antigen Retrieval buffer (Dako) at 95°C for 20 min. Slides were blocked with 2% goat serum and incubated with polyclonal rabbit anti–human CD3 (Dako) and biotinylated anti–human Foxp3 (clone 236A/E7; eBioscience) antibodies. Isotypes were stained with rabbit anti–human CD3 and Universal Negative Control mouse antibody (Dako) at 4°C overnight. Sections were stained with goat anti-rabbit–Alexa Fluor 488 and Alexa Fluor 546–streptavidin conjugates (Invitrogen), and nuclei were stained with Draq5 (Biostatus Limited). Sections were imaged using a Leica TCS-SL confocal microscope (Leica Microsystems), Z projections were made using ImageJ software (version 1.40g; http://rsb.info.nih.gov/ij/), and brightness and contrast were adjusted using Adobe Photoshop software (version 7; Adobe Systems).

Statistical analysis. To compare 2 groups when data were determined to have a normal distribution, the Student *t* test was used. If data did not have a Gaussian distribution, the Mann-Whitney *U* test was used for unpaired data, and the Wilcox signed rank test was used for paired data. Contiguous data over time was compared by repeated-measures analysis of variance; if significant, pairwise comparison was done using the Tukey-Newman test. Differences with $P < .05$ were considered to be statistically significant.

RESULTS

T_{reg} cells in lung granulomas and draining lymph nodes. The NHP model is the only animal model that mimics the spectrum of *M. tuberculosis* infection (latent and active disease) seen in humans [22]. Infection outcomes are determined by clinical criteria, and severity of disease is quantified at necropsy [22].

Cells from involved lungs and thoracic lymph nodes at necropsy were stained for CD3, CD4, CD25, and Foxp3 (Figure 1*A* and 1*B*). In tissues, not all Foxp3$^+$ cells express CD25, and not all CD25$^+$ cells express Foxp3. We further characterized CD4$^+$Foxp3$^+$ cells among PBMCs and showed that they expressed the T_{reg} cell–associated cell-surface markers CD25, CD39, CTLA-4, and GITR to varying degrees (Figure 2) and did not produce cytokines in response to *M. tuberculosis* antigens (data not shown). Thus, we used CD3$^+$CD4$^+$Foxp3$^+$ to define the T_{reg} cell population.

To address whether T_{reg} cells were preferentially localized to

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 2. Cell-surface markers on regulatory T (T_{reg}) cells.

areas of lungs containing bacilli, tissue homogenates were divided into *M. tuberculosis*–positive (involved) and *M. tuberculosis*–negative (uninvolved) categories on the basis of whether *M. tuberculosis* was cultured from the sample. Involved lung tissue (granulomas) contained a significantly higher frequency of both total $CD4^+$ T cells (Figure 1*C*) and T_{reg} cells (Figure 1*D*) than did uninvolved lungs. Similar to findings of murine experiments [18], lymphocytes localized to pulmonary sites that contained *M. tuberculosis*. Interestingly, when thoracic lymph nodes from infected NHPs were divided into *M. tuberculosis*–positive and *M. tuberculosis*–negative samples, involved lymph nodes had significantly fewer $CD4^+$ T cells than did uninvolved lymph nodes (Figure 1*E*), but there was a trend toward increased numbers of T_{reg} cells within the $CD4^+$ T cell subset (Figure 1*F*). It may be that fewer T cells are present in involved lymph nodes because of effacement by granulomas with caseous necrosis.

Immunofluorescent staining demonstrated that $CD3^+Foxp3^+$ T_{reg} cells were abundant within the lymphocyte cuff of granulomatous lungs (Figure 3) but were rare in uninvolved lungs. These data support the concept that T_{reg} cells preferentially localize to involved tissue (granulomas) and are proportionally increased in thoracic lymph nodes.

Migration of T_{reg} cells from peripheral blood to airways during early* M. tuberculosis *infection. We analyzed PBMCs from 41 NHPs (infected for other studies) for 6 months after inoculation. The frequency of T_{reg} cells decreased dramatically within the first 2 weeks and was significantly lower than preinfection frequencies 8–12 weeks after infection (Figure 4*A*), whereas $CD4^+$ T cell frequencies were unchanged (Figure 4*C*). By 16 weeks, the T_{reg} cell frequencies in all monkeys returned to baseline levels ($P > .05$ for 0 vs 16 weeks for both active and latent infection). However, as infection progressed monkeys with active disease had increasing frequencies of T_{reg} cells, compared with the steady-state or declining levels found in latently infected animals.

The reduction in peripheral T_{reg} cell frequencies during early infection suggested that T_{reg} cells were migrating to the lungs. BAL is the only relatively noninvasive way to serially sample the pulmonary environment. For 6 months, BAL cells were obtained monthly from 17 macaques and stained for T_{reg} cells (Figure 4*B*). By 4 weeks after infection, the frequency of T_{reg} cells increased in airways, corresponding to the reduction in the periphery. After an initial influx of T_{reg} cells into airways, the frequency of T_{reg} cells fluctuated until 16 weeks after infection, when levels of both T_{reg} cells and $CD4^+$ T cells (Figure 4*B* and 4*D*) increased.

Correlation between preinfection levels of T_{reg} cells among PBMCs and clinical outcome. Of 41 macaques monitored, 22 developed active disease and 19 had latent infections. Surprisingly, the monkeys that developed latent infection had significantly higher frequencies of T_{reg} cells among PBMCs before infection, compared with the monkeys that developed active disease ($P = .012$) (Figure 4*E*). Although all NHPs exhibited an initial decrease in T_{reg} cells, latently infected animals maintained higher levels of $Foxp3^+$ cells among PBMCs than did those with active disease 8 weeks after infection (Figure 4*E*). By 16 weeks, frequencies of T_{reg} cells returned to preinfection levels in all animals. Animals that developed latent infection maintained preinfection T_{reg} cell frequencies, whereas $Foxp3^+$ cells continued to increase up to 28 weeks after infection in those developing active disease. NHPs that developed latent infection demonstrated a trend toward a higher frequency of T_{reg} cells among BAL cells 4 weeks after infection, compared with those that would develop active disease (Figure 4*F*), although this was not statistically significant.

Effect of $Foxp3^+$ cells on proliferative responses in peripheral blood. To determine whether an increased frequency of T_{reg} cells correlated with a reduced proliferative response, PBMCs were stimulated with mitogen PHA or mycobacterial CFP. Before infection, PBMCs from animals that would develop latent infection demonstrated significantly less proliferation in response to PHA than did PBMCs from animals that would develop active tuberculosis (Figure 5*A*), correlating with the presence of more peripheral T_{reg} cells in monkeys with latent infection (Figure 5*E*). By 6 weeks after infection, the latent group exhibited a trend toward decreased proliferation in response to both PHA (Figure 5*A*) and CFP (Figure 5*B*). T_{reg} cells were not measured at 6 weeks, but the frequency of T_{reg} cells was significantly increased 8 weeks after infection in latently infected monkeys, compared with that in monkeys that would develop active disease. All NHPs showed increased proliferation to both stimuli by 6 weeks after infection and had significantly lower frequencies of T_{reg} cells by 8 weeks after infection relative to preinfection frequencies. These data support an association between decreased proliferation and the presence of more T_{reg} cells, suggesting that T_{reg} cells may limit T cell proliferation.

We tested whether depletion of T_{reg} cells would reverse this reduced response. No convenient method for depleting $Foxp3^+$ cells from primates exists, so $CD25^+$ cells were depleted from PBMCs (Figure 5*C*). Depletion of $CD25^+$ cells resulted in increased proliferation in 7 of 10 animals for both stimuli (Figure 5*D* and 5*E*).

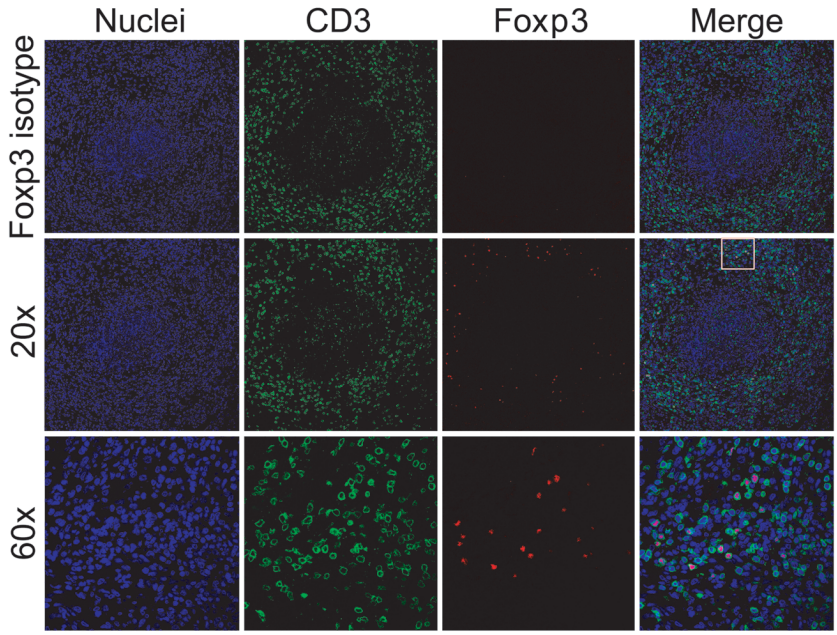

Figure 3. Regulatory T (T_{reg}) cells in granulomas. Paraffin-embedded granulomatous lung tissue was immunofluorescently stained for nuclei (*blue*), CD3 (*green*), and Foxp3 (*red*). The 60× section is an enlargement of the box indicated on the merged image for the 20× section.

DISCUSSION

The immune response against *M. tuberculosis* is a tightly controlled balance between sufficient inflammation to limit *M. tuberculosis* growth and regulatory factors that prevent damage to surrounding tissue. We sought to determine whether the frequency of T_{reg} cells in the blood and airways was associated with the outcome of infection in a macaque model of tuberculosis. We hypothesized that if T_{reg} cells were involved in modulating the immune response to *M. tuberculosis* and limiting damage to tissue, T_{reg} cells would be found at the site of infection. If T_{reg} cells contributed to the development of active disease, we would expect the following: first, animals that develop primary tuberculosis would have a higher frequency of T_{reg} cells in the blood and airways before or during early infection, compared with those that develop latent infection; second, an increased frequency of T_{reg} cells would correspond with decreased proliferation when PBMCs were stimulated with antigens. Conversely, if an increased frequency of T_{reg} cells was not a causative factor of active tuberculosis but rather a response to inflammation, a disparity in T_{reg} cells would become apparent between the 2 groups when those with active disease failed to control bacterial growth.

In healthy individuals, *M. tuberculosis* infection is predominantly localized to the lungs and thoracic lymph nodes. Bacilli and granulomas are most commonly found in lung parenchyma rather than airways, making the sampling of involved tissues difficult. In humans, the timing of infection as well as the dose and strain of inoculum are unknown, and samples from before infection are not generally available for comparison. Because these factors can contribute to the quality of the immune response and infection outcome, we used the macaque model of tuberculosis. This model resembles human tuberculosis in disease outcome and pathology, and inoculum size, strain, and timing of infection can be controlled [23]. PBMCs and BAL cells can be obtained frequently, and tissue samples are available at necropsy.

Classically, T_{reg} cells were defined as $CD4^+CD25^{hi}$ cells. However, CD25 is also found on activated effector T cells, and where immune stimulation is continuous (eg, chronic infection) one is unable to distinguish T_{reg} cells from effector cell populations with this single marker. The transcription factor Foxp3 denotes a T_{reg} cell phenotype [9], and transfection of constitutively active Foxp3 in human T cells confers the T_{reg} cell phenotype [26]. We defined our T_{reg} cell population as $CD3^+CD4^+Foxp3^+$ but used additional markers to confirm this. In blood we are most likely measuring $Foxp3^+$ natural T_{reg} cells [10]; cells found within airways and tissues may be primed before migrating to the site of infection. We have shown here that T_{reg} cells are present in involved lungs and lymph nodes and localize to the lymphocyte cuff of granulomas, suggesting that they may modulate immune responses within the granuloma.

We examined the association between T_{reg} cells in the blood and airways of infected monkeys and the development of active disease and found surprising results. Although the literature

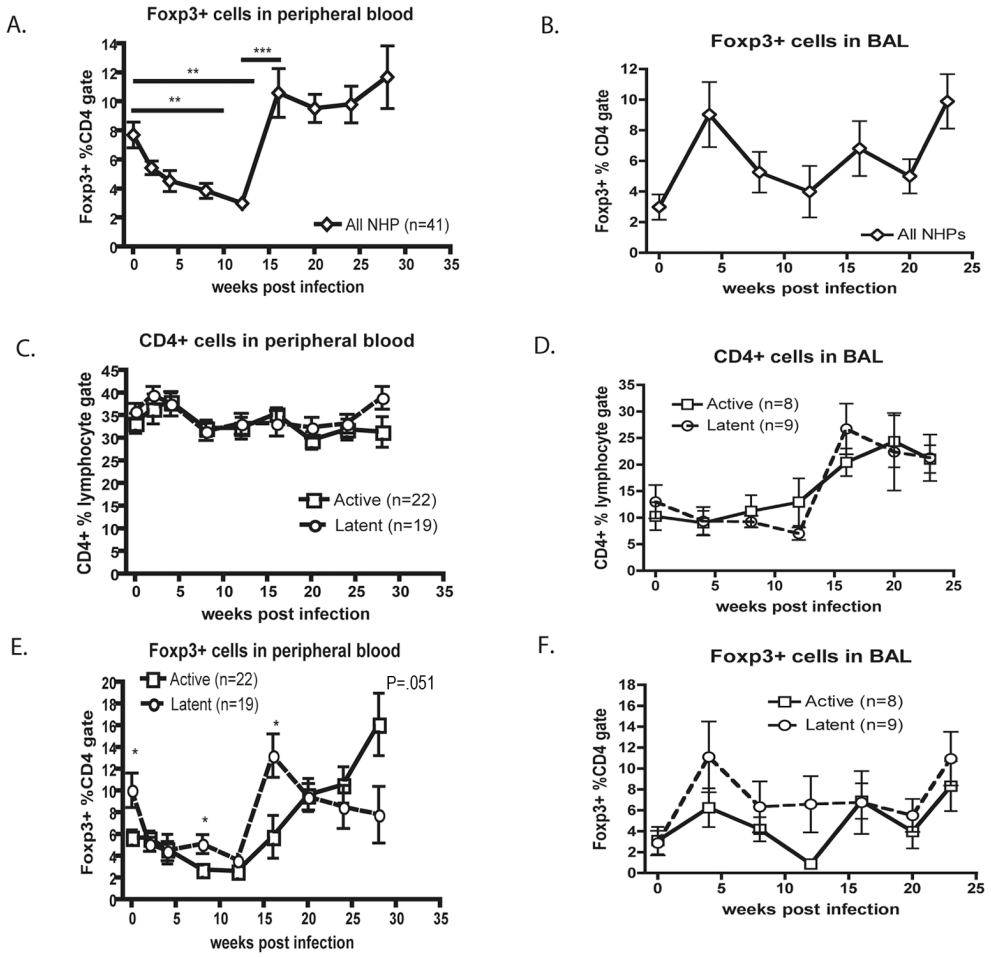

Figure 4. Regulatory T (T_{reg}) cells among peripheral blood mononuclear cells (PBMCs) and in airways during *Mycobacterium tuberculosis* infection. The frequency of T_{reg} cells ($CD3^+CD4^+Foxp3^+$) among PBMCs during *M. tuberculosis* infection ($n = 41$ monkeys) was determined by gating on live cells, lymphocytes, and $CD3^+CD4^+$ cells (*A*). Bronchoalveolar lavage (BAL) cells from infected nonhuman primates (NHPs) ($n = 17$) were stained and gated as described above (*B*). $P < .05$ was considered significant. PBMCs and BAL cells were stained and compared between groups with active disease (*squares*) and latent infection (*circles*). The frequency of $CD4^+$ T cells within the lymphocyte gate (*C* and *D*) or of $Foxp3^+$ cells within the $CD4^+$ gate (*E* and *F*) for blood (*C* and *E*) and airways (*B* and *D*) are shown. Statistical significance over time was determined by repeated-measure analysis of variance with a Tukey-Newman posttest (*A*); the Mann-Whitney *U* test was used to determined significance between each group at each time point (*C–F*). $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

[20, 21, 27–29] supports an association between active disease and increased frequencies of T_{reg} cells in the blood of humans, we found that monkeys that developed latent infection had a significantly higher frequency of T_{reg} cells among PBMCs before and during early infection, compared with those that developed active tuberculosis. The increased frequency of T_{reg} cells in monkeys that became latently infected correlated with decreased proliferation when exposed to mitogen before *M. tuberculosis* infection. When $CD25^+$ cells were depleted from PBMCs, several NHPs exhibited a modest increase in proliferation when stimulated with CFP and PHA. Our findings do not support the hypothesis that more T_{reg} cells and a less vigorous lymphoproliferative response during the initial phase of *M. tuberculosis* infection would predispose an individual to the development of primary tuberculosis. On the contrary, our data indicate that the presence of more T_{reg} cells before infection correlates with a better infection outcome.

These data led us to ask whether the increased frequency of T_{reg} cells associated with active disease is a response to increased inflammation. Regardless of the outcome of infection, the frequency of $Foxp3^+$ cells dramatically decreased in blood during the first 8 weeks. This early reduction in $Foxp3^+$ cells suggests that these cells are poised to sense inflammation and migrate quickly to the site of infection, mirroring a decline reported in

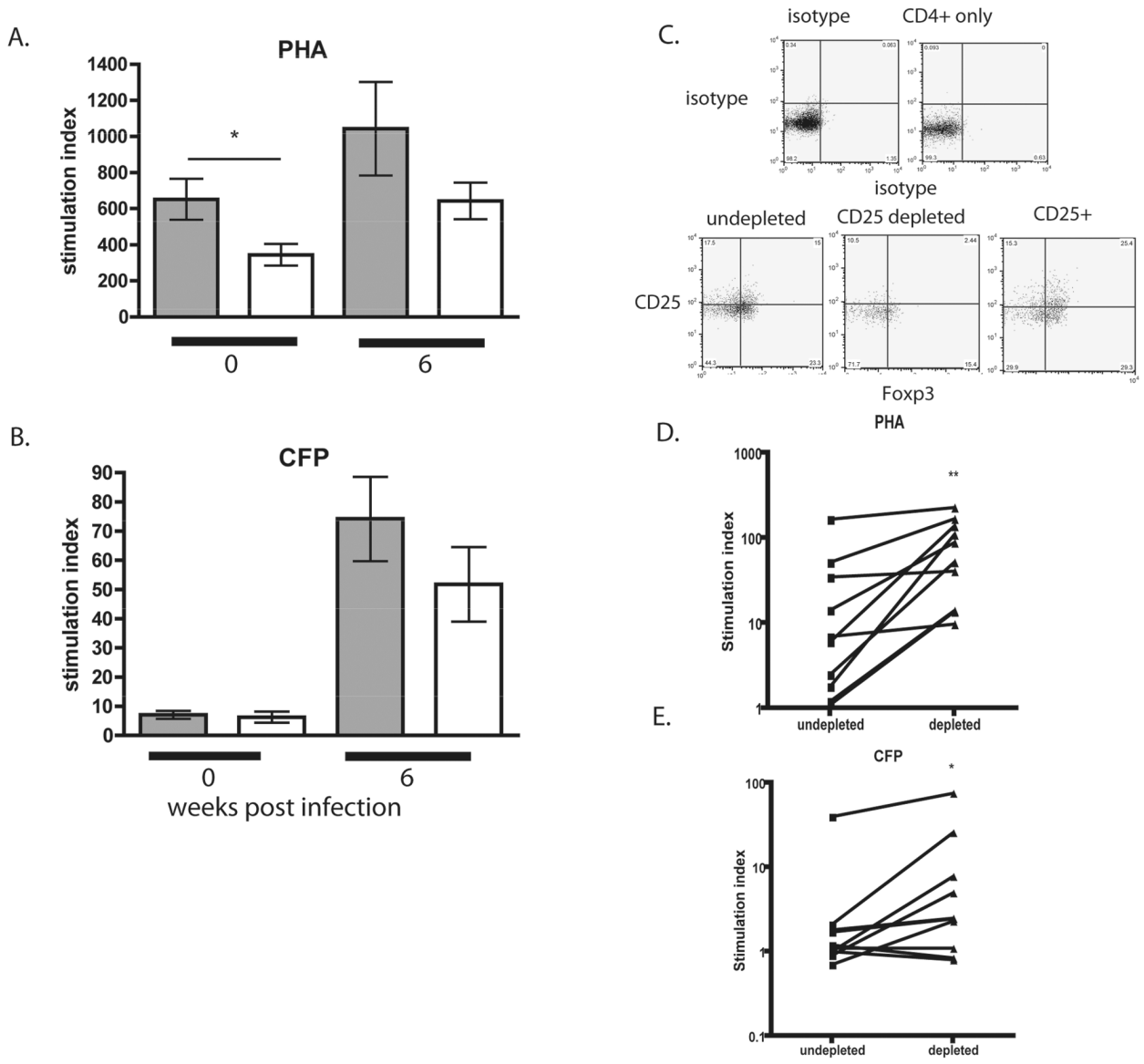

Figure 5. Inhibition of peripheral blood mononuclear cell (PBMC) proliferation by regulatory T (T_{reg}) cells. PBMCs obtained at 0 and 6 weeks after infection from animals that would develop active disease (*shaded bars*; $n = 22$) or latent infection (*white bars*; $n = 19$) were stimulated with phytohemagglutinin (PHA) (*A*) or culture filtrate protein (CFP) (*B*), and proliferation was measured. Data are reported as a stimulation index. Statistical significance was determined by the Mann-Whitney *U* test. $CD25^+$ cells were depleted from PBMCs ($n = 10$) (*C*) and stimulated as described above. Comparison of the PBMC proliferative response to PHA (*D*) and CFP (*E*) before and after CD25 depletion is reported as a stimulation index. Statistical significance was determined by the Wilcox signed rank test. $^*P < .05$; $^{**}P < .01$.

the peripheral blood of healthy human contacts in the Gambia [28]. At the same time, the frequency of T_{reg} cells increased in airways, supporting the hypothesis that these cells migrate to the lungs. In vitro experiments on lymphocytes from both humans and mice indicate that natural T_{reg} cells change their chemokine receptor phenotype more quickly than do naive resting T cells in the periphery [30]. This may be a mechanism for rapidly increasing the number of T_{reg} cells at the infection site for expansion during the initial burst of inflammation to protect surrounding tissue from damage. Although the frequencies of T_{reg} cells among PBMCs in all monkeys returned to baseline levels by 16 weeks after infection, those with latent infection remained steady, whereas T_{reg} cells in NHPs with active disease began to increase.

In summary, these data indicate that the frequency of T_{reg} cells rapidly decreases in peripheral blood after infection and are maintained at low levels in the periphery for ~4 months. When bacterial growth is under control (ie, during latent in-

fection), peripheral levels of T_{reg} cells return to preinfection levels. However, when bacterial growth is not contained and active disease develops, the population of T_{reg} cells continues to increase, perhaps acting to dampen peripheral inflammation as a protective mechanism. On the basis of this concept, we propose a model in which T_{reg} cells in peripheral blood act as important regulators poised to protect uninvolved tissues from potentially damaging antimycobacterial immune responses. On infection, T_{reg} cells migrate to the lungs and draining lymph nodes to protect "healthy" tissue and limit inflammation. T_{reg} cells are retained within granulomas and involved lymph nodes, along with effector T cells. Provided that the T cell response is sufficient to contain *M. tuberculosis* infection, T_{reg} cells control damage to surrounding tissue by limiting the proliferation of effector T cells in granulomas. In latent infection, peripheral responses are limited as a result of containment of antigen within granulomas and control of bacterial growth. However, if the immune response is insufficient to control bacterial replication, the antigenic burden increases, resulting in increased inflammation. In this instance, T_{reg} cells in the periphery may limit the inflammation associated with active disease. The increased T_{reg} cell frequency observed in the blood of people with active tuberculosis is likely a response to inflammation and bacterial burden, not a predetermining or contributing factor to active disease, at least during the initial stages of infection.

Control of *M. tuberculosis* infection is the result of a dynamic balance between inflammation and immune regulation maintained by the host. Here, we explored the role played by T_{reg} cells in the dynamics of this response during the initial stages of infection. An increased understanding of how these cells function at the site of infection as well as the signals responsible for an early exodus from the periphery will provide valuable information about the microenvironments needed to maintain control of *M. tuberculosis* infection.

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