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Leishmaniasis-Associated Membranoproliferative Glomerulonephritis With Massive Complement Deposition

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INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN) is a pattern of injury characterized by mesangial and endocapillary proliferation, double contours along the capillary walls, and lobular accentuation of the capillary tufts.[1] Based on pathophysiology, MPGN is classified into MPGN mediated by immune complexes and Igs and complement-mediated MPGN.[1,2] Immune complex/Ig–mediated MPGN most often results from an underlying infection, autoimmune disease, or monoclonal gammopathy.[3–5] Complement-mediated MPGN includes C3 glomerulopathy, which encompasses C3 glomerulonephritis and dense deposit disease.[6,7] C3 glomerulopathy is characterized by dominant staining for C3 and negative or minimal staining for Igs.[8] The staining for C3 is at least 2 orders greater in magnitude than Ig staining. C3 glomerulopathy results from dysregulation of the alternative pathway of complement with accumulation of complement factors of the alternative and terminal pathways of complement.[9,10]

Infections are an important cause of MPGN that are usually associated with an immune complex–mediated MPGN. Immune complex–mediated MPGN due to infections is characterized by the presence of Ig, usually IgG or IgM, along with complement factors of the classical and terminal pathways. In this report, we present an unusual form of MPGN characterized by negative Ig but by large deposits of complement factors of the classical and terminal pathways in a patient with leishmaniasis and HIV infection. Furthermore, *Leishmania* species amastigotes were detected within macrophages in the interstitium. This finding of both complement-mediated MPGN and interstitial inflammation associated with leishmaniasis is extremely unusual and should be kept in mind as an uncommon cause of renal disease in the immunocompromised host.

CASE PRESENTATION

A 35-year-old Hispanic man from El Salvador presented with abdominal and back pain, proteinuria, and lower-extremity edema. The patient had a history of HIV infection with AIDS and leishmaniasis involving both the skin and bone marrow. The patient was compliant with HIV treatment and had an undetected viral load, although his CD4 counts remained low at 75 cells/mm^3. He had received treatment for leishmaniasis with amphotericin B that resulted in improvement of his cutaneous lesions. The patient also had pancytopenia with a hematocrit of 21 and neutropenia (white blood cell count 1.8 $\times$ 10^3/μl and neutrophil count of 1.5 $\times$ 10^3/μl). Evaluation of kidney function revealed a normal serum creatinine of 0.9 mg/dl and significant proteinuria of 1.6 g/d. Other laboratory evaluation included low serum total protein (5.2 g/dl) and albumin (2.0 g/dl), low hemoglobin (8.6 g/l), and low platelet count (120 $\times$ 10^3/μl). The C3 level was low (63 mg/dl, normal range 90–180 mg/dl), while the C4 level was borderline high (44 mg/dl, normal range 10–40 mg/dl). Urinalysis showed 2–3+ protein, with no red blood cells. Remaining evaluation was unremarkable with negative serology for antinuclear antibody, double-stranded DNA, and hepatitis B and C. Peripheral blood flow cytometry showed mostly granulocytic and monocytic cells positive for CD11c and CD38 and unremarkable lymphocytes with no clonal or abnormal phenotype. Lymphocytes were composed of 69% T cells with a CD4/CD8 ratio of 1:1. A kidney biopsy was done to determine the cause of proteinuria.

Kidney Biopsy

The tissue for light microscopy contained 3 cores that contained 60 glomeruli, of which 13 were globally

sclerosed. The glomeruli showed an MPGN with mesangial and endocapillary hypercellularity, lobular accentuation of the glomerular capillary tufts, and double contours along the capillary walls. Interestingly, the capillary walls showed fuscinophilic deposits on trichrome stains. The deposits had a bead-like appearance and were particularly prominent on the toluidine blue stain. No necrotizing, crescentic, or thrombotic lesions were present. The interstitium showed patchy interstitial inflammation. There was moderate (30%) tubular atrophy and interstitial fibrosis (30%) present. Vessels were unremarkable. Most interestingly, aggregates of macrophages were present in the interstitium. Numerous *Leishmania* species amastigotes were detected in the cytoplasm of macrophages; the amastigote kinetoplast was best seen on Jones methenamine silver stain. Representative light microscopy findings are shown in Figure 1a–e.

There were 10 glomeruli present for immunofluorescence microscopy. The glomeruli showed bright staining for C1q (3+), C3c (3+), C3d (3+), and C4d (3+). Surprisingly, staining for Igs was negative. Immunofluorescence microscopy following pronase digestion to detect masked Igs showed mild segmental staining for IgM but was negative for IgA, IgG, and κ and λ light chains. Representative immunofluorescence microscopy findings are shown in Figure 1f–i.

Electron microscopy showed extremely dense osmiophilic deposits along the capillary walls and in the mesangium. Subepithelial, intramembranous, and subendothelial deposits were all present; the deposits were large, discrete, and almost bead-like in some areas. The deposits did not form the sausage-shaped intramembranous dense deposits seen in dense deposit disease, and substructures were not present. Electron-dense deposits along the tubular basement membranes were not present. Representative electron microscopy findings are shown in Figure 1j–m.

Kidney Biopsy Diagnosis

Primary Diagnosis

Infection-related glomerulonephritis, with extensive deposition of complement; leishmaniasis-associated interstitial nephritis (leishmania infection/clinical).[11]

Pattern of Injury

Membranoproliferative and sclerosing glomerulonephritis.

Additional Findings

Focal (22%) global glomerulosclerosis, moderate (30%) tubular atrophy and interstitial fibrosis.

Laser Microdissection and Mass Spectrometry

We performed laser microdissection of the glomeruli followed by mass spectrometry to confirm and determine the complement profile of the deposits.[12] Mass spectrometry showed very large spectra numbers for C3 and C4, moderate spectra numbers for C9, and smaller spectra numbers for C1q, C5, C6, and C7. Interestingly, small spectra numbers for IgG1 chain C region, κ chain C region, and Ig λ2 C region were also present. The mass spectrometry profile is shown in Figure 2.

Clinical Follow-up

The patient was started on monthly infusions of liposomal amphotericin B 200 mg as suppressive therapy for *Leishmania*. Since biopsy, his proteinuria has fluctuated based on the timing of the infusions. After receiving the medication, the proteinuria tends to go down, but then gradually increases. For example, the spot albuminuria test on the day of 1 of the infusions was 3072.3 mg, and 22 days later it was reduced to 1963.2 mg. He has been started on a low dose of lisinopril as well, to be titrated as his blood pressure can tolerate. His renal function thus far has remained normal, with serum creatinine of 0.8 mg/dl.

DISCUSSION

Glomerulonephritis in the setting of chronic infections is typically immune complex–mediated.[1,11,13–20] The kidney biopsy shows immune deposits along with C3 on immunofluorescence studies. The C3 is likely derived from activation of the classical pathway of complement by the immune complexes. We describe a patient with chronic, recurrent leishmaniasis in the setting of HIV and AIDS who developed glomerulonephritis with numerous deposits of complement factors of the classical pathway of complement in the absence of any significant immune complexes. In addition, moderate interstitial inflammation was present, and *Leishmania* species amastigotes were detected within macrophages in the interstitial infiltrates. Amastigotes are generally the only forms of this protozoal parasite that are seen in humans, and can be identified on routine histologic sections by their location within macrophages, round–oval shape, small size (2–4 μm in diameter), and presence of both a nucleus and a rod-shaped kinetoplast. Amastigotes are not stained using the Gomori methenamine silver stain for fungi, and thus can be easily differentiated from small intracellular yeasts such as *Histoplasma capsulatum*. Notably, the Jones methenamine silver stain using a hematoxylin and eosin counterstain provided superior resolution of the kinetoplasts than hematoxylin and eosin alone. To our knowledge, this is the first report of using the Jones methenamine silver stain for enhanced morphologic identification of *Leishmania* amastigotes.

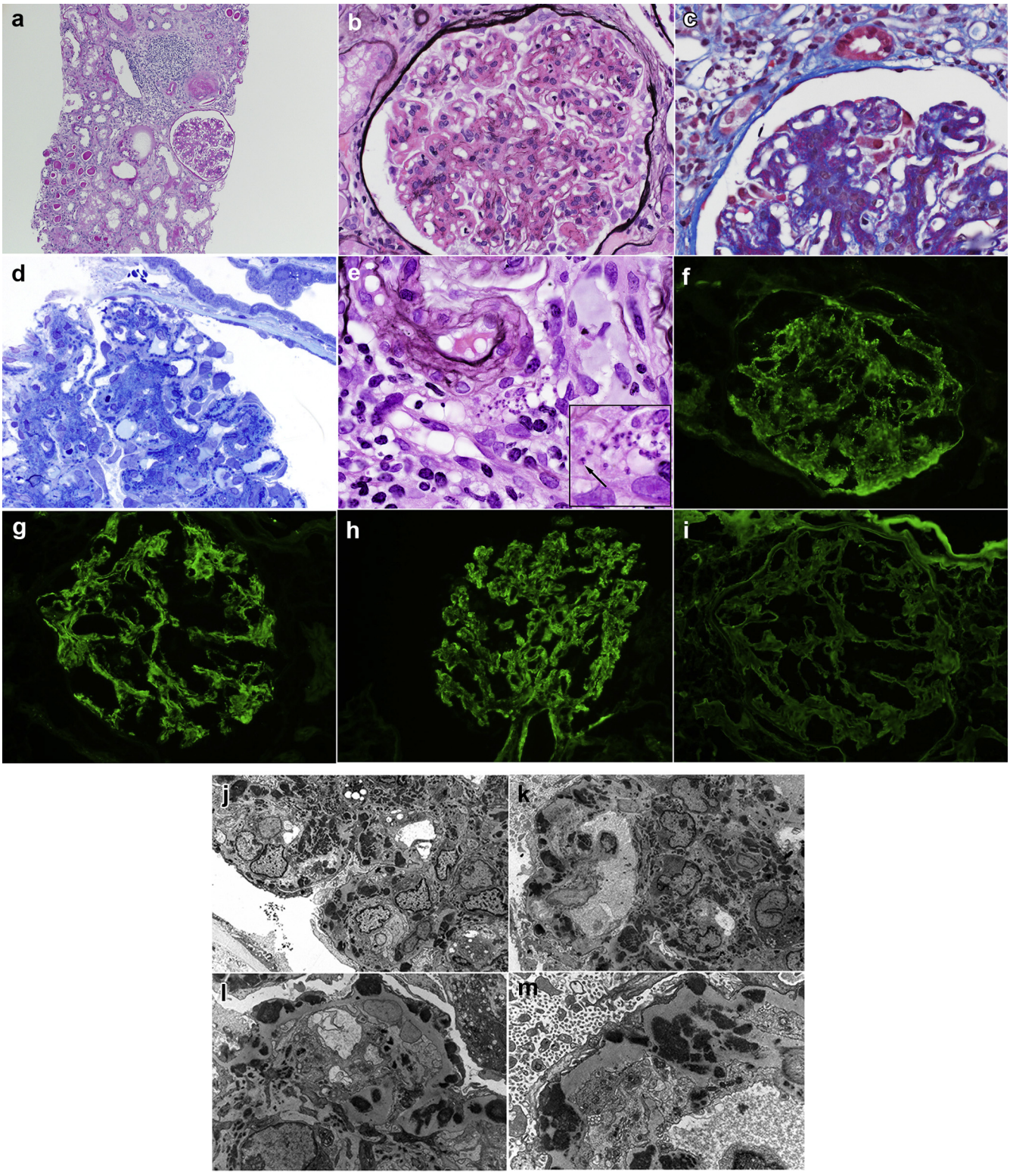
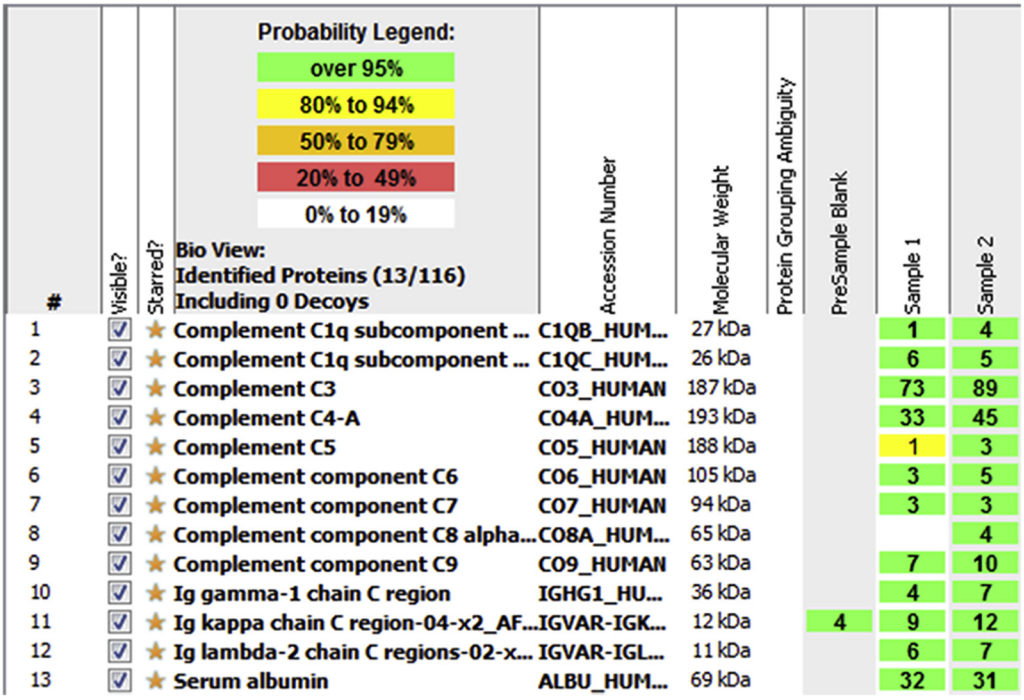


Figure 1. Complement-mediated membranoproliferative glomerulonephritis. (a–e) Light microscopy showing a membranoproliferative glomerulonephritis with silver-negative fuscinophilic deposits in the mesangium and along the capillary walls (a, periodic acid–Schiff, original magnification X10; b, silver methenamine, original magnification X40; c, trichrome, original magnification X60; d, toluidine blue, original magnification X60). Note the bead-like deposits along the capillary walls on toluidine blue stain. (e) *Leishmania* species amastigotes within tissue macrophages (Jones methenamine silver stain with hematoxylin and eosin counterstain, original magnification X1000). The characteristic features of the amastigotes, including a small round-to-oval nucleus and rod-shaped kinetoplast (arrow), are shown in the inset (original magnification approximately X2000). (f–i) Immunofluorescence microscopy showing bright staining for C1q (3+) (f), C3c (3+) (g), and C4d 3+ (h), and negative staining for IgG (i; C3d not shown). (j–m) Electron microscopy showing large and extremely dense deposits in the mesangium and along the capillary walls. The deposits are subepithelial, intramembranous, and subendothelial. Double contours are noted along the capillary walls. (j and k, original magnification X4000; l, original magnification X8000; m, original magnification X12,000.)



Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

#	Visible?	Starred?	Bio View: Identified Proteins (13/116) Including 0 Decoys	Accession Number	Molecular Weight	Protein Grouping Ambiguity	PreSample Blank	Sample 1	Sample 2
1	☑	★	Complement C1q subcomponent ...	C1QB_HUM...	27 kDa			1	4
2	☑	★	Complement C1q subcomponent ...	C1QC_HUM...	26 kDa			6	5
3	☑	★	Complement C3	CO3_HUMAN	187 kDa			73	89
4	☑	★	Complement C4-A	CO4A_HUM...	193 kDa			33	45
5	☑	★	Complement C5	CO5_HUMAN	188 kDa			1	3
6	☑	★	Complement component C6	CO6_HUMAN	105 kDa			3	5
7	☑	★	Complement component C7	CO7_HUMAN	94 kDa			3	3
8	☑	★	Complement component C8 alpha...	C08A_HUM...	65 kDa				4
9	☑	★	Complement component C9	CO9_HUMAN	63 kDa			7	10
10	☑	★	Ig gamma-1 chain C region	IGHG1_HU...	36 kDa			4	7
11	☑	★	Ig kappa chain C region-04-x2_AF...	IGVAR-IGK...	12 kDa		4	9	12
12	☑	★	Ig lambda-2 chain C regions-02-x...	IGVAR-IGL...	11 kDa			6	7
13	☑	★	Serum albumin	ALBU_HUM...	69 kDa			32	31

Figure 2. Mass spectrometry data show glomerular accumulation of large spectra numbers of C3 and C4. Small spectra numbers for C1q, C6, C7, and C9 are also present. Small spectra numbers for IgG1 γ constant region were also detected. The samples were run in duplicate, representing 2 separate dissections (samples 1 and 2). Green boxes signify >95% probability for protein identity. The numbers in the boxes represent the total number of spectra matched to the protein in a sample.

Renal disease is an uncommon complication of visceral leishmaniasis. There are only a few reports of *Leishmania* species causing renal disease in humans. In general, leishmaniasis-associated renal disease occurs in the immunocompromised host, particularly in the setting of HIV infection. Visceral leishmaniasis treatment failure is common in patients with concomitant HIV infection, particularly in patients with CD4 counts below 200 cells/μl as presented here.[21] Direct involvement of the renal parenchyma by *Leishmania* resulting in interstitial inflammation has been reported in the renal transplant.[22] In such cases, *Leishmania* species amastigotes are often detected within macrophages (as noted in our case). Rarely, chronic leishmaniasis may even be associated with renal AA amyloidosis.[23] With regard to glomerulonephritis, leishmaniasis causes an immune complex–mediated glomerulonephritis with an MPGN pattern of injury.[24] Most of these cases were previously reported as MPGN type I or type III, and IgG and IgM were the commonly detected Igs on immunofluorescence studies.[25,26] Cryoglobulins resulting in an immune complex–mediated glomerulonephritis have also been reported in the setting of leishmaniasis.[27]

The kidney biopsy in our case showed many distinctive findings. Light microscopy showed an MPGN pattern of injury that was characterized by bright staining for C1q and C3 on immunofluorescence studies indicating activation of the classical pathway of complement. On immunofluorescence studies there was no staining for Ig including IgG, IgM, IgA, or κ or λ light chains. In order to detect masked Ig deposits, we performed immunofluorescence studies for IgG, IgA, IgM, C1q, and κ and λ light chains following pronase digestion of the paraffin-embedded material (paraffin immunofluorescence), and these studies were also negative for Ig except for bright staining for C1q. Thus, both routine and paraffin immunofluorescence studies were negative for Ig deposits. We also performed immunofluorescence studies to detect end products of C4 and C3 activation by staining for C4d and C3d, respectively, both of which were brightly positive. Taken, together the presence of bright C1q, C4d, C3, and C3d indicates accumulation of the components of the classical pathway of complement. Typically, classical pathway complement factors (C1q, C3, C4d) are found in the presence of Igs (immune complex glomerulonephritis).[1,26,28] Thus, massive accumulation of complement factors of classical pathway of complement in the absence of Igs is unusual. Laser microdissection of the glomeruli followed by mass spectrometry was performed that confirmed the kidney biopsy findings.

The findings are somewhat akin to C3 glomerulopathy, where components of the alternative pathway of complement are present, with the absence or minimal deposition of Ig.[6] C4d, C3d, and C3 deposition may result from activation of the lectin pathway of complement. However, the finding of bright C1q along

with C4d, C3d, and C3 is indicative of activation of the classical pathway and not of the lectin pathway of complement. The glomerular deposition of a large amount of complement factors of the classical pathway and absence of immune deposits are suggestive of leishmania-driven fluid-phase activation of classical pathway of complement, similar to that seen in C3 glomerulopathy, where there is fluid-phase activation of the alternative pathway of complement.[29] The laboratory finding of low C3 levels was consistent with overall activation of the complement pathway. However, normal levels of C4 were contrary to the expected low C4 levels seen in activation of the classical pathway of complement. It may be related to the activity of the disease and timing of laboratory tests. However, the exact cause of this finding is not known.

The electron microscopy findings were also unique in that large extremely dense deposits were present in the mesangium and along capillary walls. The deposits were reminiscent of the deposits of dense deposit disease, except that they did not form the dense intramembranous sausage-shaped deposits, but instead formed bead-like deposits along the capillary walls. The dense deposits are indicative of deposits composed of complement factors. Indeed, laser microdissection and mass spectrometry confirmed the presence of large spectra for C3 and C4. The high average spectra number of C3 (average 81) appeared even in excess of those found in C3 glomerulopathy (average spectra of 33; our unpublished data). Similarly, large spectra numbers of C4 were present. Finally, the presence of C6, C7, and C9 indicated terminal pathway of complement activation. Small spectra numbers of IgG (average 5) were detected on mass spectrometry. However, it is difficult to draw meaningful conclusions of low spectra numbers since small spectra for IgG may be derived from the plasma in the glomeruli.

A search of the literature revealed a case similar to our case in which the authors described a 47-year-old man with HIV and leishmaniasis.[29] The kidney biopsy showed an MPGN pattern of injury, and the immunofluorescence was characterized by bright C3 and C1q, and negative Igs. Electron microscopy was not reported.

Interestingly, treatment of *Leishmania* with monthly infusions of amphotericin resulted in a decrease in proteinuria, which gradually increased again prior to the next infusion. It was felt that the amphotericin infusions may control the *Leishmania* infection but not cure the leishmaniasis. The fluctuation in proteinuria supports the finding that the glomerulonephritis is directly linked to the leishmaniasis. *Leishmania* avidly weakens the T helper cell 1 host immune response through mediation of cytokine secretion. HIV infection has a similar effect on the T helper 1 cellular immune response and promotes transition to a T helper cell 2 response, resulting in decreased macrophage anti-leishmanial activity. HIV and *Leishmania* coinfection results in a greatly elevated risk of recurrent visceral leishmaniasis and progression to AIDS.[30] The massive complement accumulation may result from decreased macrophage activity and weakened T helper cell 1 response resulting in defective clearing mechanisms.

CONCLUSION

To summarize, we present an unusual form of glomerulonephritis characterized by the presence of large amounts of complement factors of the classical pathway of complement and extremely dense deposits in the mesangium and along the capillary walls in the setting of HIV infection and leishmaniasis. Furthermore, we detected *Leishmania* species amastigotes within macrophages in the interstitium using routine hematoxylin and eosin and Jones silver stains.

DISCLOSURES

All the authors declared no competing interests.

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