Elevated Levels of Innate Immune Modulators in Lymph Nodes and Blood Are Associated with More-Rapid Disease Progression in Simian Immunodeficiency Virus-Infected Monkeys[▽]

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Cytokines and chemokines are critical for establishing tissue-specific immune responses and play key roles in modulating disease progression in simian immunodeficiency virus (SIV)-infected macaques and human immunodeficiency virus (HIV)-infected humans. The goal here was to characterize the innate immune response at different tissue sites and to correlate these responses to clinical outcome, initially focusing on rhesus macaques orally inoculated with SIV and monitored until onset of simian AIDS. Cytokine and chemokine mRNA transcripts were assessed at lymph nodes (LN) and peripheral blood cells utilizing quantitative real-time PCR at different time points postinfection. The mRNA expression of four immune modulators—alpha interferon (IFN- α), oligoadenylate synthetase (OAS), CXCL9, and CXCL10—was positively associated with disease progression within LN tissue. Elevated cytokine/chemokine expression in LN did not result in any observed beneficial outcome since the numbers of CXCR3⁺ cells were not increased, nor were the SIV RNA levels decreased. In peripheral blood, increased OAS and CXCL10 expression were elevated in SIV⁺ monkeys that progress the fastest to simian AIDS. Our results indicate that higher IFN- α , OAS, CXCL9, and CXCL10 mRNA expression in LN was associated with rapid disease progression and a LN environment that may favor SIV replication. Furthermore, higher expression of CXCL10 and OAS in peripheral blood could potentially serve as a diagnostic marker for hosts that are likely to progress to AIDS. Understanding the expression patterns of key innate immune modulators will be useful in assessing the disease state and potential rates of disease progression in HIV⁺ patients, which could lead to novel therapy and vaccine approaches.

Most worldwide human immunodeficiency virus type 1 (HIV-1) transmission events occur after the translocation of the virus across a mucosal epithelial surface such as the genital, rectal, or oral mucosa. Cells able to be infected by HIV (such as macrophages, dendritic cells [DCs], and CD4⁺ T cells) exist at these mucosal sites and likely represent the initial target cells for the virus (10, 27, 38, 65). The propensity of HIV to utilize mucosal transmission to spread to new hosts suggests that developing an effective HIV vaccine will likely require an in-depth understanding of these earliest events at the mucosal sites, as well as the subsequent events that follow at other tissue sites such as lymph nodes (LN) and blood. The simian immunodeficiency virus (SIV) infection of rhesus macaques is an excellent model to assess these different tissue sites permitting both virologic and immunologic assessment of critical tissue compartments (1, 5, 21, 40, 66). This model has been particularly useful in assessing the role of cytokine and chemokine responses in different tissue sites at early times after SIV infection (4, 5, 40). The expression of immune modulators has the potential to impact both SIV transmission and SIV-induced disease progression. The timing, location, and type of immune modulator elicited have been demonstrated to play

crucial roles in this outcome (1–3, 5, 20, 32, 55). For example, the expression of interferons (IFNs), proinflammatory cytokines, and chemokines at the mucosal challenge site can be positively associated with viral replication (4, 5). Also, increased expression of innate immune modulators in blood and other tissues (e.g., LN and lungs) has been observed during acute and chronic SIV infection (1, 5, 55). In contrast, assessment of gut-associated lymphoid tissues of SIV-infected macaques has indicated an inverse association between mRNA expression of immune modulators and viral replication, since increased gene expression was generally associated with a decrease in viral burden (20). In addition, vaccine-induced protection is associated with differential innate gene expression at tissue sites that leads to variable outcomes with regard to SIV viral levels (2, 3, 32). At the oral mucosa, it has been demonstrated that an attenuated SIV vaccine can induce protection after tonsillar inoculation of SIV in rhesus macaques, and this was associated with two types of immune cells: $\gamma\delta$ T cells and mature DCs (61).

The activation state of the immune system is likely to be critical for both the frequency of the transmission event and the rate that an SIV/HIV-infected host progresses to AIDS. During HIV infection an association between immune activation, as defined by the activation state of T-cell populations, was first described as potentially having an influence on disease progression by Ascher and Sheppard in 1988 (6) and later by Giorgi and coworkers (22, 34). These findings have been verified in more recent studies that identify a clear association

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between the activation state of the immune system and the rate at which HIV-infected patients progress to AIDS (19, 25, 31, 58, 59). Furthermore, low levels of immune activation were associated with a lack of clinical signs of AIDS in natural hosts of SIV infection, such as sooty mangabeys and African green monkeys (39, 45, 57). The activation state of the immune system also likely serves a key role for the earliest events of HIV/SIV transmission, viral spread, and acute-phase disease progression, although less is known about these earliest events postinfection.

Our laboratory has focused primarily on assessing oral transmission of HIV/SIV, which is an important route for oral-sexual transmission (9, 48, 49, 53, 63) and mother-to-child transmission (41, 44, 52). In the present study, the expression levels of several immune modulators were assessed at two tissue sites, LN and blood, during both the acute and the chronic phases of infection. These data were then compared to findings from the oral mucosa, permitting an assessment of three different tissue sites during disease progression. Our earlier studies focused primarily on mucosal tissues, oral and rectal (40), and demonstrated that early and sustained increases in antiviral cytokine alpha IFN (IFN- α), the IFN-stimulated gene 2'-5' oligoadenylate synthetase (OAS) (50), and the proinflammatory chemokines CXCL9 (Mig) and CXCL10 (IP-10) (18) at mucosal sites were associated with a slower rate of disease progression in a cohort of SIV-infected macaques (40). The study presented here determined that the elevated expression of IFN- α , OAS, CXCL9, and CXCL10 in LN are associated with a more rapid disease progression; additionally, we extended these analyses to the peripheral blood of SIV-infected monkey that progress to simian AIDS (sAIDS) at various rates. We observed that two of these innate immune modulators, OAS and CXCL10, were elevated in the peripheral blood of SIV⁺ monkeys that exhibited a more rapid course of disease, suggesting the potential use of these genes as diagnostic markers during HIV infection. These studies highlight the importance of assessing innate cytokines/chemokines in both tissues and peripheral blood since these analyses may lead to novel therapy and vaccine approaches.

MATERIALS AND METHODS

Animal inoculations and virus stock. The macaques used in these studies were colony-bred rhesus macaques (*Macaca mulatta*) housed at either the California or the Yerkes National Primate Research Center. The following macaques were housed at the California National Primate Research Center: RM11 (33291), RM12 (32167), RM13 (32174), RM14 (32296), RM15 (33353), and RM16 (32127). These macaques were orally inoculated with two 10⁵ 50% tissue culture infective doses (TCID₅₀) of SIVmac251-5/98 (24, 36) 1 h apart. They were monitored throughout infection, and blood and LN biopsy specimens were taken on days 7 or 15, 21 or 28, 45 or 56, and 85 postinfection. Macaques were euthanized after the onset of sAIDS (62). Rhesus macaques RM1 (RSm), RM2 (RTq), RM3 (RUh), RM4 (RWp), RM5 (RPc), and RM6 (RHj) were housed at the Yerkes National Primate Research Center and were intravenously inoculated with 2.5 × 10⁵ TCID₅₀ of SIVmac239 (42). Blood samples were taken during chronic infection between days 258 and 287 postinfection. Also housed at the Yerkes National Primate Research Center, macaques RCe8, RCo8, RDo8, REi9, RHk8, RiF8, RJj8, RJj9, RKb9, RNr8, ROu8, RUu8, RWi8, RWu8, and RZz8 were intravenously inoculated with 10⁴ TCID₅₀ of SIVmac239 (13). Blood samples for analysis were taken on days 7 and 168 postinfection. The sooty mangabeys used in the present study (SM1 [FFr], SM2 [FBr], SM3 [FCq], SM4 [FCs], SM5 [FRu], SM6 [FUq]) were intravenously inoculated with plasma from an SIVsmm-infected mangabey as described previously (43) and had been infected between 258 and 287 days at the time of analysis. All animals were cared

for in accordance with National Institutes of Health guidelines and local animal care and use committees.

Tissue collection and processing. Oral mucosal and LN biopsy specimens, as well as peripheral blood, were obtained from macaques under ketamine hydrochloride anesthesia (10 mg/kg). As controls, biopsy specimens were obtained from four age-matched uninfected macaques to achieve baselines. Peripheral blood mononuclear cells (PBMC) were extracted through density centrifugation with Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden). Tissue biopsies were placed in RNAlater (Ambion, TX) and stored at -80°C. To obtain LN mononuclear cells, LN biopsy specimens were physically disrupted (scalpel microdissection) and crushed through a 70- μ m-pore-size cell strainer (BD Bioscience [BD], San Jose, CA). Cells were washed 1× in phosphate-buffered saline prior to use.

Quantification of plasma viral RNA. Viral RNA in plasma was quantified by a Chiron Corp. branch DNA signal amplification assay (version 4.0) specific for SIV (37). The viral load in plasma is reported as copies of viral RNA per milliliter of plasma with a limit of detection of 125 copies per milliliter of plasma.

RNA extraction and cDNA synthesis. Total RNA was extracted from the mucosal biopsies as previously described utilizing mechanical homogenization, followed by TRIzol extraction (2). Total RNA from LN mononuclear cells and PBMC was extracted by using an Aurum Total RNA minikit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. cDNA was synthesized by using a SuperScript first-strand synthesis system for reverse transcription-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

Quantitative real-time PCR analysis of immune effector genes. Real-time PCR utilizing gene-specific primers/probes were performed on an ABI 7700 or an ABI 7300 (Applied Biosystems, Foster City, CA) sequencer, as described previously (1, 2). Changes in the expression of eight innate immune genes (IFN- α , IFN- γ , interleukin-10 [IL-10], IL-12, CXCL9, CXCL10, tumor necrosis factor alpha [TNF- α], and OAS) and the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) housekeeping gene were calculated utilizing delta cycle threshold (ΔC_T) values (2, 40).

Quantification of LN-associated viral loads. Real-time PCR with primers and probe specific for SIVgag and GAPDH was run with cDNA from LN mononuclear cells on an ABI 7300. Plasmids containing SIVgag and GAPDH sequences were used to generate standard curves from which copy numbers of SIVgag and GAPDH mRNA in LN were derived. The LN-associated viral load is expressed as the number of SIVgag RNA copies per 10⁶ GAPDH RNA copies.

Flow cytometry for phenotypic analysis of cell populations. The percentages of CXCR3 expression on CD3⁺, CD4⁺, and CD8⁺ T cells and their phenotypes were determined by flow cytometry using a LSR II flow cytometer (BD). The following α -human antibodies cross-react with rhesus macaque cells and were utilized here: CXCR3 (Alexa Fluor 488 [BD], clone 1C6/CXCR3), CD3 (allophycocyanin [APC]-Cy7 [BD], clone SK7), CD4 (APC [BD], clone SK3), CD8 (PerCP or phycoerythrin [PE; BD], clone SK1), Ki67 (PE [Dako], clone Ki-67), CCR7 (PE-Cy7 [BD], clone 3D12), CD69 (PE-Cy7 [BD], clone FN50), and HLA-DR (PerCP [BD], L243).

RESULTS

Oral SIV infection results in varied clinical outcomes. To assess correlates of disease progression, six rhesus macaques were nontraumatically orally inoculated with SIVmac251 and monitored until they exhibited signs of sAIDS. The macaques were divided into three groups: the rapid progressors (RM11, RM13, and RM14), the intermediate progressors (RM12 and RM15), and one slow-progressing macaque (RM16) that did not develop sAIDS for more than 2 years (Fig. 1A). Elevated levels of plasma viral loads were associated with more rapid disease progression: the three rapid progressors exhibiting the highest peak and set-point viral loads, whereas the slow progressing macaque (RM16) had the lowest peak and set-point viral loads (Fig. 1B). Similarly, LN-associated viral loads were generally associated with disease progression (Fig. 1C). The most rapidly progressing macaque (RM11) had persistently increasing LN-associated viral load throughout infection and had the highest LN-associated viral load of all animals at day 84 postinfection (220,000 RNA copies/10⁶ GAPDH). In con-

FIG. 1. Characterization of orally SIV-inoculated rhesus macaques. (A) Time to progression to AIDS. The most rapidly progressing macaque (RM11, dark red) developed AIDS after only 13 weeks, followed by two animals progressing to disease within 22 (orange) and 25 (pink) weeks. Two macaques, shown in gray bars (RM12 and RM15), progressed at an intermediate rate (32 and 36 weeks), whereas another macaque did not exhibit any signs of AIDS for more than 2 years (RM16, green). Same color scheme was utilized in other figures. (B) Plasma viral load, shown as viral RNA copies per mL of plasma, measured by branch DNA assay. (C) LN-associated viral loads, shown as SIVgag RNA copies per 10⁶ copies of GAPDH, measured by quantitative reverse transcription-PCR.

trast, the slow progressor (RM16) maintained low LN-associated viral loads throughout infection and exhibited the lowest viral load at day 84 postinfection (11,000 RNA copies/10⁶ GAPDH). The additional macaques exhibited intermediate LN-associated viral loads and intermediate rates of disease progression.

Cytokine/chemokine expression in peripheral blood and LN. Expression of innate immunomodulatory genes was assessed at both the peripheral LN (axillary and inguinal) and blood. Samples in both compartments were taken at similar time points: 7 or 15, 21 or 28, 45 or 56, and 84 days postinfection. LN biopsies and PBMC were assessed for mRNA levels of eight cytokines and chemokines by real-time PCR: IFN- α and OAS as antiviral modulators; the proinflammatory chemokines CXCL9 (monokine induced by IFN- γ , Mig) and CXCL10 (IFN-inducible protein of 10 kDa, IP-10); the proinflammatory cytokines IFN- γ , IL-12, and TNF- α ; and the anti-inflammatory cytokine IL-10.

The LN represent a tissue site important for both initiating immune responses and as a site of viral replication. Compared to uninfected control macaques, the expression of IFN- α , OAS, CXCL9, and CXCL10 at LN was generally upregulated in the rapidly progressing macaques at all time points assessed, as demonstrated by symbols from these macaques appearing above the two-standard-deviation cutoff (Fig. 2, within blue boxes). In contrast, these same immune modulators were not

upregulated compared to uninfected control macaques in the slow-progressing macaque. The expression levels of IFN- γ , IL-12, TNF- α , and IL-10 in LN were comparable to uninfected control macaques in the SIV⁺ macaques irrespective of the rate of disease progression (Fig. 2).

Expression of these same genes in peripheral blood did exhibit some similarities to what was observed in LN; however, there were some interesting differences as well (Fig. 3). Whereas the mRNA levels of four of these immune modulators increased in the LN of macaques with more rapid AIDS progression, only two were elevated within the peripheral blood cells: OAS and CXCL10. This increased expression of OAS and CXCL10 in the peripheral blood of the SIV⁺ macaques was generally observed within the more rapidly progressing macaques (symbols within blue boxes in Fig. 3). These immune modulators were not upregulated and were similar to uninfected controls in the slowest-progressing macaque (Fig. 3, green circles). The levels of the other five immune modulators examined in PBMC—IFN- α , CXCL9, IFN- γ , TNF- α , and IL-10—generally remained similar to those in uninfected control macaques (Fig. 3).

Presence of CXCR3⁺ cells in peripheral LN. The chemokines CXCL9 and CXCL10 function by inducing chemotaxis in cells through the binding of their cognate chemokine receptor CXCR3, which is expressed on activated T cells (particularly TH1), natural killer, and plasmacytoid DCs (8, 18, 26, 35).

FIG. 2. Fold changes in immune response gene mRNA expression in LN. Shown are the fold changes of eight immune response genes (from left: IFN- α , OAS, CXCL9, CXCL10, IFN- γ , IL-12, TNF- α , and IL-10) in LN of orally infected macaques at different time points throughout disease progression: 7 to 15 days (A), 21 to 28 days (B), 45 to 56 days (C), and 84 days (D) postinfection. The most rapidly progressing macaque is represented by the red triangles, the second fastest progressor is shown as orange diamonds, and the third is indicated by pink diamonds. The two intermediately progressing monkeys are represented by black squares, and green circles show the slow progressor. Symbols within the blue boxes represent mRNA expression levels that are increased more than two standard deviations away from the expression in uninfected animals.

Thus, high levels of CXCL9/10 in LN of rapid progressors suggest that CXCR3⁺ cells may be recruited to these LN. To determine the levels of CXCR3⁺ cells in LN, we assessed the percentages of CD4⁺ and CD8⁺ T cells expressing CXCR3 utilizing flow cytometry (Fig. 4). Despite the increased mRNA expression of CXCL9 and CXCL10 in the LN of the rapidly progressing macaques (RM11, RM14, and RM13), there was no evidence of increased recruitment of CXCR3⁺ CD4⁺ T cells (Fig. 4A) or CD8⁺ T cells (Fig. 4B) compared to the LN of the slow progressor. Furthermore, the CXCR3⁺ cells did generally not exhibit any differences with regard to activation (HLA-DR, Fig. 4C and D) or proliferation (Ki67, data not shown) when compared between the fast- and slow-progressing macaques. These findings indicate that there is no correlation between the mRNA expression of chemokines and the recruitment of their respective target cells at the LNs of SIV⁺ macaques.

OAS, CXCL10, and TNF- α mRNA expression at LN, oral mucosa, and peripheral blood during acute SIV infection. Since OAS and CXCL10 mRNA expression was upregulated in LN and PBMC of SIV⁺ macaques that progressed more rapidly to disease, a temporal assessment of these data in each of these tissues was undertaken, including the oral mucosa, which has been studied in detail previously (40) (Fig. 5). The data indicate that CXCL10 and OAS mRNA levels in LN are

generally highest at the earliest time points in the more rapidly progressing macaques (elevated beyond the two-standard-deviation range) and decline over time (Fig. 5A and B). In contrast, the CXCL10 mRNA levels at the oral mucosa are highest in the slowest-progressing macaque for each of the time points, with elevated levels of CXCL10 most evident at the last time point assessed in this macaque (70 days postinfection, 150-fold above uninfected levels) (Fig. 5D). The TNF- α mRNA levels are included here as an example of a cytokine/chemokine that did not exhibit any particular pattern with regard to disease progression; the levels are similar for all SIV⁺ macaques (generally at levels comparable to those of uninfected controls) (Fig. 5C, F, and I).

One goal of these studies was to ascertain whether the cells obtained from the peripheral blood exhibited mRNA levels of these immune modulators that were similar to either the LN or the oral mucosa. These data indicate that the mRNA levels of CXCL10, OAS, and TNF- α are strikingly similar to the data obtained from the LNs and distinct from the findings for the oral mucosa (Fig. 5G, H, and I). This would indicate that peripheral blood cells have a general upregulation of CXCL10 and OAS in the more rapidly progressing SIV⁺ macaques and lower levels of these immune modulators in the more slowly progressing macaques. TNF- α mRNA levels remain within the two-standard-deviation range and similar to those of the un-

FIG. 3. Fold changes in immune response gene mRNA expression in peripheral blood. Shown are the fold changes of seven immune response genes (from left: IFN- α , OAS, CXCL9, CXCL10, IFN- γ , TNF- α , and IL-10) in PBMC of orally infected macaques at different time points throughout disease progression: 7 to 15 days (A), 21 to 28 days (B), 45 to 56 days (C), and 84 days (D) postinfection. IL-12 mRNA levels were not included since they remained below detection in the PBMC of the uninfected and majority of the SIV-infected macaque samples. The color designations are as described for Fig. 2. Symbols within the blue boxes represent mRNA expression levels that are increased more than two standard deviations away from the expression in uninfected animals.

infected macaques, which is also more similar to the LN. The other immune modulators were also assessed from the peripheral blood, and none exhibited mRNA levels that were positively or negatively associated with the rates of disease progression (Fig. 3). These data raise the possibility that the assessment of CXCL10 and/or OAS mRNA levels from peripheral blood cells of SIV⁺ monkeys or HIV⁺ patients could provide useful information with regard to the potential for that host to progress rapidly to AIDS.

Comparison of CXCL10 and OAS expression in peripheral blood cells of chronically SIV-infected rhesus macaques and sooty mangabeys. To assess the association between peripheral blood levels of select immune modulators and rates of progression to AIDS, we undertook a retrospective assessment of OAS and CXCL10 expression in six additional chronically SIV-infected rhesus macaques (Fig. 6A and C). These macaques had been intravenously inoculated with SIVmac239 resulting in both intermediate progressors to disease (RM1, RM2, RM4, RM5, and RM6) and a nonprogressor (RM3) (42). Of the five intermediate progressors, three exhibited increased CXCL10 mRNA levels (Fig. 6A), and all five had increased OAS mRNA levels in their peripheral blood cells (Fig. 6C). Interestingly, the sixth animal, a macaque that did not exhibit any signs of AIDS even at 4 years postinfection (RM3 [Fig. 6A, star]), did not exhibit increased levels of these

two immune modulators (Fig. 6A and C). To further examine these immune modulators, we assessed sooty mangabeys, an African natural host species that replicates SIV to high levels and yet does not exhibit any clinical signs of AIDS (30, 39, 43, 45, 56, 57). Six chronically infected mangabeys were assessed, and the real-time PCR data were compared to six uninfected mangabeys to ascertain whether the SIV infection altered the mRNA levels of OAS or CXCL10 within their peripheral blood. Similar to the slowly progressing SIV⁺ rhesus macaques, the SIV infection of this natural host species did not result in any evidence for increased mRNA levels of OAS or CXCL10 within peripheral blood cells during chronic infection (Fig. 6B and D).

Comparison of CXCL10, OAS, and TNF- α expression in peripheral blood cells during acute and chronic SIV infection of rhesus macaques. The results from the chronically infected animals encouraged us to further test our hypothesis by analyzing CXCL10, OAS, and TNF- α expression in 15 additional SIV-infected rhesus macaques (Fig. 7). These macaques had been intravenously inoculated with SIVmac239 (13), resulting in both intermediate progressors to disease (REi9, RHk8, RJj8, RJi9, RKb9, RWi8, RWu8, and RZz8) and slow progressors (RCe8, RCo8, RDo8, Rlf8, RNr8, ROu8, and RUn8). We examined PBMC samples taken during acute (7 days postinfection) and chronic (168 days postinfection) infection. Inter-

FIG. 4. CXCR3⁺ cells in peripheral LN. The expression of the chemokine receptor CXCR3 was assessed by flow cytometry on the CD4⁺ T-cell subset (A) and the CD8⁺ T-cell subset (B) of LN cells. Expression of the activation marker HLA-DR was examined on the CXCR3⁺ CD4⁺ (C) and CXCR3⁺ CD8⁺ T cells (D) and is depicted as the percentage of HLA-DR-positive cells within each of these populations. Each bar represents one time point of one animal. ND, not determined.

estingly, the expression levels of both CXCL10 (Fig. 7A) and OAS (Fig. 7B) did not differ between acutely infected rhesus macaques progressing to disease at slow or intermediate rates. However, differences were observed during the chronic stage of infection at day 168 postinfection, with the slower-progressing macaques tending to exhibit lower levels of these two immune modulators. Indeed, a significant decrease was observed for OAS mRNA levels in the slow-progressing compared to the intermediate-progressing macaques. The TNF- α levels tended to remain within the range observed in uninfected controls, demonstrating that not all mRNA levels of inflammatory markers were elevated in these macaques (Fig. 7C). These results identify the further potential for utilizing mRNA levels of CXCL10 and OAS as diagnostic indicators for hosts that would be likely to proceed rapidly to AIDS.

DISCUSSION

Knowledge of the earliest events after SIV administration to mucosal sites will be critical for identifying new therapeutic and vaccine strategies. Often this information is acquired through the assessment of SIV-infected macaques that are necropsied, enabling a careful analysis of just this one time point (1, 4, 5, 20, 38, 55). The approach in the present study enabled a characterization of the innate immune response at different tissue sites and different times throughout the acute phase of the infection, while still permitting an assessment with regard to the clinical outcome in these SIV⁺ macaques. After assessing mRNA levels of eight immune modulators, compelling findings were observed in four genes with roles in innate immunity: IFN- α , OAS, and two inflammatory chemokines (CXCL9 and CXCL10). We had previ-

FIG. 5. Detailed representation of fold changes for CXCL10, OAS, and TNF- α . Fold changes of mRNA expression are shown for CXCL10 (A, D, and G), OAS (B, E, and H), and TNF- α (C, F, and I) at LN (A, B, and C), oral mucosa (D, E, and F), and PBMC (G, H, and I). Each bar represents one time point of one animal. The fold change is considered increased or decreased in infected compared to uninfected macaques when it is greater than two standard deviations (shaded area) away from the average expression in uninfected animals. ND, not determined.

ously observed that an increased expression of these immune modulators at the oral and rectal mucosa was associated with a slower disease progression to *sAIDS*, whereas low mucosal expression was associated with more rapid disease progression (40). Interestingly, macaques that progressed the fastest to disease exhibited the highest levels of these same effector molecules in the LN, whereas the slower progressors exhibited generally lower levels in the LN. In peripheral blood, heightened expression of two of these immune modulators, CXCL10 and OAS, were associated with more rapid disease progression. It is possible that the increase in mRNA levels at mucosal sites (40), in LN, or in PBMC is due to a differential increase of one or more cell types migrating to these sites and expressing these immune modulators. Alternatively, the cells present at the different sites could express

higher levels of certain immune modulators on a per-cell basis due to stimulation by SIV viral products, cytokines, or chemokines. In this context, it is important to note that the tissue types assessed here contain differing cell types. The gingival samples consist of epithelial cells and some immune cells, whereas in LN the majority of cells are comprised of T and B cells, as well as antigen-presenting cells, such as macrophages and dendritic cells. The cell types assessed in peripheral blood are mononuclear cells comprised mainly of T cells, B cells, natural killer cells, and monocytes. These differences in cell composition likely play a key role in the differential expression of immune modulators observed at these different tissue sites, as well as providing insights into the cell types expressing mRNA of the immune modulators being studied.

FIG. 6. Expression of OAS and CXCL10 in PBMC of chronically infected rhesus macaques and sooty mangabeys. The fold changes of mRNA expression are shown for CXCL10 in PBMC of chronically (258 to 287 days postinfection)-infected rhesus macaques (A) and sooty mangabeys (B), as well as the fold changes for OAS in macaques (C) and mangabeys (D). Each column represents one chronically infected animal with the star indicating a long-term nonprogressor macaque (RM3). The fold change is considered increased or decreased in SIV-infected compared to uninfected macaques (A and C) or uninfected (B and D) mangabeys when it is greater than two standard deviations (shaded area) away from the average expression in uninfected animals of the respective species.

Regardless of the cell types present at the different tissue sites, the higher levels of immune modulators at mucosal sites very early in infection might aid in an earlier initiation of adaptive immune responses. In fact, the slow-progressing macaque exhibited an earlier and stronger induction of SIV-specific antibodies than the other animals (40). In addition, the high levels of cytokines/chemokines at mucosal sites might contribute to an immune environment capable of preventing opportunistic infections, since levels stay high throughout infection. Together, this might lead to slower disease progression. It is interesting that other studies also have found benefits of immune responses at mucosal sites. Racz and coworkers showed that the presence of dendritic cells, as well as $\gamma\delta$ T cells, in the tonsils is associated with vaccine-mediated protection against pathogenic viral infection (61). These cell types might indeed contribute to the heightened cytokine and chemokine expression during slow disease progression. On the other hand, high expression of cytokines and chemokines in LN might contribute to general immune activation rather than to viral control, thus favoring rapid disease progression (15, 17, 46, 58). In addition, the paucity of CXCR3⁺ cells despite increased levels of its ligands CXCL9 and CXCL10 at the LN of rapid progressors may represent immunological dysfunction of LN, which is a characteristic of pathogenic SIV and HIV infection (7, 16, 54).

It has been previously observed that the induction of innate immune responses at the vaginal mucosa through TLR ligands prior to SIV inoculation does not protect macaques from infection and elicited a broad range of immune modulators (64). In contrast, Li et al. recently demonstrated that the specific inhibition of certain proinflammatory cytokines and chemokines such as macrophage inflammatory protein 3 α (MIP-3 α) at the vaginal mucosa prior to challenge could prevent vaginal transmission of SIV, possibly by preventing the recruitment of CCR5-bearing cells to the site of virus exposure (33). These findings, together with our observations, indicate that immune modulators at different tissue sites might enhance, whereas others might inhibit or decrease, SIV transmission and/or disease progression.

It has been shown that immune activation is a strong predictor of HIV disease progression (6, 22, 25, 34, 59). Here, our studies focus on innate immune modulators and their association with disease progression. We found that in LN and peripheral blood the expression of the IFN response gene OAS and the chemokine CXCL10 (IP-10) was associated with disease progression. Because these two immune modulators were still upregulated during chronic infection in macaques, which showed signs of sAIDS, but not in slow or nonprogressors, their expression levels may be useful as a diagnostic marker of hosts that will progress relatively rapidly to AIDS. Natural

FIG. 7. Expression of CXCL10, OAS, and TNF- α in PBMC of acutely and chronically infected rhesus macaques. Fold changes of mRNA expression are shown for CXCL10 (A), OAS (B), and TNF- α (C) in PBMC of acutely and chronically infected rhesus macaques. Expression of the three immune modulators is divided into intermediate-progressing macaques and slow-progressing macaques, with the acute time point on the left (open columns, day 7 postinfection) and the chronic time point on the right (shaded columns, day 168 postinfection) of each graph. The macaques representing the intermediate progressors are (from left to right): REi9, RHk8, RJj8, RJl9, RKb9, RWi8, RWu8, and RZz8. The macaques representing the slow progressors are (from left to right): RCe8, RCo8, RDo8, RIf8, RNr8, ROu8, and RUn8. Each column represents one time point of one infected animal. The fold change is considered increased or decreased in SIV-infected compared to uninfected macaques when it is greater than two standard deviations (shaded area) away from the average expression in uninfected macaques. The Mann-Whitney test was conducted to determine whether there was a statistical difference between the chronically infected intermediate progressors and slow progressors, and the *P* value is indicated. Acutely infected animals were not statistically different for any of the three genes between the two groups.

hosts of SIV, such as sooty mangabeys, exhibit immune activation during acute phases of infection that is transient and then maintain low levels of immune activation during chronic phases of infection and generally do not exhibit any clinical signs of sAIDS (12, 14, 23, 29, 39, 45, 56–58). Although most of these studies focus on T-cell activation, it is interesting that innate immune modulators seem to follow a similar trend as IFN-stimulated gene expression, such as OAS or CXCL10, is upregulated during acute infection in sooty mangabeys but declines back to baseline levels after 30 days postinfection (S. E. Bosinger et al., unpublished data). In addition, Sarkar et al. found that SIV⁺ macaques with high viral loads had higher numbers of CXCL10-producing cells compared to animals with low viral loads, and CXCL10 production was inversely correlated with peripheral CD4⁺ T-cell numbers (51). Chim-

panzees infected with HIV that progressed to sAIDS consistently demonstrated increased levels of CXCL10 in plasma, whereas the levels were undetectable in nonprogressing chimpanzees (28). Furthermore, there is evidence that CXCL10 (IP-10) might be increased in a specific manner in HIV⁺ patients as the CXCL10 levels in plasma rapidly increase in HIV-infected individuals and remain elevated in all patients assessed (60). In contrast, in hepatitis B virus- or hepatitis C virus-infected individuals, plasma CXCL10 (IP-10) levels rise later in infection and are not elevated in every patient (60). In addition, other researchers have suggested using high levels of CXCL10 in cerebrospinal fluid for the diagnosis of AIDS dementia complex, a common neurological disorder associated with HIV infection and AIDS (11). In a study published in the mid-1980s, HIV-infected patients with AIDS were observed to

exhibit elevated levels of OAS, prompting the authors to suggest that this immune modulator might be used as a prognostic indicator for progression to AIDS (47). These results, in combination with our findings, warrant further investigation of the use of CXCL10 (IP-10) and OAS as diagnostic markers of immune activation and disease progression in HIV-infected individuals. These analyses could in theory provide a means to identify those hosts, which are intermediate or rapid progressors exhibiting increased levels of CXCL10 and OAS, and slow or nonprogressors that do not have increased CXCL10 and OAS mRNA expression during chronic phases of infection.

In summary, the present study has expanded upon our previous work that focused on mucosal sites (40) by characterizing the response of innate immune modulators in LN and peripheral blood during both pathogenic and nonpathogenic natural SIV infection. The finding that elevated mRNA levels of certain innate immune modulators in LN and blood are associated with rapid disease progression, whereas elevations of these same immune modulators at mucosal sites are associated with slower disease progression, highlights the complexity that is inherent in primate species, including humans. These diverse outcomes indicate that assessment of multiple tissue compartments is necessary to provide a complete overview of the innate immune response post-SIV (and post-HIV) infection. However, findings in peripheral blood are still informative since they may provide markers that are useful as predictors of hosts that are likely to progress rapidly to AIDS. Our data suggest that it may be beneficial for future therapies or vaccines to induce increased expression of specific immune modulators, including IFN- α , OAS, CXCL9, and CXCL10, at mucosal sites but not the lymphoid tissues or peripheral blood. In addition, the timing and magnitude of this response may be critical to achieving the desired outcome of inhibiting HIV transmission or disease progression.

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