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Received June 22, 1960. P.S.E.B.M., 1960, v104.

Production of Acute Glomerulonephritis in Mice with Soluble Antigen-Antibody Complexes Prepared from Homologous Antibody.* (25960)

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Soluble antigen-antibody complexes, injected intravenously in antigen excess, are capable of producing the characteristic lesions of experimental serum sickness within a short period of time. When large amounts of either rabbit antiovalbumin-ovalbumin or rabbit anti-BSA-BSA (bovine serum albumin) complexes are injected into mice or rats a high incidence of acute glomerulonephritis results. In addition a focal arteritis and a valvulitis may be seen on occasion(1-4). Fluorescent labeled antibody technics have shown both the antigen and the antibody to be present at the site of the lesion. In the present study these observations have been extended to production of lesions with soluble complexes prepared from homologous mouse antibody. Antiovalbumin was produced in mouse ascitic fluid according to the method of Munoz(5) and compared with the corresponding rabbit antibody with regard to its ability to elicit glomerular lesions when administered to mice as soluble antigen-antibody complexes. The incidence and severity of anaphylaxis in both systems was studied. The effect of varying the antigen to antibody ratio in preparation of these complexes was also explored.

Materials and methods. Animals: Swiss Webster male and female mice weighing 20 to 30 g. *Antigens*: Hen ovalbumin, 3 times recrystallized, from Worthington Biochemical Co., Freehold, N. J. *Immunization*: Rabbit antisera against hen ovalbumin were prepared as previously described(3). Mice were immunized according to the method of Munoz. An emulsion containing 1 mg ovalbumin and 1 mg *Mycobacterium butyricum* per ml was prepared with Difco complete adjuvant. One ml was injected into the peritoneal cavity of each mouse on days 1, 18, and 38. Between 50 and 80% of the mice became ascitic, and between 1.0 and 15.0 cc of fluid was obtained from each animal. These collections were pooled. The various pools of antibody were analyzed for antibody protein content and antigen-antibody ratio at equivalence by the quantitative precipitin technic(6). Precipitates were analyzed for antibody protein by UV absorption at 280 mu in the case of rabbit antibody(7), and by the microKjeldahl method in the case of mouse antibody(6). Rabbit antiovalbumin. Pool I which contained 4.2 mg antibody protein/ml. Pool II which contained 4 mg antibody protein/ml. Mouse antiovalbumin Pool I which contained 8 mg antibody protein/ml. Pool II which contained 6.2 mg antibody protein/ml.

Preparation and injection of soluble complexes. Soluble complexes for injection into mice for the experiments on glomerulonephritis were prepared by direct addition of excess antigen to either the rabbit antisera or mouse ascitic fluid at room temperature. Volume was adjusted to 0.5 ml per injection and injected into the tail vein within 5 minutes. For the studies on anaphylaxis soluble complexes were prepared as previously described, by dissolving the washed immune precipitate in antigen excess(3). *Preparation of histolo-*

* Aided by grants from U. S. P. H. S. and from Kidney Disease Fn. of N. Y.

TABLE I. Incidence and Severity of Glomerulonephritis in Mice Given 3 Injections of Soluble Antigen-Antibody Complexes in 24 Hr, and Killed 24 Hr after 3rd Injection.

Material inj.	No. of mice	No. of mice with glomerulonephritis			
		Mild	Moderate	Severe	Total
Soluble complexes*	12	3	4	5	12
Antibody alone	10	0	0	0	0
Antigen "	3	0	0	0	0
Normal mice	33	0	0	0	0

* 2, 3, and 3 mg mouse antiovalbumin antibody protein/inj. as soluble complexes in 5 times antigen excess.

gic sections. The mice were sacrificed by decapitation 24 hours after last injection and tissues were fixed in 10% buffered formalin. Sections of heart, lung, liver, spleen, and kidney were routinely stained with hematoxylin and eosin.

Results. A preliminary series of experiments showed that doses of one mg or more of mouse antibody protein in the form of soluble complexes in 10 times antigen excess, produced a mortality from anaphylaxis of nearly 100%. To reduce incidence of anaphylactic deaths, animals used in the studies on glomerulonephritis were first given 0.25 mg of antibody protein in 5 times antigen excess one to 2 hours prior to administration of the larger doses. Animals so treated suffered no mortality from subsequent injections.

A group of 12 mice were given a series of 3 injections of mouse antiovalbumin in the form of soluble complexes in 5 times excess antigen. Each mouse received 2 mg antibody protein in the first injection and 3 mg in each of the 2 subsequent injections, all within 24 hours. In addition 3 animals were given antigen alone and 10 were given antibody alone in the same doses.

Incidence and severity of the lesions observed in these animals are shown in Table I. Glomerulonephritis was present in all of the experimental animals.

The nature of the glomerular lesion was similar in all of the animals. Both kidneys were uniformly involved. The glomeruli were hypercellular, had swollen cells, were relatively bloodless, and were infiltrated with neutrophiles; in severe cases karyorrhexis was present (Fig. 2). In many of the animals a striking accumulation of amorphous, eosinophilic material was seen in the glomerular capillary loops (Fig. 3).

Two animals showed a mild acute inflammation of the mitral valve. These lesions were similar to those previously described (3).

Since the intensity of the glomerular lesions was equal to or greater than that previously seen when rabbit antibody was used, an attempt was made to compare the two systems and to determine minimal effective dose of mouse antiovalbumin antibody necessary to produce renal lesions.

Two groups of 12 mice each were given a series of 3 injections of soluble complexes in 24 hours. The first group received 1 mg of antibody protein per injection as ovalbumin-rabbit antiovalbumin in 5 times antigen excess. The second group received exactly the same schedule of treatment, but mouse antiovalbumin was used instead of the rabbit antibody. A third group of 6 animals were given mouse antibody in the same manner as the second group but received only 0.25 mg antibody protein/injection. A fourth group of 3 mice were given a single injection of 2.0 mg antibody protein in the form of ovalbumin-mouse antiovalbumin complexes in 5 times antigen excess. These last animals were survivors from an anaphylaxis experiment described below, and did not receive previous in-

TABLE II. Comparative Ability of Soluble Antigen-Antibody Complexes Prepared from Mouse and Rabbit Antiovalbumin to Produce Acute Glomerulonephritis in Mice.

Soluble complexes made with	No. of mice	No. of mice with glomerulonephritis			
		Mild	Moderate	Severe	Total
Rabbit antibody 1 mg × 3*	12	4	0	0	4
Mouse antibody:					
1. 1 mg × 3*	12	5	5	1	11
2. .25 mg × 3†	6	0	0	0	0
3. 2 mg × 1‡	3	2	1	0	3

* 1 mg antiovalbumin protein/inj., 3 inj., complexes in 5 times antigen excess.

† .25 mg antibody protein/inj., in 10 times antigen excess.

‡ One inj. of 2 mg antibody protein, in 5 times antigen excess.

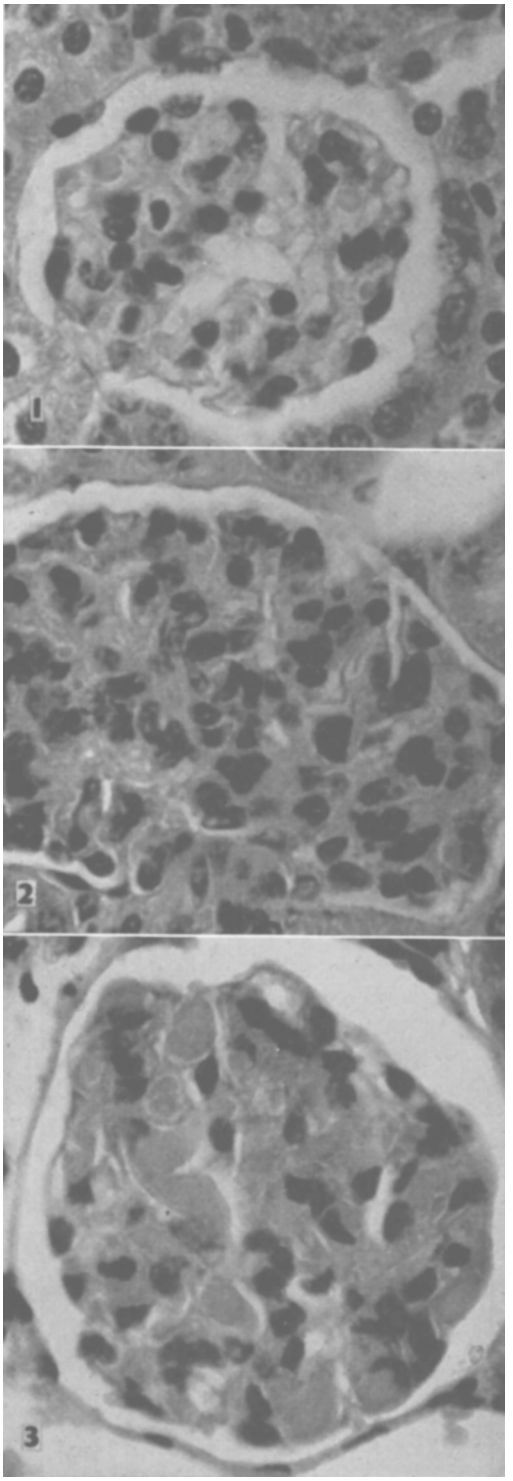

FIG. 1. Kidney of normal mouse. Hematoxylin and eosin stain. ×880.

jections. Incidence and severity of the lesions in the 4 groups of mice are indicated in Table II.

It is apparent from the results presented that soluble complexes prepared with mouse antibody are more effective than those prepared with rabbit antibody in producing glomerulonephritis in mice. Furthermore a single injection containing as little as 2 mg of mouse antibody protein in 5 times antigen excess can produce mild acute glomerulonephritis within 24 hours.

Since administration of soluble antigen-antibody complexes is invariably followed by anaphylaxis, the relative abilities of the mouse and rabbit systems to elicit the reaction was investigated. The effect of varying ratio of antigen to antibody on occurrence of anaphylaxis was also studied. The experimental data are shown in Table III.

As in production of experimental glomerulonephritis, mouse antibody also proved to be much more effective than rabbit antibody in producing anaphylaxis. In both systems, a decreased incidence of anaphylaxis was observed when either antigen to antibody ratio or dose of antibody protein was reduced.

Discussion. Intravenous administration of soluble antigen-antibody complexes to mice, using homologous antibody in the form of immune ascitic fluid, results in a very high incidence of severe acute glomerulonephritis. The lesions are more severe than those produced with equivalent amounts of rabbit antibody. The greater pathogenicity of complexes prepared with mouse antibody is further emphasized by their ability to produce the characteristic pathologic changes within 24 hours after a single injection containing 2 mg antibody protein.

FIG. 2. Kidney of a mouse given 3 inj. of ovalbumin-mouse antiovalbumin complexes, 2, 3, and 3 mg antibody protein per inj. respectively, and sacrificed 24 hr later. Glomerulus is hypercellular, bloodless, and infiltrated with neutrophiles. Hematoxylin and eosin stain. ×880.

FIG. 3. Kidney of a mouse given 3 inj. of ovalbumin-mouse antiovalbumin complexes, 2, 3, and 3 mg antibody protein per inj. respectively, and sacrificed 24 hr later. A striking accumulation of amorphous, eosinophilic material is seen in glomerular capillary loops. Hematoxylin and eosin stain. ×880.

TABLE III. Effect of Varying Ratio of Antigen to Antibody and Dose of Antibody on Production of Anaphylaxis: A comparison of rabbit and mouse systems in the mouse.

Antibody protein (mg)	Antigen excess (times)	Mouse antiovalbumin		Rabbit antiovalbumin	
		No. mice	No. deaths	No. mice	No. deaths
.25	5	5	0	5	0
	10	5	2	5	0
	20	5	3	5	0
	50	5	3	5	0
1	5	15	11	16	4
	10	18	14	5	2
	20	4	3	5	3
	50	6	6	11	8
2	5	8	5	5	3
	10	4	3	5	3
	20	2	1	5	5
	50	—	—	5	5

The appearance of the glomeruli was essentially the same in both systems except for the marked accumulation of amorphous eosinophilic material in capillary loops of those animals given complexes prepared with homologous antibody. Similar findings had been previously observed in animals given soluble rabbit complexes following pretreatment with cortisone(3). In those animals this material was shown to contain both antigen and antibody by the Coons' technic and was presumed to be precipitated complexes. In the present experiments these findings may be explained by the low levels of antigen excess used. Since ovalbumin is normally excreted by kidneys of mice, use of only slight excess antigen would favor aggregation of the circulating soluble complexes in the glomeruli.

Finally, the observations on occurrence of anaphylaxis in mice treated with soluble complexes prepared with mouse antiovalbumin in comparison with rabbit antiovalbumin indicate that homologous is considerably more effective than heterologous antibody. The data concerning variations of antigen to antibody ratios and dosage of antibody are substantially in accord with those previously reported by other investigators(8,9).

Summary. 1) Passive serum sickness was produced in mice using homologous antibodies, obtained from immune ascitic fluid, in the form of soluble antigen-antibody complexes. Lesions were similar to those obtained with rabbit antibody-antigen complexes, but, in addition, amorphous eosinophilic material was seen in many glomerular capillary loops. Incidence and intensity of lesions using homologous antibody was greater than with equal amounts of heterologous rabbit antibody. 2) Homologous antibody was more effective in producing lethal anaphylaxis than rabbit antibody when both were given as soluble complexes.

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