American Journal of Pathology, Vol. 134, No. 2, February 1989

Cellular Localization of Simian Immunodeficiency Virus in Lymphoid Tissues

I. Immunohistochemistry and Electron Microscopy

D. J. Ringler, M. S. Wyand, D. G. Walsh, J. J. MacKey, L. V. Chalifoux, M. Popovic,* A. A. Minassian,* P. K. Sehgal, M. D. Daniel, R. C. Desrosiers, and N. W. King

*From the Harvard Medical School, New England Regional Primate Research Center, Southborough, Massachusetts and the Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland**

Simian immunodeficiency virus (SIV) is a lentivirus with genetic relatedness to the human immunodeficiency viruses (HIV-1 and HIV-2). It induces a fatal syndrome in rhesus monkeys that closely parallels the clinical course of AIDS in humans. The authors used double-labeling immunohistochemical procedures on rhesus lymph node and spleen taken during different time periods after SIV infection to localize the p27 gag protein to specific cellular immunophenotypes. In animals with follicular hyperplasia, viral protein was found associated predominantly with follicular dendritic cells. Many of these cells showed ultrastructural alterations consisting of swollen dendritic processes containing electron-dense material. Lentiviral particles were found associated with this cell type only rarely. In lymphoid tissues with other histopathologic changes, macrophages and multinucleate giant cells were the predominant cell types containing detectable quantities of viral protein; smaller numbers of p27+ lymphocytes were present. Ultrastructurally, viral particles were found within the extracellular space adjacent to tissue macrophages and within membrane-bound vacuoles of giant cells and tissue macrophages. These results show that certain histologic patterns seen during the course of infection correlate with the localization of viral antigen to specific cellular immunophenotypes and that during the disease course, viral protein is preferentially localized in sections of lymph node and spleen to cells of the macrophage and dendritic cell lineages. (Am J Pathol 1989, 134:373–383)

Human immunodeficiency virus type-1 (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), selectively replicates within CD4+ cells *in vitro*,[1–3] and patients with AIDS develop a relative decrease in the numbers of CD4+ lymphocytes within the peripheral blood and lymphoid organs.[4–7] This selective tropism for helper/inducer lymphocytes is mediated by the interaction of viral envelope with the CD4 receptor, a 60 kd glycoprotein; thus, the presence of the CD4 receptor on a cell is believed to be requisite for infection by this novel retrovirus.[2,3,8,9] Other than lymphocytes, cells of the monocyte/macrophage and dendritic cell lineages express the CD4 receptor, although to a much more limited extent compared to lymphocytes.[10–12] Therefore, CD4+ lymphocytes, macrophages, and dendritic cells are potential targets for HIV infection *in vivo*.

It is advantageous to localize these targets in tissue sections using double labeling immunohistochemistry or *in situ* hybridization combined with immunohistochemical procedures, because these combined techniques permit the examination of large sections of tissue while providing objective criteria for cell type identification. Using these methods, the study of tissues sampled throughout the entire course of infection may provide insight into the pathogenesis of lymphoid depletion, immunosuppression, and evolution of histologic changes within lymphoid organs. Because of the difficulty in prospectively studying HIV-infected volunteers, however, this work may be best performed using an animal model for AIDS.

The rhesus monkey infected with simian immunodeficiency virus (SIV), a lentivirus with morphologic, genetic, and antigenic relatedness to HIV, has been shown previously to be a relevant animal model for AIDS.[13–18] As in HIV-infected people, SIV-infected rhesus monkeys develop a distinct granulomatous encephalitis and manifest profound immunosuppression, clinically characterized by multiple opportunistic infections and neoplasms.[16–18] In

Supported by NIH grants 1R29AI25644-01 and RR00168 to the New England Regional Primate Research Center.

Accepted for publication August 26, 1988.

Address reprint requests to Dr. Douglas J. Ringler, New England Regional Primate Research Center, One Pine Hill Drive, Southborough, MA 01772.

addition, both SIV and HIV preferentially replicate in CD4+ lymphocytes *in vitro,*[13,19] both cause a reduction in the number of CD4+ cells in the peripheral circulation of the respective host,[16,18,20] and both result in diminished blastogenic responses of peripheral blood lymphocytes to mitogens *in vitro.*[16,18]

In this study, we used single and double-labeling immunohistochemical techniques to localize the major *gag* protein of SIV in lymphoid tissues from infected rhesus monkeys. Our results indicate that SIV-related protein is harbored predominantly in follicular dendritic cells during hyperplastic stages and in macrophages and multinucleate giant cells in histologically normal tissue or in tissue undergoing lymphoid involution.

Materials and Methods

Animals and Virus

Nineteen rhesus monkeys were used in this study; these animals are enumerated with relevant data in Table 1. They were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. All animals except 309-78 were inoculated with uncloned SIV (SIV_{mac251}) that was originally isolated from a rhesus monkey (251-79) with lymphoma. The SIV_{mac251} used for infection was derived from infected HUT-78 cell cultures or alternatively from infected interleukin-2-stimulated human peripheral blood lymphocytes. Eighteen animals were inoculated intravenously or intramuscularly with cell-free supernatant from SIV-infected cell cultures. Experimental infection of five of these macaques (202-84, 167-84, 188-84, 392-84, and 127-83) has been described previously.[16,21] One animal (160-86) was infected by gently inserting a cotton ball saturated with 1.0 ml of SIV-containing cell-free supernatant in the vagina for 30 minutes. Another (309-78) from the colony-at-large was found to be infected naturally with SIV.[22] All animals were monitored for infection by the appearance of SIV-specific antibodies and by recovery of SIV from peripheral blood mononuclear cells, as described previously.[22] Five of the eighteen animals (221-86, 198-85, 258-85, 206-85, 205-85) had been vaccinated previously with killed preparations of SIV, as part of an unrelated study. All five mounted a humoral immune response against SIV; however, SIV was repeatedly isolated from the peripheral blood of all five after challenge with virus. They were included in this study because the results obtained from these animals did not appear to differ from unvaccinated animals.

Tissue Manipulation and Histologic Evaluation

Spleen and peripheral lymph node were harvested from 14 animals at the time of the post-mortem examination. Additionally, all major organs were collected from these animals for routine histopathological evaluation, and relevant observations and opportunistic diseases were noted (Table 1). In one of these 14 and in 5 other animals, tissue was obtained by surgical biopsy techniques. Two animals (159-86 and 126-86) were subjected to three sequential biopsy procedures early in the course of infection, whereas animal 202-84 was surgically manipulated twice (Table 1). Control tissue consisted of lymph node and spleen biopsies from six animals before SIV inoculation, as part of an unrelated study. All tissue was subdivided for routine light microscopy, electron microscopy, and immunolight microscopy.

Lymphoid tissues from the study animals were categorized according to the presence or absence of histologic alterations within lymph nodes (Table 1). We have determined the criteria for nodal histologic classifications in SIV-infected rhesus monkeys,[23] modified from the original descriptions for HIV.[24,25] Briefly, five categories were used: 1) Follicular hyperplasia—these lymph nodes contained large, sometimes confluent, germinal centers and thin mantle zones, as previously described,[17,26] 2) Follicular depletion with normal or expanded paracortices—these lymph nodes were characterized by the relative paucity of follicles containing active germinal centers or the presence of involuting follicles, and a normal or hyperplastic paracortex, 3) Depletion—these lymph nodes contained reduced numbers of lymphocytes and often only small nodules of lymphocytes scattered within the parenchymal reticular scaffolding, 4) Granulomatous lymphadenitis—these lymph nodes contained epithelioid macrophages and multinucleate giant cells individually and in clusters throughout all regions of the parenchyma. Acid-fast stains on tissues from two of the animals in this group (127-83 and 392-84) failed to reveal any mycobacterial organisms. The tissues from the third animal (249-85) contained sheets of macrophages containing numerous acid fast-positive bacilli, and 5) Normal.

With only a few exceptions, the changes within the splenic white pulp closely paralleled those seen in lymph nodes (the T cell domains of the spleen corresponding to the nodal paracortex). In those cases where differences were found, the spleen was placed in the appropriate category; these cases are indicated in Table 1.

Electron Microscopy

Tissue from selected cases was initially fixed in 3% glutaraldehyde, washed in phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon.

Table 1. *Rhesus Monkeys Infected with SIV Categorized by Nodal Microscopic Morphology*

Animal	Sex	Age at inoculation (months)	Incubation time (weeks)	Infection course
Follicular hyperplasia				
202-84*†	M	14	28 and 88	Lymphadenopathy—long-term survivor (2.5 years)
159-86*	M	19	7§ and 13	Lymphadenopathy developed between 3 and 7 weeks after inoculation
120-85	M	14	37	Lymphadenopathy; multiorgan lymphoproliferative disease
167-84*†	M	15	80	Lymphadenopathy; long term survivor (2.5 years)
221-86*†	M	12	27	
Follicular depletion with normal or expanded paracortex				
565-70	F	201	6	Pulmonary infarction and acariasis; gastritis
188-84‖	M	15	42	Gastroenteritis; proliferative pneumonia
198-85	F	14	28	Pulmonary pneumocystosis; granulomatous encephalitis
258-85	M	12	32	Pulmonary pneumocystosis, enterocolitis; glomerulonephropathy
165-86	M	14	16	Granulomatous encephalitis and myelitis; interstitial pneumonia; colitis
188-86*†	M	13	27	
309-78‖	F	NA	NA	Gastroenteritis; biliary cryptosporidiosis
Nodal depletion				
206-85	F	14	54	Interstitial pneumonia; glomerulonephropathy; gastroenteritis
160-86	F	19	12	Bacterial pneumonia; sepsis
205-85	F	14	37	Pulmonary pneumocystosis; glomerulonephropathy; colitis; adenoviral pancreatitis
Granulomatous lymphadenitis				
127-83	M	18	152	Granulomatous encephalitis; pulmonary pneumocytosis; glomerulonephropathy; colitis; *M. avium intracellulare* negative
392-84‡	F	8	39	*M. avium intracellulare* negative
249-85	M	12	78	Disseminated *M. avium intracellulare* infection
Normal				
159-86*	M	19	3	
126-86	M	19	3*, 7*, and 9	Granulomatous encephalitis at death (9 weeks after inoculation); glomerulonephropathy; leukomyelitis; sepsis

NA, not applicable, natural infection.
* Biopsy.
† Lymph node only.
‡ Spleen only.
§ Spleen considered in normal category.
‖ Spleen considered in depleted category.

Ultrathin sections were cut and stained with uranyl acetate and lead citrate. These sections were examined using a JEOL 100S transmission electron microscope.

Monoclonal Antibodies

SIV *gag* protein in tissue sections was detected by immunohistochemical staining using R1C7, a monoclonal antibody described elsewhere.[27] This antibody was produced by immunizing mice with purified disrupted human immunodeficiency virus type-2 (HIV-2). In addition to reacting with the core protein of HIV-2, it was found on the basis of Western blot analysis to recognize epitopes of the major core proteins (p24 and p27) of HIV-1 and SIV, respectively.[27] Ascitic fluid containing this antibody was found to be optimally reactive against SIV-infected H9 cells at a dilution of 1:1000, using standard immunohistochemical techniques.[28] Tissue macrophages and follicular dendritic cells were identified using EBM11 and R4/23 respectively (Dakopatts Corporation, Copenhagen, Denmark). T cells were recognized by T11/3Pt2H9, provided

by S. Schlossman (Dana-Farber Cancer Institute, Boston, MA). These cell-specific antibodies have been found previously to cross-react with rhesus monkey determinants and were used at either the manufacturer's recommended dilution or previously published concentrations.[29,30]

Immunohistochemical Procedures

Tissue specimens were embedded in OCT compound (Miles Scientific, Naperville, IL) and snap-frozen in 2-methylbutane cooled with dry ice. Six micron cryostat sections were mounted on gelatin-coated glass slides and stored dessicated at −80 C until further processing. Before staining, the sections were fixed in 2% paraformaldehyde (pH 7.2) for 10 minutes at 4 C.

Single antigen labeling for SIV protein was performed with the use of a three-layer peroxidase-antiperoxidase (PAP) procedure with diaminobenzidine (DAB) as the chromagen, as described previously.[28] Double labeling for SIV protein and cell-type specific antigens was performed using different methods, similar to the techniques described by us and others;[30,31] choice of technique depended on organ being stained and the immunoglobulin subclass of the monoclonal antibodies that were used.

Double labeling using R1C7 (IgG1) and R4/23 (IgM) in lymph node was performed by the simultaneous application of monoclonal antibodies, followed by the application of subclass-specific reagents. Sections were incubated overnight with appropriately diluted monoclonal antibodies. Subsequent 30 minute respective layers included: 1) goat anti-mouse IgG1 (Caltag Laboratories, San Francisco, CA) diluted 1:20 with biotinylated goat anti-mouse IgM (Caltag) at a dilution of 1:50, and 2) alkaline phosphatase mouse anti-alkaline phosphatase (APAAP) (Dakopatts) diluted 1:60 together with avidin-horseradish peroxidase (ABC-Per) (Vector Laboratories, Burlingame, CA) used at 5 times the concentration prescribed by the manufacturer. Visualization of each antigen was obtained by first using 0.06% red aminoethylcarbizole (AEC) (Sigma Chemical Co., St. Louis, MO) with 0.03% hydrogen peroxide in 0.1 M acetate buffer (pH 5.2) followed by blue staining with the alkaline phosphatase substrate kit (Vector) mixed in 0.05 M Tris-buffered saline (pH 8.2) with 0.002 M levamisol. The result was a blue R1C7+ cell, a red R4/23+ cell, and cells positive for both antigens were a mixture of these colors.

This technique could not be used in rhesus splenic tissue because of large quantities of endogenous alkaline phosphatase that was not blocked by levamisol. This spurious staining was eliminated by using the alkaline phosphatase squelching ability of Bouins fixative.[32] This fixative eliminated endogenous alkaline phosphatase reactivity while preserving the conjugated biotin of secondary polyclonal reagents. After incubations with the primary monoclonal reagents and secondary subclass-specific reagents as described above, subsequent respective layers included: 1) mouse monoclonal PAP complexes (Dakopatts) diluted 1:75, 2) AEC development, 3) Bouins fixative for 1 minute, 3) avidin-alkaline phosphatase (ABC-AP) (Vector) at 5 times the concentration prescribed by the manufacturer, and 4) alkaline phosphatase development as described above. The result was a blue R4/23+ cell, a red R1C7+ cell, and similar to the above technique, cells positive for both antigens were a mixture of these colors. Both of the above techniques used multiple control sections that tested for potential cross-reactivity of all of the reagents.

Double labeling using primary monoclonal antibodies of the IgG1 subclass was performed using the technique originally described by Hancock et al with modifications.[31] A four-layer PAP technique[28] using AEC as the chromagen, herein referred to as the first sequence, was used for the detection of viral protein (antibody R1C7). Sections were then labeled with a second monoclonal antibody (such as EBM11 or T11/3Pt2H9) using the APAAP technique or ABC-AP technique. Briefly, the second monoclonal antibody was applied after the rabbit anti-mouse immunoglobulins (Dakopatts) in the first sequence. After development of the first sequence with AEC, the APAAP sequence consisted of goat anti-mouse IgG1 (Caltag) at 1:50 followed by the APAAP reagent, while the ABC-AP method consisted of biotinylated goat anti-mouse IgG1 (Caltag) at 1:30 followed by Bouins fixative and finally the ABC-AP reagent. Both techniques were developed using the blue alkaline phosphatase detection kit (Vector) that was previously described. Incubation times less than 12 minutes with the blue alkaline phosphatase reagent was necessary to prevent overdevelopment and subtle cross-reaction between the first and second antibody sequences. Multiple control sections using mouse irrelevant IgG1 (Coulter Laboratories) at similar concentrations as the primary monoclonal antibodies were substituted for each monoclonal reagent individually and together in both sequences to test for cross-reaction.

Results

All study animals were unequivocally confirmed to be infected with SIV by multiple viral isolation recoveries before or at the time of tissue procurement, including the animals in which biopsies were obtained three weeks after inoculation. Fourteen of 19 animals (74%) also mounted a humoral immune response to SIV proteins by 15 weeks after inoculation using an ELISA technique.[21,22] Five animals failed to develop this response, and four of these animals died by 16 weeks after inoculation (565-70, 165-86, 160-86, and 126-86). These results are consistent with our pre-

vious observations suggesting a direct correlation of antibody titer with ability to survive infection.[21,22]

Follicular Hyperplasia

Three of the five animals had detectable quantities of viral protein localized to specific cells within the tissues. The most frequent staining pattern seen within this group was delicate reticular staining within germinal centers (Figure 1A). The percentage of germinal centers that stained within the tissue varied between animals and ranged from 30% in animal 120-85 to practically 100% in animal 167-84. Viral protein was usually localized throughout the entire germinal center; however, in animals 167-84 and 120-85, approximately half of the stained follicles had reaction product distributed in the outer third of the germinal center, closer to the mantle zone (Figure 1B). In animal 159-86, staining within germinal centers was found in the 13-week biopsy of spleen and lymph node but not in the seven week sample of lymph node. Furthermore, in lymph node tissues from both time periods from this animal, occasional small round viral antigen-positive cells were distributed throughout the mantle zone and paracortex. Similarly, in the spleen from this animal at 13 weeks, in addition to reticular staining within germinal centers, rare positive large cells were found within the red pulp. Viral protein could not be detected in animals 202-84 and 221-86.

Double labeling demonstrated that the staining within germinal centers was associated with follicular dendritic cells (Figure 1C). In animal 167-84, regions within the follicle lacking p27 reaction product (usually the central area of the germinal center) corresponded with a general paucity of this cell type. Using desmosomes and intricate plasma membrane infoldings as ultrastructural markers,[33] the dendrites of follicular dendritic cells were more plentiful in the outer region of germinal centers in animals 120-85 and 167-84, and the processes of these cells were swollen and contained homogeneous electron-dense material and membrane-bound vesicles, lamellar myelin figures, and free ribosomes. In animal 167-84, a solitary lentiviral particle was found within the follicular dendritic cell labyrinth. Viral budding from this cell type was not observed in any specimen examined.

Follicular Depletion with Normal or Expanded Paracortices/T Cell Domains

The tissues from four of the seven animals in this group (565-70, 258-85, 309-78, 188-86) contained only a rare p27+ cell. The remaining three animals had a very large viral protein load; as many as 30 p27+ large and small cells per dry high powered field (×400) could be found distributed throughout the entire parenchyma of lymph node and spleen. In spleen, these cells could be found within cords, sinusoids, as well as within white pulp (Figure 1D). Similarly, positively stained cells within lymph node were localized to paracortex, medulla, as well as the subcapsular sinus, where large histiocytic cells were intensely positive.

In spleen, the vast majority of the p27+ cells coexpressed macrophage antigens (Figure 1E, F). This phenomenon was especially true in the red pulp, where up to 80% of the p27+ cells were EBM11+, and these p27+, EBM11+ macrophages were found within sinusoids (Figure 1F) as well as within cords. p27+ macrophages were also found within the white pulp, although usually not with the same frequency as seen in the red pulp. Significantly smaller numbers of p27+, T11+ cells were found in the red and white pulp (Figure 1G), and most, but not all, of these cells were localized to white pulp domains. Staining for follicular dendritic cells using R4/23 revealed that none of the p27+ cells within the white pulp coexpressed this antigen.

Similar results were found in lymph nodes. Most of the cells that contained detectable quantities of viral protein were immunophenotypically identified as macrophages, and these cells were found in paracortex, medulla, as well as occasionally within small, depleted follicles. A smaller number of p27+, T11+ cells were found throughout the node parenchyma. Ultrastructural examination of lymph node from animal 188-84 revealed typical retroviral particles within membrane-bound vacuoles of parenchymal macrophages (Figure 2) and within the adjacent extracellular space. Viral budding into the extracellular space or into the vacuoles was not observed.

Lymphoid Depletion

Excluding the spleen from animal 205-85 that contained no SIV-positive cells, all lymphoid tissues from this group contained at least two or three cells per section that were positive for SIV core protein (Figure 1I). However, the relative number of cells containing viral protein in tissues from this group was comparatively lower than in other groups, and most high power fields that were microscopically examined did not contain a single positively-stained cell.

The p27+ cells tended to be larger cells, and despite their relative paucity, they tended to be preferentially distributed to specific microenvironments within the tissue. Most often in spleen, these cells were found either within the small Malpighian corpuscles or within the red pulp just adjacent to them. Similarly, in lymph node, these cells were localized to depleted paracortical/cortical areas or within medullary regions that bordered on these atrophic lymphoid areas.

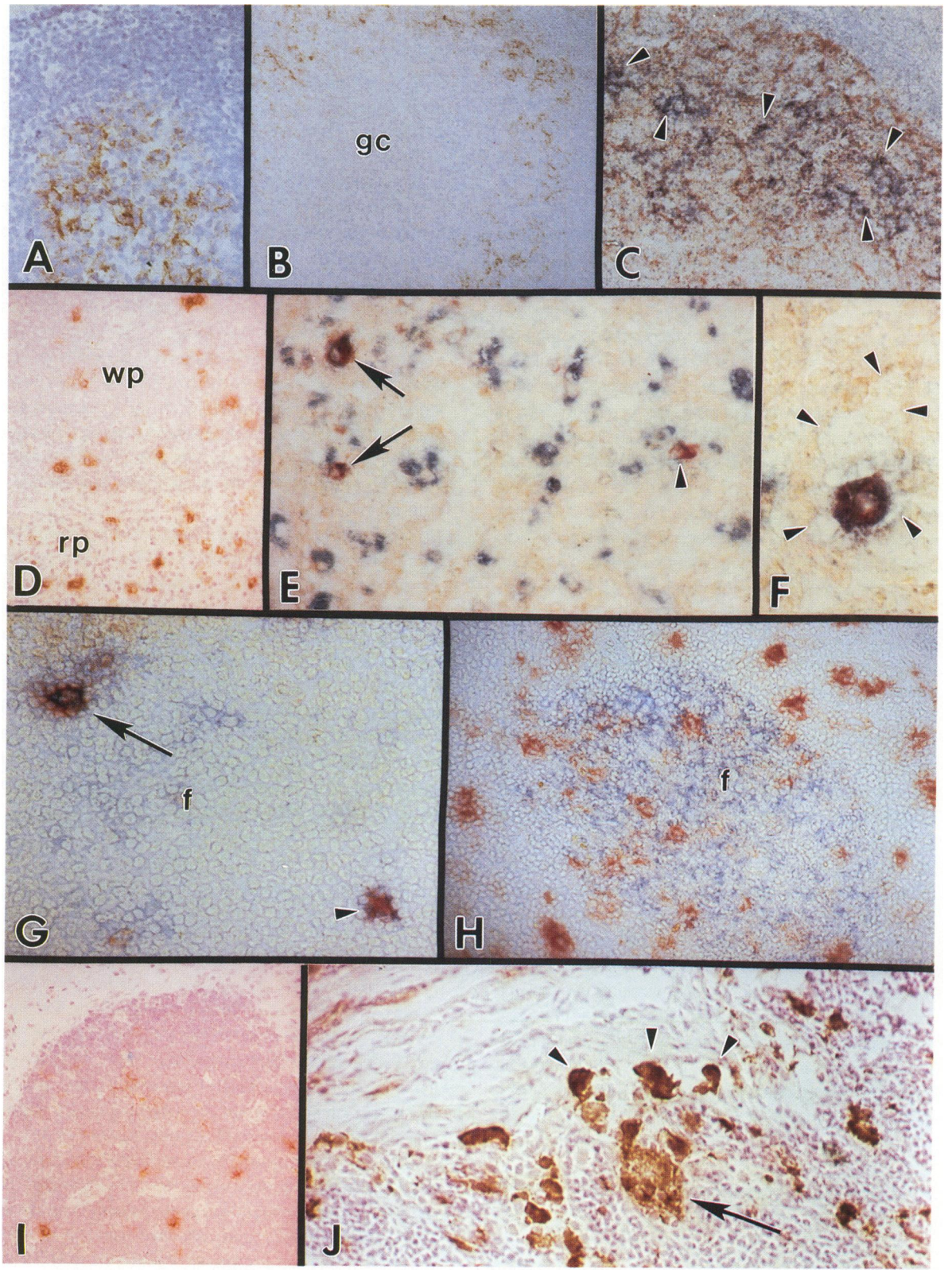
A
B
gc
C
wp
rp
D
E
F
f
G
f
H
I
J

Figure 1. *Localization of SIV-related core protein in tissue sections having varied histologic patterns.* **A, B, and C**—*167-84. Follicular hyperplasia; nodal germinal centers (gc) have delicate reticular staining for core antigen (A), often only in the regions closer to mantle zones (B). Double labeling using R1C7 (anti-p27, blue) and R4/23 (anti-follicular dendritic cells, red-brown) antibodies (C) shows that the blue p27 antigen appears to localize to a subset of the red-brown follicular dendritic cell processes (arrowheads). (PAP, A, ×165; B, ×100; ABC-Per and APAAP, C, ×244)* **D, E, F, and G**—*165-86. Nodal follicular depletion with normal or expanded T cell domains; p27+ cells are found within white pulp (wp) and red pulp (rp) of spleen (D). (PAP, ×125) E and F—Double labeling using R1C7 (red-brown; E, arrowhead) and EBM11 (anti-macrophage, blue) antibodies in red pulp. Macrophages containing SIV core protein are detected by color mixing (E, arrows; F) and are occasionally found within sinusoids (F, border of sinusoid delineated by arrowheads). (PAP and ABC-AP, E, ×300; F, ×600) G—Within a splenic follicle (f), some of the p27+ cells (red-brown, arrowhead) coexpress blue T11 antigens (arrow). (PAP and ABC-AP, ×300)* **H**—*126-86 at 9 weeks. Histologically normal tissues; there is no coexpression of SIV-related antigens (red/brown) and follicular dendritic cell antigens (blue) within splenic follicles (f). (PAP and ABC-AP, ×200)* **I**—*205-85. Depleted lymph node; only a rare cell stains for SIV core protein within residual lymphoid tissue. (PAP, ×125)* **J**—*127-83. Granulomatous lymphadenitis*—M. avium intracellulare *negative; multiple p27+ cells are seen within the cortex. Other positively stained cells include macrophages within the subcapsular sinus (arrowheads) and a multinucleate giant cell (arrow). (PAP, ×230)*

Granulomatous Lymphadenitis and Splenitis

In both avian tuberculosis positive and negative tissues, multiple foci containing clusters of epithelioid macrophages and islands of preexisting lymphoid tissue were found in both lymph nodes and spleen. In the avian tuberculosis negative tissues, multinucleate giant cells of the foreign body or Langhans' type were particularly plentiful among the epithelioid macrophages. Therefore, these tissues allowed the examination of large numbers of cells that could be identified as macrophages on the basis of morphologic criteria alone.

In the avian tuberculosis negative tissues, approximately 10% of the epithelioid macrophages expressed p27 antigen in the cytoplasm (Figure 1J). The multinucleate giant cells were almost uniformly positive (Figure 1J),

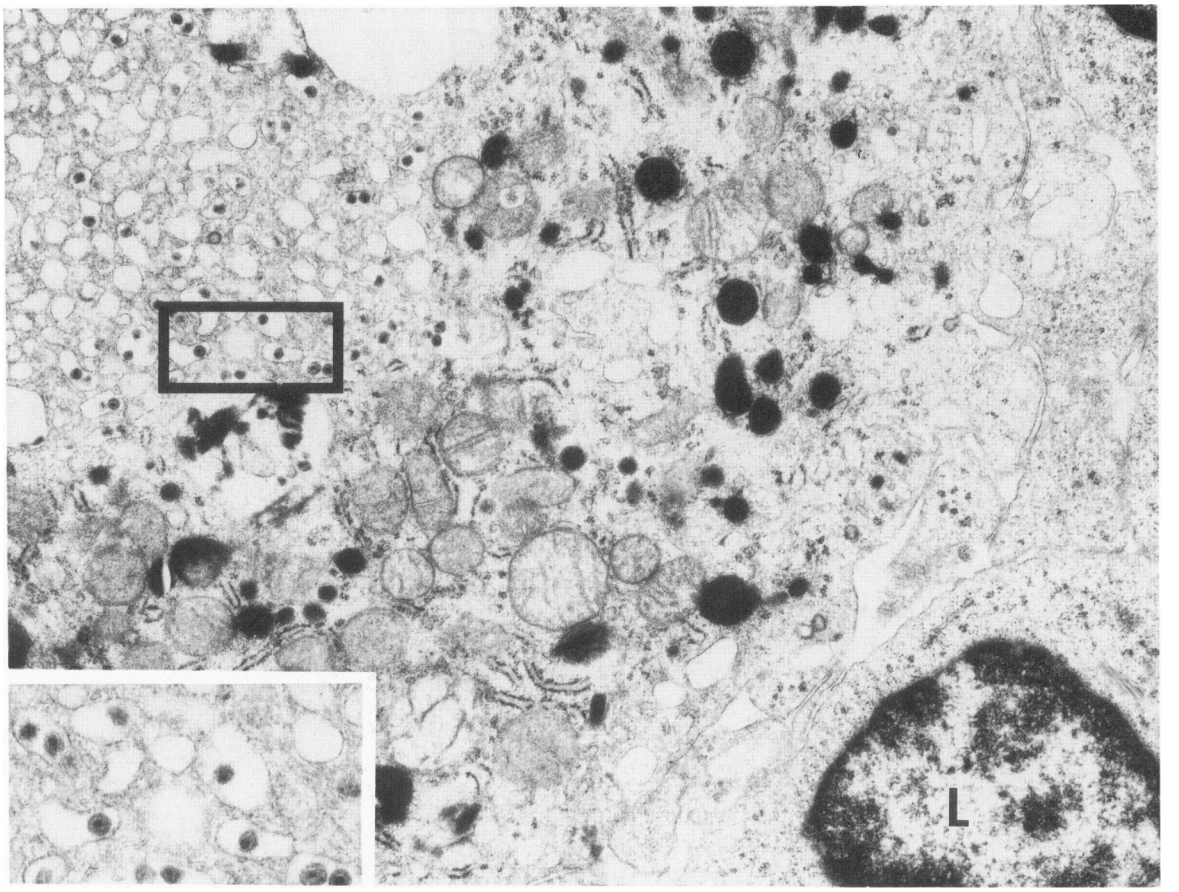

Figure 2. *188-84. Transmission electron micrograph of lymph node from a monkey infected with SIV. A macrophage is adjacent to a lymphoid cell (L). The cytoplasm of the macrophage contains multiple mitochondria, electron-dense granules and lipid droplets, and vacuoles (enclosed). Within some of the vacuoles, mature virions (enclosed and enlarged in inset) are present. These viral particles have cylindrical nucleoids and diameters of approximately 110 nm. (uranyl acetate and lead citrate, ×15,000; inset, ×37,500)*

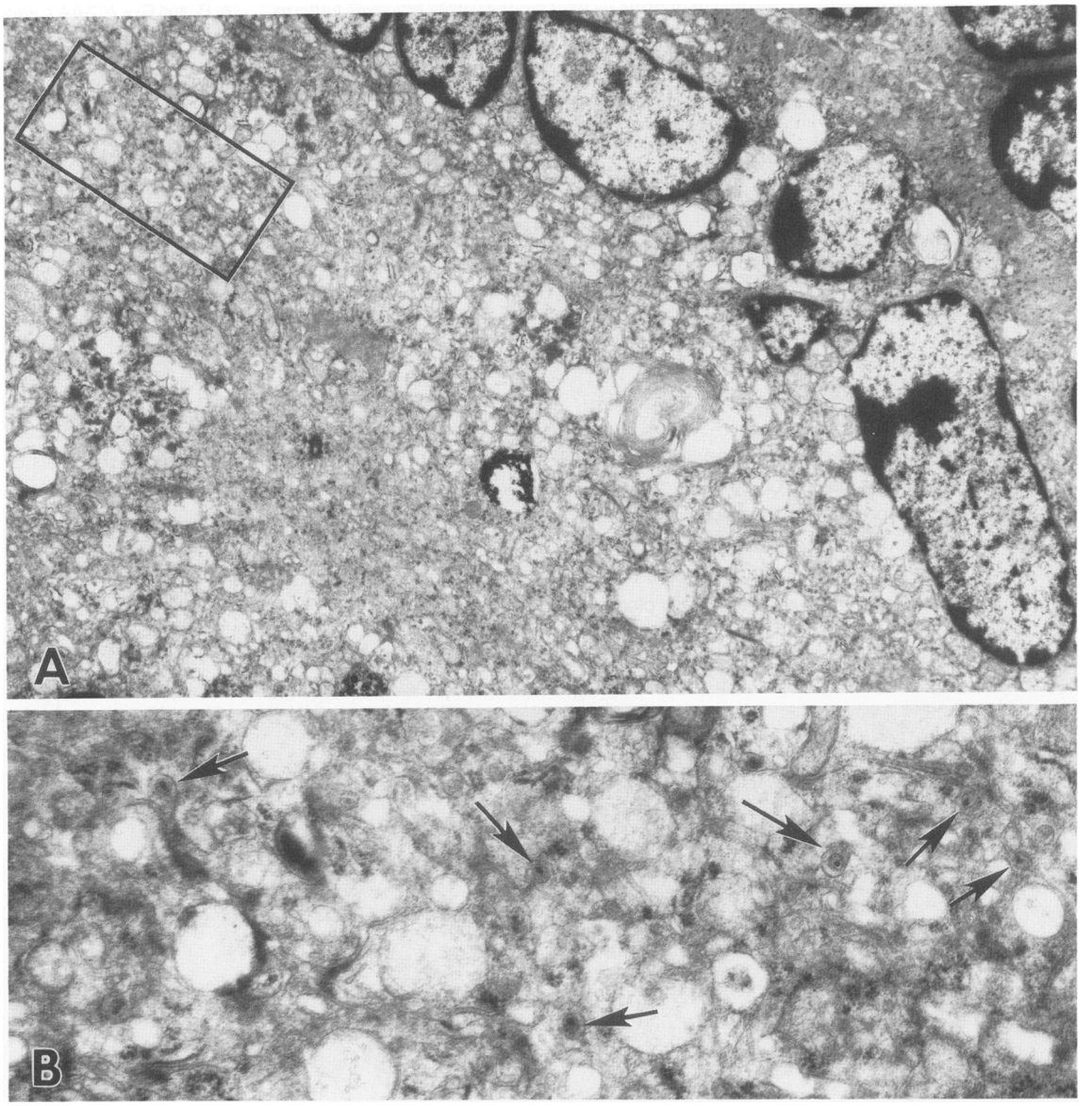

Figure 3A, B. *127-83. Transmission electron micrographs of a multinucleate giant cell from the spleen of a SIV-infected monkey. Mature viral particles (B, arrows) are contained singly within small intracytoplasmic membrane-bound vacuoles. (Enclosed in A and enlarged in B; uranyl acetate and lead citrate, A, ×6000; B, ×26,000)*

and using electronmicroscopy to examine these cells, lentiviral particles were found within cytoplasmic membrane-bound vacuoles (Figure 3A, B). In the avian tuberculosis positive tissue, the spleen was negative for viral protein, and the lymph node contained only a rare p27+ macrophage; most of the macrophages containing mycobacterial bacilli did not contain viral antigen.

Normal Lymph Nodes and Spleen

The number of p27+ cells in tissues of this group varied from case to case. The early biopsy tissues from animal 159-86 were either negative or contained only rare positive cells. Conversely, the tissues from animal 126-86 at 7 and 9 weeks post-inoculation contained up to 40 p27+ cells per high powered field. Between these two extremes, the tissues taken from this same animal at three weeks contained between 5 to 10 p27+ cells per high powered field.

Positively-labeled cells tended to be large, nondendritic cells scattered throughout the parenchyma of the tissue. In lymph node, these cells were found in medulla, paracortex, and mantle zones of follicles. Fewer cells were localized to germinal centers. In the spleen, cells with morphologic characteristics of those seen in lymph

node were distributed evenly throughout red and white pulp. These cells did not localize to any preferential location within the spleen.

Except for tissues from animal 126-86 at 3 weeks, results of double labeling experiments were similar to those of the group with follicular depletion; most of the p27+ cells coexpressed macrophage antigens. p27+ lymphocytes were less numerous and only accounted for 10–25% of the total number of p27+ cells. However, in a very early 3-week biopsy from animal 126-86, 65–75% of the p27+ cells in lymph node were T11+ lymphocytes rather than EBM11+ macrophages. In the only other biopsy taken three weeks after inoculation (animal 159-86), only a solitary p27+ cell was observed in one section of lymph node, and conclusive double labeling experiments could not be performed on this tissue. Viral antigen was not localized to follicular dendritic cells in tissues from this group (Figure 1H).

Discussion

This study shows that SIV, which causes a clinical syndrome in monkeys that closely parallels AIDS in humans, is preferentially localized in sections of lymph node and spleen to cells of the macrophage and dendritic cell lineages. Follicular dendritic cells are incriminated in the sequestration of viral protein during follicular hyperplastic stages, while tissue macrophages supercede follicular dendritic cells in this capacity during other stages. These results are similar, but not identical, to the results obtained from tissues of patients infected with HIV. In those patients with HIV-related persistent generalized lymphadenopathy (PGL), virus was exclusively localized to follicular dendritic cells, on the basis of electronmicroscopy[34–36] and immunohistochemistry.[36] In other studies involving varied nodal histologic changes, cells within germinal centers were again incriminated in viral sequestration,[37–39] although the cell type could not be definitively identified. In addition, on the basis of *in situ* hybridization[37] and immunohistochemistry,[38,39] a smaller population of viral positive cells could be found in the subcapsular sinus,[39] interfollicular cortex,[37] and paracortex.[38] In these studies, however, distribution of viral protein or nucleic acid was predominantly within the germinal center, regardless of the histologic pattern.

Tenner-Racz and coworkers[34,36] and others[24,25] have previously reported a disruption of the follicular dendritic cell network and degenerative ultrastructural alterations in follicular dendritic cells from patients infected with HIV. Similarly, our results demonstrate ultrastructural alterations to the follicular dendritic cell labyrinth and a relative paucity of these cells in the central areas of expanded follicles. Although degeneration and destruction of follicular dendritic cells may account for these results, we cannot entirely exclude expansion of B cells and immunoblasts from the follicular center without concurrent population by follicular dendritic cells. In addition, it is unlikely that these observations are specific for SIV-related lymphadenopathy since similar changes to follicular dendritic cells have been observed in mice and rats that have been experimentally sensitized and challenged with iodinated antigen.[33,40]

It is interesting to note that SIV-related antigen could be demonstrated in tissues as early as three weeks after experimental inoculation. More importantly, one early biopsy of lymph node from animal 126-86 was the only tissue in this study containing greater numbers of p27+ lymphocytes than p27+ macrophages. Taken collectively with results from tissues obtained later in the disease course, these data suggest that lymphocytes may be more important than macrophages in early events related to viral dissemination in lymphoid compartments, but that macrophages are more important in viral sequestration later in the course of infection. This possibility needs further investigation, however, not only to increase the number of cases examined, but also to address whether preparation of the SIV inoculum in lymphoid cells *vs.* macrophages influences the course of infection.

There appeared to be no correlation between the numbers or types of p27+ cells with length of survival, clinical outcome, or antibody response. Therefore, it will be important to examine other factors, such as the strength of the cellular immune response, for potential effects on viral replication within specific cells and systemic viral antigenic load. More studies are also necessary to determine through what cell type, if any, the virus gains entry and disseminates systemically, and if the route of infection has any correlation with viral localization, replication, and eventual clinical outcome.

It cannot be entirely excluded that the localization of SIV core protein to particular cell types represents either phagocytosis of extracellular virions or entrapment of SIV-related antigen-antibody complexes. However, for tissue macrophages, this scenario is unlikely because rhesus monocyte-derived macrophages[41] and alveolar macrophages (Ringler DJ, personal observation) actively support the replication of SIV *in vitro,* and using electron microscopy, macrophages within the encephalitic brains of SIV-infected monkeys have been previously demonstrated to contain viral particles budding into intracytoplasmic vacuoles.[42] At present, the role follicular dendritic cells play in SIV infection is uncertain, because only a rare viral particle, without evidence of budding, was seen associated with this cell type using electron microscopy, and in a related study, SIV RNA was not significantly localized to germinal centers using *in situ* hybridization.[43] This issue is addressed more completely by Wyand and coworkers in a companion study.[43]

Lastly, this study corroborates the importance of macrophages and other histogenetically-related cell types in the localization of lentiviral-specific products. Gendelman, Narayan, and coworkers have previously demonstrated infection of macrophages with the related ovine-caprine lentiviruses.[44-46] Similarly, replication of HIV within macrophages and dendritic cells *in vitro* has been described,[47-50] and these cells appear to escape, at least in large part, the lytic phase of infection that is characteristic of HIV-infected lymphocytes.[49,50] Because macrophages are the source of a number of active cytokines, it is possible that these long-lived infected cells may mediate localized inflammatory sequelae as well as systemic altered immunoregulatory phenomena. In this way, lentiviral-infected macrophages residing in normal microenvironments, such as splenic sinusoids, as well as macrophages within inflammatory sites, such as the brain, may both play a role in the devastating clinical outcome observed in SIV-infected monkeys and patients with AIDS.

References

1. Klatzmann D, Barre-Sinoussi F, Nugeyre MT, Dauguet C, Vilmer E, Griscelli C, Brun-Vezinet F, Rouzioux C, Gluckman JC, Chermann J-C, Montagnier L: Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science 1984, 225:59–63
2. Dalgleish AG, Beverly PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA: The CD4(T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 1984, 312:763–767
3. Klatzmann D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercend T, Gluckman J-C, Montagnier L: T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 1984, 312:767–768
4. Wood GS, Burns BF, Dorfman RF, Warnke RA: *In situ* quantitation of lymph node helper, suppressor, and cytotoxic T cell subsets in AIDS. Blood 1986, 67:596–603
5. Mildvan D, Mathur U, Enlow RW, Romain PL, Winchester RJ, Colp C, Singman H, Adelsberg BR, Spigland I: Opportunistic infections and immune deficiency in homosexual men. Ann Intern Med 1982, 96:700–704
6. Morris L, Distenfeld A, Amorosi E, Karpatkin S: Autoimmune thrombocytopenic purpura in homosexual men. Ann Intern Med 1982, 96:714–717
7. Friedman-Kien AE, Laubenstein LJ, Rubinstein P, Buimovici-Klein E, Marmor M, Stahl R, Spigland I, Kim KS, Zolla-Pazner S: Disseminated Kaposi's sarcoma in homosexual men. Ann Intern Med 1982, 96:693–700
8. McDougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A, Nicholson JKA: Bonding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. Science 1986, 231:382–385
9. Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R: The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell 1986, 47:333–348
10. Hancock WW, Atkins RC: Immunohistologic analysis of the cell surface antigens of human dendritic cells using monoclonal antibodies. Transplant Proc 1984, 16:963–967
11. Wood GS, Turner RR, Shiurba RA, Eng L, Warnke RA: Human dendritic cells and macrophages: *In situ* immunophenotypic definition of subsets that exhibit specific morphologic and microenvironmental characteristics. Am J Pathol 1985, 119:73–82
12. Wood GS, Warner NL, Warnke RA: Anti-Leu-3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. J Immunol 1983, 131:212–216
13. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, Kanki PJ, Essex M, Desrosiers RC: Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 1985, 228:1201–1204
14. Kanki PJ, McLane MF, King NW Jr, Letvin NL, Hunt RD, Sehgal P, Daniel MD, Desrosiers RC, Essex M: Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. Science 1985, 228: 1199–1201
15. Chakrabarti L, Guyader M, Alizon M, Daniel MD, Desrosiers RC, Tiollais P, Sonigo P: Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature 1987, 328:543–547
16. Letvin NL, Daniel MD, Sehgal PK, Desrosiers RC, Hunt RD, Waldron LM, MacKey JJ, Schmidt DK, Chalifoux LV, King NW: Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science 1985, 230:71–73
17. Chalifoux LV, Ringler DJ, King NW, Sehgal PK, Desrosiers RC, Daniel MD, Letvin NL: Lymphadenopathy in macaques experimentally infected with the simian immunodeficiency virus (SIV). Am J Pathol 1987, 128:104–110
18. Chalifoux LV, King NW, Daniel MD, Kannagi M, Desrosiers RC, Sehgal PK, Waldron LM, Hunt RD, Letvin NL: Lymphoproliferative syndrome in an immunodeficient rhesus monkey naturally infected with an HTLV-III-like virus (STLV-III). Lab Invest 1986, 55:43–50
19. Kannagi M, Yetz JM, Letvin NL: *In vitro* growth characteristics of simian T-lymphotropic virus type III. Proc Natl Acad Sci USA 1985, 82:7053–7057
20. Kannagi M, Kiyotaki M, Desrosiers RC, Reimann KA, King NW, Waldron LM, Letvin NL: Humoral immune responses to T cell tropic retrovirus simian T lymphotropic virus type III in monkeys with experimentally induced acquired immune deficiency-like syndrome. J Clin Invest 1986, 78:1229–1236
21. Daniel MD, Letvin NL, Sehgal PK, Hunsmann G, Schmidt DK, King NW, Desrosiers RC: Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus. J Gen Virol 1987, 68:3183–3189
22. Daniel MD, Letvin NL, Sehgal PK, Schmidt DK, Silva DP, Solomon KR, Hodi FS Jr, Ringler DJ, Hunt RD, King NW, Desrosiers RC: Prevalence of antibodies to 3 retroviruses in a captive colony of macaque monkeys. Int J Cancer 1988, 41: 601–608
23. Wyand MS, Chalifoux LV, Desrosiers RC, Daniel MD, Letvin NL, Ringler DJ, King NW: Histologic categorization of lymph nodes from macaques infected with simian immunodeficiency virus. Manuscript in preparation.

24. Biberfeld P, Porwit-Ksiazek A, Bottiger B, Morfeldt-Mansson L, Biberfeld G: Immunohistopathology of lymph nodes in HTLV-III infected homosexuals with persistent adenopathy or AIDS. Cancer Res 1985, 45(suppl):4665s–4670s
25. Diebold J, Marche CI, Audouin J, Aubert JP, Le Tourneau A, Bouton CI, Reynes M, Wizniak J, Capron F, Tricottet V: Lymph node modification in patients with the acquired immunodeficiency syndrome (AIDS) or with AIDS related complex (ARC). Path Res Pract 1985, 180:590–611
26. Chalifoux LV, King NW, Letvin NL: Morphologic changes in lymph nodes of macaques with an immunodeficiency syndrome. Lab Invest 1984, 51:22–26
27. Minassian AA, Kalyanaraman VS, Gallo RC, Popovic M: Monoclonal anibodies against HIV-2 core proteins: Cross-reactivity with HIV-1 and SIV. Proc Natl Acad Sci 1988, 85: 6939–6943
28. Hancock WW, Becker GJ, Atkins RC: A comparison of fixatives and immunohistochemical technics for use with monoclonal antibodies to cell surface antigens. Am J Clin Pathol 1982, 78:825–831
29. Ringler DJ, Walsh DG, MacKey JJ, Hunt RD, King NW: Immunophenotypic characterization of mononuclear phagocytes and dendritic cells in lymphoid organs of the rhesus monkey. Clin Immunol Immunopathol 1988, 49:349–364
30. Ringler DJ, Hancock WW, King NW, Letvin NL, Daniel MD, Desrosiers RC, Murphy GF: Immunophenotypic characterization of the cutaneous exanthem of SIV-infected rhesus monkeys: Apposition of degenerative Langerhans cells and cytotoxic lymphocytes during the development of acquired immunodeficiency syndrome. Am J Pathol 1987, 126:199–207
31. Hancock WW, Lord RH, Colby AJ, Diamantstein T, Rickles FR, Dijkstra C, Hogg N, Tilney NL: Identification of IL 2R+ T cells and macrophages within rejecting rat cardiac allografts, and comparison of the effects of treatment with anti-IL 2R monoclonal antibody or cyclosporin. J Immunol 1987, 138:164–170
32. Bulman AS, Heyderman E: Alkaline phosphatase for immunocytochemical labelling: Problems with endogenous enzyme activity. J Clin Pathol 1981, 34:1349–1351
33. Hanna MG Jr, Szakal AK: Localization of ^{125}I-labeled antigen in germinal centers of mouse spleen: Histologic and ultrastructural autoradiographic studies of the secondary immune reaction. J Immunol 1968, 101:949–962
34. Tenner-Racz K, Racz P, Dietrich M, Kern P: Altered follicular dendritic cells and virus-like particles in AIDS and AIDS-related lymphadenopathy. Lancet 1985, 1:105–106
35. Armstrong JA, Horne R: Follicular dendritic cells and virus-like particles in AIDS-related lymphadenopathy. Lancet 1984, 2:370–372
36. Tenner-Racz K, Racz P, Bofill M, Schulz-Meyer A, Dietrich M, Kern P, Weber J, Pinching AJ, Veronese-Dimarzo F, Popovic M, Klatzmann D, Gluckman JC, Janossy G: HTLV-III/LAV viral antigens in lymph nodes of homosexual men with persistent generalized lymphadenopathy and AIDS. Am J Pathol 1986, 123:9–15
37. Biberfeld P, Chayt KJ, Marselle LM, Biberfeld G, Gallo RC, Harper ME: HTLV-III expression in infected lymph nodes and relevance to pathogenesis of lymphadenopathy. Am J Pathol 1986, 125:436–442
38. Baroni CD, Pezzella F, Mirolo M, Ruco LP, Rossi GB: Immunohistochemical demonstration of p24 HTLV III major core protein in different cell types within lymph nodes from patients with lymphadenopathy syndrome (LAS). Histopathology 1986, 10:5–13
39. Ward JM, O'Leary TJ, Baskin GB, Benveniste R, Harris CA, Nara PL, Rhodes RH: Immunohistochemical localization of human and simian immunodeficiency viral antigens in fixed tissue sections. Am J Pathol 1987, 127:199–205
40. Nossal GJV, Abbot A, Mitchell J, Lummus Z: Antigens in immunity. XV. Ultrastructural features of antigen capture in primary and secondary follicles. J Exp Med 1968, 127:277–290
41. Banapour B, Marthas M, Pedersen N, Luciw P: Simian immunodeficiency virus from a molecular clone productively infects rhesus macaque macrophages in vitro. (Abstr). IV International Conference on AIDS, 1988, Stockholm, Sweden
42. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW: Simian immunodeficiency virus-induced meningoencephalitis: Natural history and retrospective study. Ann Neurol 1988, 23(suppl):S101–S107
43. Wyand MS, Ringler DJ, Naidu YM, Mattmuller M, Chalifoux LV, Daniel M, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid organs. II. *In situ* hybridization. Am J Pathol 1989, 134:385–393
44. Gendelman HE, Narayan O, Molineaux S, Clements JE, Ghotbi Z: Slow, persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow. Proc Natl Acad Sci USA 1985, 82:7086–7090
45. Narayan O, Wolinsky JS, Clements JE, Strandberg JD, Griffin DE, Cork LC: Slow virus replication: The role of macrophages in the persistence and expression of visna viruses of sheep and goats. J Gen Virol 1982, 59:345–356
46. Narayan O, Kennedy-Stoskopf S, Sheffer D, Griffin DE, Clements JE: Activation of caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. Infect Immun 1983, 41:67–73
47. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth M, Janotta F, Aksamit A, Martin MA, Fauci AS: Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science 1986, 233:1089–1093
48. Patterson S, Knight SC: Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. J Gen Virol 1987, 68:1177–1181
49. Salahuddin SZ, Rose RM, Groopman JE, Markham PD, Gallo RC: Human T lymphotropic virus type III infection of human alveolar macrophages. Blood 1986, 68:281–284
50. Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M: The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 1986, 233:215–219

Acknowledgment

The authors thank Ms. Beverly Blake for editorial assistance and Mrs. Debbie Brosseau for manuscript preparation. Dr. Wayne Hancock from Farmington, CT provided expert advisory help.