Human Anti-IgM Iso-Antibodies: Detection of IgM Allotypic Markers

(IgG/Waldenström macroglobulins/allotypic marker)

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ABSTRACT IgG class antibodies against human IgM were detected in a human subject up to a titer of 1:1024 against 5 of a group of 23 monoclonal Waldenström macroglobulins. Inhibition studies confirmed at least one antigenic determinant shared by the 5 IgMs. The determinant was present on the μ chain but required tertiary structure for its full expression. Inhibition of the reaction between the antibodies to IgM and erythrocytes coated with 2 of the 5 IgMs was obtained at titers of 1:2 to 1:16 with 51.0% of 300 normal sera (85.2% of sera from 122 Caucasian, 26.1% of sera from 23 Negro, and 27.7% of sera from 155 Japanese individuals). This inhibition was independent of the serum IgM concentration. Family studies indicated that the determinant was inherited according to basic Mendelian laws and appears to be the first allotypic marker on human serum IgM. We have designated it Mm 1.

Numerous studies have been reported on the inherited allotypic markers on the γ -heavy chains of IgG molecules (Gm markers) and on κ -type light chains (Inv markers) (1,2). An allotypic marker on the α -heavy chains of IgA molecules was studied with a human serum containing antibodies against one of a group of IgA myeloma proteins and erythrocytes coated with the specific IgA myeloma protein by the chromic chloride method (3). This marker was originally designated Am₁ and is probably identical to the marker Am₂ on IgA₂ molecules (4). It has been redesignated A₂m₁ with the discovery of its allele designated A₂m₂, by Van Loghem *et al.* (submitted for publication).

Attempts by several laboratories over the past 10 years to detect IgM allotypic determinants by similar techniques have been less fruitful (5). Anti-IgM antibodies were detected in an earlier study in only 16 of 378 sera (4.2%), including subjects who had had multiple blood transfusions or several pregnancies (5). These antibodies were of low titer, directed in each case against only one of a group of 15 Waldenström macroglobulins, and slightly inhibited in two cases by some normal sera (6). We report here studies on a subject whose serum contained IgG-class antibodies in high titer against five of a group of 23 monoclonal IgM macroglobulins. Inhibition experiments indicated a specificity against one or more determinants shared by the five IgM proteins, and the evidence indicates that this shared determinant represents an inherited characteristic ("allotype").

MATERIALS AND METHODS

The chromic chloride method (6) was used to coat erythrocytes from a group O, Rh-positive subject with purified pro-

teins from a group including 23 Waldenström macroglobulins. As controls, each experiment also included tests with cells coated with κ light chains, λ light chains, normal IgG, and normal IgA. IgG was prepared from serum R.K. by DEAE-cellulose chromatography [17.5 mM phosphate buffer (pH 7.8)], and IgM by starch block electrophoresis and gel filtration on Sephadex G-200; their purity was confirmed by standard immunologic techniques with monospecific antisera (5). Polypeptide fragments and chains were prepared from selected monoclonal IgM proteins as follows: heavy (H) and light (L) chains by reduction with 0.2 M 2-mercaptoethanol, alkylation, and gel filtration on Sephadex G-100 (7); F(ab')₂ and Fc_μ by trypsin digestion (8); Fab_μ by pepsin digestion (9); and IgM 7S monomers by mild reduction with 0.1 M 2-mercaptoethanol and alkylation. In the latter three sets of experiments, the fragments were isolated by gel filtration on Sephadex G-200. Solutions of IgM proteins and their polypeptide fragments were used at a concentration of 1 mg/ml in the coating procedure with CrCl₃ (5).

The hemagglutination (HA) and hemagglutination inhibition (HAI) assays were performed in V-shaped disposable Microtiter trays; the incubation period was 2 hr at 37°. Inhibition of agglutination was performed with both (i) solutions of the coating IgM proteins in serial 2-fold dilutions from 1.0 mg/ml, and (ii) normal serum specimens in dilutions of 1:2, 1:4, 1:8, and 1:16.

RESULTS

Subject R.K. is a 30-year old Caucasian female. Antibodies to human IgM have been detected in her serum since 1967; on six subsequent occasions her serum contained antibodies

TABLE 1. *Titers of antibodies to purified IgM coats in seven serum specimens from R.K.*

Coat no.	Source of protein	Type of light chain	Titer of anti-IgM antibodies*							
			May 1967	Sept 1967	Nov 1967	Nov 1968	June 1969	Jan 1971	April 1971	
7	Gi	κ	512	128	512	1024	1024	256	512	
8	Pr	κ	64	32	64	128	128	64	64	
10	Pi	κ	64	32	64	128	128	64	64	
11	Ka	λ	512	128	256	256	512	256	512	
21	Bu	κ	512	128	512	512	512	256	512	

* Titers given as reciprocal of lowest dilution with agglutination; all other coats and controls were negative.

Abbreviation: L and H chains, light and heavy chains, respectively.

TABLE 2. Incidence of inhibitors among normal human sera from different races for the reaction between serum R.K. antibodies and erythrocytes coated with IgM 7 or 11

Sera tested	Number tested	Inhibited both IgM 7 and 11		Inhibited only IgM 7		Inhibited only IgM 11		Inhibited neither IgM 7 nor 11	
		No.	%	No.	%	No.	%	No.	%
Caucasian	122	104	85.2	4	3.3	1	0.8	13	10.7
Negroid	23	6	26.1	3	13.0	0	0	14	60.9
Japanese	155	43	27.7	9	5.8	1	0.6	102	65.9
Total	300	153	51.0	16	5.3	2	0.7	129	43.0

against the same five IgM coats (Table 1). All other coats were negative. In January 1971 she gave birth to a daughter (B.K.) after a normal primiparous pregnancy; screening of the infant's cord serum detected antibodies at a titer of 1:32 against each of three IgMs 7, 11, and 21. Testing of IgG isolated from serum R.K. confirmed that the anti-IgM antibodies were of the IgG class. Each of the five IgM proteins inhibited the reaction between two agglutinating units of serum R.K. and each of the five protein coats. Limiting protein inhibitory concentrations ranged from 1 to 64 μ g/ml, and IgMs 7, 11, and 21 were more potent inhibitors. All other proteins were negative in inhibition experiments, namely, κ light chains, λ light chains, normal IgG, normal IgA, normal IgM from serum R.K., the other 18 IgMs, bovine IgM, bovine colostrum, and goat serum. These results were consistent with those showing IgM antigenic determinant(s) common to these five IgMs and reacting with IgG antibodies in serum R.K.

Structural localization of the determinants on the IgM molecule was sought in two ways after the different polypeptide fragments were prepared from each of three "positive" proteins 8, 10, and 11 and from one control "negative" protein 16 (see *Methods*). First, each fragment was coated on erythrocytes, and serum R.K. was tested for agglutinating activity. Only the 19S IgMs reacted with R.K. antibodies 8, 10, and 11. Second, the polypeptide fragments were tested for inhibitory activity against cells coated with 19S protein and two agglutinating units of serum R.K. Inhibitory activity was found with 7S monomers of IgMs 8, 10, and 11 but not with isolated H-chains. No inhibitory activity was detected in the other fragments or in the L-chains.

Data on the inhibition by normal human sera of the reaction between serum R.K. and cells coated with IgM 7 or 11 are summarized in Table 2. Inhibition was obtained in many of the sera from Japanese individuals at a dilution of only 1:2; the majority of inhibitory sera were effective at a dilution of 1:4 or 1:8. The differences in the percentages of inhibitory sera from Caucasians compared with those from Negroes and Japanese were statistically significant. The percentages of inhibitors in sera from Negro and Japanese individuals were not significantly different. The majority of sera were comparable in their inhibitory titers against both proteins or their inability to inhibit both proteins. However, 18 of 300 sera (6.0%) demonstrated discordance in that they inhibited one protein but not the other. Sixteen subjects inhibited only IgM 7 and two inhibited only IgM 11; the inhibitory titers

ranged from 1:2 to 1:8. Six of 306 sera (2.0%) were excluded from inhibitory studies as they contained anti-IgM antibodies.

Table 3 summarizes data on the presence of the postulated IgM determinant among 69 children in 37 Japanese families. In 17 families where both parents were negative for the determinant, none of the 29 children was positive. Serum IgM concentrations were measured in 25 members (14 positive, 11 negative) in six Japanese families; there was no statistically significant correlation between the serum IgM concentration and the presence of the determinant. The serum concentration of IgM ranged from 32–140 mg/100 ml (mean 65 mg/100 ml) in positive sera and from 53–130 mg/100 ml (mean 84 mg/100 ml) in negative sera.

DISCUSSION

The pathogenesis of these anti-IgM antibodies is unknown. Subject R.K. has no history of infusion of parenteral blood, plasma, or blood proteins and no history of earlier pregnancy that might account for initial sensitization. The patterns of inhibition and specificity of the anti-IgM antibodies in her infant suggest that the infant received the anti-IgM antibodies by transfer of maternal IgG across the placenta.

The ability of each of the five IgMs to inhibit the reaction of serum R.K. antibodies with each of the same five IgM coats strongly suggests at least one determinant common to these proteins. The determinant is not dependent on the L-chain type, and the failure of polymer IgA myeloma proteins to inhibit the reaction excluded J-chain. It appears that μ and L chains are required in their normal association as the 7S subunits of IgM to express the determinant although we feel that it is located in the Fc region of the μ chain. The dependence of antigenicity on the association of H and L chains with intact disulfide bonds has been described for γ chain allotypic markers Gm(b²), Gm(f), and Gm(z) on IgG molecules (1, 2) and for an individual antigenic specificity on the μ chain of a monoclonal IgM M ϕ (11). We have thus far been unable to verify the location of the determinant in the Fc μ region since the isolation procedures for the fragments apparently destroy its antigenicity in our test system.

There are several points that indicate that this system is more complex than that of a single determinant. The titers against proteins 7, 11, and 21 were consistently higher, and these higher titers apparently accounted for the transfer of only these specificities into the serum of the infant. The titer differences might reflect various degrees of response to a common antigenic determinant with minor steric or conformational changes between proteins 7, 11, and 21 and proteins 8 and 10. Alternatively, they might reflect additional antibody specificities against additional determinants restricted

TABLE 3. Inheritance of postulated IgM determinant Mm1 among 37 Japanese families with 69 children

Mm1 determinant		No. of couples	No. of children	Mm1 determinant in children	
Father	Mother			Positive	Negative
+	+	2	5	4	1
+	—	13	27	13	14
—	+	5	8	1	7
—	—	17	29	0	29

to some but not all of the five IgM proteins. Thus, 18 of 300 normal sera (6.0%) inhibited IgM 7 or IgM 11 but not both proteins, while six other sera (2.0%) contained antibodies against either IgM 7 or IgM 11. With IgG allotypic determinants, Gm(x) specificity may be found in subjects positive for Gm(a) but Gm(x) is only rarely found in subjects negative for Gm(a) specificity (1, 2).

The significantly different incidences of sera from different races with inhibitory activity against both IgM 7 and IgM 11 are consistent with genetic polymorphism for an inherited determinant common to these proteins. We propose Mm 1 as its tentative designation. Studies of its incidence in different races have proved difficult as sera were inhibitory at a dilution of only 1:2 in many of the Japanese individuals. The inhibitory titers were usually 1:4 or 1:8 in Caucasians. Tests for Gm and Am determinants on IgG and IgA molecules, respectively, are usually performed at dilutions of 1:10, 1:20, and 1:40 (2, 3). The serum concentration of IgM is about 0.5 that of IgA and 0.1 that of IgG, and the lower IgM serum concentration would contribute to the lower sensitivity of the inhibition system for IgM determinants. Perhaps more important is the complex three-dimensional structure of the IgM pentamer with a greater likelihood of masking of antigenic determinants.

Further studies with R.K. anti-IgM antibodies have been restricted by the small volume of available R.K. serum. Rabbit and goat antisera to IgMs 7, 8, 10, and 11 were therefore prepared and absorbed with Mm 1-negative IgMs to

remove anti-IgM-class and L-chain specificities. Thus far, antibodies have not been found in heterologous species or in other humans with the specificities of the anti-IgM antibodies in serum R.K.

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