Secondary Macroglobulin Antibody Responses to Plasma Proteins in Rabbits.* (30710)

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Recent studies have created the impression that the exuberant production of antibody in the secondary response is characteristically a phenomenon of γ-G (7S) immunoglobulin synthesis. Production of γ-M (19S) antibodies has generally exhibited little, if any, more vigor in the secondary than in the primary response.

With bovine gamma globulin and bovine serum albumin (BSA) in rabbits, for example, secondary responses were characterized by great augmentation of γ-G synthesis, whereas γ-M production was not increased (1,2). With poliovirus in rabbits(3) and bacteriophage φX 174 in guinea pigs(4) enhanced synthesis of γ-M neutralizing antibodies was observed following a second injection of antigen, but only if the second dose was given within a few days after the peak of primary γ-M production; while with longer intervals adequate doses elicited profuse secondary synthesis of γ-G antibodies, the γ-M titers were equal or inferior to those seen in the primary response.

Unlike the above, many systems exhibit exclusively or predominantly γ-M antibody synthesis. Forssman antigens in rabbits, for instance, are reported to elicit mostly or entirely γ-M hemolysins(5). Similarly, a preponderance of γ-M immunoglobulins is reported in the successive responses of chickens to BSA(6). These systems do not show secondary responses like those typical of soluble proteins and many other antigens. The induction period following second injections of antigen may be shortened, but there is little or no increase in peak titers. Only the work of Borel *et al* with erythrocyte antigens indicates that in special situations some increase in γ-M antibody production may occur with repeated injection of antigen(7).

The common feature of the reports summarized is that γ-M antibody formation does not show vigorous secondary responses. The studies presented here, on the other hand, demonstrate that under some circumstances the secondary response may exhibit pronounced enhancement of γ-M antibody production.

Materials and methods. New Zealand white or Polish rabbits were injected intravenously with bovine or human serum albumins (BSA, HSA) in aqueous solution. The tests for antibody utilized a semi-micro procedure for agglutination of antigen-coated, tannic acid-treated sheep erythrocytes, with dilutions performed by an automatic machine(8). Reported titers were based on the last dilution showing at least one-plus agglutination according to Stavitsky(9). Such titers are generally 4- to 8-fold lower than those based by some workers on the last dilution showing any agglutination whatever.

Differentiation of antibody activity associated with the 7S and 19S globulins was made by both 2-mercaptoethanol (2-ME) and sucrose density gradient ultracentrifugation. Sera were treated with 2-ME by incubating (45 minutes at 37°C) a mixture of equal parts of 1:4 serum in saline and 0.01 M 2-ME. The resulting 1:8 dilution was used as the initial dilution for the titration, compared with a control serum treated in the same way with saline solution instead of 2-ME.

The density gradient ranged from 10 to 40% sucrose as determined by refractive indexes. Onto a gradient of total volume 5.2 ml was placed 0.2 ml of a 1:2 or 1:8 dilution of serum in saline. After 18 hours' centrifugation (swinging-arm head) at 35,000 rpm, the gradient was divided into 13 fractions, each of which served as the initial dilution for a titration.

Calculations of composite titers for each of the two chief classes of immunoglobulins were based on the assumption that quantities of agglutinating activity in several fractions may

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TABLE I. Sample Determination of Composite 19S and 7S Hemagglutinin Titers to HSA in a Polish Rabbit.

Gradient fraction	Reciprocal titer	Composite titer	Titers by 2-ME
Top	1		
12	1		
11	±	*7S*	
10	4	210	160*
9	16		
8	16		
7	4		
6	4		
5	32	*19S*	
4	32	395	480†
3	8		
2	2		
Bottom	1		

* Titer of serum treated with 2-ME.
† Titer of untreated serum less titer of serum treated with 2-ME.

be added. The reciprocal titers from all fractions containing 19S antibody were summed and multiplied by an overall dilution factor (2.5 times the dilution of serum placed on the gradient). The value obtained is considered equal to the reciprocal titer which would be obtained if 19S antibody alone could be determined on the original whole serum. Similar computation gave a composite titer for 7S antibody. Table I illustrates a sample situation. Titers from the gradient fractions on an antiserum (from those presented below) are shown along with the composite titers and the titers obtained with 2-ME treatment. The good agreement seen here between results with the two methods is typical of all the sera studied.

Results. Fig. 1 presents representative data on primary and secondary responses of Polish rabbits to 20 mg HSA. Complete or partial data from 5 additional animals in this experiment indicate that the individual rabbit shown here was typical. The rabbits received the second dose of antigen 6 months after the first, when the primary antibody was no longer detectable by hemagglutination. It is clear that vigorous production of both 19S and 7S antibodies occurred in the secondary response. The 19S antibody appeared much earlier than in the primary response. In the animal shown here it attained a titer more than 20-fold greater than that observed in the primary response. In other animals in the series the increase ranged up to 100-fold.

Partial data from New Zealand white rabbits injected with BSA agree with those presented here. As in the experiment summarized above, the secondary responses exhibited much more rapid and abundant production of both 19S and 7S hemagglutinins than did the primary responses.

Discussion. These data apparently conflict with the previous studies summarized above (1-6). The types of antigens or the experimental animals employed can hardly be responsible, since some of the results which differ from ours utilized BSA in rabbits(2). Dispute over the relative hemagglutinating capacity of different classes of immunoglobulins (see 6) does not seem pertinent, for the data presented here contrast the production of each class of antibody in the secondary response with the primary production of the same class of antibody. Nicholas and Johnson have independently obtained results similar to ours with human gamma globulin in mice.

One possible explanation for the apparent discrepancy revolves about the reported suppression of 19S antibody production by circulating 7S antibody(10). In our work there was no detectable antibody at time of reinjection. In most of the previous studies discussed the second injection of antigen took place while primary antibody was still present and possibly capable of suppressing 19S globulin production.

Summary. Rabbits reinjected with human

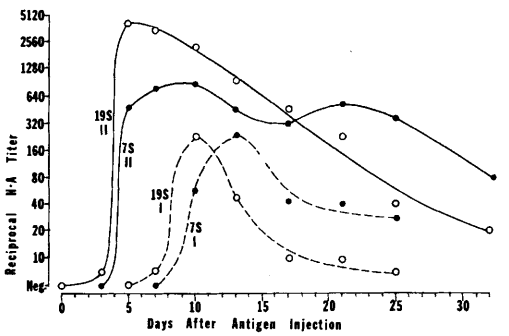

FIG. 1. Course of hemagglutinin titers in 7S and 19S fractions of sera from a Polish rabbit undergoing primary (I) and secondary (II) responses to HSA.

or bovine serum albumins showed vigorous secondary-type responses in both 19S macroglobulin and 7S gamma globulin antibody production. The data indicate that capacity for a secondary response is not confined to 7S gamma globulin synthesis.

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Parainfluenza 2 Virus: Increase in Hemagglutinin Titer on Treatment With Tween-80 and Ether.* (30711)

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Treatment of myxoviruses with ether results in loss of infectivity and the release of hemagglutinin (HA)(1,2,5,7). The amount of recoverable hemagglutinin measured as guinea pig red cell agglutinin titer varies for different viruses. Compared with the HA titer of the virus preparation prior to ether treatment, there is a slight increase for Newcastle disease virus and a marked fall for parainfluenza 1 and mumps viruses(8). On the other hand, treatment with a detergent and ether results in moderate elevation of the HA titer for influenza A & B(4), Sendai and Newcastle disease viruses (fowl red cells)(9) and a marked elevation for measles virus(6).

Because of the frequent use of parainfluenza and mumps viruses in serodiagnosis by hemagglutination inhibition (HI) titration, these viruses were treated with a detergent (Tween-80) and ether in an attempt to enhance their HA titer. The results are shown in Table I. This paper reports in detail studies done on parainfluenza 2, because of the marked enhancement of HA titer as shown in Table I.

TABLE I. Tween 80-Ether (T-E) Treatment of Myxoviruses.

Virus	HA titer before T-E treatment	HA titer after T-E treatment	Rise in titer
Uninfected monkey kidney cells	<1:2	<1:2	—
Parainfluenza 1	1:8	1:8	0
" 3	1:64	1:128	× 2
Mumps	1:128	1:512	× 4
Parainfluenza 2	1:16	1:512	×32

Parainfluenza 2 virus was obtained from Drs. Helen Coates and Robert M. Chanock (Parainfluenza 2 (CA) MK_5MH_3—9-27-63—Bethesda). In our laboratory it was passed once in primary rhesus monkey kidney cell monolayers (Flow Laboratories) maintained at 37°C in medium 199 in Earle's BSS without serum. On the sixth day, the virus was harvested by scraping the cell sheet down and triturating vigorously. Tween-80 was added to a final concentration of 0.125% and mixed well by shaking at room temperature for 5

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