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Nonpathogenesis of Simian Immunodeficiency Virus Infection Is Associated with Reduced Inflammation and Recruitment of Plasmacytoid Dendritic Cells to Lymph Nodes, Not to Lack of an Interferon Type I Response, during the Acute Phase▽

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Divergent Toll-like receptor 7 (TLR7) and TLR9 signaling has been proposed to distinguish pathogenic from nonpathogenic simian immunodeficiency virus infection in primate models. We demonstrate here that increased expression of type I interferon in pathogenic rhesus macaques compared to nonpathogenic African green monkeys was associated with the recruitment of plasmacytoid dendritic cells in the lymph nodes and the presence of an inflammatory environment early after infection, instead of a difference in the TLR7/9 response.

Type I interferons (IFNs) are synthesized in response to a variety of pathogens, including bacteria, protozoa, and viruses. DNA and RNA viruses induce type I IFN expression by plasmacytoid dendritic cells (pDCs) through stimulation of Toll-like receptor 7 (TLR7) and TLR9 (46). The IFN response is an early event in simian immunodeficiency virus (SIV)-infected monkeys (1, 21, 28, 41). SIV infection of nonhuman primates remains an invaluable animal model for studies of AIDS pathogenesis, therapeutics, and vaccines. In particular, understanding the basis of pathogenic and nonpathogenic host-virus relationships in nonhuman primates is likely to provide important clues regarding AIDS pathogenesis (20). It has recently been reported that pDCs from the nonpathogenic model of SIV infection, sooty mangabey, produce markedly less IFN-α in response to SIV and other TLR7 and TLR9 ligands than pDCs from pathogenic rhesus macaques (RMs) of Indian origin (29). Therefore, it has been proposed that chronic stimulation of pDCs by SIV and human immunodeficiency virus (HIV) in non-natural hosts may drive the unrelenting immune system activation and dysfunction associated with AIDS progression (29). However, the detailed dynamics of IFN-α production in these monkeys was not addressed. Furthermore, a peak of plasma levels of type I IFN has been observed early after SIV infection in African green monkeys (12)—a nonpathogenic model of SIV infection—in contrast to the previous hypothesis. In addition, transcriptional gene profiling in nonpathogenic SIV-infected monkeys has revealed the presence of genes related to type I and type II IFN responses (23).

pDCs migrate through high endothelial venules during viral and bacterial infections following the release of inflammatory cytokines and chemokines (4). Therefore, we have analyzed more thoroughly the question of changes in IFN-α synthesis and the relationship with dynamics of pDC accumulation and viral replication in peripheral lymph nodes (LNs) during the acute phase of different models of SIV infection in nonhuman primates (9). Indeed, few studies have focused on LNs. We performed a longitudinal study of 8 Indian and 20 Chinese RMs experimentally infected with the SIVmac251 strain (among these latter 7 were noncontrollers [NC-RMs] and 13 controllers [C-RMs]). In addition, six Chinese RMs were experimentally infected with a *nef* deletion SIVmac251 strain (SIVΔ*nef*), and six African green monkeys were infected with SIVagm-sab.

MATERIALS AND METHODS

Monkeys, virus, and samples. RMs (*Macaca mulatta*) were seronegative for simian T leukemia virus type 1, SRV-1 (type D retrovirus), herpesvirus B, and SIVmac. Animals were housed and cared for in compliance with existing French regulations (9). Macaques were inoculated intravenously with 10 50% animal infectious doses of the SIVmac251 strain (provided by A. M. Aubertin, INSERM U74, Strasbourg, France). Axillary and inguinal LNs were collected before infection and at days 7, 11, 14, and 60 postinfection. Half of the LNs were frozen and stored at −70°C for subsequent analysis by *in situ* hybridization or immunohistochemistry, while the other half was processed for flow cytometric analyses, as previously described (9). Six African green monkeys (AGMs) (*Chlorocebus aethiops sabeus*) were experimentally infected with 300 50% tissue culture

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infective doses of the SIVagm.sab92018 strain. This virus preparation was never passaged *in vitro*.

Quantitative analysis of productively infected cells and IFN-expressing cells. Productively infected cells (SIV^+ RNA cells) were assessed in LNs by *in situ* hybridization as previously described (9). Infected cells were detected and counted in the paracortical zone on a minimum of three sections by using a Nikon-FXA microscope. IFN-α1 gene expression (which is one of the main IFN-α subtypes produced) was detected by *in situ* hybridization as described previously (21). Briefly, a fragment of 600 bp of the IFN-α1 gene specific for RMs and AGMs was introduced into a plasmid (pSP65), while the antisense riboprobes were generated from the SP6 promoter. The plasmids were then labeled with ^{35}S-labeled UTP. To enhance the penetration of the probes into tissue sections, the ^{35}S-labeled RNA were subjected to mild alkaline hydrolysis in order to obtain a majority of fragments in the 150- to 200-nucleotide range. The number of positive cells was then divided by the surface of the entire LN section, and the results were expressed as the number of positive cells per 2-mm^2 section. The mean count was calculated for three slides of the same LNs obtained in a blinded fashion by two different investigators.

Immunophenotyping of pDCs. LN cells were incubated with a cocktail of monoclonal antibodies for 30 min at 4°C in the dark. We used a cocktail of fluorescein isothiocyanate (FITC)-conjugated antibodies, namely, lineage (Lin) (6, 45), comprising anti-CD3 (FN-18), anti-CD14 (MΦP9), anti-CD20 (L27), in combination with phycoerythrin (PE)-conjugated anti-CD123 (7G3) antibodies, with peridinin-chlorophyll protein (PerCp)-conjugated anti-human leukocyte antigen (HLA)-DR (L243), and finally with biotinylated CD11c (3.9) antibodies. All monoclonal antibodies were purchased from BD Biosciences, except for the anti-CD3 and anti-CD11c antibodies obtained from Biosource (Nivelles, Belgium) and eBioscience (Clinisciences, Montrouge, France), respectively. Cells were washed and incubated in the presence of allophycocyanin (APC)-coupled streptavidin for 30 min in the dark at 4°C and then fixed. At least 500,000 events corresponding to mononuclear cells by forward- and side-scatter characteristics were acquired by using a FACSCalibur and further analyzed by using CellQuest analysis software (BD Biosciences). However, pDCs from SIV-infected RMs of Indian origin were not assessed due to import restriction of these species for research in Europe. Fresh cells from RMs of Indian origin and AGMs to test cytokines were kindly provided by F. Wanert, Centre de Primatologie, Université de Strasbourg.

Ex vivo PBMC stimulation with TLR agonists. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation of peripheral blood from SIV^- monkeys. PBMC (500,000 cells per well) were incubated in duplicate wells with 10 ng of lipopolysaccharide (LPS; TLR4 agonist)/ml, 1 μg of CLO97 (TLR7)/ml, and 1 μM ODN M362 (TLR9; InvivoGen) for 18 h at 37°C. After stimulation, supernatants were collected and assessed for cytokine detection. PBMC after stimulation were incubated with brefeldin A (5 μg/ml; Sigma-Aldrich) and then surface stained for CD123, CD11c, and Lin and fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS). Cells were then permeabilized with 0.5% saponin in PBS-fetal calf serum, followed by incubation with FITC-labeled anti-IFN-α2 antibody (eBiosciences clone 225.C) as previously described (6) and as described in other reports (29). Finally, the cells were washed and analyzed by flow cytometry.

Quantification of IFN-α and IL-8. Type I IFN was measured by an enzyme-linked immunosorbent assay (ELISA; PBL Biomedical laboratories) as previously described (29, 45) and, as shown here, was able to detect type I IFN levels in both RMs and AGMs. Interleukin-8 (IL-8) was measured by using an inflammatory cytokine cytometric bead array kit (BD Biosciences).

Statistical analyses. Friedman test with Dunn's correction was used to determine whether differences in means from sequential samples were significant. Correlations were evaluated by using the Spearman's test. Statistical significance between the different groups was analyzed by the nonparametric Mann-Whitney test. Prism version 3.0 (GraphPad Software) was used for statistical analyses.

RESULTS

By *in situ* hybridization, we found that cells expressing IFN-α1 (IFN-$\alpha1^+$), one of the major subtypes of type I IFN, expanded at days 7 and 11 and then declined at day 14 (Fig. 1A and 2A). Whereas the dynamics were qualitatively similar between the different groups analyzed, the magnitude of the response was clearly distinct. RMs of Indian origin (RMIs) infected by SIV produced, at the peak (day 11), higher levels of IFN-α1 (median, 16 IFN^+ cells/2 mm^2) than that observed in

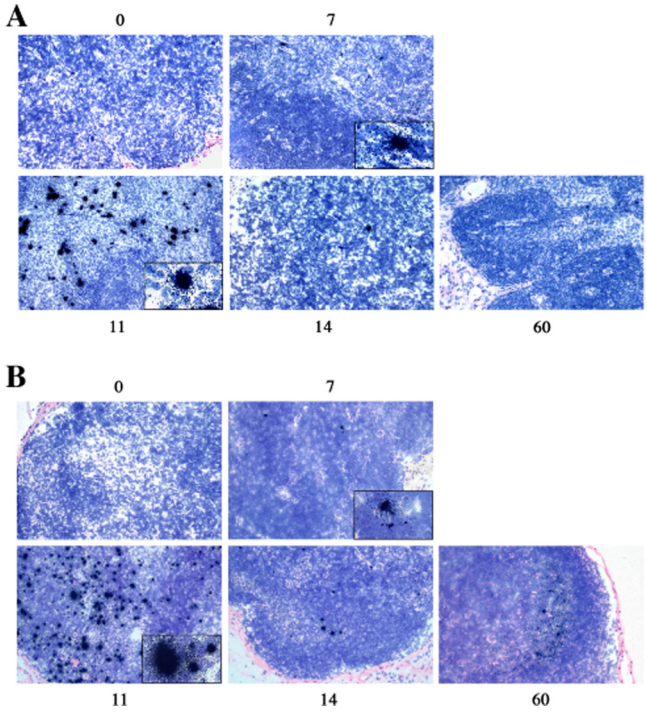

FIG. 1. Detection of IFNα1^+ cells and productive SIV infection in LNs from RMs of Indian origin. (A) Detection of IFN-α1^+ cells in LNs from one RM of Indian origin at 7, 11, 14, and 60 days after infection by *in situ* hybridization of (magnification, ×200). (B) Detection of virus-replicating cells (SIV RNA^+ cells) in LNs at 7, 11, 14, and 60 days after infection by *in situ* hybridization of the same monkey as shown above (magnification, ×200).

NC-RMs of Chinese origin (7.5 IFN^+ cells/2 mm^2; RMIs versus NC-RMs, $P = 0.04$), and C-RMs (2.5 IFN^+ cells/2 mm^2; RMIs versus C-RMs, $P = 0.0006$; NC-RMs versus C-RMs, $P = 0.003$) compared to uninfected monkeys (0.5 IFN^+ cells/2 mm^2). The levels at the peak were less than 2.0 IFN^+ cells/2 mm^2 in SIVΔ*nef*-infected RMs and in SIV-infected AGMs. At 2 months (Fig. 2A), the numbers of IFN-$\alpha1^+$ cells were higher in monkeys progressing faster to AIDS (RMIs [4.8 IFN^+ cells/2 mm^2] and NC-RMs of Chinese origin [3.2 IFN^+ cells/2 mm^2]; RMIs versus NC-RMs, $P = 0.02$) than that observed in nonprogressors (C-RMs [0.98 IFN^+ cells/2 mm^2] and AGMs [0.4 IFN^+ cells/2 mm^2]; NC-RMs versus C-RMs [$P = 0.0003$] and C-RMs versus AGMs [$P = 0.01$]). The levels of type I IFN detected in the plasma (Fig. 3) were consistent with the dynamics of IFN-$\alpha1^+$ cells in LNs and revealed at the peak higher levels in animals progressing faster to AIDS compared to nonprogressors (NC-RMs versus C-RMs [$P = 0.017$] and NC-RMs versus AGMs [$P = 0.001$]). Moreover, no significance difference was observed between C-RMs and AGMs (P = not significant). Our data also revealed a significant correlation between the numbers of IFN-$\alpha1^+$ cells at the peak and disease outcome (months of survival) (Fig. 2B). In order to analyze whether the Indian group had an impact on the correlation, we excluded this group, and found a positive correlation between the expression of type I IFN and the months of survival, including only NC- and C-RMs ($r^2 = 0.53$, $P = 0.0002$). Animals have been followed up for disease outcome

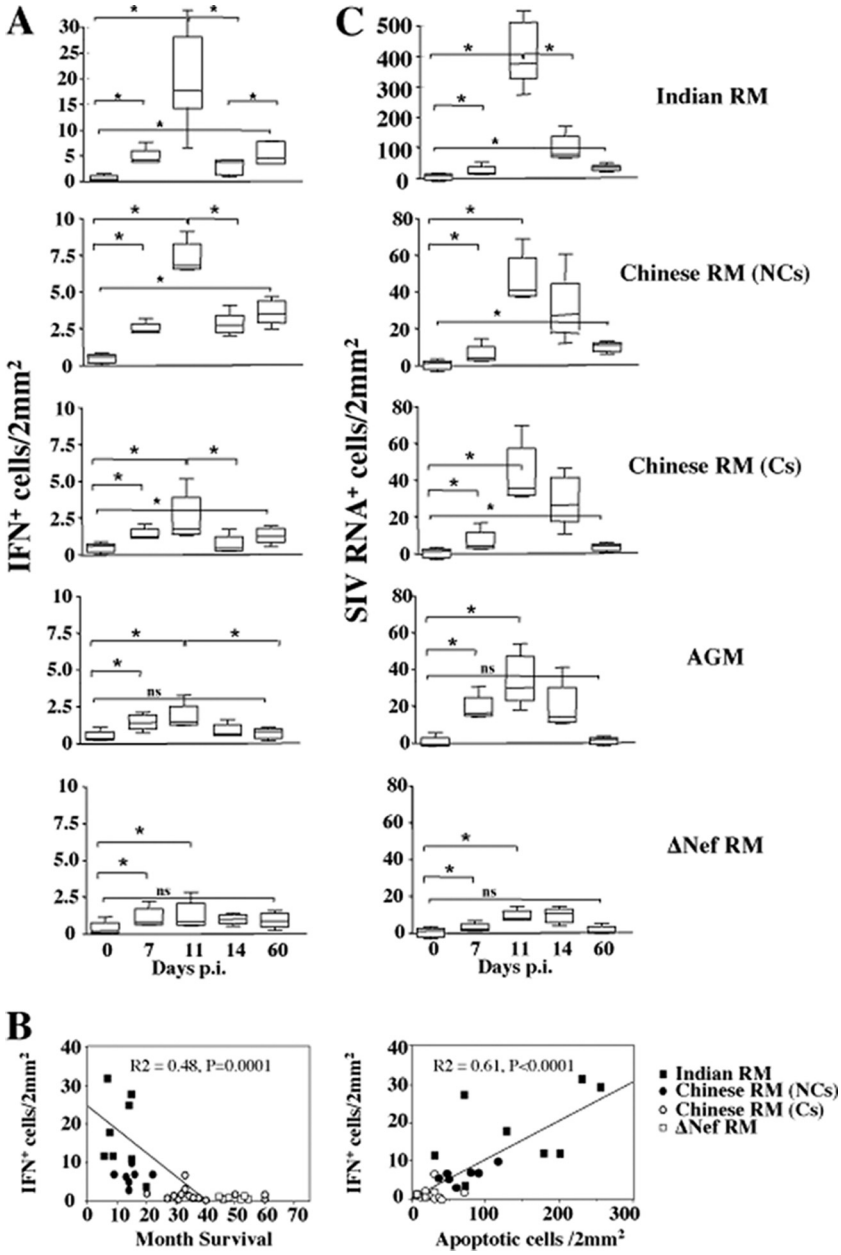

FIG. 2. Comparison of IFN-α1$^+$ cells expression in pathogenic and nonpathogenic SIV models. (A) Dynamics of IFN-α1$^+$ cells in LNs during primary SIV infection. IFN-α1-expressing cells (shown as IFN-α1$^+$ cells/2 mm^2) were detected by *in situ* hybridization at days 0, 7, 11, 14, and 60 in the LNs of Indian RMs, Chinese RMs (noncontrollers, NCs; controllers, Cs), AGMs, and Δ*nef* SIV-infected RMs. IFN-α1$^+$ cells were quantified, and means ± the standard deviations (SD) are shown. Statistical significance: ∗, $P < 0.05$; ns, not significant. (B) IFN-α1-expressing cells in LNs predict AIDS correlation between the number of IFN-α1$^+$ cells at day 11 and survival (months of survival) and the extent of apoptosis quantified by the TUNEL method at day 14. Each symbol represents one individual. (C) Dynamics of productive SIV infection in LNs during primary SIV infection. Virus-replicating cells (shown as SIV$^+$ cells/2 mm^2) were detected by *in situ* hybridization in the LNs of Indian RMs, Chinese RMs (noncontrollers, NCs; controllers, Cs), AGMs, and Δ*nef* SIV-infected RMs at 7, 11, 14, and 60 days after SIVmac251 infection. SIV-RNA$^+$ cells were quantified, and means ± the SD are shown. Statistical significances: ∗, $P < 0.05$; ns, not significant.

and killed at different time points postinfection corresponding to a wasting syndrome as previously described (9, 10, 33, 35, 36). The extent of apoptosis—a predictive factor of disease outcome (20, 49)—quantified in the tissues at the peak, also correlated with the levels of IFN-α1$^+$ cells (Fig. 2B).

A straightforward explanation would be that increasing viral replication in these tissues induced higher levels of IFN-α1$^+$ cells. Although we found that RMIs displayed higher numbers of SIV$^+$ RNA cells compared to RMs of Chinese origin at the peak (~10-fold higher, Fig. 2A), there was no significant difference in the extent of SIV$^+$ cells, as well as in plasma viral load between NC-RMs, C-RMs, and SIV-infected AGMs at the same time (Fig. 2C). Lower levels of SIV$^+$ cells and lower plasma viral load compared to the other groups of macaques

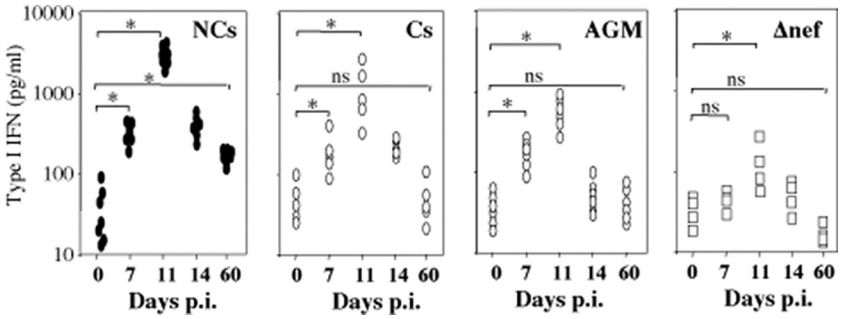

FIG. 3. Detection of type I IFN in the blood of SIV-infected monkeys. Type I IFN-α was assessed by ELISA. Each symbol represent an individual monkey. Statistical significance: *, $P < 0.05$; ns, not significant.

were detected only in SIVΔ*nef*-infected RMs. Consistent with previous reports (9, 27), these findings suggest that the level of viral replication at the peak does not discriminate between pathogenic and nonpathogenic primate models of SIV infection (RMIs nevertheless showed higher frequencies of infected cells and faster disease progression than Chinese RMs). These results also indicate that the extent of viral replication detected in the LNs was not the only factor driving higher levels of IFN-$\alpha1^+$ cells in the tissues at the peak of viral replication between RMs and AGMs.

Thereafter, the viral load declined. Thus, at day 60, the numbers of SIV^+ RNA cells were higher in Indian than in Chinese RMs (15.9/2 and 7.4/2 mm^2, $P = 0.001$) (Fig. 2C). However, it should be noted that at day 60, the number of SIV^+ RNA cells in LNs was extremely low in AGMs (0.5/2 mm^2), despite a detectable viral load (see Fig. 6A). Viral load in the blood also declined in the blood to reach a steady state (days 60 to 180). Higher levels in NC-RMs than in C-RMs and AGM ($P = 0.001$) were observed. Thus, the level of IFN-$\alpha1^+$ cells detected in the LNs at day 60 is more consistent with the notion that persistence of SIV replication in LNs was associated with higher levels of IFN-$\alpha1^+$ cells. These results are consistent with another report indicating that the expression of genes related to the IFN response in peripheral LNs was higher in progressors than in nonprogressors RMs (32).

Differential IFN-$\alpha1^+$ expression in pathogenic and nonpathogenic models, despite similar levels of viral replication during the acute phase, suggests an alternative explanation such as a difference in TLR signaling. However, in contrast to the previous report on sooty mangabeys (29), we found that *in vitro* stimulation of PBMC with TLR7 and TLR9 ligands induced similar levels of type I IFN-α as detected by ELISA from PBMC between both RMs of Indian and Chinese origins and AGMs (Fig. 4A).

Consistent with the notion that $CD123^+$ and $CD11c^-$ pDCs are potent producers of type I IFNs (4), we observed, by flow cytometry, that the predominant source of IFN-α2 was pDCs both in healthy RMs of Chinese origin and in AGMs (Fig. 4B).

Therefore, we next examined the dynamics of pDCs (Fig. 5) (6, 45). The percentage of pDCs in healthy RMs exhibited a mean of 0.17% (range, 0.057 to 0.30%) in the LNs. This percentage increased early after infection by days 7 and 11 coincident with the peak of type I IFN-expressing cells. The percentage of pDCs then drastically decreased and returned to baseline levels at day 14. Interestingly, the percentages of pDCs were higher in the LNs of NC-RMs than in LNs of C-RMs both at day 11 (0.6% ± 0.11% versus 0.31% ± 0.07%, $P = 0.005$) and at day 60 (0.25% ± 0.07% versus 0.11% ± 0.06%, $P = 0.01$), a finding consistent with the dynamics of IFN-α^+ cells. In contrast, we found no major changes in the dynamic of pDCs in LNs both in SIV-infected AGMs and SIVΔ*nef*-infected RMs. We cannot exclude that other cells produce type I IFN in the LNs, such as macrophages which may produce 10-fold less type I IFN than pDCs. However, during the acute phase, IFN-α^+ cells were essentially localized in the paracortical T-cell zone (Fig. 1) but not in the germinal centers, which contain a majority of B cells (37), and most of the IFN-α^+ cells were $CD123^+$ as determined by immunochemistry (data not shown), which is consistent with another report (25).

The production of chemokines and cytokines in an inflammatory environment participates in the recruitment of pDCs. Interestingly, the level of IL-8, which is a general marker of inflammation, was increased early after infection at day 7 in both RMs and AGMs (RMs of Indian origin, 300 ± 36 pg/ml; RMs of Chinese origin, 200 ± 25 pg/ml; AGMs, 220 ± 35 pg/ml) (Fig. 6B). Thereafter, IL-8 was highly expressed only in pathogenic SIV-infected RMIs (3,000 ± 232 pg/ml at day 14), as well of NC-RMs of Chinese origin (2,100 ± 270 pg/ml at day 14), compared to C-RMs (200 ± 45 pg/ml at day 14, $P = 0.0003$) and nonpathogenic SIV models (AGMs, 110 ± 29 pg/ml at day 14, $P = 0.0006$) (Fig. 6B). Other reports have also shown an increased level of IL-8 early after infection of RMs of Indian origin (5, 25). At day 60, the levels of IL-8 remained higher in monkeys progressing faster to AIDS (5,000 ± 269 pg/ml in RMIs, and 4,600 ± 320 pg/ml in NC-RMs) compared to AGMs (120 ± 14 pg/ml, $P = 0.0003$) and C-RMs (110 ± 23 pg/ml, $P = 0.0006$). However, the absence of IL-8 detection in the nonpathogenic AGM model was not related to defective TLR signaling (Fig. 6C). Thus, the inflammatory response is uncoupled from the extent of viral replication at the peak but characterizes pathogenic models of SIV infection.

DISCUSSION

This study showed an early elevation of IFN-α1 expression in peripheral LNs, as well type I IFN in the blood during the acute phase of SIV infections in both pathogenic and nonpathogenic primates. The magnitude during the acute phase

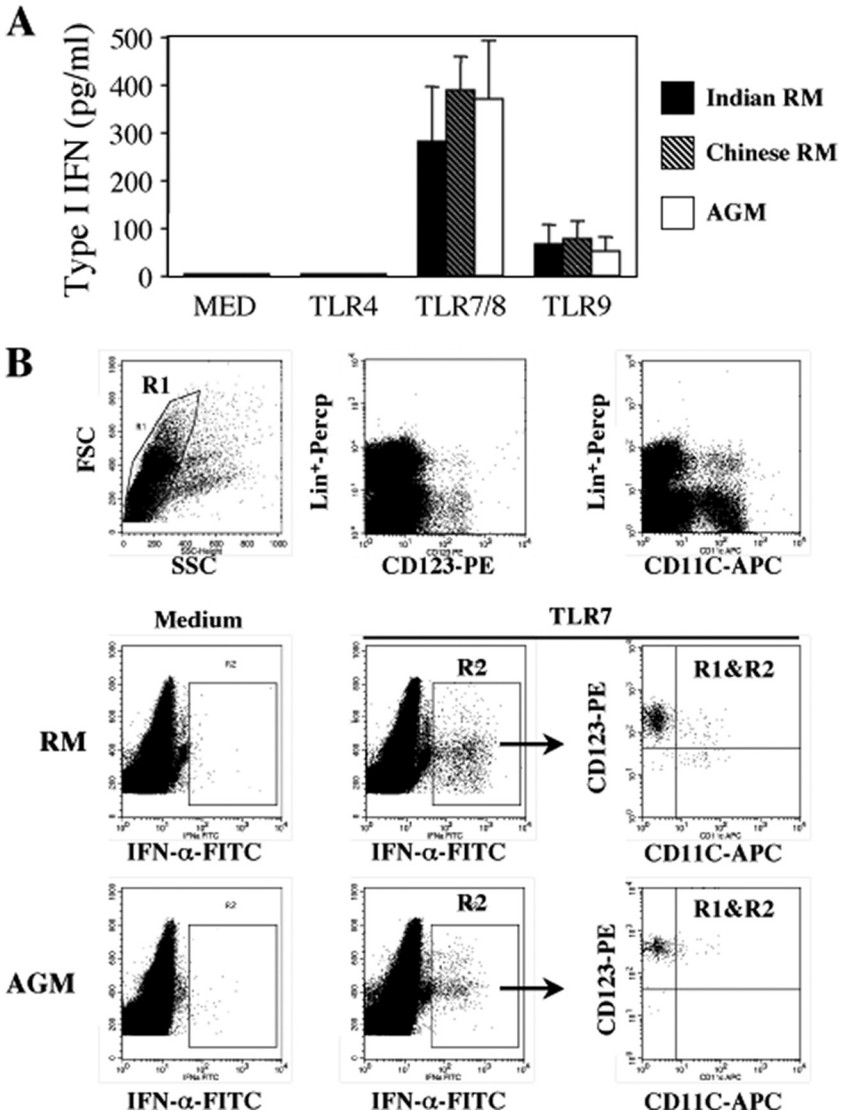

FIG. 4. IFN-α production after TLRs stimulation. (A) PBMC isolated from healthy monkeys were stimulated overnight in the absence (Med) or presence of the TLR agonists: LPS, 10 ng/ml (TLR4); CLO97, 1 μg/ml (TLR7); and CpG-DNA, 1 μM (TLR9). Supernatants were collected and assessed for the detection of type I IFN-α by ELISA. Values are means ± the SD ($n = 7$). (B) Phenotype of IFN-α2a-expressing cells by flow cytometry. PBMC isolated from monkeys (RM versus AGM) were stimulated overnight in the absence (Med) or presence of CLO97 at 1 μg/ml (TLR7). Cells were stained with antibodies directed against IFN-α2a, CD11c, and CD123 and included a lineage marker (Lin^+). The gating strategy is shown. Similar data have been obtained with four healthy monkeys of each species.

and the persistent expression of IFN-α1 at the set point is associated with a more rapid progression toward AIDS. The extent of viral replication in the tissues may participate in the direct elevation of type I IFN-α1; in fact, RMs of Indian origin exhibited increased expression of type I IFN-α1 and increased levels of viral replication compared to RMs of Chinese origin and macaques infected with the Δ*nef* SIVmac251 strain. However, despite similar levels of viral replication within LNs at the peak in AGMs and C- and NC-RMs of Chinese origin, the level of IFN-α1 expression was higher only in RMs that progress toward AIDS, compared to C-RMs and AGMs. This discrepancy was not due to a defect in TLR signaling between the different species, in contrast to an earlier report in sooty mangabey (29). Our data revealed that recruitment of pDCs, which represent the main cell type producing type I IFN (4), occurs early after SIV infection concomitantly with the peak of type I IFN. Higher recruitment of pDCs within LNs is observed in monkeys progressing more rapidly to AIDS and associated with greater inflammatory response (as measured by the presence of circulating IL-8). Recently, the examination of global gene expression changes of pathogenic (pigtail monkeys) and nonpathogenic (AGMs) SIV infections (23) has revealed in LNs that the greatest differential regulation of genes is related to (i) genes associated with cell death, which is consistent with the extent of apoptosis detected by the TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) method in the tissues of SIV-infected monkeys (9, 14, 33, 43, 49); (ii) inflammatory and chemotaxis genes,

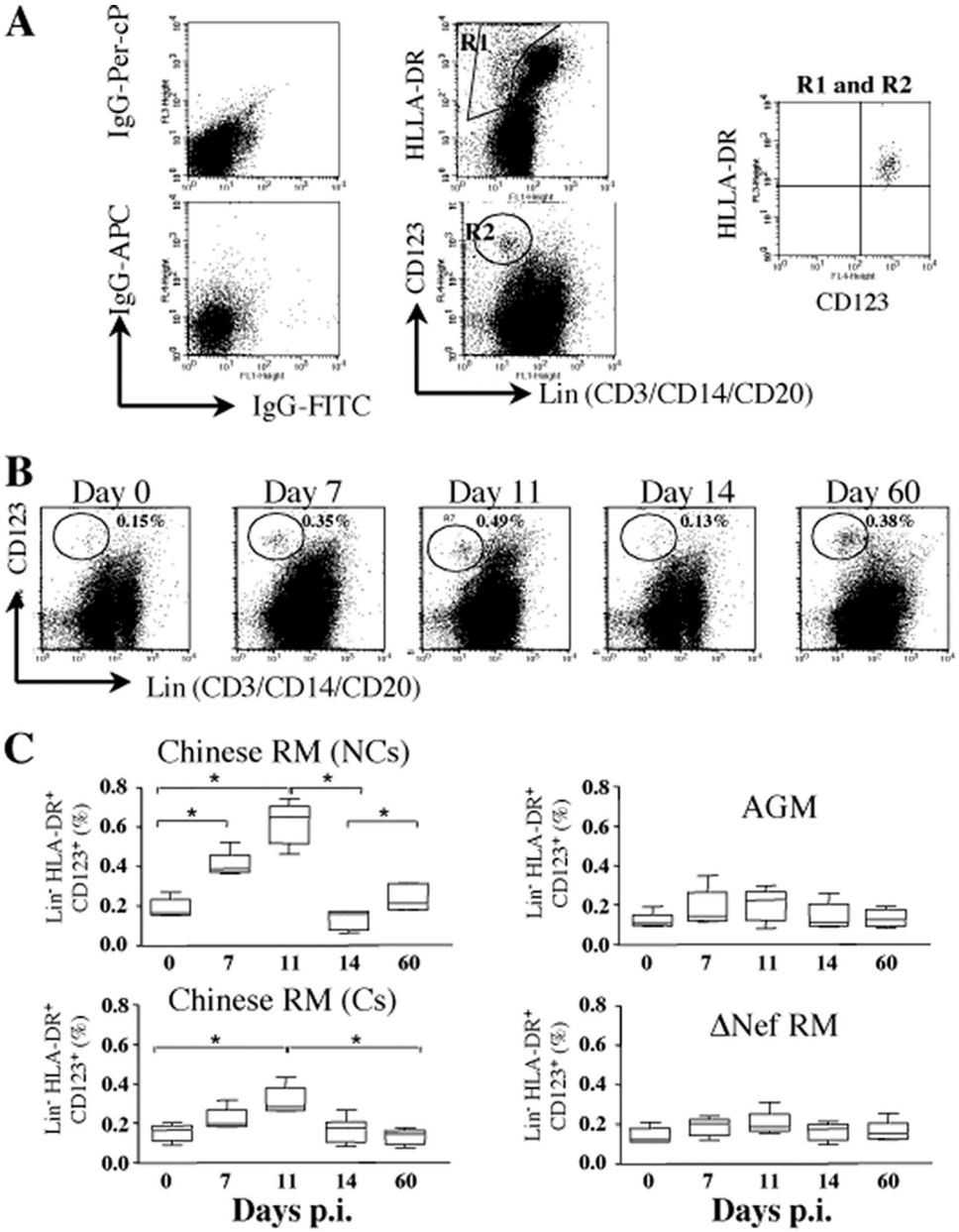

FIG. 5. Dynamics of pDCs in LNs during primary SIV infection. (A) Gating strategy used to analyze pDCs in LNs as defined by Lin^- $CD123^+$ $HLA\text{-}DR^+$ cells. (B) The results for one representative RM analyzed at different time points after infection are shown. (C) pDCs were defined as Lin^- $CD123^+$ $HLA\text{-}DR^+$ cells and analyzed by flow cytometry. Means ± the SD are shown in NC-RMs (NCs, $n = 6$) and C-RMs (Cs, $n = 6$), SIVΔ*nef*-infected RMs ($n = 6$), and SIV-infected AGMs ($n = 6$). Statistical significance: *, $P < 0.05$; ns, not significant.

which is consistent with the detection of IL-8 and other reports (1, 25, 40, 42); (iii) higher levels of immune activation consistent with previous studies showing that in NCs the extent of CD8 activation is greater than in CS RMs of Chinese origin (34, 36); and (iv) genes associated with the IFN response (1, 21, 41). Consistent with our data, Milush et al. (32) found that expression of type I IFN expression in LNs was more highly expressed in NC-RMs than in C-RMs. However, these authors observed that genes associated with the IFN response were differently expressed in mucosal tissues and peripheral LNs. Thus, these genes were more highly expressed in the LNs of RMs that do not control infection (NC-RMs), whereas the genes were expressed less in the colon, and vice versa, in nonprogressors (C-RMs). Lederer et al. (23) also observed higher levels of IFN gene in the colon of nonpathogenic AGMs compared to pathogen SIV-infected pigtail monkeys. More recently, mRNA expression of four immune modulators—IFN-α, oligoadenylate synthetase, CXCL9, and CXCL10—was positively associated with disease progression within the LN tissues of SIV-infected RMs of Indian origin (13) and consistent with the profile of mRNAs observed in lymphatic tissue of untreated HIV-infected persons during the acute phase (26). Thus, depending on the anatomical location, the levels of expression of IFN-associated genes may distinguish pathogenic and nonpathogenic infections.

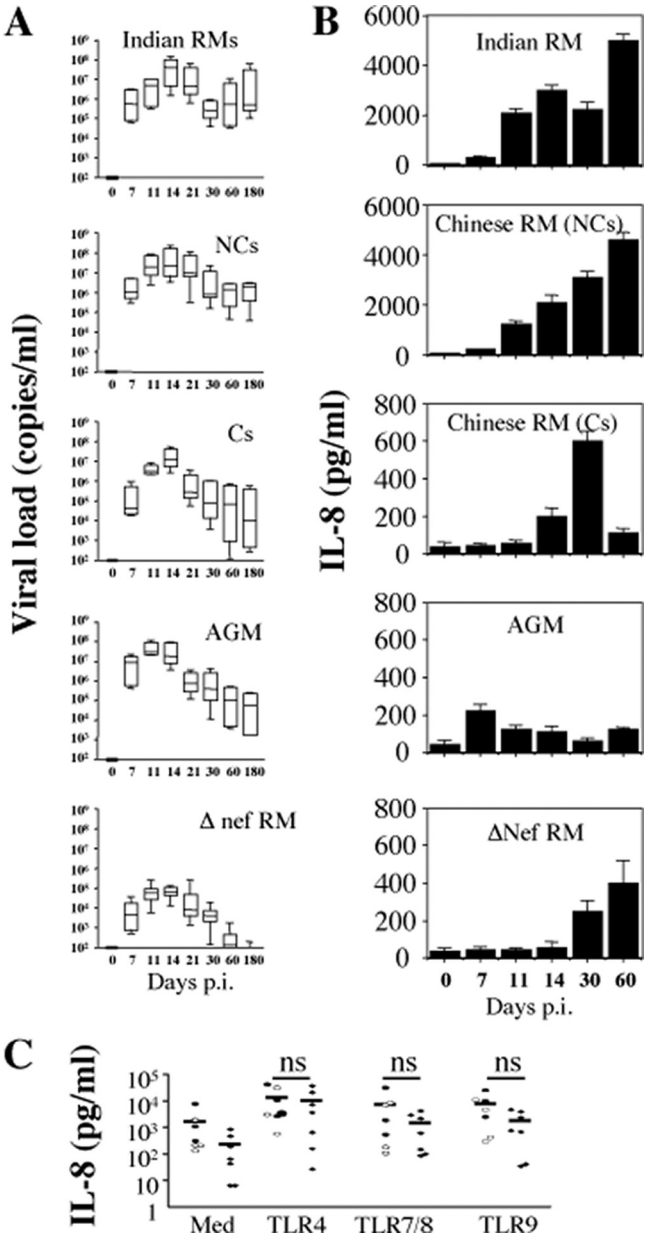

FIG. 6. Dynamics of IL-8 during primary SIV-infection. (A) Viral load was quantified in peripheral blood of SIV-infected Indian RMs, Chinese NC- and C-RMs, SIV-infected AGMs, and SIVΔ*nef*-infected RMs at different days postinfection. (B) The levels of IL-8 were detected in the sera by ELISA at days 0, 7, 14, 21, 30, and 60 after inoculation. Values are means ± the SD ($n = 6$). (C) IL-8 production after TLRs stimulation. PBMC isolated from healthy monkeys (solid ovals, Indian RMs; open ovals, Chinese RMs; solid diamonds, AGMs [$n = 7$]) were stimulated overnight in the absence (Med) or presence of the following TLR agonists: LPS, 10 ng/ml (TLR4); CLO97, 1 μg/ml (TLR7); and CpG-DNA, 1 μM (TLR9). Supernatants were collected and assessed for the detection of IL-8 by ELISA. Each symbol represents one individual.

A further observation was the persistence of IFN-α expression and pDC recruitment in the peripheral LNs of animals progressing to AIDS. These findings provide evidence that productive infection persists despite a prolonged IFN-α response within peripheral LNs. This was consistent with gene expression (23), since most of the significant changes between days 10 and 45 postinfection in the LNs and the colon involved genes associated with IFN responses and were significantly attenuated during nonpathogenic SIV infection (AGMs) compared to pathogenic SIV infection (pigtail monkeys). The decreased expression of these genes and the lower levels of IFN-$\alpha 1^+$ cells detected in the LNs of AGMs may in part be due to decreased viral replication in these LNs, despite similar levels of viral load in the blood. However, we do not know whether this also occurs in the colon. Thus, it has been assumed that the strong and early IFN signaling in the colons of SIV-infected AGMs should be a mechanism for limiting SIV pathogenesis, and such early attenuation of the inflammatory response in AGMs may help to reduce damage (23). Similar observations have been reported in macaques that progress slowly (32). Thus, depending on the anatomical location, the production of type I IFN should be beneficial or detrimental, controlling or favoring an inflammatory response.

Activities and therapeutic uses of type I IFNs have clearly established a wide breadth of biological activities. In particular, it has been reported in the past that low-dose IFN-α treatment exerts an anti-inflammatory control action associated with a significant reduction in tissue infiltration of neutrophils, macrophages, and lymphocytes. In contrast, high doses can directly induce proinflammatory and pyretic responses in humans, and a common adverse reaction is lymphopenia associated with flulike symptoms (for reviews, see references 2 and 38).

The notion that type I IFN could be more detrimental than beneficial during HIV infection was originally proposed a decade ago; in fact, it has been demonstrated that type I IFN in the sera of chronically HIV-infected patients was associated with disease progression, instead of resolution (17, 22), and the loss of uninfected T cells *in vitro* due to HIV-1 is mediated by IFN-α (50). It has also been reported that IFN-α derived from pDCs, following *in vitro* exposure to HIV, was implicated in the killing of T cells (19). Thus, type I IFNs may lead to immune deviation by altering CD4 Th-cell differentiation through the promotion of CD4 T-cell apoptosis during the acute phase (9, 14, 24, 33, 43, 49). Indeed, the events occurring early after infection play a key role in conditioning further disease evolution in particular the loss of memory CD4 T cells (24, 30, 35, 39, 44, 48). However, it remains to be determined whether pDCs from SIV-infected monkeys are capable of inducing CD4 T-cell death.

Moreover, models of viral infection in mice, such as infection with lymphocytic choriomeningitis virus or measles virus, have established that there is induction of a generalized immune suppression in their natural hosts. This occurs early after infection concomitantly with a change in the balance of type I IFN and IL-12 expression (3, 11, 18) in which type I IFN antagonizes IL-12 production (8, 31). IL-12 is a cytokine essential in the development of cell-mediated immunity (47) and cell survival (7, 15, 16). The peak of type I IFN observed in our study early after infection may lead to decreased IL-12 expression in LNs during the acute phase. Thus, the recruitment of pDCs in the peripheral LNs during the acute phase of SIV infection could have a detrimental indirect effect on CD4 T-cell function. It remains to be determined whether the balance

of IL-12 expression is affected during the acute phase in both pathogenic and nonpathogenic primate models.

In conclusion, our data demonstrate that the increased number of IFN-α1$^+$ cells in the peripheral LNs of RMs of Chinese origin pathogenically infected with SIV is at least related to an early elevation in the level of recruitment of pDCs and associated with an increased inflammatory environment during the acute phase. Conversely, reduced recruitment of pDCs and reduced expression of IFN-α1 in the peripheral LNs is correlated with nonpathogenic disease outcome, despite an intense peak of viral replication.

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