Role of Aggregated Bovine Serum Albumin in Pathogenesis of Serum Sickness Nephritis in Rabbits.* (32056)

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The serum sickness model of experimental glomerulonephritis in rabbits has many features similar to those of acute poststreptococcal glomerulonephritis in man. The latent period(1), the fall in complement(2,3), similar histologic, immunofluorescent and ultrastructural findings(4) in both situations suggest that the pathogenetic mechanisms are similar. It is believed that glomerular injury in the experimental and clinical disease is mediated by glomerular deposition of antigen-antibody complexes(5-8).

Although nephritis of experimental serum sickness and acute poststreptococcal glomerulonephritis are similar, a degree of disparity exists because of the large doses of bovine serum albumin required to produce the experimental glomerular lesions.

It is the purpose of this paper to attempt a dissection of the immunologic processes responsible for serum sickness nephritis produced in rabbits with bovine serum albumin. We initially postulate that only a very small portion of the bovine serum albumin (BSA) exists in the form of spontaneously occurring aggregates (probably dimers, trimers and polymers), that this aggregated component of the BSA is related to the antigenicity of this protein and provides the stimulus for antibody poduction. After antibody formation soluble complexes are formed by reacting with the soluble non-aggregated components of the bovine serum albumin that remain in the circulation and have not participated in induction of antibody formation. This leads to the glomerular deposition of the complex and results in glomerular injury as originally pointed out by Germuth(7) and Dixon *et al* (6). The experiments to be presented here show that heat-aggregated BSA, although inducing regular antibody production in rabbits, does not lead to serum sickness. However, in animals so stimulated a subsequent injection of soluble BSA leads to the antigen antibody interaction in the circulation, immune elimination of the injected BSA and the development of lesions of glomerulonephritis.

Materials and methods. Animals: New Zealand white rabbits weighing one to two kilograms were used in all experiments. Materials: Three preparations of bovine serum albumin (Armour, Kankakee, Illinois) were used. (a) Unmodified BSA—prepared as a 6 g % solution in a .01 M phosphate buffered saline pH 7.3. (b) Deaggregated BSA—prepared as a 10 g % solution in phosphate buffered saline and centrifuged in a Spinco model L preparative ultracentrifuge in 35 cc aliquots at 105,000 × *g* for 5 hours. The upper two-thirds of each aliquot was used as the deaggregated preparation. This preparation was analyzed in the analytical ultracentrifuge‖ and compared with a sample taken from the lower third of the tubes. Only a slowly sedimenting peak representing BSA was observed in both preparations. The absence of a more rapidly sedimenting peak suggested that aggregates of BSA were not present in sufficient concentration to be detected by this method. (c) Aggregated BSA —prepared as a 6 g % solution of BSA and heat aggregated by the method of Iio and Wagner(9).

Antigen clearance was monitored every 24 to 48 hours by analysis of serum samples from the experimental animals using the capil-

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TABLE I. Effect of Modifying BSA Solutions on the Incidence of Glomerular Localization of IgG and BSA, on Antigen Elimination and on Formation of Anti-BSA in Rabbits.

	BSA administration		Glomerular fluorescent microscopy		Antigen elimination		Serum anti-BSA antibodies	
	Day 0	Day 7	IgG	BSA	Day	No. rabbits	Day	No. rabbits
Experiment 1								
Group A	deaggregated 250*	—	2/12†	0/12	10–12	12/12	12	10/11
Group B	unmodified 250	—	5/12	4/12	10–12	12/12	12	10/12
Experiment 2								
Group C	aggregated 25	unmodified 100	5/11	5/11	11–15	11/11	10	0/10
							14	4/4
Group D	aggregated 25	aggregated 100	0/9	0/9	8	10/10	11	7/9
Group E	aggregated 25	unmodified 100	4/12	3/12	11–15	11/11	not tested	
					17	1/1		
Group F	unmodified 25	unmodified 100	1/12	1/12	12–14	8/12	14	4/8
					16	4/4	21	5/12

* BSA dose in mg per kg body wt.
† The numerator denotes the number of positive animals and the denominator denotes the number tested.

lary precipitin technique with rabbit anti-BSA serum. At the time of complete antigen elimination open renal biopsies were performed under pentobarbital sodium anesthesia, using a clean, non-sterile technique. The tissues were stained with rabbit anti-bovine serum albumin and goat antirabbit gamma-globulin antisera conjugated with fluorescein-isothiocyanate; preparation of antisera and immunofluorescent methods employed in this study are similar to those previously described from this laboratory(4,10). Glomerular localization of rabbit IgG and BSA was used as evidence of glomerular injury. Following antigen elimination, presence of circulating antibody to BSA was detected by the capillary precipitin technique.

Experimental groups. Twelve rabbits were used in each group (Table I). All BSA preparations were given intravenously by the marginal ear vein.

Experiment I. The rabbits in Group A received 250 mg per kg body weight of deaggregated BSA on day 0. Those in Group B received 250 mg per kg of unmodified BSA on day 0.

Experiment II. The rabbits in Group C received 25 mg per kg of aggregated BSA on day 0 and 100 mg per kg of unmodified BSA on day 7. Those in Group D received a similar dose of aggregates on day O, but received 100 mg per kg of aggregated BSA on day 7. Renal biopsies in this group were not performed at the time of antigen elimination (day 8) but were done on days 11, 12 and 14. Group E rabbits received 25 mg per kg of aggregated BSA on day 0 and 100 mg per kg of unmodified BSA on day 8. Rabbits in Group F received 25 mg per kg of unmodified BSA on day 0 and 100 mg per kg of unmodified BSA on day 7.

Results. The effect of the 3 preparations of BSA upon the subsequent development of experimental nephritis is summarized in Table I. Glomerular localization of rabbit IgG occurred in only 2 of 12 animals in Group A, but in 5 of 12 in Group B. The localization of antigen was not seen in any animals of Group A, but was noted in 4 of 5 animals with IgG localization in Group B. Antigen elimination occurred however at about the same time in Groups A and B, and antibody was subsequently detected in the sera of all animals.

In Group C, glomerular localization of rabbit IgG and BSA occurred in 5 of 11 animals. One animal died of anaphylaxis, after receiving the unmodified BSA on day 7. In Group D, rabbit IgG could not be detected in any of the 9 animals examined; 3 animals died of anaphylaxis after receiving the aggregated BSA on day 7. In Group E glo-

merular localization of rabbit IgG occurred in 4 of 12 animals, whereas this was seen in only 1 of 12 animals in Group F.

Antigen elimination from the circulation occurred between days 11 and 15 in 22 of 23 rabbits of Groups C and E. In Group D where only aggregated BSA was used, no BSA was detected 24 hours after its administration. In Group F although glomerular injury was present in only 1 of 12 rabbits, antigen elimination by day 14 was found in 8 of 12 rabbits. A similar disparity was observed above in Group A where antigen elimination occurred in all animals by day 12 but glomerular localization of IgG was observed in 2 of 12 rabbits.

Discussion. Thorbecke, Maurer and Benacerraf have clearly demonstrated that modification of BSA by denaturation and aggregation will enhance the rate of its removal by the reticuloendothelial system(11). Work by Dressser, Claman and Battisto and Miller has shown that prior centrifugation of BGG will increase the ability of the supernatant to induce tolerance(12-14). This would be consistent with the concept that centrifuged BGG is less avidly phagocytized and does not induce a primary immune response. The necessity for phagocytosis of antigen in the production of an immune response is well demonstrated in a recent report by Frei, Benacerraf and Thorbecke(15). In their study the phagocytized portion of a BSA preparation (spontaneous protein aggregates) was removed by using a "filter" animal. They were able to demonstrate a marked impairment of of antibody production to form antigen-antimals which subsequently received the "filtered" BSA.

We have attempted to extend these observations to serum sickness nephritis in rabbits induced by BSA. Our results are consistent with the hypothesis that a very small part of the total BSA consists of spontaneously occurring aggregates of BSA which are rapidly phagocytized. In order to produce serum sickness nephritis there must be both aggregated BSA for induction of antibody response and BSA circulating free at the time of antibody production to form antigen-antibody complexes.

Our attempt to remove the portion of BSA which is phagocytized, by ultra-centrifugation was only partially successful. Antibody production and immune elimination appeared to occur in a similar fashion in both Groups A and B. This suggests that the deaggregated BSA preparation must still have contained a sufficient concentration of aggregates to induce a primary response. However the group receiving unmodified BSA (Group B) had a higher incidence of glomerular localization of rabbit gamma globulin.

We have demonstrated that both aggregated and free BSA are necessary for the production of serum sickness nephritis (Group C and E). Free circulating BSA (Group F) or aggregated BSA (Group D) alone are insufficient. While unmodified BSA administered on day 7 in Groups C and E does contain a small amount of spontaneously aggregated antigen, it seems unlikely that this was responsible for the antibody production at the time of antigen elimination.

In both Group A and Group F the experimental conditions were manipulated in an effort to reduce the amount of aggregated antigen presented to the animals. Although the majority of the animals eliminated the free BSA between days 10-14, there was a marked reduction in the incidence of glomerular injury. These observations may reflect an alteration in the quantity, nature and kinetics of both antibody synthesis and antigen-antibody complex formation.

These data provide an additional explanation for the first part of the 3-phased curve of antigen elimination from the circulation as reported by Dixon, Vazquez, Weigle and Cochrane(6). In addition to being due to equilibration of antigen between intra and extra vascular compartments it may also reflect the rapid removal by cells of the reticuloendothelial system of the spontaneously aggregated BSA. The antigenic component of BSA may indeed be a very small fraction of the total BSA.

One of the difficulties in accepting serum sickness nephritis in rabbits as the experimental counterpart of acute poststreptococcal glomerulonephritis in man, is the massive doses of BSA (250 mg/kg) needed to produce

the experimental disease. Although we were able to induce nephritis in experimental Groups C and E with a total dose of BSA half that previously used by other workers(4,5,7) it is necessary to emphasize that following the administration of unmodified BSA on day 7, the amount of BSA present in the circulation is either the same or more than that present at this time in the experiments of the other investigators. Hence, the amount of soluble antigen-antibody complexes formed in previous experiments may well be the same as those involved in this study. The total dose of 125 mg BSA/kg was arbitrarily chosen and conceivably much smaller doses of aggregated and unmodified BSA, given at the appropriate times would produce the same incidence of nephritis. It would then be possible to lower the dose of antigen to a level more comparable to the situation in poststreptococcal glomerulonephritis in man.

Summary. 1. A working hypothesis concerning the nature of serum sickness involving a dissection of the antigen into "antigenic" component and "combining" component of the antigen is presented. 2. Evidence is presented that injection of aggregated BSA in amounts capable of inducing an immune response does not lead to the renal lesions of serum sickness in the rabbit. If, however, this stimulus was combined with subsequent administration of soluble BSA serum sickness nephritis occurred. 3. BSA centrifuged for 5 hours at 105,000 $\times$ *g* had lesser capacity to induce serum sickness than did an unmodified BSA. 4. These observations are consistent with the view that the production of serum sickness nephritis can be dissected into two separate components which can be separately manipulated.

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Antiviral Activity of Higher Plants and Propionin on Lymphocytic Choriomeningitis Infection. (32057)

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In a previous paper(1) it was shown that two higher plants emerged as antiviral drugs from a screening program of Chinese medicinal agents. They were active at the animal level against lymphocytic choriomeningitis (LCM) infection. In this paper, we report further studies of the two plants, and of propionin, an antibiotic, which has newly been found active against LCM virus in mice. These natural products have also been compared with methotrexate which was reported to have antiviral action by Hass and Stewart (2) and Sidwell *et al*(3).

Materials and methods. 1. *Higher plants.*