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Early Events in Tissues during Infection with Pathogenic (SIVmac239) and Nonpathogenic (SIVmac1A11) Molecular Clones of Simian Immunodeficiency Virus

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The extent of virus replication, tissue distribution, localization of virus within tissues, and the presence of pathological lesions was examined early after experimental infection of rhesus monkeys with simian immunodeficiency virus (SIV). Three strains of SIV were used: molecularly cloned pathogenic SIVmac239; molecularly cloned nonpathogenic SIVmac1A11; and uncloned pathogenic SIVmac. The major targets of infection in all animals at 2 weeks postinoculation were the thymus and spleen. The distribution of virus within lymphoid organs varied with the viral inoculum: nonpathogenic SIVmac1A11 was present primarily within lymphoid follicles and in the thymic cortex; SIVmac239 was present primarily within periarteriolar lymphoid sheaths in the spleen, the paracortex of lymph nodes, and the medulla of the thymus; uncloned SIVmac was present in all these areas but tended to parallel the distribution of SIVmac239. Animals inoculated with nonpathogenic SIVmac1A11 had fewer SIV-positive cells by in situ hybridization and after 13 weeks postinoculation, virus was undetectable in any tissue from these animals. No significant pathological abnormalities were recognized in animals inoculated with this nonpathogenic virus. In contrast, nearly half of the animals inoculated with either SIVmac or SIVmac239 developed significant pathological lesions, including opportunistic infections by 13 weeks postinoculation, highlighting the virulence of these viruses. Our results indicate marked differences in tissue distribution between pathogenic and nonpathogenic molecular clones of SIV during the acute phase of infection. The most striking differences were the absence of SIVmac1A11 from the central nervous system and thymic medulla. The prominent early involvement of the thymus suggests that infection of this organ is a key event in the induction of immune suppression by SIV. (Am J Pathol 1994, 145:428–439)

Immunosuppressive lentiviruses in humans, macaques, and cats (HIV, SIV, and FIV, respectively) share many genetic, immunological and pathological features, including a primary acute flulike illness occurring within the first few weeks of infection, followed by a variable "asymptomatic" phase, and a terminal phase of profound immune deficiency.[1–6] While much is known about the terminal phase of acquired immune deficiency syndrome (AIDS) in all of these species, relatively little is known about the acute phase of infection that probably plays a major role in determining the disease outcome.

In HIV infection, the viral load early in infection is high with active viral replication (for review see ref. 1). Viral dissemination during this time may be important in determining disease course and the relative involvement of various organs such as the brain and gastrointestinal tract. This early, rampant viral repli-

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cation decreases coincident with the onset of the host immune response.[1] The strength of this immune response has been correlated with survival time.[4,7] A thorough understanding of the early events of infection with immunosuppressive lentiviruses is important for understanding the pathogenesis of these viruses and designing appropriate vaccines and interventive strategies. These early events are difficult to study in HIV-infected humans because it is usually months or years before individuals are aware of their infection status. Thus, animal models are critical for examining the primary events in infection with these viruses.

The simian immunodeficiency virus (SIV) macaque model of AIDS has been used extensively to examine pathogenesis, vaccine strategies, and viral determinants of disease.[2,4,8–10] The availability of defined molecular clones of varying cellular tropism and virulence makes this model system particularly useful.[4,11] The objective of this study was to examine early events, particularly those occurring in tissues, in the pathogenesis of SIV, using molecular clones of markedly different virulence. We reasoned that comparing clones of different virulence would allow us to make inferences as to the importance of infection of various tissues to disease outcome.

In this prospective study, rhesus macaques were inoculated with either pathogenic (SIVmac239)[12] or nonpathogenic (SIVmac1A11)[13] molecular clones of SIV and compared to macaques inoculated with an uncloned biological isolate of SIV (SIVmac). The biological isolate has been used extensively in previous pathogenesis and vaccine experiments.[14–16] In addition to obvious differences in virulence, the biological properties of SIVmac239 and SIVmac1A11 have been further characterized *in vitro* as well as in experimentally infected macaques. SIVmac239 replicates and causes cytopathology in cultures of macaque T lymphocytes but not macrophages,[17–19] whereas SIVmac1A11 replicates and causes cytopathology in both cell types.[18–20] The inability of SIVmac239 to cause cytopathology in macaque macrophages is due to restricted replication in macrophages that is determined by the *env* gene but is not due to restricted entry into macrophages.[21] Furthermore, the ability to replicate in macrophages can be conferred on SIVmac239 by as few as nine amino acid changes in *env*, and these amino acid changes occur in approximately 30% of infected macaques over the course of infection.[22,23] *In vivo*, pathogenic SIVmac239 (as well SIVmac) establish persistent cell-free and cell-associated viremia and persistent infection of multiple tissues leading to fatal AIDS-like disease,[11] including SIV-encephalitis and enteropathy.[16,23–25] In contrast, SIVmac1A11 causes a transient cell-associated viremia and is nonpathogenic, despite greater than 98% homology at the nucleotide level with SIVmac239.[11,26,27] The viral determinants responsible for the biological differences between these two molecular clones are complex and scattered throughout the genome.[11,27]

Animals inoculated with these viruses were serially sacrificed and examined histologically and by *in situ* hybridization to determine viral distribution. Our results indicate that the markedly different virulence and distinct biological characteristics of the two molecular clones are reflected in their distribution within tissues and that infection of the thymic medulla may be a key event in determining progression to AIDS. Furthermore, within the first 13 weeks of infection, there were a surprising number of significant histopathological lesions in animals inoculated with pathogenic virus.

Materials and Methods

Animals and Viral Infections

Twenty-four male rhesus macaques (*Macaca mulatta*) aged 22 to 41 months were obtained from the D retrovirus-free and SIV-free colony at the California Regional Primate Research Center. The animals were divided randomly into three groups of eight and housed in accordance with standards of the American Association for Accreditation of Laboratory Animal Care. The investigators adhered to the Guide for the Care and Use of Laboratory Animals prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council. Before use, the animals were negative for antibodies to HIV-2, SIV, type D retrovirus, and simian T-cell leukemia virus type 1 (STLV-1).

The three groups of eight animals were inoculated intravenously with cell-free stocks of SIV. One group was inoculated with 10^4 50% tissue culture infectious doses of the pathogenic molecular clone, SIVmac239;[12] a second group was inoculated with 10^4 50% tissue culture infectious doses of the nonpathogenic molecular clone, SIVmac1A11;[13,23] and the third group of eight animals was inoculated with $10^{2.5}$ 50% tissue culture infectious doses of uncloned SIVmac (derived from SIVmac251 kindly provided by R. Desrosiers, New England Regional Primate Research Center). These doses of SIVmac239 and SIVmac have induced disease in the majority of animals by six months after inoculation.[15,27] The dose of SIVmac1A11 was chosen because it induces viremia in 100% of inoculated animals and was equal to the dose of SIVmac239. While SIVmac239 and

SIVmac1A11 behave very differently *in vivo*, they are quite similar at the nucleotide level. The genetic differences between these two molecular clones are scattered throughout the genomes.[11] Salient genetic differences include premature truncation of the *vpr* gene and the transmembrane domain of *env* in SIVmac1A11, whereas the *nef* gene of SIVmac239 has an in-frame stop codon that rapidly reverts in rhesus monkeys.[29] Sequence differences are also present in variable region 1 (V1) in the surface domain of the *env* gene. Full proviral sequences of SIVmac1A11 and SIVmac239 have accession numbers M76764 and M33262 respectively, in the GenBank database resource.[11,30]

Virus Isolation

Peripheral blood and cerebrospinal fluid (CSF) were collected before inoculation and at 1, 2, 4, 6, 8, 11, 13, 21, and 23 weeks after inoculation. Infectious virus was isolated from blood by cocultivation of 5×10^6 CEMX174 cells with at least 4×10^6 peripheral blood mononuclear cells (PBMCs) as described previously.[15] For isolation of SIV from CSF, 10^6 CEMX174 cells were pelleted and resuspended with 0.5 ml of whole CSF and incubated at room temperature for 30 minutes. Ten ml of RPMI 1640 tissue culture media was then added, and the cells cultured as above. Culture supernatants were tested biweekly for the presence of SIV by p27 capture enzyme-linked immunosorbent assay (ELISA).[31] Cultures were considered positive if culture supernatants yielded 10 ng/ml or more of SIV p27 at two consecutive time points. All cultures were maintained for 8 weeks and tested for SIV p27 by ELISA before being scored as virus-negative. This assay could detect one SIV-infected cell per 10^6 PBMCs.

SIV-Specific Antibody Titers and Plasma Antigenemia

SIV-specific antibody titers were determined by ELISA, using sucrose gradient-purified SIVmac grown in HUT 78 cells and peroxidase-conjugated anti-macaque immunoglobulin G, as described previously.[27] The ELISA was performed on serial twofold dilutions of serum from 1:100 to 1:204,800. All dilutions of each sample and the positive control (serum from an SIV-infected animal) were assayed in duplicate and the mean values of optical density were calculated. Data are presented as the inverse of the highest sample dilution that exceeded 50% of the maximum positive control optical density.

The amount of SIV-p27 per ml of plasma was determined using a commercial SIV-p27 antigen capture ELISA kit (Coulter Immunology, Hialeah, FL) according to the manufacturer's recommendations. Antigen values of greater than or equal to 0.10 ng/ml of plasma were considered to be SIV antigen-positive.

Tissue Collection and Histological Evaluation

Two animals from each group were killed at 2, 8, 13, and 23 weeks postinoculation (pi) by intravenous overdose of sodium pentobarbital. Tissue specimens obtained from these animals at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 6 μ for *in situ* hybridization to determine the distribution of SIV nucleic acid. Sections were also cut at 6 μ and stained with hematoxylin and eosin for histopathological examination. Lymphoid tissues from the animals were categorized according to the presence or absence of histological alterations (Table 1). The criteria used to categorize changes in the lymph nodes and spleen have been previously described.[32] For the thymus, we modified a previously published histological classification used in SIV-infected macaques.[33] Five categories were used to classify thymic morphology to maintain consistency with the grading scheme for lymph nodes and spleen: 1) mild lymphoid depletion equal to approximately 20 to 40% reduction in cortical thickness; 2) moderate lymphoid depletion equal to approximately 40 to 60% reduction in cortical thickness; 3) severe depletion with lobular collapse and no distinction between cortex and medulla; 4) giant cell thymitis—infiltrates of multinucleated giant cells in the thymus; and 5) normal.

Localization of SIV-Infected Cells by In Situ *Hybridization*

In situ hybridization was performed using a ^{35}S-labeled SIV DNA probe on formalin-fixed, paraffin-embedded tissue sections as previously described,[16] except that tissues were denatured so that both RNA and DNA would be detected. Radioactive probes were produced with a specific activity of 1×10^8 cpm/μg or greater by nick-translation of a 9-kb fragment of the SIVmac genome (obtained from P. Sonigo, Institute Pasteur) consisting of the entire *gag*, *pol*, and *env* regions.[34] Controls included: 1) SIV-infected and uninfected cultured T cells (HUT78); 2)

Table 1. *Lymphoid Morphology and Localization of SIV by in Situ Hybridization*

			Thymus		Spleen		Axillary lymph node	
			ISH	Histopath	ISH	Histopath	ISH	Histopath
Virus	Time pi	Animal no.	Cortex/ Medulla		Follicles/PALS/ Red pulp		Follicles/ Paracortex	
SIVmac	2 weeks	24318	+/+++	2	+++/++/–	1	+/++	2
		24354	+/+++	1	++/+++/–	3	+/+	3
	8 weeks	24260	–/–	1	++/–/–	2	+/–	2
		24634	+/+	5	+/+/–	2	–/–	2
	13 weeks	24373	+/+	5	++/+/–	1	+/++	1
		24377	–/–	5	+/–/–	1	+/–	1
	23 weeks	24891*	–/+	3	–/–/+	3	+/++++	4
		25003	–/+	5	+/–/–	1	+/++	1
SIVmac239	2 weeks	23894	NA	NA	+/++/–	2	+/++	1
		23930	NA	NA	+/+++/+	2	NA	NA
	8 weeks	24231	+/++	5	+/++/–	1	+/++	1
		24255	+/++++	5	+/++++/+	1	+/+++	1
	13 weeks	24289	–/+	5	–/++/–	1	+/–	2
		24219	+/–	5	–/–/–	1	–/–	1
	23 weeks	24242	NA	NA	++/+/–	1	+/+	1
		24263	–/–	5	+/–/–	1	+/+	1
SIVmac1A11	2 weeks	24120	++/–	5	+/–/–	5	–/–	5
		24777	NA	NA	+/–/–	1	+/–	2
	8 weeks	24029	+/–	5	++/–/–	5	+/–	5
		24209	++/–	5	+/–/+	5	+/–	5
	13 weeks	24062	+/–	5	–/–/–	1	–/–	5
		24172	++/–	5	++/–/–	5	–/–	5
	23 weeks	24091	–/–	5	–/–/–	5	–/–	5
		24272	–/–	5	–/–/–	5	–/–	5

ISH = *in situ* hybridization. PALS = periarteriolar lymphoid sheaths. Results of *in situ* hybridization were quantified as follows: no positive cells = negative; one to five positive cells per section = 1+; five to 10 positive cells per 10 × field = 2+; 10 to 15 positive cells per 10 × field = 3+; greater than 15 positive cells per 10 × field = 4+.

Histopath = histopathological grading of lymphoid morphology. For lymph nodes and spleen grade 1 = follicular hyperplasia; grade 2 = follicular depletion with normal or expanded paracortices; grade 3 = generalized lymphoid depletion; grade 4 = giant cell lymphadenitis; and grade 5 = normal. For the thymus the grading system was slightly modified: grade 1 = 20 to 40% reduction in cortical thickness; grade 2 = 40 to 60% of reduction in cortical thickness; grade 3 = severe atrophy with lobular collapse and no visible cortex; grade 4 = giant cell thymitis, and grade 5 = normal.

* Animal 24891 was euthanatized at 22 weeks pi with terminal simian AIDS.

NA = not available for study.

matched tissues from two uninfected rhesus monkeys and one type D retrovirus-infected rhesus monkey; and 3) hybridization with a nick-translated probe containing only the pSP64 plasmid vector. Sections were examined microscopically and subjectively quantified as follows: no positive cells = negative; one to five positive cells per section = 1+; five to 10 positive cells per 10× field = 2+; 10 to 15 positive cells per 10× field = 3+; greater than 15 positive cells per 10× field = 4+.

Results

Virus Isolation

Infectious virus was isolated from PBMCs of all animals inoculated with pathogenic virus (SIVmac or SIVmac239) by 1 week pi with the exception of one animal inoculated with SIVmac239 (Table 2). By two weeks pi, all animals inoculated with pathogenic virus were persistently viremic. Animals inoculated with nonpathogenic SIVmac1A11 were all viremic by week 2, but the viremia was transient (Table 2). The cessation of viremia by 8 weeks pi is a consistent feature of infection with SIVmac1A11.[26–28]

In the CSF, virus was isolated from seven of eight animals inoculated with SIVmac and four of eight animals inoculated with SIVmac239 by 1 week pi, and from 100% of animals inoculated with either of these pathogenic viruses by 2 weeks pi (Table 2). In contrast, virus was never isolated from CSF of animals inoculated with SIVmac1A11 (Table 2). By 8 weeks pi with SIVmac or SIVmac239, the CSF in only one animal in each group remained virus-positive, but SIV could again be isolated from CSF at 21 and 23 weeks pi in the four remaining SIVmac- or SIVmac239-inoculated animals (Table 2). Thus, virus was present in the CSF very early (before week 4) and late (weeks 21 and later) after inoculation with pathogenic virus but was inconsistently isolated at other times.

Table 2. *Isolation of SIV from Peripheral Blood and Cerebrospinal Fluid*

Virus	Animal no.	Weeks pi: 1 PBMCs/CSF	2 PBMCs/CSF	4 PBMCs/CSF	8 PBMCs/CSF	11 PBMCs/CSF	13 PBMCs/CSF	21 PBMCs/CSF	23 PBMCs/CSF
SIVmac	24318	+/+	+/+						
	24354	+/+	+/+						
	24260	+/+	+/+	+/–	+/–				
	24634	+/+	+/+	+/+	+/–				
	24373	+/NA	+/+	+/+	+/–	+/–	+/–		
	24377	+/+	+/+	+/+	+/–	+/–	+/–		
	24891*	+/+	+/+	+/+	+/+	+/+	+/NA	+/+	+/+
	25003	+/+	+/+	+/+	+/–	+/–	+/–	+/+	+/+
SIVmac239	23894	+/+	+/+						
	23930	+/–	+/+						
	24231	–/–	+/+	+/+	+/+				
	24255	+/–	+/+	+/–	+/–				
	24289	+/–	+/+	+/+	+/–	+/–	+/–		
	24219	+/+	+/+	+/–	+/–	+/–	+/–		
	24242	+/+	+/+	+/+	+/–	+/+	+/+	+/+	+/+
	24263	+/+	+/+	+/+	+/–	+/–	+/–	+/+	+/+
SIVmac1A11	24120	NA/–	+/–						
	24777	NA/–	+/–						
	24029	NA/–	+/–	+/–	–/–				
	24209	NA/–	+/–	–/–	–/–				
	24062	NA/–	+/–	+/–	–/–	–/–	–/–		
	24172	NA/–	+/–	–/–	–/–	–/–	–/–		
	24091	NA/–	+/–	–/–	–/–	–/–	–/–	–/–	–/–
	24272	NA/–	+/–	+/–	–/–	–/–	–/–	–/–	–/–

* Animal 24891 was euthanatized at 22 weeks pi with terminal simian AIDS.
NA = not available.

SIV-Specific Antibody Titers and Plasma Antigenemia

All of the animals inoculated with SIVmac or SIVmac239 were antigenemic at 2 weeks pi (Table 3) with the exception of 24219 (inoculated with SIVmac239). By 13 weeks pi, SIV-specific immunoglobulin had been detected in five of six SIVmac-infected and five of six SIVmac239-infected animals that were alive at least 8 weeks pi (Table 3). The two animals inoculated with SIVmac or SIVmac239 that did not develop antibodies and remained antigenemic were 24891 and 24255. Animal 24891 (inoculated with uncloned SIVmac) was diagnosed with simian AIDS at 22 weeks pi (Table 4). This association of short disease course and lack of an early humoral immune response has been described previously.[4,35,36] Animal 24255 (inoculated with SIVmac239) was probably on a similar disease course but was randomly assigned for necropsy at 8 weeks pi (where it was found to have cryptosporidiosis, see Table 4 and below) and thus later time points are not available. In contrast, animals inoculated with the nonpathogenic SIVmac1A11 developed lower antibody titers than animals inoculated with pathogenic virus, were slower to seroconvert and were never antigenemic (Table 3).

Histopathological Lesions Occur soon after Infection with Pathogenic SIV

Even though all the animals except one (24891) were killed early in the disease course, before the development of clinically evident disease, there were a number of significant morphological changes in lymphoid tissue and lesions in other organ systems (Tables 1 and 4) in animals inoculated with SIVmac or SIVmac239. Most animals inoculated with SIVmac1A11 had normal lymphoid tissue (Table 1) and none of them had lesions in other organ systems.

Within lymphoid organs, it was interesting to note the asynchronous development of morphological changes in thymus versus lymph nodes and spleen (Table 1). The latter two organs generally had similar morphological changes, which occurred rapidly. In contrast, few major changes in thymic morphology were noted, except in the one animal that died from simian AIDS (24891) in the 24-week course of this study. Similar findings have been reported in two previous studies where thymic depletion in clinically healthy animals was generally not evident before 24 weeks pi.[33,37]

Of the 16 animals inoculated with either SIVmac or SIVmac239, 11 had significant lesions in nonlym-

Table 3. *SIV-Specific Antibody Titers and Plasma Antigenemia*

Virus	Animal no.	2 weeks pi		8 weeks pi		13 weeks pi		23 weeks pi	
		Ab	Ag	Ab	Ag	Ab	Ag	Ab	Ag
SIVmac	24318	<100	+						
	24354	<100	+						
	24260	<100	+	6,400	–				
	24634	<100	+	1,600	–				
	24373	<100	+	<100	+	12,800	+		
	24377	<100	+	51,200	–	51,200	–		
	24891*	<100	+	ND	ND	<100	+	<100	+
	25003	<100	+	ND	ND	3,200	+	3,200	+
SIVmac239	23894	<100	+						
	23930	<100	+						
	24231	<100	+	6,400	–				
	24255	<100	+	<100	+				
	24289	<100	+	800	–	6,400	–		
	24219	<100	–	800	–	800	–		
	24242	<100	+	ND	ND	6,400	–	6,400	–
	24263	<100	+	ND	ND	25,600	–	51,200	–
SIVmac1A11	24120	<100	–						
	24777	<100	–						
	24029	<100	–	<100	–				
	24209	<100	–	<100	–				
	24062	<100	–	100	–	100	–		
	24172	<100	–	<100	–	100	–		
	24091	<100	–	ND	ND	200	–	200	–
	24272	<100	–	ND	ND	100	–	200	–

Ab = anti-SIV antibody titer in plasma presented as the inverse of the highest sample dilution that exceeded 50% of the maximum positive control optical density. Ag = SIV major core (p27) antigen in plasma.
* Animal 24891 was euthanatized at 22 weeks pi with terminal simian AIDS.

Table 4. *Pathological Findings*

Virus	Time pi	Animal no.	Major pathological diagnoses
SIVmac	2 weeks	24318	Meningitis, mild; cytomegalovirus enteritis
		24354	Meningoencephalitis, lymphocytic with perivascular cuffs and glial nodules
	8 weeks	24260	NSL
		24634	Interstitial pneumonia, multifocal
	13 weeks	24373	Cytomegalovirus pneumonia
		24377	Meningitis and mild choroid plexitis, pulmonary arterial thrombosis and recanalization
	23 weeks	24891*	Cryptosporidiosis of duodenum and pancreatic ducts: cytomegalovirus enteritis. Disseminated giant cell disease: SIV encephalitis, giant cell pneumonia, giant cell lymphadenitis etc.
		25003	NSL
SIVmac-239	2 weeks	23894	Meningitis, mild
		23930	Meningitis, choroid plexitis and rare lymphocytic perivascular cuffs. Mineralization, perivascular, basal ganglia
	8 weeks	24231	Meningitis, choroid plexitis and rare lymphocytic perivascular cuffs. ***Pneumocystis carinii*** **pneumonia**
		24255	Cryptosporidiosis, small intestine and pancreatic ducts
	13 weeks	24289	Meningomyelitis, lymphocytic with perivascular cuffs and glial nodules
		24219	NSL
	23 weeks	24242	NSL
		24263	NSL

* Animal 24891 was euthanatized at 22 weeks pi with terminal simian AIDS. Animals inoculated with SIVmac1A11 had no significant pathological findings. NSL = no significant lesions identified.

phoid tissue (Table 4). Among these are cytomegalovirus-induced enteritis and pneumonia, *Pneumocystis carinii* pneumonia, cryptosporidiosis of the small intestine and pancreatic ducts, perivascular mineralization in the basal ganglia, and thrombosis and recanalization of the pulmonary arteries. These are all lesions commonly seen in SIV-infected animals in the terminal stages of disease.[8,38–42] However, in

this study, all but one of these animals (24891) was killed before the terminal stage. The rapidity with which these lesions occurred highlights the virulence of these isolates in rhesus macaques.

The central nervous system (CNS) was particularly interesting because at 2 weeks pi, four of four animals inoculated with SIVmac or SIVmac239 had a mild meningitis, two of four had perivascular cuffs of lymphocytes in cerebral white matter, and one of four had glial nodules consistent with previous reports of acute infection with uncloned SIV.[43,44] These lesions contained viral nucleic acid (see below) and occurred concurrently with onset of plasma antigenemia and uniform isolation of SIV from CSF. At later time points, CNS lesions were less common as was viral isolation from CSF (Tables 2 and 4). Moreover, this was generally associated with the disappearance of plasma antigenemia (Table 3). However, these associations did not always hold as exemplified by 24289, an animal inoculated with SIVmac239 that had CNS lesions at necropsy 13 weeks pi but no viral isolation from the CSF after 4 weeks pi. Conversely, animal 24242, also inoculated with SIVmac239, was persistently virus-positive in the CSF but had no CNS lesions at necropsy 23 weeks pi.

Pathogenic and Nonpathogenic Molecular Clones Have Different Tissue Distributions

At 2 weeks pi, abundant virus was present in tissues coincident with the onset of plasma antigenemia (Figures 1 and 2 and Table 3). The major targets in all animals at this early time point were the thymus and spleen (Figure 1 and Table 1). Less virus, based on the number of positive cells seen by *in situ* hybridization, was present in lymph nodes, and other tissues. The distribution of virus within lymphoid organs varied with viral inoculum: nonpathogenic SIVmac1A11 was present primarily within lymphoid follicles in lymph nodes and spleen, and in the thymic cortex (Figure 1, a to c); SIVmac239 was present primarily within periarteriolar lymphoid sheaths in the spleen, the paracortex of lymph nodes, and the medulla of the thymus (Figure 1,, d to f); uncloned SIVmac was present in all these areas but tended to parallel the distribution of SIVmac239. Two animals (24255 and 24242) infected with SIVmac239 also had a diffuse signal in scattered germinal centers (Figure 2) similar to what has been described in HIV-infected humans.[45]

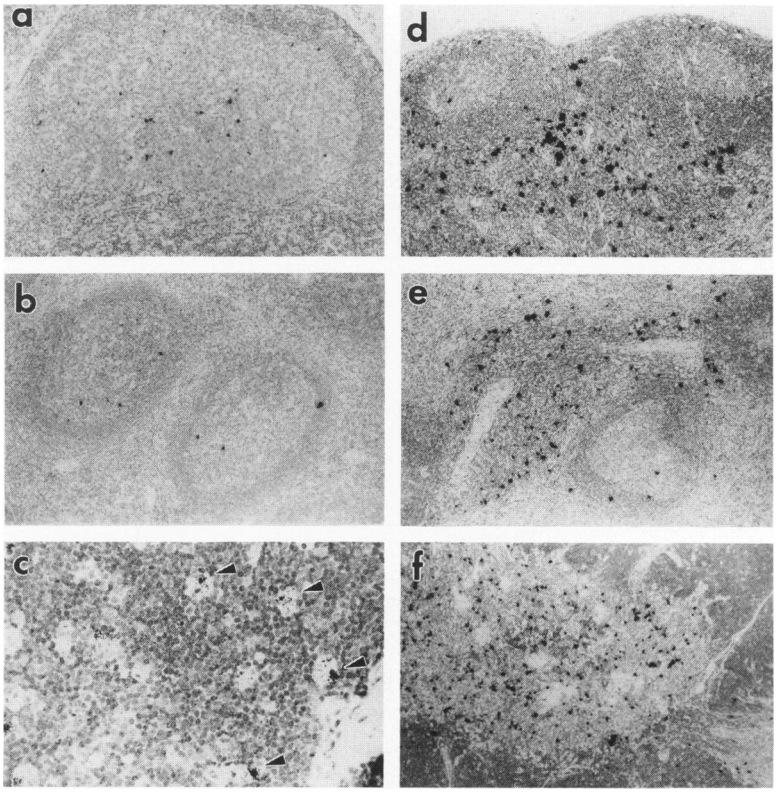

Figure 1. In situ *hybridization for SIV in peripheral lymph node* (**a,d**) *spleen* (**b,e**), *and thymus* (**c,f**) *from animals inoculated with nonpathogenic SIVmac1A11* (**a** *to* **c**) *or pathogenic SIVmac239* (**d** *to* **f**). *SIVmac1A11 was present primarily within lymphoid follicles in lymph nodes* (**a**) *and spleen* (**b**) *and in scattered cells in the thymic cortex (arrowheads,* **c**). *In contrast, SIVmac239 was detected primarily within the paracortex of lymph nodes* (**d**), *periarteriolar lymphoid sheaths in the spleen* (**e**), *and the medulla of the thymus* (**f**). *Original magnification:* **a**, ×*100;* **b**, ×*50;* **c**, ×*200;* **d** *to* **f**, ×*50.*

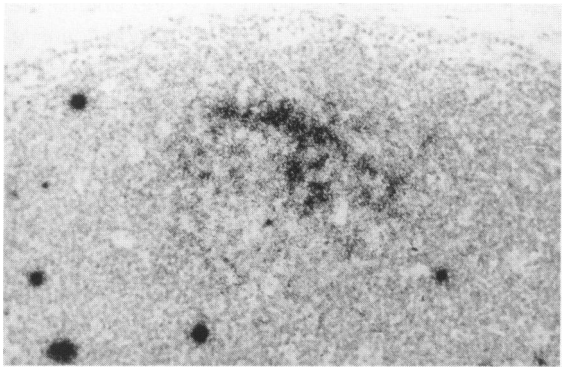

Figure 2. In situ *hybridization for SIV in a peripheral lymph node from animal 24255 inoculated with SIVmac239. In addition to scattered individual positive cells, there is diffuse labeling in the germinal center. Magnification: ×125.*

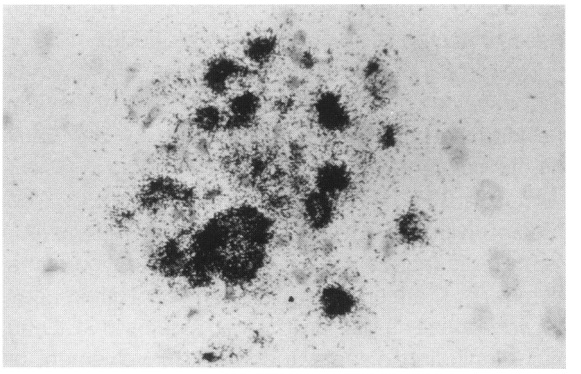

Figure 3. In situ *hybridization for SIV on brain of 24891 (inoculated with SIVmac) demonstrating abundant SIV nucleic acid in a multinucleated giant cell lesion. Magnification: ×250.*

By 13 weeks pi, the number of SIV-positive cells by *in situ* hybridization in all tissues of animals inoculated with either SIVmac or SIVmac239 had decreased. This drop in the number of SIV-positive cells was roughly coincident with the onset of an anti-SIV immune response (Tables 1 and 3). A similar correlation between plasma viremia, onset of an anti-SIV immune response and viral load in peripheral lymph nodes as assessed by *in situ* hybridization has recently been described by Reimann et al.[46] In the current study, the most striking decrease in the number of SIV-positive cells was seen in the thymus of SIVmac and SIVmac239-infected animals. This could not be attributed to lymphoid depletion because there were few animals that had thymic atrophy (Table 1). Similarly, relatively short-lived bursts of viral replication in the thymus have been observed in SCID-hu mice infected with HIV.[47,48]

In animals inoculated with nonpathogenic SIVmac1A11, the relative number of SIV-positive cells by *in situ* hybridization remained unchanged at 13 weeks, however, all the animals in this group were aviremic by this time, suggesting a latent infection. By 23 weeks pi, no virus was detectable in any tissue from these animals.

No significant differences were seen in the animals terminated at 23 weeks pi, with the exception of one animal that did not develop a significant humoral immune response and developed a disseminated giant cell disease including SIV-encephalitis (24891). Large numbers of SIV *in situ* hybridization-positive cells were present in the parenchyma of multiple organs of this animal, including the CNS, lung, and intestine (Figure 3).

Discussion

This study demonstrates that there is abundant replication of SIV in lymphoid organs and other tissues within 2 weeks of inoculation. During this period of abundant viral replication, before the onset of a detectable humoral immune response, marked differences in tissue distribution between closely related pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones were seen. These differences suggest that infection of distinct morphological compartments of the immune system are important for induction of immune suppression and organ-specific disease. The most striking differences were the absence of nonpathogenic SIVmac1A11 from the thymic medulla, T-cell areas of the spleen and lymph nodes, and CNS. The reasons for these differences are not clear but may be related to the different cellular tropism of the isolates and the inability of SIVmac1A11 to produce a cell-free viremia.

In the thymus, SIVmac1A11 was confined to cells morphologically compatible with cortical macrophages, whereas pathogenic SIVmac239 was present primarily within the thymic medulla, where mature T cells reside. In addition, the thymus seemed to be a major early target of viral replication. After 8 weeks pi, very little virus could be detected in the thymus. This occurred concurrently with onset of an anti-SIV immune response and the disappearance of plasma antigenemia. The decrease in the relative number of SIV-infected cells in the thymus after 8 weeks pi was not due to thymic depletion. Whereas severe thymic depletion is a common finding in terminal simian AIDS, it apparently does not occur until the late stage of disease as seen in this and previous studies.[33,37] Furthermore, it is interesting to note that in this study, the progression of changes in thymic

morphology apparently occurred independently from those observed in spleen and lymph nodes.

The prominent early involvement of the thymus suggests that infection of this organ is a key event in the induction of immune suppression by SIV. Early infection of the thymus is a common finding in similar studies in SIV-infected macaques,[33,37] and in mice infected with the ts1 mutant of Moloney murine leukemia virus (a possible model of HIV infection of the thymus).[49] There are, however, significant differences between these studies and related studies in SCID-hu mice, including disagreement as to involvement of thymic epithelium,[33,37,50] quantity of virus in the thymus,[33,37,47,49] and whether the medulla[33,51] or cortex[47,48] contains more virus. Regardless, all the studies indicate that the thymus is a major early target of infection. Furthermore, our study suggests that infection of T cells in the thymic medulla is correlated with virulence, as the nonpathogenic molecular clone (SIVmac1A11) was confined to the thymic cortex.

In lymph nodes and spleen, SIV was present throughout the time course of this study, although the distribution of the pathogenic and nonpathogenic clones within the lymph nodes and spleen was distinctly different. SIVmac239 was present primarily within T-cell areas (periartiolar lymphoid sheaths of the spleen, paracortex of lymph nodes), whereas the nonpathogenic SIVmac1A11 was primarily in B-cell areas (germinal centers) in individual cells. The immunophenotype of the infected cells was not determined, but the SIV-infected cells within germinal centers are probably macrophages or CD4+ lymphocytes. A diffuse signal for SIV in germinal centers suggestive of follicular dendritic cells was only detected in two animals (24255, 24242). This is consistent with previous studies in SIV-infected macaques,[52] but is in contrast to reports in HIV-infected humans where HIV nucleic acid is commonly seen associated with follicular dendritic cells in hyperplastic germinal centers.[45,53] The reason for the lower frequency of SIV-positive germinal centers is not clear, especially because the distribution of viral antigen in germinal centers of SIV-infected macaques and HIV-infected humans is identical.[32,52,54]

The speed with which significant pathological lesions were seen in animals in this study was surprising (see Table 4). By 13 weeks pi, 10 of 12 animals inoculated with SIVmac or SIVmac239 had significant lesions including opportunistic infections (four animals), pulmonary arterial thrombosis, interstitial pneumonia, perivascular mineralization of the basal ganglia, and meningitis with or without encephalitis. These are all lesions commonly described in simian AIDS.[8,38–42,55] After 13 weeks pi, it is interesting to note that with the exception of the one animal that developed simian AIDS (24891), no significant lesions were detected in any animal. This suggests that the lesions seen during the first 13 weeks of this study may be a result of rapid viral replication with concomitant high viral loads and early immune suppression.

The CNS lesions were particularly interesting because all of the animals terminated at 2 weeks pi that were inoculated with either SIVmac or SIVmac239 had SIV-infected cells in the CNS by *in situ* hybridization associated with small vessels in the meninges and parenchyma. These animals also had meningitis, and one of the animals (24354) had an encephalitis with scattered glial nodules that were SIV-positive. This is consistent with previous reports and further emphasizes the speed with which pathogenic isolates of SIV invade the CNS.[43,44] However, it raises the question of why only 20% to 50% of animals infected with SIV develop SIV encephalitis if most if not all of the animals have virus in the CSF and brain within the first few weeks of infection.[8,42] The data in this study suggest that SIV enters the CNS early and late in infection at times of high viral load. In the intervening asymptomatic period, where there is a SIV-specific immune response, viral load drops and virus becomes difficult to isolate from CSF or detect within the brain. The mechanisms of viral entry into the CNS are unknown and may be different at different stages of disease. However, the consistent association of infected cells with small venules, in conjunction with our previous observations on the role of cellular adhesion molecules in SIV encephalitis, suggest that cell trafficking into the CNS is critical for infection of the CNS and the development of subsequent SIV encephalitis.[56,57]

This study demonstrates that closely related pathogenic and nonpathogenic molecular clones of SIV have distinct tissue distribution and that viral dissemination occurs within 2 weeks of infection. The differences in tissue distribution especially within the thymus suggest that the thymus is a major early target of SIV infection and may play a key role in the induction of immune suppression and organ-specific diseases such as SIV encephalitis. A similar association between thymic infection and organ-specific disease has been described for the ts1 mutant of Moloney murine leukemia virus.[58]

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References

1. Pantaleo G, Graziosi C, Fauci AS: Mechanisms of disease: the immunopathogenesis of human immunodeficiency virus infection. N Engl J Med 1993, 328:327–335
2. Niu MT, Stein DS, Schnittman SM: Primary human immunodeficiency virus type 1 infection: review of pathogenesis and early treatment intervention in humans and animal retrovirus infections. J Infect Dis 1993, 168:1490–1501
3. Yasutomi Y, Reimann KA, Lord CI, Miller MD, Letvin NL: Simian immunodeficiency virus-specific CD8+ lymphocyte response in acutely infected rhesus monkeys. J Virol 1993, 67:1707–1711
4. Desroslers RC: The simian immunodeficiency viruses. Annu Rev Immunol 1990, 8:557–578
5. Pedersen NC, Barlough JE: Clinical overview of feline immunodeficiency virus. JAVMA 1991, 199:1298–1305
6. Pedersen NC, Ho EW, Brown ML, Yamamoto JK: Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 1987, 235:790–793
7. Sheppard HW, Ascher MS, McRae B, Anderson RE, Lang W, Allain J-P: The initial immune response to HIV and immune system activation determine the outcome of HIV disease. J Acq Immun Def Synd 1991, 4:704–712
8. Lackner AA, Dandekar S, Gardner MB: Neurobiology of simian and feline immunodeficiency virus infections. Brain Pathol 1991, 1:201–212
9. Warren JT, Dolatshahi M: First updated and revised survey of worldwide HIV and SIV vaccine challenge studies in nonhuman primates: progress in first and second order studies. J Med Primatol 1993, 22:203–235
10. Cranage MP, Polyanskaya N, McBride B, Cook N, Ashworth LAE, Dennis M, Baskerville A, Greenaway PJ, Corcoran T, Kitchin P, Rose J, Murphey-Corb M, Desroslers RC, Stott EJ, Farrar GH: Studies on the specificity of the vaccine effect elicited by inactivated simian immunodeficiency virus. AIDS Res Hum Retroviruses 1993, 9:13–22
11. Luciw PA, Shaw KE, Unger RE, Planelles V, Stout MW, Lackner JE, Pratt-Lowe E, Leung NJ, Banapour B, Marthas ML: Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). AIDS Res Hum Retroviruses 1992, 8:395–402
12. Kestler H, Kodama T, Ringler D, Marthas M, Pedersen N, Lackner A, Regier D, Sehgal P, Daniel M, King N, Desrosiers R: Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 1990, 248:1109–1112
13. Marthas ML, Banapour B, Sutjipto S, Siegel ME, Marx PA, Gardner MB, Pedersen NC, Luciw PA: Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. J Med Primatol 1989, 18: 311–319
14. Sutjipto S, Pedersen NC, Miller CJ, Gardner MB, Hanson CV, Gettie A, Jennings M, Higgins J, Marx PA: Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. J Virol 1990, 64:2290–2297
15. Miller CJ, Alexander NJ, Sutjipto S, Lackner AA, Hendrickx AG, Gettie A, Lowenstine LJ, Jennings M, Marx PA: Genital mucosal transmission of simian immunodeficiency virus: animal model for heterosexual transmission of human immunodeficiency virus. J Virol 1989, 63:4277–4284
16. Lackner AA, Smith MO, Munn RJ, Martfeld DJ, Gardner MB, Marx PA, Dandekar S: Localization of simian immunodeficiency virus in the central nervous system of rhesus monkeys. Am J Pathol 1991, 139:609–621
17. Naidu YM, Kestler HW, Li Y, Butler CV, Silva DP, Schmidt DK, Troup CD, Sehgal PK, Sonigo P, Daniel MD: Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. J Virol 1988, 62:4691–4696
18. Banapour B, Marthas ML, Munn RJ, Luciw PA: *In vitro* macrophage tropism of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). Virology 1991, 183:12–19
19. Banapour B, Marthas ML, Ramos RA, Lohman B, Unger R, Gardner MB, Pedersen NC, Luciw PA: Identification of viral determinants of macrophage tropism for simian immunodeficiency virus (SIVmac). J Virol 1991, 65:5798–5805
20. Unger RE, Marthas ML, Pratt LE, Padrid PA, Luciw PA: The nef gene of simian immunodeficiency virus SIVmac 1A11. J Virol 1992, 66:5432–5442
21. Mori K, Ringler DJ, Desrosiers RC: Restricted replication of simian immunodeficiency virus strain 239 in macrophages is determined by *env* but is not due to restricted entry. J Virol 1993, 67:2807–2814
22. Mori K, Ringler DJ, Kodama T, Desrosiers RC: Complex determinants of macrophage tropism in *env* of simian immunodeficiency virus. J Virol 1992, 66:2067–2075
23. Desrosiers RC, Hansen-Moosa A, Mori K, Bouvier DP, King NW, Daniel MD, Ringler DJ: Macrophage-tropic variants of SIV are associated with specific AIDS-related lesions but are not essential for the development of AIDS. Am J Pathol 1991, 139:29–35
24. Heise C, Vogel P, Miller CJ, Lackner AA, Dandekar S: Distribution of SIV infection in the gastrointestinal tract of rhesus macaques at early and terminal stages of AIDS. J Med Primatol 1993, 22:187–193

25. Heise C, Vogel P, Miller CJ, Halsted CH, Dandekar S: Simian immunodeficiency virus infection of the gastrointestinal tract of rhesus macaques: functional, pathological and morphological changes. Am J Pathol 1993, 142:1–13
26. Marthas ML, Miller CJ, Sutjipto S, Higgins J, Torten J, Lohman BL, Unger RE, Ramos RA, Kiyono H, McGhee JR, Marx PA, Pedersen NC: Efficacy of live-attenuated and whole-inactivated simian immunodeficiency virus vaccines against vaginal challenge with virulent SIV. J Med Primatol 1992, 21:99–107
27. Marthas ML, Ramos RA, Lohman BL, Van Rompay KKA, Unger RE, Miller CJ, Banapour B, Pedersen N, Luciw PA: Viral determinants of simian immunodeficiency virus (SIV) virulence in rhesus macaques assessed by using attenuated and pathogenic molecular clones of SIVmac. J Virol 1993, 67:6047–6055
28. Marthas ML, Sutjipto S, Higgins J, Lohman B, Torten J, Luciw PA, Marx PA, Pedersen NC: Immunization with a live, attenuated simian immunodeficiency virus (SIV) prevents early disease but not infection in rhesus macaques challenged with pathogenic SIV. J Virol 1990, 64:3694–3700
29. Kestler HW, Ringler DJ, Mori K, Panicali DL, Sehgal PK, Daniel MD, Desrosiers RC: Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. Cell 1991, 65:651–662
30. Regier DA, Desrosiers RC: The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus. AIDS Res Human Retroviruses 1990, 6:1221–1231
31. Lohman B, Higgins J, Marthas M, Marx P, Pedersen N: Development of simian immunodeficiency virus isolation, titration, and neutralization assays which use whole blood from rhesus monkeys and an antigen-capture enzyme-linked immunosorbent assay. J Clin Microbiol 1991, 29:2187–2192
32. Ringler DJ, Wyand MS, Walsh DG, MacKey JJ, Chalifoux LV, Popovic M, Minassian AA, Sehgal PK, Daniel MD, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid tissues. I. Immunohistochemistry and electron microscopy. Am J Pathol 1989, 134:373–383
33. Baskin GB, Murphey-Corb M, Martin LN, Davison-Fairburn B, Hu F-S, Kuebler D: Thymus in simian immunodeficiency virus-infected rhesus monkeys. Lab Invest 1991, 65:400–407
34. 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
35. Zhang J, Martin LN, Watson EA, Montelaro RC, West M, Epstein L, Murphey-Corb M: Simian immunodeficiency virus/delta-induced immunodeficiency disease in rhesus monkeys: relation of antibody response and antigenemia. J Infect Dis 1988, 158:1277–1286
36. 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
37. Müller JG, Krenn V, Schindler C, Czub S, Stahl-Henning C, Coulibaly C, Hunsmann G, Kneitz C, Kerkau T, Rethwilm A, terMeulen V, Müller-Hermelink HK: Alterations of thymus cortical epithelium and interdigitating dendritic cells but no increase of thymocyte cell death in the early course of simian immunodeficiency virus infection. Am J Pathol 1993, 143:699–713
38. Chalifoux LV, Simon MA, Pauley DR, MacKey JJ, Wyand MS, Ringler DJ: Arteriopathy in macaques infected with simian immunodeficiency virus. Lab Invest 1992, 67:338–349
39. Simon MA, Chalifoux LV, Ringler DJ: Pathologic features of SIV-induced disease and the association of macrophage infection with disease evolution. AIDS Res Hum Retroviruses 1992, 8:327–337
40. Lackner AA: Pathology of simian immunodeficiency virus induced disease. Current Topics in Microbiology and Immunology: Simian Immunodeficiency Virus. Edited by Desrosiers RC, Letvin N. Berlin, Springer Verlag, 1994, pp 35–64
41. Baskerville A, Ramsay A, Cranage P, Cook N, Cook RW, Dennis MJ, Greenaway PJ, Kitchin PA, Stott EJ: Histopathologic changes in simian immunodeficiency virus infection. J Pathol 1990, 162:67–75
42. Baskin GB, Murphey-Corb M, Watson EA, Martin LN: Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 1988, 25:456–467
43. Chakrabarti L, Hurtrel M, Maaire M, Vazeux R, Dormont D, Montagnier L, Hurtrel B: Early viral replication in the brain of SIV-infected rhesus monkeys. Am J Pathol 1991, 139:1273–1280
44. Sharer LR, Michaels J, Murphey-Corb H, Hu F-S, Kuebler DJ, Martin LN, Baskin GB: Serial pathogenesis study of SIV brain infection. J Med Primatol 1991, 20: 211–217
45. Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, Fox CH, Orenstein JM, Kotler DP, Fauci AS: HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993, 362:355–358
46. Reimann KA, Tenner-Racz K, Racz P, Montefiori DC, Yasutomi Y, Lin W, Ransil BJ, Letvin NL: Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. J Virol 1994, 68:2362–2370
47. Aldrovandi GM, Feuer G, Gao L, Jamieson B, Kristeva M, Chen ISY, Zack JA: The SCID-hu mouse as a model for HIV-1 infection. Nature 1993, 363:732–736
48. Bonyhadi ML, Rabin L, Salimi S, Brown DA, Kosek J, McCune JM, Kanesha H: HIV induces thymus deple-

tion in vivo. Nature 1993, 363:728–732
49. Stoica G, Floyd E, Illanes O, Wong PKY: Temporal lymphoreticular changes caused by tsl, a paralytogenic mutant of Moloney murine leukemia virus TB. Lab Invest 1992, 66:427–436
50. Stanley SK, McCune JM, Kaneshima H, Justement JS, Sullivan M, Boone E, Baseler M, Adelsberger J, Bonyhadi M, Orenstein J, Fox CH, Fauci AS: Human immunodeficiency virus infection of the human thymus and disruption of the thymic microenvironment in the SCID-hu mouse. J Ex Med 1993, 178:1151–1163
51. Namikawa R, Kasneshima H, Liberman M, Weissman IL, McCune JM: Infection of the SCID-hu mouse by HIV-1. Science 1988, 242:1684–1686
52. Wyand MS, Ringler DJ, Naidu YM, Mattmuller M, Chalifoux LV, Sehgal PK, Daniel MD, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid tissues. II. In situ hybridization. Am J Pathol 1989, 134:385–393
53. Fox CH, Tenner-Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS: Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. J Infect Dis 1991, 164:1051–1057
54. Tenner-Racz K, Bofill M, Schulz-Meyer A, Dietrich M, Kern P, Weber J, Pinching AJ, Veronese-Dimarzo F, Popovic M, Klatzmann D, Gluckman JC, Jasnossy 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
55. Baskin GB, Murphey-Corb M, Martin LN, Soike KF, Hu F-S, Kuebler D: Lentivirus-induced pulmonary lesions in rhesus monkeys (*Macaca mulatta*) infected with simian immunodeficiency virus. Vet Pathol 1991, 28: 506–513
56. Sasseville VG, Newman WA, Lackner AA, Smith MO, Lausen NC, Beall D, Ringler DJ: Elevated vascular cell adhesion molecule-1 in AIDS encephalitis induced by simian immunodeficiency virus. Am J Pathol 1992, 141:1021–1030
57. Sasseville VG, Newman W, Brodie SJ, Hesterberg P, Pauley D, Ringler DJ: Monocyte adhesion to endothelium in simian immunodeficiency virus-induced AIDS encephalitis is mediated by vascular cell adhesion molecule-1/$\alpha 4\beta 1$ integrin interactions. Am J Pathol 1994, 144:27–40
58. Saha K, Wong PKY: Rudimentary thymus of SCID mouse plays an important role in the development of retrovirus-induced neurologic disorders. Virol 1993, 195:211–218