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NEPHROLOGY FORUM

Light at the end of the TUNEL: HIV-associated thrombotic microangiopathy

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CASE PRESENTATION

A 45-year-old man with long-standing HIV infection presented to the emergency room at his local medical center. Complications of his HIV infection in the past had consisted of numerous opportunistic infections including toxoplasmosis, pneumocystis pneumonia, cytomegalovirus retinitis, chronic vertebral osteomyelitis resulting from infection with an atypical mycobacterial organism (*M. avium intracellulare*), and rectal herpes. He had received multiple combinations of anti-retroviral therapy for his primary infection as well as therapies directed more specifically to the various infections he had encountered. Other specific details of his HIV infection history such as blood counts, lymphocyte subsets, and measurements of viremia were unavailable. Other than complications of his HIV infection, the patient's medical history included a renal stone that underwent successful basket retrieval five years previously and extraction of a cataract approximately seven months prior to the current presentation. He had had renal insufficiency (serum creatinine of approximately 2.0 mg/dL) approximately one year prior to the current presentation, but he declined further workup at that time. The patient reportedly had normal blood pressure at that time and reportedly had no history of hypertension that might have been identified during his care for the noted infections.

The patient's chief complaints were nausea and vomiting. He had more long-standing symptoms of generalized weakness, malaise, and chronic headache. He disclosed a list of current and/or recent medications that included rifabutin, ethambutol, clarithromycin, nelfinavir, stavudine, lamivudine, trimethoprim-sulfamethoxazole, loratadine, and amlodipine. Compliance had been a concern, and the patient recently had initiated a self-imposed drug holiday. At presentation, his physical examination was noteworthy for a blood pressure of 180/120 mm Hg; heart rate ranging from 84 to 112 beats/min; and temperature, 96.9°F. The remainder of the physical examination was noncontributory.

Laboratory values obtained at presentation included a serum creatinine of 9.5 mg/dL; BUN, 86 mg/dL; uric acid, 7.6 mg/dL; sodium, 135 mEq/L; potassium, 4.7 mEq/L; chloride, 106 mEq/L; total CO_2, 18 mEq/L; total protein, 6.1 g/dL; albumin, 2.3 g/dL; total bilirubin, 0.6 mg/dL; glucose, 104 mg/dL; and calcium, 8.2 mg/dL. Urinalysis revealed 1+ blood and 3+ protein; microscopic examination of the urine sediment showed 6 to 8 white blood cells and 4 to 8 red blood cells/high-power field and no casts. More quantitative assessment of the degree of proteinuria was not performed. Urine cultures grew no organisms. A complete blood count revealed a hematocrit of 26.3%; hemoglobin, 8.8 g/dL; and white blood cell count, 3600/mm^3, with 75% neutrophils, 14% lymphocytes, 7% monocytes, 3% eosinophils, and 1% band forms. The platelet count was 76,000/mm^3. A peripheral blood smear revealed red cell macrocytosis and schistocytes.

Therapy aimed at achieving acute control of the patient's blood pressure was instituted, but the serum creatinine and BUN did not improve over the subsequent two days. Because of issues related to the recently recognized renal insufficiency and the desire to exclude treatable causes of renal failure and plan for institution of dialysis, a renal biopsy was performed and referred to the University of Washington for evaluation.

Renal biopsy microscopic description

Sections of the renal cortex contained greater than 75 glomeruli, of which all but three or four per level section were completely sclerosed. Several of the intact glomeruli showed prominence of intracapillary foam cells with foci of mesangiolysis characterized by dissolution of the adjacent mesangial matrix. These glomeruli showed focal thickening and reduplication of peripheral capillary basement membranes. The glomerular tufts also showed segmental prominence of glomerular

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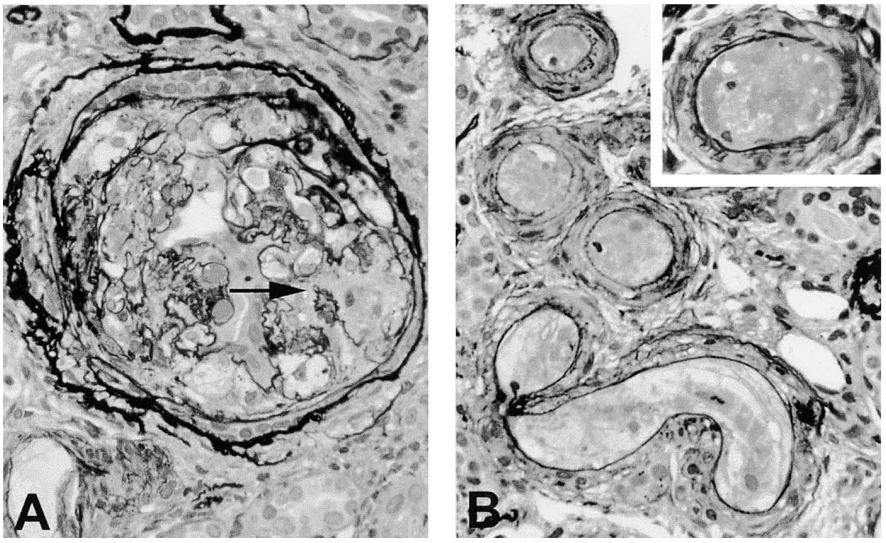

Fig. 1. Pathologic features of the kidney in TMA. (*A*) A partially scarred glomerulus demonstrates features of mesangiolysis (arrow) and segmental peripheral capillary wall changes including thickening and splitting of basement membranes. (*B*) Small arterial and arteriolar vessels containing intraluminal thrombi, some of which contain red cell fragments and fibrinous material [inset].

visceral epithelial cells, which frequently appeared swollen and contained prominent intracytoplasmic protein reabsorption droplets. Features of overt segmental sclerosis or glomerular capillary collapse were not identified. The tubular parenchyma showed broad areas of interstitial fibrosis with broad areas of tubular atrophy, which was focally pronounced. Some tubular segments exhibited prominent dilation. A prominent interstitial inflammatory infiltrate was composed predominantly of mononuclear cells. Arterial vessels demonstrated intimal sclerosis without evidence of vasculitis. Several smaller arterial and arteriolar vessels showed features of intraluminal thrombosis (Fig. 1).

Immunofluorescence studies revealed nondescript segmental staining of some glomeruli for C3 only. Deposits of IgG, IgM, IgA, C1q, kappa and lambda chains, fibrinogen, or albumin were not identified.

Ultrastructural examination of two glomeruli disclosed prominent expansion of mesangial regions due primarily to accumulations of increased matrix. Peripheral capillary walls showed irregular thickening. Overlying epithelial cells revealed focal effacement of foot processes, but some of these remained preserved. Features of overt thrombosis were not identified within the glomerular capillary loops. Electron-lucent widening of the subendothelial space was not clearly identified. Glomerular endothelium was focally swollen, but was without prominent tubuloreticular structures.

Table 1. Causes of thrombotic microangiopathy

Hemolytic–uremic syndrome
Thrombotic thrombocytopenic purpura
Malignant hypertension
Eclampsia/pre-eclampsia
Post-partum renal failure
Oral contraceptives
Infections
Allograft rejection
Scleroderma
Systemic lupus erythematosus
Anti-phospholipid antibody syndrome
Heredity
Radiation
Disseminated intravascular coagulation
Drugs/toxins
HIV infection

DISCUSSION

Dr. Charles E. Alpers (*Professor of Pathology, Department of Pathology, University of Washington Medical Center, Seattle, Washington, USA*): This case is an example of thrombotic microangiopathy (TMA) in a patient infected with HIV. The case is ambiguous, and all of us can recognize that alternate interpretations of the clinical findings are possible. Key clinical and laboratory data that would enable us to unequivocally establish the diagnosis and its pathophysiologic basis are lacking. Only some features of TMA, mesangiolysis and microvascular thromboses, are present in the renal biopsy, and characteristic changes of TMA involving glomerular capillary walls are not prominent. The presence of severe, nearly end-stage renal injury might be unrelated to the acute intravascular thromboses that are present. Although TMA is a nonspecific term, it can encompass a wide variety of diseases and initiating events (Table 1) [1]. Nonetheless, if we make use of the clinical and pathologic details available, this case offers us an opportunity to consider the relationship of HIV infection and thrombotic microangiopathy. It allows me a particular opportunity to link two long-standing research interests of my laboratory: the mechanisms underlying glomerular mesangiolysis and its repair, and the development of relevant models of HIV-associated renal injury.

Thrombotic microangiopathy involving the kidney was first described in an AIDS patient by Boccia et al in 1984 [2]. Subsequently TMA has been reported in several hundred HIV-infected humans worldwide and

appears to be the most common microvascular injury associated with HIV infection [3–14]. The review by Bell et al documents the increasing recognition of HIV-associated TMA encompassing reports published through mid-1996 [11].

In humans, as in rodent models, TMA injury involves damage to the endothelial lining of capillaries and arterial microvessels, with activation of the plasma coagulation cascade causing thromboses in these vessels [1, 15]. The kidney is perhaps the organ most susceptible to injury from TMA [15, 16] and typically demonstrates pathologic findings of occlusive thrombi in small arteries and arterioles, detachment of glomerular endothelial cells from the basement membrane, glomerular mesangiolysis, widening of the subendothelial space of glomerular capillaries by electron-lucent material presumed to be derived from plasma, and reduplication of capillary basement membranes as a consequence of either new basement membrane synthesis and/or "splitting" of the capillary walls by interposed cells and/or insudated plasma and plasma proteins [15]. Accordingly, patients with HIV-associated TMA often present with hemolytic-uremic syndrome (HUS), in which clinically evident organ dysfunction is limited to the kidneys and blood and which is manifest as renal insufficiency, hemolytic anemia, platelet consumption, and fragmentation of red blood cells (schistocyte formation). When the TMA process results in severe extrarenal manifestations, the term thrombotic thrombocytopenic purpura (TTP) has been employed to denote the systemic nature of the microvascular injury and clotting processes.

An immediate area of uncertainty concerns the magnitude of the problem. Some data suggest that HIV-associated TMA is an under-recognized clinical entity. The diagnosis of this injury can be difficult if the clinical findings are largely limited to the kidney and the entity is unsuspected. At times, HIV-associated TMA might be recognized only by characteristic findings in renal biopsies and hence might be underdiagnosed because of physicians' frequent reluctance to perform such biopsies in patients with HIV infection. Two studies that are helpful in defining the prevalence of this entity include a multicenter autopsy study in which 15 of 214 patients (7%) whose deaths were attributable to AIDS had evidence of TMA at the time of death [17]; and a study from France by Peraldi et al, in which the most common etiologic basis for acute or rapidly progressing renal failure in patients with HIV infection was HUS, the diagnosis having been established by renal biopsy in the great majority of cases [13]. This diagnosis was more prevalent than the typical form of HIV-associated nephropathy or allergic- or infection-related acute interstitial nephritis and involved 32 of 92 patients in this large study. It is not clear that a similar proportion of patients with TMA would be found among the HIV-infected patient population with new or progressive renal insufficiency at other centers, but these findings do indicate that the number of affected patients is substantial and therefore this condition might well be under-recognized. From my review of published case reports to date, no specific clinical feature of HIV infection (for example, blood counts, infection with specific organisms, therapy with specific antiviral agents) particularly predisposes to the development of TMA.

Pathogenesis

The pathogenesis of TMA revolves around direct endothelial damage. Whether cells are injured directly by toxins, shear stress, vasoactive factors, or immune/inflammatory injury, the endothelial response produces a local state in which coagulation (through such mechanisms as secretion or increased surface expression of procoagulant molecules, decreased fibrinolytic activity, platelet activation, exposure of subendothelial thrombogenic matrices, release of von Willebrand factor multimers, and leukocyte activation) and intravascular thrombosis are favored. This injury appears to be unrelated to any form of immune-complex deposition in glomeruli or blood vessels. Extrapolating from a similar injury that occurs in some patients with systemic lupus erythematosus, and on the basis of surveys of blood samples of asymptomatic HIV-infected patients [18], some researchers have postulated a role for circulating anti-phospholipid autoantibodies in initiating this injury [19–24]. Uthman and Gharavi recently pointed out that anti-phospholipid/anti-cardiolipin antibodies are a common finding when sought in the HIV-infected population but are of unknown significance because the majority of individuals with such findings (antibodies) lack evidence of clinically relevant thrombotic events [19]. A particularly relevant study of 85 patients infected with HIV found a 40% prevalence of anti-phospholipid antibodies, the presence of which could not be correlated with either thrombotic events or other manifestations of AIDS [20]. Studies by one French group indicate that circulating levels of one molecule important in the regulation of fibrinolysis, plasminogen activator inhibitor type 1 (PAI-1), is not altered in HIV-infected patients with TMA [21]. Based on this finding, Peraldi et al concluded that HIV-associated TMA is unlikely to result from decreased fibrinolytic activity. Studies of cultured human endothelial cells suggest that exposure to plasma of patients with TMA, including one patient with HIV-1 infection, causes endothelial cell injury and apoptosis, which in turn could promote TMA directly or by exposure of thrombogenic matrix underlying dead and detached endothelial cells [22, 23]. A 1993 report cited evidence of direct HIV infectivity of endothelium, which could lead to phenotypic changes promoting coagulation, but these data have yet to be substantiated [7]. In the absence of CD4 expression, the major receptor mediating HIV infection of leukocytes, it has been difficult to define

a mechanism for HIV infectivity of endothelial cells, although one study in simian models has demonstrated that endothelial cells of brain capillaries, a recognized site of TTP/TMA injury, can be infected by immunodeficiency viruses utilizing chemokine receptors independently of CD4 receptors [24].

Because direct renal microvascular infection by HIV has not been demonstrated, it behooves us to consider alternate mechanisms that might cause microvascular injury and subsequent TMA. Several promising studies have been published recently. Studies of human umbilical vein endothelial cells (HUVEC) in culture demonstrate that these cells can undergo apoptotic injury when exposed to intact HIV virions or subunit peptides [25]. The relevance of this finding for the renal microvasculature is uncertain because HUVECs can express the chemokine receptors CCR5 and CXCR4, important co-receptors with CD4 in mediating HIV infection of susceptible cells, unlike renal endothelium. Evidence indicates that the Tat (transactivator of viral replication) peptide produced by HIV-1 can cause apoptosis of cultured human microvascular endothelial cells [26], but postulating a role for Tat as a cause of TMA must be tempered by the experience to date with Tat transgenic mice, which can develop multiple types of tumors, including one resembling Kaposi's sarcoma, but which do not appear to develop TMA. Finally, data suggest that the HIV envelope protein gp120 can induce expression of tissue factor, an important procoagulant molecule, in cultured human arterial smooth muscle cells [27]. Such a process, if confirmed in vivo, could be an important contributory mechanism to the development of HIV-associated TMA in patients.

Despite these intriguing studies, the pathogenesis of HIV-associated TMA remains poorly understood. One central question remains: does the virus directly infect intrinsic microvascular or other renal cells in the course of this disease? Until recently, the evidence that HIV could directly infect renal parenchyma was scanty, resting primarily on an in situ hybridization study demonstrating viral genomic material in podocytes [28]. We and others found this evidence difficult to replicate (abstract; Pardo et al, *FASEB J* 5:A907, 1991) [29–31]. Additional evidence for infection of renal cells came from a study by Kimmel et al, who used polymerase chain reaction (PCR) amplification techniques to demonstrate HIV viral DNA in microdissected glomeruli, tubules, and leukocytes from renal biopsies of HIV-infected patients [32]. However, identification of this genomic material did not clearly correspond to manifestations of HIV-associated disease in these patients. Because of the risk of leukocyte contamination in such difficult microdissection procedures, the exquisite sensitivity of PCR amplification, and the lack of an identifiable receptor for HIV on most renal parenchymal cells, an element of uncertainty accompanied these findings. More recently, a series of studies from Paul Klotman and his colleagues, utilizing sophisticated in situ PCR techniques to detect circular forms of viral DNA, has provided evidence that podocytes and entire tubular segments can be infected with HIV in vivo [33–36]. These studies have not identified viral infection of renal microvascular cells. In view of the persistent difficulty in identifying HIV peptide products or other evidence of a productive infection in these infected epithelial cells, it remains difficult to define the scenario by which this cellular infection leads to the parenchymal changes of HIV-associated nephropathy (HIVAN) or other HIV-associated pathologies. Obviously this presents a major challenge for individuals engaged in the investigation of HIV-associated renal disease.

The ability of HIV to directly infect intrinsic renal and renal microvascular cells also has been investigated in cell culture. We have been unable to demonstrate productive infection of human mesangial cells, a cell type considered to be a variant of vascular smooth muscle cells, despite exposure of these cells to a variety of wild-type and laboratory-adapted strains of HIV-1 and HIV-2 [37]. In a recently published letter, Conaldi et al reported successful infection of mesangial cells [38], but many of the details needed to establish this finding were not provided, and we await an expanded description of this work. One other group has reported HIV infectivity of human glomerular endothelial cells in vitro and of a small proportion of mesangial cells but not epithelial cells [39]. A viral receptor or other mode of entry of virus into these cells was not identified in this study, and the study design suggested that the observed findings were the result of transfection rather than infection of the virus. In toto, the results of cell culture studies to date have not provided clearly defined mechanisms of renal microvascular infection by HIV.

Our understanding of HIV infectivity is further complicated by our limited knowledge concerning possible receptors for viral entry into specific microvascular renal cell types. The importance of the leukocyte surface antigen CD4 as a principal receptor mediating entry of HIV into cells has been long established. Chemokine receptors have been identified as essential co-receptors for human immunodeficiency virus infection in mammalian cells [40, 41]. The chemokine receptor CCR5 serves as the major co-receptor together with CD4 for macrophage-tropic strains of HIV-1 [42, 43], whereas T-lymphocyte-tropic strains of HIV-1 most often utilize the chemokine receptor CXCR4 as the principal co-receptor required for infection [40, 44]. The expression of CCR5 and CXCR4 in HIV-associated TMA renal disease is unknown. Our laboratory published data showing that constitutive expression of these receptors in human and macaque renal parenchyma and in the renal microvasculature is undetectable using probes for mRNA that code for CCR5 and CXCR4 [45, 46]. In the case of CCR5, this finding has

been substantiated by corroborative studies of CCR5 protein expression by immunohistochemical techniques [47]. To date, no evidence supports constitutive expression in vivo of CD4 by intrinsic renal cells. Furthermore, we were unable to detect up-regulated expression of these receptors on intrinsic renal cells in renal biopsies from patients with HIV-associated nephropathy [13]. Absent the presence of known receptors that can mediate infection of cells by HIV, it becomes more difficult to construct the early events by which HIV enters renal cells and/or causes renal injury, unless the pathogenetic basis for injuries associated with HIV is indirect and involves other systemic or locally produced mediators such as cytokines. A recent abstract suggested the possibility that additional HIV co-receptors (in this case, GPR15/Bob) are present in the kidney and might contribute to infection of renal cells (abstract; Clayton et al, *Lab Invest* 82:276A, 2002). This study also contained evidence that the glycosphingolipid Gb3 (globotriaosyl ceramide), already known to be present in the kidney and implicated in the TMA injury induced by Shiga toxin [48], might have the ability to bind surface proteins (gp120) of some strains of HIV-1. If these findings are substantiated, characterization of new pathways of viral infectivity could help resolve some of the uncertainties about the linkage between renal infection and renal injury.

The pathogenesis of TMA occurring outside the setting of HIV infection is multifactorial. Some causative agents have been well defined, including products of bacterial infection (verotoxins such as Shiga toxin from *Shigella dysenteriae* and Shiga-like toxins from *E.coli* O:157, and bacterial and viral neuraminidases), certain drugs (for example, mitomycin, cyclosporine, tacrolimus, and oral contraceptive agents), radiation, and circulating antiphospholipid antibodies [1, 15]. Certain disease settings also predispose patients to this form of injury, including malignant hypertension (a relevant consideration in the patient presented), scleroderma, and eclampsia. A small minority of affected individuals has a familial, inherited disorder of von Willebrand factor multimers, which predisposes them to relapsing episodes of TMA [1, 15]. In a significant number of individuals with idiopathic TMA, the pathogenesis remains undetermined, although two reports indicate a pathogenesis resulting from the presence of circulating antibodies that act as inhibitors of von Willebrand factor-cleaving protease [49, 50].

Laboratory studies

Researchers have developed a number of animal models of thrombotic microangiopathy (Table 2). Working with colleagues formerly at the University of Washington, Richard Johnson and Masaomi Nangaku, and their fellows and visiting scientists, we have focused on the anti-Thy 1 antibody rat model of mesangiolysis and repair, and on a model of injury induced by anti-endothelial cell antibodies. The anti-Thy 1 model has been extensively studied by numerous investigators [51]. The major findings in this model can be summarized: the initial injury of mesangiolysis is induced by an antibody directed against the Thy 1 antigen present on the surface of mesangial cells and is followed by a reparative response that includes mesangial cell proliferation and migration, which are mediated at least in part by growth factors FGF-2, PDGF-B, and PDGF receptor β; a capillary regenerative response likely mediated at least in part by VEGF [52–54]; formation of a provisional matrix mediated by TGF-β; and restoration of glomerular normocellularity through apoptosis, a process in which the key regulatory factors remain unknown [55].

Table 2. Animal models of thrombotic microangiopathy

Induction by anti-mesangial cell antibodies
Induction by anti-endothelial antibodies
Induction by mesangial toxins (mitomycin, Habu snake venom)
Hypertension (DOCA salt)
Shiga toxin
HIV infection

The second rodent model produces a similar lesion of mesangiolysis, albeit by a different mechanism. In this case, mesangiolysis and TMA follow the administration of anti-endothelial antibodies. This model demonstrates a predictable sequence of endothelial injury, intracapillary thrombosis, mesangiolysis, widening of the glomerular subendothelial space, and complete resolution of the glomerular injury with morphologic findings remarkably similar to the pathologic findings in human cases of TMA [56]. This predictable sequence of injury, induced solely by an initial insult to the endothelium, is highly relevant to our current understanding of TMA associated with HIV infection. Together, the two rodent models illustrate how features of TMA can arise either from an injury to the mesangium or to the glomerular capillary endothelium. These models suggest how diverse and perhaps multifactorial pathogenetic events can lead to a common pattern of renal injury.

Our interest in the problem of HIV infection-associated TMA originated in part from our group's attempt to establish a primate model of renal disease that resembled human HIVAN. We believed that an animal model that closely mimicked the lesions seen in human HIV expression would provide the best opportunity for clarifying many questions that have arisen regarding the significance of this renal lesion. Our previous studies of primates infected with simian immunodeficiency virus (SIV), simian retrovirus type D (SRV), HIV-1, or HIV-2 have taught us that macaques infected with SIV, HIV-2, and possibly HIV-1 can develop all the manifestations of human HIV infection including features such as lymphocyte depletion, lymphoproliferative disorders, encephalitis, and opportunistic infections. Further, these manifestations depend some-

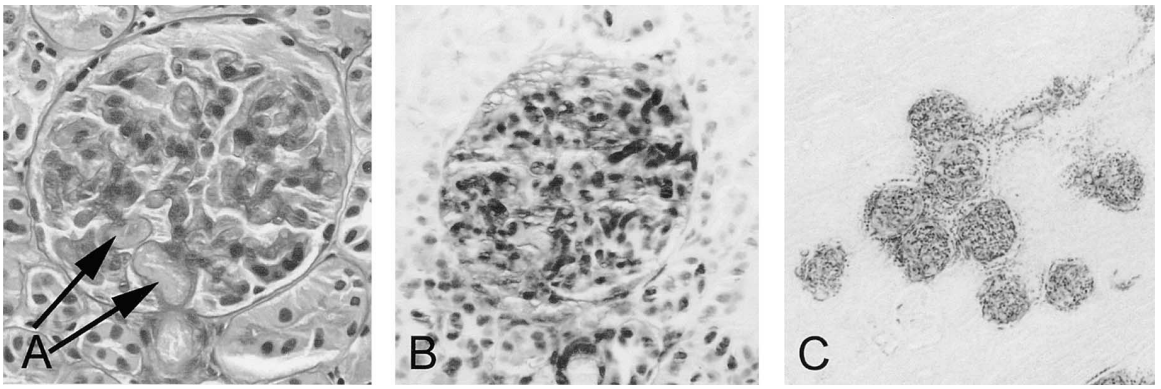

Fig. 2. (*A*) Glomerulus from a macaque with HIV-2 infection-associated thrombotic microangiopathy. Note the intracapillary thromboses (arrow). (*B*) Same glomerulus as in A, in a replicate tissue section, showing widespread cellular staining (dark, homogeneous nuclear staining) with the TUNEL procedure. (*C*) Lower-power view of histologic section of kidney from a macaque with TMA, stained by the TUNEL technique. Note the staining of cells comprising glomeruli and the vasculature in an area involved by the injury process, while other portions of the kidney remain completely unaffected.

what on the viral strain used for infection and on variations in host species [57–59]. We have learned from these models that it is very difficult to demonstrate direct viral infection of host epithelial or mesenchymal cells, including vascular cells; that expression of the viral receptor CD4 generally remains limited to cells of hematopoietic origin; and that a few macaques get renal lesions resembling HIVAN [57], but that overall a robust model for this particular injury has not been established either in our center or in other primate centers working with various simian models of AIDS. However, we now know that macaques infected with the HIV-2_{287} virus clone have an approximate 25% incidence of TMA within a one month period of infection following inoculation with the virus [60].

Although the exact origin of HIV-2 remains unidentified, Hirsch et al suggested that HIV-2 probably originated as a zoonotic disease transmitted from feral sooty mangabey monkeys found throughout western Africa [61]. One strain of HIV-2 (HIV-2_{287}), originally derived from a human AIDS patient, is not only readily infectious in macaques but also highly pathogenic, progressing to a clinical AIDS syndrome within an accelerated time frame of 6 to 12 months [62]. We demonstrated that approximately 25% of macaques inoculated intravenously with this strain of HIV-2 and sacrificed in a prospective fashion develop pathologic manifestations of TMA (Fig. 2) [59]. These animals show a decline in peripheral CD4 and blood cells but do not develop overt features of AIDS or renal insufficiency within the time period studied. The animals with TMA developed thromboses in both renal and extrarenal sites, and the histologic and ultrastructural pathologic findings (thromboses, mesangiolysis, detachment of endothelial cells from glomerular basement membranes) were remarkably similar to those of human TMA.

I have cited evidence obtained from in vitro studies that exposure to plasma of HIV-1-infected patients with TMA can lead to endothelial cell injury and apoptosis. An interesting study by Bodi et al identified increased apoptosis of tubular epithelial cells in renal biopsies of patients with HIVAN but did not reveal similar apoptotic injury within the microvasculature [63]. Studies of human proximal tubular epithelial cells in culture also have provided evidence of increased susceptibility to apoptosis in the setting of HIV-1 infection [64]. We have evidence, first obtained by electron microscopic examination of tissue from macaques with TMA, that this disease process involves endothelial and vascular smooth muscle cell injury morphologically resembling, but not equivalent to, apoptosis (Fig. 3). This injury is characterized by condensation of nuclear chromatin into dense aggregates in cells of the microvasculature affected by TMA and cells enmeshed within thrombi, but not in other portions of the same tissue, including similar vascular structures that are uninvolved by the TMA process. This last finding, and the absence of such changes in numerous uninfected primates and in primates infected with SIV, indicates that this form of endothelial injury is not an artifact or an unrelated finding common to macaques. This ultrastructural appearance is unique and differs from the homogeneous clumping of nuclear chromatin commonly accepted as pathognomonic of apoptosis, although the evidence that links apoptosis to TMA raised the possibility that the HIV-2-associated injury might be similar to apoptosis. Further investigation of this injury process by Frank Eitner and Stephan Segerer in my laboratory, who used a variety of morphologic techniques that characterize apoptosis, revealed a unique pattern of tissue injury in which vascular structures involved by the TMA process, as well as nearby cells comprising other tissue compartments, demonstrate widespread cell labeling by the TUNEL procedure in histologic sections

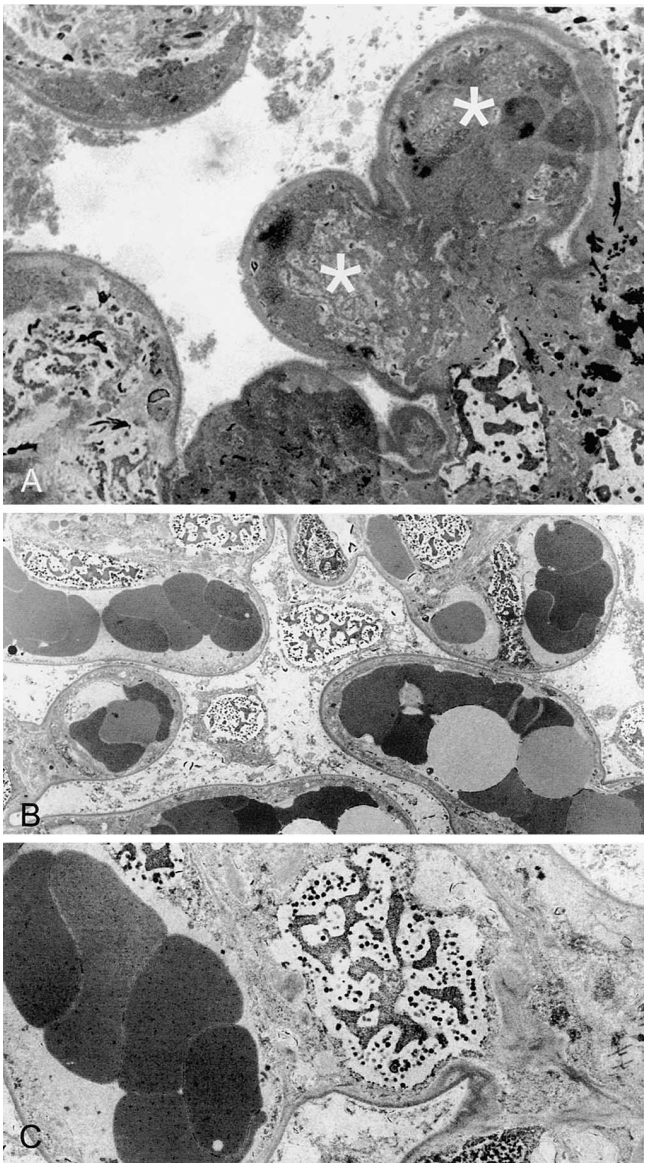

Fig. 3. Ultrastructural appearance of macaque glomeruli involved by HIV-2-associated TMA. (*A*) Intracapillary thromboses are present (asterisks). (*B*) Another glomerulus demonstrating characteristic ultrastructural nuclear changes in capillary endothelial cells, mesangial cells, and visceral epithelial cells. (*C*) High-power view of a glomerular mesangial cell with characteristic nuclear alterations. Glomerular cells demonstrate a unique, dispersed, speckled pattern of chromatin organization within the nuclei.

(Fig. 3) [65]. The TUNEL procedure stains loose single-stranded fragments of DNA and is considered a sensitive, although not necessarily specific, marker for apoptosis. The finding of areas of TUNEL-positive cells was confined to macaques with evidence of TMA. This feature of cell injury corresponded to the cells with altered histologic nuclear detail associated with TMA and with other morphologic markers that are considered somewhat specific for apoptotic cell injury, such as in situ end labeling (ISEL) of single-stranded DNA and staining with a monoclonal antibody that detects single-stranded DNA [66]. There was strong concurrence in the detection of cell injury utilizing these techniques.

Because of the striking demonstration of discrete areas of injury marked by TUNEL positivity, we informally refer to this injury as tunelosis (abstract; Segerer et al, *J Am Soc Nephrol* 11:551A, 2000). As detailed in Table 3, tunelosis differs from apoptosis by a number of features, notably including the involvement of groups of cells within tissue compartments and the unique nuclear changes (Fig. 3). On the other hand, tunelosis also has features that distinguish it from necrosis, which can involve groups of cells and tissue compartments but differs in the morphologic appearance of affected cells and does not result in positive labeling by techniques that identify single strands and breaks of nuclear DNA [67, 68].

We currently believe that tunelosis is a unique form of cellular injury, one certainly linked to the pathogenesis of TMA occurring in HIV-infected macaques. We do not yet know whether such an injury occurs in human disease or what features of HIV infection in macaques produce such a phenotype. These issues are currently being explored. Renal biopsies from patients with TMA, both HIV-associated and not, are being investigated by the same techniques used to characterize injury in the macaques. Cell culture systems using microvascular endothelial cells have been set up to evaluate changes in these cells that might be induced by HIV virions or peptides as well as cytokines and other molecules that might be circulating in the blood of infected patients and nonhuman primates. We are hopeful that these studies will provide clues to the pathogenesis of HIV-associated TMA.

Treatment

Before concluding, I'd like to return to the patient presented. This patient had limited treatment options because of the background of end-stage sclerosing renal injury. Unfortunately, even without such a compromised background, patients with this disorder have limited options. There are sporadic reports of treatment of affected patients with prednisone and/or plasmapheresis, but the limited number of patients seen in most centers precludes controlled clinical trials to establish the efficacy of such treatments. Plasmapheresis has perhaps been the most widely employed therapeutic modality, but it has had only limited success [69]. Available reports do not offer a compelling case for any therapy beyond what might be employed in TMA arising in other disease states.

QUESTIONS AND ANSWERS

Dr. Nicolaos E. Madias (*Executive Academic Dean, Tufts University School of Medicine, Boston, Massachusetts*): Collapsing focal glomerulosclerosis occurs in non-HIV infected individuals. Also, thrombotic microangiopathy has been associated with a number of viral diseases,

Table 3. Comparison among the pattern of injury in HIV-2-infected macaques and the features of apoptotic and oncotic necrosis

	Necrosis	Apoptosis	Cellular injury in HIV-2-infected macaques
Nuclei	Swelling Pale Karyolysis	Shrinkage Dense Karyorrhexis	Swelling Pale
Cells	Swelling Disintegration	Segregation into "apoptotic bodies"	No distinctive features other than nuclear changes
Chromatin	Irregular clumps (coarse strand)	Condensation into blebs near the nuclear envelope	Condensation into a dense center, surrounded by diffusely distributed small particles
Distribution	Groups of cells	Single cells	Groups of cells
Inflammation	Yes	No	No
TUNEL	Usually negative	Positive	Positive
ssDNA	Usually negative	Positive	Positive

including cytomegalovirus and parvovirus. Might it be that both the renal disease and the microangiopathy in HIV-infected individuals are actually due to superinfection with a different viral agent?

Dr. Alpers: The idea that collapsing glomerulopathies might be caused by viral infections, perhaps as a result of direct infection of glomerular epithelial cells, has some appeal on clinical grounds and some supporting evidence as well. Indirect evidence is based on clinical observations that patients with collapsing glomerulopathy often have a prodrome that seems to be a manifestion of some unspecified flu-like viral illness. Isolation of viral DNA from renal biopsy specimens from affected patients and localization of viral DNA by in situ hybridization have yielded more specific evidence that parvovirus B19 is associated with collapsing glomerulopathy [70]. Other studies have shown that parvovirus B19 DNA is commonly found in renal tissue from patients with a variety of renal diseases, and was not limited to focal and segmental glomerulosclerosis and collapsing glomerulopathy [71]. So while some evidence suggests that parvovirus could play a role in this disorder, we have insufficient evidence to establish causality or specificity of this virus for this disorder. Other viral infections probably are good candidates for causing this lesion. It is possible that some thrombotic microangiopathies are also a result of an infection by an as-yet-unrecognized virus, perhaps one in which the pathogenicity is strengthened by concurrent HIV infection. However, unlike the case of collapsing glomerulopathy, even one such virus that could be a candidate for direct microvascular infection leading to this injury has yet to be identified. It is also possible that systemic infection with unspecified viruses can affect microvascular cells in ways that we are not able to recognize. Your question introduces the multiplicity of changes in the host that occur in the setting of a systemic infection independent of viral infection of specific renal tissues. There is a host-cytokine response to infection with alterations in circulating cytokines, as well as locally produced cytokines, all of which can participate in microvascular injury and lead to thrombotic microangiopathy. The unknown variables involved in such a process are many.

Dr. Madias: How much do we know about factors that determine susceptibility of HIV-infected patients to thrombotic microangiopathy? Do these patients have abnormalities in factor H, von Willebrand factor-cleaving protease, or polymorphisms in cytokine genes that might contribute to decreased thromboresistance?

Dr. Alpers: This is a critical but currently unanswerable question. Few studies to detect these kinds of abnormalities in HIV-infected patients have been done. I did cite some studies from several groups that have looked at the issues of circulating anti-phospholipid antibodies and plasminogen activator inhibitor-1 in the setting of HIV-associated TMA, but the literature contains little work that extends these studies to the kinds of specific abnormalities you refer to. One problem is the lack of a defined and sufficiently large group of patients who can be studied for such alterations. It would be nice to have a registry of these individuals so that sufficient numbers of patients can be studied to make meaningful conclusions about risk factors and pathogenetic mechanisms. Several laboratories, including Remuzzi's group, have looked at the presence of specific metalloprotease activity against von Willebrand factor multimers that is linked to TTP and allows differentiation from other forms of thrombotic microangiopathy [48, 49]. Such studies would be of value, but to the best of my knowledge, these studies have not been done in the HIV-infected patient population. Kelton's group has suggested that this metalloprotease activity might not be as specific for TTP or even thrombotic microangiopathy as first believed [72]. Most studies bearing on susceptibility have used in vitro model systems to test their hypotheses. Another recent example is a study in which the HIV envelope protein gp120 activated human arterial smooth muscle cells and induced the expression of tissue factor, an important procoagulant molecule [73]. It has been difficult to test the importance of this type of result in patients or in a relevant in vitro model.

Dr. John T. Harrington (*Dean, Tufts University School of Medicine*): What techniques do you use to protect pathology lab workers who are handling HIV specimens?

Dr. Alpers: I share with our hospital epidemiologist and hospital infection control staff the presumption that all patients could be HIV infected. We didn't know in advance about the HIV status of today's patient; the history was obtained at the time we received the biopsy specimen. We try to treat all patients and their tissues the same (that is, we use universal precautions), regardless of how much we know about their status with respect to HIV or other infectious agents. However, in reality, if we know that somebody has HIV, we do a few other things to protect ourselves. In pathology, we have a set of instruments and sectioning equipment dedicated to potentially infectious tissues. These are disinfected with bleach, and autoclaved and decontaminated after each use. Only experienced personnel are allowed to cut histologic sections. All personnel use double gloves when handling tissue specimens, and metal gloves are available for those who prefer them. We minimize the use of unfixed tissues, and in turn fix tissues promptly. Complete facial protection—masks, eye protection, and at times respirators—are a requirement when handling tissues.

Dr. Harrington: My second question regards the diagnosis of HUS either after HIV infection or after transplantation. It seems to me that in the past, HUS or TTP was relatively easy to diagnose clinically. Cutaneous findings, renal abnormalities, and microangiopathy were present, and the platelet count was very low. Now, we're seeing patients with few hematologic findings, yet the renal pathology in many patients in whom the disease was not suspected turns out to be thrombotic microangiopathy. Did we just miss these patients in the past or has something changed?

Dr. Alpers: This is an important question, and one that comes up often in clinical practice when a diagnosis of TMA is made by renal biopsy. It is my experience, and I think that of many others, that renal biopsies are rarely obtained from patients with renal abnormalities in whom pathognomonic findings of TMA such as microangiopathic hemolytic anemia and thrombocytopenia are present. However, TMA is a fairly common renal biopsy diagnosis, and the patients who have this diagnosis established by biopsy usually do not have overt or prominent hematologic abnormalities. These are patients in whom the diagnosis cannot be established from looking at a peripheral blood smear and identifying fragmented red blood cells. In my experience, it is not unusual for hematologists who are brought into consultation at that point in a patient's evaluation to resist any diagnosis of HUS or TTP, absent these hematologic abnormalities. For individuals of this persuasion, the kind of patient you are describing conceptually does not exist. Yet, there are clearly syndromes of renal insufficiency that are associated with characteristic microvascular changes and thrombi in small blood vessels, and with characteristic glomerular changes of mesangiolysis and capillary loop expansion with basement membrane splitting and intracapillary thrombosis, that occur without much evidence of anemia or any other systemic finding. As you indicate, such cases are clearly those of a renal-limited form of TMA, and the only way I know to establish the diagnosis of a primarily renal-limited form of TMA is by renal biopsy. What I don't know is whether there is a changing incidence of TMA within renal biospy practice in general, or how changes in community standards about the thresholds for performing renal biopsy have affected the perceived incidence of TMA. My own sense is that the frequency of TMA in renal biopsy practice is increased as a consequence of transplantation-related injuries (for example, calcineurin inhibitor toxicity) but that no major changes in frequency exist in the non-transplant renal patient population. For example, sporadic increases can result from infections or new drug toxicities, and we might see a diminishing incidence of radiation nephropathy and severe hypertension, with other entities more or less being the same. This is admittedly a crude assessment, and it does not take into account caveats like variability in clinician thresholds for biopsy.

Dr. Andrew S. Levey (*Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Have you searched for these markers of tunelosis, for instance, in your rat antibody-induced mesangiolysis or anti-endothelial cell antibody model of TMA, or in the transgenic mice that Dr. Klotman used to investigate HIV infection and its renal affects? Also, you raised the interesting question of whether the injury is related to HIV infection per se.

Dr. Alpers: We have not evaluated our markers in the transgenic mouse models that you refer to. It might be an interesting thing to do. To the best of my knowledge, these mice develop features of glomerulosclerosis and tubular injury, but they do not develop features of a TMA. Some studies have looked at apoptosis in these mice, but broad areas of tissue injury, as exemplified in our HIV-2 infected macaques, have not been reported in these models. Apoptosis has been studied by TUNEL staining techniques and by ultrastructural morphology in both the anti-Thy-1 and anti-endothelial cell antibody injury models. Apoptotic injury can be identified in these models, principally in mesangial regions during the reparative process and also in some portions of the tubulointerstitial parenchyma, but no broad tissue areas of injury correspond to what we have seen in the macaque infection model. Apoptosis, when present, mostly appears to involve individual or very small groups of cells within specific tissue compartments. We have a study underway to see whether this phenomenon can be broadly

extended to TMA as it occurs in human kidneys, including studies both of individuals with HIV-2 infection as well as those whose TMA is due to various causes of this injury (Table 1). Our initial studies have not identified a process comparable to that in the monkey model. We have had the opportunity to study only two cases of human HIV-2-associated TMA in this manner.

Dr. Madias: Have endothelial function and the nitric oxide system of HIV-infected patients or animals been examined?

Dr. Alpers: I know of no tests of endothelial function that have been directly linked to HIV infection, nor am I aware of such studies in relevant animal models. It is known that treatment of HIV-infected patients with protease inhibitors can cause hyperlipidemia, lipoprotein abnormalities, and hyperglycemia. Recognizing that these are risk factors for cardiovascular disease, some investigators have tested endothelial function in large arteries (brachial artery responses measurable by ultrasound) and demonstrated general endothelial functional impairments in HIV-infected patients taking this class of drugs [74]. One other recent study showed diminished circulating levels of leukocyte adhesion molecules (soluble VCAM-1) and von Willebrand factor, both released by damaged endothelium, in HIV-infected patients taking various regimens of antiretroviral therapy [75]. It is not clear whether this was an effect of the actual therapy or its efficacy in reducing viral load. It is also not clear what role the HIV infection played in causing the release of endothelial cell products into the circulation in the first place.

There are conflicting reports on the roles of the NO system in TMA in general, and I don't know of studies applicable specifically to HIV-associated TMA. Remuzzi's group reported increased NO activity in patients with recurrent TMA [76]. Studies in experimental TMA in rodents suggest that endothelial nitric oxide synthase (eNOS) in particular is protective in TMA renal injury [77, 78].

Dr. V. Balakrishnan (*Division of Nephrology, New England Medical Center*): My understanding is that endothelial cells are relatively slow-growing, long-lived cells that bear *Fas* ligand. You alluded to some data that HIV turns on *Fas* expression in endothelial cells. Did you look for *Fas* expression in the HIV-infected macaque model?

Dr. Alpers: We did not. We tried to adapt some reagents that detect human *Fas* using immunohistochemistry for similar use in monkeys, but we found that they did not work well. I can't say that we've worked extensively at it. In view of the data in human TMA that I discussed earlier, it seems that a renewed effort to test the importance of *Fas* pathways in TMA would be worthwhile.

Dr. Madias: Regarding the co-receptor molecules you referred to, are those only important for HIV entry into cells, or do they also influence cellular response to infection?

Dr. Alpers: I would hesitate to make definitive statements about that. Most of what we know about the function of the chemokines and their receptors is their ability to recruit specific classes of leukocytes to sites of injury and hence modulate specific immune and inflammatory responses. They can be important in organ development; as an example, CSCR4 and its chemokine ligand SDF-1 have been shown to be important in the development of the immune, circulatory, and central nervous systems [79]. How certain chemokine receptors came to their roles as co-receptors of viral entry into cells is something of a mystery. Activation of chemokine receptors does lead to intracellular signaling events, and I am aware of studies that demonstrate that engagement of these receptors by the HIV envelope protein, for example, leads to intracellular signaling events [73], but I don't know that the significance of such interactions has been well worked out. I also don't know how specific such engagement of relevant chemokine receptors with downstream signaling is for different viruses, virus subtypes, or viral peptides. I have to plead ignorance for much of your question.

Dr. Harrington: To the best of your knowledge, is nitric oxide, or its lack, involved in the TMA destructive process?

Dr. Alpers: I don't think anybody really knows for certain. Rick Johnson's group at Baylor has taken the glomerular anti-endothelial cell antibody model and looked at a number of factors by which the injury might be ameliorated or exacerbated. They found that administration of L-NAME, an inhibitor of nitric oxide synthesis, exacerbated endothelial injury and resulted in more apoptosis and thrombosis [78]. Their data offer some evidence of nitric oxide involvement in TMA injury. As an aside, this group also has provided evidence that vascular endothelial growth factor (VEGF) can be protective of renal injury in this rat model [80].

Dr. Harrington: You mentioned that the TMA injury in the Thy-1 model was reversible, at least in some instances. Has reversible disease been shown in humans? If so, to what extent?

Dr. Alpers: The basic anti-Thy-1 antibody model, if you don't modify it or administer multiple doses of anti-Thy-1 antibody, is typically reversible just like the anti-endothelial cell antibody in the rat. The human disease is potentially reversible as well. Typical syndromes of infection-related hemolytic-uremic syndrome in children have a self-limited course with a return to normal renal function. I have direct experience from my renal biopsy practice that thrombotic microangiopathies resulting from toxicity of drugs, such as cyclosporine, can be reversed with removal of the offending drug from the patient's immunosuppression regimen. We believe that the anti-Thy-1 and anti-endothelial cell antibody experimental systems are particularly good models for these kinds

of self-limited thrombotic microangiopathic injuries in humans, which begin with relatively defined onset and initiating stimuli. That is not to say that all such cases in humans are reversible. Unfortunately for the patient we discussed today, he presented with advanced renal disease. The thrombotic microangiopathy was an insuperable additional injury that pushed the patient to end-stage renal disease. He never recovered from this episode of TMA and went on to requiring maintenance dialysis.

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