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Variable Course of Primary Simian Immunodeficiency Virus Infection in Lymph Nodes: Relation to Disease Progression

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To investigate the dynamics of spread of simian immunodeficiency virus (SIV) in the lymphoid organs, we sequentially analyzed the viral burden in lymph nodes (LN) of eight rhesus macaques inoculated intravenously with a high or low dose of the pathogenic SIVmac 251 isolate. For each animal, four axillary or inguinal LN were collected during the first weeks of infection and a fifth LN was taken 6 or 8 months later to estimate disease progression. Measurement of SIV RNA by in situ hybridization showed that all of the macaques studied had a phase of acute viral replication in LN between 7 and 14 days postinoculation which paralleled that observed in the blood. In a second phase, productive infection was controlled and viral particles were trapped in the germinal centers that developed in LN. While the peaks of productive infection were similar for the eight animals, marked differences in the numbers of productively infected cells that persisted in LN after primary infection were seen. Differences were less pronounced in the blood, where productive infection was efficiently controlled in all cases. The persistence of productively infected cells in LN after primary infection was found to be associated with more rapid disease progression, as measured by the decrease of the T4/T8 ratio and the occurrence of clinical signs. However, the persistence of a significant level of viral particles in germinal centers was observed even in animals that remained healthy over a 1- to 2-year observation period. This study indicates that the course of primary SIV infection in LN is variable, and it suggests that the initial capacity of the host to control productive infection in LN may determine the rate of disease progression.

The central role of lymphoid organs in the pathogenesis of human immunodeficiency virus (HIV) infection is being increasingly recognized. Lymph nodes (LN) harbor a viral reservoir during the asymptomatic stage of the infection, in the form of viral particles trapped at the surface of follicular dendritic cells (FDC) (1, 3, 5, 15, 24, 32, 35, 36). Even during clinical latency, HIV infection is never completely silent. Productively infected cells are detected at a higher frequency in LN than in peripheral blood, emphasizing the progressive nature of HIV infection in lymphoid organs (21, 23). In addition, a significant percentage of LN lymphocytes are latently infected and constitute a possible source for reactivation of virus production (12). These findings point to the fact that HIV is widely disseminated in lymphoid tissue during asymptomatic infection. Little is known, however, about the initial phase of infection in LN. The mechanisms of viral dissemination into lymphoid tissues remain to be determined, as does the transition between the primary and clinically latent stages. In particular, it is not known whether the viral load reached in LN during primary infection correlates with that observed in chronic infection and whether it is predictive of the course of the disease. Clinical studies suggest that the severity of primary HIV infection is associated with the rapidity of disease progression (20, 33, 37). However, the relationship between early viral burden and disease progression is not clear, as measurement of the HIV RNA copy number in the blood at seroconversion did not distinguish between rapid progression and slow progression (17). As the viral burden in lymphoid organs may differ from that in the blood at an early stage, the possible correlation between early- and late-stage infections remains an open question.

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Because of the difficulty in obtaining human specimens early after HIV infection, a relevant animal model appears to be indispensable for a better understanding of the seeding of the virus in LN. Infection of rhesus macaques with simian immunodeficiency virus (SIV) provides such a model, as SIV is a retrovirus closely related to HIV in pathogenicity (10, 22) and genetic structure (6). In particular, SIV infection induces progressive LN changes similar to those seen in human HIV infection, with a phase of follicular hyperplasia, followed by follicular involution and lymphoid depletion (8, 18, 28). Target cells are also similar, as SIV can be detected at the surface of FDC in hyperplasic LN and causes widespread infection of macrophages and lymphocytes at later stages (27, 39).

In this study, lymph nodes were taken sequentially during the first weeks of SIV infection of macaques to determine the dynamics of viral spread in the lymphoid organs. Primary SIV infection in LN was found to consist of two phases, with an initial burst of virus replication, followed by accumulation of viral particles in the germinal centers. The viral burden that persisted in LN after the acute phase varied markedly among the animals, indicating that differences in susceptibility to SIV were apparent in the early stages of infection. The persistence of productively infected cells in LN, rather than that of viral particles, was found to be associated with rapid disease progression.

MATERIALS AND METHODS

Virus inoculation. Eight adult rhesus monkeys (*Macaca mulatta*) were included in this study. Prior to inoculation, they were demonstrated to be seronegative for simian T-cell leukemia virus type 1, simian retrovirus type 1 (type D retrovirus), herpesvirus B, and SIVmac. All procedures with animals were performed after anesthesia with ketamine (Imalgène; Mérieux). The animals were infected with the pathogenic SIVmac 251 isolate provided by R. Desrosiers (10). In the first

experimental series, four monkeys were inoculated intravenously with a high dose of SIVmac 251 consisting of 3×10^3 50% tissue culture-infective doses. In the second experimental series, four other monkeys were inoculated with a lower dose corresponding to 10 50% animal-infective doses of an in vivo-titrated stock of SIVmac 251 provided by Anne-Marie Aubertin (Université Louis Pasteur, Strasbourg, France). Previous titration experiments had shown that 10 50% animal-infective doses correspond to 0.32 50% tissue culture-infective doses (1a). Thus, it can be estimated that the low-dose group received 10^4 times less virus than the high-dose group.

LN collection. Four axillary or inguinal LN were collected from each animal during the early stages of infection, between days 7 and 68 postinoculation (p.i.), and a fifth LN was taken 6 or 8 months later to estimate disease progression. LN were taken surgically and frozen in isopentane cooled in liquid nitrogen. After being embedded in O.C.T. compound (Tissue-tek; Miles), LN were cryostat sectioned at 4-μm intervals and the sections were stored at −20°C until use.

Preparation of peripheral blood mononuclear cells (PBMC). Heparinized blood was collected from the macaques, diluted two times in RPMI 1640, and separated on a J-prep (J. Bio, Les Ulis, France) density gradient (1.077 g/ml). PBMC were washed two times in RPMI 1640 and resuspended at 10^7/ml in RPMI 1640 supplemented with 10% fetal bovine serum. PBMC were spotted onto slides coated with 3-aminopropyltriethoxysilane (Sigma), at a density of 2×10^5 per spot. The slides were air dried for a minimum of 2 h, fixed for 10 min in acetone, and kept frozen before processing by in situ hybridization.

Serologic assays. Viremia was detected by SIV p27 Gag antigen measurement in monkey sera with a SIVmac antigen capture enzyme-linked immunosorbent assay (ELISA) (Coulter). The antibody response to SIV was monitored with an HIV-2 ELISA (Diagnostics Pasteur) performed on serum samples at a 1/100 dilution.

Determination of T4 and T8 lymphocyte subsets. Lymphocyte subsets were monitored by flow cytometry after labeling with anti-CD4 (OKT4; Ortho-mune) and anti-CD8 (Leu2a; Becton Dickinson) monoclonal antibodies conjugated with phycoerythrin. EDTA-treated blood was incubated for 15 min with monoclonal antibodies added at a 1/20 dilution. Erythrocytes were lysed with Lyse & Fix reagents (Immunotech). The samples were washed three times in phosphate-buffered saline and analyzed on a FACScan flow cytometer (Becton Dickinson).

Preparation of ^{35}S-labelled RNA probes. RNA probes were derived from the transcription vector Bluescript (Stratagene) into which a fragment of the SIV mac 142 plasmid clone spanning the *nef* gene (nucleotides 8718 to 8234) (6) was inserted. This SIV-specific riboprobe was chosen because all types of SIV RNAs, genomic as well as singly or doubly spliced, hybridize to this region of the genome. The antisense riboprobe used to detect SIV RNA was generated from the T7 promoter by in vitro transcription of 1 μg of a plasmid template with 50 U of T7 RNA polymerase in the presence of 50 μCi of [^{35}S]UTP and [^{35}S]ATP. Two labeled nucleotides were included in the transcription reaction to increase the specific activity of the probe. After incubation for 1 h at 37°C, the DNA template was digested with 10 U of DNase I for 15 min at 37°C. To enhance the penetration of the probe into tissue sections, the ^{35}S-labeled RNA was subjected to mild alkaline hydrolysis in 80 mM $NaHCO_3$ and 120 mM Na_2CO_3 at 60°C. The hydrolysis time was optimized to obtain a majority of fragments in the 150- to 200-nucleotide range. After neutralization with 600 mM Na acetate and 167 mM acetic acid, the probe was purified by phenol chloroform extractions and ethanol precipitation. Specific activity ranged between 1×10^8 and 2×10^8 dpm/μg of input DNA.

In situ hybridization. The hybridization techniques used were based upon published procedures (4, 34). Hybridization was carried out on tissue sections recently cut on a cryostat to minimize RNA degradation during storage. Sections were fixed in 4% paraformaldehyde and acetylated in acetic anhydride-triethanolamine (pH 8) to minimize the background. Sections were denatured in 70% formamide at 70°C for 2 min to enhance the accessibility of nucleic acids. The hybridization mixture contained the ^{35}S-labeled riboprobe at 50,000 dpm/μl, 50% formamide, 10% (wt/vol) dextran sulfate, 0.3 M NaCl, 20 mM Tris (pH 7.5), 5 mM EDTA, 10 mM NaH_2PO_4, 1× Denhardt's solution, 0.5 mg of yeast tRNA per ml, and 100 mM dithiothreitol. The mixture was heated at 80°C for 2 min and applied to slides. Coverslips were mounted and sealed with rubber cement, and hybridization was carried out at 45°C overnight in a humid chamber. Slides were rinsed successively in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10 mM dithiothreitol for 1 h at room temperature, in 50% formamide–2× SSC–10 mM dithiothreitol for 30 min at 60°C, in 2× SSC for 30 min at room temperature, and in 0.1× SSC for 1 h at room temperature and then dehydrated in ethanol with 0.3 M ammonium acetate. Slides were coated with NTB2 nuclear track emulsion (Kodak) diluted 1:1 with 0.6 M ammonium acetate and autoradiographed for 20 days at 4°C. The long exposure time ensured that weak hybridization signals, such as those present in germinal centers, would be detected. After exposure, slides were treated with Kodak D-19 developer, fixed, and stained with hematoxylin-eosin. Controls included hybridization of SIV-infected and uninfected, cultured lymphocytes, hybridization of LN tissue from uninfected monkeys, and hybridization with a control human T-cell leukemia virus type I RNA probe unrelated to SIV.

Image analysis. Hybridization in germinal centers was quantified with a computerized image analysis system. LN sections were viewed at a final magnification of ×1,000, and three measurements were made in distinct germinal centers. For each measurement, the image was acquired through a 3-CCD video camera attached to a Nikon-FXA microscope. The image was digitized and analyzed with Optilab software, version 2.1 (Graftek, Mirmande, France). The threshold was set so as to distinguish the silver grains from the lymphoid tissues. The surface occupied by silver grains was automatically measured on a 100-μm^2 region. Background hybridization was evaluated for each section and subtracted. The mean percentage of the germinal center area covered by silver grains is reported (see Fig. 2B).

Productively infected cells in LN and PBMC were counted manually on a 2-mm^2 grid. The mean count obtained for three slides is indicated (see Fig. 2A and 4B).

RESULTS

Variable course of early SIV infection in LN. To characterize the initial stages of SIV infection in lymphoid organs, we determined the viral load in LN obtained sequentially from eight rhesus monkeys inoculated with the pathogenic SIVmac 251 isolate. To evaluate the dose effect on the kinetics of LN infection, four of the animals were inoculated intravenously with a high dose of virus, while four others received a low dose (10 50% animal-infective doses) of SIVmac 251. The viral load in LN sections was analyzed by in situ hybridization by using a radioactive riboprobe specific for SIV RNA.

The evolution of early infection in LN is shown in Fig. 1 for

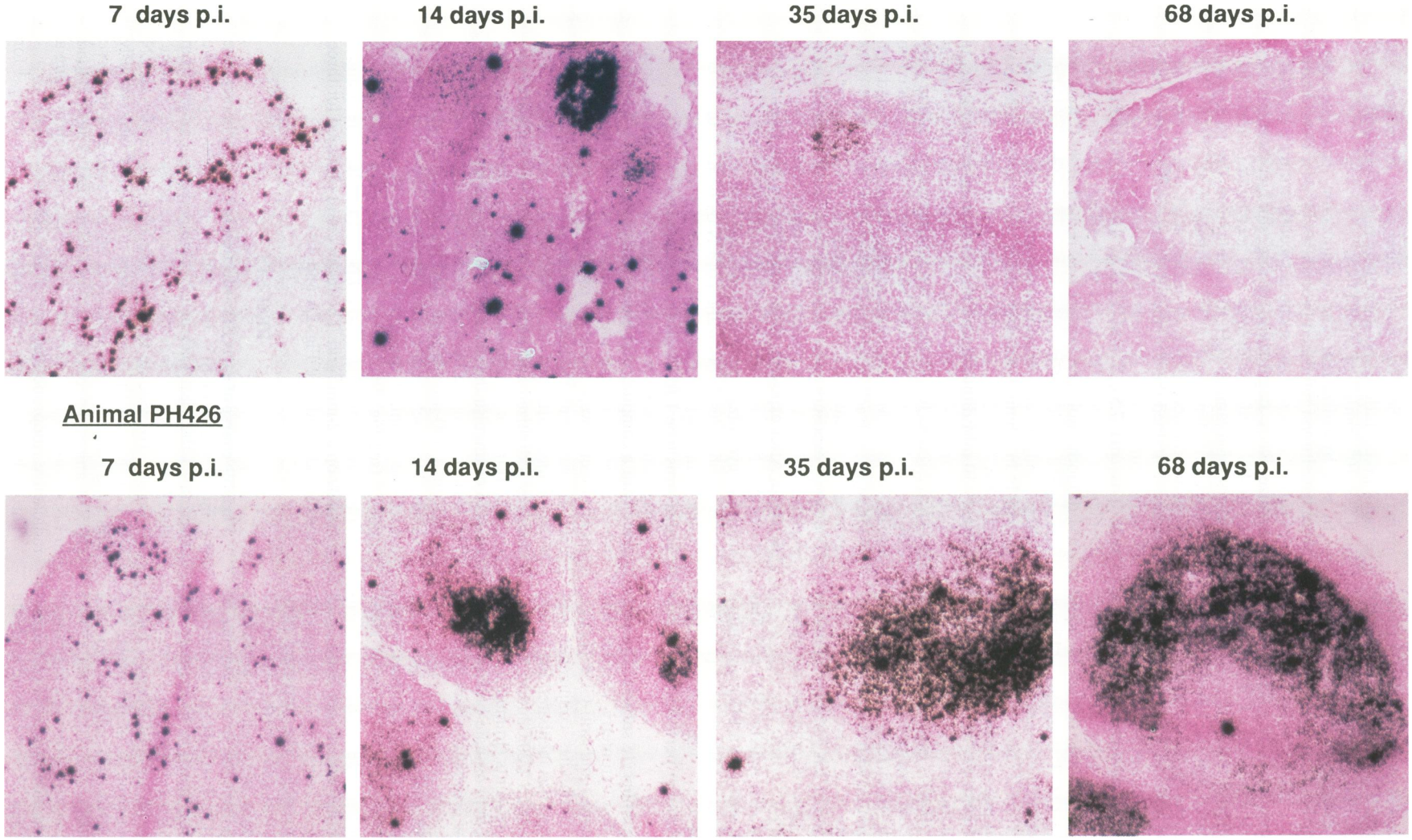

FIG. 1. Comparison of the courses of early SIV infection in LN of two rhesus macaques. LN obtained sequentially during early SIV infection were analyzed by in situ hybridization. Viral RNA was detected with a SIV-specific riboprobe labeled with ^{35}S. Hybridizations are shown for two animals of the high-dose group, P4 and PH426 (magnification, ×200). Spots corresponding to productively infected cells were detected in LN at 7 days p.i. The concentration of spots at the border of the tissue section (animal P4) indicates the presence of infected cells in LN sinuses. At later stages, a more diffuse signal, corresponding to accumulation of viral particles in the germinal centers, was detected.

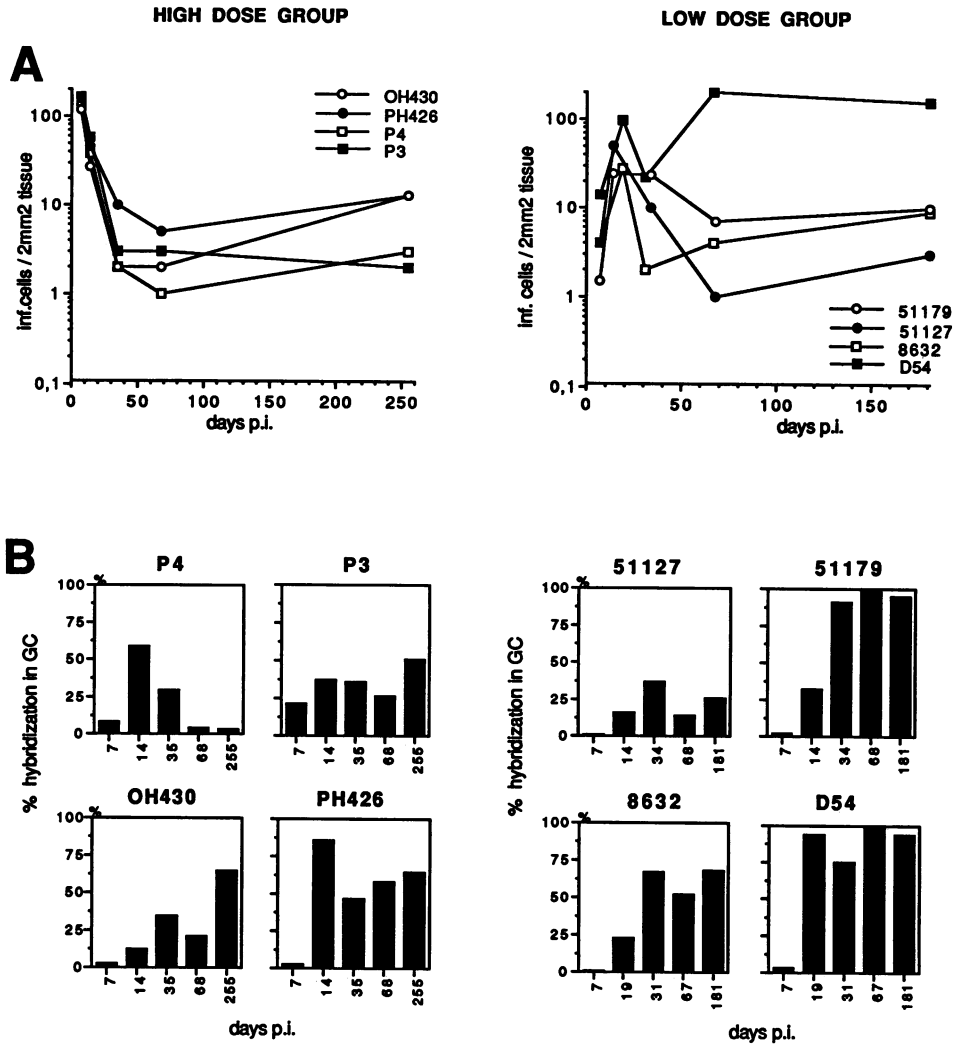

FIG. 2. Quantitation of the viral load detected by in situ hybridization in LN of SIV-infected macaques. SIV RNA was detected in LN obtained from the four macaques of the high-dose group (left) and the four macaques of the low-dose group (right). Two types of hybridization signals were quantified. The number of productively infected cells, detected as spots, was counted on a 2-mm^2 area of the paracortex (A). The mean count obtained for three sections is indicated. The diffuse signal in the germinal centers was quantified by image analysis on a 100-μm^2 area (B). Each solid bar symbolizes the percentage of the area covered by silver grains. The percentage indicated represents the mean obtained for three measurements in distinct germinal centers.

two representative macaques of the high-dose group, P4 and PH426. A large number of infected cells was detected in the LN of both animals as early as 7 days p.i. These cells were found scattered throughout the LN tissue and contained a large amount of SIV RNA, a finding indicative of productive viral replication. A shift in the infection pattern was observed at 2 weeks p.i., with a concentration of SIV RNA in the germinal centers of the follicles. At this stage, two types of hybridization signals were simultaneously detected: spots, corresponding to isolated infected cells, and a more diffuse signal localized to the germinal centers. We had previously characterized the nature of the target cells in SIV-infected LN by using combined in situ hybridization-immunohistochemistry (7). The spots were identified as infected macrophages and T4 lymphocytes, while the diffuse signal corresponded mostly to accumulation of viral particles on the FDC present in the germinal centers. At 1 month p.i., a difference between the LN of the two animals was detected. P4 LN had a low viral load with few productively infected cells and a reduced amount of viral RNA in the germinal centers. In contrast, PH426 LN had a relatively high viral load with persistence of productively infected cells and of a large amount of viral RNA in the germinal centers. As can be seen in Fig. 1, the difference became even more apparent at 68 days p.i., as the virus was barely detectable in the LN of P4 while both infected cells and diffuse viral RNA persisted in the LN of PH426. In addition, PH426 LN already showed pathological signs at this stage, such as marked follicular hyperplasia and infiltration of CD8$^+$ cells in the germinal centers (data not shown). Thus, comparison of these two animals revealed that SIV infection could evolve differently in macaques which initially had similar phases of acute infection in lymphoid organs.

Quantification of the viral load in LN. To evaluate the viral load in LN better, we quantified the signal detected by in situ hybridization for each of the animals studied. The number of infected cells, detected as spots, in 2 mm^2 of the paracortical

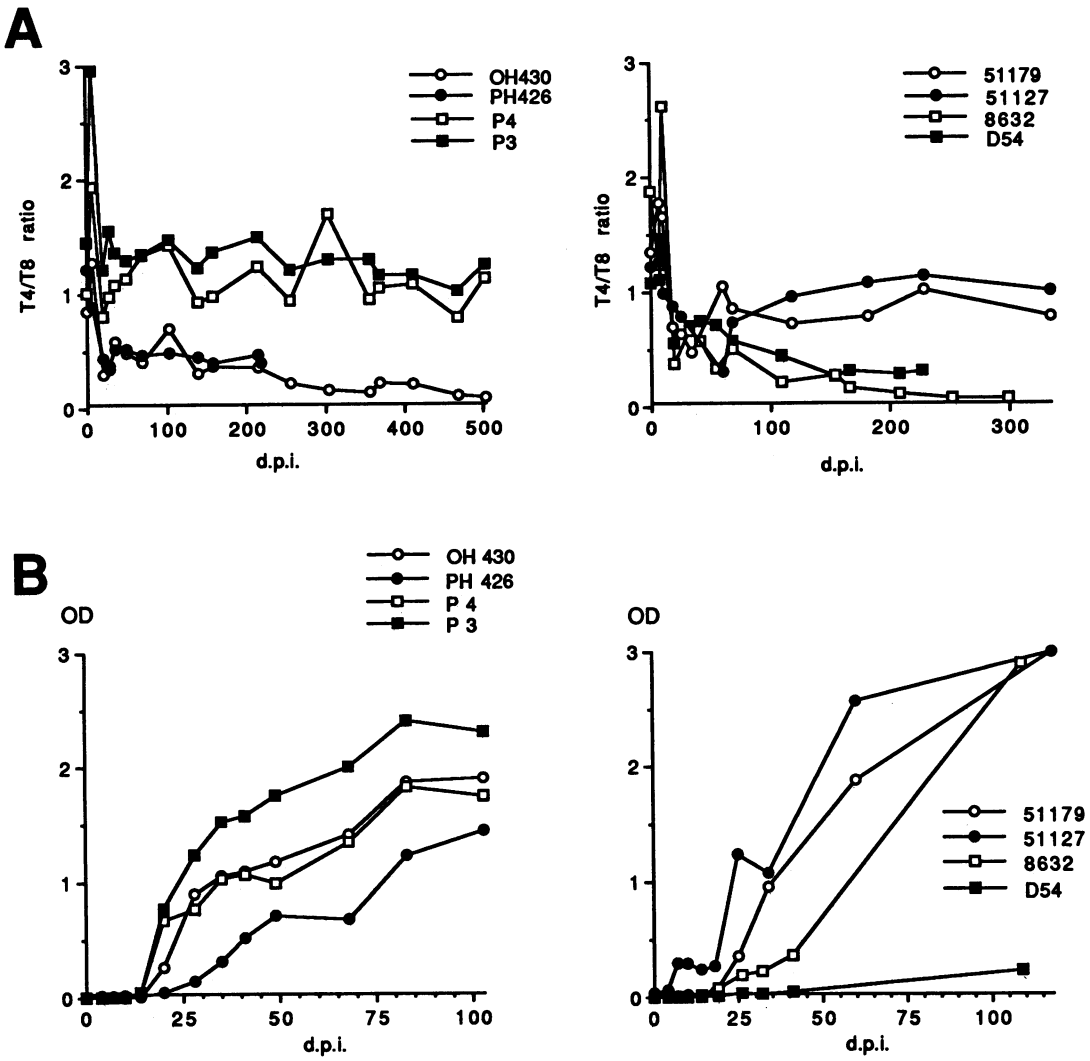

FIG. 3. Evolution of immunological parameters in the course of SIV infection. (A) Evolution of the T4/T8 lymphocyte ratio. Peripheral blood samples were labeled with phycoerythrin-conjugated OKT4 (anti-CD4) and Leu2a (anti-CD8) monoclonal antibodies and analyzed by flow cytometry. (B) Development of the antibody response against SIV. Macaque sera diluted 1:100 were tested with a HIV-2 antibody ELISA that cross-reacts with SIV. d.p.i., days p.i.

area of LN tissue sections was counted. The four animals infected with a high dose of SIV had equivalent numbers of infected cells during the primary phase of infection, ranging from 100 to 200 cells per 2 mm^2 at 7 days p.i. (Fig. 2A, left). This number rapidly dropped to less than 10 cells per 2 mm^2 at 1 month p.i. The control of productive infection was not complete, however, and differed among the animals. Between one and six infected cells per 2 mm^2 persisted at 2 months p.i. (animals P4 and PH426, respectively). At later stages, an increase of productive infection was noted for two of the animals (PH426 and OH430), while two others (P4 and P3) maintained a low level of chronic infection.

The four animals inoculated with a low dose of SIV had unexpectedly high viral loads in LN during primary infection (Fig. 2A, right). At the peak, the number of productively infected cells was only two to five times less than that of the high-dose group. In comparison, the inocula were estimated to differ by a factor 10^4 between the two groups. Thus, the intensity of acute infection did not depend strictly on the viral dose. The kinetics of infection was delayed, however, as the peak was observed 1 week later in the low-dose group, at 14 instead of 7 days p.i. Comparison of the four animals in the low-dose group showed limited variability in the peak value of productively infected cells (24 to 100 cells per 2 mm^2). In contrast, remarkable variability in the control of productive infection occurred. The number of productively infected cells that persisted in LN at 2 months p.i. ranged from 1 to 200 cells per 2 mm^2. Animal 51127 maintained a low viral load, animals 51179 and 8632 were intermediate, and animal D54 appeared to be particularly susceptible to SIV, with a prolonged phase of virus replication in LN. The level of productive infection observed at 2 months p.i. was maintained or slightly increased at 6 months p.i., suggesting the establishment of a relatively stable phase of chronic viral replication in LN.

The other component of the viral load in LN, which corresponded to the amount of virus present in the germinal centers, was also quantified (Fig. 2B). The portion of the surface of the germinal centers covered by silver grains was

TABLE 1. Markers of clinical progression[a]

Group and animal	T4/T8 ratio	Clinical progression
High dose[b]		
P4	1.13	No sign
P3	1.24	No sign
OH430	0.09	No sign
PH426	(0.4)	Thrombocytopenia, intestinal necrosis, death at 255 days p.i.
Low dose[c]		
51127	1.01	No sign
51179	0.79	No sign
8632	0.08	Lymphoma, death at 311 days p.i.
D54	(0.32)	Wasting pancreatitis, death at 227 days p.i.

[a] The last T4/T8 lymphocyte ratio determined and the clinical status are indicated for the eight macaques studied. The observation times were 500 and 300 days for the high- and low-dose groups, respectively. The values in parentheses are T4/T8 ratios at the time of death.
[b] The T4/T8 ratios of this group were determined at 500 days p.i.
[c] The T4/T8 ratios of this group were determined at 300 days p.i.

determined by using a video-based image-analyzing system. Virus accumulation in the germinal centers peaked between 14 and 35 days p.i. and thus appeared to follow in time the peak of productively infected cells. This observation supports the notion of trapping of viral particles previously produced during the acute phase of infection. All of the animals except P4 maintained detectable amounts of virus in the germinal centers after 35 days p.i. However, these levels differed markedly among the animals. In particular, four monkeys (PH426, 8632, 51179, and D54) had a persisting high viral load while the other three showed a down-regulation at 68 days p.i., followed by yet another increase in virus at later stages. Comparison of Fig. 2A and B shows that animals with a high number of productively infected cells in LN tissue also harbored a high viral load in the germinal centers. The reciprocal was not true, however, as some animals had a significant amount of virus in the germinal centers but few productively infected cells in LN. Animal P3, for example, had only two infected cells per 2 mm^2 at 255 days p.i. while 51% of the germinal center area was covered by silver grains. This suggested that virus presence in the germinal centers was not necessarily associated with active infection.

Taken together, the data show that viral spread in LN occurred in two phases, with a uniform peak of intense viral replication, followed by a more variable stage of viral down-regulation and virus trapping in the germinal centers.

Comparison of clinical and immunological parameters. We next asked whether the evolution of SIV infection in LN was predictive of the rate of progression towards disease for the eight animals studied. The T4/T8 ratio (Fig. 3A) and the clinical status (Table 1) were monitored over periods of 500 and 300 days for the high- and low-dose groups, respectively. Three animals showed rapid disease evolution accompanied by a marked decrease in the T4/T8 ratio. These animals had a high viral load in germinal centers and a high (PH426 and D54) or intermediate (8632) level of infected cells in LN. In addition, D54 and PH426, which had the highest viral load in LN, rapidly developed disease and had to be sacrificed at days 227 and 255 because of intestinal necrosis associated with thrombosis and pancreatitis, respectively. Animal 8632 also became immunodeficient and was sacrificed at day 311 because of lymphoma. Two animals experienced intermediate disease evolution, with absence of clinical signs but a marked (OH430) or limited (51179) decrease in the T4/T8 ratio. These two animals had a high viral load in germinal centers and an intermediate level of infected cells in LN. Three animals (P4, P3, and 51127) showed no signs of T4 depletion. All three had a low level of infected cells in LN, but two of them had detectable virus in the germinal centers. Taken together, these data indicated that persistence of productively infected cells in LN after the primary phase of infection was associated with more rapid disease progression. However, the accumulation of viral particles alone was not indicative of rapid progression.

Development of the anti-SIV humoral response was monitored by ELISA (Fig. 3B). Anti-SIV antibodies were detected between days 7 and 14 p.i. in most of the animals studied. However, in the high-dose group, PH426 had a delayed appearance of anti-SIV antibodies compared with the other three animals. PH426 antibodies persisted at lower levels throughout the infection. In the low-dose group, this phenomenon was even more marked for D54, which developed almost no detectable antiviral antibodies during the period of infection. It is noteworthy that the two animals with more rapid disease evolution were characterized by a lower level of anti-SIV antibodies. This suggested that greater susceptibility to the virus was associated with a less efficient humoral response.

Quantification of the viral load in the blood. To determine the level of viral particles circulating in the blood, macaque sera were tested with an antigen capture assay specific for SIV (Fig. 4A). All of the animals had a peak in antigenemia during acute infection which occurred simultaneously with the peak in infected cells in LN at 7 and 14 days p.i. for the high- and low-dose groups, respectively. The heights of the peaks in antigenemia differed strikingly among the animals and were reflected in the variable viral load observed later on in LN. Three animals (PH426, D54, and 8632) had a very high peak in antigenemia. These were the animals that developed AIDS in the course of the study, which suggested that the level of antigenemia during acute infection was an early indicator of susceptibility to the virus. During the asymptomatic stage of the infection, only D54 maintained detectable antigenemia, which was associated with a high level of productively infected cells in LN. The other animals remained aviremic, although some of them harbored a significant viral load in LN.

To assess productive infection of circulating cells, PBMC samples were spotted onto slides and analyzed by in situ hybridization for SIV RNA. In the high-dose group, up to 4% of PBMC were found to be productively infected during primary infection (Fig. 4B). In the low-dose group, this value ranged from 1 to 3%, which confirmed that the inoculated dose had little effect on the viral load developed during primary infection. In a previous study that included dissociation of SIV-infected LN, 1 to 2% of productively infected cells were detected in LN during the acute stage (7). The rate of LN infection was expected to be similar in the high-dose group of the present study, as the inoculum and the kinetics of infection were identical. Thus, the initial rate of LN cell infection can be estimated to be in the same range as that of PBMC infection. At later stages, the rate of productively infected PBMC was generally maintained between 0.01 and 0.1%, with minor differences among the eight animals. The viral load was found to be more variable in LN than in blood. At 255 days p.i., PH426 had the same rate of infected PBMC as P4, while PH426 had 10 times more infected cells in LN. In the low-dose group, D54 had three times more infected PBMC than the other three animals at 181 days p.i., while the ratio of infected cells in LN ranged from 15 to 50. These data underscore the

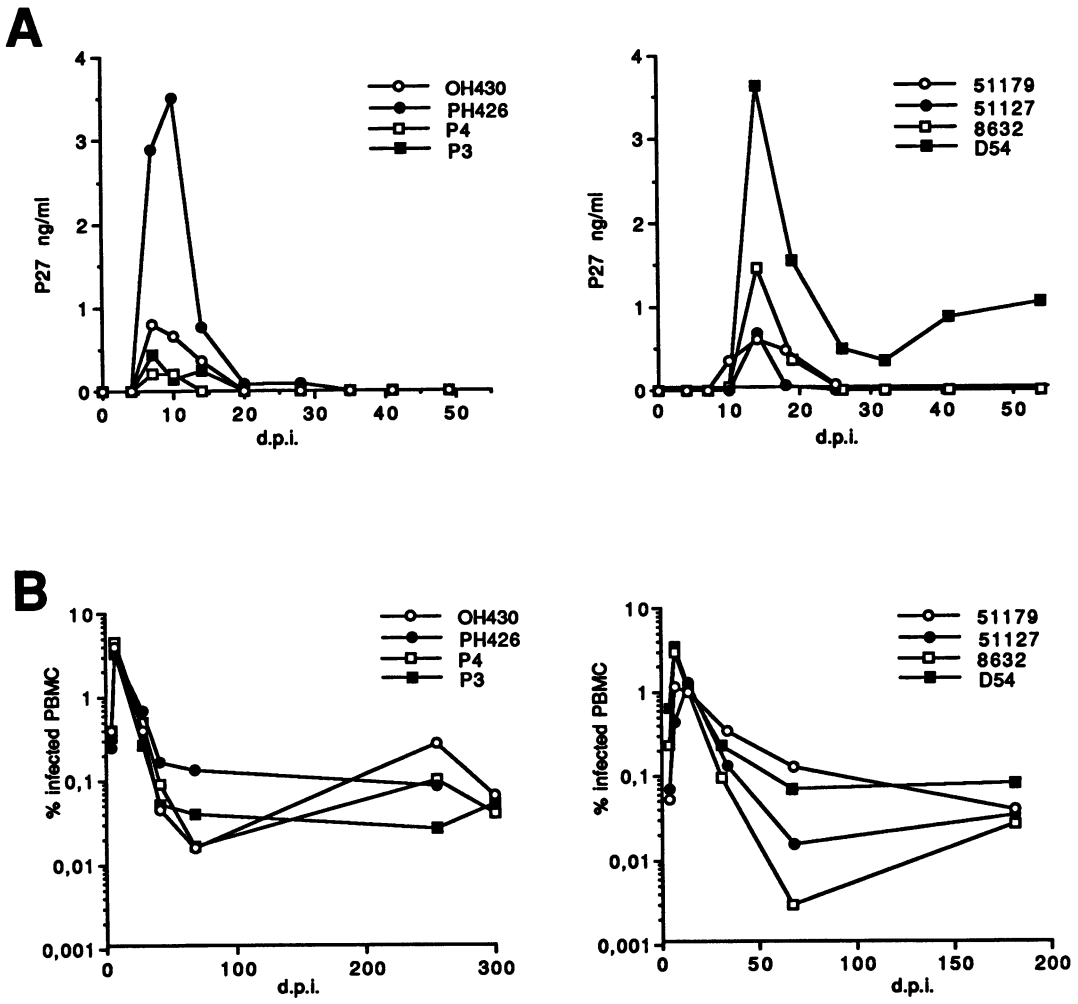

FIG. 4. Viral load in peripheral blood during primary SIV infection. (A) Viremia was determined with serum samples by using an antigen capture ELISA specific for SIV Gag p27. (B). The percentage of productively infected cells in the blood was determined by in situ hybridization. PBMC samples were spotted onto slides and hybridized with a SIV-specific riboprobe. The percentage of PBMC positive for SIV RNA is plotted. The initial time point studied corresponds to 4 days p.i. (d.p.i.). The peaks of infected PBMC were observed at 7 days p.i. in the high-dose group (left side) and between 7 and 14 days p.i. in the low-dose group (right side).

preferential localization of infected cells in LN rather than in blood.

DISCUSSION

Multiple findings indicate that the slow pathological process that ultimately leads to AIDS takes place primarily in lymphoid organs (reviewed in references 2, 13, 14, and 16). To understand HIV-induced pathogenesis better, it is important to determine how the virus initially propagates in lymphoid organs. In this study, we used the SIV-macaque model to characterize the sequence of events leading to viral persistence in LN. Primary SIV infection in LN was found to consist of an initial phase of massive viral replication, followed by a decrease in the number of productively infected cells and accumulation of viral particles in the developing germinal centers. The peaks in productive infection were identical for the four animals in the high-dose group and showed limited variability in the low-dose group. This suggested that the intrinsic ability of the virus to replicate in host target cells was equivalent for these animals. In contrast, the outcome of the primary infection in LN varied markedly among the animals. Differences in viral burden became apparent as the host immune response developed, which strongly suggested that susceptibility to SIV depended on the efficacy of the host immune system. The ability to control the number of productively infected cells in LN was identified as the key indicator of clinical progression, whereas the degree of persistence of viral particles in the germinal centers was less informative.

The eight animals studied showed a peak of productively infected cells in LN between 7 and 14 days p.i., corresponding to the peak of infected cells in the blood. Wide seeding of the virus occurred in LN, which may explain how a large percentage of LN lymphocytes can be latently infected during the asymptomatic stage of the infection (12). A concentration of infected cells was seen in the sinuses at the border of LN sections, which suggested that peripherally infected cells infiltrated into LN (Fig. 1). As signs of immune activation can be detected during primary HIV and SIV infections (25, 37), it is

likely that activated cells home into tissues and migrate through the lymph, thus representing a major route of entry of the virus into lymphoid organs.

Sequential analysis of LN revealed the biphasic nature of primary SIV infection. The peak of infected cells scattered in LN tissue was followed by a concentration of viral RNA in the germinal centers. FDC are known to trap antigen-antibody complexes at their surface (31) and were found to be the site of viral particle accumulation in LN during SIV and HIV infections (1, 3, 5, 15, 24, 32, 35, 36). We previously showed that SIV DNA could not be detected in the germinal centers, although SIV RNA was abundant (7). This confirmed that SIV was present mostly in the form of RNA-containing viral particles, although limited replication of the virus in FDC could not be excluded. In this study, we observed that virus trapping peaked during the first month of infection and followed the peak in productively infected cells by 1 to 2 weeks. Thus, it appeared that viral particles produced during the burst of primary infection were progressively trapped in the germinal centers as these structures developed in the lymphoid tissues. The mechanism of virus trapping is not entirely clear. The fact that the virus became detectable in the germinal centers during establishment of the anti-SIV humoral response suggested a role for antibodies in this phenomenon. However, the two animals which had the slowest appearance of antiviral antibodies in serum (PH426 and D54) were those with the more massive and rapid accumulation of virus in the germinal centers. One explanation may be that specific antibodies were actually produced but were titrated by the large amount of viral particles, leading to retention of antigen-antibody complexes in the germinal centers. Another possibility is that complement molecules alone can mediate virus trapping. Indeed, FDC were shown to express three types of complement receptors at their surface, while Fc receptors were barely detectable (31). As HIV particles can be coated by complement in the absence of antibodies (11), the possibility of a trapping of antigen-complement complexes at the surface of the FDC should be considered.

As the asymptomatic phase of the infection was established, SIV RNA remained detectable in the LN of all of the animals studied, which emphasized the role of lymphoid organs as a reservoir of the virus. At least a few productively infected cells were detected in LN tissues, which indicated that SIV infection was never completely silent. The level of viral persistence varied among the animals, as the count of infected cells ranged from 1 to 200/2 mm^2 2 months after inoculation. The level of virus accumulation in the germinal centers also varied markedly, ranging from apparent clearance of the virus (animal P4) to persistence at levels similar to those of the primary infection. In the asymptomatic stage, no direct relationship was observed between the count of infected cells and the amount of SIV RNA in the germinal centers. Animals with intermediate or high infected-cell counts had high viral loads in the germinal centers. However, two of three animals with low infected-cell counts also harbored significant viral loads in the germinal centers (P3 and 51127). Thus, the presence of virus trapped within the germinal centers did not exclusively reflect the ongoing viral replication in LN. This could be attributed to the ability of FDC to retain antigens at their surface for extended periods of time, a property which is thought to play a key role in the maintenance of B-cell memory (31). Similarly, FDC may retain for months a part of the particle stock produced during acute infection.

To evaluate the rate of disease progression, both clinical parameters and T4-T8 lymphocyte subsets were monitored over periods of 500 and 300 days, in the high- and low-dose groups, respectively. In the dose range studied, a smaller inoculum did not lead to a slower rate of disease progression. In particular, two of the three macaques in which the disease developed rapidly belonged to the low-dose group. Individual susceptibility to SIV, rather than the size of the SIV inoculum, appeared to be the crucial factor in determining the clinical outcome. Animals which had high numbers of productively infected cells in their LN at the beginning of the asymptomatic stage (D54 and PH426) showed a more rapid disease evolution. In contrast, animals with the lowest count of infected cells at 2 months p.i. (P4 and 51127) showed neither clinical signs nor T4 depletion in the course of the study. These data suggested that the relative susceptibility to the virus was expressed early in infection and was associated with the level of viral replication in the asymptomatic stage. However, a statistically significant correlation between productive infection in LN at 2 month p.i. and T4 decline could not be established in the high-dose group, as the differences in productive infection remained limited. Studies conducted with a larger number of animals are needed to confirm this relationship.

The notion that increases in viral transcription levels are predictive of clinical deterioration is supported by longitudinal studies of HIV-infected patients (9, 30). Our data suggest that determination of the viral burden in LN, rather than in blood, may be informative early in the asymptomatic stage of the infection. An important point is that viral replication should be considered rather than viral particle accumulation in LN. In this study, two animals (P3 and 51127) had significant virus levels in the germinal centers and nonetheless remained healthy throughout the study period. Thus, the amount of virus trapped in the germinal centers was not predictive of clinical progression for these animals. This observation raises the issue of the infectious potential of viral particles trapped in the germinal centers. It is generally hypothesized that FDC constitute a viral reservoir that continuously transmits infection to T4 lymphocytes that traffic through the germinal centers (16, 35). However, the observation that persistence of viral particles is not necessarily associated with progressive infection could mean that trapped particles represent viral dead ends rather than infectious virus. Whether viral particles trapped in the LN microenvironment and coated with antibodies or complement molecules are still able to recognize the CD4 receptor and fuse with target cells remains to be determined.

This study allowed us to compare the evolution of the SIV burden in blood with that in LN. Initially, a close parallel was observed between the productive infection in blood and that in LN. The peak in productively infected PBMC was uniform in the high-dose group and exhibited limited variability in the low-dose group. Interestingly, the p27 antigenemia levels demonstrated much greater variability. One reason may be that, in addition to virus production, the differences in antigenemia also reflect various degrees of efficiency in the very early immune responses that participate in the clearance of the virus. Individual differences in complement-mediated opsonization of viral particles or in nonspecific macrophage activation may influence the control of p27 levels in plasma. The fact that antigenemia constitutes the earliest marker of susceptibility to SIV points to the possible involvement of the nonspecific immune response in determining the course of infection.

At later stages, a dissociation between the viral load in the blood and that in the LN was observed. From 2 months p.i. onward, the rate of productively infected PBMC stabilized at low levels, generally between 0.01 and 0.1%. In contrast, LN showed a persistence of both productively infected cells and viral particles in the germinal centers. In particular, it was

striking that animal D54 had as many infected cells in LN at 2 month p.i. as during primary infection, while the number of infected PBMC was reduced by a factor of 30 during the same period. Thus, productively infected cells were concentrated in LN rather than in the blood, a phenomenon which has also been described in the asymptomatic stage of HIV infection in humans (23) and chimpanzees (29). These data emphasize the need to study lymphoid organs to assess the viral status of an individual.

In conclusion, this study shows that the SIV burden in lymphoid organs is determined early in infection and that it influences disease evolution. The basis for the heterogeneity observed in LN infection is not fully understood. Anti-SIV cytotoxic T lymphocytes can be detected in LN as early as 7 days p.i. (26, 27a), and low cytotoxic responses have been associated with increased viral burdens (38). In this study, we also confirmed the finding that low titers of circulating anti-SIV antibodies are predictive of rapid disease progression (19, 40). However, the possibility that low titers reflect trapping of antibodies in LN rather than a defect in antibody response needs to be considered. Nonspecific effector cells, such as macrophages and NK cells, may also be essential in the containment of acute SIV infection because of their rapidly inducible antiviral activities. Further studies will help to elucidate the kinetics and respective roles of nonspecific, humoral, and cellular immune responses in the control of early SIV infection.

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