THE FORMATION OF MACROGLOBULIN ANTIBODIES. I. STUDIES ON ADULT HUMANS *

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The occurrence of antibodies of different molecular size in man and experimental animals has long been known (1). In recent years increasing attention has been focused on the group with high molecular weight (19S), which includes not only a number of well defined antibodies but, in addition, certain substances with similar electrophoretic and ultracentrifugal characteristics that are not firmly established as antibodies. Both groups have been discussed by Kunkel, Fudenberg and Ovary (2, 3).

Although numerous immunoglobulins in human serum are found either in the 7S or 19S class of γ -globulins, certain antibody activities are found in both classes. Among these are the isoagglutinins (3), Wassermann antibodies (4), and lupus factors (5). In addition, antibodies to typhoid H antigen have recently been found in both the 7S and the 19S fractions in the sera of neonatal infants and adults (6, 7).

In the course of an investigation of the immune response to typhoid and paratyphoid antigens (8) in patients with a variety of diseases, it was observed that the individuals tested could be placed in one of three categories with respect to the size of the antibodies produced. One group produced only the 7S type; a second, only the 19S type; and a third, a mixture of the two. This distribution is similar to that found for saline anti-A isoagglutinins in different individuals (3). This study was undertaken to elucidate the factors governing the formation of one or the other type of antibody and the interrelationship, if any, of the two types.

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MATERIALS AND METHODS

Most of the individuals were immunized by three injections at weekly intervals of mixed typhoid and paratyphoid vaccine (Lederle), some of 0.5 ml intramuscularly, and others of 0.1 ml intradermally. Blood was drawn 1 week after the last injection, allowed to clot at 37° C, and the serum collected and stored in a freezer until ready for fractionation and testing.

Five ml of serum was dialyzed against 3L of 0.01 M Na₂HPO₄, pH 8.5 to 9.0, for 2 days before chromatographic fractionation. Chromatographic separation was carried out on diethylaminoethyl (DEAE)-cellulose columns as described previously (9). The fractions comprising each peak were pooled and tested for antibodies to typhoid O, typhoid H, and paratyphoids A and B by standard agglutination procedures (10). Antibody activities associated with the first (pH 7.0, 0.01 M) and the last (pH 5.0, 0.3 M) chromatographic peaks were considered to contain 7S and 19S y-globulins, respectively, on the basis of the behavior of these proteins on DEAE-cellulose (9, 11, 12). Although the first, or 7S, peak contains no 19S globulins, the last peak is contaminated by 7S protein, which could account for part of the antibody titers associated with this fraction. The results of treatment with mercaptoethanol indicate, however, that virtually all of the activity of the pH 5 peak is in the 19S fraction.

The effect of 2-mercaptoethanol (ME) on agglutinin activity was tested at a number of concentrations and at different temperatures and times. The following procedure was finally adopted. Isotonic saline-phosphate buffer, pH 7.0, containing 0.05 M 2-ME was prepared. All dilutions of sera and chromatographic fractions for testing were carried out in this buffer. The suspensions of test organisms were also diluted 1:10 in the same buffer. Agglutination tests without ME were carried out simultaneously.

Some subjects were given booster injections of the mixed vaccine 3 to 15 months after the initial series. In these cases blood was obtained before administration of the antigen, as well as 1 week later.

RESULTS

The chromatographic pattern of the serum from a normal individual obtained 1 week after the last

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FIG. 1. CHROMATOGRAPHIC PATTERN OF SERUM FROM A NORMAL INDIVIDUAL (J.L.) 1 MONTH AFTER IMMUNIZA-TION WITH TYPHOID-PARATYPHOID VACCINE. Five ml serum on 7.5 g DEAE-cellulose. Peak I, fractions 1–20 eluted at pH 7, 0.01 M phosphate. Peak II, fractions 21-42, eluted at pH 7, 0.025 M phosphate. Peak III, fractions 43-75, eluted at pH 6, 0.1 M phosphate. Peak IV, fractions 76-99, eluted at pH 5, 0.15 M phosphate + 0.15 M NaC1.

of three weekly intramuscular injections of 0.5 ml of mixed typhoid-paratyphoid vaccine is shown in Figure 1. Essentially all of the agglutinin activity for each of the antigens was found in the pooled 19S-containing fraction (peak 4). The distribution of antibody activity seen in Figure 1 represents one of three types seen in an initial survey of different individuals. The distribution of titers in three characteristic subjects is shown in Table I. One of these (J.B.), who produced almost entirely 7S antibody, had been initially immunized 15 years earlier. He had received a booster injection 2 years previously and a second booster injection of 0.1 ml of mixed vaccine intradermally. Another (J.Be.) produced both 7S and 19S antibodies 1 week after a similar booster injection. He had been immunized initially 8 years earlier. The third (P.C.) produced only 19S antibody 1 month after a single dose of 0.1 ml vaccine administered intradermally. She had not been previously immunized.

In order to investigate the causes of the heterogeneity of antibody size seen in Table I, a group of subjects was immunized under different conditions and by different routes. In 9 normal individuals and 21 patients with a variety of diseases who were immunized for the first time, the antibodies produced 1 week after the last of three intramuscular or intradermal injections were predominantly of the 19S class (Table II). There was no history of typhoid or paratyphoid fever in these subjects. The titers in Table II indicate that, while high titers of 19S antibodies against all four antigens were present, little 7S antibody was produced in the 1-month period after start of immunization.

In all of these, no qualitative difference was observed between subjects immunized by different routes (i.m. or i.d.). The preponderance of 19S antibody at this stage of immunization has also been observed in the neonatal infant (6, 7). It should also be pointed out that the antibodies to typhoid O have been consistently of the 19S type, whereas considerable variability in the distribution of the other three antibodies has been observed, as noted above.

Thirty-two subjects, 11 normal and 21 with a variety of disorders, were investigated to determine the effect of previous immunization or exposure on the size of the antibody produced. In these subjects booster injections, intramuscular or

Chromatographic fraction	Initial immunization P.C.				Booster after 8 yrs J.Be.			Booster after 15 yrs J.B.				
	0	Н	Α	В	0	н	А	В	0	Н	Α	В
pH 7.0, 0.01 M (contains 7S antibody)	0	0	0	0	0	160	320	320	0	160	160	80
pH 7.0, 0.025 M	0	0	0	0	0	0	0	0	0	0	0	0
pH 6.0, 0.1 M	0	20	0	0	0	20	10	0	0	0	0	0
pH 5.0, 0.3 M (contains 19S antibody)	80	320	80	160	20	40	80	160	20	10	0	10

 TABLE I

 Patterns of typhoid-paratyphoid agglutination tests*

* Mean reciprocal titers, 1 week after immunization.

Subjects	0		н		Α		В	
	75	19S	7S	19S	75	19S	75	19S
Normals	0	43	14	289	20	320	37	382
[9]		(0-80)	(0–80)	(40-640)	(0-80)	(80–640)	(0–160)	(80–1,280)
Patients	0	35	20	302	36	450	42	420
[21]		(0–160)	(0–160)	(20–640)	(0–160)	(20–1,280)	(0-320)	(20–1,280)
Combined	0	36	18	300	31	412	41	410
[30]		(0–160)	(0–160)	(20–640)	(0–160)	(20–1,280)	(0–320)	(20–1,280)

TABLE II Distribution of typhoid-paratyphoid agglutinins after initial immunization *

* Mean reciprocal agglutinination titers. Number of subjects in brackets.

intradermal, were given 1 or more years after the initial immunization. Sera from these subjects were collected 1 week after the booster injections. The results (Table III) indicate that the antibodies produced in this group in response to boosters given long after initial immunization were predominantly of the 7S variety for typhoid H and paratyphoid A and B antigens. Again, the anti-O activity was confined to the 19S fraction.

The data shown in Tables II and III for typhoid H and paratyphoid A and B antigens suggested that the three different patterns of antibody distribution illustrated in Table I represented different stages of immunization in which macroglobulin antibody initially produced was in time followed by 7S antibody. In no subject (Table II) was the 7S antibody titer higher than the 19S at the end of 1 month. It was of considerable interest, therefore, to determine whether this sequence of events could be demonstrated and the time intervals determined. In Table IV are shown the results obtained in six normal subjects at 1 and 4 to 6 months after initial immunization. Samples drawn before and after a booster injection given at 4 to 6 months show the change in the character of the response from that observed after initial immunization. It is seen that 7S antibody appeared in significantly higher concentration in response to the secondary or "booster" immunization, while there was no significant rise in the amount of 19S fraction. For purposes of comparison, a similar study was conducted in six hospitalized patients over the same time period with similar results. It is noteworthy, in both the normal individuals and in the patient group, that while high titers of the 19S agglutinins were still present after the 4 to 6 months' booster, they were, in all cases, smaller than those present 1 month after immunization.

In a group of premature infants (7), there was almost a complete transition of 19S to 7S antibody within a 3-month period after initial immunization. Other differences have been reported by Smith, Eitzman and Miller (6).

Effect of treatment with 2-mercaptoethanol. The susceptibility of some 19S macrogammaglobulins to degradation by sulfhydryl compounds such as 2-mercaptoethanol has been well documented (3, 13-19). Sera from nine adults obtained immediately after initial immunization with the typhoid-paratyphoid antigens were tested, therefore, after treatment with this compound. The results obtained before and after treatment with ME are compared in Table V. It is seen

		0	н		Α		в	
Subjects	7S	19S	7S	19S	75	19S	75	19S
Normals	0	29	180	44	193	35	147	37
[11]		(0-80)	(80–320)	(40–160)	(40–320)	(0–320)	(20–320)	(0–160)
Patients	0	24	216	74	252	28	201	71
[21]		(0–160)	(40–640)	(10–160)	(40–1,280)	(0–80)	(20–1,280)	(0–320)

 TABLE III

 Typhoid and paratyphoid agglutinins in previously immunized subjects *

* See footnote, Table II.

Time after immunization	0		н			A	В	
	75	19S	7S	19S	78	19S	75	195
months								
1	0	47 (40–80)	8 (0–20)	267 (160–320)	27 (10–80)	373 (320–640)	53 (20–160)	480 (160–1,280)
4–6 (Prebooster)	0	33 (20-40)	5 (0–20)	227 (80-320)	11 (0–40)	140 (40–320)	18 (0–40)	123 (20–160)
4–6 (Postbooster)	0	30 (20-40)	55 (10–160)	· 152 (80–160)	77 (20–160)	173 (80–320)	73 (40–160)	130 (20–320)

TABLE IV Changes in size of typhoid-paratyphoid agglutinins with time in six normal subjects

that the agglutinating activity of the macroglobulin antibodies tested decreased markedly after treatment with ME. Where 7S antibodies were present, the titers were not significantly affected by this treatment.

DISCUSSION

Changes in the physical properties of antibodies during the course of immunization have been observed in the past (20–25). Of particular interest has been the evidence (21–27) that rabbit antibodies, produced early after immunization with sheep erythrocytes, are associated with γ -globulins of greater electrophoretic mobility than are those produced later in the course of immunization. It was also shown in these investigations that soon after immunization the antibodies were of the 19S type, and those formed later fell into the 7S category (26). These observations recently have been confirmed and extended by Bauer and Stavitsky for a number of antigens in the rabbit (28).

In the present studies, antibodies directed against typhoid-paratyphoid antigens appear to be of the 19S variety early after immunization, with additional 7S-type antibodies to the H, A, and B antigens appearing in higher concentrations in adult humans after 4 to 6 months have elapsed. This observation is based upon the chromatographic properties of the agglutinins found in sera obtained at various times after immunization of 1) individuals followed serially and 2) groups of individuals examined at specific times after immunization.

Supporting evidence for the 19S character of early antibodies was obtained by means of agglutination tests performed in the presence of 2mercaptoethanol. Agglutinins found in sera obtained shortly after immunization were almost completely inactivated by treatment with ME, while sera obtained later contained sulfhydryl-resistant 7S agglutinins. These findings support the chromatographic data indicating the presence of 19S and 7S antibodies in eluates obtained at pH 5, 0.3 M, and pH 7, 0.01 M, respectively. Thus, if the fraction containing 19S also contains 7S globulin, the results obtained after ME treatment indicate that this can be only a very small amount and that most of the agglutinins found in this fraction are of the 19S type. More precise characterization of these antibodies would, of course, entail use of the density gradient centrifugation technique.

The appearance of 19S antibodies followed by those of the 7S variety was observed in response

.		0	н		Α		В	
tested	7S	19S	75	19S	75	19S	75	19S
Without	0	49	8	293	20	382	41	462
ME		(20–160)	(0-40)	(80–640)	(0-80)	(80–640)	(0–160)	(80–1,280)
With	0	6	3	19	10	27	23	21
ME		(0–20)	(0–10)	(0-40)	(0–40)	(0-80)	(0-80)	(0-40)

 TABLE V

 Effect of 2-mercaptoethanol (ME) on typhoid-paratyphoid agglutinins

to the typhoid H and paratyphoid A and B antigens, but this sequence did not occur with typhoid O antigen. In the latter case, the agglutinins formed at the outset, as well as those in patients followed for more than 2 years, were entirely of the 19S variety. In no serum of more than 150 individuals studied was any 7S agglutinin to typhoid O found. Moreover, in individuals given booster injections of typhoid-paratyphoid vaccine more than 15 years after initial immunization, typhoid O agglutinins were entirely in the 19S fraction, whereas the other antibodies tested were found chiefly among the 7S γ -globulins.

The underlying reasons for the phenomena described above are not understood. One of the fundamental questions is why some antigens elicit the formation of only 19S antibodies, while others, either directly or after the elaboration of a 19S type of antibody, result in 7S antibody formation. Of the known antibodies that remain macroglobulins, most appear to be formed in response to particulate antigens with a relatively high carbohydrate content, e.g., typhoid O antibody, red cell isoagglutinins, and heterophile antibody. The agglutinins tested in these experiments, including typhoid O antibody, were also elicited by administration of a series of particulate antigens. It is not clear whether the 19S and 7S antibodies produced against paratyphoid B, for example, arise in response to different antigens, or whether two separate and distinct antibody-forming processes involving different cell populations (reacting with the same or different antigens) are involved. The evidence from these and companion studies on premature infants (7) suggests that the latter possibility may obtain. In these studies, initial immunization resulted in formation of 19S antibodies with little or no 7S antibodies, and secondary immunization was followed by formation of predominantly 7S antibody. The presence of only 7S antibodies and little or none of the 19S type after booster immunization of some individuals long after primary immunization indicates that the secondary response was restricted to 7S antibody formation. The decreased tendency to produce 19S antibodies in these individuals seems best attributed to the stimulation of a cell population producing 7S rather than 19S antibodies against the same antigen. It is possible that a secondary 19S antibody rise is not generally observed at this

time because at the particular time after immunization that 19S serum antibodies to their corresponding antigens are not detectable, intracellular or tissue-fixed antibodies may be present in sufficient quantity to bind injected antigens and thus inhibit the anamnestic response. Finally, it is possible that the 19S antibody response is easily suppressed by very small concentrations of serum antibody of the 19S type, or even of the 7S type, according to the mechanism proposed by Uhr and Baumann (29). This would tend to eliminate the 19S antibody response and permit 7S antibody formation to proceed preferentially.

A salient feature of the antibody response has been observed in corollary studies on premature infants (7). The presence in the infant of maternally transferred antibodies to the H, A, and B antigens completely inhibited the response to injected vaccine. While these studies are, as yet, incomplete, the results to date indicate that the antibodies that traverse the placenta into the infant circulation, which are entirely of the 7S variety, effectively prevent an immune response to administered vaccine so that neither 19S nor 7S antibody is produced. It would be expected, if two antigen groups of significantly different characteristics were involved, that the formation of 19S serum antibodies would not be inhibited by the 7S antibody present. These results suggest that in humans the antibodies formed at different stages of immunization, although different in size, are directed against the same or very similar antigens. Similar results were obtained in the case of antibodies produced to sheep erythrocytes and other antigens in rabbits (21-28), and to pneumococcus polysaccharides in the horse (30).

It is also of interest that the change in antibody from the 19S to the 7S type, at least in regard to the antigens used in this investigation, requires a considerable length of time in adults and is rarely complete. In the group of individuals studied from the outset of immunization, blood drawn 1 to 2 years after initial inoculation, while rich in 7S antibody, still contained appreciable titers of 19S antibodies. Although, in some individuals examined 10 to 15 years after immunization, agglutinins produced on booster injection were chiefly 7S globulins with little or no activity present in the 19S fractions, this type of subject represented the exception, rather than the rule. In most, while the secondary response consisted, as far as could be detected, essentially of the formation of 7S antibody, there was usually demonstrable 19S activity. The final distribution of antibodies may be governed by factors similar to those that determine the types of saline isoagglutinins in adult humans (3).

In contrast, the production of 7S agglutinins following the 19S variety was considerably more rapid in newborn infants, especially after booster immunization. At a point 4 to 6 months after initial immunization, the antibodies in a group of adults were still predominantly in the 19S fraction (Table IV), whereas the same agglutinins were found chiefly in the 7S fraction in infants at the same time. Older infants showed an adult-type response. The underlying reasons for the differences between the newborn and the adult are unclear. A possible explanation is discussed elsewhere (7).

The results obtained in these studies with antibodies to the O antigens confirm the previous description of these antibodies as members of the 19S class of globulins (31). On the other hand our data suggest that previous reports, indicating that typhoid H antibodies are 7S proteins (5, 32), are oversimplifications.

The present findings demonstrate that both man and experimental animals produce at least two different types of antibody to some antigens, on the basis of physicochemical criteria. Yet others, like typhoid O, may elicit only 19S antibodies even after many years. The relationship of antigenic structure and size to antibody size is as yet unexplored. Differences between types of antigens, which may be responsible for the phenomena observed here, are currently under investigation. Information of this type may lead to an understanding of the events leading to formation of macroglobulin and 7S antibodies.

SUM MARY

1) Normal and diseased subjects produce high molecular weight (19S) antibodies and little or no 7S antibody after initial immunization with typhoid-paratyphoid antigens. Different routes of immunization, whether intramuscular or intradermal, gave qualitatively similar results.

2) After booster injection of subjects immunized at some time in the past, antibodies to typhoid H and paratyphoids A and B are found predominantly in the low molecular weight (7S) fraction.

3) Typhoid O agglutinins have been found exclusively in the 19S fraction, and no change in the size of these antibodies occurs with time.

4) The change in the typhoid H and paratyphoid A and B antibodies from 19S to predominantly 7S type in adults requires more than 6 months, and few subjects show complete change. In most subjects, 19S agglutinins are present even 1 to 2 years after initial immunization, although 7S antibodies predominate.

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