JOURNAL OF VIROLOGY, Apr. 1994, p. 2362–2370
0022-538X/94/$04.00+0

Vol. 68, No. 4

Immunopathogenic Events in Acute Infection of Rhesus Monkeys with Simian Immunodeficiency Virus of Macaques

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Received 30 September 1993/Accepted 5 January 1994

Infection of the rhesus monkey with simian immunodeficiency virus of macaques (SIVmac) was employed to explore the early immune events associated with the initial containment of an acute AIDS virus infection. In nine rhesus monkeys infected intravenously with uncloned SIVmac strain 251, high-level p27 plasma antigenemia was usually detected transiently from approximately day 7 through day 21 following virus inoculation. SIVmac replication in lymph nodes measured by in situ RNA hybridization closely paralleled the time course and magnitude of viremia. The containment of SIVmac spread by 3 to 4 weeks following infection suggests an efficient, early immune control of this virus infection. Anti-SIVmac antibodies were first detected in the blood at approximately day 14. At the time antigenemia was decreased or cleared, SIVmac neutralizing antibodies were present. A rise in circulating and lymph node $CD8^+$ T cells also occurred coincident with the clearance of antigenemia and persisted thereafter. These $CD8^+$ lymphocytes in lymph nodes had increased expression of both major histocompatibility complex class II and the adhesion molecule LFA-1; they also demonstrated decreased expression of the naive T-cell-associated CD45RA molecule. SIVmac-specific cytotoxic T-lymphocyte precursors were detected in both blood and lymph node by 7 days post-virus inoculation. These studies indicate that both virus-specific humoral and cellular immune mechanisms in blood and lymph node are associated with the clearance of viremia that occurs within the first month of infection of rhesus monkeys with SIVmac.

The early virologic and immunologic events following infection may have important consequences for the eventual clinical course of human immunodeficiency virus (HIV)-induced disease. A picture of the immunopathogenic events in the acutely HIV-infected individual is beginning to emerge. At the time of clinical presentation with fever, lethargy, pharyngitis, and skin rash, the acutely infected person has readily measurable plasma viral antigen reflecting a high viral replication. The early development of virus-specific cytotoxic T lymphocytes (CTL) and an emerging neutralizing antibody response appear to correlate with the clearance of this plasma antigenemia as the individual enters a period of clinical latency (2–4, 21). Because the acutely infected person comes to clinical attention weeks or even months following viral exposure if at all, it is difficult to study the immunologic mechanisms responsible for the clearance of this acute viremia in humans.

Despite the apparent clearance of plasma antigen soon after acute infection, important virologic events appear to be ongoing in the clinically asymptomatic, HIV-infected individual. Lymph nodes (LNs) have been implicated as a reservoir of HIV infection (7, 18, 19) and important in virus spread (19, 20). In situ DNA PCR studies have shown a higher proviral load in LNs than in the blood of these individuals (6, 12). In situ assessments of viral RNA (vRNA), an indicator of actively replicating virus, also suggest a high degree of viral replication in these LNs (12). It is important to determine the nature of the virus-specific immune responses in the LNs of infected individuals since it is these responses that should contain the spread of HIV. These regional immune responses to replicating virus may be of particular importance in the setting of acute infections.

The rhesus monkey infected with simian immunodeficiency virus of macaques (SIVmac) provides a unique system for studying the immunopathogenesis of AIDS. SIVmac, a lentivirus with genetic homology to the HIVs, infects CD4-bearing lymphocytes and macrophages and induces a disease in macaque monkeys with significant similarities to HIV-induced disease in humans (5, 9). Studies with SIVmac-infected rhesus monkeys have already provided valuable insights into virologic and immunologic events following acute AIDS virus infections (5, 9, 15, 16, 23).

In the present studies, we have employed the SIVmac model to assess immunopathologic events in LNs following an acute AIDS virus infection. These studies demonstrate that an initial burst of virus replication in LNs in the immediate weeks following acute infection is contained with remarkable success. The containment of this infection appears to correlate temporally both with the expansion of the CD8 cell subset and the emergence of a virus-specific CTL response in the peripheral blood lymphocytes (PBL) and LNs and with the appearance of SIVmac-specific antibodies in the infected animals.

MATERIALS AND METHODS

Animals. The rhesus monkeys (*Macaca mulatta*) used in this study were maintained in accordance with the guidelines of the

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Committee on Animals for the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (11a). Monkeys were infected by intravenous inoculation with 1 to 3 animal infectious doses of uncloned SIVmac, strain 251, which was propagated in human PBL. Monkeys were anesthetized with ketamine-HCl for all blood sampling and biopsies.

SIVmac antigen and antibody assays. Serum or plasma from heparinized blood was collected and stored at −70°C until analyzed. SIVmac p27 antigen in serum or plasma was assayed in duplicate with a commercial SIV p27 antigen capture enzyme-linked immunosorbent assay (ELISA) kit (Coulter Corporation, Hialeah, Fla.).

The presence of antibodies to SIVmac in serum or plasma was detected by an indirect immunofluorescence assay. SIVmac-infected H9 cells (10^5) were incubated with 100 μl of plasma or serum diluted 1:10 in phosphate-buffered saline (PBS)–0.1% bovine serum albumin (BSA) for 30 min at 37°C. Samples were then washed with 1 ml of PBS and incubated for 20 min at room temperature with 100 μl of fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulin G (IgG)-IgM-IgA (Jackson ImmunoResearch, West Grove, Pa.) diluted 1:50 in PBS–1% BSA. This antibody cross-reacts with rhesus monkey Ig. Samples were again washed with PBS and fixed with PBS–0.1% formalin. As a control, serum or plasma was reacted similarly with uninfected H9 cells. Samples were then analyzed for FITC fluorescence on a flow cytometer and scored positive for SIV-specific antibody when mean fluorescence on SIV-infected H9 cells exceeded fluorescence on uninfected H9 cells by twofold.

SIVmac neutralizing antibodies were measured in 96-well plates as previously described (11) except that CEMx174 cells were used in place of MT-2 cells (8). Briefly, twofold dilutions of heat-inactivated (56°C, 1 h) plasmas were made in triplicate in a total of 100 μl of growth medium per well. Fifty microliters of SIVmac251 (2,000 50% tissue culture infective doses) was added to all wells except for one row of cell control wells, which received growth medium in place of virus. Virus and plasmas were incubated together for 1 h at 37°C before CEMx174 cells (10^5 in 100 μl of growth medium) were added to each well. Viable cells were quantified colorimetrically by vital dye (neutral red) uptake 3 days later when >70% virus-induced cell killing was present in the virus control wells. Percent protection was defined as the difference in absorption between test wells (cells plus plasma plus virus) and virus control wells (cells plus virus) divided by the difference in absorption between cell control wells (cells only) and virus control wells. Neutralizing titers are the reciprocal of the last dilution to provide at least 50% protection from cytopathicity. SIVmac was obtained from culture supernatants of acutely infected CEM cells. Virus-containing culture fluids were clarified of cells by low-speed centrifugation and filtration through 0.45-μm-pore-size cellulose-acetate filters (Millipore Corp., Bedford, Mass.) and stored at −70°C in aliquots. The concentration of virus in the inoculum was determined by titration on CEMx174 cells with serial threefold dilutions of virus made in sets of eight wells per dilution. The reciprocal dilution at which cells in at least half the wells showed cytopathic effects after 2 weeks defined the titer (1 50% tissue culture infective dose).

Lymphocyte immunophenotyping. LN biopsies were placed in Hanks balanced salt solution with 2.5% fetal calf serum and gently teased with forceps to place cells in suspension. This suspension was then filtered through 112-μm-pore-size nylon mesh, and cell number was adjusted to 2×10^6 nucleated cells per ml in Hanks balanced salt solution–2.5% fetal calf serum. Both the LN lymphocytes and PBL were stained with cell-specific monoclonal antibodies and analyzed by flow cytometry.

A total of 50 μl of EDTA anticoagulated blood or LN cell suspension was added to 100 μl of PBS–0.1% BSA which contained an appropriate amount of monoclonal antibody. Monoclonal antibodies used were anti-CD4 (OKT4-FITC; Ortho Diagnostic Systems, Raritan, N.J.), anti-CD8 (T8-FITC, T8-RPE; Dako Corporation, Carpinteria, Calif.), anti-CD11a (CD11a-FITC; AMAC, Inc., Westbrook, Maine), anti-CD18 (anti-LFA-1 alpha; Dako, Inc.), anti-CD45RA (2H4-RD1, Coulter Corp.), anti-major histocompatibility complex (MHC) class II (I3-RD1, Coulter Corp.), and anti-CD45 (LCA; Dako Corp.). Cells were incubated for 30 min at room temperature and then washed once with PBS. In the stains for CD45, an indirect staining technique was used. Cells were incubated, additionally, with a 1:50 dilution of FITC-conjugated goat anti-mouse Ig [$F(ab')_2$ fragment] (Jackson ImmunoResearch) in PBS–0.1% BSA for 20 min at room temperature and then washed with PBS. Erythrocytes in all samples were lysed with a commercial erythrocyte lysing kit (Whole Blood Lysing Kit; Coulter Corp.), washed in PBS, and resuspended in 0.4 ml of PBS–1% formalin. Samples were analyzed routinely on an EPICS-C flow cytometer with forward light scatter, 90° light scatter, and CD45 fluorescence to identify lymphocytes.

CTL assays. PBL of a rhesus monkey which expressed the MHC class I allele *Mamu-A*01* were isolated from heparinized blood by density gradient centrifugation over Ficoll-Hypaque (Ficopaque; Pharmacia Chemical Co., Piscataway, N.J.). LN lymphocytes were simultaneously obtained from the same monkey by the method described above. These cells were cultured for 3 days at 10^6 cells per ml in concanavalin A (5 μg/ml) or at 2.5×10^6 cells per ml in synthetic SIV Gag peptides (5 μg/ml) and human recombinant interleukin 2 (400 U/ml). These cells were then assayed for their ability to lyse autologous B-lymphoblastoid cell lines which were incubated with SIV Gag peptides as described previously (10).

In situ hybridization of SIV RNA. LN biopsies were fixed in 10% neutral buffered formalin and embedded in paraffin. Five-micrometer-thick sections were placed on slides coated with 3-amino-propyl-triethosilane. Four sections from each node per time point were hybridized with a ^{35}S-labeled single-stranded (antisense) RNA probe of SIVmac239 (Lofstrand Laboratories, Gaithersburg, Md.). The clone was obtained in collaboration with Susanne Gartner (Jackson Laboratories, Rockville, Md.) through the National Institutes of Health Research and Reference Program. The probe was composed of fragments of 1.4 to 2.7 kb in size and collectively represented approximately 90% of the SIV genome.

Dewaxed sections were treated with 0.2 N HCl for 20 min at room temperature and rinsed in water containing 2% diethyl pyrocarbonate. The sections were digested with proteinase K (0.01 mg/ml in 10 mM Tris-HCl buffer [pH 7.4]–2 nM $CaCl_2$; Sigma, Munich, Germany) at 37°C for 15 min. After being washed in two changes of diethyl pyrocarbonate-treated water, the sections were acetylated in 0.25% acetic anhydride–0.1% triethanolamine, pH 8.0, at room temperature for 5 min. Sections were prehybridized in cocktail containing 50% formamide, 0.5 M NaCl, 10 mM Tris HCl (pH 7.4), 1 mM EDTA, 0.02% Ficoll-polyvinylpyrrolidone-BSA, and 2 mg of tRNA per ml for 2 h at 45°C. The hybridization mixture consisted of prehybridization cocktail, 10% dextran sulfate, and 2×10^6 dpm of radiolabeled probe per ml. This was heated in boiling water for 60 s and chilled. Then, 4 μl per square-centimeter section was layered on, and sections were then covered with a coverslip, sealed with rubber cement, and placed in a 45°C moist chamber overnight.

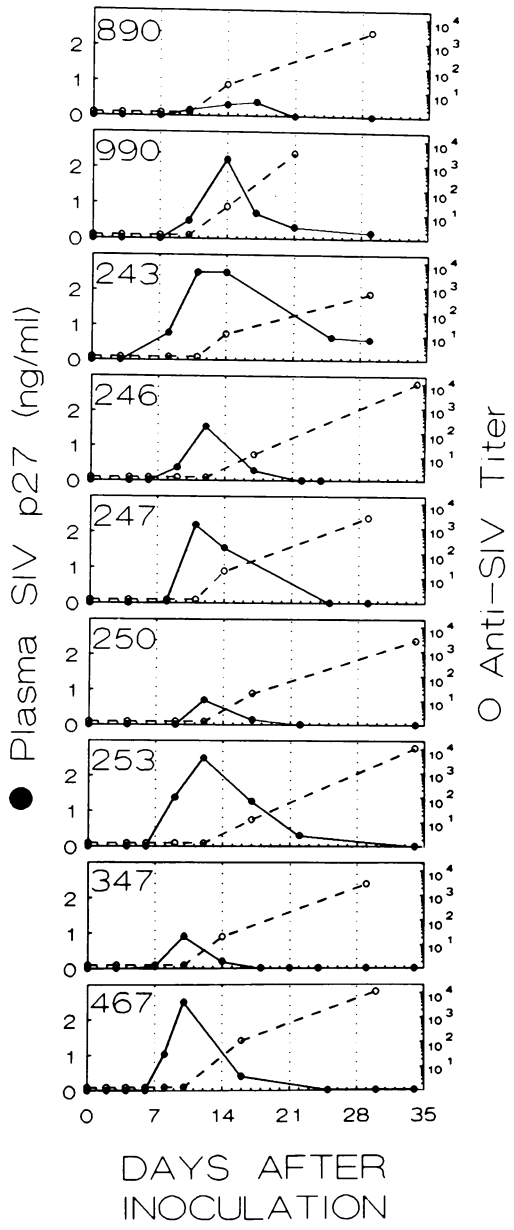


FIG. 1. After acute infection with SIVmac, rhesus monkeys exhibit a transient viral antigenemia which is cleared coincident with developing SIVmac-specific antibody responses. Serum or plasma samples obtained after experimental infection of nine rhesus monkeys with SIVmac were analyzed for SIV p27 antigen by an antigen capture ELISA (●). Linear range of the assay was from 0.01 to 2.5 ng/ml. The same serum or plasma samples were analyzed for SIVmac-specific antibody by an indirect immunofluorescence assay on SIVmac-infected H9 cells (○).

Sections were washed sequentially for 15 min with three changes each in 50% formamide–50% 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), then in 2× SSC–0.1% Triton, and finally in 0.1× SSC. Sections were then digested with RNase (Boehringer-Mannheim, Mannheim, Germany) at 37°C for 40 min, washed again in 2× SSC, and dehydrated in 0.3 M ammonium acetate–70 to 90% ethanol. The slides were dipped in Kodak NTB-2 emulsion, exposed for 4 days at 4°C, and then developed in Kodak D-19 developer, counterstained with hemalaun, and mounted.

TABLE 1. Neutralizing antibody titers after primary infection with SIVmac

Monkey	Titer at days after infection:			
	14–17	29–34	50–52	>100
890	<12	6,144	12,288	
990	12	1,536		
243	<12	768	1,536	
246	<12	12,500		24,576
247	48	3,072	3,072	12,288
250	<12	3,072		6,144
253	<12	6,144		
347	48	768	1,536	
467	192	768		6,144

As a positive control, cytospin preparations of peripheral blood mononuclear cells infected with SIVmac were hybridized with the same probe. As a negative control, one section per LN for each time point was hybridized with a radiolabeled sense-strand probe. The sections were examined with an Axiophot Zeiss microscope equipped with epiluminescent illumination. Cells with at least 20 silver grains were scored as vRNA positive. This corresponded to a sixfold excess of silver grains over background.

Statistical analysis. Scatterplots were constructed from the relative or absolute number of lymphocytes over time, together with their mean values at all time points where >2 datum points were collected (see Fig. 2, 3, and 5). The plots were visually compared for qualitative differences and similarities in the time course patterns. Statistically significant differences ($P < 0.05$) in expression of cell surface molecules on $CD8^+$ lymphocytes between time points (see Fig. 6) were detected with a random analysis of variance model and were compared on a pairwise basis by the least significant difference test.

RESULTS

Viral antigen was cleared from plasma in rhesus monkeys by 3 weeks after acute SIVmac infection. A period of high viral antigen in plasma occurs in both HIV-infected humans and SIV-infected macaques shortly after an acute AIDS virus infection (2, 4, 21, 23). We sought to characterize the virologic and immunologic events in LNs during this early antigenemia and the immune mechanisms responsible for AIDS virus clearance. Nine naive rhesus monkeys were infected by intravenous inoculation with 1 to 3 animal infectious doses of uncloned SIVmac251. SIVmac p27 could be first detected in the plasma of these animals 6 to 12 days following virus inoculation; by days 21 to 25, six of the nine monkeys had cleared this plasma antigen, and by days 29 to 34, seven of nine had cleared plasma antigen (Fig. 1). In the two monkeys in which p27 antigenemia was still detectable after day 29, the level of antigenemia had declined 5- to 10-fold from its peak level.

Anti-SIVmac antibody was detected in the monkeys by 14 days following virus inoculation. These acutely infected animals were assessed for evidence of an emerging SIVmac-specific antibody response. Indirect immunofluorescence, employing chronically SIVmac-infected H9 cells, demonstrated seroconversion by days 12 to 17 in all nine animals, and high-titer antibody responses were clearly demonstrable by days 29 to 34 (Fig. 1). Thus, the emergence of an anti-SIVmac antibody response correlated with the decrease or clearance of

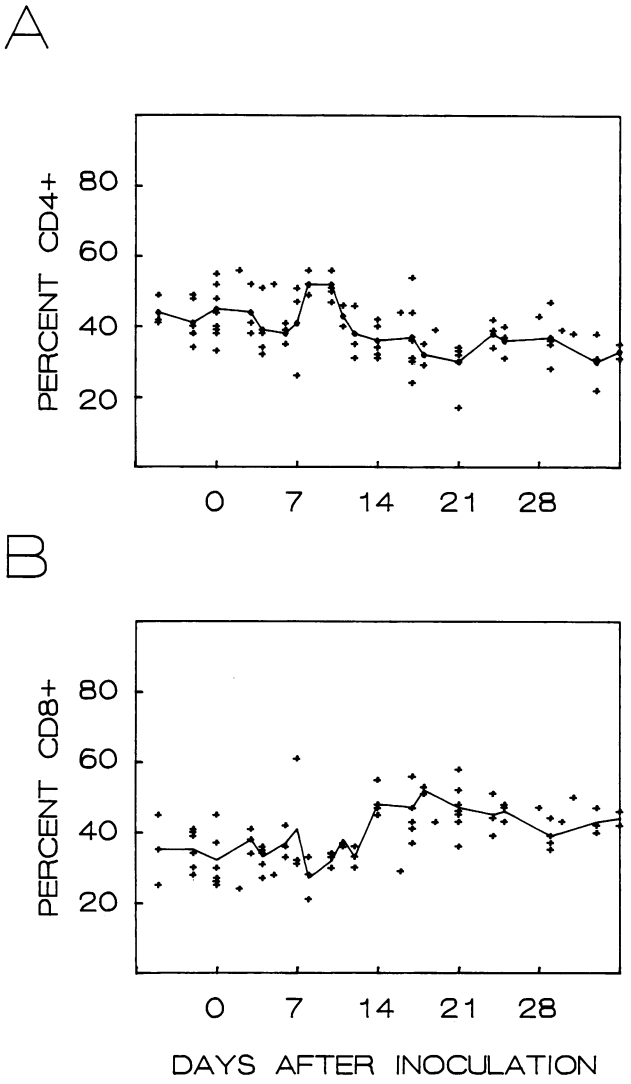


FIG. 2. Acute infection with SIVmac results in an increase in percent circulating $CD8^+$ lymphocytes. Percent $CD4^+$ (A) or $CD8^+$ (B) lymphocytes in peripheral blood was determined for nine rhesus monkeys before or immediately following experimental inoculation with SIVmac.

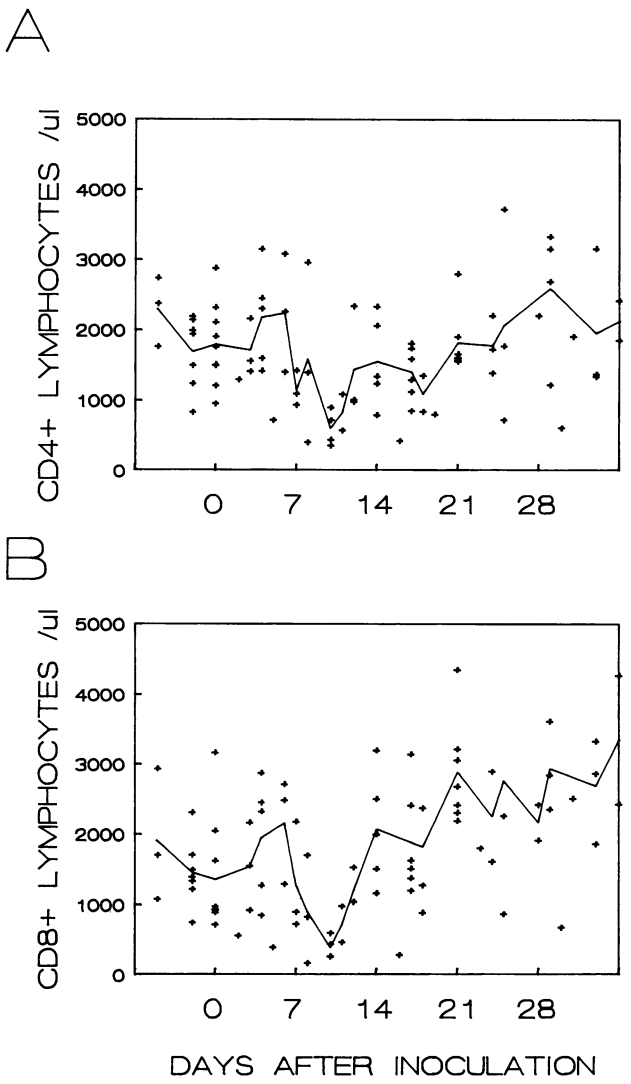


FIG. 3. $CD4^+$ and $CD8^+$ lymphopenia corresponds with plasma antigenemia followed by a $CD8^+$ lymphocytosis. Absolute numbers of $CD4^+$ (A) and $CD8^+$ (B) lymphocytes were determined for nine monkeys before or immediately after they were experimentally inoculated with SIVmac.

plasma antigenemia in these animals. When they were assayed for virus neutralization, neutralizing antibody titers were detected at low levels within the first 17 days in four of nine animals, and all had significant neutralizing antibody titers by days 29 to 34 (Table 1). The magnitude of the neutralizing antibody response did not correlate with magnitude or duration of viral antigenemia.

A $CD8^+$ lymphocytosis occurred coincident with the onset of plasma antigenemia in the monkeys. Cellular immune responses were then characterized in these acutely infected monkeys. Most monkeys experienced a lymphopenia coincident with the onset of plasma antigenemia. Absolute numbers of both $CD4^+$ and $CD8^+$ PBL decreased during antigenemia. Following the period of antigenemia, percent $CD4^+$ PBL declined while percent $CD8^+$ PBL increased (Fig. 2). However, during this time, absolute PBL number increased so that by 30 to 35 days postinoculation, absolute $CD4^+$ PBL number had nearly returned to normal despite a decrease in percent $CD4^+$ PBL. In contrast, both the absolute number and the percent $CD8^+$ PBL increased during this period following antigenemia (Fig. 3).

SIVmac RNA expression in LN T cells paralleled p27 antigenemia in the acutely infected monkeys. Previous findings suggest that LNs of AIDS virus-infected individuals are important reservoirs for virus (7, 18–20) and contain higher virus loads than blood mononuclear cells (6, 12). We, therefore, sought to assess the extent of viral replication in LNs during the period immediately following SIVmac infection. LNs from three monkeys were assessed by in situ hybridization with an SIVmac probe for vRNA expression. LNs removed prior to SIVmac infection contained no vRNA sequences. Low numbers of productively infected cells appeared as early as 5 to 8 days postinfection (Table 2 and Fig. 4A). They were scattered in the extrafollicular parenchyma and/or sinuses and contained

TABLE 2. Correlation of virus replication in LNs assessed by in situ hybridization with plasma p27 antigen and anti-SIVmac antibody responses

Animal	Days post-infection	SIV RNA$^+$ cells/field[a]	SIV RNA signal/cell[b]	p27 antigen[c]	Anti-SIV antibody[d]
467	0	0	−	0	−
	5	0–3	+	0	−
	10	>90	++++	>2.50	−
	16	26–40	+++	0.40	+
	30	0–3	+	0	+
347	0	0	−	0	−
	8	0–3	+	0.06	−
	14	14–25	++	0.20	+
	21	4–13	+	0	+
	40	0–3	+	0	+
890	0	0	−	0	−
	8	0–3	+	0	−
	14	14–25	++	0.32	+
	21	4–13	+	0	+
	40	0–3	+	0	+

[a] Number of cells per magnification field (×100) showing positive signal for SIV RNA.

[b] SIVmac RNA signal per cell was semiquantified by estimating the number of silver grains present per positive cell. − to ++++, fewest to most grains.

[c] SIVmac p27 antigen (nanograms per milliliter) in plasma was quantified by an ELISA in which the limit of detection was 0.01 ng/ml.

[d] Antibodies against SIVmac were detected by an indirect immunofluorescence assay in which a positive (+) titer was considered ≥1:10.

20 to 35 silver grains per cell. However, not all cutting levels contained such cells.

At days 10 to 14, a dramatic increase of vRNA-positive cells occurred. There was considerable variation in the magnitude of this increase from animal to animal (Table 2 and Fig. 4B). Monkey 467 had the highest number of productively infected cells with 90 or more positive cells per field. In addition, the signal intensity was greatest in this animal with >50 silver grains per cell. The two other animals contained fewer positive cells (14 to 25 per field), and the intensity of signal was lower. They exhibited 25 to 40 silver grains per cell. In all animals, the vRNA-positive cells had the morphologic appearance of lymphocytes and macrophages and were mainly in the extrafollicular parenchyma and sinuses. Several germinal centers or mantle zones also contained one or two vRNA-positive cells despite the small, inactive state of these germinal centers.

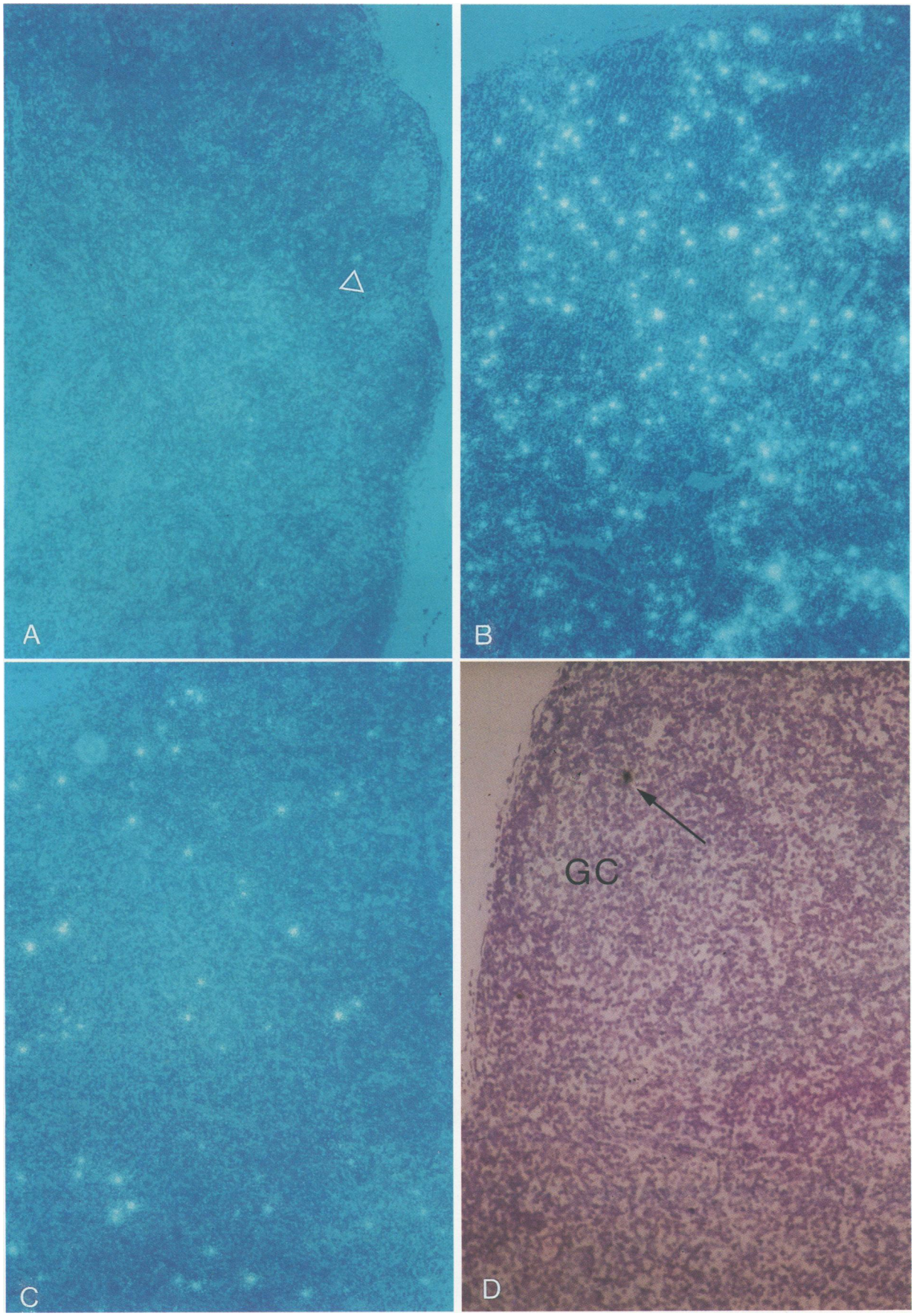
In biopsies taken 21 days after infection, the distribution of productively infected cells remained the same (Table 2 and Fig. 4C). However, the number of infected cells declined in all monkeys. In addition, in animal 467, the hybridization signal per cell decreased. LNs sampled 30 to 40 days postinfection were almost completely free of vRNA-positive cells (Table 2). Positive cells were not seen in many of the four sections examined. On some cutting levels, however, a few productively infected cells (one to three) could be visualized either in the extrafollicular parenchyma or in the germinal centers (Fig. 4D). The rapid emergence and equally rapid down-regulation of viral replication in these LN cells paralleled to a remarkable degree the plasma antigenemia in these animals (Table 2).

T-lymphocyte phenotypic changes in LNs paralleled those in PBL following acute SIVmac infection. To assess T-lymphocyte changes in peripheral LNs of these acutely infected animals, phenotypic changes in the T-cell compartments of these nodes were assessed. Coincident with the clearance of plasma antigen, a drop in the percent CD4$^+$ LN T cells together with a rise in the percent CD8$^+$ LN T cells was documented (Fig. 5). Since lymphadenopathy was clinically apparent in these animals by approximately 1 week following SIVmac infection, this rise in percent CD8$^+$ cells probably represented an absolute increase in LN CD8$^+$ cells.

Phenotype changes in LN CD8$^+$ cells correlated with the clearance of p27 antigenemia in the acutely infected monkeys. These observations indicated that an increase in circulating and LN CD8$^+$ T cells correlated with the clearance of acute antigenemia in this cohort of monkeys. We sought to characterize further this CD8$^+$ lymphocyte population by phenotypic analysis. PBL were examined before, during, and following antigenemia; assessed were their state of activation (MHC class II expression), memory status (CD45RA expression), and adhesion potential (CD11a-CD18 expression) (Fig. 6). Interestingly, only minimal changes in PBL CD8$^+$ lymphocyte expression of MHC class II and CD11a-CD18 occurred coincident with the clearance of plasma antigenemia. However, in T cells from LNs of these animals, the expression of both MHC class II and CD11a-CD18 molecules was increased on CD8$^+$ lymphocytes following antigenemia. Moreover, the expression of CD45RA was decreased on peripheral blood and LN CD8$^+$ T cells following antigenemia.

SIVmac Gag-specific CTL precursors were demonstrable in LNs and PBL of the infected animals by day 7 following infection. These studies indicated the emergence of a CD8$^+$ lymphocyte population in the LNs and peripheral blood of the acutely infected animals coincident with the clearance of plasma antigenemia and replicating virus in LNs. The phenotype was consistent with a cytotoxic effector cell (13, 22). We sought to document the time course for the generation of SIVmac-specific CTL in these acutely infected animals. We have previously shown that Gag- and Nef-specific CTL were demonstrable in PBL by 14 days following infection, while CTL effector precursors were seen in PBL of acutely SIVmac-infected rhesus monkeys by 4 to 6 days following virus infection (23). These studies were done in monkeys with a defined MHC class I haplotype. We have previously shown that rhesus monkeys expressing the MHC class I molecule *Mamu-A*01* develop SIVmac Gag-specific CTL that recognize a 9-amino-acid epitope of the Gag protein termed p11C. One *Mamu-A*01*$^+$ rhesus monkey was selected from this cohort of nine animals for prospective evaluation of SIVmac Gag-specific CTL activity in peripheral blood and LN T-cell populations. We assessed both lectin- and p11-stimulated T cells from this animal on a weekly basis following infection for CTL activity. In both peripheral blood and LNs, p11C-stimulated T cells from this animal exhibited p11-specific effector function by day 7 following infection. By day 14 after infection, both p11C- and Con A-stimulated T cells exhibited such effector function

FIG. 4. The number of productively SIVmac-infected LN cells correlates with the magnitude of plasma antigenemia. Shown are the results of in situ hybridization of LNs for SIVmac vRNA. (A) Five days postinoculation, only a few vRNA-positive cells are seen (see arrow). (B) Ten days postinoculation, the number of productively infected cells increases dramatically. (C) Sixteen days postinoculation, the number of productively infected cells begins to decline. (D) On day 30, vRNA-positive cells are only rarely seen (see arrow). (A to C) Epipolarized illumination; vRNA-positive cells appear as light areas; magnification, ca. ×90. (D) Transillumination; GC, germinal center; magnification, ca. ×200.


A
B
C
D
GC

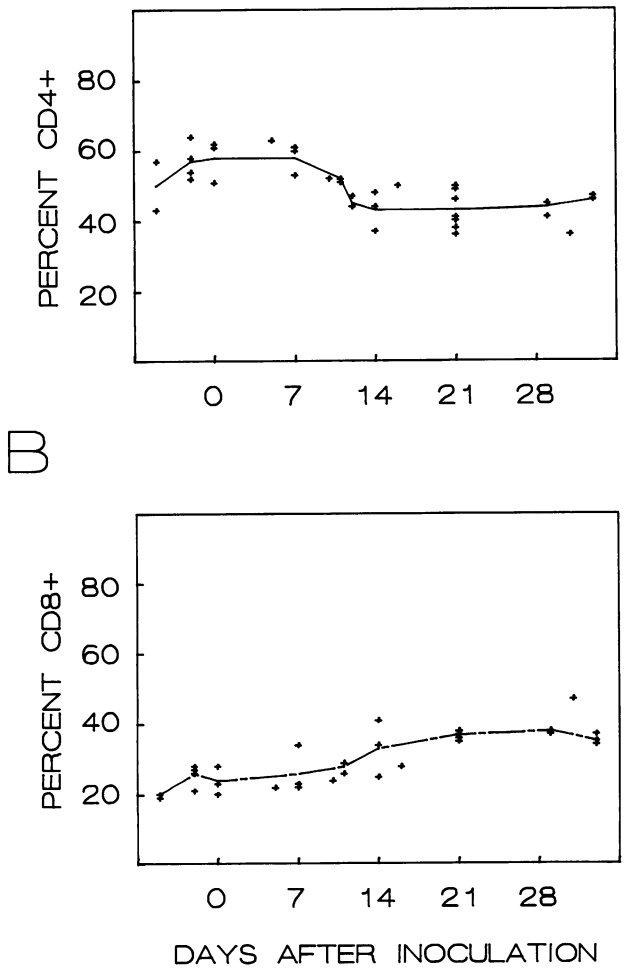


FIG. 5. Phenotypic changes in LN lymphocytes paralleled those seen in the peripheral blood. LN biopsies were obtained from nine rhesus monkeys before or immediately following acute infection with SIVmac and immunophenotyped for CD4 (A) and CD8 (B).

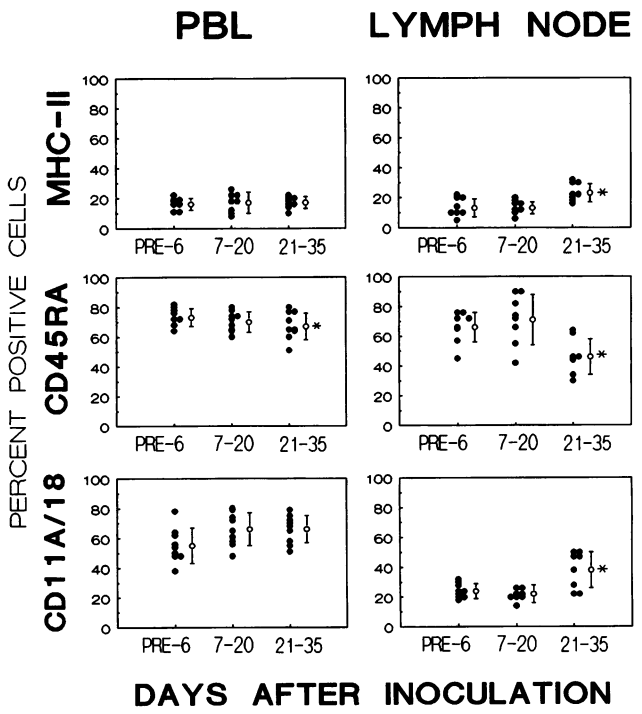


FIG. 6. LN CD8$^+$ lymphocytes change expression of CD11a-CD18, MHC class II, and CD45RA coincident with clearance of SIVmac antigenemia. PBL or LN cells obtained from nine rhesus monkeys before or immediately following acute infection with SIV were immunophenotyped for coexpression of CD18-CD11a, CD45RA, and MHC class II on CD8$^+$ lymphocytes. Asterisk denotes significant difference from preantigenemic (PRE-6) values. Open circles denote mean values ± standard deviation.

(Table 3). Thus, CD8$^+$ CTL precursors were demonstrable not only in peripheral blood but also in LN T-cell populations within the first week following SIVmac infection. Moreover, SIVmac Gag-specific CTL were present in both LN and PBL T-cell populations at a high enough frequency to be detected without specific peptide restimulation by 14 days after infection. The emergence of this effector T-cell response correlated in this animal with the clearance of plasma antigenemia and viral replication in LNs.

DISCUSSION

Events following acute intravenous infection of rhesus monkeys with SIVmac parallel those seen in acutely HIV-infected humans who have been studied. Within weeks of infection, lymphadenopathy and skin rash are seen in both. Moreover, acute antigenemia occurs within days to weeks following SIVmac or HIV infection. In both, virus-specific antibodies and MHC class I-restricted CD8$^+$ CTL responses are generated. An inversion in the CD4/CD8 lymphocyte subset ratio occurs, and the early, high virus load is cleared.

The findings in the present study contribute to the further definition of the immunopathogenic events following an acute AIDS virus infection. The high frequency of cells in peripheral LNs harboring replicating virus as early as 2 weeks following acute infection can certainly account for the extremely high

TABLE 3. Appearance of SIVmac-specific cytotoxic lymphocytes in peripheral blood and LN after virus inoculation

Day[a]	Cell type[b]	% Specific lysis[c]							
		Con A–IL-2 expanded at indicated E/T ratio:				Gag peptide p11 expanded at indicated E/T ratio:			
		40:1	20:1	10:1	5:1	40:1	20:1	10:1	5:1
7	PBL	0	0	0			10	8	1
	LN	0	0	0			25	16	6
14	PBL	30	26	26	21	47	51	48	43
	LN	23	25	13	12	52	56	51	45
28	PBL	76	62	53	42				
	LN	57	54	39	34				

[a] Days after inoculation with SIVmac.
[b] Source of effector cells.
[c] Lysis of SIVmac expressing autologous target cells as described in Materials and Methods. Effector cells were expanded for 4 to 7 days in Con A–IL-2 or SIV Gag peptide p11–IL-2. E/T, effector cells/target cells.

plasma viral antigen loads documented in individuals during this stage of infection. This finding provides further evidence for the importance of the LN as a site of viral replication in the AIDS virus-infected individual.

Virtually all viral replication in the LNs of these monkeys occurred in the paracortex. It is important to note, however, that different isolates of SIVmac may have different cell tropisms and, therefore, may have different cellular compartmental distributions in vivo immediately following infection. Thus, while there was no evidence in the LNs of these rhesus monkeys infected with uncloned SIVmac251 for many productively infected cells in germinal centers, other SIVmac isolates may exhibit different anatomic distributions during this early phase of infection.

The importance of the immune response in containing an acute lentivirus infection is highlighted by studies of equine infectious anemia virus-infected horses. While equine infectious anemia virus infection eventually results in a chronic asymptomatic carrier state in normal horses, Perryman et al. have shown that a rapidly progressive fatal disease without evidence of viral clearance develops in equine infectious anemia virus-infected Arabian foals with a genetically determined combined immunodeficiency disease (14). The sudden disappearance of vRNA-expressing cells in the LNs of the rhesus monkeys in the present study and the coincident fall in plasma antigenemia suggest that the early immune response against the infecting AIDS virus may be quite effective. It will be important to determine whether preexisting vaccine-induced AIDS virus-specific immune responses or early antiviral therapy can accelerate the containment of this early viral infection.

The clearance of plasma viral antigenemia and the disappearance of vRNA in LN cells correlate with both the emergence of anti-SIVmac antibodies and an SIVmac-specific CTL response. Early-appearing SIVmac-specific antibodies, which usually preceded neutralizing antibodies, may promote viral immune complex formation with complement and lead to virus clearance through the mononuclear phagocytic system independent of the classical neutralizing antibodies (1, 17). Previously, we have shown that SIVmac-specific CTL precursors appear in PBL within days after experimental infection (23). In addition, an activated $CD8^+$ lymphocyte appears in LNs by 2 months after infection (16). In the present study, we show that the emergence of these cellular responses correlates with the clearance of viremia and suppression of virus replication. While these studies do not distinguish between the contribution made by cellular immunity and that made by humoral immunity in the control of this early burst of viral replication, they do provide further evidence for the likely importance of the cell-mediated immune response in clearing the early AIDS virus infection.

Distinct SIVmac isolates may have very different tropisms and cause disease with different clinical courses in rhesus monkeys. Similarly, the dose or route of virus inoculation in the monkeys may also dramatically alter the virologic and immunologic events following initial infection. The SIVmac-rhesus monkey model provides a powerful system for exploring issues such as these in the immunopathogenesis of AIDS. The SIVmac-infected rhesus monkey will also provide an extremely useful model for assessing the impact of immunologic and antiretroviral interventions on the acute phase of AIDS virus infection.

ACKNOWLEDGMENTS

We thank Birgit Guhlk and Kirstin Evers for technical assistance. Data organization and analysis were performed on the PROPHET system, a national computer resource sponsored by the National Center for Research Resources, National Institutes of Health.

This work was supported by NIH grants AI-20729, CA-50139, and RR-000168. Additional funds were provided by Bundesministerium fur Forschung und Technologie, Bonn, Germany, grant FKZ BGA III 006-89/FVP 6, and the Korber Foundation, Hamburg, Germany. K.A.R. is the recipient of a Special Emphasis Research Career Award, RR-00055.

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