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Premature Induction of an Immunosuppressive Regulatory T Cell Response during Acute Simian Immunodeficiency Virus Infection

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Abstract

Here we report the results of an investigation into the possibility that one mechanism responsible for the establishment of persistent human immunodeficiency virus infection is an early regulatory T (T_{reg}) cell response that blunts virus-specific responses. Using the simian immunodeficiency virus (SIV)–infected rhesus macaque model, we show that, indeed, viral replication and immune activation in lymphatic tissue drive a premature immunosuppressive response, with dramatic increases in the frequencies of $CD4^+CD25^+FOXP3^+$ T_{reg} cells, transforming growth factor–$\beta1^+$ cells, interleukin–10^+ cells, and indoleamine 2,3-dioxygenase$^+CD3^+$ cells.When we compared SIV infection with rhesus cytomegalovirus (RhCMV) infection, we found that the frequency of T_{reg} cells paralleled the magnitude of immune activation during both infections but that the magnitude of immune activation and of the T_{reg} cell response were lower and peaked much later during RhCMV infection. Importantly, the frequency of T_{reg} cells inversely correlated with the magnitude of the SIV-specific cytotoxic T lymphocyte response. We conclude that an early T_{reg} cell response during acute SIV infection may contribute to viral persistence by prematurely limiting the antiviral immune response before infection is cleared.

Generally, viral infections activate the immune system, which generates large numbers of immune cells, cytokines, and other effectors that are needed to terminate the infection by eradicating infected cells and virus [1]. This initial expansion stage is followed by a contraction stage, during which, it is currently thought, regulatory T (T_{reg}) cells play a prominent role in maintaining the delicate balance between an immune response that is sufficiently robust to clear the infection and the immunopathological consequences of sustained immune activation and inflammation [2, 3].

Similarly, during the early stages of HIV and simian immunodeficiency virus (SIV) infection, viral replication in lymphatic tissue activates the immune system, and viral levels decrease in concert with increasing numbers of virus-specific cytotoxic T lymphocytes (CTLs) [4, 5]. However, HIV and SIV infections are typically persistent, with viral replication continuing because of the incomplete clearance of productively infected cells. Among the many mechanisms that contribute to the inability of CTLs to eliminate all of the productively infected cells, there is increasing evidence (particularly from the SIV-infected

rhesus macaque [RM] model) indicating that the CTL response is too little, too late [6, 7]. Because several studies have shown that the magnitude of effector T cell immune responses to acute viral infections can be limited by Treg cells [8–10], and because T_{reg} cells may enable some pathogens to establish chronic infection by blunting the immune response, we hypothesized that an immunosuppressive T_{reg} cell response might be one mechanism that delays and limits the CTL response during acute lentiviral infection, resulting in virus-specific responses of insufficient magnitude to clear these infections. In this hypothesis, the immunosuppressive T_{reg} cell response is untimely or premature in that it kicks in to regulate the massive immune activation and inflammation in lymphatic tissue so quickly that it dampens the CTL response before infected cells have been completely eliminated. We tested this hypothesis using the SIV-infected RM model, in which we could examine, during the acute stage of infection, the relationship between viral replication and immune defense and immunoregulatory responses. We show that, in lymphatic tissue, T_{reg} cells and immunosuppressive mediators are induced so early in response to immune activation that SIV-specific CTL responses are constrained at just the time when they are most needed to clear the infection.

Materials and Methods

Animals and experimental SIV and rhesus cytomegalovirus (RhCMV) infections. To characterize the early T_{reg} cell response in lymphatic tissue, we studied tissues from 3 different experiments in SIV-infected RMs and from a separate study of primary RhCMV infection. In the first study, lymph node (LN) tissues were obtained at necropsy from 4 adult female Indian RMs 1–12 days after atraumatic intravaginal (ivag) infection, as described elsewhere [11]. In the second study, LN tissues were obtained at necropsy from 5 adult female RMs 2 h–28 days after ivag inoculation of $TCID_{50}$ of SIV_{mac251} 2×10^5 or SIV_{mac239} [12]. In the third study, which was longitudinal, LN biopsy samples were obtained from 4 adult female RMs before and 7 and 28 days after intravenous (iv) infusion of 1 MID_{50} of SIV_{mac239}.

In the fourth study, 4 CMV-negative juvenile RMs were subcutaneously inoculated with 1×10^6 pfu of CMV strain 68.1 at 2 sites. LN biopsy samples were obtained 21 days before and 7, 14 (2 RMs), 28 (2 RMs), and 70 days after infection. Tissues were snap-frozen in liquid nitrogen and embedded in OTC for long-term storage. All animal studies were approved by the appropriate institutional review board.

Immunohistochemical staining, in situ hybridization, quantitative image analysis, and tetramer staining. Single immunohistochemical staining, in situ hybridization, and quantitative image analysis were performed as described elsewhere [11, 13]. Antibodies used were rabbit and goat anti–transforming growth factor (TGF)–β1, goat anti-FOXP3, mouse and rabbit anti–interleukin (IL)–10 (all from Santa Cruz Biotech), rabbit anti-FOXP3 (gift from A. Y. Rudensky, M. A. Gavin, and P. deRoos, Howard Hughes Medical Institute, University of Washington), rabbit anti-CD3 (DakoCytomation), mouse anti– indoleamine 2,3-dioxygenase (IDO; Chemicon International), mouse anti-CD4 (Novocastra Laboratories), and mouse anti-CD25 (NeoMarkers). Anti-FOXP3 (Santa Cruz Biotech) specificity was determined by incubating the antibody with the FOXP3 peptide used as the immunogen at a peptide:antibody ratio of 20:1 before incubation with tissue sections. Although the peptide-blocking treatment did not completely abrogate FOXP3 staining in all tissue samples, it did dramatically reduce the detection of FOXP3 by use of antibody (data not shown). Dual-label immunofluorescence confocal microscopy was performed as described elsewhere [13]. Tetramer analysis on CTLs from lymphatic tissue was performed as described elsewhere [14].

Statistical methods. For quantitative image-analysis data, the mean for each RM was derived from at least 18 randomly acquired high-power images. The graphs show either individual means (for groups with $n = 1$) or group means (derived by averaging all individual RM means ± SEs). To assess increases in the frequency of TGF-$\beta1^+$ and $FOXP3^+$ cells during infection, we fit linear models for each protein, pooling all of the data from the RMs into 1 model. Linearity was assessed graphically for those RMs with multiple measurements and was also more formally assessed by incorporating a quadratic termin the models; both of these strategies suggested that the linearity of change over time was reasonable. In a linear model that allowed for correlation within measurements from the same RM, model parameters were estimated using maximum likelihood (using S-plus [version 3.1, release 1], a statistical program from Math-Soft). To test whether the magnitude of the responses was increasing, we conducted a hypothesis test on the slope parameter in the linear model. This test indicated that the changes observed for both TGF-$\beta1^+$ cells and $FOXP3^+$ cells were statistically significant ($P < .0001$, for both). To test for an association between the frequency of T_{reg} cells and the percentage of SL8 tetramer$^+$CD8$^+$ T cells, a random-effects model was used (using restricted maximum likelihood, as implemented in the varcomp function in S-plus). This model allows for testing an association when multiple measurements are taken from individual subjects and one wants to combine these measurements. This model was also used to investigate the effect of LN location on the association; however, because no such effect was found, the data were aggregated.

Results

In recent experiments investigating the CTL response to SIV after ivag infection, we found that the response to immunodominant epitopes in SIV was too late in the sense that it was not detectable until several days after the peak of virus replication in lymphatic tissue [7]. Moreover, the

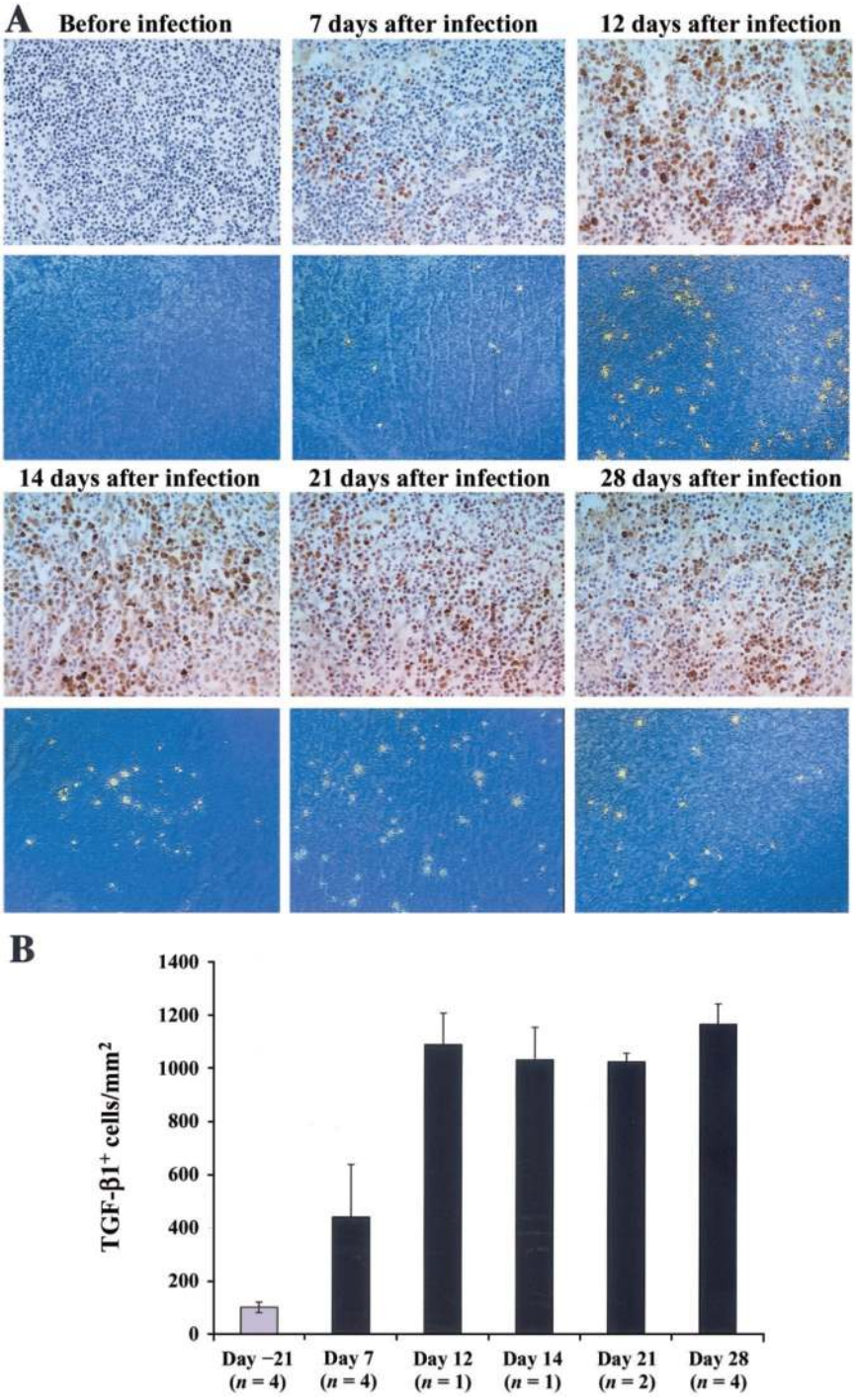

Figure 1. Dramatic increase in transforming growth factor (TGF)–β1 expression in the paracortical T cell zones of lymph nodes during acute simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs). *A,* Images showing a significant increase in the frequency of TGF-β1$^+$ cells *(upper panels)* in the paracortical T cell zones of secondary lymphatic tissue shortly after both intravenous (iv) and intravaginal (ivag) SIV infection (TGF-β1$^+$ cells are indicated by brown staining; original magnification, ×400). Productively infected cells *(lower panels)* were detected by in situ hybridization within 7 days of iv infection, and the level peaked at 28 days after iv infection, whereas the level of productively infected cells peaked at 12 days and remained high until 21 days after ivag infection (original magnification, ×100). *B,* Enumeration of the frequency of TGF-β1$^+$ cells. TGF-β1$^+$ cells were enumerated in at least 18 randomly acquired high-power images for each RM, and the graphs show either individual means (for groups with $n = 1$) or group means (derived by averaging all individual RM means ±SEs); the no. of RMs at each time point is shown in parentheses. To determine whether the increase in TGF-β1 expression was statistically significant over time, the data were fit into a model that incorporated data from all RMs and time points, as outlined in Materials and Methods. This model indicated that the change observed for TGF-β1 expression was statistically significant ($P < .0001$).

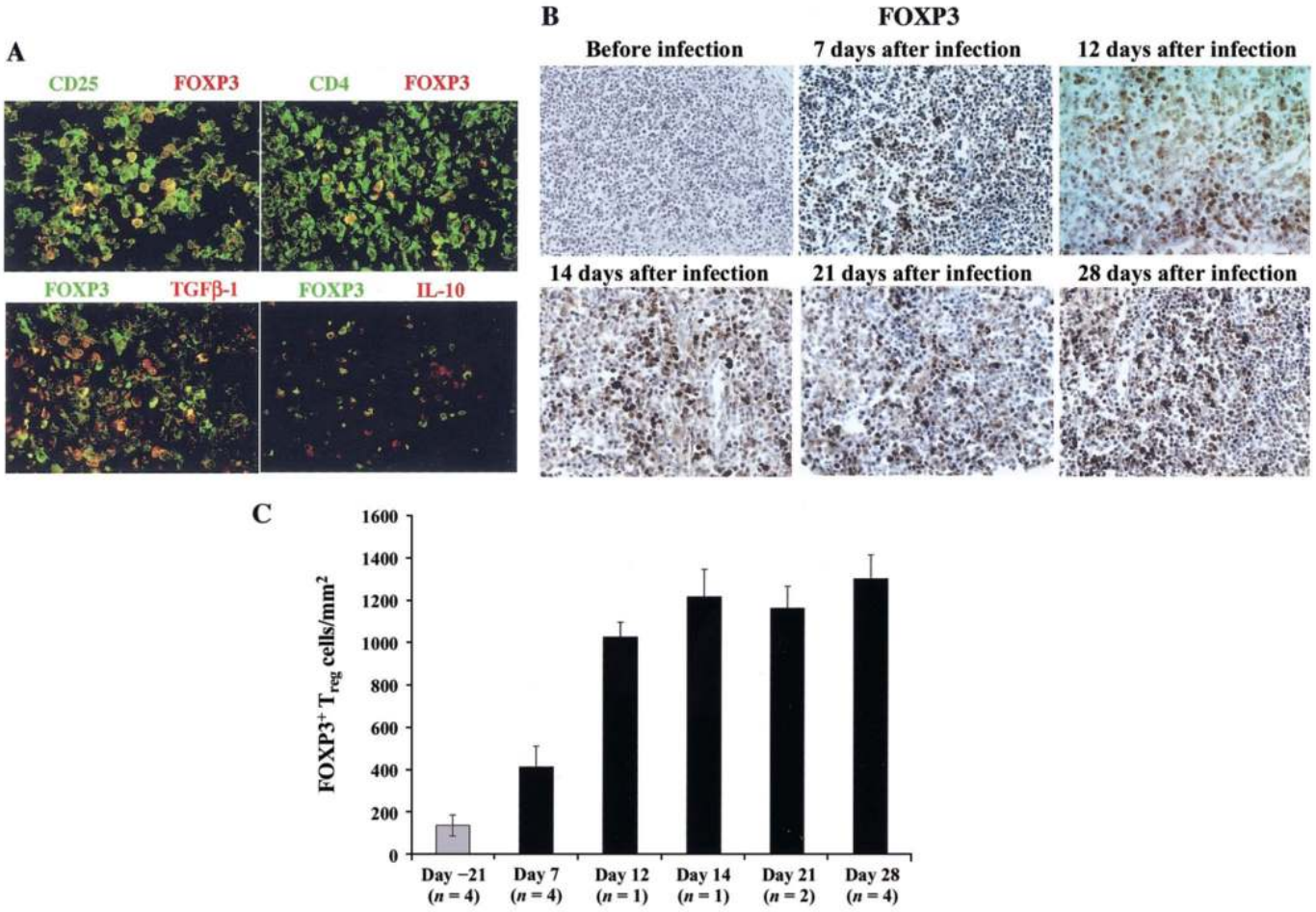

Figure 2. Dramatic increase in FOXP3$^+$ regulatory T (T_{reg}) cells in the paracortical T cell zones of lymph nodes during acute simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs). *A*, Expression of FOXP3 by CD4$^+$CD25$^+$ T_{reg} cells, phenotypically identifying them as natural T_{reg} cells. The majority of FOXP3$^+$ cells were transforming growth factor–β1 and interleukin–10 positive (original magnification, ×640). *B*, Rapid increase in the frequency of FOXP3$^+$ T_{reg} cells (brown staining) in the paracortical T cell zones of secondary lymphatic tissue after both intravenous and intravaginal SIV infection (original magnification, ×400). *C*, Frequency of FOXP3$^+$ T_{reg} cells. The frequency of FOXP3$^+$ T_{reg} cells was determined and plotted as described in figure 1. To determine whether the increase in FOXP3 expression was statistically significant over time, the data were fit into a model that incorporated data from all RMs and time points, as outlined in Materials and Methods. This model indicated that the change observed for FOXP3 expression was statistically significant ($P < .0001$).

response was too little in the sense that the number of SIV-specific CTLs decreased from peak levels even while productively infected cells remained in lymphatic tissue. To investigate the possibility that one reason for the failure to clear infection was an early immunosuppressive response that prematurely limited the immune response before the infection had been cleared, we examined lymphatic tissue obtained during acute SIV infection, to evaluate the relationship that might exist between the timing and magnitude of immunosuppressive responses and viral replication.

Induction of TGF-β1$^+$ cells during acute SIV infection. We began by assaying TGF-β1, a potent immunosuppressive soluble mediator and major effector molecule used by CD4$^+$CD25$^+$ T_{reg} cells. Analyzing the period from the onset of viral replication in lymphatic tissue (~7 days after infection), through the peak (10–14 days) and subsequent decrease of replication, and to the time of persistent infection (28 days), during which sustained levels of replication are characteristic [12], we quantified TGF-β1 expression in pooled LN biopsy samples from separate studies of acute infection after inoculation of SIV by iv and ivag routes.

In LN samples obtained sequentially from the same RMs that had been iv infected, there were few TGF-β1$^+$ cells before infection, but, by 7 days after infection, the frequencies of TGF-β1$^+$ cells in the paracortical T cell zones had increased >4-fold, and, by 28 days after infection, they had increased >11-fold (figure 1*A* and 1*B*). There was a similar rapid increase in LN samples obtained at necropsy from RMs 12, 14, and 21 days after ivag infection—the frequencies of TGF-β1$^+$ cells were >10- fold higher in infected RMs than in uninfected control RMs (figure 1*A* and 1*B*).

The rapidity of the TGF-β1 response was consistent with the hypothesis that the early peak of viral replication

Table 1. In vivo phenotype of regulatory T (T_{reg}) cells expressed in lymph nodes from simian immunodeficiency virus–infected rhesus macaques 14 days after infection.

Cell type	FOXP3$^+$	TGF-β1$^+$	IL-10$^+$	IDO$^+$
Natural T_{reg} cells (FOXP3$^+$)	…	67.5 ± 13.8 (36.1–93.9)	58.3 ± 7.5 (46.8–70.6)	83.0 ± 11.9 (62.2–100)
Th3 cells (TGF-β1$^+$)	76.2 ± 16.7 (53.2–97.2)	…	ND	ND
Type 1 T_{reg} cells (IL-10$^+$)	67.9 ± 9.2 (51.5–89.4)	ND	…	ND

Data are the mean ± SD (range) percentage of cells in the left column expressing the antigen listed at top. Eighteen randomly acquired high-power confocal images were obtained and processed in Photoshop, and quantitative image analysis was performed to determine the colocalization of the 2 different antigens of interest. IDO, indoleamine 2,3-dioxygenase; IL, interleukin; ND, not determined; TGF, transforming growth factor.

was driving this immunoregulatory response. Indeed, we found that the peak of the TGF-β1 response occurred soon after the peak of virus replication, from days 10 to 14. More importantly, the frequency of TGF-β1$^+$ cells remained high when there were still significant numbers of productively infected cells in lymphatic tissue (figure 1*A*, lower panels), a finding that is consistent with the hypothesis that this immunosuppressive response is premature with respect to the host defense's attempt to eradicate the infection.

Induction of CD4$^+$CD25$^+$FOXP3$^+$ T_{reg} cells during acute SIV infection. We next established that the TGF-β1 response is just one manifestation of an early general immunosuppressive response composed of T_{reg} cells and potent immunosuppressive mediators. We quantified the T_{reg} cell response by staining tissue sections for the transcription factor that controls the generation of natural T_{reg} cells, FOXP3 [15, 16], and for CD4 and CD25. The majority of the FOXP3$^+$ cells (mean ± SD, 93.4% ± 4.2%) that accumulated rapidly in the paracortical T cell zones of secondary lymphatic tissue shortly after SIV infection were CD4$^+$CD25$^+$ T_{reg} cells (figure 2*A*). In addition, we found that most of the TGF-β1$^+$ cells (mean ± SD, 76.2% ± 6.7%) also coexpressed FOXP3 and were CD4$^+$CD25$^+$ T_{reg} cells (figure 2*A* and table 1), with the rest presumably being Th3 cells. Importantly, the increased frequency of FOXP3$^+$ T_{reg} cells in secondary lymphatic tissue (figure 2*B* and 2*C*) was approximately equal to the increased frequency of TGF-β1$^+$ cells. Because human T_{reg} cells are highly susceptible to HIV infection in vitro [17], we reasoned that the dramatic increase in the frequency of T_{reg} cells reported here could provide a highly susceptible population of target cells for further SIV propagation, and we investigated the possibility that the increase in the frequency of T_{reg} cells was limited by productive infection. Surprisingly, at the peak of viral replication (10–14 days after infection), we found that, on average, only 13% of FOXP3$^+$ T_{reg} cells in secondary lymphatic tissue were infected (SIV RNA$^+$; data not shown), indicating that, in vivo, productive infection of T_{reg} cells does not greatly contribute to limiting the expansion of this population.

Induction of IL-10$^+$ and IDO$^+$CD3$^+$ T_{reg} cells during acute SIV infection. Because IL-10 has been shown to be a potent immunosuppressive mediator in a variety of settings and can be expressed by both natural T_{reg} cells and type 1 T_{reg} cells, we investigated changes in the frequency of IL-10$^+$ cells (figure 3*A*). We found that the frequency of IL-10$^+$ cells increased as early as 7 days after infection with a dramatic increase by 28 days, which was similar in magnitude and timing to the increased frequency of TGF-β1$^+$ cells and FOXP3$^+$ T_{reg} cells. Moreover, we also found increased frequencies of cells expressing IDO (figure 3*B*), an interferon (IFN)–γ inducible enzyme that generates such highly immunosuppressive byproducts as n-formylkynurenine by oxidative degradation of tryptophan.We suspected that tolerogenic dendritic cells (DCs) might be the source of the IDO, on the basis of the study by Munn et al. demonstrating the existence of a subset of monocyte-derived DCs that use this enzyme to suppress T cell proliferation in vitro [18]. However, we found that, during acute SIV infection, most of the IDO$^+$ cells were CD3$^+$ T cells (figure 3*B*) and that most were, in fact, FOXP3$^+$ T_{reg} cells (mean ± SD, 83.1% ± 10.6%). Moreover, a detailed analysis of the phenotype of T_{reg} cells at peak induction revealed a highly heterogeneous T_{reg} cell population that included natural T_{reg} cells (FOXP3$^+$), Th3 cells (FOXP3$^-$TGF-β$^+$), and type 1 T_{reg} cells (FOXP3$^-$IL10$^+$) (table 1). On the basis of the strong correlation between the frequency of CD4$^+$FOXP3$^+$ cells and the frequencies of TGF-β1$^+$, IL-10$^+$, and IDO$^+$CD3$^+$ cells, we conclude that a heterogeneous population of T_{reg} cells expressing several powerful immunosuppressive mediators is induced during acute SIV infection.

Kinetics of induction of T_{reg} cells parallel kinetics of immune activation but differ during SIV and RhCMV infection. We hypothesized that the immune activation that accompanied viral replication in SIV-infected LNs was driving the early induction of the immunosuppressive response, and we tested this hypothesis in 2 ways: (1) by characterizing the relationship between the kinetics of the induction of T_{reg} cells and the kinetics of immune activation during SIV infection and (2) by comparing this relationship with that for infection with another virus, RhCMV, during which immune activation occurs later and in response to antigenic stimulation only, rather than to the combined effect of antigenic stimulation and viral infection in lymphatic tissue, as is the case for SIV. Consistent

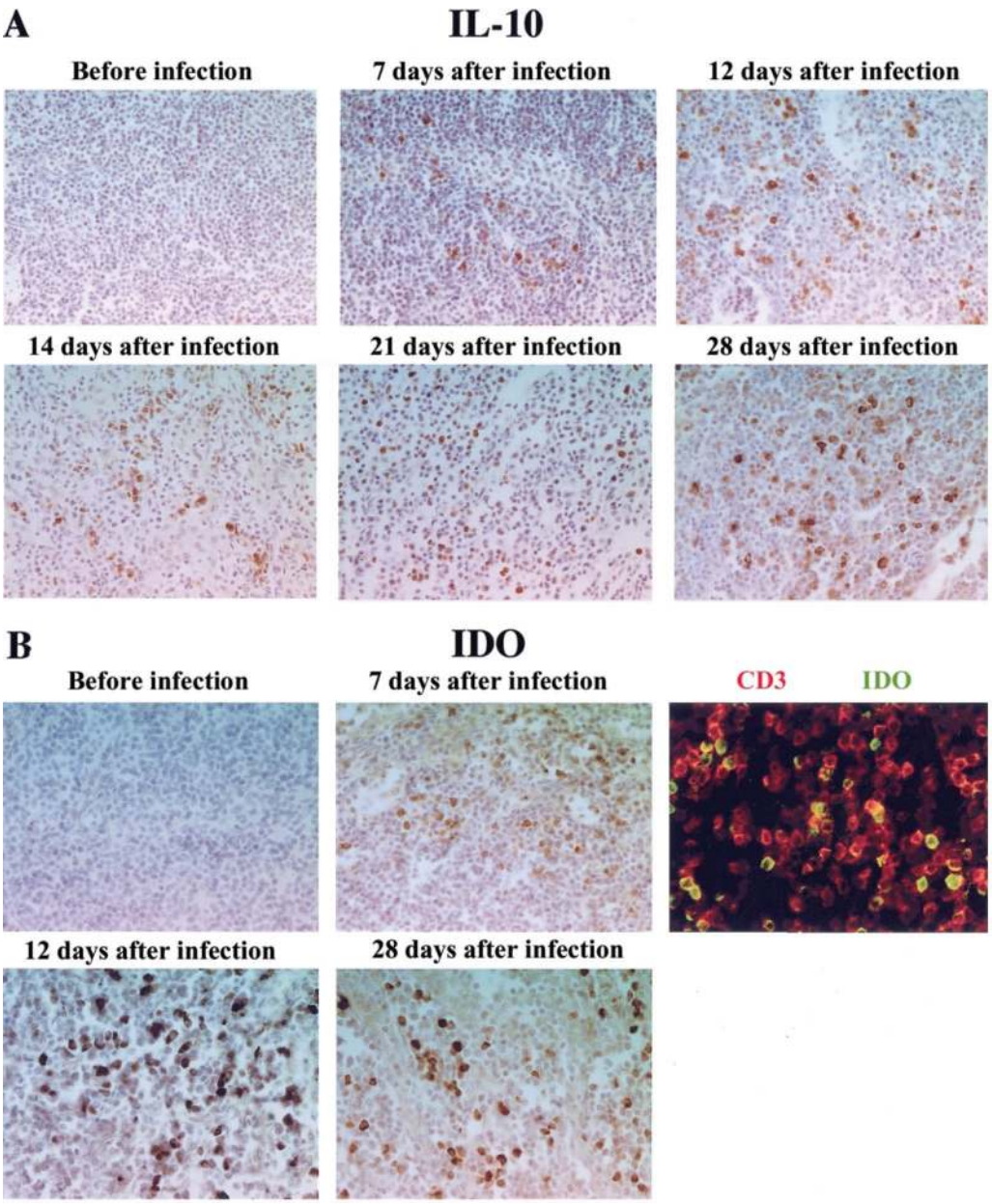

Figure 3. Rapid induction of immunosuppressive interleukin (IL)–10 and indoleamine 2,3-dioxygenase (IDO) in the paracortical T cell zones of lymph nodes during acute simian immunodeficiency virus (SIV) infection of rhesus macaques. The frequencies of both IL-10$^+$ cells *(A)* and IDO$^+$ cells *(B; left panels)* increased shortly after both intravenous and intravaginal SIV infection. Most IDO$^+$ cells were CD3$^+$ T cells *(B; right panel)*, not dendritic cells (as determined on the basis of phenotypic analysis of dual-stained tissue at peak expression of IDO) (original magnification, ×400).

with this hypothesis, we found that the kinetics of the induction of FOXP3$^+$ T_{reg} cells paralleled the kinetics of immune activation (Ki67$^+$ cells) in the paracortical T cell zones of secondary lymphatic tissue from 4 RMs after iv SIV infection (figure 4*A*). In addition, we found that, although there was a positive correlation between the frequency of T_{reg} cells and that of SIV RNA$^+$ cells ($r = 0.5969$), there was a much stronger relationship between the frequency of T_{reg} cells and the magnitude of the immune activation ($r = 0.8967$) (figure 4*B*). To examine how the frequency of SIV RNA$^+$ cells and the magnitude of immune activation simultaneously impact the frequency of T_{reg} cells, we fit a multiple regression model to the data on all RMs using all variables. The results of this model indicated that, although the frequency of SIV RNA$^+$ cells was related to the frequency of T_{reg} cells, when considering the effect of SIV infection on the frequency of T_{reg} cells given the effect of immune activation, there was not a statistically significant relationship. However, there was a statistically significant relationship between the magnitude of immune activation and the frequency of T_{reg} cells given the effect of SIV infection ($P = 0006$). Therefore, this model indicates that the

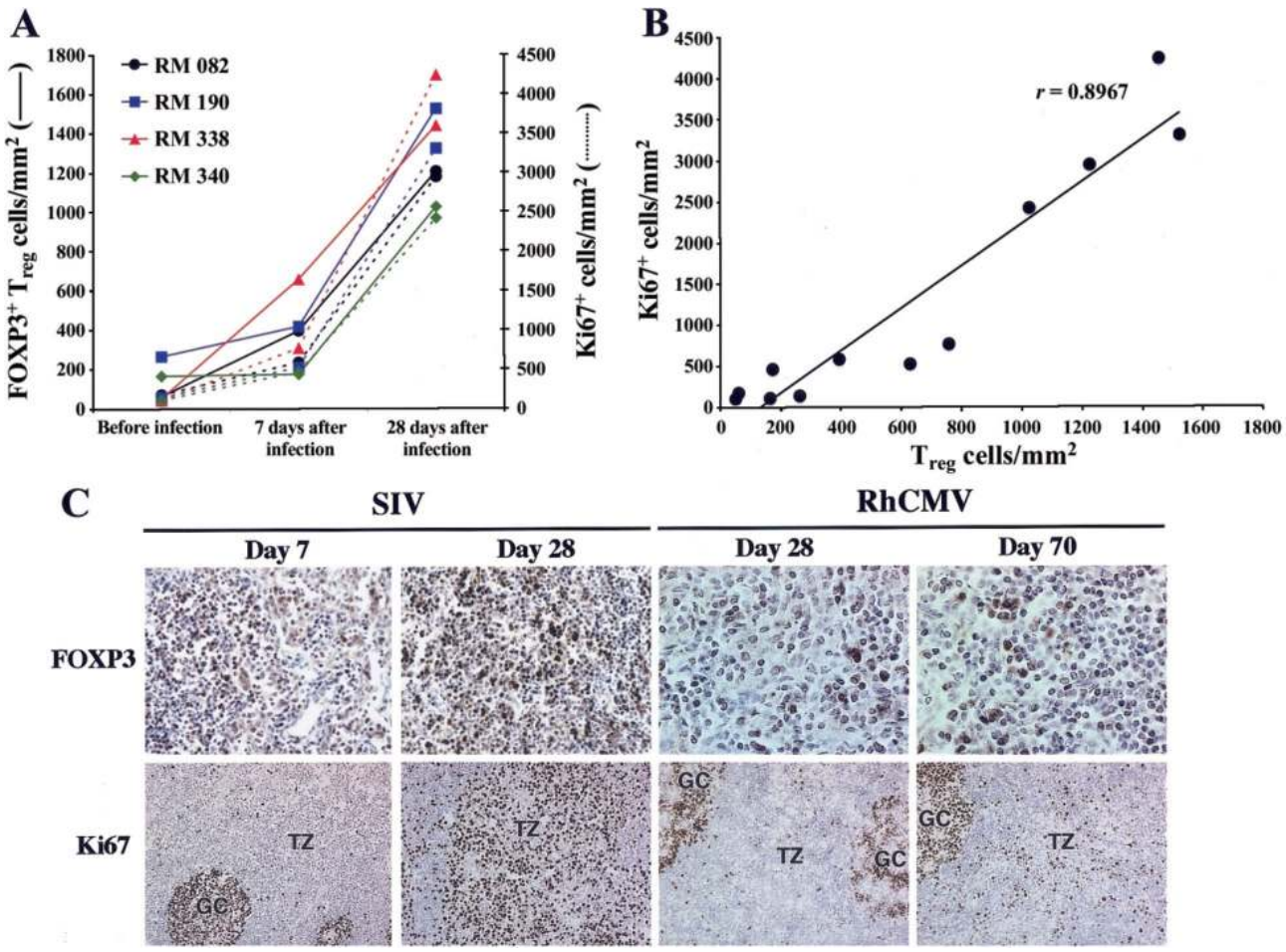

Figure 4. Regulatory T (T_{reg}) cell generation is driven by immune activation in the paracortical T cell zones of lymph nodes during acute simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs). Changes in the frequency of Ki67$^+$ cells mirrored the level of T_{reg} cell expression from 7 to 28 days after intravenous infection *(A)*, and a strong relationship existed between the frequency of T_{reg} cells and the magnitude of immune activation *(B)*; in contrast, primary rhesus cytomegalovirus (RhCMV)–infected RMs showed a delayed induction of FOXP3$^+$ T_{reg} cells associated with T cell zone immune activation (Ki67$^+$ cells) (*C*; original magnification, ×400 for FOXP3 and ×100 for Ki67; "TZ" indicates T cell zones, and "GC" indicates germinal centers).

weaker relationship we observed between the frequency of SIV RNA$^+$ cells and the frequency of T_{reg} cells reflects the stronger association between the frequency of T_{reg} cells and the magnitude of immune activation and the relationship between the magnitude of immune activation and the level of SIV infection. We think that these data support the hypothesis that viral replication indirectly drives the induction of T_{reg} cells by inducing a massive state of immune activation.

In contrast, in the RhCMV-infected RMs, immune activation within the paracortical T cell zones was not evident until 70 days after infection, and the peak T_{reg} cell response was correspondingly delayed and of lower magnitude (figure 4*C*). Immune activation in the RhCMV-infected RMs likely occurred in response to persistent systemic infection at other anatomic sites, because we did not detect any IE-1$^+$ (immediate early–1 gene product) or pp65$^+$ (matrix phosphoprotein–65 late gene product) productively RhCMV-infected cells in the LN samples by immunohistochemistry or sensitive polymerase chain reaction techniques (data not shown). Thus, for both infections, immune activation is correlated with a counterbalancing T_{reg} cell response, but the timing is different. Most importantly, the replication- driven immune activation that occurs early during SIV infection induces a rapid T_{reg} cell response that has the potential to negatively impact the host cellular immune response to infection while replication continues.

Impact of T_{reg} cells on the CTL response during acute SIV infection. To test the hypothesis that the early induction of the T_{reg} cell response to counter immune activation and inflammation in SIV-infected lymphatic tissues might, in fact, detrimentally affect SIV-specific immune responses, we compared the frequency of FOXP3$^+$ T_{reg} cells to the percentage of SIV-specific CTLs that recognized SL8 (aa 28–35), an immunodominant epitope in Tat that has been shown to exert strong selective pressure on SIV (figure 5) [19]. We quantified the SL8 tetramer$^+$CD8$^+$ T cell responses in 5 Mamu-A*01 major histocompatibility complex

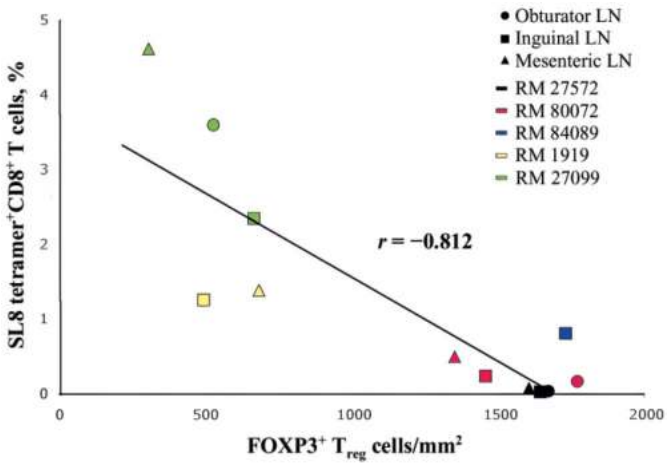

Figure 5. Significant inverse correlation between the frequency of FOXP3$^+$ regulatory T (T_{reg}) cells and the magnitude of the cytotoxic T lymphocyte (CTL) response to the Tat immunodominant epitope SL8. Tetramer analysis was performed on CTLs that had been isolated from a portion of the obturator *(circles)*, inguinal *(squares)*, and mesenteric *(triangles)* lymph nodes (LNs) from 5 Mamu-A*01 major histocompatibility complex class I allele–positive rhesus macaques (21–28 days after infection). The frequency of T_{reg} cells was determined on the basis of tissue sections from the same LNs, as described in Materials and Methods. The relationship was highly significant ($P < .01$), as determined on the basis of a test of the regression slope in a random effects model, which is described in detail in Materials and Methods.

class I allele–positive RMs, and we detected significant percentages (1.25%–4.61%) of SL8 tetramer$^+$ CD8$^+$ T cells in 2 of them (RM 1919 and RM 27099). In contrast, the SL8 responses were low (0.01%–0.8%) or undetectable in the other 3 RMs. There was a significant inverse correlation between the frequency of T_{reg} cells and the percentage of SL8 tetramer$^+$CD8$^+$ T cells at 21 and 28 days after infection ($r = 0.812$; $P < .01$) (figure 5), which is consistent with the idea that T_{reg} cells inhibit the CTL response to the immunodominant epitope SL8, which has been shown to play such an important role in the initial partial control of infection.

Discussion

Shortly after infectious challenges, specific host adaptive immune responses are initiated to combat these pathological threats. Most pathogens are effectively contained and then eliminated from the host by these responses; in some cases, however, the infectious agent is only partially controlled and is not eradicated, resulting in long-term persistent infections, immune hyperactivation, and immune-mediated tissue damage [20]. Understanding why the immune system fails to control these infectious pathogens is of great interest, particularly for persistent viral infections that result in high morbidity and mortality, such as infection with hepatitis C virus and HIV. Here, we provide evidence that one mechanism for lentiviral persistence may be a massive immunosuppressive regulatory response induced by the host shortly after viral exposure in secondary lymphatic tissues in response to corresponding viral replication and immune activation.

There is a growing body of evidence that T_{reg} cells suppress effector immune responses by a variety of mechanisms [8, 9, 15, 20–31]. In addition, it has recently been shown that T_{reg} cells not only regulate herpes simplex virus–specific CTL responses but may also suppress HIV-specific T cell responses during chronic infection, thus diminishing the magnitude of the immune response to these viral pathogens [8, 32–38]. Recently, Haeryfar et al. [39] showed that depleting T_{reg} cells significantly enhanced the magnitude of the CTL response to influenza A virus–, vaccinia virus–, and simian virus 40–transformed cells in vivo. These and our present data support a role for T_{reg} cells in controlling the magnitude of CTL responses in vivo, likely through inhibiting T cell proliferation and clonal expansion. To add to the growing evidence for control of pathogenic immune responses by T_{reg} cells, we show here for the first time that, during acute SIV infection, the magnitude of the SIVspecific CTL response to the Tat immunodominant epitope SL8 is inversely related to the magnitude of the immunosuppressive T_{reg} cell response. In addition, preliminary results indicate that RMs with high frequencies of T_{reg} cells in secondary lymphatic tissues have significantly lower frequencies of both IFN-γ^+ cells and IL-12$^+$ cells, compared with those in RMs with low frequencies of T_{reg} cells (authors' unpublished data). Furthermore, the present study indicates that the anatomical distribution of T_{reg} cells in vivo is primarily contained within the paracortical T cell zones of secondary lymphatic tissue, the immune inductive site for the stimulation and activation of effector T cell responses.

Our data suggest that the early, sustained immunosuppressive response is composed, in large part, of TGF-$\beta1^+$ T_{reg} cells. In the SIV-infected RM model, there are currently no experimental approaches to show in tissue that the initial T_{reg} cell response is antigen specific, as has been shown both in vitro and in vivo in other systems [15, 16, 40, 41], but we reason that the early T_{reg} cell response generated shortly after SIV exposure is a consequence of immune activation of resident SIV-specific T_{reg} cells in secondary lymphatic tissue. However, it is likely that, by the peak of this T_{reg} cell response, the production of immunosuppressive effector cytokines (such as TGF-β1) by antigen-specific T_{reg} cells may drive the induction of nonspecific inducible "bystander" T_{reg} cells, further amplifying the immunosuppressive response.

What roles TGF-β1 and IL-10 play in the in vivo function of CD4$^+$CD25$^+$ natural T_{reg} cells are still quite controversial. Although most in vitro studies have shown that CD4$^+$CD25$^+$ T_{reg} cells do not mediate immunosuppression via soluble factors, several in vivo studies have demonstrated the importance of TGFb1 and IL-10 in immunosuppression mediated by CD4$^+$CD25$^+$ T_{reg} cells [42–47]. The present study, which demonstrated the expression of

TGF-β1 and IL-10 by $CD4^+CD25^+FOXP3^+$ T_{reg} cells, provides further evidence indicating that natural T_{reg} cells likely use either or both TGF-β1 and IL-10 as mechanisms for immunosuppression in vivo and shows for the first time that most natural T_{reg} cells also express the highly immunosuppressive enzyme IDO, which represents another mechanism for immunosuppression mediated by T_{reg} cells.

The induction of T_{reg} cells during acute SIV infection likely parallels the induction of immune activation in the paracortical T cell zones of secondary lymphatic tissue as a response by the host to control immune-mediated pathological damage due to immune hyperactivation. However, this T_{reg} cell response dose not appear to be capable of limiting the massive hyperactivation in the lymphatic compartments during acute SIV infection in RMs. In contrast, a recent study by Kornfeld et al. indicates that, during the course of natural infection in African green monkeys (AGMs) infected with SIV_{agm}, a very rapid immunosuppressive response is induced and peaks during the first week of infection, followed by a decease to baseline levels [48]. The much more rapid immunosuppressive response in AGMs abrogates immune hyperactivation, resulting in a far more benign disease outcome in comparison with that in RMs. Thus, the timing of the T_{reg} cell response in relation to immune activation and the adaptive immune response is the critical determinant of outcome. The T_{reg} cell response in SIV-infected RMs is too late to counterbalance and prevent the immunopathological consequences of sustained immune activation and is too early and untimely with respect to immune control, as it down-regulates important effector T cell responses before immune control is achieved. In AGMs, the T_{reg} cell response is also too early to prevent a persistent infection, with viral replication at levels comparable with those in SIV-infected RMs, but is sufficiently early to prevent the immunopathological effects of sustained immune activation.

In summary, we have shown that SIV infection elicits an early T_{reg} cell response in secondary lymphatic tissue, one likely driven by the immune activation associated with infection. Although this immunosuppressive response may limit the immunopathological effects of sustained immune activation and inflammation, the T_{reg} cell response may also limit SIV-specific immune responses at just the time when they are critically needed to clear an infection. We think that a successful vaccine will need to establish a better balance between these beneficial and adverse effects of T_{reg} cell responses, and we speculate that there may be a role for anti-inflammatory treatment during acute infection, to moderate the T_{reg} cell response.

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