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Aortic Endothelium in HIV-1 Infection

Chronic Injury, Activation, and Increased Leukocyte Adherence

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Clinical and serological studies provide evidence for a pathogenetically relevant vasculopathy in acquired immune deficiency syndrome (AIDS); however, the morphological status of the endothelium under conditions of human immunodeficiency virus (HIV)-1 infection is only sparsely documented. In this study we adapted an en face preparation technique of endothelium for use in immunohistochemistry and investigated the aortic endothelium of pre-AIDS and AIDS patients (n = 32) in comparison with an HIV-negative group (n = 17). The control group showed a regular pattern of evenly distributed aortic endothelial cells, whereas the endothelial cell pattern in the HIV-1-infected patients was clearly disturbed. Simultaneously, the degree of leukocyte adherence on the aortic endothelium increased significantly. These changes were accompanied by an up-regulation of the vascular cell adhesion molecule-1 (VCAM-1) and E-selectin (ELAM-1). The endothelium turnover increased, and one-half of the HIV-1-infected patients exhibited HLA-DR (major histocompatibility complex class II) antigen in the aortic endothelium. Our results provide evidence for a profound and repeated injury with regeneration and activation of the endothelium in HIV-1 infection. Injury as well as activation of the endothelium impairs its normal regulatory properties. This could have consequences for the maintenance of the blood-brain barrier; it might influence the immunologically important interaction of the endothelium with T cells; and it might trigger Kaposi's sarcoma. (Am J Pathol 1996, 149:1887–1898)

The endothelium is a regulatory organ that participates in immune responses and plays a critical role in the progression and outcome of infectious diseases. In this framework it can be important in the spread of virus, in T-cell function, and in the initiation of neoangiogenesis.[1–4] In human immunodeficiency (HIV)-1 infection and acquired immune deficiency syndrome (AIDS), the endothelium is under the combined influence of deregulated immune effector cells, increased concentrations of circulating antigens, and immune complexes.[5] It is chronically exposed to imbalanced concentrations of cytokines and other activating factors such as the HIV Tat protein.[6–8] Consequently, the normal function of the endothelium may be impaired under the conditions of HIV-1 infection. Indeed, clinical indications for a disturbance of vascular function are becoming increasingly apparent. An HIV-associated ocular microangiopathic syndrome is well established.[9–14] Other groups reported on an altered brain perfusion with a reduced cerebral blood flow already in the early stages of HIV-1 infection.[15,16] Joshi et al[17] demonstrated an artheriopathy in children with AIDS. Plasma levels of diverse endothelial cell markers such as von Willebrand factor antigen (vWF), tissue-type plasminogen activator, plasminogen activator inhibitor, fibronectin, angiotensin-converting enzyme, and endothelin increase in the course of HIV-1 infection.[18–22] An elevated von Willebrand factor plasma value as a presumed marker of endothelial cell damage correlated inversely with the $CD4^+$ cell

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counts.[20,21] High von Willebrand factor levels are also regarded as an adverse prognostic factor in AIDS.[20] These data indicate an endothelium injury and a systemic vasculopathy under the conditions of HIV-1 infection.

In this study we evaluated the normal pattern of the aortic endothelium in a healthy control group and compared it qualitatively and quantitatively with the endothelium of HIV-1-infected patients. Presumably, more information is obtained by observing endothelial cells *en face* than from sections cut perpendicularly or obliquely to the surface. For this reason we used an endothelium *en face* preparation method. This technique offers far better possibilities for the assessment of endothelial alterations than conventional paraffin histology does. The adaption of this method to immunohistochemistry enabled us to determine endothelial parameters such as adhesion molecules and major histocompatibility complex (MHC) class II antigens. Counting Ki-67-positive cells, we could compare the endothelium proliferation rate of the healthy group with that of the HIV-1-infected patients.

Materials and Methods

Patient Characteristics and Autopsy Data

Extensive autopsy and histological examinations of all patients included in this study were carried out. Serum testing before autopsy by enzyme-linked immunosorbent assay and, if required, by additional Western blotting confirmed the HIV status. From death to autopsy all persons had been kept in a cold-storage chamber at 4°C. Patients with a higher degree of atherosclerosis were not included in this study and only macroscopically inconspicuous areas of the aorta without fatty streaks or manifest atherosclerosis were chosen for *en face* preparation. The extent of overall atherosclerosis did not differ between the groups. The material for *en face* preparation was obtained from the ascending and the abdominal aorta. No differences could be observed in the degree of structural and immunohistochemical results between the ascending and the abdominal aorta. Surrounding areas of vascular ostia were omitted for *en face* preparation.

The anamnestically healthy persons in the HIV-negative control group (n = 17; 7 females and 10 males; average age, 39 years with a range from 12 to 57 years) died instantly due to suicide or a road accident without any period of intensive care. The average time interval between death and collection of specimens was 18 hours (4 to 30 hours) at 4°C. Postmortem investigation ruled out infections, neoplasms and other diseases. Toxicological tests of blood and urine by fluorescence polarization immunoassay on barbiturates, benzodiazepines, morphine derivatives, amphetamines, tricyclic antidepressant drugs, cocaine, Methadone, and cannabis excluded drug abuse in these patients.

The HIV group (n = 32; 2 females and 30 males; average age of 42 years with a range from 7 to 60 years) was composed of pre-AIDS patients (Centers for Disease Control (CDC) stage A,[23] n = 6) and patients dying of AIDS (CDC stage C,[23] n = 26). The average time interval between death and collection of specimens was 20 hours (11 to 24 hours) at 4°C.

All of the pre-AIDS patients (n = 6 males; average age of 41 years with a range from 34 to 45 years) died an unnatural death (suicide or accident). From anamnestical data, these patients were not in medical care and not on medication. Most of them obviously did not know of their infection while alive. The first HIV-1 test was carried out at autopsy. Infections, neoplasms, and drug abuse were ruled out as in the control group.

Four of the six pre-AIDS patients exhibited an HIV-associated generalized lymphadenopathy with follicular hyperplasia.

The AIDS collective (n = 26; 2 females and 24 males; average age of 42 years with a range from 7 to 60 years) showed one or more opportunistic infections and/or Kaposi's sarcoma as well as typical HIV-associated morphological changes to the lymphatic system. Most AIDS patients were on intensive medical care with multi-drug therapy for their infections and/or Kaposi's sarcoma. The type and number of opportunistic infections/malignancies proven by autopsy as well as the class and combination of drugs administered in the last weeks before death did not show a measurable influence on the degree of the endothelial changes described in this study.

Preparation of Endothelium

The method of endothelium *en face* preparation by means of a nitrocellulose film was established more than 50 years ago. For this study we used the technique in Freudenberg's modified form.[24,25] For obtaining endothelial cell monolayers with this method, the aorta was purged of blood for 2 minutes in physiological sodium chloride and then freed of fat and connective tissue and opened longitudinally. The specimens were spread, mounted on a piece of cork with the endothelial layer facing upward. Specimens were fixed in 70% ethanol overnight at 4°C. After dehydration in graded ethanol, the specimens were

Table 1. *Monoclonal Mouse Antibodies Used in this Study Applied to Antigens Listed*

Antigen	MAb clone	Source	Dilution of MAb
ELAM-1	1.2 B 6	Dianova-Immunotech (Hamburg, Germany), 1243	1/5*
HLA-DR	CR3/43	Dako, M 775	1/100*
IL-1β	FIB-3	Dianova (Hamburg, Germany), M 400	1/10
Ki-67	MIB-1	Dianova (Hamburg, Germany), DIA 505	1/10*
VCAM-1	1G11	Dianova-Immunotech, 1244	1/5*

*Microwave pretreatment.

dissected into pieces of 1 cm^2 and immersed in a mixture of ethanol/ether (1:1, v:v). A grease-free glass slide was coated with a thin colloidon film (Cedukol, Kollodium, and glycerine from Merck (Darmstadt, Germany) and Pro Celloidin from Fluka (Bŭchs, Switzerland)) and dipped into a mixture of ethanol/ether (1:1, v:v). Pieces of the arterial wall (1 cm^2) were then placed on the colloidon film with the endothelium facing downward. The endothelium was pressed evenly onto the colloidon film. Sixty seconds after placing the vessel on the coated slide the tunica media and adventitia were pulled away. The endothelial cell monolayer, which adhered to the colloidon film, was cut out, lifted off the slide, air dried, and stored at room temperature. In each case at least six endothelial cell preparations were stained with hematoxylin and eosin (H&E), Giemsa stain, and chloroacetate esterase histochemical stain.[26] Depending on the availability of additional suitable *en face* preparations, immunohistochemical studies were performed using standard immunohistochemical techniques[27,28] and a panel of monoclonal mouse antibodies against human endothelial cell antigens (Table 1). Antibodies to ELAM-1, VCAM-1, HLA-DR, and IL-1β were applied to demonstrate different endothelial activation parameters and adhesive leukocytes. An antibody to Ki-67 (MIB-1) was used for visualization of cells undergoing mitosis. The avidin-biotin complex method was applied for detection of VCAM-1, ELAM-1, and HLA-DR antigens (Vectastain, Vector Laboratories, Burlingame, CA). The alkaline phosphatase anti-alkaline phosphatase method was chosen for IL-1β (FIB-3) and Ki-67 (MIB-1) antigens (Dako, Copenhagen, Denmark).[27,28]

During all steps of the staining procedures the *en face* preparations have to float loosely in reaction fluids and buffers to guarantee dyes and antibodies complete passage through the endothelial monolayer. Pretreatment and dilution of antibodies differed between regular paraffin material and *en face* preparations and had to be adapted specifically for the individual antigens (Table 1). To ensure specificity and for control of background staining, controls were included in all staining runs with the primary antibody replaced by bovine serum albumin. No staining was observed in these controls.

To quantify alterations of the structural endothelial pattern, the following grading system was used: regular structure, evenly distributed endothelial cells without phenotypical changes; low-grade disturbance, major parts of the *en face* preparations with a regular structure, with only small areas with disturbance of pattern and minor phenotypical changes to the endothelial cells; intermediate-grade disturbance, some undisturbed areas beneath fields with clear alterations; high-grade disturbance, predominance of a disturbed endothelial cell pattern, with frequently phenotypical changes of the endothelial cells (Table 2).

The mean number of adhesive leukocytes was determined as the percentage of adhering leukocytes compared with the total intimal cell count (<5, <10, <20, and >20%). The mean of all *en face* preparations of each case was calculated (Table 3).

The documentation of immunohistochemical staining was evaluated according to Remmele using an immunoreactive score.[29] The score was calculated from the staining intensity in four categories (0 to 3), and the percentage of positive cells was calculated in four categories (0 to 3) by multiplying staining intensity and percentage of positive cells. Through this method of keeping a semiquantitative score, the immunohistochemical results were rated negative (N), low (1+), intermediate (2+), or highly immuno-

Table 2. *Aortic Endothelium Cell Pattern and Extent of Disturbance*

Pattern	Number of cases (n)	
	HIV-negative control group	HIV-positive patients
N	13	0
LD	2	2 (1)
ID	1	4 (2)
HD	1	26 (3)

Disturbance of endothelial pattern in gradations: N, normal endothelial pattern; LD, low-grade disturbance; ID, intermediate-grade disturbance; HD, high-grade disturbance. n, number of pre-AIDS patients.

Table 3. *Evaluation of Leukocyte Adherence on the Aortic Endothelium*

Leukocyte adhesion	Number of cases (n)	
	HIV-negative control group	HIV-positive patients
0–5%	8	1 (1)
5–10%	6	1 (1)
10–20%	3	17 (2)
>20%	0	13 (2)

Leukocyte adhesion is presented as the number of adherent cells as a percentage of the total number of intimal cells. n, number of pre-AIDS patients.

reactive (3+) (Table 4). The numbers of Ki-67-positive nuclei were counted per 1-cm^2 area of *en face* preparations: 0 to 1 positive cells/cm^2, 1 to 2 positive cells/cm^2, 2 to 3 positive cells/cm^2 and >3 positive cells/cm^2 (Table 5).

Results

We compared the aortic endothelium of HIV-1-infected patients and HIV-negative control persons with regard to cellular pattern, proliferation, leukocyte adhesion, and activation of gene expression (VCAM-1, ELAM-1, HLA-DR, and IL-1β). These different parameters were qualitatively and quantitatively evaluated to characterize the biological status of the endothelium under the conditions of HIV-1 infection.

Morphological Alteration of the Aortic Endothelium under Conditions of HIV-1 Infection (Table 2)

In 76% (13 of 17) of non-HIV-infected, healthy persons, the aortic endothelium formed a regular cell layer of flattened, fairly uniform mononuclear cells. Endothelial cells were oriented longitudinally along the vessel with their nuclei uniformly aligned in parallel (Figure 1A). This regular pattern of the normal aortic endothelial cell layer was clearly disturbed in 94% (30 of 32) of the HIV-infected patients. Most obvious was an aberrant irregular cell pattern with variability in cellularity as well as nuclear size, number, and staining (Figure 1, B and C). In detail, the aortic endothelium in HIV-1-infected patients showed two types of phenotypic changes: 1) the presence of smaller cells with rounded nuclei and slightly increased chromatin (Figure 1C) and 2) multinucleated, sometimes bizarre-shaped endothelial cells (Figure 1, B and C) often located near small denuded areas. Frequently, nuclear pyknoses of endothelial cells were evident (Figure 1D). One-half of the pre-AIDS patients (3 of 6) exhibited a high-grade disturbance in the structure of the endothelium.

Increased Adherence of Leukocytes to Aortic Endothelium under Conditions of HIV-1 Infection (Table 3)

The number of nonintimal cells was less than 10% of all intimal cells in 82% (14 of 17) of the non-HIV-infected persons. Thus, some mononuclear cells and granulocytes were found to be regular constituents of the normal endothelium. In 18% (3 of 17) of control patients, a degree of leukocyte adherence between 10 and 20% of all intimal cells was revealed.

HIV-1-infected patients exhibited clearly increased numbers of nonendothelial cells in the aortic intima. In 94% (30 of 32) of the HIV-1-infected patients, the number of leukocytes exceeded 10% of the total number of intimal cells (Figure 2, A and B). Two patients of the pre-AIDS group showed lower numbers of adherent leukocytes.

Only a few areas with clear structural changes were lacking significant mononuclear cell adhesion (Figure 1B). Most *en face* preparations showed an increased leukocyte adhesion in parallel with the structural alterations of the aortic endothelium.

Using the chloroacetate esterase reaction[26] in the HIV-1-infected group, the amount of granulocytes was determined to be less than 5% of the nonintimal cell population. Therefore, it can be concluded that the majority of adhesive cells on the endothelium must be mononuclear cells. Ongoing experiments with the macrophage-associated antibody MAC 387 (Dako-

Table 4. *Antigen Expression and Immunoreactive Score of the Aortic Endothelium*

	HIV-negative control group				HIV-positive patients (n)			
	N	1+	2+	3+	N	1+	2+	3+
VCAM-1	6	1	0	0	0	6 (2)	8	9 (2)
ELAM-1	7	0	0	0	3 (1)	2 (1)	5	0
HLA-DR	6	0	0	0	8 (2)	4	2 (1)	2
IL-1	5	1	0	0	0	3	9 (1)	12 (4)

N, no positive cells (HLA-DR and IL-1β) or basic weak and patchy expression in single cells (VCAM-1 and ELAM-1); 1+, low immunoreactive score; 2+, intermediate immunoreactive score; 3+, high immunoreactive score. n, number of pre-AIDS patients.

Table 5. *Ki-67 Index of Aortic Endothelium*

Number of Ki-67-positive cells per cm^2 of endothelium *en face* preparation	Number of cases	
	HIV-negative control group	HIV-positive patients (n)
0–1	6	0
1–2	0	8 (2)
2–3	0	3 (1)
>3	0	4

n, number of pre-AIDS patients.

patts, Glostrup, Denmark) reveal the majority of adhesive mononuclear cells in HIV infection to be of the monocyte/macrophage lineage (data not shown).

Increased Expression of Adhesion Molecules in the Aortic Endothelium under Conditions of HIV-1 Infection (Table 4)

The total number of suitable *en face* preparations that can be obtained is limited and differs from patient to patient. Therefore, additional immunohistochemical studies could not be carried out in all cases. The number of immunohistochemically investigated patients varied for the diverse antibodies between 6 and 24 patients (Table 4, n = 6 to 24). To elucidate the cause of the increased adhesion of monocytes/macrophages to the aortic endothelium in HIV-1 infection we examined the expression of adhesion molecules.

VCAM-1

In 6 of 7 investigated HIV-negative control patients, only a few endothelial cells (<5% of the total number of endothelial cells) exhibited a weak and patchy VCAM-1 expression, whereas the majority of cells were clearly negative. This expression pattern was rated as normal (Table 4, N). Just 1 person (of 7) in the control group and 6 (of 23) HIV patients showed a low-grade VCAM-1 immunoreaction that exceeded the weak and focal constitutional expression. Of the HIV patients, 74% (17 of 23) displayed a clearly increased VCAM-1 expression (Table 4, 2+/3+). More than one-half of this group exhibited strong immunostaining (Table 4, 3+; Figure 3C). VCAM-1-positive cells are often found around denuded areas of the endothelium. After subdividing the HIV group, we found that one-half of the investigated pre-AIDS patients (2 of 4) showed a high-grade immunoreaction (Table 4, 3+).

ELAM-1

In the healthy control group, ELAM-1-specific antibodies revealed a patchy and faint expression of this adhesion molecule in a few cells or small cell clusters of the aortic endothelium. This low synthesis has been described as being constitutional in aortic endothelium and was graded as normal or basic expression (Table 4, N).[30] In 50% (5 of 10) of the HIV-1-positive group, a clearly increased synthesis of ELAM-1 antigen was observed (Table 4, 2+; Figure 3D). From the two pre-AIDS patients investigated, one exhibited a low-grade immunoreaction, which exceeded that of the control persons.

Additional Endothelium Activation Parameters under Conditions of HIV-1 Infection (Table 4)

Besides the expression of adhesion molecules, we also looked for additional signs of an endothelial activation with antibodies to HLA-DR and IL-1β.

HLA-DR

The endothelial cells of HIV-negative patients did not display any expression of the HLA-DR gene (MHC class II), whereas HLA-DR synthesis was up-regulated in 50% (8 of 16) of the HIV-1-infected patients (Figure 3, A and B). In particular, areas with increased leukocyte adhesion displayed a strong HLA-DR staining with phenotypical changes of the endothelial cells (Figure 3B). Some multinucleated cells were also positive for HLA-DR (Figure 3, A and B). In areas with lower numbers of adhesive leukocytes, the regularly formed endothelial cells exhibited a granular immunoreaction with the HLA-DR antibody (Figure 3A). After subdividing the HIV group, we found that 2 (of 3) pre-AIDS patients were negative for HLA-DR, whereas 1 patient showed an up-regulated synthesis.

IL-1β

The immunostaining with an antibody to IL-1β was either negative or very weak in the control specimens (5 of 6). A clearly increased staining of IL-1β antigen (Table 4, 2+/3+) was observed in the aortic endothelium of the HIV-1-infected patients (21 of 24, Figure 3E). Of these patients, 5 (of 5) were pre-AIDS patients. Endothelial cells as well as parts of the nonintimal cell population showed a positive immunostaining with this antibody. The differences in the degree of immunohistochemical parameters such as

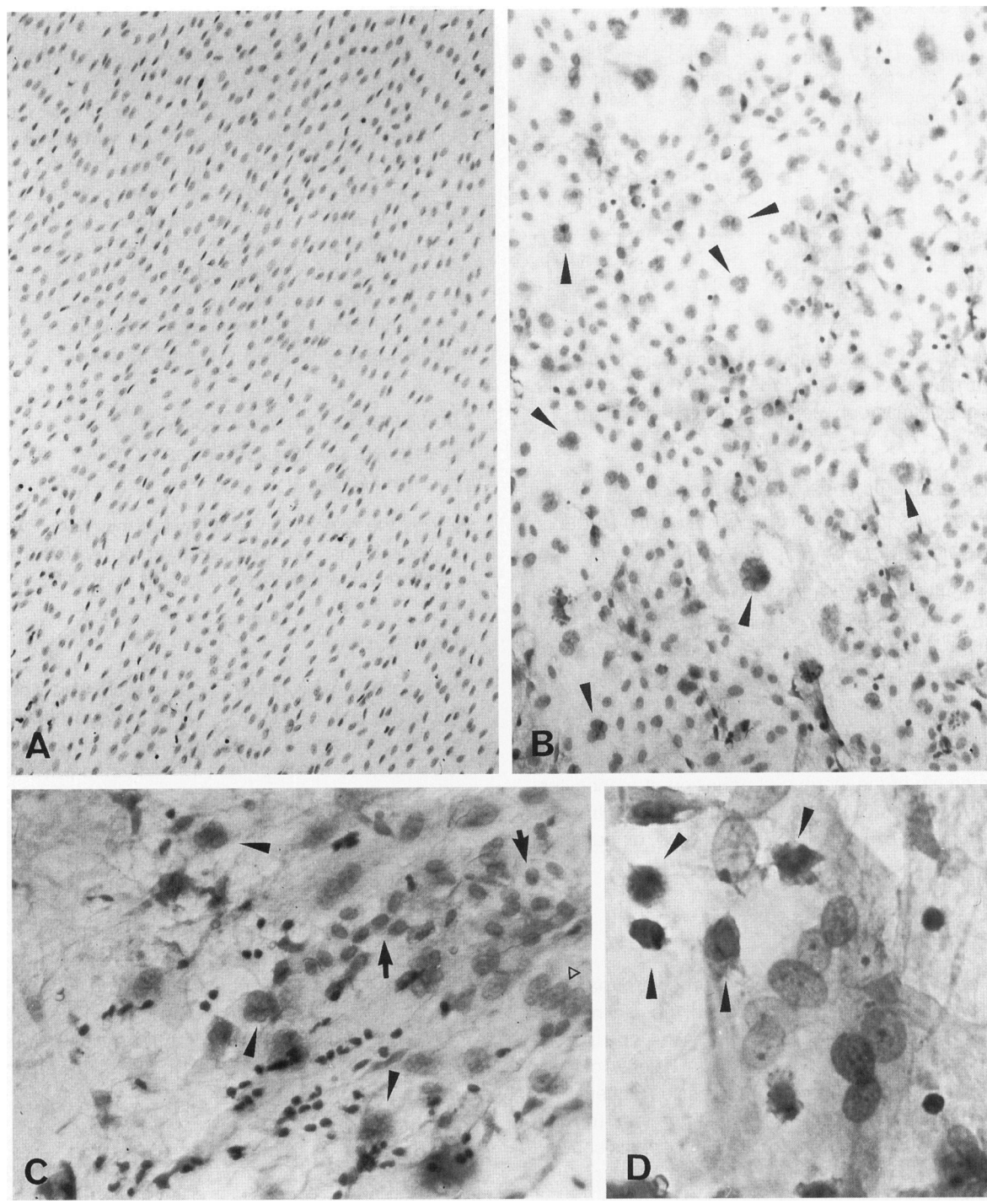


Figure 1. *Morphological pattern of aortic endothelium in* en face *preparations of aortic endothelium.* A: *HIV-negative control group, showing regular continuous aortic endothelial pavement and fairly uniform endothelial cells without any relevant leukocyte adherence. H&E; magnification, ×120.* B: *HIV-positive patient, with high-grade structural disturbance of endothelium. Note the frequent formation of multinucleated cells in association with small denuded areas* (arrowheads). *Giemsa stain; magnification, ×180.* C: *Area of extensive endothelial disturbance with some regular endothelial cells* (open arrowhead), *multinucleated cells* (black arrowheads) *and increased numbers of smaller endothelial cells with rounded nuclei and slightly more condensed chromatin, which are often arranged in cell groups* (arrows). *Giemsa stain; magnification, ×320.* D: *Nuclear pyknosis of single endothelial cells* (arrowheads). *Giemsa stain; magnification, ×740.*

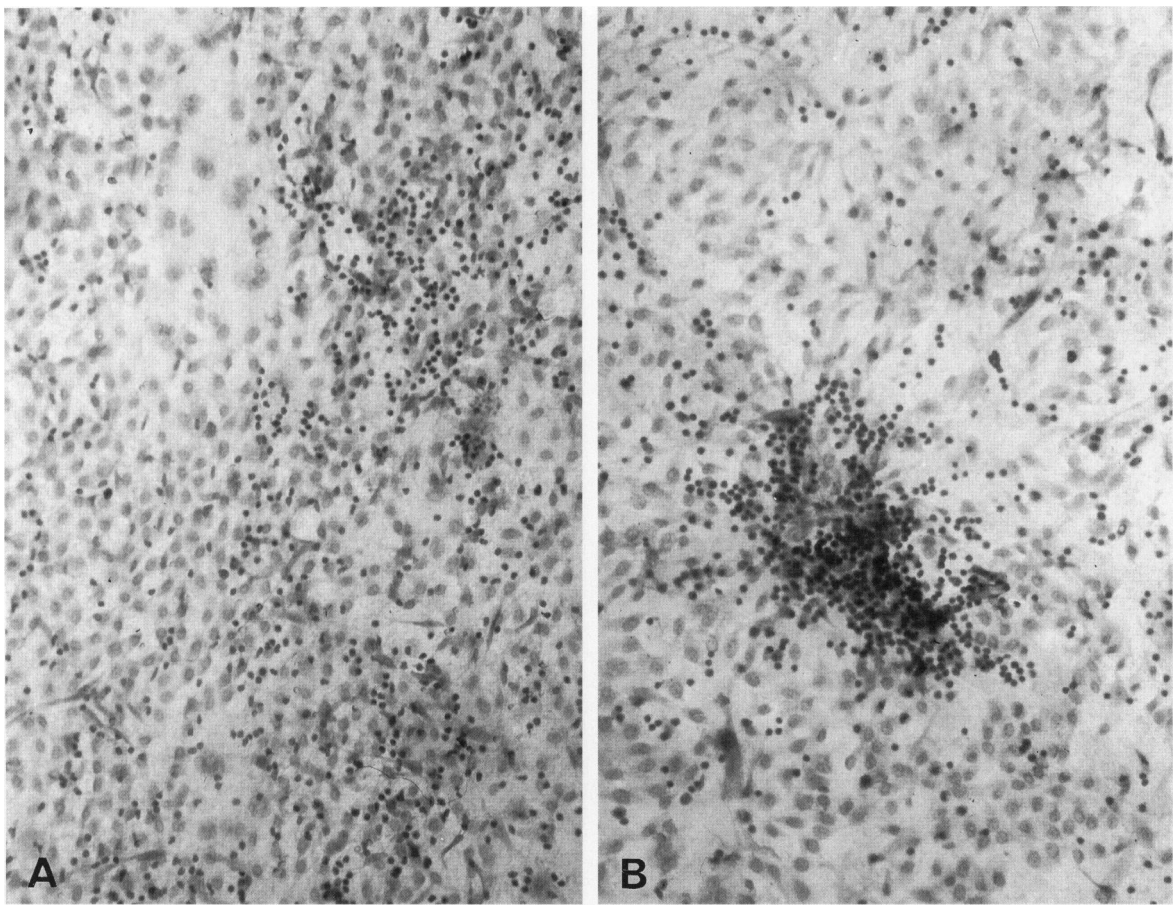


Figure 2. *Adherence of leukocytes to aortic endothelium in* en face *preparations of aortic endothelium. Increased adhesion of mononuclear cells is seen in a diffuse* (A) *and focally accentuated* (B) *pattern. In both micrographs the number of leukocytes exceeded 20% of the total number of intimal cells. Giemsa stain; magnification, ×180* (A) *and ×200* (B).

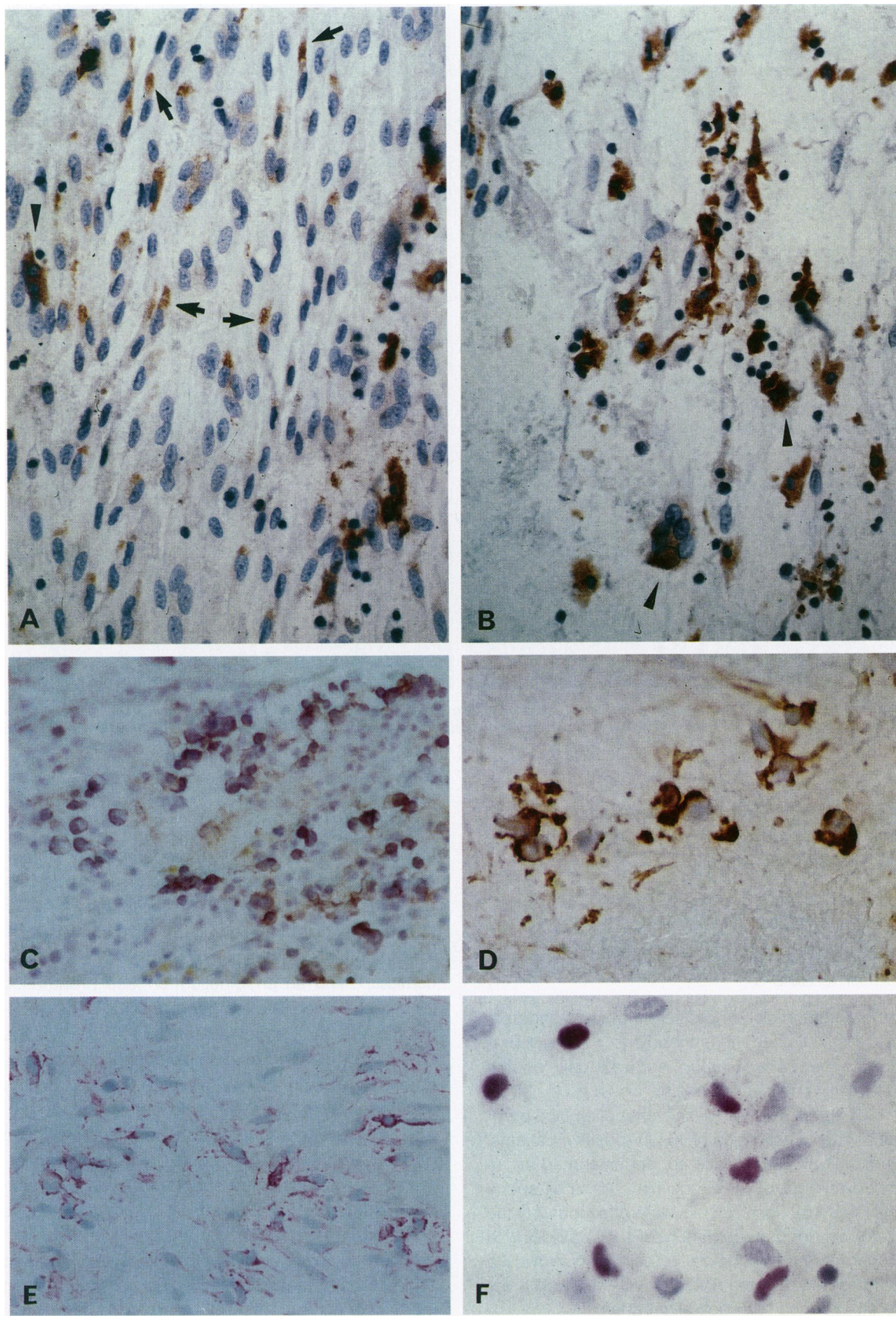
VCAM-1, ELAM-1, and IL-1 might be associated with different temporal patterns of expression of these molecules after stimulation.

Increased Proliferation Index of the Aortic Endothelium under Conditions of HIV-1 Infection (Table 5)

For the determination of proliferating aortic endothelium cells we used an antibody to Ki-67 (MIB-1) antigen. The control group showed only sparse Ki-67-positive cells. On average, we found less than one Ki-67-positive cell in 1-cm^2 area of *en face* preparation. The investigation of 15 HIV-positive patients, including 3 pre-AIDS patients, demonstrated an increased number of Ki-67-positive cells in all specimens (Table 5). Positive cells were often found at the edge of denuded areas (Figure 3F). All patients with high numbers of Ki-67-positive cells also showed a high-grade immunoreactivity for VCAM-1 and a severe disturbance of endothelial pattern.

Discussion

Clinical findings of a microangiopathy and serologically elevated endothelial cell factors in AIDS suggest repeated endothelial cell damage.[9–14,18–22] Morphological information on the status of the endothelium in HIV-1 infection is limited and needs clarification. Using an endothelium *en face* preparation technique, which offers a good possibility for studying the structural endothelial cell pattern *in vivo*, we could show clear morphological changes of the aortic endothelium in the HIV-1-infected group. All HIV-infected patients exhibited a disturbance of the normally regular endothelium cell pattern and phenotypic changes of the endothelial cells with formation of multinucleated endothelial cells. From experimental research in animals it is known that these changes in the structure of the aortic endothelium are morphological equivalents of vascular injury and endothelial regeneration.[31,32] The detection of an increased number of Ki-67- and VCAM-1-positive


A
B
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D
E
F

cells around denuded areas of endothelium also indicates morphological signs of injury as demonstrated by Lindner and Collins.[33]

Demonstrating an up-regulation of the inducible adhesion molecules VCAM-1 and ELAM-1 as well as MHC class II antigens such as HLA-DR, this study also provided support for an activated endothelial cell status.[4]

Taking all of these results into account, it can be concluded from our data that the aortic endothelium of the HIV-infected patients exhibits clear signs of injury, regeneration, and activation. This was accompanied by increased numbers of adherent leukocytes on the endothelium.

Causes of the Endothelial Injury in HIV-1 Infection

Due to the immense antigen shift of the virus and triggered by simple stress factors, pre-AIDS patients already exhibit ongoing and serologically measurable episodes of a repeatedly increased viral burden with HIV-1.[34] This continuous immunological challenge finds a morphological equivalent in HIV-associated lymphadenopathy, which we also found in four of our six pre-AIDS patients. In more advanced stages of the disease with more frequent episodes of virus-associated endothelial injury, one may expect a higher degree of endothelial changes. Therefore, it is interesting that neither in the evaluation of endothelial structure and leukocyte adherence nor in the immunohistochemical results were we able to show essential differences between the HIV-1-infected persons in the CDC stage A and the patients with full-blown AIDS (CDC stage C).[23] The absence of essential differences between the HIV groups may be due to the high regeneration capacity of the endothelium in the intervals between the repeated episodes of viremias. As demonstrated by Ki-67 immunohistochemistry, the HIV-positive patients exhibited a high regeneration capacity of the endothelium, which is obviously similar in pre-AIDS and AIDS patients.

There are several ways in which the repeated viral burden of HIV-1 may effect the endothelium. Some groups found a direct HIV infection of endothelial cells.[35,36] HIV infection of mononuclear cells with derangement of immunocompetent cells and disturbance of the local cytokine balance might even be more important for the genesis of endothelial changes.

It is well known that inflammatory cytokines activate cultured endothelial cells to synthesize and express leukocyte adhesion molecules.[4,37–41] Cytokines such as tumor necrosis factor-α, IL-1β, and interferon-γ are elevated in the serum of HIV-1-infected patients.[6,42] They may up-regulate the synthesis of the adhesion molecules VCAM-1 and ELAM-1 and, as a consequence, may be the cause of an increased adhesion of leukocytes to the endothelium. Adherence of leukocytes to the endothelium can cause an additional increase of cytokine concentrations in locally restricted areas. This may result in patches of adherent leukocytes, as was observed in this study. Toxic products released by monocytes are known to damage the endothelium. Indeed, in the HIV group, we observed a positive correlation between the number of adherent cells and the degree of endothelial disturbance. Additionally, the HIV-1 Tat protein is known to induce adhesion molecules on endothelial cells.[8] Changes may also be due to HIV-1-associated direct complement pathway activation and to other factors such as circulating immune complexes with HIV-1 antigens.[5,43,44]

Consequences of the Endothelial Injury in HIV-1 Infection

Injury and activation of the endothelial cells disrupt the normal regulatory mechanisms and result in morphological and functional alterations commonly defined as endothelial dysfunction.[4] In this regard, our findings might indicate a dysfunction of the aortic endothelium in HIV-1 infection, although we did not measure function. Is such an injured endothelium a localized phenomenon in the aorta or are there arguments for a systemic event? Indeed, clinical and morphological studies of the eyes and the brains from HIV-1-infected patients confirmed an increased endothelial permeability and a vascular leakiness.[45–48] In this context, it is interesting that brain capillaries as well as aortic endothelium in mice show prolonged VCAM-1 expression after stimulation.[49] A prolonged elevation of VCAM-1 combined with a vascular leakage triggered by a dysfunctional endothelium may influence the leukocyte trafficking

Figure 3. *Immunohistochemical expression pattern in HIV-positive patients in* en face *preparations of aortic endothelium.* **A:** *HLA-DR expression. Note the perinuclear granular staining of endothelial cells* (**arrows**) *and staining of a multinucleated cell* (**arrowhead**). *Magnification, ×300.* **B:** *Area with extensive structural disturbance and strong HLA-DR expression. Note the phenotypic cell changes in strongly positive cells and the immunoreaction in multinucleated cells* (**arrowheads**). *Magnification, ×300.* **C:** *Expression of VCAM-1. Magnification, ×250.* **D:** *Immunostaining of ELAM-1. Magnification, ×600.* **E:** *Immunolocalization of IL-1β. Magnification, ×300.* **F:** *Nuclear staining of Ki-67 antigen. Magnification, ×700.*

to the central nervous system and the integrity of the blood-brain barrier in AIDS.[48] This in turn may be important for the development of HIV-associated encephalopathy and dementia.

Injury, activation, and leukocyte adhesion of endothelial cells may play a role in the genesis of HIV-associated microvascular proliferation and Kaposi's sarcoma. With regard to Kaposi's sarcoma, the increased endothelial cell proliferation and factors produced from the adherent monocytes might participate in the initiation of the early angiomatoid Kaposi's sarcoma.[50–53] Viruses of the herpes type in particular can interact with the endothelium.[1] The association of Kaposi's sarcoma and a new human γ-herpes virus, which has been given the trivial descriptive name Kaposi's sarcoma-associated herpesvirus with its formal classification likely to be human herpesvirus-8 is by now well documented.[54]

Most importantly, a chronically repeated injury of the endothelium might disrupt the normal immunological features of endothelial cells such as phagocytosis, antigen presentation, and induction of lymphocyte proliferation and maturation.[4] Endothelial cells are able to stimulate primary and secondary T-cell responses and they offer co-stimulatory signals such as VCAM-1 expression to functional T-cell activation.[2,3,39,55] Whereas normal vascular endothelial cells enhance T-cell responses by augmenting IL-2 concentrations,[56] HIV-exposed endothelial cells were consistently defective in promoting IL-2 secretion *in vitro*.[57,58]

In conclusion, our study revealed injury, regeneration, and activation of the aortic endothelium in HIV-positive patients. This may play a more active role in the pathogenesis and progression of AIDS than assumed so far, as it may have an affect on the contribution of the endothelium to the immune network, on its regulatory properties for T-cell function, and on maintenance of the blood-brain barrier; it may also be relevant for the initiation of Kaposi's sarcoma.

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