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The Effect of 7S and 19S Antibodies on the Primary Response to *Salmonella typhi* Antigens

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Summary. The effect of IgM and IgG antibodies on the primary response to *Salmonella typhi* antigen has been studied. IgG antibodies given before antigenic challenge or combined with antigen in antibody excess profoundly suppressed the formation of agglutinins in rabbits and humans. IgM antibodies appeared to have little or no inhibitory effect when given as complexes in antibody excess or when infused in relatively small amounts. Larger amounts do inhibit agglutinin formation in rabbits. It is suggested that the antibodies exert their inhibitory effect by combining with antigen and removing it as a stimulant for antibody formation.

INTRODUCTION

In newborn infants the presence of maternal 7S (IgG)† antibody to individual antigens has been shown to inhibit production of antibodies in response to those antigens (Osborn, Dancis and Julia, 1952; Vahlquist, 1958; Fink, Miller, Dorward and LoSpalluto, 1962). When antigens complexed with excess IgG antibodies were administered to experimental animals, no antibody response was observed (Uhr and Baumann, 1961). It has been demonstrated both in man and in experimental animals that in the primary response, 19S (IgM)‡ antibodies comprise a large part of the total antibody present within 1 week following antigenic challenge and, within a few weeks, this type of antibody diminishes in the circulation as IgG antibodies increase in concentration (Smith, 1960; Bauer and Stavitsky, 1961; LoSpalluto, Miller, Dorward and Fink, 1962; Fink *et al.*, 1962; Uhr, Dancis, Franklin, Finkelstein and Lewis, 1962; Svehag and Mandel, 1964a, b).

The present work was prompted by the observation that in the course of the primary response, IgG antibodies were formed at a time when titres of IgM antibodies were high (LoSpalluto *et al.*, 1962). It appeared, therefore, that in contrast to the inhibitory effect exerted by IgG antibody, little or no effect on the subsequent formation of IgG antibody could be attributed to the IgM antibodies formed early in the primary response. This report is devoted to a study of the relative inhibitory effects of IgG and IgM antibodies on the response to *Salmonella* antigens. A preliminary communication has appeared elsewhere (LoSpalluto and Fink, 1964).

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† In this paper, 7S and IgG will be used interchangeably.
‡ 19S and IgM will be used interchangeably.

MATERIALS AND METHODS

Experiments with rabbits

Preparation of purified 19S and 7S rabbit antibodies. Adult rabbits with no detectable serum agglutination titres were given 0·1 ml of typhoid H (flagellar d) febrile antigen (Lederle No. 2481-31) in each footpad and bled several times within the first 10 days following immunization. All sera in this and subsequent experiments were heated at 56° for 30 minutes. The sera were pooled and fractionated by addition of 4 M ammonium sulphate solution; the fraction precipitating at 1·8 M, which contained γ-globulins chiefly, was further purified by chromatography on diethylaminoethyl(DEAE)-cellulose as previously described (LoSpalluto *et al.*, 1962). The fractions containing IgM typhoid H agglutinins were pooled, dialysed against 0·01 M phosphate buffer, pH 7·0, and lyophilized.

Two months after the initial immunization, each rabbit received two 0·1 ml booster injections of antigen one week apart. One week following the second booster injection, high titres of IgG agglutinins could be demonstrated in the sera. Rabbits were bled, sera pooled, fractionated as previously indicated, and the chromatographic fractions containing IgG agglutinins were harvested and lyophilized. Samples of the purified IgG and IgM antibody preparations dissolved in saline buffer (pH 7·0, 0·01 M phosphate plus 0·15 N NaCl) were then layered over a 10–40 per cent sucrose gradient and centrifuged at 100,000 ***g*** in a Spinco Model L ultracentrifuge using the SW39 swinging bucket rotor (Britten and Roberts, 1960). Serial fractions were collected through a needle inserted into the bottom of the tube and these fractions were tested for agglutinating activity. Each antibody pool was found to contain either 19S or 7S agglutinins but not both.

Groups of young rabbits with no serum agglutination titres, matched for weight (approximately 700–800 g), were given protein fractions containing 7S typhoid H antibody, 19S antibody, or an equivalent amount of normal rabbit serum protein by intravenous injection. The quantity of antibody globulin administered to each animal was established from the volume of distribution of the antibodies as calculated from the weight of each individual animal. It was assumed that the blood compartment constitutes approximately 10 per cent of the total body weight. It was also assumed that all of the 19S but only 50 per cent of the 7S antibodies remained in the circulation (Cohen and Freeman, 1960). Twenty-four hours after antibody infusion, a serum sample was tested for agglutination titre. Animals with sufficiently high titres were then challenged either subcutaneously or intraperitoneally with 0·03 ml of typhoid H antigen suspended in 0·5–1·0 ml of normal saline. Blood samples were obtained at 9 days and at weekly intervals thereafter for varying periods of time. Some animals received an additional injection of 0·03 ml antigen in saline 7 weeks later.

For the experiments in which antigen–antibody complexes were administered, these were prepared by adding 0·03 ml of the typhoid H antigen, *in vitro*, to a large excess of either IgG or IgM antibody. The presence of excess antibody was verified by tests on the supernatant solutions following removal of agglutinates. After washing five times with 3 ml of saline, the complexes suspended in saline were administered intraperitoneally into matched groups of animals. Another group of rabbits, also matched for weight, was given 0·03 ml of antigen alone.

Following administration of free or complexed antigens, blood samples were obtained from each animal at various time intervals. All sera were allowed to clot at 37° and then heated at 56° for 30 minutes. Each serum was then fractionated on columns of DEAE-cellulose in order to separate IgG from IgM antibodies (LoSpalluto *et al.*, 1962). Aliquots

of the column effluents were then tested for antibodies using standard agglutination procedures (Kolmer, Spaulding and Robinson, 1951). Titres were referred back to the volume of serum from which fractions were obtained.

Observations in newborn infants

It was possible to study the influence of IgG, IgM and mixture of both *Salmonella* antibodies, acquired in the course of an exchange transfusion, on the response to these antigens in newborn infants who had no circulating antibody to the *Salmonella* antigens. Blood samples were obtained from each subject before and after exchange transfusion as well as from the donor. Sera were heated at 56° for 30 minutes. Both the post-exchange and donor were tested and shown to have similar titres. Within 48 hours following exchange transfusion, the infants received by intramuscular injection 0·25 ml of a typhoid–paratyphoid (TPT) vaccine (Lederle) containing 10^9 organisms of *Salmonella typhi* per ml and $2{\cdot}5 \times 10^8$ *paratyphi* A and B per ml. Injections were repeated at weekly intervals and blood was obtained 1 week following the third injection.

An aliquot of each serum, equilibrated with 0·01 M phosphate buffer, pH 9·0, by dialysis, was fractionated and tested as described for the rabbit sera.

RESULTS

PASSIVE TRANSFER OF ANTIBODY INTO RABBITS

Four groups of young rabbits with no pre-immune antibody titres were given either IgG typhoid H antibody, two levels of IgM typhoid H antibody or normal rabbit serum

TABLE 1

PRODUCTION OF ANTI-TYPHOID H AGGLUTININ BY RABBITS GIVEN IgG OR IgM ANTIBODY PRIOR TO ANTIGENIC CHALLENGE

Group	Days after challenge	Reciprocal agglutination titre					
		IgG fraction			IgM fraction		
		No. with titre	Mean	Range	No. with titre	Mean	Range
Controls	0	0/12	0	–	0/12	0	–
	9	3/11	4	0–20	11/11	197	10–640
	16	11/12	65	0–320	12/12	113	20–320
	23	11/12	108	0–320	12/12	57	10–80
	51	3/3	147	40–320	3/13	60	20–80
	58*	3/3	240	80–320	3/13	93	40–160
IgG	0	17/17	28	10–80	0/17	0	–
	9	1/17	~1	0–10	12/17	32	0–80
	16	2/17	~2	0–20	10/17	31	0–160
	23	2/17	~3	0–40	14/17	24	0–80
	51	5/8	26	0–80	7/8	22	0–40
	58*	7/8	171	0–640	8/8	185	40–640
IgM_1	0	0/10	0	–	10/10	20	10–40
	9	3/10	8	0–40	10/10	148	80–320
	16	10/10	33	20–80	10/10	120	80–160
	23	10/10	74	20–160	9/10	80	0–160
	51	10/10	110	40–160	10/10	28	20–40
	58*	10/10	416	160–640	10/10	115	80–160
IgM_2	0	0/14	0	–	14/14	126	80–320
	9	1/14	~1	0–10	8/14	20	0–80
	16	5/14	9	0–40	9/14	39	0–320
	23	9/14	39	0–80	9/14	19	0–80
	30	5/8	16	0–40	5/8	10	0–20

* Samples obtained 1 week following booster injection of 0·03 ml antigen.

by intravenous injection. Twenty-four hours later serum samples obtained from each animal were tested for agglutination titre. Each animal was then challenged with antigen. The response of the four groups of animals is shown in Table 1. The average titres of the seventeen rabbits who had received IgG agglutinins and of the ten who had received low levels of IgM agglutinins were 1 : 28 and 1 : 20, respectively, prior to antigen administration. At 9 and 16 days following challenge with typhoid H antigen the agglutinin titres in the control animals and the low level IgM antibody recipients were similar, whereas those of the IgG recipients were considerably lower. It is also apparent that formation of IgG agglutinins was suppressed and delayed in those animals receiving IgG antibodies. Low levels of IgM appeared to have no appreciable effect on subsequent antibody formation, but some inhibitory effect in formation of 19S and 7S agglutinins was seen when higher levels were pre-infused. The data in Table 1 also indicate that 1 week following booster injection of antigen at 51 days, all animals had a similar secondary response with a sharp rise in IgG agglutinin titres.

PRODUCTION OF AGGLUTININS INDUCED BY IgG OR IgM ANTIBODY ANTIGEN COMPLEXES

In this experiment, rabbits were given intraperitoneally 0·03 ml of the typhoid H antigen which had been previously agglutinated *in vitro* with a large excess of either IgG or IgM antibodies. Supernatant solutions from such agglutinates had titres of 1 : 320 or more, indicating antibody excess. Control animals received an equivalent amount of free antigen suspended in saline.

The results (Table 2) show essentially no production of IgG agglutinins in the animals

TABLE 2

PRODUCTION OF ANTI-TYPHOID H AGGLUTININ BY RABBITS GIVEN IgG OR IgM ANTIBODY–ANTIGEN COMPLEXES

Group	Days after challenge	Reciprocal agglutination titre					
		IgG fraction			IgM fraction		
		No. with titre	Mean	Range	No. with titre	Mean	Range
Controls	0	0/5	0	–	0/5	0	–
	9	0/5	0	–	5/5	168	40–640
	16	4/5	20	0–40	5/5	64	20–160
	23	4/5	78	0–320	5/5	56	20–80
	65	1/4	<3	0–10	2/4	7	0–20
	72*	3/4	65	0–160	3/4	17	10–40
IgG complexes	0	0/10	0	–	0/10	0	–
	9	0/10	0	–	5/10	13	0–40
	16	0/10	0	–	7/10	25	0–80
	23	0/10	0	–	8/10	26	0–40
	65	0/10	0	–	0/10	0	–
	72*	7/10	154	0–1280	8/10	140	0–640
IgM complexes	0	0/12	0	–	0/12	0	–
	9	3/12	12	0–80	7/12	82	0–320
	16	4/12	47	0–320	12/12	73	10–160
	23	6/12	48	0–160	12/12	54	10–160
	65	5/12	88	0–640	3/12	14	0–80
	72*	9/12	174	0–1280	10/12	75	0–320

* Samples obtained 1 week following injection of 0·03 ml antigen.

receiving IgG complexes. These animals did, however, produce rather low titres of IgM agglutinins. In the group receiving IgM complexes, both IgG and IgM agglutinins were produced in titres not unlike those in the control group. One week following the 65-day booster injection of antigen alone, both types of agglutinins were produced in all groups. The titres of the control group cannot be considered significantly different from the other groups due to the small number of individuals in this group.

OBSERVATIONS IN NEWBORN INFANTS

In a limited number of newborn infants, it was possible to observe the effect on the response to *Salmonella* antigens acquired exclusively by exchange transfusion. The results, Table 3, indicate that IgG antibodies, with or without IgM antibodies, present in the

TABLE 3

PRODUCTION OF ANTIBODIES IN NEWBORN INFANTS GIVEN AGGLUTININS BY EXCHANGE TRANSFUSION

Group	Days after antigen TPT	Reciprocal agglutination titre					
		IgG fraction			IgM fraction		
		No. with titre	Mean	Range	No. with titre	Mean	Range
				Typhoid H agglutinins			
Exchanged with blood	−1*	2/2	10	–	0/2	0	–
containing anti-typhoid H	21	1/2	5	0–10	0/2	0	–
antibodies (flagellar D)	−1	6/6	22	10–40	6/6	18	10–40
	21	3/6	5	0–10	1/6	2	0–10
	−1	0/2	0	–	2/2	15	10–20
	21	0/2	0	–	2/2	800	320–1280
				Paratyphoid A agglutinins			
Exchanged with blood	−1	6/6	13	0–20	0/6	0	–
containing paratyphoid A	21	0/6	0	–	0/6	0	–
antibodies (flagellar A)	−1	1/1	40	–	1/1	40	–
	21	0/1	0	–	0/1	0	–
				Paratyphoid B agglutinins			
Exchanged with blood	−1	5/5	34	10–80	0/5	0	–
containing paratyphoid B	21	2/5	12	0–40	0/5	0	–
antibodies (flagellar B,	−1	0/1	0	–	1/1	80	–
1, 2)	21	0/1	0	–	1/1	160	–
Control:	0	0/31	0	–	0/13	0	–
no exchange (2–5 weeks)	21	17/31	24	0–160	31/31	523	20–5120
Control: exchanged	−1	0/15	0	–	0/15	0	–
with non-immune blood	21–35	6/15	37	0–320	14/15	355	0–2560

* In each case, 0·25 ml TPT antigen (Lederle) was administered 1 day following exchange. Titres on day 0 or −1 were levels before antigen administration.

circulation prior to antigenic challenge, effectively suppress the immune response. This is seen in eight infants who had received anti-typhoid H, seven with anti-paratyphoid A and five with anti-paratyphoid B. In three infants who had received IgM antibodies alone, little or no inhibition of agglutinin formation could be observed. In those subjects where levels of both IgG and IgM agglutinins were present prior to challenge, which include six with typhoid H agglutinins and one with paratyphoid A agglutinins, no significant immune response could be observed.

DISCUSSION

Inhibition of the primary response by passively administered primary and secondary 7S antibodies has been demonstrated in the guinea-pig (Uhr and Bauman, 1961) and rabbit (Sahiar and Schwartz, 1964). In their studies of anti-ΦX 174 antibody formation in guinea-pigs, Finkelstein and Uhr (1964) have shown that 7S antibodies administered 3 days following antigen suppressed the formation of IgM antibodies partially and IgG antibodies completely. The results obtained in the experiments reported herein confirm and extend their observations in another system. When antibodies were administered 24 hours before antigen, it became apparent (Table 1) that IgG antibodies at a 1 : 28 level effectively inhibited 7S agglutinin formation but did not completely suppress 19S agglutinin production. Titres of IgM antibodies similar to those of IgG antibodies (Table 1, IgM_1), had little or no effect on subsequent formation of agglutinins. Since 19S antibodies to antigens similar to those employed in this study have been shown to possess as much as twenty-two times more agglutinating activity than 7S antibodies on a molar basis (Robbins, Kenny and Suter, 1965), considerably smaller amounts of antibody protein had been infused to produce a 1 : 20 titre of IgM than the 1 : 28 titre of IgG (Table 1). In order to compare effects of equal amounts of the IgM and IgG antibody protein, titres of IgM some twenty times higher would be required (e.g. 1 : 560). Because of a rather limited supply of IgM, antibody titres of only 1 : 126 could be achieved (IgM_2, Table 1). In this instance, some inhibition of both 19S and 7S antibody formation was observed. It seems reasonable to conclude that still higher titres of 19S agglutinins would exhibit inhibitory effects approaching those of the 7S type. This suggests that differences between the inhibitory capacity of the two kinds of antibody are due largely to the somewhat shorter half-life of IgM antibodies (Uhr and Finkelstein, 1963; Svehag and Mandel, 1964a, b). In addition, affinities of 19S antibodies for their antigens, which are lower than the affinities of early- or late-appearing 7S antibodies, would result in less effective combination with antigen and, hence, less inhibition of the immune response.

It was of interest that the 7S antibodies at a titre of 1 : 28 almost completely suppressed IgG agglutinin formation but incompletely suppressed IgM agglutinins. The 7S typhoid H antibodies used in this study were produced only 2 months after the initial injection of antigen and as such can be regarded as antibodies with relatively lower affinity than those IgG antibodies produced in the secondary response (Eisen and Siskind, 1964). Since synthesis of IgM or IgG antibody depends upon the presence of antigen (Uhr and Finklestein, 1963; Svehag and Mandel, 1964b), inhibition of synthesis by passively administered antibody is quite likely due to removal of antigen by the acquired antibody so as to make the antigen unavailable as a stimulus for the immune response. If the concentration and affinity of the infused IgG antibody for antigen is sufficiently low, amounts of free antigen adequate for stimulation of formation of 19S antibody but not of 7S antibody become available. Svehag and Mandel (1964b) have noted that after challenge with sufficiently small amounts of antigen, only 19S antibodies and no 7S antibodies are formed to polio virus. Higher concentrations of IgG antibody or similar titres of high affinity secondary IgG antibody would be expected to completely suppress antibody formation. This is discussed further below.

The results obtained after intravenous injection of antibodies are supported by those obtained after administration of antibody–antigen complexes (Table 2). It is seen that antigen administered in the form of an IgG antibody excess complex induced no 7S

agglutinins and low but significant titres of IgM agglutinins. IgM–antigen complexes induced formation of both 7S and 19S agglutinins in titres similar to those obtained with antigen alone. Since IgM antibodies appear to be less 'avid' (Robbins *et al.*, 1965), and since they have a considerably shorter half-life than IgG antibodies (Svehag and Mandel, 1964b), antigen may have been released in amounts sufficient to induce a normal primary response with IgM complexes but not with the IgG complexes. The fact that 19S antibodies, unlike 7S antibodies, are confined to the vascular compartment (Cohen, 1963) has been considered as a contributing factor to the less effective inhibiting capacity of the 19S antibodies (Finkelstein and Uhr, 1964). The data shown in Table 2 would indicate that this is not an important factor since even when 19S antibodies were administered as complexes formed *in vitro*, little or no inhibition of the primary response was observed.

Although rather meagre, the data obtained in studies on newborn infants agree with those obtained with rabbits. Here again (Table 3), 19S antibodies, where present, had little or no effect on subsequent antibody formation. The presence of 7S antibodies generally had a marked suppressive effect on both 19S and 7S antibody formation. In these, as well as in our previous studies with maternally transferred antibodies (Fink *et al.*, 1962), the transferred IgG agglutinins could be considered of sufficiently high affinity to completely bind and effectively remove the administered antigen as a stimulus for antibody formation. Complete suppression of antibody formation by some 7S antibodies has also been observed by others (Uhr and Bauman, 1962; Finkelstein and Uhr, 1964) in humans as well as experimental animals.

Finally, it should be pointed out that in discussing the results obtained, no direct role has been postulated for the IgG or IgM antibodies in regulation of their synthesis. This regulation has been attributed primarily to antigen levels. The possibility that a true feedback control mechanism does in fact exist has not been excluded but, at present, has little direct evidence to support it.

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REFERENCES

BAUER, D. C. and STAVITSKY, A. B. (1961). 'On the different molecular forms of antibody synthesized by rabbits during the early response to a single injection of protein and cellular antigens.' *Proc. nat. Acad. Sci. (Wash.)*, **47**, 1667.

BRITTEN, R. J. and ROBERTS, R. B. (1960). 'High-resolution density gradient sedimentation analysis.' *Science*, **131**, 32.

COHEN, S. (1963). 'Properties of the peptide chains of normal and pathological human γ globulins.' *Biochem. J.*, **89**, 334.

COHEN, S. and FREEMAN, T. (1960). 'Metabolic heterogeneity of human γ globulin.' *Biochem. J.*, **76**, 475.

EISEN, H. N. and SISKIND, G. W. (1964). 'Variations in affinities of antibodies during the immune response.' *Biochemistry*, **3**, 996.

FINK, C. W., MILLER, W. E., Jr., DORWARD, B. and LOSPALLUTO, J. (1962). 'The formation of macroglobulin antibodies. II. Studies in neonatal infants and older children.' *J. clin. Invest.*, **41**, 1422.

FINKELSTEIN, M. S. and UHR, J. W. (1964). 'Specific inhibition of antibody formation by passively administered 19S and 7S antibody.' *Science*, **146**, 67.

KOLMER, J. A., SPAULDING, E. H. and ROBINSON, H. W. (1951). *Approved Laboratory Technic*, 5th edn. Appleton–Century Crofts, New York.

LOSPALLUTO, J. and FINK, C. W. (1964). 'Effect of prior administration of 7S and 19S antibody on antibody formation.' *Arthr. and Rheum.*, **7**, 324.

LOSPALLUTO, J., MILLER, W., Jr., DORWARD, B. and FINK, C. W. (1962). 'The formation of macroglobulin antibodies. I. Studies on adult humans.' *J. clin. Invest.*, **41**, 1415.

OSBORN, J. J., DANCIS, J. and JULIA, J. F. (1952). 'Studies of the immunology of the newborn infant. II. Interference with active immunization by passive transplacental circulating antibody.' *Pediatrics*, **10**, 328.

ROBBINS, J. B., KENNY, K. and SUTER, E. (1965). 'The isolation and biological activities of rabbit γM- and γG-anti-Salmonella typhimurium antibodies.' *J. exp. Med.*, **122**, 385.

SAHIAR, K. and SCHWARTZ, R. S. (1964). 'Inhibition of 19S antibody synthesis by 7S antibody.' *Science*, **145**, 395.

SMITH, R. T. (1960). 'Response to active immunization of human infants during the neonatal period.' *Cellular Aspects of Immunity, Ciba Foundation Symposium*, pp. 348–368. Little-Brown, Boston.

SVEHAG, S. E. and MANDEL, B. (1964a). 'The formation and properties of poliovirus-neutralizing antibody. I. 19S and 7S antibody formation: differences in kinetics and antigen dose requirement for induction.' *J. exp. Med.*, **119**, 1.

SVEHAG, S. E. and MANDEL, B. (1964b). 'The formation and properties of poliovirus-neutralizing antibody. II. 19S and 7S antibody formation: Differences in antigen dose requirement for sustained synthesis, anamnesis, and sensitivity to x-irradiation.' *J. exp. Med.*, **119**, 21.

UHR, J. W. and BAUMANN, J. B. (1961). 'Antibody formation. I. The suppression of antibody formation by passively administered antibody.' *J. exp. Med.*, **113**, 935.

UHR, J. W., DANCIS, J., FRANKLIN, E. C., FINKELSTEIN, M. S. and LEWIS, E. W. (1962). 'The antibody response to bacteriophage ΦX 174 in newborn premature infants.' *J. clin. Invest.*, **41**, 1509.

UHR, J. W. and FINKELSTEIN, M. S. (1963). 'Antibody formation. IV. Formation of rapidly and slowly sedimenting antibodies and immunological memory to bacteriophage γX174.' *J. exp. Med.*, **117**, 457.

VAHLQUIST, B. (1958). 'The transfer of antibodies from mother to offspring.' *Advanc. Pediat.*, **10**, 305.