Brief Definitive Report

DETERMINANTS RECOGNIZED BY MURINE RHEUMATOID FACTORS: MOLECULAR LOCALIZATION USING A PANEL OF MOUSE MYELOMA VARIANT IMMUNOGLOBULINS*

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It is now well established that rheumatoid factor (RF)-like anti-IgG autoantibodies frequently occur in the mouse (1–6).

The development of RF-secreting hybridomas derived from polyclonally activated spleen cells or obtained from animals spontaneously producing RF made it possible to study the fine specificity of these autoantibodies. It appeared that, whatever their origin, the majority of mouse monoclonal RF were specific for a single IgG subclass: most were directed against IgG1 or IgG2a, some against IgG2b. Competition experiments with heat-aggregated immunoglobulins showed that subclass specificity was also characteristic of the polyclonal RF found in the serum of autoimmune strains, NZB and MRL/MpJ-lpr (7). In addition, many IgG2a-specific RF derived from BALB/c (Igh^a) or 129/Sv (Igh^a) mice also displayed allotype specificity in the sense that they failed to react with IgG2a of the "b" allotype.

To localize the determinants involved in these RF-IgG interactions, we have investigated the reactivity of RF, both polyclonal and monoclonal with immunoglobulins carrying abnormal heavy chains (8, 9).

The results indicate that both the allotypic and isotypic determinants recognized by mouse RF are located in the C-terminal region of γ1, γ2a, and γ2b heavy chains.

Materials and Methods

Rheumatoid Factors. RF-secreting hybridomas were derived from spleen cells of 129/Sv, BALB/c, CBA/Ht, or C57Bl/6 mice as described (5, 7). Both cell culture supernatants and purified proteins were used as a source of monoclonal RF. RF-containing sera were obtained from 20-wk old 129/Sv, MRL/MpJ-lpr and NZB/BinJ mice. The former were maintained in our colony; the latter two were obtained from The Jackson Laboratory (Bar Harbor, ME).

Proteins. Hen egg white lysozyme was purchased from Koch-Light Laboratories Ltd

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TABLE I

Binding of IgG1-specific Monoclonal RF to IgG1 Mutants and F(ab')$_2$ Fragments*

Strains	Monoclonal RF	MOPC 21‡	Mutant MOPC 21‡		Anti-lysozyme IgG1§	
			IF1	IF2	Native	F(ab')$_2$
129/Sv	1302G3	31	0	31	28	0
	1312E8	23	2	29	33	0
	1413A3	25	0	30	28	0
	1415D9	13	0	30	33	0
	8816C7	19	0	29	28	0
	8817D7	27	0	33	34	1
	A5701A4	25	0	29	33	0.7
	A7206F3	27	0	30	33	0.4
	B2001A7	23	0	25	34	0
	B2003G5	20	0	23	33	0
	B1202A5	22	23	30	34	0
BALB/c	B2701B11	28	0	28	37	0
C57B1/6	B2806C3	26	0	26	33	0.3
	B2801F11	27	0	28	35	0
	B2802D1	30	0	29	27	0
CBA/Ht	A5503D10	20	2	26	19	1
	A5501C3	17	2	29	31	0.4

* Measured by solid phase RIA as described in (7) and expressed in cpm × 10^{-3} after subtraction of cpm bound to wells coated with BSA. The latter never exceeded 10% of the specific binding. Binding of IgG1-specific RF to F(ab')$_2$ fragments was studied with wells coated overnight with lysozyme (30 μg/ml) and incubated for 2 h at 37°C with 10 μg/ml monoclonal IgG1 anti-lysozyme antibody (A7202E11) or its F(ab')$_2$ fragments. When checked with ^{125}I-labeled affinity-purified rabbit antibodies against mouse Fab fragments, the binding of F(ab')$_2$ fragments to lysozyme-coated wells was found to be equivalent to that of native antibody.

‡ Adsorption of proteins onto polyvinyl wells was checked with ^{125}I-labeled rabbit anti-mouse Fab antibody. Wells incubated with IF1 bound approximately two times more anti-Fab antibody than either MOPC21 and IF2. Experiments were run in triplicate.

§ Experiments run in duplicate.

(Berkshire, U.K.). MPC11 (γ2b, κ) and variant proteins were purified from ascites fluids as described (8). MOPC21 (γ1, κ), MOPC173 (γ2a, κ), and hybridoma proteins, A7202E11 (γ1, κ), 1103G4 (γ2a, κ), 308A8 (γ2b, κ), and A7708F11 (γ2a, κ) were purified from ascites fluid by chromatography on Protein A Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden), and mutant proteins IF1 and IF2 were isolated by ammonium sulfate precipitation and sucrose gradient ultracentrifugation. A7202E11, an IgG1 anti-lysozyme antibody of 129/Sv origin, was submitted to pepsin digestion (0.1 mg/mg antibody) at pH 3.6 for 24 h at 37°C. F(ab')$_2$ fragments were purified by chromatography on AcA44 Ultrogel (LKB, Bromma, Sweden), and their molecular weight and purity checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Coating and Agglutination of Polystyrene Particles. Carboxylated polystyrene particles (0.8 μm diameter, Rhône-Poulenc, Courbevoie, France) were coated with purified monoclonal proteins by the carbodiimide method as described (7).

Results

Determinants Recognized by Monoclonal IgG1-specific RF. We first tested the binding of IgG1-specific RF to F(ab')$_2$ fragments derived from a monoclonal IgG1 anti-lysozyme antibody (A7202E11). Typical results are shown in Table I. Each of 71 monoclonal anti-IgG1 RF tested, strongly reacted when intact antibody was complexed to lysozyme but not when F(ab)'$_2$ fragments were used. In a second set of experiments we examined the binding of these monoclonal RF to mutant IgG1 molecules, IF1 and IF2, which are derived from the MOPC21 cell line and carry γ1 chains lacking the C_H3 and C_H1 domains, respectively. Typical results are given in Table I. All RF optimally bound to IF2, whereas 70 of 71 completely failed to react with IF1, which strongly suggests the C_H3 location of the determinants involved in these reactions. The RF that reacted

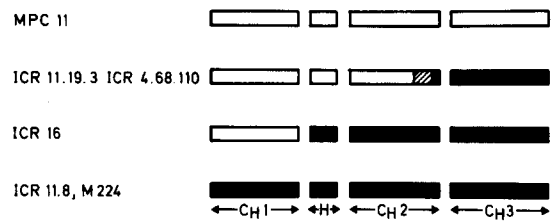

FIGURE 1. Heavy chain constant region organization of MPC11 and of 3 groups of mutant proteins. γ2b segments are denoted (▭) and γ2a segments (▬). The hatched area (▨) represents a sequence (N316-339) comprising 24 aminoacids that are identical between γ2a and γ2b chains. The proteins used in the present experiments are indicated.

TABLE II

*Reaction of Monoclonal RF with IgG2b Variant Proteins: Agglutination of Polystyrene Particles**

Monoclonal RF	% Agglutination of particles coated with:							
	IgG2a	IgG2b		Variant IgG2b				
	1103G4	308A8	MPC11	ICR 11.19.3	ICR 4.68.110	ICR 16	M224	ICR 11.8
Anti-IgG2a								
A5701B4	90	0	0	85	80	94	96	94
A5705B8	91	0	0	93	94	95	97	86
A5705A5	92	0	0	66	68	93	94	75
A6507A10	88	5	3	92	83	93	93	88
A6504C12	80	0	4	83	89	88	92	88
A6503G8	65	4	0	87	89	92	92	86
Anti-IgG2a‡								
A6107G6	90	6	0	89	83	94	96	92
A6506G[illegible]	88	4	5	90	90	83	92	55
A7105D4	88	0	0	91	89	90	96	94
A8601A2	90	0	0	57	55	88	94	88
A8603A11	91	5	4	51	61	92	96	93
A8602B4	91	3	2	60	70	88	96	88
A8305H6	87	0	0	0	8	75	83	90
Anti-IgG2b								
A4403A3	0	74	84	0	17	3	8	0
A4401F8	0	83	85	0	0	0	9	7
A4403H6	0	83	89	0	9	0	0	0
B2801D11	0	76	82	0	3	0	8	0

* Culture supernatants were incubated for 1 h at 37°C with polystyrene particles. Nonagglutinated particles were then instrumentally counted as described in Materials and Methods.

‡ Unlike anti-IgG2a RF, anti-IgG2a^a RF do not bind to IgG2a^b.

with IF1 did not bind to F(ab')$_2$ fragments and therefore probably recognized determinants in the C_H2 domain.

Determinants Recognized by Monoclonal IgG2a- and IgG2b-specific RF. To localize the determinants recognized by anti-IgG2a and anti-IgG2b RF, we examined their binding to mutant proteins produced by variants derived from the MPC11 (γ2b, κ) cell line.

On the basis of peptide maps, charge, and assembly characteristics, these mutant proteins fall into three groups (10). Representatives of each group have been studied further by amino acid sequence analysis and nucleic acid studies (9, 11–13). The structures of the heavy chain constant regions of the variant proteins used in the present work are schematically summarized in Fig. 1.

The binding of 4 IgG2b-specific and 13 IgG2a-specific monoclonal RF to these variant proteins as well as to normal IgG2a and IgG2b was tested by agglutination of IgG-coated polystyrene particles. Anti-IgG2a RF included autoantibodies

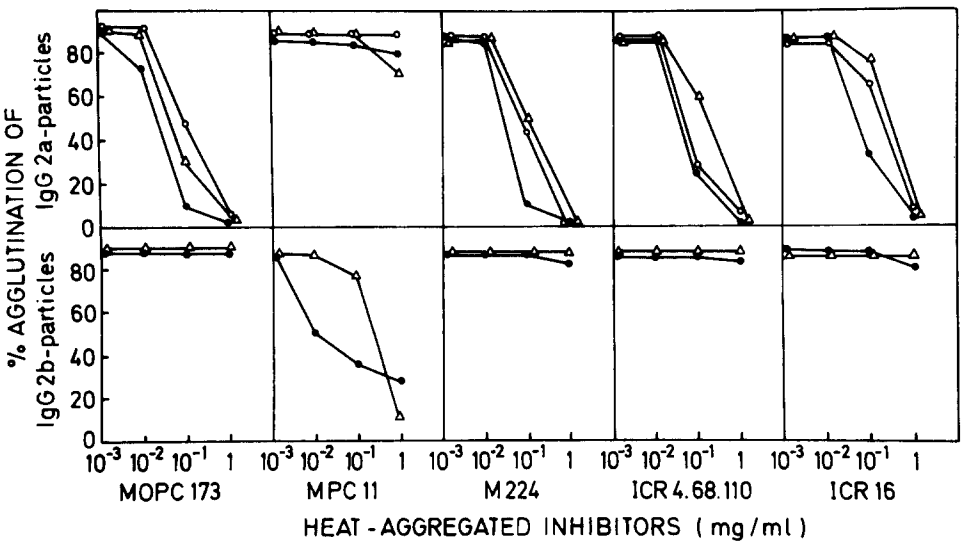

FIGURE 2. Localization of the determinants recognized by polyclonal RF of MRL/l (△), NZB/BinJ (●) and 129/Sv (○) mice. Inhibition of IgG2a- and IgG2b-specific RF by heat-aggregated IgG2a (MOPC173), IgG2b (MPC11), and IgG2b mutant proteins (M224, ICR 4.68.110, and ICR 16). Appropriately diluted sera were incubated with inhibitors for 1 h at 37°C before addition of polystyrene particles coated with IgG2a (1103G4) or with IgG2b (308A8). Residual agglutinations were measured as described in (7).

directed against isotypic and allotypic determinants. The results shown in Table II indicate that most of these RF reacted only in the presence of the proper segment between N-340 and the C-terminus. Of the 17 monoclonal RF tested, only one IgG2a-specific RF (A8305H6) apparently reacted with a structure located in the C_H2 domain or in the hinge region because it agglutinated particles coated with ICR16 but failed to react with ICR 11.19.3 or ICR 4.68.110. These results were confirmed by solid phase radioimmunoassay with IgG-coated wells (not shown).

Determinants Recognized by Polyclonal IgG2a- and IgG2b-specific RF. It was previously shown that sera of autoimmune strains, MRL/MpJ-lpr and NZB/BinJ, contain both IgG2a- and IgG2b-specific RF (7). Because of the simultaneous presence in these sera of anti-IgG2a and anti-IgG2b autoantibodies, it was not possible to map the antigenic determinants involved by direct binding or agglutination assays. Competition experiments with heat-aggregated variant proteins were used instead. For 129/Sv sera, which lack IgG2b-specific but have high titers of IgG2a-specific RF, these experiments confirmed the data already obtained with monoclonal RF derived from this strain. With MRL/MpJ-lpr and NZB/BinJ sera, heat-aggregated ICR 11.19.3 and ICR 4.68.110 completely inhibited the agglutination of IgG2a-coated particles and not at all that of IgG2b-coated particles (Fig. 2).

Discussion

RF-like anti-IgG autoantibodies have now been detected in many species, but the determinants recognized by RF have so far only been mapped for human IgG. Using limited digestion of human IgG fragments with pepsin, it was

concluded that RF binding sites were distributed in both C_H3 and C_H2 domains (14).

Previous attempts to localize the determinants recognized by mouse monoclonal RF by using papain fragments of various mouse IgG subclasses have met with little success, especially for anti-IgG1 RF. The latter, which represent the major form of RF produced after polyclonal activation, indeed failed to react with both IgG1 Fab or Fc fragments (5). This observation led to the tentative conclusion that the determinants involved were located in the hinge region; however, in the absence of any positive identification, it was difficult to exclude the possibility that conformational changes altering determinants located elsewhere in the molecule were responsible for the observed lack of reaction with Fab and Fc. It seemed thus important to analyze the determinants involved in RF-IgG interactions with intact immunoglobulins. Variant immunoglobulins generated by mutagenic treatment of MPC11 and carrying various hybrid γ2a-γ2b heavy chains have been used before to determine which parts of the mouse γ2b heavy chain react with Fc receptors of macrophages and to localize allotypic determinants recognized by monoclonal alloantibodies (15, 16). In the present experiments, these proteins permitted clear-cut mapping of the determinants recognized by IgG2a- and IgG2b-specific RF to a segment of the heavy chain spanning the C-terminal eight residues of the C_H2 domain and the complete C_H3 domain. Presence of a γ2a sequence in this part of the heavy chain completely abolished all reactions with IgG2b-specific RF and was sufficient to optimally bind most IgG2a-specific RF, be they allotype-specific or not.

Various spontaneous somatic mutants derived from the MOPC21 myeloma cell line have extensively been characterized (9). Two of them, IF1 and IF2, which lack the C_H3 and C_H1 domains of the γ1 heavy chain, respectively, were of particular interest to map the determinants recognized by anti-IgG1 RF. These variant immunoglobulins have been used before to demonstrate the importance of the C_H3 domain for the binding of IgG to mouse lymphocytes (17). Of 71 monoclonal IgG1-specific RF obtained from 4 different strains under a variety of conditions including polyclonal activation in vivo or in vitro, only 1 bound to IF1, whereas all reacted equally well with IF2 and normal IgG1. Together with the absence of reaction between IgG1-specific RF and IgG1 $F(ab')_2$ fragments, these observations strongly suggest that, like IgG2a- and IgG2b-specific RF, anti-IgG1 autoantibodies also preferentially recognize determinants located in the C-terminal region of the heavy chain.

Summary

The structures recognized by monoclonal anti-IgG1 rheumatoid factors (RF) were localized by testing their reactivity with mutant immunoglobulins carrying γ1 chains that lacked either the C_H1 or the C_H3 domains. While optimal binding was observed in the absence of C_H1, deletion of C_H3 completely abolished the reactivity of all but one of the 71 monoclonal RF tested.

Similar experiments were carried out with IgG2a- and IgG2b-specific RF by using variant immunoglobulins carrying various hybrid γ2a-γ2b heavy chains. It was found that both the polyclonal RF produced by autoimmune strains, MRL/MpJ-lpr and NZB/BinJ, and most of the monoclonal RF derived from normal strains, BALB/c, C57Bl/6, and 129/Sv, were directed against determinants

located in a segment spanning the C-terminal 8 residues of the C_H2 domain and the complete C_H3 domain.

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