AIDS Vasculopathy

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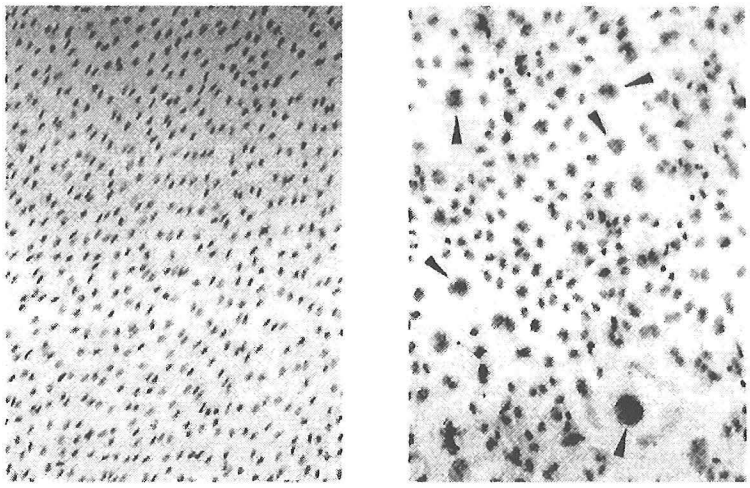
Introduction

The vascular endothelium, far from being an inert, passive barrier, has proven to serve an increasing number of diverse functions vital to the proper homeostasis of virtually all organs. Accordingly, illnesses which diffusely affect the endothelium such as Diabetes Mellitus, sepsis, and atherosclerosis cause widespread, protean and often severe manifestations of disease. The endothelium is a silent target of Human Immunodeficiency Virus-1 (HIV-1), and infection of individuals with HIV-1 results in just such a diffuse vascular process which is frequently not recognized. Aortic endothelium of AIDS patients demonstrate marked dysmorphic changes with chaotic cell distribution, atypia with giant cell formation, pyknotic nuclei, bare denuded patches, and focally increased surface expression of endothelial adhesion proteins such as E-selectin and VCAM-1 (1). Microcirculatory blood flow, assessed in vivo by nail fold microscopy, is severely disturbed (2). In addition, endothelium directly or indirectly affected by HIV-1 may contribute to the pathogenesis and expression of many common HIV-linked diseases not generally thought to be vascular in nature.

Primary Vascular Syndromes

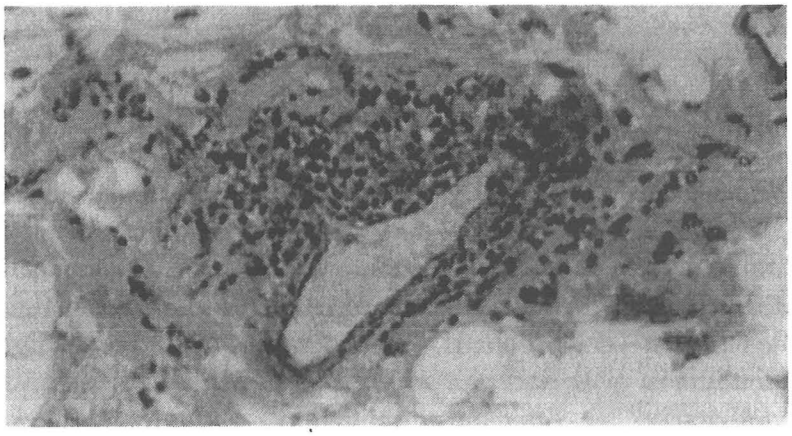
A number of primary vascular diseases can be found with increased incidence in the HIV-infected population. A propensity for thrombosis causes a variety of clinical problems. For instance, widespread digital ischemia with gangrene is an uncommon but dramatic presentation of HIV infection (3), and malignant atrophic papulosis, which causes infarctive thrombosis of the skin and viscera, has been reported (4). Thrombotic microangiopathy, perhaps the second most common renal lesion seen in AIDS, causes intraglomerular and extraglomerular arteriolar thrombus formation, and is the hallmark histologic lesion of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (5-7).

Systemic vasculitis has also been reported by a number of investigators, and the clinical and histologic patterns have resembled necrotizing arteritis, non-necrotizing arteritis,



Normal **AIDS**

Aortic endothelium from control and HIV-1-infected individual demonstrating marked cellular atypia in the AIDS patient. From ref. 1.

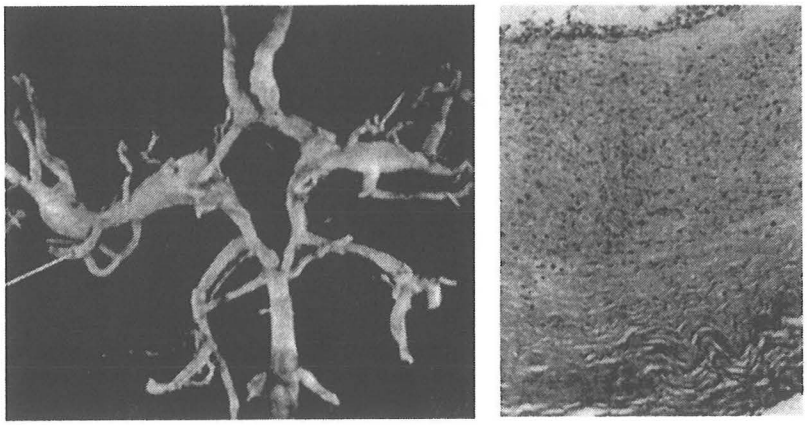


Necrotizing vasculitis involving a perimysial venule. From Gherardi et al, Human Pathol. 1991;22:1187.

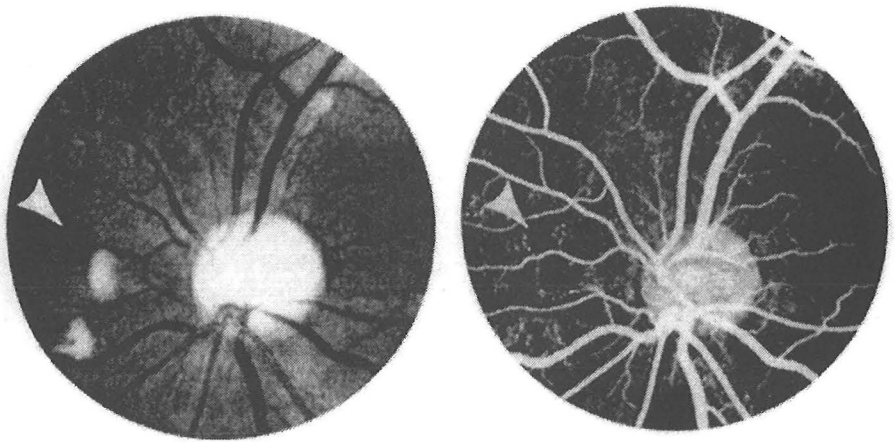
polyarteritis nodosa, Henoch-Schonlein purpura, and hypersensitivity vasculitis (8, 9). In the spleen, arteriolar deposition of para-amyloid material has been consistently noted (10), although the significance and origin of this material is uncertain.

Cerebrovascular lesions are seen at autopsy in 25% of pediatric AIDS cases, and include a distinct form of fusiform aneurysmal dilation of the large arteries leaving the Circle of Willis (11, 12). This is a highly lethal condition in which the arterial intima becomes markedly hyperplastic, the media becomes acellular and fibrotic, and the intervening internal elastica lamina is degraded. Interestingly, this histologic pattern of vasculopathy is mirrored in an HIV proviral transgenic mouse model, in which HIV transgenes missing *gag, pol,* and much of the *env* genes is expressed (13). In this model, the intimal hyperplasia can be so exuberant as to compromise the patency of the conduit artery. If nothing else, this model serves to demonstrate the ability of the HIV accessory proteins to derange vascular cell growth and behavior.

Primary pulmonary hypertension is another vascular lesion seen with increased frequency in the AIDS population, and the pathogenesis remains cryptic. Although the plexiform lesions themselves do not appear to harbor HIV-1, perivascular inflammatory infiltrates can be found (14), similar to what is observed in rhesus monkeys infected with



Gross and microscopic appearance of intracranial aneurysms in an 18 y/o HIV-infected patient showing multiple fusiform dilations and extensive intimal hyperplasia. From ref. 12.



In vivo funduscopic exam showing cotton wool infarcts and fluorescein angiogram demonstrating uneven perfusion and microaneurysms. From ref. 18.

simian immunodeficiency virus (SIV) (15). Another potential clue is the current paradigm of primary pulmonary hypertension as evolving from a primary, monoclonal expansion of endothelial cells within the intima of any given early plexiform lesion (16). This derangement in endothelial growth signaling can be reconciled with the ability of certain HIV-1 accessory proteins such as Tat and Nef to regulate proliferation signals and even transform cells under certain conditions.

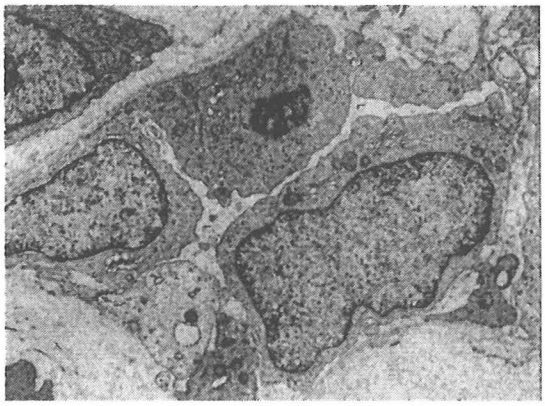
Finally, HIV-1 infected patients nearly universally have retinovascular changes both grossly and microscopically. Cotton wool infarcts and retinal hemorrhages are common even in asymptomatic patients. Loss of retinal capillary cells and focal occlusions of small vessels are common, and fluorescein angiography reveals numerous ruptured or unruptured microaneurysms, vascular breaches, and telangiectasias (17, 18). Importantly, the retinal endothelium has been shown to be capable of supporting productive HIV-1 infection in vivo (19). The ramifications of CNS vascular cell infection for HIV-related neurologic syndromes are extensive.

Kaposi's Sarcoma

Given the pervasive nature of the primary vascular diseases with which HIV-1 is associated, it should not be surprising to find that the clinical expression of other, more common diseases may also derive from the virus' interaction with the endothelium. A likely example of this may be found in the pathogenesis of Kaposi's Sarcoma (KS). The clinical hallmarks of this disease are its aggressive course, its multifocal nature, and its frequent appearance early in the course of HIV-1 infection. In fact, KS represents the initial manifestation of AIDS in 25% of HIV-1 infected patients.

The origin of the hallmark KS spindle cell is not definitively understood, but abundant data would suggest that the cell is derived from abnormal endothelium. Many, though not all, endothelial-specific surface markers appear on KS cells *in vitro* and *in vivo*.

Some of the earliest changes to skin parenchymal cells appears to be the appearance of abnormal vascular structures such as thick-walled capillaries and dilated, thin walled blood-filled spaces (20). Spindle cells are absent in such early lesions. Ultrastructurally,



Early KS lesion showing abnormal capillary with hypertrophied endothelial cells and destruction of basal lamina. From ref. 21.

endothelial cells appear hypertrophied to the point of closing off lumina, with poor intercellular junctions and degraded basement membranes. In addition, endothelial cells escape capillaries, migrate, and make incomplete attempts to form abnormal lumina (21). Of additional interest, uninvolved perilesional skin also displays the same endothelial abnormalities, suggesting that the endothelial cell is a target which responds to soluble mediators, generating a field effect. Indeed, KS lesions have been likened in histologic appearance to granulation tissue, in which nascent endothelial cells are actively invading matrix and attempting to form vascular structures.

In this model, KS arises not from the metastatic spread of a monoclonal transformed spindle cell, but rather results from a multicentric and polyclonal expansion of endothelial cells responding to abnormal growth signals. Indeed, analysis of advanced nodular KS lesions in HIV-infected women suggests a polyclonal inactivation of the highly polymorphic, X-linked human androgen receptor gene, demonstrating that spindle cells from a given lesion do not arise from a common ancestor (22). At a later stage, however, it is likely that a certain subpopulation of spindle cells acquire a malignant phenotype.

The role of the KS-associated human herpesvirus (HHV8) in causing lesions is unclear. Although HHV8 possesses viral homologs of potentially transforming genes encoding such products as IL-8R, Bcl-2, and cyclin D, such genes are poorly expressed, and with rare exception, HHV8 does not transform cells *in vitro* (see ref (23) for a notable exception, however).

Rather than directly transforming cells, it may be more likely that stable infection of vascular cells with HHV8 would incite an inflammatory response, subjecting these cells to chronic cytokine exposure. Indeed, KS most often arises prior to the collapse of the immune system, and early KS lesions are infiltrated with CD8+ cells expressing IFNγ and other cytokines (24). These observations are consistent with the notion that KS arises in an inflammatory environment, much like granulation tissue. It is highly suggestive that normal human endothelial cells, when exposed to a mixture of cytokines found in the KS lesion (largely IFNγ, TNFα, and IL-1β), assume a spindle morphology and, when then injected under the skin of a nude mouse, temporarily form a KS-like lesion (24, 25).

The effect of cytokines on endothelial cell behavior may in large part result from induction of the potent angiogenic growth factor basic fibroblast growth factor (bFGF).

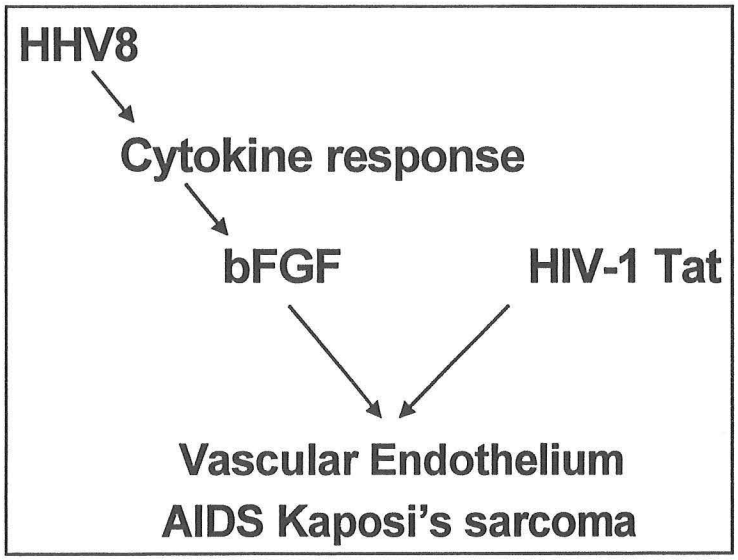
Cytokines which induce spindle cell formation also induce bFGF *in vivo* and *in vitro* (24, 26, 27), and high levels of bFGF are found in human KS lesions (28). KS spindle cells produce high levels of bFGF, which acts as a potent autocrine and paracrine growth factor *in vitro* (29, 30). This appears to be true *in vivo* as well, since antisense bFGF treatment of KS cells causes these cells to loose their ability to form KS lesions in mice (31).

However, the presence of HHV8 does not completely explain the pathogenesis of AIDS KS. First, HHV8 is neither necessary nor sufficient for KS histogenesis. In a cohort of HIV-1 negative individuals with African endemic KS, only 49% were HHV8 positive (32). In addition, HHV8 is found in a number of benign and malignant lymphoproliferative and vascular lesions such as primary effusion lymphoma, Castleman's disease, angiolymphoid hyperplasia with eosinophilia, angiosarcoma, and hemangioma (33, 34). More importantly, an explanation for the heightened aggressiveness in HIV-infected patients is lacking.

An interesting link with HIV-1 was made with the finding that Tat-expressing transgenic mice develop KS-like lesions. Interestingly, expression of the Tat transgene was limited to the skin, yet the spindle cells did not express Tat stably but rather appeared to be paracrine targets of the protein, consistent with the existence of a field effect. *In vitro*, Tat promotes KS spindle cell proliferation (35), and causes normal endothelial cells to migrate and invade matrix (36).

In vivo, Tat initiates angiogenesis (37). This important property appears to be mediated by the mid-region basic domain which mimics basic growth factors. Specifically, the Tat basic domain ligates and causes autophosphorylation of FLK-1, one of two cognate receptors for the strongly angiogenic vascular endothelial growth factor (VEGF) (38). Again, however, the presence of a single factor, in this case Tat, is insufficient to explain the histogenesis of KS, which resembles angiogenesis gone awry.

A view that AIDS KS arises from a cooperation between the effects of the two viruses, HHV8 and HIV-1, has been supported by the observation that Tat and bFGF synergistically act to cause the formation of KS-like lesions in mice (28). In these studies,



Potential basis for cooperative effect between HHV8 and HIV-1 in the pathogenesis of AIDS KS.

an additional motif found towards the carboxyl terminus of Tat, the RGD region, plays a significant role in the formation of KS lesions. Therefore, competing RGD tripeptides block the synergistic ability of Tat and bFGF to cause KS lesions in mice (26). The RGD region of Tat can be shown to bind with high affinity to two important endothelial integrins, $\alpha_v\beta_3$ and $\alpha_5\beta_1$, which normally anchor the cell to fibronectin and vitronectin (39).

The cooperation of Tat with bFGF may potentially occur at several levels. First, the basic domain of Tat acts at low concentrations as a cationic competitor to retrieve bFGF bound to anionic heparan sulfated matrix residues (40). Second, bFGF increases the synthesis of the fibronectin and vitronectin integrin subunits to which Tat binds (26, 39, 41), thus potentially increasing Tat signaling, and third, ligation of these integrins permits growth factor signaling, conceivably by both bFGF and the Tat basic domain itself (28, 40).

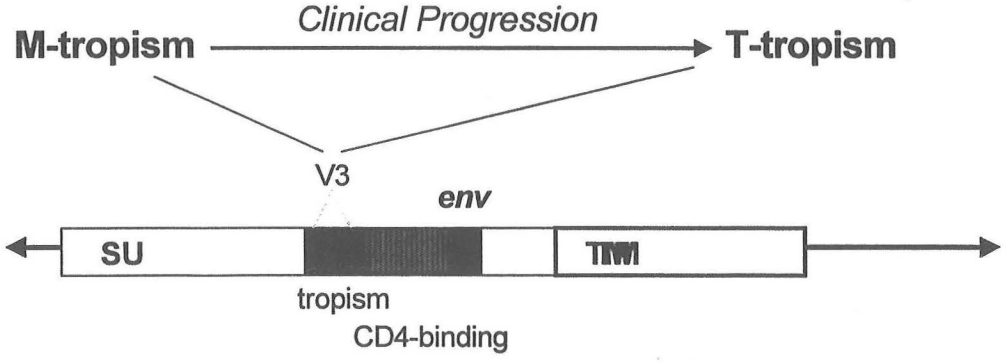
Although compelling, the current model of AIDS KS pathogenesis is nonetheless incomplete. It is as yet unclear, for instance, why endothelial cells are induced to initiate the first phase of angiogenesis involving proliferation and invasion, but are not allowed to complete this differentiation program. It is also not clear whether and how the spindle cells eventually acquire a malignant phenotype. In addition, it does not explain a gender bias which may transcend the male homosexual demographic, since classical KS occurs in men also. Oddly enough, in the original Tat transgenic strains, only male mice developed KS lesions (42).

HIV encephalitis

In addition to provoking endothelial pathology indirectly through soluble viral proteins, HIV-1 may also affect the clinical expression of diseases through direct infection of the endothelium. Potential examples of this phenomenon can be found in the pathogenesis of AIDS dementia and AIDS lymphoma.

CNS infection by HIV-1 is common and apparently occurs early in the course of HIV-1 illness. In contrast, the clinical hallmarks of HIV encephalitis, dementia and sensorimotor deficits, occur late and in only about 20% of patients.

The mode of entry of the virus into the CNS is uncertain but the process is necessary for the development of dementia. As with all macromolecules, HIV-1 cannot freely enter the brain compartment without specific

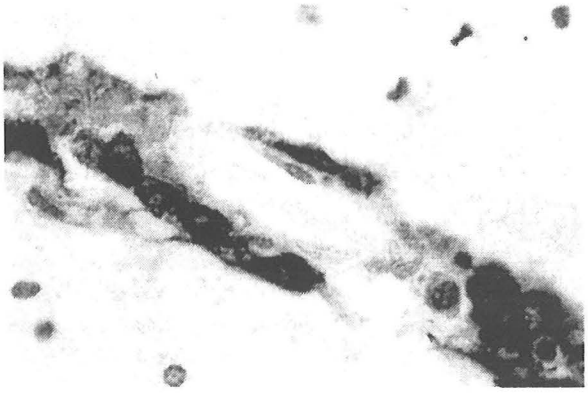


interactions with the brain endothelium comprising the blood-brain barrier. More is understood about the route of viral entry into immune effector cells. In this paradigm the viral surface protein gp120 interacts first with the host cell CD4 protein, causing viral tethering and conformational changes of gp120 which allow interaction with one of several chemokine receptors. These G-protein linked serpentine receptors facilitate viral entry. Importantly, certain viral strains can only enter macrophages (M-tropic), others only T-cells (T-tropic), and others both. An important determinant of host cell tropism is contained within gp120 in the third variable (V3) region, separate from the CD4 binding domain. The V3 loop presents a surface which apparently selects specific chemokine receptors and therefore determines host cell tropism (43).

In an interesting study by Korber and others (44), the tropism-determining region of a number of simultaneous blood and brain HIV isolates was sequenced in several patients, and the brain-derived viral sequences were found to cluster relative to the blood isolates, suggesting a strong evolutionary pressure in the brain to conserve this region. In particular, the V3 region displayed a brain signature pattern which was highly predictive for macrophage tropism. This finding was consistent with the histologic finding that the principal HIV host cell in brains of encephalopathic patients is the brain macrophage (45-47), and with the virologic finding that brain isolates replicate more efficiently in macrophages than T-cells (48). In fact, the severity of dementia has been found to correlate highly with the degree of macrophage infiltration (49).

Importantly, both infected and uninfected brain macrophages are frequently found clustered around and in contact with the microvascular and venular endothelium (50, 51), highlighting the importance of the endothelium in controlling viral entry into the brain. The precise mechanism by which HIV crosses the blood-brain barrier is not known, but there are at least two non-exclusive scenarios proposed.

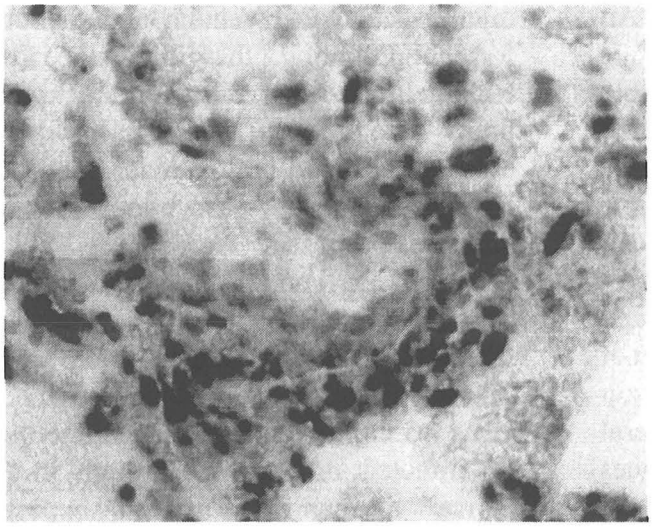
In the first, HIV-infected mononuclear cells are thought to alter brain endothelium to facilitate monocyte attachment, diapedesis, and migration into the brain parenchyma. The virus would therefore be carried into the CNS by infected monocytes, through a "Trojan Horse" mechanism. Indeed, exposure of human brain endothelial cells *in vitro* to HIV-infected monocytes causes dramatic increases in expression of endothelial adhesion proteins such as E-selectin and VCAM-1, with concomitant monocyte hyperadherence (52).



Perivascular macrophages staining for HIV gp41 encasing a brain microvessel. From ref. 49.

Simultaneously, infected monocytes express increased surface levels of adhesive β2 integrins, secrete the protease MMP-9, and incite retraction and desquamation of human endothelial cells *in vitro* (53, 54), suggesting a mechanism of monocyte penetration across the endothelium. In a simian model of AIDS dementia, neurovirulent strains of SIV cause brain endothelial cell apoptosis and perivascular edema (55). Correlative studies in brains of AIDS patients have shown an increase in expression of adhesion proteins and viral products in demented compared with asymptomatic HIV-infected patients, and a strong histologic association between macrophage infiltration and endothelial cell adhesion protein expression (52). Normal monocytes also display enhanced VCAM-1 and E-selectin-dependent binding to encephalitic brain tissue slices *ex vivo* (52).

An alternative to the Trojan Horse hypothesis has been presented which proposes that an important first step involves direct infection of the brain endothelium by HIV-1. The capacity of human brain endothelium to undergo productive infection by HIV-1 has been demonstrated by the expression of viral proteins in the CNS endothelium *in vivo* (19, 44, 56) and by direct infection of cultured human brain endothelium by HIV-1 (57). In the latter studies, viral infection appears to be non-lytic. Since brain endothelium does not express CD4, viral entry proceeds via a non CD4-dependent process which appears to employ the tropism-determining V3 region of HIV gp120. This parallels the mechanism by which neurovirulent, dementia-causing SIV strains enter simian brain endothelium, in which the virus uses the chemokine receptor CCR5 as a primary viral receptor (58). Once infected, the brain endothelium would be subjected to a positive feed back cycle since the viral protein Tat increases brain endothelial CCR5 expression (59).



PCR-driven in situ hybridization demonstrating HIV-1 DNA in both endothelium and adjacent perivascular macrophages, from the brain of an encephalopathic patient. From ref. 44.

The basis for endothelial tropism is not clear, and there are likely several determinants. One determinant appears to reside in the transmembrane protein gp41, which anchors gp120 (60). In HIV-1, endothelial tropism also maps separately from M-tropism, and may lie in the C1 region of gp120 (61).

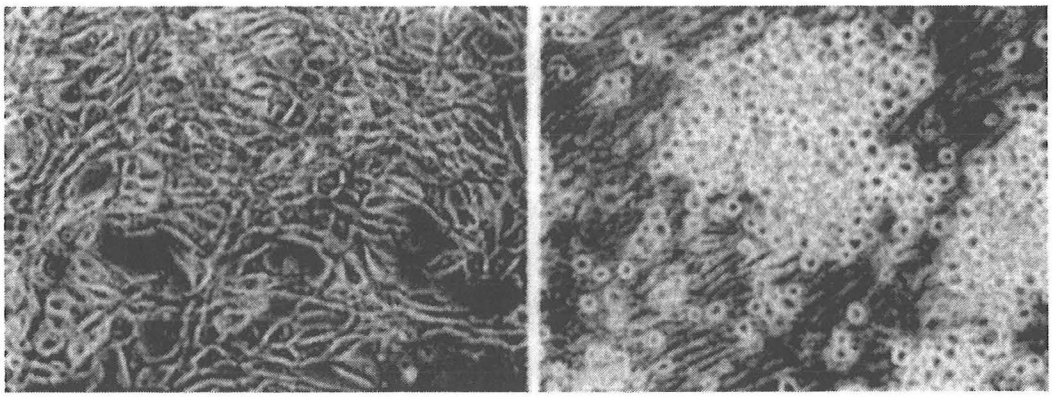
The importance of CNS endothelial infection to HIV encephalitis is clearer in the simian model of AIDS dementia. In this model, studies employing differing strains of SIV have revealed that as with the human

situation, only M-tropic viruses enter the brain and cause dementia. However, tropism for macrophages alone is insufficient for neurovirulence; dual tropism for both macrophages and brain endothelium appears to be required to induce simian dementia (60, 62). The brain endothelium thus acts as a filter of sorts which selects neuropathogenic strains. It may be that dual tropic viruses possess a pathogenic advantage by using the brain endothelium as a high surface area viral reservoir, efficiently passing virus to resident microglial cells or newly arrived brain macrophages. Indeed, HIV-1 can latently infect endothelial cells *in vitro* and be rescued later by susceptible T-cell lines (63, 64), confirming the infectious capacity of HIV-1-containing endothelium. Although the necessity of dual tropism for human dementia has not been investigated, it is interesting to note that the tropsim-determining V3 regions of gp120 are different in brain-derived HIV isolates from demented and non-demented patients (65).

Lymphoma

Another common illness from which AIDS patients suffer is lymphoma. Generally B-cell non-Hodgkin's lymphomas, these malignancies are particularly aggressive in their biologic behavior and have a propensity for extranodal involvement. In particular, brain involvement is quite common, and ~20% of AIDS lymphoma patients have primary brain lymphoma.

The frequency of extranodal lymphomas in AIDS patients suggests that homing mechanisms responsible for the sequestration of lymphocytes in lymph nodes, intact even in most lymphoma cells, becomes deranged. Normal lymphocytes interact extensively with endothelial cells to direct homing behavior. In particular, interactions between lymphocytes and bone marrow stromal endothelial cells are thought to be important for normal B-cell growth, lymphopoiesis, and homing, and by virtue of



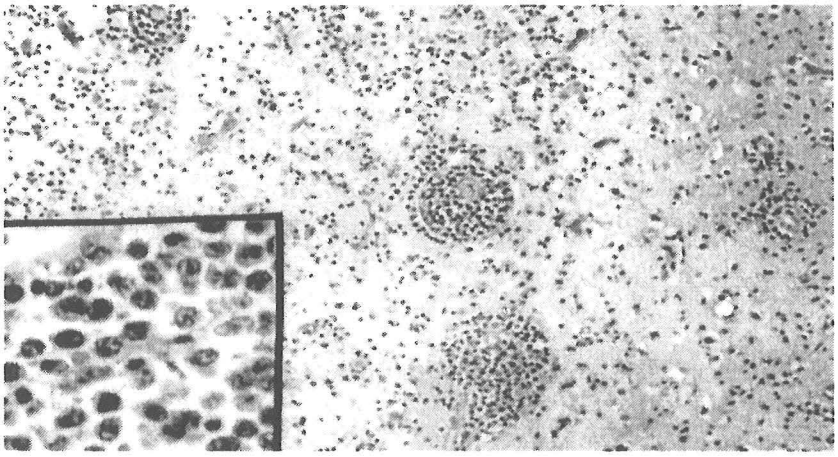
In vitro infection with HIV-1 of bone marrow stromal cultures induces spontaneous outgrowth of B-lymphoma cells. Left: uninfected; Right, HIV-infected bone marrow stroma. From ref. 66.

cytokine and adhesion-dependent signals, for the development of B-cell lymphomas.

In light of the crucial nature of B-cell--bone marrow endothelial cell interactions, it is highly noteworthy that this vascular bed, like that of the brain, can and does become infected with HIV-1 (66, 67). These studies strongly suggest that HIV-infected bone marrow endothelium greatly enhances the proliferation of B-lymphoma cells. Endothelial cell-enriched bone marrow stroma from non-HIV infected lymphoma patients will not support the proliferation of autologous lymphoma cells unless infected with HIV-1 *in vitro*, and bone marrow endothelium cultures from an HIV-infected patient supported the spontaneous outgrowth of autologous lymphoma cells (66). Therefore HIV-1 can promote the growth of malignancies through infection of supportive accessory cells such as the endothelium.

Importantly, HIV-1 infection of brain endothelium also strongly promotes both the attachment and proliferation of B-lymphoma cells (66), and purified preparations of the HIV protein Tat can stimulate migration of lymphoma cells *in vitro* (68). This may explain not only the high frequency with which primary brain lymphomas occur, but also the striking propensity of such lymphomas to grow in an angiocentric fashion, as though nurtured by endothelial factors.

The mechanism for stimulation of lymphoma cell growth by infected endothelial cells is not clear, although a cooperative interaction between endothelial CD40 and VCAM-1 and lymphocyte CD40L and VLA-4 may trigger lymphoma cell proliferation. In addition, close proximity of the two cells may allow viral accessory proteins such as Tat and Nef to promote such adhesion-dependent growth signals.



Primary CNS lymphoma in brain of HIV-infected patient demonstrating angiocentric nature of the malignancy. From Burns, DK, J.Child.Neurol. 1992;7:332.

Conclusions

Admittedly, this review presents a great deal of experimental and observational data linked by speculative syntheses. However, the consistent themes which have endured the previous 10-15 years of AIDS research are that infection of humans with HIV-1 causes marked functional and morphologic changes in the vascular endothelium. Besides causing primary vasculopathies of varying sorts, endothelial

alterations may also greatly alter the clinical expression of common HIV-associated diseases such as dementia, lymphoma, and Kaposi's sarcoma. The precise mechanisms underlying these endothelial derangements are not well understood, but such knowledge may suggest new treatment options for both AIDS and non-AIDS associated vascular conditions.

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