Renal Ultrastructural Markers in AIDS-Associated Nephropathy

PRAVEEN CHANDER, MD, ANITA SONI, MD, ANURADHA SURI, MD, RAVI BHAGWAT, MD, JINIL YOO, MD, and GERHARD TRESER, MD

Renal tissues from two groups of patients with acquired immune deficiency syndrome (AIDS) were examined: Group A had severe proteinuria and varying degrees of renal insufficiency, designated AIDS-associated nephropathy (AAN), and Group B had no renal involvement. Control Group C consisted of patients with heroin-associated nephropathy (HAN) with proteinuria comparable to patients in Group A but without AIDS or its related complex (ARC). The most frequent finding, common to both AAN and HAN, was focal glomerular sclerosis. In contrast to HAN, AAN tissue showed mesangial hypercellularity, sparse interstitial infiltrates, severe tubular degenerative changes, tubular microcystic ectasia, Bowman’s space dilatation, and presence of multiple complex inclusions both in the nuclei and cytoplasm in a variety of cells. Abundant tubuloreticular inclusions were found in the endothelial and occasionally in the interstitial cell cytoplasm. Nuclear bodies (NBs) were seen in greater frequency, complexity, size, and heterogeneity, and of budding configuration in Group A as compared with Groups B and C; NBs in Group C were mostly of simple types (I and II). In addition, a peculiar granulofibrillary transformation in many tubular and interstitial cell nuclei was observed in Group A. This transformation was rarely present in Group B and was never seen in Group C. Because complex NBs (Types III to V) and various intracytoplasmic and intranuclear inclusions present in Group A are often associated with viral invasion, their presence in kidneys of AIDS patients with proteinuria suggests a viral etiology for AAN. (Am J Pathol 1987, 126:513-526)

RENAL DISORDERS have been reported in over one-third of an unselected group of patients with the acquired immune deficiency syndrome (AIDS). The AIDS-associated renal abnormalities include acute tubular necrosis, focal nephrocalcinosis, interstitial nephritis, intrarenal infection, and proteinuria. Various hemodynamic factors, infection by opportunistic pathogens, and administration of drugs and other toxic substances can be implicated in the pathogenesis of many of these abnormalities. The occurrence of severe proteinuria, accompanied by variable renal insufficiency, however, is difficult to explain. The spectrum of glomerular lesions reported in such cases includes focal to diffuse mesangial hypercellularity, diffuse proliferative glomerulonephritis, membranoproliferative glomerulonephritis, and focal and segmental glomerulosclerosis (FGS). Although FGS was reported as the dominant morphologic pattern in AIDS patients with severe proteinuria by Rao et al and termed “AIDS-associated nephropathy” (AAN), no single lesion was considered characteristic of AIDS by other investigators.

In order to assess the renal morphology of AAN, renal biopsy and/or autopsy material from 7 AIDS patients with greater than 2 g/24 hr of proteinuria was compared with that of 7 AIDS patients without significant proteinuria. Because illicit drug use may be a common risk factor and glomerular lesions seen in heroin-associated nephropathy (HAN) were described as strikingly similar to those of AAN, renal tissue of 7 heroin users with severe proteinuria but without AIDS was also studied.

Materials and Methods

Between 1983 and 1984, 68 patients with AIDS were identified at Lincoln Medical Center. The diagnosis of AIDS in patients with a history of homosexuality or intravenous drug abuse was established by the
reversal of the T-helper versus-suppressor cell ratio (less than 0.8) and the presence of opportunistic infections. T-lymphocyte subsets were quantitated by indirect immunofluorescence with the use of commercially available monoclonal antibodies (OKT 3 for T cells, OKT 4 for T-helper/inducer cells, and OKT 8 for T suppressor/cytotoxic cells). The diagnosis of opportunistic infections was established by culture or direct identification of the etiologic agent in body fluids and tissue specimens. The diagnosis of Kaposi’s sarcoma was based on established histologic criteria. Hepatitis B surface antigen (HBsAg) was measured by radioimmunoassay. Renal function and urinary protein excretion were determined on every patient by serial blood urea nitrogen and serum creatinine determinations, routine urinalysis, and 24-hour urine collection for estimation of protein excretion. Seven patients had more than 2 g protein/24 hr and were labeled Group A. Percutaneous renal needle biopsies were performed in 2 of these patients. Renal tissue was obtained at autopsy from 6 patients in this group, including 1 patient who had had a kidney biopsy previously, and from 7 AIDS patients with comparable clinical signs but without proteinuria. The latter were labeled Group B. In addition, renal morphology of the AIDS patients was compared with that of 7 age-matched patients with HAN and a comparable degree of proteinuria but without evidence of AIDS (Group C). Renal biopsies were performed on patients with AAN and HAN for diagnostic indications and not for the purpose of this study.

Renal biopsy tissue was fixed in alcoholic Bouin’s solution for histologic study, snap-frozen for immunofluorescence examination, and fixed in 2.5% glutaraldehyde in phosphate buffer for electron microscopy. The autopsy material fixed in 10% buffered formalin was processed for light microscopy and electron microscopy only.

Paraffin-embedded 2–3-μ tissue sections were stained with hematoxylin and eosin, Masson’s trichrome, Jones’ silver methenamine, and periodic acid–Schiff stains. The entire sections were examined at ×400. Direct immunofluorescence studies were performed on 2-μ cryostat sections of the snap-frozen tissue with fluorescein isothiocyanate conjugated IgG fractions of goat antiserum against human IgG, IgA, IgM, IgE, C3, C1q, fibrin/fibrinogen and albumin (CalBiochem). The sections were examined with a Zeiss Universal fluorescence microscope. For electron microscopy, tissues were postfixed in osmium tetroxide and embedded in Epon. Survey sections were cut at 1 μ and stained with toluidine blue. Ultrathin sections were cut from a total of 2–4 representative blocks per case containing 2–4 glomeruli. These were studied with a Siemens 101 electron microscope at 80 kv. Contiguous areas of the entire ultrathin section from every block of Groups A, B, and C were photographed at a magnification of ×3500 and printed at a magnification of ×8750. At least 40 electron micrographs were obtained per patient for purposes of counting the number of total cell nuclei (all cell types inclusive), total nuclear bodies, total cell nuclei with nuclear bodies, nuclei with multiple nuclear bodies (>1 nuclear body/nucleus), and nuclear bodies apparently present as budding forms. On average, 120 cells per patient were examined. All nuclear bodies were typed from I to V, mostly by electron micrographs photographed at ×8000 to ×20,000 and printed at ×20,000 to ×50,000.

Nuclear bodies, first described by deThe et al in 1960 and later by Weber et al., are intranuclear inclusions of diverse morphology and measure approximately 0.5–1.5 μ in size. These bodies were classified by Bouteille et al. The simplest types are composed of a granular concentric fibrillar matrix surrounded often by a clear halo (Type I). Type II consists of concentrically arranged fibrils with an onion-skinline architecture with no granules or sparse granules in the center. The more complex forms of nuclear bodies are characterized by fewer small (Type III) to several large (Type IV) dense osmiophilic granules or dense globules (Type V) surrounded by a concentric microfibrillar cortex.

Results

Clinical and laboratory data from Groups A and B are summarized in Table 1. Among the 7 AIDS patients with severe proteinuria, 5 were male and 2 female; the 7 without proteinuria were all male. One patient in Group A was homosexual and denied drug abuse, and the remainder in both groups were heroin abusers. Age at the time of diagnosis of AIDS ranged from 19 to 41 years (average, 32.5) and was comparable in the two groups. The diagnosis of AIDS was established concomitantly with the detection of severe proteinuria in 6/7 patients, while 1/7 presented with nephrotic syndrome 4 months before the diagnosis of AIDS was confirmed. Proteinuria ranged from 2 to 12 g/24 hr (average 5.7 g). Six of 7 patients from Group B had neither proteinuria nor abnormal urinary sediments, 1 had 10–15 red blood cells and 8 white blood cells per high-power field on urinalysis. All 14 died of opportunistic infections. The follow-up period ranged from 2 to 10 months (average, 4.5) and was comparable in the two groups. None developed end-stage renal failure requiring dialysis; however, intermittent deterioration of renal function was ob-
Table 1 — Clinical Data on Group A and Group B Patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>24-hour urine protein</th>
<th>Serum creatinine</th>
<th>Kaposi's sarcoma</th>
<th>Opportunistic infections</th>
<th>Drugs administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>M</td>
<td>8 g</td>
<td>1.3</td>
<td>5.6</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>M</td>
<td>12 g</td>
<td>4.0</td>
<td>4.5</td>
<td>Neg</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>M</td>
<td>8 g</td>
<td>4.0</td>
<td>5.5</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>M</td>
<td>2 g</td>
<td>1.8</td>
<td>3.2</td>
<td>Pos</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>M</td>
<td>2.5 g</td>
<td>1.0</td>
<td>2.1</td>
<td>Pos</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>F</td>
<td>4 g</td>
<td>1.8</td>
<td>1.5</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>F</td>
<td>3 g</td>
<td>1.2</td>
<td>1.5</td>
<td>Pos</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>M</td>
<td>Neg</td>
<td>0.7</td>
<td>2.1</td>
<td>Pos</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>M</td>
<td>Neg</td>
<td>0.8</td>
<td>1.5</td>
<td>Pos</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>M</td>
<td>Neg</td>
<td>0.7</td>
<td>1.1</td>
<td>Pos</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>M</td>
<td>Neg</td>
<td>0.8</td>
<td>0.9</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>41</td>
<td>M</td>
<td>Neg</td>
<td>1.2</td>
<td>3.1</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>M</td>
<td>Neg</td>
<td>0.7</td>
<td>0.9</td>
<td>Neg</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>33</td>
<td>M</td>
<td>Neg</td>
<td>0.7</td>
<td>1.0</td>
<td>Neg</td>
<td>No</td>
</tr>
</tbody>
</table>

Pathology

Because the specimens in Group C were all biopsy specimens, no gross pathologic findings are available. Small acute renal infarcts were noted in 1 subject each in Groups A and B and one kidney from Group B revealed multiple small venous thrombi.

Light Microscopy (Table 2)

The dominant glomerular findings in Group A patients (6/7) were the presence of focal segmental and global glomerulosclerosis affecting 18–55% glomeruli. One Group A and 4 Group B specimens revealed
in frequent global glomerulosclerosis (2–3%). In addition, 10–35% of the non-sclerotic glomeruli in 4 Group A cases displayed global collapse of glomerular capillary tufts, resulting in wrinkling and thickening of glomerular capillary walls, narrowing of the capillary lumens, and apparent (secondary to globally collapse capillary tufts) and sometimes real dilatation (actual widening of Bowman’s space over and above that due to collapsed capillary tufts) of Bowman’s spaces filled with proteinaceous material. Widening of Bowman’s spaces was present in 10–50% glomeruli in 5/7 Group A cases. Segmental synechiae to Bowman’s capsule were common in glomeruli undergoing solidification. In most Group A cases, visceral epithelial cells were segmentally ballooned and engorged with eosinophilic proteinaceous material and displayed discrete proliferation overlying the sclerotic lobules. The mesangium was normal to hypocellular, sometimes with mild swelling of the mesangial matrix. Glomeruli in 5 Group B cases were unremarkable except in 2 cases with positive serologic results for HBsAg, which showed moderate segmental to diffuse mesangial proliferation. Five Group A kidneys revealed foci of enormously ectatic tubules, sometimes to microcystic proportions, filled with proteinaceous material affecting 25–75% of the tubules. In addition, extensive degenerative and focal regenerative changes were present in tubular epithelial cells, predominantly in the proximal tubules. Marked swelling and vacuolization of distal tubular and collecting duct cells were some of the other unusual features noted in the 2 biopsy specimens from Group A kidneys. Although tubular degenerative and focal regenerative changes were seen in Group B, they were not as severe as in Group A. None of the Group B kidneys displayed the other changes listed above. Mild focal nephrocalcinosis was more frequent in Group B (4/7) than in Group A (1/7) subjects. Interstitial edema of variable degree was noted in both groups, with conspicuously sparse cellular inflammatory infiltrates, which were present predominantly in the small subcapsular vascular scars. The renal interstitial cells appeared to be reduced in Group A kidneys. The post-mortem renal findings were noteworthy for the absence of the common opportunistic pathogens in spite of disseminated infections elsewhere, except for the presence of cryptococci in glomeruli and interstitium in one specimen from Group A. The absence of renal cellular response in this patient was similar to the lack of granuloma formation observed in other organs also affected by cryptococci. The characteristic intranuclear inclusions of cytomegalovirus were not seen in any of the 14 specimens.

Glomerular histologic changes in patients with heroin-associated nephropathy (Group C) were comparable to those of AIDS patients in Group A in the extent of segmental and global sclerosis (range, 29–60%, in 6/7 cases), globally collapsed capillary tufts (range, 8–25%, in 4/7), and apparent widening of the Bowman space, present in some cases but less frequent. In contrast to Group A, Group C showed mild, focal mesangial hypercellularity, rather than hypocellularity. Real dilatation of Bowman space was not evident, and in 3/7 biopsies focal thickening of Bowman’s capsule was observed. Tubular atrophy with accompanying interstitial fibrosis was more prominent than in Group A or Group B, which suggested a more chronically progressive disease. Tubu-

Table 2—Comparison of Light-Microscopic Findings* in Groups A, B, and C

<table>
<thead>
<tr>
<th>Histologic findings</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal, segmental and global glomerulosclerosis, % glomeruli affected</td>
<td>18–55 (6/7)</td>
<td>2–3 (4/7)</td>
<td>29–60 (6/7)</td>
</tr>
<tr>
<td>Global collapse of glomerular tufts, % glomeruli affected</td>
<td>10–35 (4/7)</td>
<td>NP</td>
<td>8–25 (4/7)</td>
</tr>
<tr>
<td>Dilatation of Bowmans space, % glomeruli affected‡</td>
<td>10–50 (5/7)</td>
<td>NP</td>
<td>2–25 (3/7)</td>
</tr>
<tr>
<td>Hypocellular mesangium</td>
<td>(4/7)</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Hypercellular mesangium</td>
<td>NP</td>
<td>+ (2/7)</td>
<td>+/+ (4/7)</td>
</tr>
<tr>
<td>Bowman capsule thickening</td>
<td>NP</td>
<td>NP</td>
<td>+ (3/7)</td>
</tr>
<tr>
<td>Ectatic tubules filled with proteinaceous material</td>
<td>+++ (5/7)</td>
<td>NP</td>
<td>++/+ (4/7)</td>
</tr>
<tr>
<td>Swelling and vacuolization of distal tubular and collecting duct cells‡</td>
<td>+++ (2/2 biopsies)</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>+/+ (5/7)</td>
<td>+ (3/7)</td>
<td>+ (2/7)</td>
</tr>
<tr>
<td>Interstitial inflammatory cells</td>
<td>±/+ focal (5/7)</td>
<td>±/+ focal (2/7)</td>
<td>+/+ (6/7)</td>
</tr>
<tr>
<td>Hypocellular interstitium</td>
<td>(3/7)</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Nephrocalcinosis</td>
<td>+ focal (1/7)</td>
<td>+ focal (4/7)</td>
<td>NP</td>
</tr>
<tr>
<td>Pathogens in renal parenchyma</td>
<td>cryptococci (1/7)</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

*Some of the histologic findings are graded semiquantitatively from 0 to ++++.†Apparent dilatation of Bowman’s space in Group C primarily due to collapse of glomerular capillary tufts; true as well as apparent widening of Bowman’s space.
‡Tubular cells not assessed for these changes in autopsy material.
§Number of cases affected in parentheses.
NP, Not present or negative.
lar dilatation, although present, was not as enormous as in Group A, with a similar degree of proteinuria. Cellular swelling and vacuolization of distal tubular and collecting duct cells were not apparent. Interstitial edema was mild and only rarely observed (2/7), whereas a dense mononuclear interstitial infiltrate was prominently present in most kidneys (6/7).

**Immunofluorescent Microscopy (IF)**

Both biopsy specimens from Group A showed segmental, coarsely granular, mild to moderate glomerular staining with antisera to C3. All biopsies from Group C revealed coarsely granular segmental to diffuse moderate staining with antisera for C3. IgM and C1q were demonstrated segmentally in 3 cases. In addition, minimal to mild amounts of IgG and IgA were seen in 2 cases each. IF was not performed on autopsy material.

**Electron Microscopy**

Autolytic changes were comparable in autopsy specimens of Groups A and B and in 5 control kidney postmortem specimens from patients with renal diseases other than AAN and HAN, examined for the occurrence of intranuclear inclusions. The ultrastructural findings were similar in all Group A subjects. Pronounced effacement (30–95% of the external capillary surface) of the visceral epithelial cell foot processes was associated with foci of degeneration and/or detachment from the lamina densa of capillary walls, particularly overlying segmentally sclerotic areas. Glomerular basement membranes were focally wrinkled and thickened in sclerotic areas, while being of normal thickness elsewhere. Similar glomerular changes were often observed in biopsies from patients from Group C. Segmental glomerulosclerosis, thickened GBM, and focal detachment of foot processes were generally absent in glomeruli from Group B.

Electron-dense mesangial deposits, occasionally massive, sometimes associated with subendothelial, subepithelial, and intramembranous deposits were observed in 3 specimens from Group A (2/3 serologically positive for HBsAg) and 2 specimens from Group B (both serologically positive for HBsAg). Two of seven biopsy specimens from Group C revealed mild to moderate mesangial deposits but were serologically negative for HBsAg. In spite of positive serologic results for this antigen, no deposits were observed in the renal tissue from 2 other patients (one each from Groups A and B). The mesangium often displayed hypocellularity in some of the glomeruli of Group A specimens, and mesangial cells focally showed changes of degeneration (Figure 1).

Numerous large (protein) phagolysosomes were focally observed in proximal tubular cells in specimens from Groups A and C. The distal tubular and collecting duct cells in both biopsy specimens of Group A were markedly swollen because of hypertrophy of intracellular organelles, particularly of the rough endoplasmic reticulum. Similar changes were not present in any of the biopsy specimens from Group C patients. Biopsy material for comparison was not available from Group B patients.

Both biopsy specimens from Group A patients revealed numerous tubuloreticular inclusions (TRIs) in the endothelial cells of glomerular and sometimes interstitial capillaries as well as occasionally in the cytoplasm of the renal interstitial cells (Figure 2). Although tubuloreticular complexes, structures related to TRIs, were observed in glomerular endothelial cells in a single Group B case, endothelial cell cytoplasm was not preserved well enough in the autopsy material for us to ascertain the presence or absence of TRIs in most cases. A search at the ultrastructural level of biopsies from Group C patients did not yield TRIs in endothelial or interstitial cells. Numerous parallel stacks of closely apposed membranes with electron-dense material in the middle, consistent with lamellar stacks of confronting cisternae (Figure 3), were observed in tubular cells in 3/7 Group A and 1/7 of Group B specimens. Some of the other unusual features in Group A specimens included cylindrical confronting cisternae, also referred to as test tube and ring-shaped forms (TRFs), in the cytoplasm of tubular epithelial and interstitial fibroblast cells (3/7) (Figure 4).

In addition to the cytoplasmic changes, many unusual intranuclear inclusions were observed, particularly in the renal interstitial cells. These included filamentous crystalline and fibrillary inclusions (Figure 5) (2/7 Group A, 2/7 Group B), membranous lamellae or profiles (1/7 Group A, 1/7 Group B) (Figure 6), vacuolar, lipid, and granular vesicles (Figure 7) (1/7 Group A, 1/7 Group B), and test tube and ring-shaped forms (1/7 Group A, 1/7 Group B) (Figure 8). Two Group A and 5 Group B specimens did not reveal any of these unusual intracytoplasmic and intranuclear inclusions. The cell frequency of these inclusions was also slightly greater in Group A specimens. In addition, 6 Group A specimens displayed peculiar changes in nuclear chromatin of unknown nature in many cells. These consisted of coarsely granular material the size of interchromatin granules, albeit more fibrillary in nature, diffusely distributed throughout the nucleoplasm in some cells (Figure 9), while in others the nuclei were only partially affected (Figure 10). The granulofibrillar material apparently her-
Figure 1—Patient 2 (biopsy). Mesangial areas are hypocellular. Mesangial cells show degenerative changes and contain many lipid vacuoles. The glomerular basement membranes are of normal thickness. Marked effacement of the foot processes and villous hypertrophy of the visceral epithelial cells are present. (×3500)

Figure 2—Patient 3 (autopsy). TRIs within interstitial cell cytoplasm. (×12,800)

Figure 3—Patient 4 (autopsy). Proximal tubular cell with several parallel stacks of confronting cisternae in close proximity to degenerating mitochondria. (×12,000)
niated through the outpouches of the nuclear membrane, occasionally spilling into the cell cytoplasm due to focal disruptions of the membrane (Figures 5, 9, and 10). These changes were present in both autopsy and biopsy material but were more prevalent in the former. The peculiar nuclear granulofibrillary change described above did not resemble karyolysis associated with diffuse rarification of chromatin or karyorrhexis, in which clumping and peripheral margination of chromatin are seen. Although these findings were most prominent in the interstitial cells, all cell types, particularly tubular and rarely glomerular, mesangial, endothelial, and epithelial cells, were also affected. This peculiar chromatin change was largely absent in most of the tubular cells from Group B; few interstitial cells in two specimens, however, displayed similar findings in this group (Table 3). None of these intranuclear and intracytoplasmic inclusions described earlier were seen in Group C kidneys. In addition, several autopsy kidney specimens obtained from cases with comparable time between death and fixation to serve as controls for Groups A and B also did not reveal these structures, which indicated that the above-described findings are not related to postmortem changes.

Nuclear bodies (Table 3) were seen in 17–32% (average, 24%) of the cell nuclei in the renal tissue of Group A, whereas only 3–19% (average, 11%) of cell nuclei of Group B kidneys revealed nuclear bodies.
Although all types of nuclear bodies were represented in Groups A and B, most nuclear bodies in both groups were of Type III (Figure 11) with Types IV (Figure 12) and V (Figure 13) mostly in Group A. The number of cell nuclei containing nuclear bodies in Group C amounted to 7–27% (average, 13%), which were almost exclusively of Types I and II. From 4% to 14% (average, 9%) of the cell nuclei from Group A specimens contained multiple nuclear bodies, sometimes as many as 8 nuclear bodies per nucleus (Figure 14). Some of these were joined together in a budding configuration (Figure 11), whereas others were accompanied by the granulofibrillary transformation in the nuclear chromatin. Multiple nuclear bodies were infrequent in Group C (range, 2–7%; average, 5%) and only occasionally seen in Group B (range, 0–6%; average, 1.6%). Budding configuration of nuclear bodies (Figure 11) as a percentage of total nuclei was far more frequent in Group A (range, 0.6–3.6%; average, 2.8%), as compared with Groups B (range, 0–1.3%; average, 0.35%) and C (range, 0–0.9%; average, 0.3%). Budding forms in Group A were mostly of Type III nuclear bodies, whereas in Groups B and C the few budding nuclear bodies observed were mostly of Type I and Type II. Moreover, nuclear bodies in Group A appeared larger than those present in Groups B and C.

**Discussion**

Several renal disorders associated with AIDS have been recognized, including severe proteinuria with varying degrees of renal insufficiency. Opportunistic infections, various drugs, including nephrotoxic antibiotics, intravenous heroin use, Kaposi's sarcoma, and hepatitis B, may contribute to renal damage in AIDS. These factors, however, do not appear to be solely responsible for proteinuria of the so-called AIDS-associated nephropathy, because our AIDS patients with and without proteinuria were exposed to the same factors for approximately the same length of time, suggesting, therefore, additional or alternate pathogenetic mechanisms. A morphologic study comparing renal tissues from patients with AIDS with proteinuria (Group A), without proteinuria (Group B), and with heroin-associated nephropathy.
thy (Group C) revealed that focal and segmental glomerular sclerosis (FGS) and global glomerulosclerosis were the most frequent, common findings in “AIDS-associated nephropathy” and “heroin-associated nephropathy” and were virtually indistinguishable. Several histologic and ultrastructural findings, however, were observed that could help to distinguish between these two groups. Histologic differences in Group A included paucity of interstitial cellular infiltrates in kidneys of AIDS patients, even when there was invasion by infectious organisms such as cryptococci, mesangial hypocellularity, particularly in glomeruli with global capillary tuft collapse, prominent Bowman’s capsular dilatation, and tubular ectasia sometimes to microcystic proportions. In contrast, renal tissue from patients with HAN (Group C) often showed interstitial fibrosis, marked interstitial lymphoplasmacytic infiltrates, and thickening of Bowman’s capsule. Mesangial hypercellularity of variable degree was frequent.

The most noteworthy ultrastructural finding in Group A was the increased frequency, size, complexity, and heterogeneity of nuclear bodies in a variety of renal cells, particularly in the interstitial and tubular epithelial cells. Although the number of nuclear bodies in Groups B and C was similar, Group C nuclear bodies were mostly of simple types (I and II); whereas in Group B more Type III and occasional Type IV forms were seen. Multiple complex (Types III to V) and budding forms of the nuclear bodies were significantly more frequent in Group A, in comparison with the other groups.

Simple nuclear bodies (Types I and II) are ubiquitous in most cell types,8,12 under varying physiologic conditions, and have been reported in normal kidney cells.13,14 While different forms of nuclear bodies probably represent developmental phases of a single fundamental process, increased frequency, size, heterogeneity, and complexity of nuclear bodies as present in renal tissue from Group A are generally observed in association with hormonal,15–17 drug-induced,18 immunologic,19,20 or viral stimulation of the cells.21,22 Complex nuclear bodies are also frequently found in a variety of tumors, some of which are proven or suspected to be virus-induced23–26 and in tissue or tissue cultures infected with viruses.22 Some examples include adenovirus, herpes simplex virus, cytomegalovirus, polyoma, vaccinia, and SV40 vi-
Figure 10—Patient 3 (autopsy). The nuclear chromatin pattern is normal in two of the five interstitial cell nuclei, one of which contains three nuclear bodies (top right). Two other nuclei (top left and right bottom) display partial granulofibrillary transformation, with herniation of the transformed material into the cytoplasm in one (top left). A Type V nuclear body is present in the nontransformed part of the other nucleus (right lower). (X8000; inset, X16,000)

Because there were no known differences in the immunologic or hormonal status between AIDS patients from Group A and those from Group B and medications administered were similar, it can be speculated that renal damage may be secondary to direct viral invasion of the kidney. Additional supporting evidence for a viral etiology of “AIDS nephropathy” is provided by the greater frequency, pertaining both to cell and to case number, of many unusual intracytoplasmic and intranuclear inclusions. The cytoplasmic structures present in Group A tissues, which may be associated with viral infections,

Table 3—Incidence of Nuclear Bodies (NB) and Granulofibrillary Transformation (GFT) in Groups A, B, and C

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cell nuclei counted</th>
<th>Range (average) % total number of cells with NBs</th>
<th>Range (average) % number of cells with multiple NBs</th>
<th>Range (average) % number of cells with budding NBs</th>
<th>Types of NBs as % of total NBs</th>
<th>Range (average) % of nuclei with GFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>134</td>
<td>17–32 (24)</td>
<td>4–14 (9)</td>
<td>0.6–3.6 (2.8)</td>
<td>91670.54.53.5–39.4 (19)</td>
<td>3.5–39.4 (19)</td>
</tr>
<tr>
<td>Group B</td>
<td>88</td>
<td>3–19 (11)</td>
<td>0–6 (1.6)</td>
<td>0–1.3 (0.35)</td>
<td>1931482</td>
<td>0–13.3 (3)</td>
</tr>
<tr>
<td>Group C</td>
<td>132</td>
<td>7–27 (13)</td>
<td>2–7 (5)</td>
<td>0–0.9 (0.3)</td>
<td>64323.50.5</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
include TRIs in the endothelial cells of glomerular and interstitial capillaries and occasionally in the renal interstitial cells (fibroblasts) and cylindrical (TRFs) and parallel stacks of confronting cisternae in interstitial fibroblasts and tubular cells. TRFs and TRIs have been observed in several cell types in different organs of AIDS patients.28-32 Thus far, TRFs have not been described in the kidney. TRIs have been reported mainly in lymphocytes, monocytes, and endothelial cells, including renal endothelial cells, but not in renal interstitial cells. TRFs are not seen as frequently as TRI and among various cell types occur mostly in lymphocytes, bone marrow macrophages, ciliated bronchial epithelial cells, and marrow sinus endothelial cells.29 Intranuclear inclusions found in our patients, such as filamentous crystalline and fibrillary inclusions, vacuolar, lipid, and granular vesicles, and membranous profiles, have also not yet been reported in any organ in AIDS. These inclusions are not indicative of dying cells. They represent heightened cellular metabolic activity, particularly the budding forms of nuclear bodies, which were often seen in Group A tissues, indicating proliferative changes in the nuclei. All of the inclusions are possibly a reaction to the infectious agent, perhaps viral footprints, because none of them are known to contain viral proteins per se.33-35 Some of the intracytoplasmic and intranuclear inclusions present in the kidneys of our AIDS patients have been observed in a variety of human viral infections such as fibrillary inclusions in influenza pneumonia,36 test tube- and ring-shaped forms, and confronting cisternae in hepatocytes in non-A, non-B hepatitis.37-39 TRFs in lei-
kemic cells in human T-cell leukemia virus (HTLV I)-associated lymphocytic leukemia.\textsuperscript{40} Interferon has been implicated in the induction of TRIs\textsuperscript{41-43} and possibly of cylindrical confronting cisternae.\textsuperscript{44} TRIs, in our experience, are only rarely observed on routine electron microscopy except in renal tissue obtained from patients with systemic lupus erythematosus (SLE). While in SLE numerous TRIs may be present in the cytoplasm of endothelial cells in glomerular capillaries and occasionally in interstitial capillaries, we detected TRIs in AIDS patients not only in these locations but also in the interstitial cell cytoplasm. None of the AIDS patients had clinical or serologic evidence of SLE, however. Renal tissue from Group C patients did not reveal TRIs or any other unusual cytoplasmic inclusion in any cell type. The presence of TRIs was thus distinct in FGS associated with AIDS versus FGS associated with HAN. Therefore, in the absence of clinical, laboratory and morphologic findings supporting the diagnosis of SLE, the presence of numerous TRIs in renal tissue may be considered a useful marker for “AIDS-associated nephropathy,” particularly in an intravenous drug user presenting with proteinuria. One of our patients and 7 others reported in the literature presented with severe proteinuria before the diagnosis of AIDS was established.\textsuperscript{45}

We were also intrigued by a peculiar partial to total granulofibrillary transformation of several cell nuclei, particularly those of tubular and interstitial cells predominantly seen in Group A patients in both the biopsy and autopsy specimens and not observed in Group C. This granulofibrillary material does not have the characteristics of normal heterochromatin, but a similarity with interchromatin granules is apparent. Herniation of the nucleoplasm containing these granulofibrillary structures into the cytoplasm through partially ruptured nuclear membranes was seen. We do not believe that autolysis is responsible for such findings because the characteristic changes of karyolysis and karyorrhexis were not present and autopsy material from several control cases with comparable postmortem delay did not show the changes described above. Nucleoplasm and chromatin of the neighboring cells were normal, and the cytoplasm of the affected cells did not reveal greater degenerative changes, but rather nuclear hyperactivity, indicated by the simultaneous occurrence of multiple complex and budding nuclear bodies in the same nuclei. Drug toxicity may not explain these findings either, because the types and dosages of drugs administered were comparable in Groups A and B. Although the etiology of the above described nuclear changes is not clear, invasion by viruses, suggested also by intracytoplas-
mic and intranuclear inclusions described earlier, may result in nuclear transformation and perhaps cytolyis. This may explain glomerular and interstitial hypocellularity, a frequent feature in Group A patients, resulting in loss of supporting cells, which could be responsible for some of the histologic findings such as glomerular capillary tuft collapse, Bowman capsular dilatation, and tubular ectasia.

Since most of the ultrastructural changes observed in our AIDS patients are considered morphologic markers of viral infection, rather than specific viral proteins per se, it would be appropriate to consider also that certain host or viral factors may be responsible for some of the morphologic changes. Severe renal disease can be induced in an experimental model simply by the intraperitoneal injection of interferon.\textsuperscript{46-48} It is conceivable that presence of viruses results in the production of interferon and some other factors, which in turn may induce glomerular disease and proteinuria. Increased glomerular permeability induced by lymphokines or certain other unknown factors elaborated by lymphocytes have been implicated in the pathogenesis of idiopathic nephrotic syndrome, which\textsuperscript{49-53} bears certain morphologic similarities to AAN.

Certain strains of adenoviruses have been isolated from the urine of AIDS patients with relatively high frequency.\textsuperscript{54} HTLV III on rare occasions has also been isolated.\textsuperscript{55} Because no viral isolation studies were performed on the urine of any of our patients, the assumption of direct viral invasion is speculative, and this report is meant to provoke and initiate work on the possible viral etiology of AIDS-associated nephropathy.

References

51. Moorothy AV, Zimmerman SW, Burkholder PM: Inhibition of lymphocyte blastogenesis by plasma of patients with minimal change nephrotic syndrome. Lancet 1976, 1:1160–1162

Acknowledgments

We appreciate the cooperation of Dr. Elliott Gross and Dr. Josette Montes, Office Chief Medical Examiner of New York. We thank Ms. R. Lopez for secretarial assistance and Ms. Maria Demeri, Ms. Usha Ganju, Ms. Julie Rotta, Mr. Alfred Revzin, and Mr. Alfred Holmes for technical assistance.