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 (Ighª) or 129/Sv (Ighª) 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 1

| Strains       | Monoclonal RF | MOPC 21* | Mutant MOPC 21* | Anti-lysozyme IgG1 |
|---------------|---------------|----------|-----------------|--------------------|
|               |               | IF1      | IF2             | Native             | F(ab')2            |
| 129/Sv        | 1302G3        | 31       | 31              | 28                 | 0                  |
|               | 1312E8        | 23       | 2               | 29                 | 0                  |
|               | 1415D9        | 13       | 30              | 33                 | 0                  |
|               | 8816C7        | 19       | 29              | 34                 | 1                  |
|               | 8817D7        | 27       | 33              | 34                 | 1                  |
|               | A5701A4       | 25       | 33              | 35                 | 0                  |
|               | A7206F5       | 27       | 30              | 35                 | 0.4                |
|               | B2001A7       | 23       | 25              | 34                 | 0                  |
|               | B2003G5       | 20       | 23              | 33                 | 0                  |
|               | B1202A5       | 22       | 28              | 35                 | 0.7                |
|               | B2701B11      | 28       | 28              | 37                 | 0.5                |
| BALB/c        | B2806C3       | 26       | 26              | 35                 | 0.5                |
| C57B1/6       | B2801F11      | 27       | 28              | 35                 | 0                  |
|               | B2802D1       | 30       | 29              | 37                 | 0                  |
| CBA/Ht        | A5505D10      | 20       | 22              | 26                 | 19                 |
|               | A5501C3       | 17       | 2               | 29                 | 31                 | 0.4                |

* Measured by solid phase RIA as described in (7) and expressed in cpm $\times 10^{-5}$ 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 125I-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 125I-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 1. 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 Cα3 and Cα1 domains, respectively. Typical results are given in Table 1. All RF optimally bound to IF2, whereas 70 of 71 completely failed to react with IF1, which strongly suggests the Cα3 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 amino acids 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 | IgG2a  | IgG2b  | Variant IgG2b |
|---------------|--------|--------|---------------|
|               | 1103G4 | 308A8  | ICR 11.19.3   |
| Anti-IgG2a    |        |        | ICR 4.68.110 |
| A5701B4       | 90     | 0      | 95            |
| A5705B8       | 91     | 0      | 95            |
| A5705A5       | 92     | 0      | 95            |
| A6507A10      | 88     | 5      | 93            |
| A6504C12      | 80     | 4      | 89            |
| A6503G8       | 65     | 4      | 92            |
| A6107G6       | 90     | 6      | 96            |
| A6506C7       | 88     | 4      | 95            |
| A7105D4       | 88     | 0      | 96            |
| A8601A2       | 90     | 0      | 95            |
| A8605A11      | 91     | 5      | 94            |
| A8602B4       | 91     | 3      | 95            |
| A8305H6       | 87     | 0      | 86            |
| Anti-IgG2b    |        |        | M224 ICR 11.8 |
| A4403A3       | 90     | 6      | 94            |
| A4401F8       | 88     | 4      | 95            |
| A4403H6       | 88     | 0      | 94            |
| B2801D11      | 76     | 82     | 94            |

* 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 RF do not bind to IgG2a*.

with IF1 did not bind to F(ab')2 fragments and therefore probably recognized determinants in the C4d 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.
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 Cα2 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 CH3 and CH2 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 MPC 11 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 CH2 domain and the complete CH3 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 CH3 and CH1 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 CH3 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 CH1 or the CH3 domains. While optimal binding was observed in the absence of CH1, deletion of CH3 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, BA1.B/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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References

1. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J.
    McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine
    lupus-like syndromes. *J. Exp. Med.* 148:1198.
2. Van Snick, J. L., and P. L. Masson. 1979. Age-dependent production of IgA and
    IgM autoantibodies against IgG2a in a colony of 129/Sv mice. *J. Exp. Med.* 149:1519.
3. Van Snick, J. L., and P. L. Masson. 1980. Incidence and specificities of IgA and IgM
    anti-IgG autoantibodies in various mouse strains and colonies. *J. Exp. Med.* 151:45.
4. Dresser, D. W., and A. M. Popham. 1976. Induction of an IgM anti-(bovine)-IgG
    response in mice by bacterial lipopolysaccharide. *Nature (Lond.)*. 264:552.
5. Van Snick, J. L., and P. Coulie. 1982. Monoclonal anti-IgG autoantibodies derived
    from lipopolysaccharide-activated spleen cells of 129/Sv mice. *J. Exp. Med.* 155:219.
6. Dziarski, R. 1982. Preferential induction of autoantibody secretion in polyclonal
    activation by peptidoglycan and lipopolysaccharide. 1. In vitro studies. *J. Immunol.*
    128:1018.
7. Van Snick, J. L., V. Stassin, and B. de Lestré. 1983. Isotypic and allotypic specificity
    of mouse rheumatoid factors. *J. Exp. Med.* 157:1006.
8. Francus, T., and B. K. Birshtein. 1978. An IgG2a-producing variant of an IgG2b-
    producing mouse myeloma cell line. Structural studies in the Fc region of parent and
    variant heavy chains. *Biochemistry.* 17:4324.
9. Adetugbo, K., C. Milstein, and D. S. Secher. 1977. Molecular analysis of spontaneous
    somatic mutants. *Nature (Lond.)*. 265-299.
10. Francus, T., B. Dharmgrongartama, R. Campbell, M. D. Scharff, and B. K. Birshtein.
    1978. IgG2a-producing variants of an IgG2b-producing mouse myeloma cell line. *J.
    Exp. Med.* 147:1535.
11. Eckhardt, L. A., S. A. Tilley, R. B. Läng, K. B. Marcu, and B. K. Birshtein. 1982.
    DNA rearrangements in MPC 11 immunoglobulin heavy chain class switch variants.
    *Proc. Natl. Acad. Sci. USA.* 79:3006.
12. Birshtein, B. K., R. Campbell, and B. Diamond. 1982. Effects of immunoglobulin
    structure on Fc receptor binding: a mouse myeloma variant immunoglobulin with a
    γ2b-γ2a hybrid heavy chain having a complete γ2a Fc region fails to bind to γ2a Fc
    receptors on mouse macrophages. *J. Immunol.* 129:610.
13. Birshtein, B. K., R. Campbell, and M. L. Greenberg. 1980. A γ2b-γ2a hybrid
    immunoglobulin heavy chain produced by a variant of the MPC 11 mouse myeloma
    cell line. *Biochemistry.* 19:1730.
14. Natvig, J. B., and M. V. Turner. 1971. Localization of Gm markers to different
    molecular regions of the Fc fragment. *Clin. Exp. Immunol.* 8:685.
15. Diamond, B., B. K. Birshtein, and M. D. Scharff. 1979. Site of binding of mouse
    IgG2b to the Fc receptor on mouse macrophages. *J. Exp. Med.* 150:721.
16. Oi, V. T., L. A. Herzenberg, and B. K. Birshtein. 1983. Localization of murine Igh-1a
    allotypic determinants using a panel of mouse myeloma variant immunoglobulins.
    *J. Immunol.* 130:1967.
17. Ramasamy, R., D. S. Secher, and K. Adetugbo. 1975. C_{H3} domain of IgG as binding
    site to Fc receptor on mouse lymphocytes. *Nature (Lond.)*. 153:656.