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The Thymus in SIV Infection[1]

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The thymus plays a crucial role in the development of the T cell system, as seen in inborn thymic aplasia which leads to severe immunodeficiency. Thymectomy as well as destruction of the thymus in postnatal life make the recovery of the T cell repertoire impossible, e.g. in allogeneic bone marrow transplantation experiments [1, 2]. The human immunodeficiency virus (HIV) leads to the depletion of the peripheral CD4+ T cells. Morphological changes in the thymus have been found in adults [3] and children [4] in the late stage of HIV infection (acquired immunodeficiency syndrome; AIDS). However, little is known about the mechanisms of HIV-induced thymus alterations. Some authors suggest a direct damage by the virus whereas others were unable to detect differences in the thymus pathology of AIDS patients and patients with other thymus alterations [5]. A major problem is the severe and probably nonspecific atrophy of the human thymus obtained at postmortem investigations of AIDS patients [3–6].

The thymus would be an ideal target for HIV infection because all thymic T cells are transiently CD4-positive and follow a rapid proliferation scheme. All maturation stages of thymocytes were successfully infected in vitro [7, 8], but investigations of thymi in HIV or simian immunodeficiency virus (SIV) infection showed only low amounts of virus particles and/or proviral DNA [5, 9].

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Table 1. Experimental design

M. mulatta	Country of origin	Virus inoculum	Duration of infection, weeks	Age at death
Infected				
Early phase	China	100×32H[a]	1, 3, 6, 12, 24	3–4 years
(no AIDS)	India	10^{3-4}×239[b]	31	3 years
Late phase	China	100×32H	66, 124	4–6 years
(with AIDS)	India	100×32H	20, 31, 36, 44	2–5 years
Noninfected				
Orphan infant	India			3 months
Juvenile	India			2 and 3 years
Adult	India			8, 17 and 18 years

[a] 1 ml i.v. of 100 MID_{50} of $SIV_{mac251-32H}$.
[b] 1 ml intrathecal of 10^3–10^4 $TCID_{50}$ of SIV_{mac239}.

Since normally stem cells in concert with the thymus microenvironment regenerate a peripheral loss of T cells quite effectively, it is difficult to understand how the virus could destroy the T cell repertoire without affecting the thymus itself. The goal of our study, therefore, was to investigate the nature of SIV-induced alterations at defined time points in the early phase of infection. These early alterations could determine the further involvement of the immune system and finally result in clinically overt AIDS. The macaque model was used because it represents all aspects of HIV-induced disease in humans. These include a prodromal phase with generalized lymph node swelling and a late phase with CD4 defects, opportunistic infections, lymphomas and encephalopathy. Although SIV and HIV are genetically distinct, they share the same genome organization, replication scheme and cell tropisms [10]. Another advantage of the SIV system in morphological investigations is the close resemblance of macaque and human tissues.

Material and Methods

To investigate the pathology in the early phase of SIV infection, 5 juvenile rhesus monkeys *(Macaca mulatta)* were sacrificed at defined time points after infection. The observations were compared to those in 6 monkeys with AIDS (table 1). These animals were inoculated intravenously with 100 MID_{50} (monkey infectious dose) of the 32H isolate of SIV_{mac251} [11]. One additional monkey was inoculated intrathecally with 10^3–10^4 TCID

(tissue culture infection dose) of SIV_{mac239}. SIV-induced thymus atrophy was compared with accidental involution in 1 orphan baby monkey sacrificed at the age of 3 months because of severe starvation. Age-matched controls consisted of 2 juvenile and 3 adult monkeys.

Animals were sacrificed in deep anesthesia by exsanguination. A postmortem examination was done immediately after death and tissues were collected for virus culture, flow cytometry, immunological studies, histology, electron microscopy and immunohistochemistry. Paraffin sections were stained with hematoxylin-eosin, periodic acid-Schiff, Giemsa and Gordon-Sweet. For immunohistochemistry, antibodies generated against CD3, CD4, CD8, CD22, macrophages (KiM8), cytokeratin 8 (35βH11) and proliferating cells (Ki67) as well as SIV env (KK8; produced by K. Kent and kindly provided by the AIDS Reagent Project) were used. PAP, APAAP, immunofluorescence and three-color flow cytometry were applied according to standard protocols. For electron microscopy the tissues were fixed in glutaraldehyde, Epon-embedded and subsequently cut for semithin and ultrathin sections. Photomicrographs of the complete ultrathin sections were then assembled to represent the corresponding area in the semithin section in order to localize and characterize the different cell types.

Results and Discussion

SIV infection induces specific morphological alterations in the thymus. The earliest event is a change in the size of the cortex. This is first observed 12 and 24 weeks after infection when the width of the cortex is reduced to approximately one half (fig. 1). The process of size reduction continues to a complete loss of the thymus cortex in animals with AIDS. At earlier times (1, 3 and 6 weeks after infection) the size of the cortex is comparable to that of age-matched controls. The cellular alterations which end up in a narrowed thymus cortex consist of a reduced number of proliferating thymocytes, a reduced number of lymphoblasts within the cortex and an increased amount of plasma cells (fig. 2). However, no increase in the number of pycnotic thymocytes and no increase in the number of macrophages were observed. By ultrastructural analysis severe alterations of the cells of the thymus microenvironment were found, consisting of severe alterations and destruction of the cortical epithelium, and severe changes and loss of the interdigitating reticulum cells.

Infection of susceptible cells or thymocyte cultures with HIV or SIV in vitro lead to cell death characterized by either necrosis (apoptosis) or by the induction of syncytia [7, 8]. This cytopathogenic effect could not be observed in vivo. No increase in the number of necrotic cortical thymocytes and no increase in phagocytosis was found in vivo as compared to controls. The lack of cytopathogenic effects correlates well with a low virus load in the thymus as confirmed by different techniques.

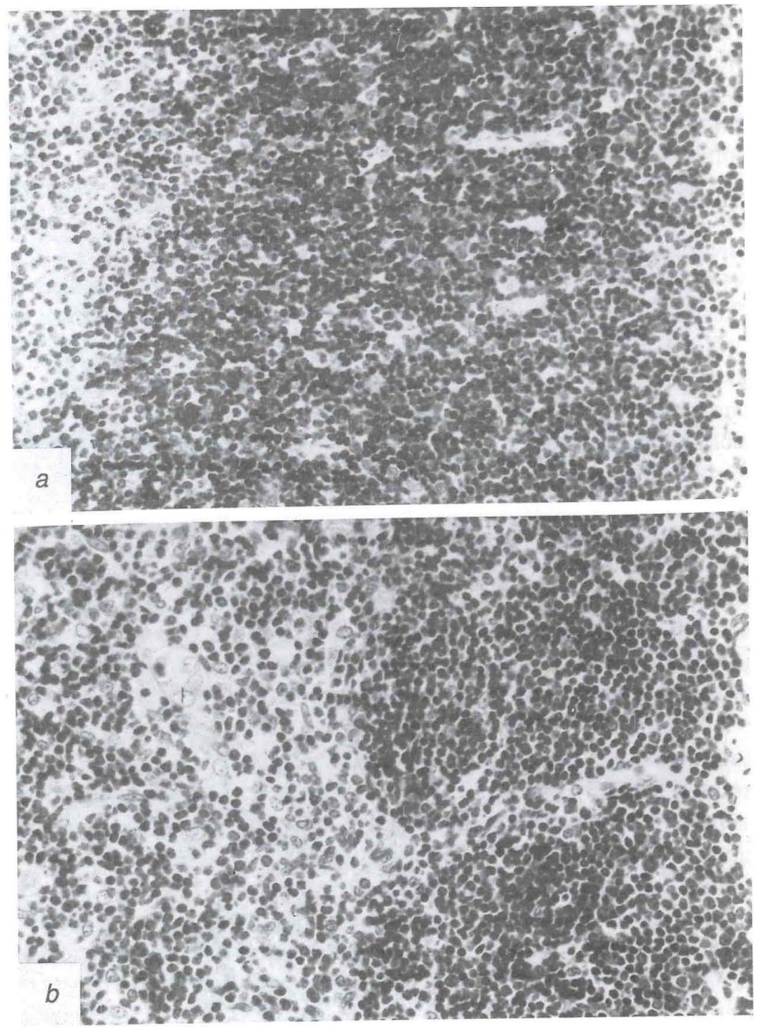

Fig. 1. a Size of the cortex in a juvenile control monkey. *b* Reduction of the size of the cortex to approximately one half in an SIV-infected monkey 24 weeks after infection. Paraffin, Giemsa. ×310.

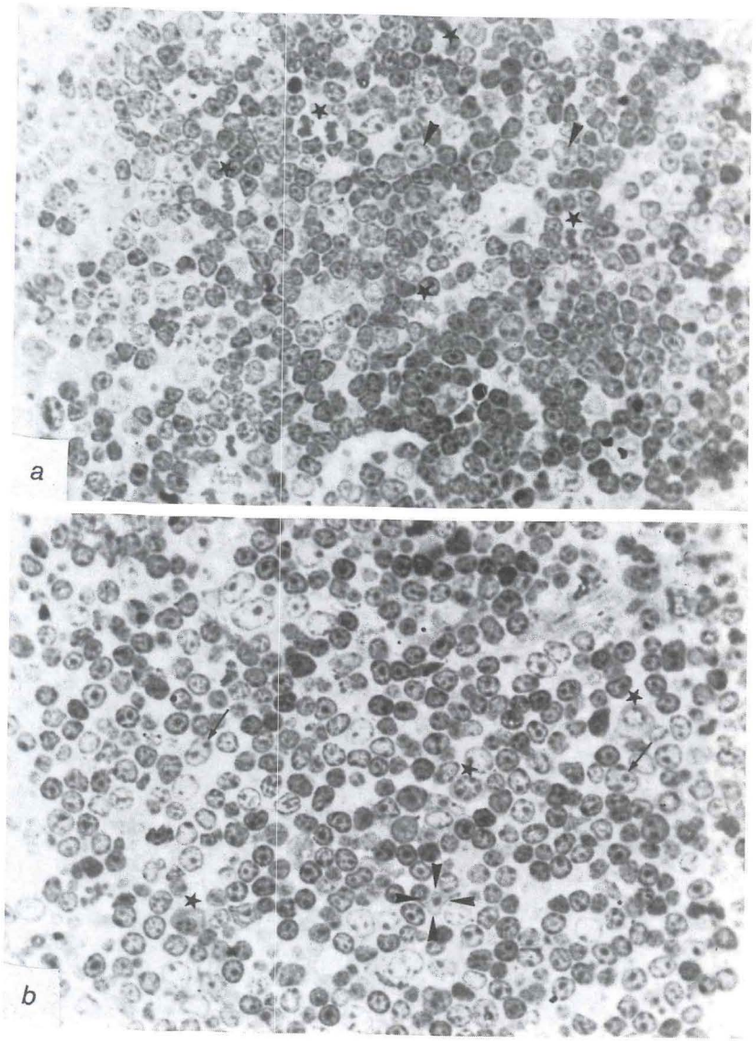

Fig. 2. *a* Cytology of the normal cortex with mitotic figures (stars) and numerous lymphoblasts (arrowheads). *b* Cytology of cortex 24 weeks after infection with reduced numbers of lymphoblasts (arrows), one degenerated epithelial cell (arrowheads) and an increased number of plasma cells (stars). Epon, semithin, toluidineblue. ×600.

Immunohistochemistry revealed that only few cells in the thymus carry viral protein. Electron-microscopic studies gave a similar result and no cytopathogenic effect was induced when homogenized thymus suspensions of infected animals were added to a susceptible cell line (C8166). Only after separation of viable thymocytes ex vivo and their polyclonal stimulation (interleukin-2 and phytohemagglutinin) a delayed cytopathogenic effect was induced. There was only a small amount of virus in the SIV-infected thymus and only few thymocytes contained proviral DNA. This is in agreement with results generated by polymerase chain reaction and in situ hybridization [9, 12]. All those data suggest that the cortical narrowing observed in animals 12 and 24 weeks after infection is not due to a direct cytopathogenic effect of SIV.

By ultrastructural analysis severe alterations of the thymus microenvironment were observed. The cortical epithelial cells in the monkeys 24 and 31 weeks after infection exhibited a vacuolization of the cytoplasm and a loss of the cytoplasmic processes (fig. 3). In some cases there was also pycnosis of the nuclei and large secondary lysosomes within the cytoplasm. These alterations occurred in the epithelial cells of the outer cortex and they were different from those epithelial cells in the corticomedullary junction with condensed cytoplasm occurring also in the controls (epithelium type 4 according to von Gaudecker [13]). The network of the cortical epithelium was investigated by immunofluorescence staining of cytokeratins; it showed a generalized loss of cortical epithelial cells in those monkeys with a partial atrophy of the cortex similar to the results of Savino et al. in man [6].

At the same time points the interdigitating cells of the medulla exhibited a loss of cell organelles and a loss of cytoplasmic processes. This destruction of the cells of the thymus microenvironment was selective. The cortical epithelial cells and the interdigitating cells exhibiting severe alterations in the early course of the SIV infection were finally completely lost, whereas some of the subcapsular and medullar epithelium as well as some Hassall corpuscles remained intact even in the most severe atrophic thymi of the monkeys with AIDS. The cause leading to the destruction of the cells of the thymic microenvironment is still unknown. Until now no virus has been demonstrated in these cells in vivo, but in vitro investigations by Numazaki et al. [14] showed that thymus epithelial cells become infected by HIV.

In parallel to the changes of the cortical epithelial cells there was an alteration in the composition of the cortical thymocytes. The total amount of immature thymocytes was decreased whereas the relative amount of the mature cells was increased. The number of proliferating thymocytes was reduced as demonstrated by immunohistochemistry (Ki67) and by counting

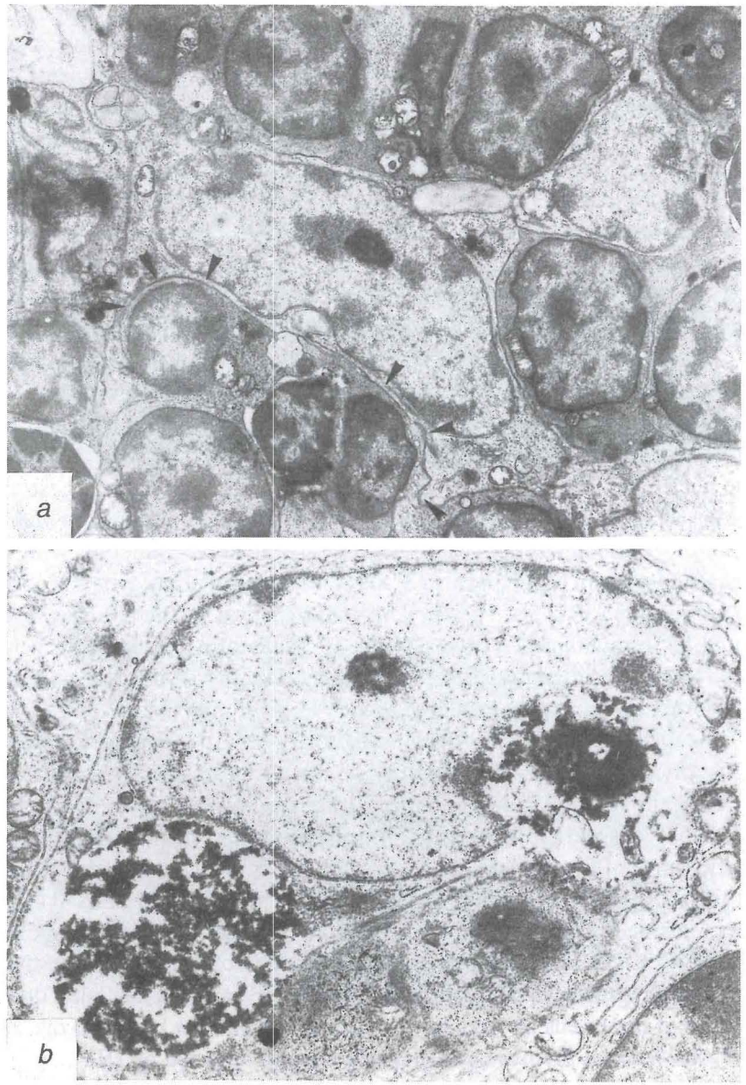

Fig. 3. *a* The cortical epithelium in age-related atrophy shows long cytoplasmic processes which surround adjacent thymocytes (arrowheads). ×4,800. *b* Ultrastructure of degenerating cortical epithelium 24 weeks after infection with vacuolization of the cytoplasm. ×8,000.

mitotic figures in semithin sections (fig. 2). The reduction in the number of lymphoblasts correlated with the results of flow cytometry. In the animals at 12 and 24 weeks after infection only 60% of all CD3+ cells are CD4+/CD8+, whereas in the control animals 80% of CD3+ cells were double-positive. These results indicated that the proliferation and the maturation of the cortical thymocytes were altered in this early phase of infection, and comparative evaluation showed that this alteration of the thymus preceded the atrophy of the lymph node paracortex and the depletion of blood T cells.

These animals and the animals with full-blown AIDS showed an increase in the amount of plasma cells in the thymus cortex, medulla and perivascular space. In human AIDS thymi, an increase in the number of plasma cells has been reported as well [3, 6]. It is unknown whether they indicate autoimmune phenomena [3].

The SIV-related thymic atrophy is quite distinct from other forms of thymus involution. Acute accidental involution follows for example the injection of high doses of corticosteroids or is due to starvation. It is characterized by death of the vast majority of the cortical thymocytes within 12–24 h and subsequent phagocytosis of the cellular debris. This starvation-induced atrophy, therefore, clearly differs from the SIV-induced form. The cortical epithelium and the other cells of the thymic microenvironment remain largely intact in acute accidental involution [15]. However, long-term malnutrition will also result in a loss of the mature cortical epithelium [16]. This was seen in a starved baby macaque where atrophy was due to massive cell death of cortical thymocytes and while macrophages with large amounts of secondary lysosomes were left (fig. 4).

The pathological pattern in age-related thymic atrophy is also different from the SIV-induced form. In age-related atrophy the cortex and the medulla were slowly reduced in size and replaced by large amounts of fat. This fat closed the gap between the former outline of the thymic capsule and the remaining 'miniature' thymic parenchyma. Analysis of the remaining parenchyma showed a well-preserved corticomedullary stratification. In the cortex, subcapsular and cortical epithelial cells remained intact and resembled those in juvenile monkeys (fig. 3a, b). Cortical thymocytes still showed proliferation. Thus, age-related thymus atrophy of the monkeys resembled that of humans [13, 17]. Both were characterized by a reduction in the absolute size of the thymic lobules but a largely unaltered structure and cellular composition.

Cortical thymocytes and the cortical epithelium form functional cortical complexes in which both participating cells depend on each other. The corti-

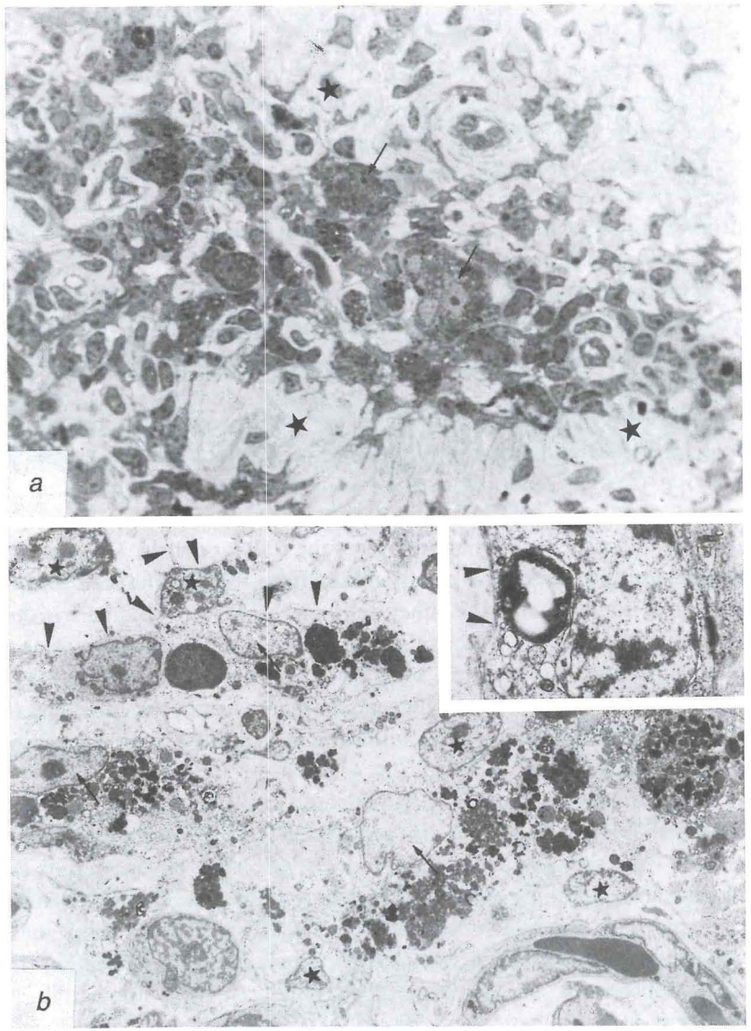

Fig. 4. a Accidental thymus involution in an orphan baby monkey with loss of the cortex and severe shrinkage and folding of the thymus capsule (stars). Increased amount of macrophages (arrows) is seen. Epon, semithin, toluidine blue. ×605. *b* Ultrastructure of *a,* with subcapsular epithelium (stars and inset, ×6,300), the folded thymic capsule (arrowheads), and numerous macrophages with secondary lysosomes (arrows). ×2,000.

cal epithelium plays a role in the positive selection of the immature thymocytes [18], and the absolute number of this type of epithelial cell may determine the size of the whole organ. This is seen in nude mice or in the DiGeorge syndrome with missing or reduced numbers of epithelial cells or in cases with neoplastic or heterotopic cortical epithelium [19]. Alterations of the cortical epithelium, therefore, may not only disturb the process of positive selection but may influence the size of the thymus cortex as well. On the other hand, the cortical thymocytes could also influence the differentiation of the cortical epithelium.

The loss of the specific epithelial cells of the thymus cortex is considered to be of primary importance in the SIV-induced thymus atrophy. Their damage would explain the breakdown of the maturation in the lymphoid compartment. A substantial contribution by SIV infection of the thymocytes in the thymus atrophy is unlikely since the amount of infected thymocytes is very low. Furthermore, even under normal conditions the natural fate of 90% of these cells would be death by apoptosis. Therefore, destruction of epithelial cells either by direct pathogenic effects of by (auto-)immune destruction may be the leading cause for SIV- and HIV-induced thymus atrophy and precedes the peripheral depletion of the T cell compartment. Furthermore loss of cells involved in intrathymic positive selection (cortical epithelial cells) and negative selection (interdigitating cells) in T cell maturation could favor the peripheral autoimmune phenomena observed in SIV and HIV infections.

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