Naturally Occurring Immune-Complex Glomerulonephritis in Monkeys (Macaca irus)

I. Light, Immunofluorescence and Electron Microscopic Studies

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Light, immunofluorescence and electron microscopic studies were carried out on renal biopsies from 32 randomly selected adult monkeys *(Macaca irus)*. Histopathology was limited to glomeruli and consisted of mild to moderate segmental increases in mesangial cells, mesangial matrix, and/or glomerular basement membrane (GBM) thickness in 41% of the animals. Granular deposits of IgM were present in the mesangial region and along the GBM in 72% of the monkeys, whereas IgG, C1q, C4 and C3 were detected in approximately 30%. Electron-dense deposits were seen predominantly in epithelial foot processes adjacent to the GBM and, to a lesser extent, in the mesangium. Those monkeys with the heaviest IgM deposition were found to have decreased serum levels of C3, IgM and IgA. Follow-up biopsies over a period of 3 to 11 months revealed that the disease process was persistent yet nonprogressive. No correlation with age or sex was noted. All animals examined were clinically healthy and had normal renal function. This is the first documented occurrence of spontaneous immune-complex glomerulonephritis in a large monkey population. It appears to be a persistent disease which does not progress to renal insufficiency and which may serve as an investigative model for mild nonprogressive forms of human glomerulonephritis (Am J Pathol 76:145–164, 1974).

SPONTANEOUS GLOMERULONEPHRITIS has been reported in several nonprimate species, including mice,[1–4] mink,[5] cats,[6–8] dogs,[9–13] sheep,[14,15] goats,[15] hogs,[16] steers [15] and horses.[17] In all of these species, the demonstration of granular deposits of host γ-globulin and complement along the glomerular basement membrane (GBM) as well as electron-dense deposits associated with the GBM and/or mesangium has supported the conclusion that the renal disease is mediated by glomerular deposition of circulating antigen–antibody complexes. Although the nature of the antigens in these putative immune complexes is generally unknown, viral antigen–antibody complexes are believed to be responsible for the glomerulonephritis observed with Gross leu-

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kemia,[2] lymphocytic choriomeningitis [3] and coxsackie B [4,18] infection in mice, cholera in hogs,[16] equine infectious anemia in horses,[17] and leukemia in cats.[6] Parasitic antigens and red blood cell antigens have also been implicated in immune-complex nephritis of dogs [9] and mice,[1] respectively.

In contrast to the above studies, there is little information in the literature concerning spontaneous glomerulonephritis in subhuman primates. Kaur *et al* [19] in a study of 150 wild rhesus monkeys *(Macaca mulatta)* reported glomerulonephritis in only 1 animal, whereas Burkholder and Bergeron [20] described an apparent immune-complex nephritis in several species of the prosimian primate *Galago*. To our knowledge, no information is available concerning spontaneous glomerulonephritis in the cynomolgus monkey *(Macaca irus)*, a close relative of the rhesus monkey used less frequently for experimental work. During routine light and immunofluorescence microscopy of control renal biopsies for an unrelated study, we were surprised by the finding of granular deposits of IgM and IgG in the glomeruli of several of these clinically healthy monkeys. To evaluate the possibility of a subclinical spontaneous glomerulonephritis in this species, renal biopsies were performed on 37 randomly selected cynomolgus monkeys, constituting 65% of our colony, for examination by light, immunofluorescence and electron microscopy.

Materials and Methods

Isolation of Monkey IgG, IgM, Fibrinogen, and Human C1q

The IgG fraction of cynomolgus monkey serum was isolated using DEAE cellulose and eluting with the equilibrating buffer, 0.01 M K_2HPO_4, pH 8.0, as described by Fahey.[21] The eluate was concentrated by negative pressure ultrafiltration to a protein concentration of 10 mg ml and screened for IgG, IgA and IgM by radial immunodiffusion with specific antiserums to human immunoglobulins (Hyland Immunoplates). The preparation was shown to contain only IgG. Monkey IgM was isolated by a four-step procedure involving euglobulin precipitation, ultracentrifugation, DEAE cellulose chromatography and Sephadex G-200 gel filtration as described by Chaplin *et al.*[22] The only alteration from the original method was the use of a nine-chamber varigrad for the DEAE gradient elution.[21] The IgM fraction from the final Sephadex step was concentrated to 10 mg ml and was shown to be free of contaminating IgG or IgA by radial immunodiffusion. Immunoelectrophoresis against anti-whole monkey serum revealed an unidentified contaminating protein in the beta region in addition to IgM. Monkey fibrinogen was isolated from pooled fresh plasma by a Blomback modification of the Cohn fractionation technic.[23] The final preparation, which was greater than 90% clottable with thrombin, was adjusted to a concentration of 10 mg ml. The C1q component of human complement was isolated from serum by precipitation with aggregated gamma globulin according to the method of Müller-Eberhard and Kunkel.[24]

Production of Fluorescein-Conjugated Antiserums

One-milliliter samples (10 mg) of monkey IgG, IgM and fibrinogen were mixed with an equal volume of Freund's incomplete adjuvant and injected subcutaneously into rabbits at 2-week intervals for 8 weeks. Ten days after the last injection, serum was collected weekly, titered against the respective immunogen, and pooled according to potency. Antiserum to C1q was raised in a similar manner, although the concentration of each dose was considerably less (50 to 100 μg). Antiserums were rendered monospecific by absorption as follows: a) anti-IgG was gamma chain specific, as assessed by double diffusion and immunoelectrophoresis and, thus, absorption was not necessary; b) anti-IgM was absorbed with monkey IgG to remove light chain reactivity and with a DEAE fraction of normal monkey serum containing the contaminating beta protein to remove the corresponding antibody; c) antifibrinogen was absorbed with pooled monkey serum until only one precipitin line remained in double diffusion with pooled plasma and until no reactivity could be detected with serum; and d) antiserum C1q was absorbed with C1q-deficient serum (R11S) until only one line remained in double diffusion against pooled human and monkey serum. The antiserum formed a line of complete identity with a known C1q antiserum, kindly supplied by Dr. Edmund Lewis, University of Chicago. The IgG fraction of each antiserum was isolated by DEAE cellulose chromatography as described above and conjugated with fluorescein isothiocyanate according to the method of Clark and Shepard.[25] Final protein concentration and fluorescein:protein ratio for each antiserum were as follows: a) anti-IgG, 7.2 mg/ml with an F:P ratio of 1.2; 2) anti-IgM, 4.8 mg/ml, F:P ratio of 2.1; 3) antifibrinogen, 10 mg/ml, F:P ratio of 1.6; 4) anti-C1q, 11.4 mg/ml, F:P ratio of 1.2. Fluorescein-conjugated antiserum to human C4 and C3 were obtained from Meloy Laboratories, Springfield, Va, and their specificity verified by immunoelectrophoresis and double immunodiffusion. All conjugated antiserums were absorbed sequentially with lyophilized monkey kidney and liver prior to use, with the exception of the antifibrinogen, which was absorbed with kidney only.

A comparison of the relative concentration of antibody in the anti-IgG and anti-IgM reagents was determined by titering in double diffusion against pure monkey IgG and IgM standards of 2 mg/ml. The anti-IgG titered to 1:2 and the anti-IgM to 1:4. Fluorescein-conjugated antiserum to human IgG (10 mg/ml, F:P ratio of 3.5) and human IgM (10 mg/ml, F:P ratio of 3.0) were obtained from Meloy Laboratories and likewise titered against the pure monkey IgG and IgM standards. The anti-IgG titered to 1:2, whereas the anti-IgM titered to 1:8.

Preparation of Renal Biopsy Tissue

Renal tissue was obtained from 32 adult and 5 juvenile cynomolgus monkeys (*Macaca irus*) by percutaneous biopsy with a Menghini needle. Tissue was divided into three portions for light, immunofluorescence and electron microscopy. Tissue for fluorescent staining was snap frozen in a dry ice-acetone bath and sectioned at 4 to 6 μ on a −20 C cryostat. Fixation and staining were carried out as previously described.[26] Specificity of staining was confirmed by inhibition of fluorescence when conjugated antiserum was incubated with antigen prior to staining and when tissue sections were preincubated with unconjugated antiserum. Tissue sections were examined with a Zeiss photomicroscope equipped with an HBO 200 W high pressure mercury lamp, a dark field condenser, a UG-1 exciter filter and a 47 65 barrier filter. Photomicrographs were recorded on 35-mm high speed Ektachrome film. Tissue for light microscopy was fixed in 10% formalin, embedded in paraffin, cut at 3 to 5 μ on an American Optical-Spencer microtome, and stained

with hematoxylin and eosin. Tissue for electron microscopy was fixed in 3.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.35, postfixed in 1% osmium tetroxide, and embedded in Epon 812 (Ladd Research). Tissue was cut on a Reichert ultramicrotome at 600 to 800 Å with a Dupont diamond knife. Sections were picked up on 3.0-mm copper grids and stained with a saturated solution of uranyl acetate in 50% ethanol for 15 to 20 minutes at 24 C, and then in lead citrate (0.08 M) at pH 12 for 30 minutes at 24 C. Tissue was examined and photographed on an RCA EMU-3D electron microscope.

Quantitation of Immunoglobulins and Complement Components

Serum IgG, IgA, IgM, C1q, C4, C3 and C3-proactivator (C3PA) were quantitated by radial immunodiffusion. IgG, IgA and IgM radial plates were purchased from Hyland Laboratories; C3 and C4 plates from Meloy Laboratories; and C3PA plates from Custom Reagent Laboratories. C1q radial plates were prepared in this laboratory according to the method of Mancini *et al*[27] utilizing our anti-C1q antiserum.

Elution Studies

Cryostat sections of two renal biopsies which demonstrated 1+ linear IgG or IgM staining were eluted in 0.02 M citrate buffer, pH 3.2, according to the method of Feltkamp and Boode.[28] Following elution, tissue sections were examined for residual IgG and IgM. Eluted immunoglobulins were quantitated by radial immunodiffusion and anti-glomerular basement membrane activity assessed by indirect immunofluorescence on sections of normal monkey kidney.

Additional Laboratory Studies

Serum total protein, albumin, calcium, cholesterol, glucose, BUN, creatinine, bilirubin, alkaline phosphatase, LDH and SGOT levels were determined by "Autoanalyzer" SMA-12/60. Hematocrit, hemoglobin, platelet count, white blood cell count and differential leukocyte count were determined by standard methods. Serum was examined for autoantibodies to IgG, thyroglobulin and deoxyribonucleoprotein (DNP) by means of commercially available latex agglutination tests (Hyland Laboratories). The indirect immunofluorescence test, utilizing monkey liver and kidney substrate, was also employed for detecting antinuclear antibody, as well as antibody to GBM and to the antigen(s) of proximal tubule cells (PTA). Serum was tested for cryoglobulins by incubation at 4 C for 72 hrs. Urine was collected by atraumatic urethral catheterization, tested for protein with Labstix®, and centrifuged for sediment analysis.

Experimental Animals

All cynomolgus monkeys used in this study were housed in the primate facilities of the United States Army Medical Research Laboratory, Fort Knox, Kentucky. They were procured in the Philippine Islands by professional trappers, skin tested upon arrival for tuberculosis, and fed a standard Purina diet with fresh fruit supplements and water *ad libitum*.

Results

Immunofluorescence Studies

The renal localization of IgG, IgM, fibrinogen/fibrin, C1q, C4 and

C3 randomly selected adult monkeys is presented in Table 1. Since IgM was the predominant immunoglobulin detected, the animals are arranged in order of increasing glomerular IgM fluorescence (0 to 4+). Significant amounts of IgM (*ie*, 1+ or greater) were seen in 23 monkeys (72%) and were present generally as granular deposits both in the mesangium and along the GBM (Figures 2 and 3). The degree of IgM deposition was not age associated, and the presence or absence

Table 1—Renal Immunofluorescence Studies

Monkey No.	Sex	Age (yrs)	IgM	IgG	Fibrin	C1q	C4	C3
26	F	2–3	0	0	0	0	0	TBM
16	F	2–3	0	0	0	ND	ND	ND
11	M	2–3	0	0	0	0	0	0
160	F	7–8	Tr	0	0	ND	0	ND
84	M	7–8	Tr	0	0	0	1 +M	ND
29	M	2–3	Tr	0	1 + I	0	Tr	ND
27	M	2–3	Tr	0	0	0	0	ND
23	F	2–3	Tr	0	0	0	Tr	0
15	M	2–3	Tr	0	0	0	0	ND
170	F	7–8	1 + G	0	0	0	0	ND
169	M	7–8	1 + G	0	0	0	0	0
113	M	7–8	1 + L	0	0	ND	ND	ND
24	F	2–3	1 + G	0	0	0	0	0
14	M	2–3	1 + G	0	1 + I	ND	ND	ND
148	M	7–8	1 + L	0	3 + I	ND	ND	ND
21	F	2–3	1 + G	Tr	0	0	ND	0
98	M	7–8	1–2 + G	0	2 + I	ND	ND	ND
18	F	2–3	2 + L	0	0	ND	ND	ND
124	F	7–8	2 + G	0	0	0	ND	ND
19	F	2–3	2 + G	Tr	0	0	1 + L	ND
25	F	2–3	2 + G	Tr	2 + I	0	0	0
115	F	7–8	2 + G	1 + M	2 + G, 3 + I	0	0	TBM
61	M	7–8	2–3 + G	0	0	ND	ND	ND
157	F	7–8	2–3 + G	1 + L	0	ND	1 + G	ND
13	M	2–3	3 + G	0	0	ND	ND	ND
10	M	2–3	3 + G	1 + L	0	ND	ND	ND
132	M	7–8	3 + G	1 + M	3 + G	0	2 + G	2 + G
20	M	2–3	3 + G	1–2 + G	0	1 + G	1 + L	Tr
162	M	7–8	3 + G	2 + G	1 + I	0	2 + G	ND
52	F	7–8	3–4 + G	1 + M	1 + I	0	1–2 + G	0
17	M	2–3	4 + G	1–2 + G	0	2 + G	2 + G	ND
28	M	2–3	4 + G	1–2 + G	2 + G	Tr	2 + G	2 + L
Percent positive			72	28	31	10	43	33

Tr = trace in certain glomeruli, G = granular GBM and mesangial deposits, L = linear GBM deposition, M = mesangial deposits only, I = interstitial, TBM = deposition along tubular basement membranes, ND = not done.

of IgM was not related to the sex of the animals. However, 7 of 8 monkeys with the heaviest IgM deposition (3+ or 4+) were male. IgG was detected in 9 of 32 animals (28%) in the mesangium and in granular and linear patterns along the GBM and was present only in those animals with moderate to heavy IgM. Although by immunodiffusion the anti-IgM reagent was one dilution stronger than the anti-IgG, it was felt that this difference would not have accounted for the marked increase in glomerular IgM detected as compared to IgG. Furthermore, two renal biopsies demonstrating 1 to 2+ IgG were examined with a high quality anti-human IgG reagent (Meloy) and demonstrated essentially the same degree of fluorescence.

C1q was seen in significant quantity in only 2 of 21 animals examined (10%), both of which had heavy IgM deposits. C4 was detected in 9 of 21 animals (43%), generally in a granular pattern along the GBM and, like IgG, was associated with heavier IgM deposition. Tissue for C3 staining was available from only 12 monkeys, 2 of which showed significant GBM deposits. Two other animals had linear deposition of C3 along the basement membrane of isolated proximal tubules. Staining for fibrin was positive in 10 of 32 monkeys (31%). In 3 it was localized to the mesangium and GBM, and in 7 it was present exclusively as focal deposits in the interstitial tissue.

Follow-up biopsies or necropsies were performed on 6 monkeys at times ranging from 3 to 11 months in an effort to determine the persistent or transient nature of the glomerular immunoglobulin and complement deposits. As seen in Table 2, in general there was no significant change in the quantity of immunoglobulin and complement from the initial biopsy. However, there was a tendency for the deposits to become more distinctly granular and in three instances (C4 in monkey 20, C3 in monkey 28, IgM in monkey 148), the pattern of fluorescence changed from linear to granular.

Since all of the 32 animals examined ranged in age from 2 to 8 years (10 years being the normal life expectancy for *Macaca irus*), 5 animals under 2 years of age born in captivity were biopsied for immunofluorescence studies. Granular deposits of IgM were present in 4 of these animals (2+ in 2 monkeys and 1+ in the other 2). IgG and complement components were generally absent except for C3, which was present in 3 monkeys along basement membranes of isolated proximal tubules.

Light Microscopy

Suitable tissue for light microscopy was available from 29 adult

Table 2—Follow-up Renal Biopsies

Monkey No.	Time between biopsies (mons)	Biopsy No.	IgM	IgG	Fibrin	C1q	C4	C3
17	3	1	4 + G	1-2 + G	0	2 + G	2 + G	ND
		2	4 + G	1-2 + G	0	2 + G	2 + G	1 + G
20	3	1	3 + G	1-2 + G	0	1 + G	1 + L	Tr
		2	3 + G	2 + G	0	0	2 + G	3 + G
28	4	1	4 + G	1-2 + G	2 + G	Tr	2 + G	2 + L
		2	3 + G	1-2 + G	1 + G	0	2 + G	2 + G
15	5	1	Tr	0	0	0	0	ND
		2	Tr	0	0	0	0	0
16	5	1	0	0	0	ND	ND	ND
		2	0	0	0	ND	ND	ND
148	11	1	1 + L	0	3 + I	ND	ND	ND
		2	1 + G	0	2 + I	ND	Tr	ND

Tr = trace in certain glomeruli, G = granular GBM and mesangial deposits, L = linear GBM deposition, I = interstitial, ND = not done.

monkeys. Pathology was limited to glomeruli (Table 3) and consisted of mild to moderate segmental mesangial hypercellularity in 8 (28%), mild to moderate increase in mesangial matrix in 5 (17%) and focal GBM thickening in 6 (17%). Twelve of 29 (41%) demonstrated at least one of these changes. As shown in Table 3, there was no correlation between the histopathologic changes and the quantity of glomerular IgM. For example, monkey 28 with 4+ IgM had essentially normal glomeruli, whereas monkey 17, also with 4+ IgM, demonstrated both hypercellularity and focal GBM thickening (Figure 1). On the other hand, monkey 11, who had no detectable immunoglobulin or complement deposits, showed increased cellularity, matrix and GBM thickening.

Electron Microscopy

Renal tissue from 5 animals with 3+ or 4+ IgM was examined by electron microscopy. All five demonstrated a generalized increase in mesangial cellularity and matrix. The GBM was of normal, uniform thickness and contained occasional intramembraneous electron-dense deposits. The greatest concentration of dense deposits, however, was seen in the epithelial cells and in those portions of the foot processes adjacent to the GBM (Figures 4 and 5). Similar deposits were seen occasionally in the mesangial matrix. Proximal and distal tubules as well as interstitial tissue appeared normal.

In contrast to these 5 animals, monkey 160, whose kidneys showed

Table 3—Relationship of Glomerular Histopathology to IgM Deposition

Monkey No.	IgM	Cellularity	Mesangial matrix	GBM
26	0	2+	N	N
16	0	N	N	N
11	0	1+	2+	1+
160	Tr	N	N	N
84	Tr	N	N	N
29	Tr	N	1+	1+
27	Tr	N	N	N
23	Tr	N	N	N
15	Tr	1+	N	1+
170	1+	N	N	N
169	1+	N	N	N
113	1+	N	N	N
24*				
14	1+	N	N	N
148	1+	N	N	N
21†				
98	1-2+	N	2+	N
18	2+	N	N	N
124	2+	2+	N	N
19	2+	N	N	N
25	2+	N	N	N
115	2+	2+	N	N
61	2-3+	N	2+	N
157†				
13	3+	2+	N	2+
10	3+	N	N	N
132	3+	N	1+	1+
20	3+	1+	N	N
162	3+	N	N	N
52	3-4+	N	N	N
17	4+	1+	N	1+
28	4+	N	N	N
Percent abnormal		28	17	20

* Only one glomerulus in specimen.
† Medulla obtained.
N = normal.

trace IgM and normal histology, had neither electron-dense deposits nor increased mesangium.

Serum Studies

In an attempt to correlate clinical studies with the degree of glomerular IgM deposition, 16 monkeys were divided into three groups in the following manner: group I (6 animals) with zero or trace IgM; group II (5 animals) with 1+ or 2+ IgM; and group III (5 animals) with 3+ or 4+ IgM. Routine blood chemistry studies, as determined

by Autoanalyzer SMA 12/60, were within the normal range for cynomolgus monkeys.[29] In particular, BUN and creatinine values gave no indication of renal functional impairment. Since there are no normal values reported in the literature for individual immunoglobulins and complement components in *Macaca irus*, group I served as a control for these determinations (Table 4). Although human radial immunodiffusion plates were employed, the high degree of cross-reactivity between human and monkey serum proteins permitted their use. As compared to group I, there was a significant decrease in group III for IgM ($P < 0.05$), IgA ($P < 0.05$) and C3 ($P < 0.005$) and an increase in C3PA ($P < 0.025$). The only value in group II significantly different from the control group was an increased C3 ($P < 0.025$). Latex agglutination tests for antibodies to IgG, thyroglobulin and DNP were negative for all 16 animals, as were the indirect immunofluorescence tests for antinuclear, anti-GBM and anti-PTA antibodies. No cryoglobulins were detected in any of the serums.

Elution Studies

Examination of cryostat sections eluted with 0.02 M citrate buffer demonstrated that all IgG and/or IgM had been removed. However, neither IgG nor IgM could be detected by radial or double diffusion in the two renal biopsy eluates. Furthermore, no anti-GBM activity could be demonstrated by indirect immunofluorescence. Since only 3 of 23 monkeys had linear IgM deposits and 2 of 9 had linear IgG, plus the fact that transition from a linear to a granular pattern was observed in 3 monkeys on follow-up biopsies, it was felt that the linear deposits most likely represented a granular pattern too fine to be discerned.

Hematologic Studies

The mean values for hematocrit, hemoglobin, white blood cell count and differential leukocyte count for group I (Table 5) were within

Table 4—Immunoglobulin and Complement Values

Test	Group I	Group II	Group III
IgG (mg/100 ml)	1930 ± 198	1820 ± 254	1570 ± 506
IgM (mg/100 ml)	75 ± 30	110 ± 47	49 ± 3
IgA (mg/100 ml)	1045 ± 474	1850 ± 936	620 ± 215
C1q (μg/ml)	355 ± 50	435 ± 96	375 ± 60
C4 (mg/100 ml)	28 ± 8	42 ± 17	21 ± 11
C3 (mg/100 ml)	310 ± 25	345 ± 24	235 ± 26
C3PA (μg/ml)	300 ± 74	350 ± 96	445 ± 126

The results of group I are expressed as the mean of 6 animals ± SD; results of groups II and III are the mean of 5 animals ± SD.

Table 5—Hematologic Studies

	Group I	Group II	Group III
Hematocrit	37 ± 4	37 ± 3	41 ± 3
Hemoglobin	11.2 ± 1.2	10.8 ± 0.7	12.4 ± 1.0
Platelets	435.000 ± 108.000	436.000 ± 135.000	356.000 ± 90.000
WBC	13.700 ± 2100	8200 ± 1800	8100 ± 1400
PMNs	44 ± 1	26 ± 16	29 ± 9
Bands	8	18	4
Lymphocytes	38 ± 6	38 ± 22	59 ± 13
Monocytes	9	10	6
Eosinophils	2	9	2
Basophils	0	1	0

The results of group I are expressed as the mean of 6 animals ± SD; results of groups II and III are the mean of 5 animals ± SD.

the normal range for cynomolgus monkeys.* Significant differences from group I were a) the decreased white cell count in group II and III ($P < 0.005$ for both); b) the decrease in polymorphonuclear leukocytes in groups II and III (P 0.05, $P < 0.005$, respectively); and c) the increase in lymphocytes in group III ($P < 0.01$).

Urinalysis

Two animals in group I, 2 in group II and 3 in group III showed trace protein. Two animals in group I gave a 1+ reaction. No abnormalities suggestive of glomerulitis were seen in any urine sediment examined.

Discussion

The occurrence of spontaneous glomerulonephritis in several nonprimate species [1,5,11,16,17] has provided unique opportunities for studying the pathogenic mechanisms and etiologic agents involved in this spectrum of disease. The similarities between these animal models and several forms of human nephritis, notably systemic lupus erythematosus (SLE) [30] and the disease of New Zealand hybrid mice,[31] have helped clarify the role of circulating antigen–antibody complexes in mediating glomerular injury. Examples of immune–complex glomerulonephritis in nonprimates are plentiful [5,8,12,14–17,30] but appear to be uncommon in subhuman primates.[19] The only documented occurrence of apparent immune-complex glomerulonephritis in a subhuman primate is a retrospective study of several species of the prosimian primate

* Comparison was made with a cynomolgus colony at the Oregon Regional Primate Research Center, Beaverton, Oregon. Information kindly supplied by Dr. David L. Hess.

Galago,[20] in which γ-globulin, complement and subepithelial electron-dense deposits were detected in several animals with the histologic diagnosis of progressive sclerotic glomerulonephritis. All renal tissue, however, was obtained during routine pathologic examination of animals that had died from various acute or chronic illnesses. Consequently, no assessment of the prevalence of glomerulonephritis in the four species of *Galago* could be made.

In the present study, percutaneous renal biopsies performed on 32 randomly selected adult cynomolgus monkeys have provided evidence for an immune-complex glomerulonephritis affecting a large percentage of our cynomolgus colony. By light microscopy, the pathology was restricted to glomeruli and was characterized by mild to moderate segmental increases in mesangial cells, mesangial matrix or GBM thickness in 41% of the animals examined. No correlation was found between age or sex and the presence of glomerular abnormalities.

Immunofluorescence microscopy revealed granular deposits of IgM in the mesangium and along the GBM in 72% of the monkeys. IgG and complement components C1q, C4 and C3 were also present as granular mesangial and GBM deposits in approximately 30% of the animals and, with few exceptions, were present in those animals with the greatest IgM deposition. The presence of glomerular fibrinogen/fibrin in only 10% of the cases would indicate that the immunoglobulin and complement components detected were the result of deposited immune complexes rather than nonspecific adherence of plasma proteins to previously damaged glomeruli. This is further supported by the ultrastructural identification of electron-dense deposits in the epithelial foot processes adjacent to the GBM and, to a lesser extent, in the mesangial matrix. As with the light microscopic findings, no correlation existed between age or sex and the presence or absence of IgM, although a male predominance was observed in those animals with the heaviest IgM deposition. Follow-up biopsies in several adult animals indicated that this immune-complex disease was not a transient phenomenon but reflected a relatively stable nonprogressive disease process, at least during the 3- to 11-month period of observation.

Since the immunofluorescence and electron microscopic findings indicated an immune-complex form of glomerulonephritis, an attempt was made to determine whether certain autologous antigens were involved in the disease and if the disease process was reflected in alterations of serum complement components. Renal deposition of antigen–antibody complexes containing nuclear antigens,[30,31] thyroglobulin,[32–34] IgG [35,36] or renal proximal tubule antigen [37,38] is believed to be

responsible for certain forms of glomerulonephritis in both humans and animals. However, antibodies to these antigens were not detected in the serum of any of the 16 monkeys examined, rendering it unlikely that these autologous antigens were involved in the disease process. Alterations in serum levels of immunoglobulins and complement components were seen, however, in those monkeys with the heaviest IgM deposits (*ie*, group III). As a group, they had significantly decreased levels of C3, IgM and IgA. Low C3 levels may reflect increased utilization by antigen–antibody complexes analogous to that which occurs in poststreptococcal glomerulonephritis and SLE.[39] Hypocomplementemia resulting from activation of the alternate complement pathway,[40,41] appears unlikely in view of the elevated level of C3-proactivator (C3PA), one of the primary components of the alternate pathway.[42] The decreased IgM may also reflect heavy renal deposition; however, since hypogammaglobulinemia is not a feature of immune-complex disease in humans, the low IgM and IgA may be merely coincidental and unrelated findings.

In view of the reduction in the white blood cell count, the percentage of neutrophils, and the elevation in the percentage of lymphocytes in group III, serum antibody titers and renal elution studies are currently in progress in an attempt to determine whether persistent viral, bacterial or parasitic infection is related to the immune-complex glomerulonephritis.

In conclusion, we have described what appears to be a spontaneous, immune-complex glomerulonephritis with the following characteristics in cynomolgus monkeys: a) mild segmental increases in mesangial cells, mesangial matrix and/or GBM thickness; b) granular deposits of IgM, IgG, C1q, C4 and C3 in the mesangium and along the GBM; c) epithelial and mesangial electron-dense deposits; and d) reduced serum levels of C3, IgM and IgA. The disease is persistent yet nonprogressive, unrelated to age or sex, and is accompanied by normal renal function. To our knowledge, this is the first report of naturally occurring immune-complex glomerulonephritis in monkeys, and may serve as a useful model of mild nonprogressive forms of human glomerulonephritis.

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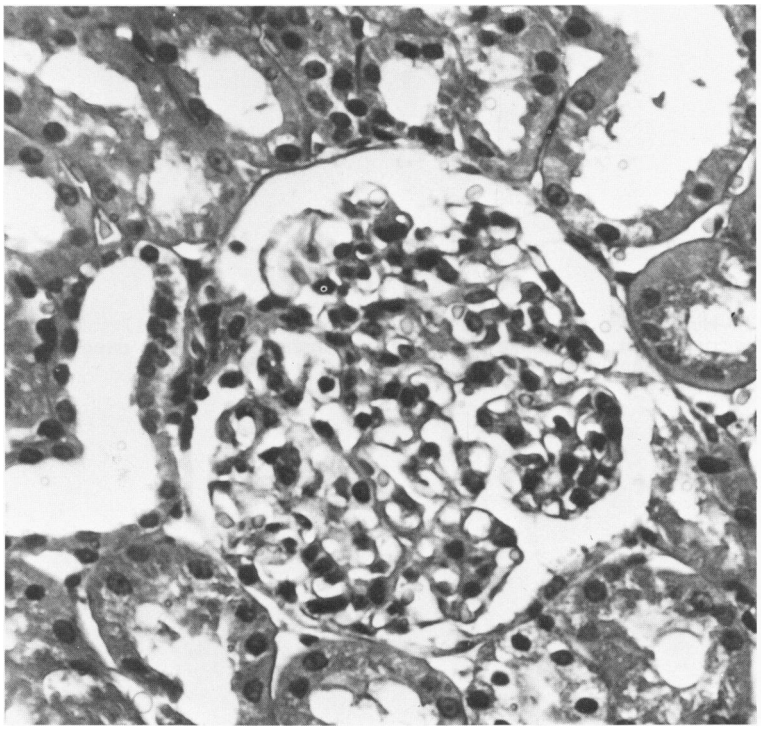
[*Illustrations follow*]



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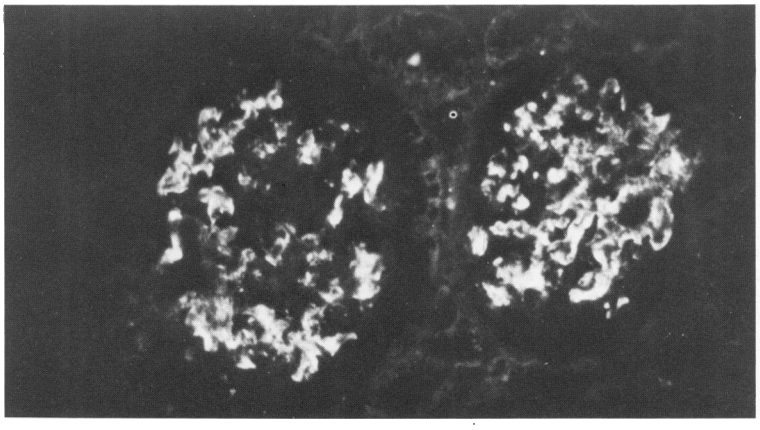
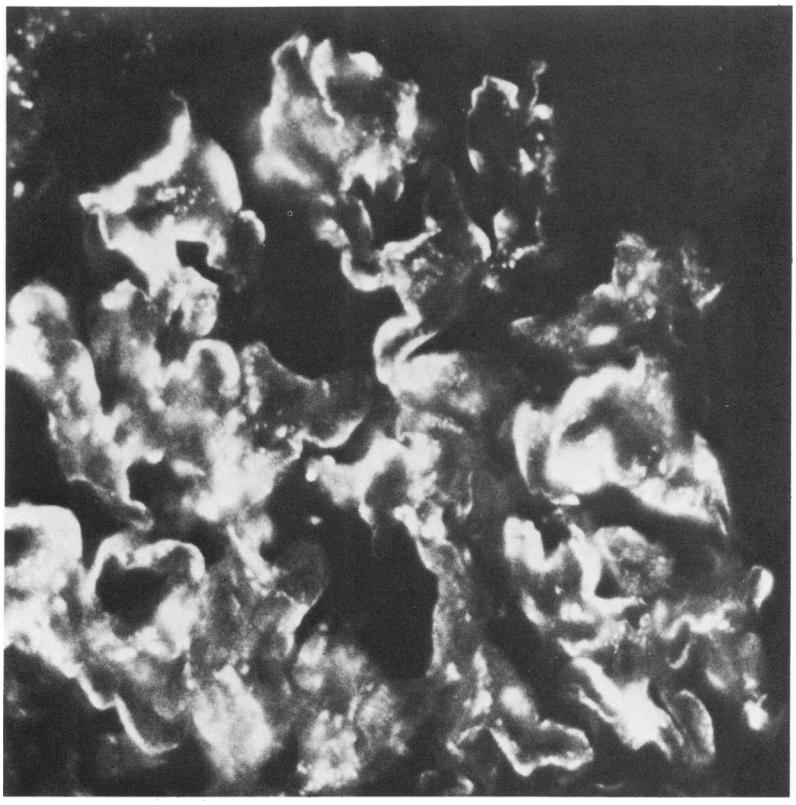


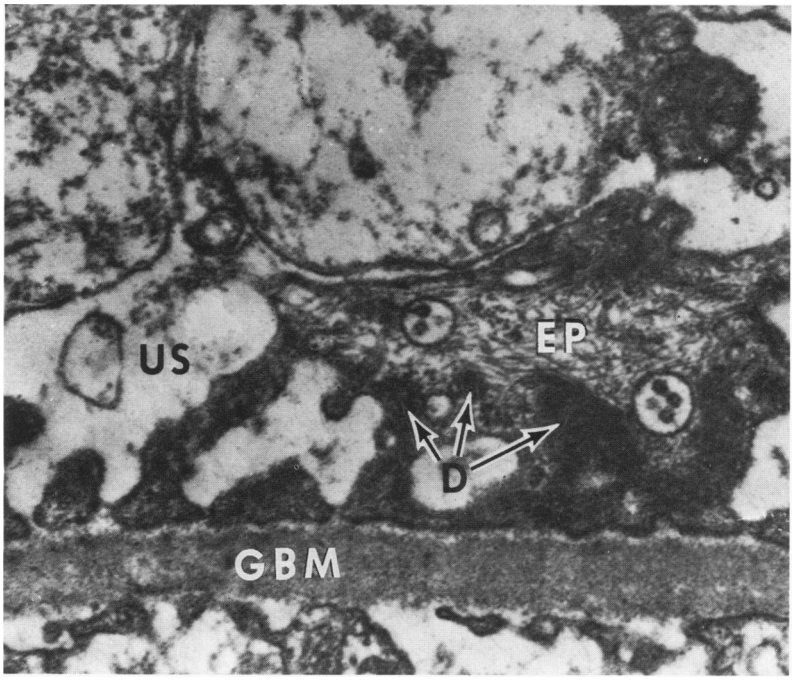
2

Fig 1—Light microscopic picture of renal biopsy specimen from monkey 17 demonstrating segmental hypercellularity and mild GBM thickening (H&E, × 200). **Fig 2**—Two glomeruli from monkey 17 positive for IgM after staining with fluorescein-conjugated anti-monkey IgM (× 100).

Fig 3—High power photograph of a glomerulus from monkey 17 stained with fluorescein-conjugated anti-monkey IgM and demonstrating granular deposits in capillary walls and mesangium (× 400).

Fig 4—Electron micrograph of a glomerulus from monkey 52 in which electron-dense deposits (*D*) are seen in epithelial foot processes adjacent to the GBM. Glomerular basement membrane (*GBM*) is of normal thickness. *EP*=epithelial cell with prominent microtubules, *US*=urinary space (× 32,000).




US
EP
D
GBM

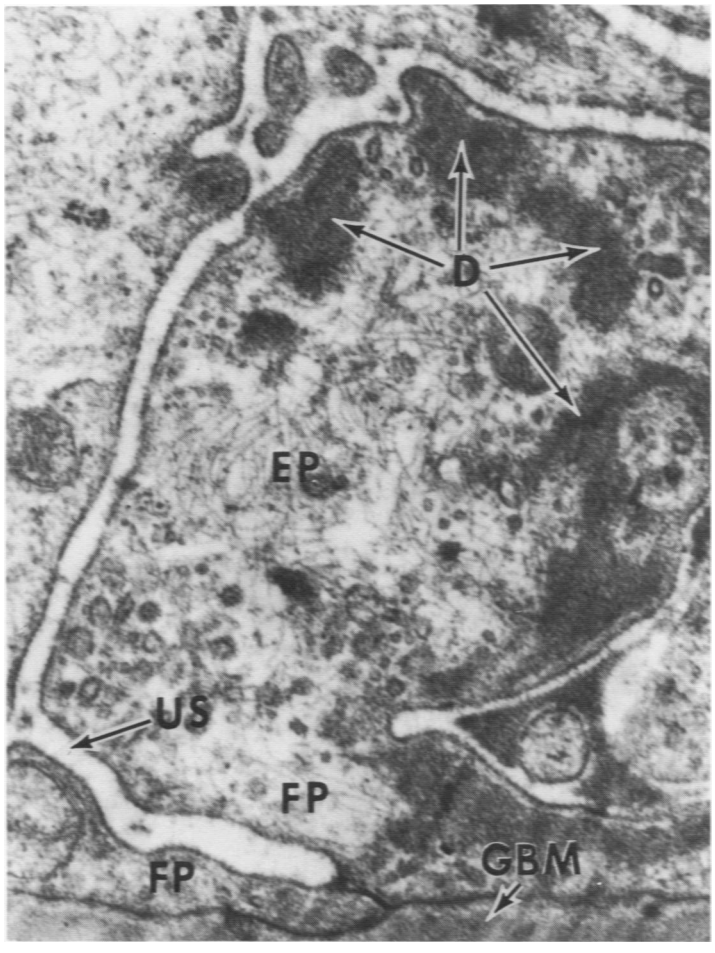


Fig 5—Higher power electronmicrograph of a glomerulus from monkey 52 demonstrating several prominent electron dense deposits (*D*) within an epithelial cell (*EP*). Foot processes (*FP*) are flattened and fused. *GBM*=glomerular basement membrane, *US* =urinary space (× 40,000).