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Neuraminidase Treated Homologous IgG and Immune Deposit Renal Disease in Inbred Rats[1] (38680)

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We have previously reported a high incidence of cryoglobulins in glomerulonephritis (1) and demonstrated that the IgG component of the cryoglobulin lacked sialic acid (2, 3) which may render the immunoglobulin autoimmunogenic and lead to the production of an autologous immune complex disease, the complex consisting of immunoglobulins of different classes. Neuraminidase is found in bacterial and viral organisms and chemical alteration of immunoglobulins by the removal of sialic acid from the molecule induced by this enzyme may ultimately lead to immune deposit disease and glomerulonephritis. Studies (4) on a glomerulonephritic kidney using sialic acid depleted IgG from a mixed cryoglobulin lend support to this hypothesis. This study was designed to further examine this theory in experimental animals.

Materials and Methods. Inbred Lewis rats were used for the study. 1. Immunoglobulin preparation: Rat immunoglobulin was prepared using fresh pooled serum from Lewis rats by half saturation with ammonium sulfate, Sephadex G 200 chromatography (5) and ion-exchange chromatography on Diethyaminoethyl (DEAE) cellulose (6). Neuraminidase Type IV from Clostridium perfringens 1.3 units/mg was obtained from Sigma Chemical Co. (St. Louis, MO.).

2. Treatment of rat IgG with neuraminidase: one gram rat IgG was treated with 10 mg of neuraminidase in 100 cc 0.02 *N* acetate buffer at pH 5.0 and incubated at 37° for 45 h. Following incubation the solution was dialyzed against phosphate buffered saline and purified by half saturation with ammonium sulfate followed by Sephadex G 200 gel chromatography and DEAE cellulose ion exchange chromatography. The solution was concentrated by pressure dialysis and centrifuged at 20,000 *g* to remove aggregated gamma globulin.

3. Preparation of control solutions of rat IgG.

a. IgG was incubated in acetate buffer and repurified under the same conditions except in the absence of neuraminidase.

b. IgG dissolved in acetate buffer as well as in phosphate buffered saline (PBS) pH 7.4.

Animals were divided into several groups.

Group I—Ten Lewis rats 100–150 g, received a single intravenous dose of neuraminidase treated IgG (NIgG) 25 mg.

Group II—Ten Lewis rats 100–150 g, received 25 mg of NIgG intravenously followed in 2 wk by 5 mg of NIgG intravenously.

Group III—Ten Lewis rats 100–150 g, received 25 mg of NIgG with complete Freund's adjuvant.

Group IV—Ten Lewis rats 100–150 g, received 25 mg of NIgG with complete Freund's adjuvant intraperitoneally followed by 5 mg of the same preparation administered by the same route in 2 wk.

Group V—Ten animals 100–150 g, received a single intravenous injection of 25 mg of IgG in acetate buffer.

Group VI—Ten animals 100–150 g, received intravenously 25 mg of IgG in acetate buffer followed in two weeks by 5 mg of intravenous IgG in acetate buffer.

Group VII—Ten animals 100–150 gm, received 25 mg of IgG in acetate buffer with

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Freund's adjuvant intraperitoneally as a single dose.

Group VIII—Ten animals 100–150 g, received 25 mg of IgG in Freund's adjuvant intraperitoneally followed by 5 mg of same preparation by the same route in 2 wk.

Group IX—Ten animals 100–150 g, received a single dose of 0.1 cc of neuraminidase in acetate buffer intravenously.

Group X—Ten animals 100–150 g, received 0.1 cc of neuraminidase in acetate buffer intravenously followed by 0.1 cc

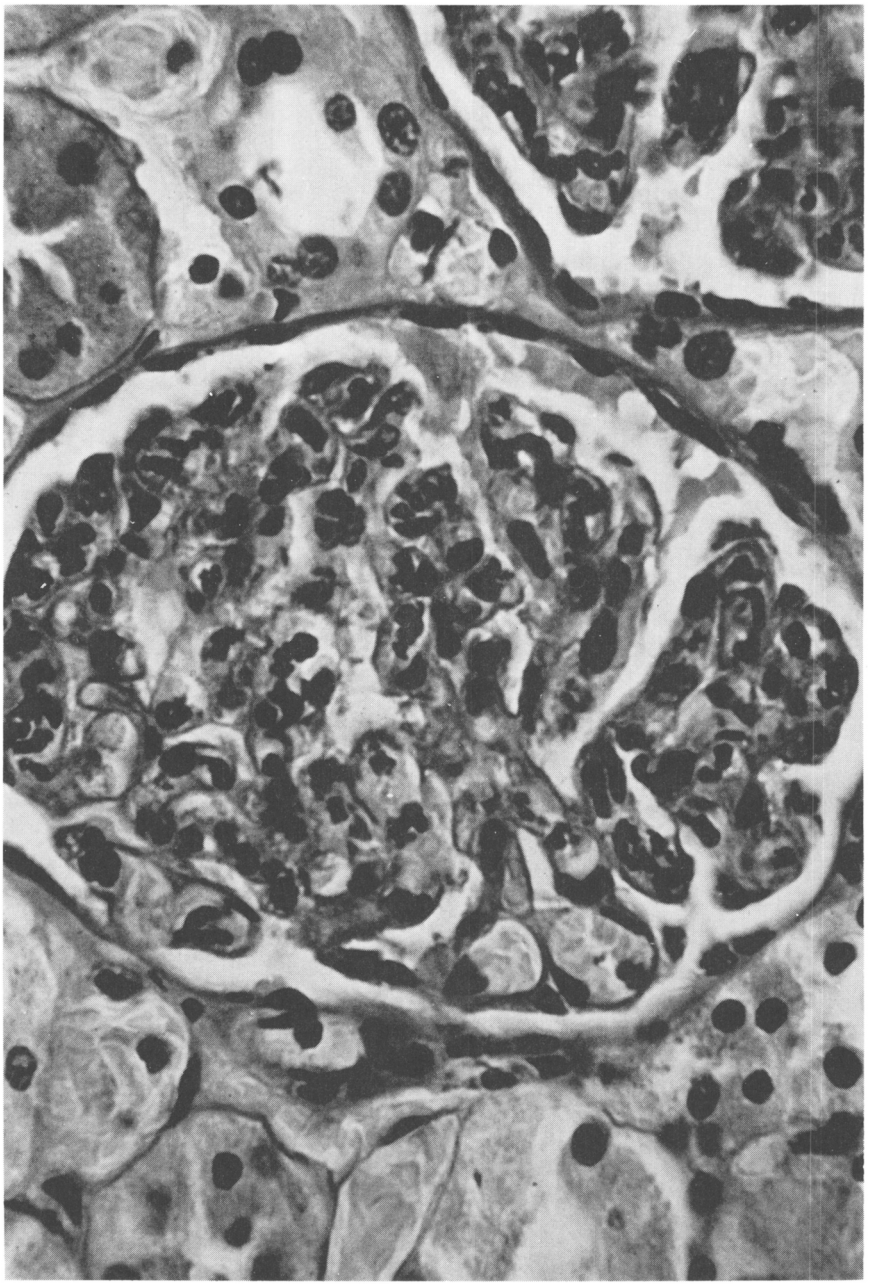

FIG. 1. Representative glomerulus from an animal from Group II showing moderate focal proliferation and glomerular basement membrane thickening. Haematoxylin and eosin.

neuraminidase in acetate buffer intravenously in 2 wk.

Groups XI and XII same as IX and X except injections were intraperitoneal and Freund's adjuvant was used.

Group XIII—Ten animals 100–150 g, received 25 mg IgG in PBS intravenously.

Group XIV—Ten animals 100–150 g, received 25 mg IgG in PBS intravenously followed by 5 mg in 2 wk.

Group XV and XVI—same as XIII and XIV except injections were intraperitoneal and preparations was mixed with Freund's adjuvant.

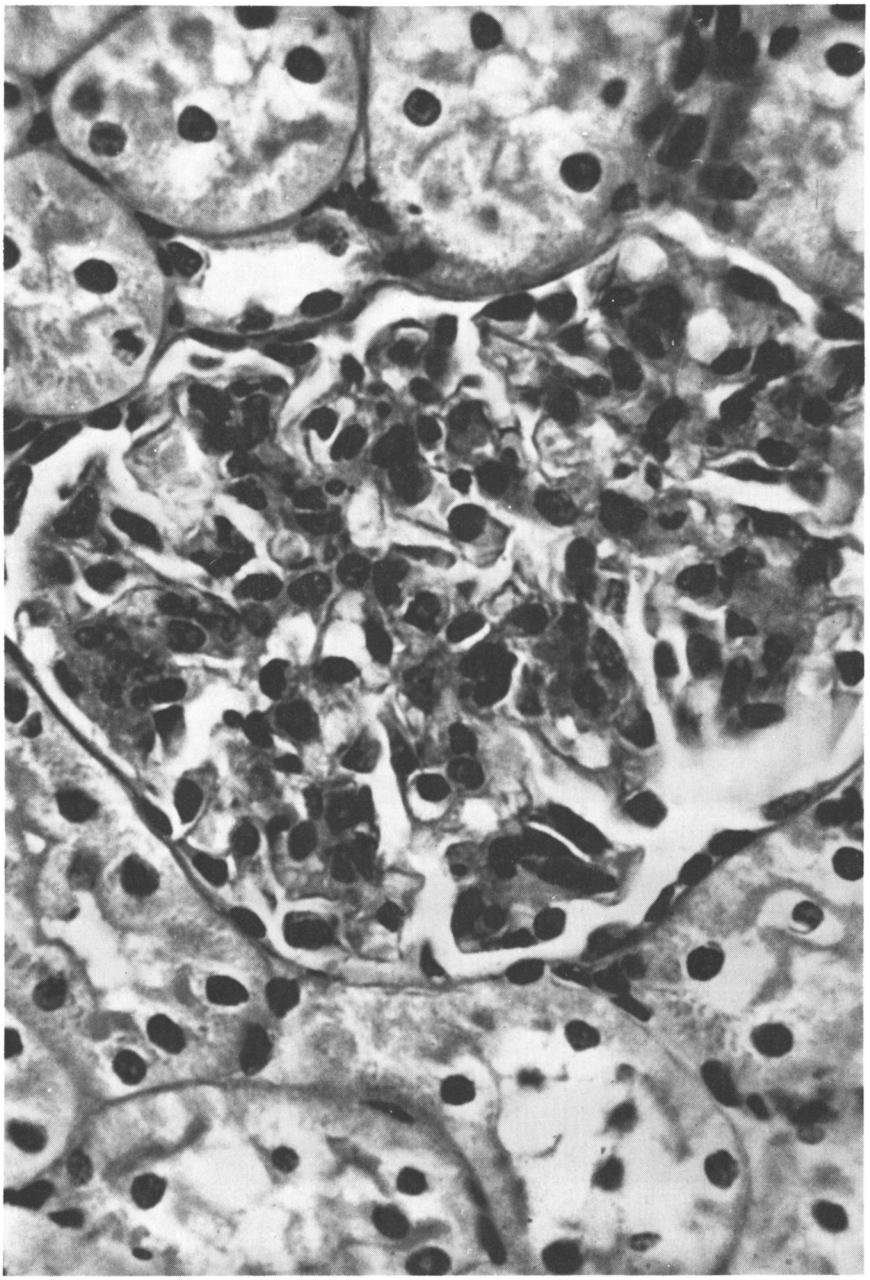

FIG. 2. Representative glomerulus from an animal from Group I showing mild focal glomerulonephritis. Periodic acid-Schiff.

Sialic acid content of all IgG preparations was measured by methods previously described (2). There was a 90% decrease in the sialic acid of IgG after neuraminidase treatment. No significant differences in sialic acid content of the nonneuraminidase control treated preparations were observed and the quantity was similar to IgG before neuraminidase treatment.

Morphologic and immunohistologic studies. All animals were sacrificed 6 wk following the onset of the experiment. Tissue was saved in formalin for light microscopic studies (H&E and P.A.S.).

Sections of cortex were placed in isopentane, quick frozen in liquid nitrogen and stored at −70° for immunohistologic studies using antisera to rat IgG, C^3, and fibrinogen.

Antisera to rat IgG was obtained from Pentex Labs. Fibrinogen was isolated by the method of Shinowara (7) and antisera produced by serial immunization of rabbits. C^3 was prepared from fresh rat serum (8) and antisera was produced by serial immunization of rabbits. Monospecific antisera was labelled with Fluorescein isothiocyanate (FITC). Immunofluorescent methods have been previously described (1). Sera obtained from animals from each group at intervals and at sacrifice were tested for the presence of antibody to non-treated and neuraminidase treated IgG by capillary precipitin tests, Ouchterlony immunodiffusion in agar gel and immunoelectrophoresis.

Results. Animals receiving single or repeated injections of NIgG either intrave-

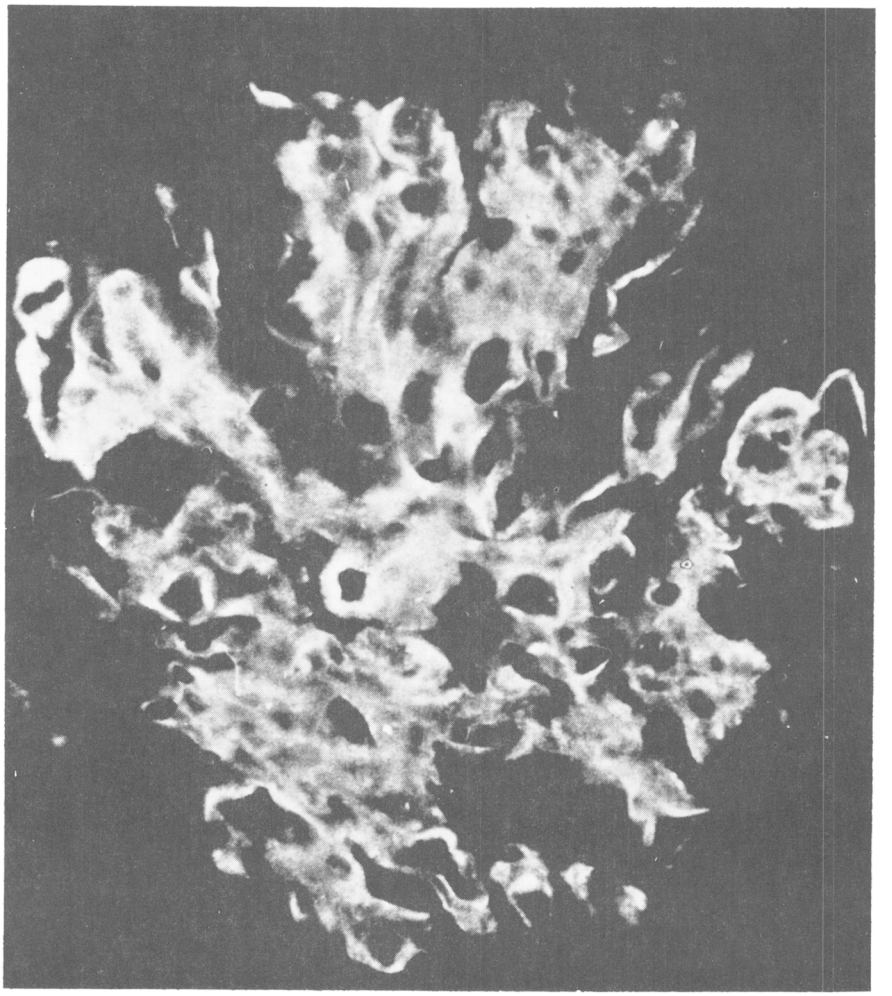

FIG. 3. Glomerulus from an animal from Group II stained with FITC conjugated rabbit antisera to rat IgG.

TABLE I

Group	Materials Used	No. of Animals	Morphologic changes	Immunohistologic changes
I	NIgG I.V. single dose	10 animals	5	10
II	NIgG I.V. two doses	10 animals	7	10
III	NIgG with Freund's single dose ip	10 animals	10	10
IV	NIgG with Freund's two doses ip	10 animals	10	10
V	IgG with acetate buffer single dose iv	10 animals	0	0
VI	IgG with acetate buffer two doses iv	10 animals	0	0
VII	IgG with acetate buffer Freund's single dose ip	10 animals	0	0
VIII	IgG with acetate buffer Freund's two doses ip	10 animals	0	0
IX	Neuraminidase in acetate buffer single dose iv	10 animals	2	2
X	Neuraminidase in acetate buffer two doses iv	10 animals	3	3
XI	Neuraminidase in Freund's single dose ip	10 animals	0	0
XII	Neuraminidase in Freund's two doses ip	10 animals	0	0
XIII	IgG in PBS single dose iv	10 animals	0	0
XIV	IgG in PBS two doses iv	10 animals	0	0
XV	IgG in PBS in Freund's single dose ip	10 animals	0	0
XVI	IgG in PBS in Freund's two doses ip	10 animals	0	0

nously or intraperitoneally in Freund's adjuvant showed morphological and immunohistologic changes (Figs. 1–3). Group IX and X animals receiving injections of Neuraminidase either as single or multiple dosages showed less marked alterations. All other groups showed no abnormalities. Table I summarizes the number of animals with alterations in each group. The extent of morphological or immunohistologic changes in Groups with alterations is summarized in Table II. Glomerular deposition of IgG and C^3 were nodular on the glomerular capillary walls and in the mesangium. Fibrinogen (F) was localized in the mesangium.

Antibodies to both neuraminidase treated IgG and untreated IgG were detected only in animals receiving NIgG. No antibody activity to either was observed in the various control groups.

Discussion. The observation (4) that immune complexes of IgM and IgG depleted of sialic acid composed certain cryoglobulins, the possible role of such complexes in glomerulonephritis and the high incidence of cryoglobulins in glomerulonephritis led to speculation that IgG depleted of sialic acid without other alterations in carbohydrate or amino acid composition may be autoimmunogenic. Several forms of glomerulonephritis are secondary to deposition of immune complexes of autologous (9) or foreign protein (10) antigens and their specific antibody. Altered immunoglobulins may be autoantigenic inciting formation of antibodies of another immunoglobulin class and leading to immune complexes which result in immune deposit renal disease.

These studies suggest that neuraminidase-treated homologous IgG, and neuraminidase administered to rats *in vivo* are associated with a significant percent of animals developing mild to moderate focal glomerular proliferation and mild glomerular basement membrane thickening with immunoglobulin deposits. Adequate controls were not associated with renal lesions. The use of inbred animals for isolation of IgG and for the studies, the variety of control solutions and the careful and controlled conditions used in handling of immunoglobulins together with the absence of pathology in control groups suggest that the disease observed was related to modification of IgG by removal of sialic acid with neuraminidase. The possibility that these changes were due to aggregation of IgG or alterations in the molecule other than those produced by neuraminidase is diminished.

Complexes of immunoglobulin antigen

TABLE II[a]

Group	Morphology[b]		Immunohistology[b]		
	GBM[c]	Proliferation	IgG	B_1C	F
I	+	++	2+	2+	1+
	+	++	2+	3+	1+
	0	++	1+	2+	1+
	0	++	1+	2+	1+
	+	+	1+	1+	1+
	0	0	1+	1+	1+
	0	0	1+	1+	1+
	0	0	1+	1+	1+
	0	0	1+	1+	1+
	0	0	1+	1+	1+
II	+	++	1+	2+	1+
	0	0	1+	2+	1+
	0	+	1+	2+	1+
	0	0	1+	2+	1+
	0	++	2+	3+	1+
	0	0	1+	1+	1+
	+	++	2+	3+	1+
	0	++	2+	3+	1+
	0	+	1+	1+	1+
	+	++	1+	1+	1+
III	+	++	2+	2+	1+
	0	++	1+	3+	1+
	+	+	1+	1+	neg
	0	+	1+	1+	neg
	0	+	1+	1+	1+
	+	++	2+	3+	2+
	0	+	1+	1+	neg
	0	+	1+	2+	1+
	0	+	1+	1+	1+
	0	+	1+	1+	1+
IV	0	+	1+	1+	1+
	+	++	2+	3+	1+
	0	+	1+	1+	1+
	+	++	1+	2+	1+
	0	+	1+	1+	neg
	+	++	1+	2+	1+
	0	+	1+	1+	1+
	0	+	1+	1+	1+
	0	+	1+	1+	1+
	+	++	2+	3+	1+
IX	1+	1+	1+	1+	neg
	0	1+	1+	1+	neg
X	1+	1+	1+	1+	neg
	1+	1+	1+	1+	neg
	1+	1+	1+	1+	neg

[a] Table II summarizes the morphologic and immunohistologic changes in animals with alterations.

[b] Graded from 0 to 4+.

[c] Basement membrane thickening.

and antibody especially rheumatoid factors have been implicated in a variety of immune mediated diseases and often appear in the serum as multicomponent cryoglobulins (3). We have produced immune deposit nephritis in rabbits by introduction of autologous serum and autologous IgG chemically modified by reduction in hexoses and sialic acid by treatment with a culture of B hemolytic streptococcus (10). In this model rheumatoid factor polyclonal single component cryoglobulins were detected in the serum.

These preliminary observations raise speculation that microorganisms with enzymes such as neuraminidase may incite immune deposit nephritis by deposition of complexes of altered immunoglobulins and their specific antibody. Investigations of the antigenicity of sialic acid free autologous IgG by the humoral and cellular antibody responses are being conducted. In addition, the incidence and nature of cryoglobulins and rheumatoid factors in a similar model is being studied to determine the nature of immune complexes.

Summary. Immune deposit renal disease followed intravenous or intraperitoneal injections of neuraminidase treated homologous IgG or neuraminidase alone. No alterations were associated with several groups of controls. This preliminary study suggests that one mechanism by which microorganisms may be involved in the development of immune renal disease is by chemical alteration of immunoglobulin.

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