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RENAL DISEASE IN CHRONIC EXPERIMENTAL *TRYPANOSOMA GAMBIENSE* INFECTIONS*

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Abstract. Two recently isolated stocks of *Trypanosoma brucei gambiense* of human origin gave rise to a moderate to severe proliferative or membranoproliferative glomerulonephritis in 40 or 44 NMRI and C57BL/6J mice infected for 7–22 weeks. Extensive granular deposits of C3, IgG1 and IgG3 were found in the mesangium, together with smaller quantities of IgG2a, IgG2b, and IgM. No trypanosomal antigen could be detected in the deposits though specific anti-trypanosoma antibodies were found in kidney eluates. By electron microscopy, a conspicuous proliferation of mesangial and endothelial cells was observed and electron-dense deposits were seen in a mesangial and subepithelial localization. With one of these trypanosome stocks, four of seven Wistar rats infected for 9–15 weeks developed morphologically similar glomerular lesions. Four other trypanosome stocks did not evoke renal alterations in 17 other rats infected for 13–56 weeks. Experimental infection in mice or rats appears to be a suitable model for the study of renal disease in chronic African sleeping sickness.

Glomerulonephritis lesions with deposits of complement and of immunoglobulins have been described in acute or subacute experimental *Trypanosoma brucei*[1] and *Trypanosoma rhodesiense*[2–4] infections. In these studies, data obtained by light microscopy, electron microscopy and immunofluorescence point to deposition of immune complexes as the causative factor.

The present paper deals with findings in kidneys from rats and mice chronically infected by human *Trypanosoma gambiense* isolates of different geographical origins. An account of the parasitological data and of general histopathological findings has already been given.[5–6]

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MATERIALS AND METHODS

Animals

Young adult outbred Wistar rats were used. Mice were either young adult outbred NMRI mice or inbred C57BL/6J mice.

Parasites

Five different stocks of *T. gambiense,* whose pedigrees are outlined below, were employed.

MONGO: primary isolation from positive human blood in 1968, Lower Zaire. After three subinoculations in guinea pigs, this stock was stabilized in 1969.

MOERBEKE 1: primary isolation in 1969 from positive blood, Lower Zaire. After three subinoculations in guinea pigs, stabilized in 1969.

MBA: primary isolation in 1974 at Mai Ndombe, Bandundu province, Zaire, from positive blood. Inoculated into cyclophosphamide-treated mice. Stabilized in 1974 after 21 subinoculations in mice. Seven rat passages in 1975 and stabilized in the same year.

GEMENA: primary isolation from positive blood into guinea pigs in 1974. Stabilized in 1974 after six subinoculations in mice.

AYL: primary isolation into mice in 1975. Stabilized after eight mouse passages. Reinoculated in mice (passages 9 to 16) and finally in rats for stabilization.

Stabilates were chosen as close as possible to the primary isolation and injected into a mouse or rat which was then used as a donor for the experimental infection.

Composition of experimental groups

Composition of experimental groups and duration of infection at the time of death are given in Table 1. Groups of animals were sacrificed when overt clinical disease, e.g. paralysis, became apparent. The animals included in the study were killed by exsanguination from a carotid artery under deep ether narcosis.

Tissue preparation

One kidney half was fixed in Bouin's fluid. After embedding in paraffin wax, 2–3 μm thick periodic-acid-Schiff (P.A.S.)-stained sections were obtained for light microscopy.

For electron microscopy, small specimens from the renal cortex were fixed in cacodylate buffered (pH 7.4) 2% glutaraldehyde, washed overnight in cacodylate buffer, postfixed in buffered 1% osmiumtetroxide, dehydrated in graded ethanol series and embedded in Epon 812. After prelocation of glomeruli on toluidine blue stained semi-thin sections, ultra-thin sections were cut on a LKB Ultrotome III, mounted upon uncoated copper grids and stained with uranylacetate and lead citrate. Sections were examined on a JAM 100 Electron Microscope operated at 80 KV.

For immunofluorescence, unfixed kidney specimens were snap-frozen by soaking in liquid nitrogen. Four-micron thick cryostat sections were layered with appropriate dilutions of specific antisera after brief acetone fixation and washing with phosphate buffered saline, pH 7.2. Commercial* fluorescein isothiocyanate labeled (FITC) antisera against rat IgG, IgM and C3 were used on rat kidney sections.

For mouse kidneys, commercial* FITC or tetramethyl rhodamine isothiocyanate (TRITC) labeled antisera against mouse C3, fibrinogen, Ig, IgA, IgG1, IgG3, IgM and in some cases against IgG2a and IgG2b, were used. Trypanosomal antigen in glomeruli was searched for by the indirect immunofluorescent technique using immune patient serum or rabbit hyperimmune sera. Rabbits were immunized with *T. gambiense* culture forms and serum was absorbed with calf serum. Sections were examined on a Leitz Orthoplan microscope equipped with the Ploemopak epifluorescence device and using filter blocks I_2 (FITC) and N_2 (TRITC). Immunofluorescence in glomeruli was graded on a 0 to 4+ scale according to its extent. Microphotographs were obtained on Agfachrome 50 S Professional film.

* Nordic Immunological Laboratories, Tilburg, The Netherlands.

TABLE 1

Composition of experimental groups in study of renal pathology in T. gambiense *infection*

Animal		No. of animals	*T. b. gambiense* stock	Weeks of infection at the time of examination
Species	Strain			
Rat	Wistar	8	MONGO	24–30
Rat	Wistar	3	GEMENA	14–56
Rat	Wistar	4	MOERBEKE	27–35
Rat	Wistar	7	AYL	9–15
Rat	Wistar	2	MBA	13–17
Mouse	NMRI	31	AYL	8–22
Mouse	C57BL/6J	7	AYL	7
Mouse	NMRI	6	MBA	8
Mouse	NMRI	6	—	—

Kidney eluates

Elution studies were performed on kidneys of 23 NMRI mice infected for 7 weeks with the AYL-stock and on kidneys of 23 age-matched intact control mice. When killed the animals from the infected group were showing incipient clinical central nervous system involvement. After exsanguination under ether narcosis and severing of the inferior caval vein, underneath the renal vein, mice were perfused in toto by injection of saline into the left heart ventricle until bleaching of the kidneys. Pooled kidneys of either group were then homogenized in 20 ml PBS at 0°C in a Potter tissue grinder. The homogenate was repeatedly centrifuged, 1,640 × g for 8 minutes at 0°C, until the supernate remained clear. The pellet was resuspended in 0.02M citrate buffer, pH 2.3, and kept for 1 hour at 4°C under constant stirring. After centrifugation for 10 min at 4,000 × g, the sediment was discarded and the supernate readjusted to pH 7.0 with 1 M NaOH. Any precipitate that

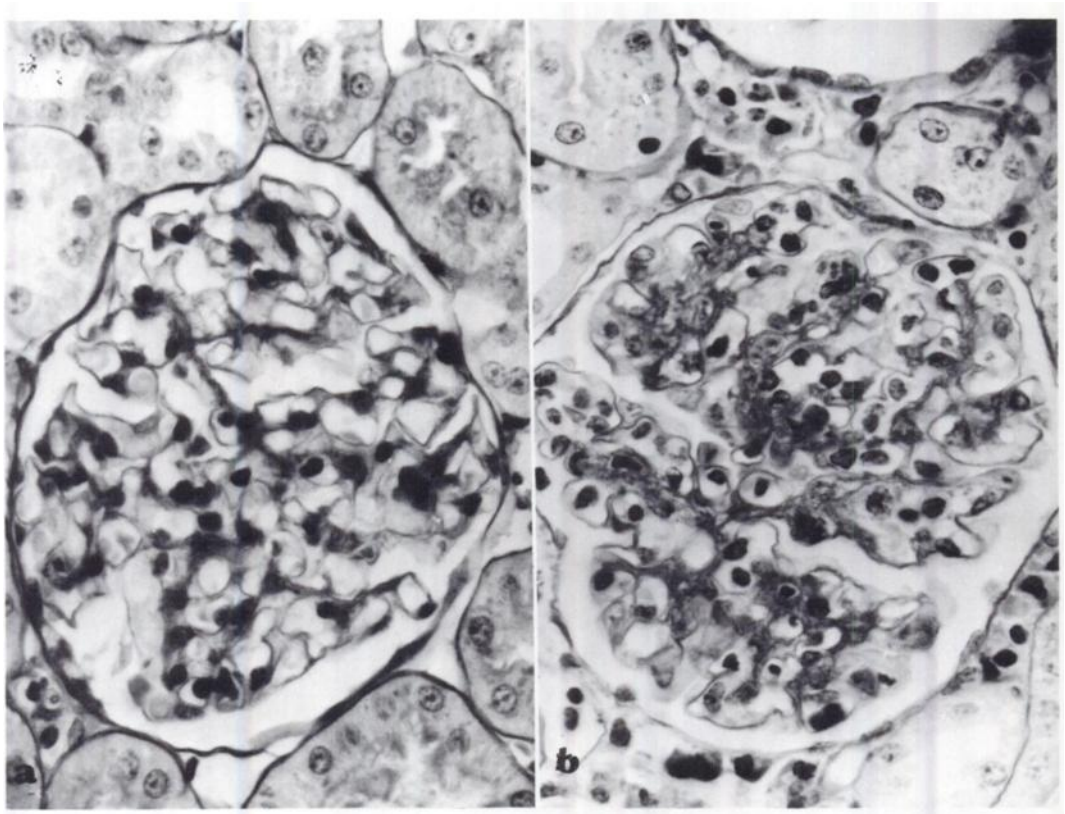

FIGURE 1. Histologically normal glomerulus from a rat infected for 26 weeks with the MONGO stock of *T. gambiense* (**a**) as compared to hypercellular glomerulus from a rat infected for 15 weeks with the AYL stock (**b**). PAS, ×790.

formed was eliminated by centrifugation. The supernate was used in an indirect immunofluorescent test on unfixed blood smears from rats infected with *T. brucei* with either FITC or TRITC labeled anti-mouse Ig, IgG1, IgG2a, IgG2b, IgG3, IgA and IgM sera in the second step. Additional control slides were treated with conjugate alone.

Antinuclear antibodies were assessed on acetone fixed cryostat sections of mouse livers by indirect immunofluorescence as for the detection of anti-trypanosome antibodies.

TABLE 2

Light microscopic changes in Wistar rats infected with Trypanosoma brucei gambiense

T. b. gambiense stock	Light microscopic changes	
	Present	Absent
MONGO	0	8
GEMENA	0	3
MOERBEKE	0	4
AYL	4	3
MBA	0	2

TABLE 3

Light microscopic glomerular changes in mice infected with Trypanosoma brucei gambiense

Mouse strain	*T. b. gambiense* stock	Light microscopic changes	
		Present	Absent
NMRI	MBA	6	0
C56BL/6J	AYL	7	0
NMRI	AYL	27	4
NMRI	—	0	6

Determination of circulating immune complexes

Immune complexes in serum of infected or intact control mice were quantitated with a ^{125}I-C1q binding test, which was basically carried out as described by Zubler et al.[7] As samples, 25 μl mouse serum mixed with 25 μl heat inactivated (56°C, 30 min) normal human serum were used. Standard samples of heat aggregated (63°C, 30 min) human IgG were used to calculate μg-equivalent quantities of the immune complexes in the test samples.

RESULTS

Light microscopy

In rats, as shown in Table 2, no histological alterations appeared in the animals infected with the MBA, GEMENA, MOERBEKE 1 and MONGO stocks. However, four of seven rats infected with the AYL stock had definite lesions, which were essentially confined to the glomeruli, though in one animal a slight pyelitis was also present. The most striking feature was a pronounced and diffuse proliferation of mesangial and endothelial cells. A few neutrophils were seen. The mesangium appeared only slightly widened through P.A.S.-positive fibrillar material. Glomerular capillary walls were not thickened (Fig. 1a, 1b).

Table 3 summarizes histological findings on mouse kidneys. Whereas intact, uninfected NMRI mice showed unaltered kidneys, almost all of the infected mice displayed glomerular lesions. In C57BL/6J and NMRI mice infected with the AYL stock, similar lesions were seen.

They were mainly characterized by a sometimes very important expansion of the mesangium which contained a rather homogeneous P.A.S.-positive material. In four of seven C57BL/6J mice and in eight of 27 NMRI mice the P.A.S.-positive material in the mesangium was so abundant as to cause obliteration of glomerular capillaries and distortion of the capillary pattern (Fig. 2).

In all cases with glomerular lesions, a variable but definite degree of mesangial and endothelial cell proliferation existed.

All six NMRI mice infected with the MBA stock had glomerular lesions, which were very severe in three of them. In the latter cases, light microscopical examination disclosed, in addition to the features seen in other groups, thickening of the glomerular capillary wall in some areas of the glomerular tuft. Tubular or interstitial lesions were not observed in infected mice.

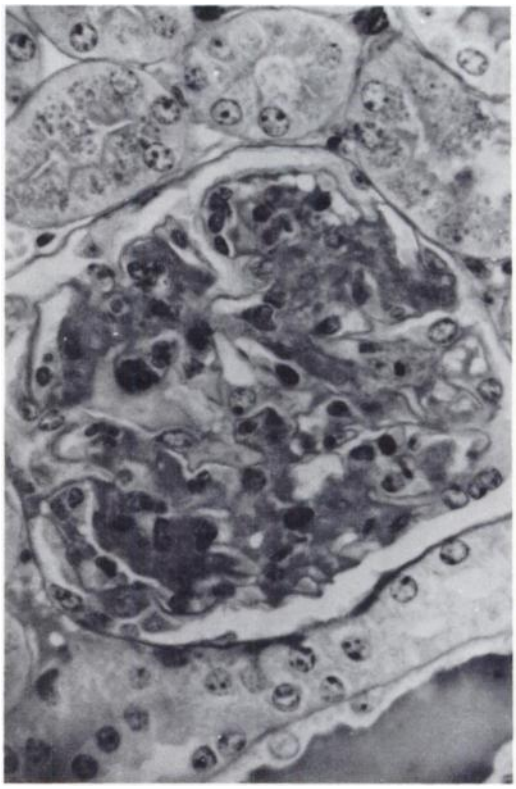

FIGURE 2. Distortion of the capillary pattern due to the deposition of PAS-positive material in the mesangium in a glomerulus from a mouse infected for 14 weeks with the AYL stock of *T. b. gambiense*. PAS, ×750.

In one NMRI mouse infected with the AYL stock, glomerular lesions, including even an occasional glomerulus with crescent formation, were very severe and gave rise to concomitant tubular atrophy without interstitial inflammatory lesions.

Immunofluorescence

In rats, positive reactions (2+ or 3+) were obtained only in the four animals which showed light microscopical alterations. In these animals granular deposits of C3 were detected in the mesangium (Fig. 3) whereas IgG and IgM were only present in trace amounts.

All infected mice had very important (mostly 4+, some 2+ or 3+) mesangial deposits of C3, IgG1 and IgG3 (Fig. 4), in contrast to the control

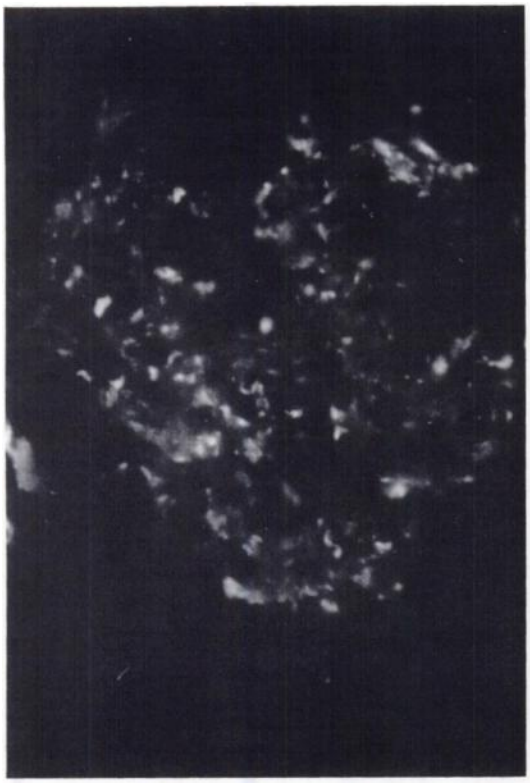

FIGURE 3. Deposits of C3 in glomerulus of rat infected with *T. b. gambiense*. Same animal as in Figure 1b. Immunofluorescence, ×735.

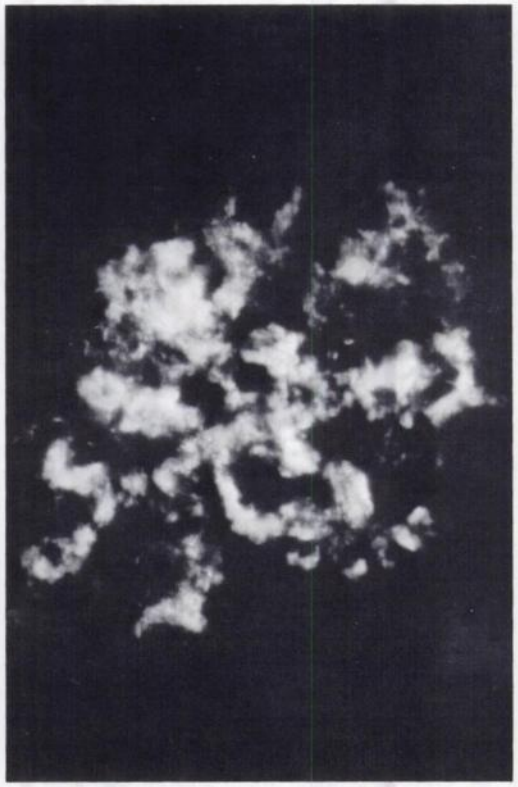

FIGURE 4. Mesangial deposits of IgG in the glomerulus of a mouse infected for 18 weeks with the AYL stock of *T. b. gambiense*. Immunofluorescence, ×735.

animals which showed no significant immune deposits. Table 4 shows the extent of the deposits in NMRI mice infected with the MBA stock compared to those found in age-matched intact control NMRI mice. NMRI and C57BL/6J mice infected with the AYL stock compared well with the NMRI mice infected with the MBA stock. The deposits were, though still extensive, somewhat less marked for IgG1 and IgG3 in C57BL/6J mice than in infected NMRI mice. Deposits of IgA, IgM, IgG2a and IgG2b were either absent or very faint in all groups. Capillary outlining by granular deposits of C3 or immunoglobulin was sometimes superimposed upon the mesangial pattern in NMRI mice infected with the MBA stock. Fibrinogen appeared deposited in a focal way and was limited to one or a few glomeruli.

The most extensive immune deposits were found in cases which showed the most severe light microscopic alterations. Some of the infected animals had, however, no detectable light microscopic lesions even in the presence of immune deposits.

Using the above-mentioned antisera, trypanosomal antigen could not be visualized within the deposits of infected rat or mouse kidneys. In several cases, however, circulating trypanosomes were demonstrated in this way in glomerular or peritubular capillaries. Elution of the sections with citrate buffer pH 2.3 before performing the immunofluorescent reaction gave the same negative results.

Electron microscopy

Electron microscopy confirmed the light microscopical results in rats. Four rats infected with the AYL stock showed proliferation of mesangial and endothelial cells. The mesangial matrix was slightly expanded and contained small electron dense deposits.

In infected mice very large electron dense de-

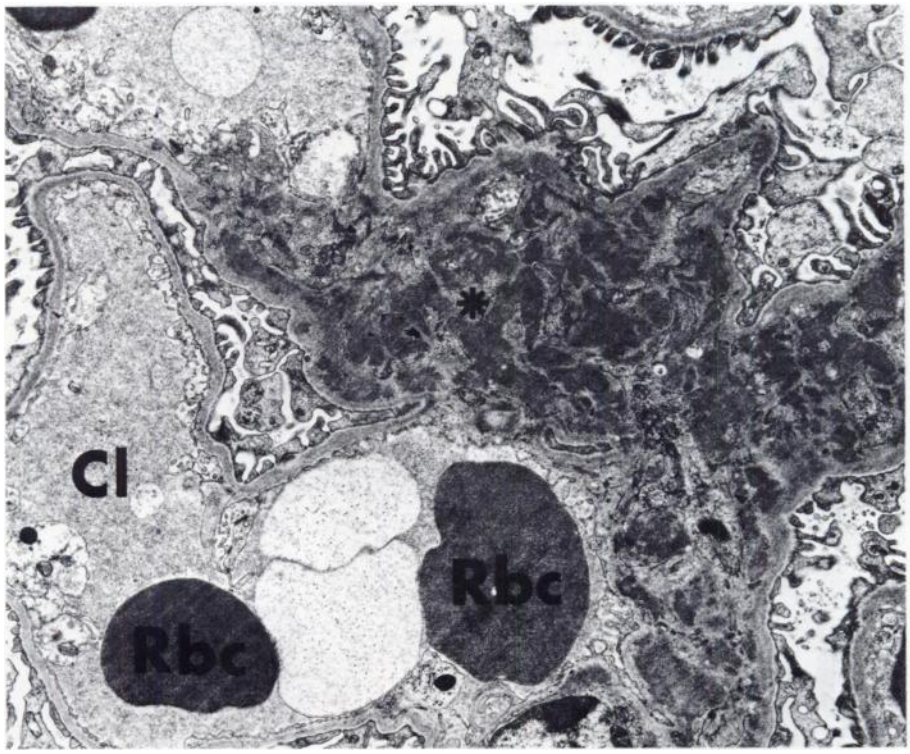

FIGURE 5. Electron micrograph of glomerulus from a mouse infected for 22 weeks with the AYL stock of *T. b. gambiense*, showing electron-dense deposits in the mesangium (*). RBC, red blood cell; Cl, capillary lumen. ×6,333.

posits were found in the mesangium (Fig. 5). The deposits sometimes extended underneath the endothelium of glomerular capillaries. There was an increase in the number of mesangial cells. Endothelial cells frequently appeared swollen, partially obliterating the capillaries. In some cases, numerous circulating trypomastigotes were seen in capillary lumina (Fig. 6). Basal membrane

TABLE 4

Immune deposits in glomeruli of NMRI mice infected with the MBA stock of Trypanosoma brucei gambiense *and of age-matched intact NMRI mice**

Identification no. of animal	Ig	IgA	IgG1	IgG2a	IgG2b	IgG3	IgM	C3
Infected group								
330	4+	0	3+	1+	1+	3+	0	3+
331	4+	3+	3+	0	1+	3+	2+	3+
333	2+	1+	0	0	0	3+	1+	1+
337	4+	0	4+	3+	4+	4+	3+	3+
338	4+	3+	3+	1+	2+	3+	2+	3+
339	4+	3+	3+	2+	2+	4+	1+	3+
Control group								
CT1	ND	0	2+	ND	1+	1+	0	1+
CT2	ND	0	0	ND	0	0	0	0
CT3	ND	1+	1+	ND	0	0	0	0
CT4	ND	1+	1+	ND	1+	0	0	0
CT5	ND	2+	0	ND	0	0	1+	0
CT6	ND	2+	1+	ND	0	1+	2+	0

* 0, negative; 1+, minimal or trace amounts; 4+, maximal (according to the extent of the immunofluorescence); ND, not done.

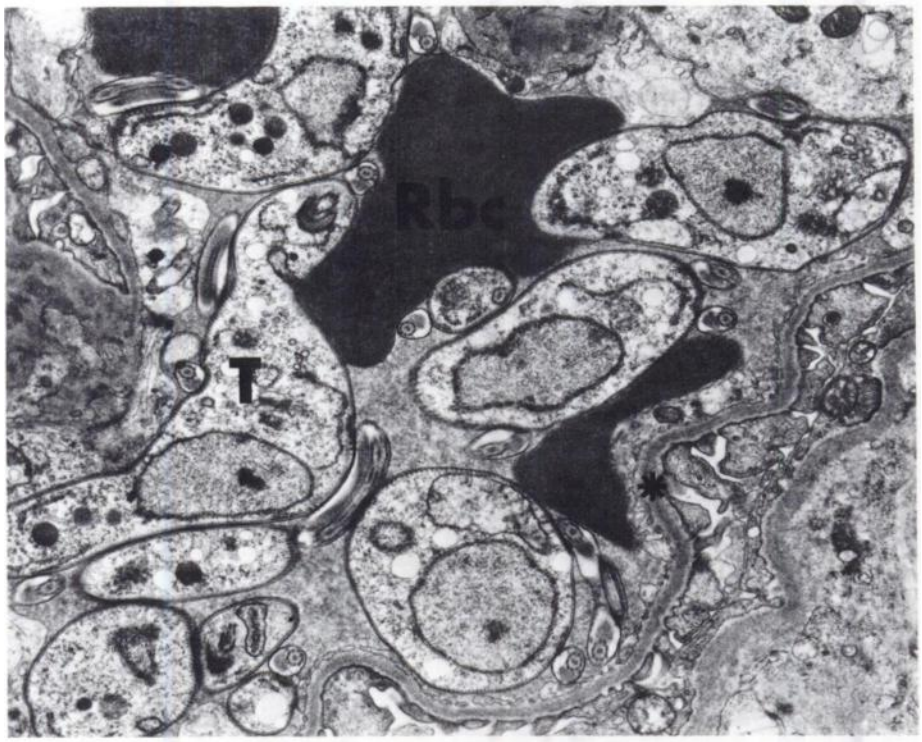

FIGURE 6. Electron micrograph of circulating trypanosomes in glomerular capillary lumen. One trypanosome is marked (T). Asterisk indicates basal membrane of glomerular capillary. Rbc, red blood cell. Same animal as in Figure 4. ×9,760.

changes were absent, except in animals infected with the MBA stock where subepithelial electron-dense deposits with fusion of overlying epithelial cell foot processes were often seen (Fig. 7).

Elution studies

Specific anti-trypanosome antibodies belonging to immunoglobulin classes IgG3, IgG2a and IgM were found in the eluates of infected mice kidneys. The eluates of kidneys from intact control animals gave entirely negative results. Control sections treated with conjugate alone were also negative. No antinuclear antibodies were revealed in either eluate.

Circulating immune complexes

Circulating immune complexes were consistently detected in infected mouse sera, as is shown in Figure 8. Control sera gave negative results. The C1q binding activity, however, did not closely parallel the gravity of morphological lesions, low activities being sometimes associated with severe renal alterations and vice versa.

DISCUSSION

In the present study it was shown that glomerular lesions do occur in rats and mice infected with certain stocks of *T. gambiense*. Their occurrence was clearly related to the advent of peaks of parasitemia in the course of the infection.[5] Rats infected with the MOERBEKE 1, MONGO and GEMENA stocks did not develop detectable peaks of parasitemia or glomerulonephritis. In these groups parasites remained barely detectable in peripheral blood but severe central nervous system lesions were shown at time of death.[6]

In contrast, in both rats and mice infected with the AYL stock, and in mice infected with the MBA stock, up to four peaks of parasitemia were observed in the course of the infection. Classical central nervous system lesions were also seen. The

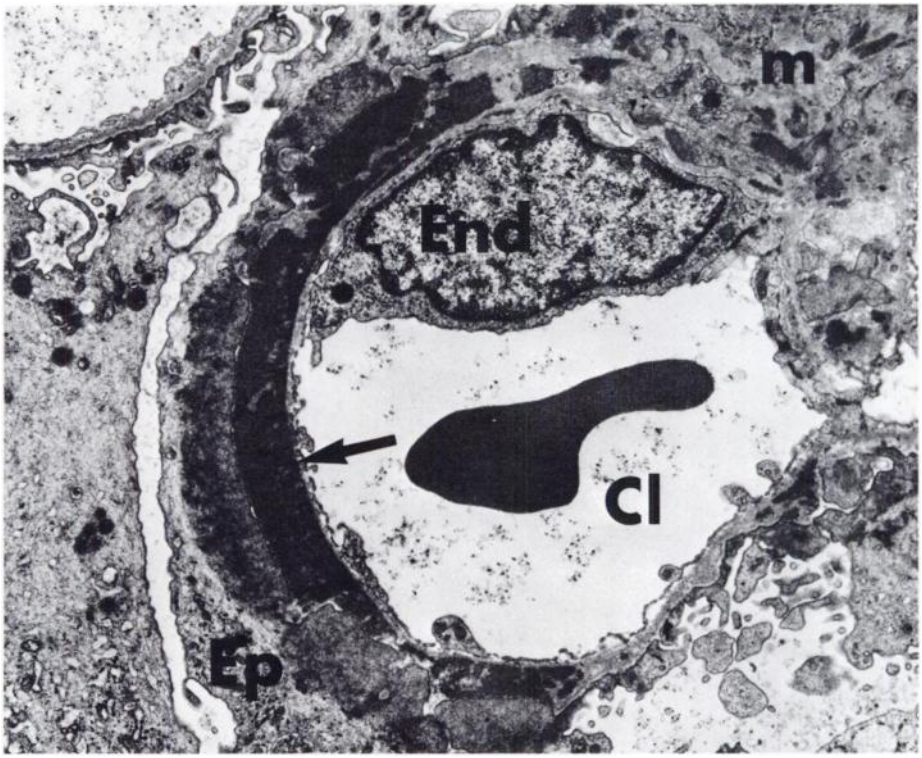

FIGURE 7. Electron micrograph of subepithelial electron-dense deposits (arrow) in glomerulus from a mouse infected for 8 weeks with the MBA stock of *T. b. gambiense*. Ep, epithelial cell; End, endothelial cell; Cl, capillary lumen; m, mesangium. ×7,600.

latter trypanosome stocks elicited with a high frequency a glomerulopathy, which can be considered as an epiphenomenon unrelated to the central nervous system lesions.

In mice, the described renal lesions have all characteristics of a glomerulonephritis mediated by the deposition of immune complexes. The morphological alterations differ, however, from those mentioned by other workers in *T. brucei*[1] or *T. rhodesiense*[2–4] infections both by their magnitude and by the high frequency with which they occur. The chronicity of the *T. gambiense* model probably accounts for this difference.

The presence of purely mesangial immune deposits in the infections with the AYL stock and of mixed subepithelial-mesangial immune deposits with the MBA stock possibly reflects differences in the molecular weight of the respective complexes involved.[8] No close correlation was found between the gravity of the lesions and the amount of immune complexes detectable in serum at sacrifice, but it has to be pointed out that the level of circulating immune complexes might fluctuate considerably in the course of the infection. Since specific anti-trypanosome antibodies were eluted from infected mouse kidneys it can be assumed that the antigen moiety of the immune complexes is of parasitic origin. The failure to detect trypanosome antigen in glomeruli could also be due to the large excess of antibodies in the complexes, leaving no antibody-binding sites uncovered. It is alternatively possible that no trypanosome antigen is actually present. In this respect it is worthwhile to recall that in African trypanosomiasis auto-antibodies with various specificities have been reported,[9–12] and that in the present groups of animals a high proportion of infected mice had antinuclear antibodies.[5]

Recently, evidence was presented for the generation of various auto-antibodies as a consequence of nonspecific polyclonal B cell activation in *T. brucei*-infected mice.[13] Though antinuclear

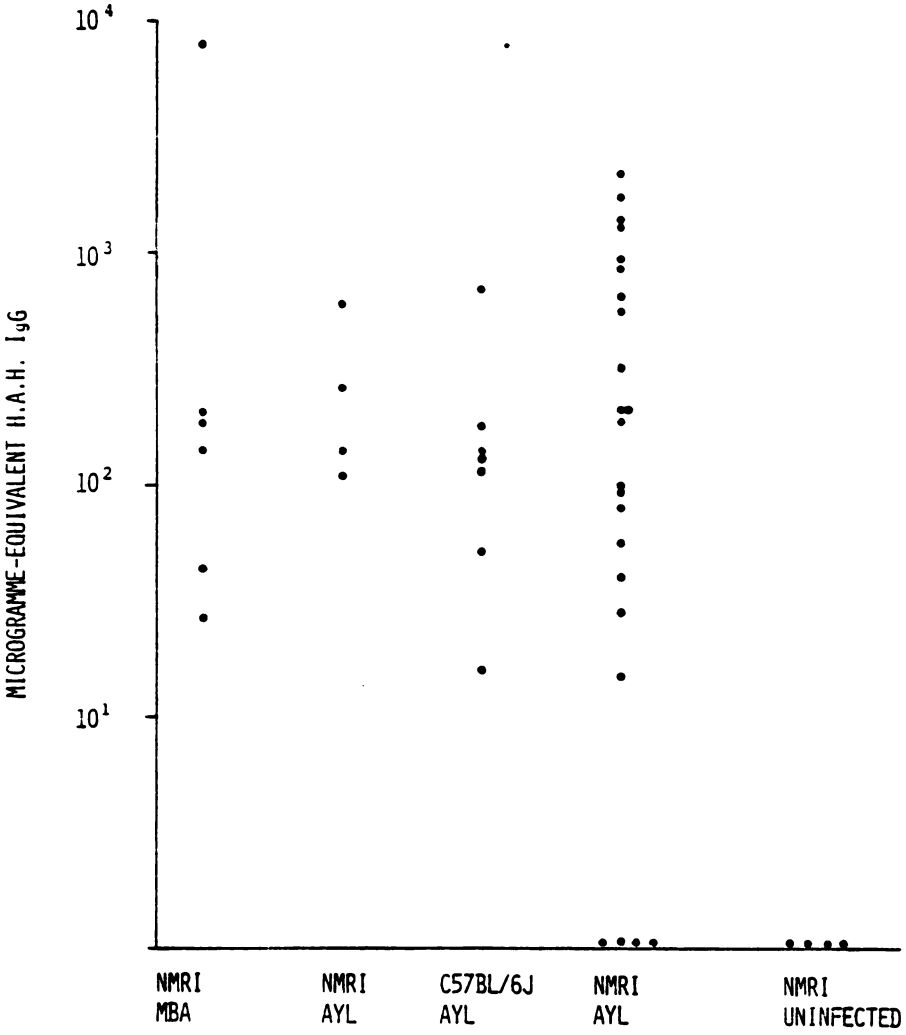

FIGURE 8. Levels of circulating immune complexes in serum of *T. b. gambiense*-infected mice at the time they were killed.

antibodies were not detected in the eluates of infected mice kidneys, yet a role for immune complexes, in which auto-antibodies of other specificities are involved, may not be discarded. In rats, the immunofluorescence findings suggest that complement activation via the alternate pathway came into play, rather than deposition of immune complexes. Facer et al.[2] and Nagle et al.[4] have shown that in rabbits and in rhesus monkeys infected with *T. rhodesiense* this event actually takes place. More work is needed to clarify the differences noticed between rats and mice, including the collection of data on complement profiles in both animal species and elution studies on rat kidneys.

The association of human African sleeping sickness with a nephropathy has hitherto not been confirmed,[14] or has not been fully documented.[15] It would certainly be unwise to extrapolate these experimental findings to the human situation. In any event, we think that the model depicted above, using chronic infections with *T. gambiense* stocks of human origin, is a valuable tool for the study of glomerulopathies mediated by parasites.

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