Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 94–98, January 1992
Immunology

Monoclonal IgM rheumatoid factors bind IgG at a discontinuous epitope comprised of amino acid loops from heavy-chain constant-region domains 2 and 3

(autoantibody/antibody structure/rheumatoid arthritis)

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Communicated by Matthew D. Scharff, September 16, 1991 (received for review May 12, 1991)

ABSTRACT **A combination of site-directed mutagenesis and exon exchange has been used to further define the structure on IgG recognized by monoclonal IgM rheumatoid factors (RFs) from patients with Waldenstrom macroglobulinemia. Most of these RFs bound IgG1, -2, and -4 but not IgG3. For these RFs, His-435 is a critical residue for binding and replacing it with arginine, the residue present in IgG3, destroys or reduces RF binding. However, additional polymorphic sequences in both the heavy-chain constant-region domains (C_H) 2 and 3 are important for RF binding. Among the important residues in C_H2 are amino acids 252–254 and 309–311, which are conserved among IgG isotypes and comprise two loops of amino acids on the surface of the domain. Therefore, at least three regions, two from C_H2 and one from C_H3, contribute significantly to the epitope recognized by the RFs. Although this epitope contains many of the same residues as the staphylococcal protein A binding site on IgG, the binding specificities of staphylococcal protein A and monoclonal RFs are not identical. Sera from patients with rheumatoid arthritis contain antibodies directed not only at this epitope but also at other sites on IgG.**

Rheumatoid factor (RF) autoantibodies bind to the constant regions of IgG and are thought to contribute to synovial inflammation in patients with rheumatoid arthritis (RA) by forming complement-activating immune complexes with autologous IgG (1). Although RFs can be detected in the sera of a majority of RA patients (2) and the presence of RFs in RA patients correlates with more active disease (3), RFs are not specific for RA and are found in many other disease states (4–6). RFs also occur in apparently healthy individuals (6, 7), and RFs are elicited by antigen–antibody complexes as part of the normal immune response (8, 9). Understanding the function of RFs and the mechanisms of their induction requires a molecular characterization of the RF–IgG interaction and a precise definition of the epitopes on IgG recognized by RFs.

Previous studies evaluated RF-binding specificities by measuring RF binding to myeloma IgG. To define more precisely the epitopes on IgG recognized by RF autoantibodies, we have used chimeric IgG antibodies consisting of murine variable (V) regions fused to human constant (C) regions expressed from cloned genes in transfected cells as targets for RF binding (10). Polyclonal IgM RFs from RA patients bind chimeric IgG1, -2, and -4 strongly; chimeric IgG3 is also bound, although generally less well (11). The polyclonal nature of RFs in RA limits their analysis; therefore, monoclonal RFs from patients with lymphoproliferative diseases have been used as a model for studying RF specificities, V gene usage, and idiotypes. The majority of monoclonal IgM RFs from patients with Waldenstrom macroglobulinemia, a plasma cell dyscrasia, bind chimeric IgG1, -2, and -4 only (11).

Binding sites on IgG for both polyclonal and monoclonal RFs were previously localized to the interface of the heavy-chain C-region domains (C_H) 2 and 3 by measuring RF binding to proteolytically cleaved fragments of IgG (12–14) and by performing competitive inhibition studies with staphylococcal protein A (SPA) (12, 13), a protein known from x-ray crystallographic studies to bind IgG at the C_H2–C_H3 interface (15). By shuffling heavy-chain C-region exons between IgG4 and IgG3 and creating hybrid IgG4/IgG3 antibodies, we showed that the binding of monoclonal RFs to IgG4 and not IgG3 is attributable to sequence variation in C_H3 (11). We have now used site-directed mutagenesis to produce chimeric IgG antibodies with amino acid substitutions to define further the amino acids in C_H2 and C_H3 that comprise the epitope on IgG recognized by monoclonal RFs.

MATERIALS AND METHODS

Mutagenesis and Expression of Chimeric IgG. Human C-region genes for IgG3, IgG4, and a hybrid IgG4/IgG3 gene were cloned into the *Sal* I and *Bam*HI sites of the M13mp19 polylinker. Site-directed mutations were introduced in these genes either by the two-primer method (16) or by the uracil-containing template method (17). Mutations were verified by dideoxynucleotide chain-termination sequencing (18). IgG3 mutants containing His-435 were generated by subcloning C_H3 from mutated hybrid IgG4/IgG3 genes into the IgG3 heavy-chain gene (11). Mutated C-region genes were subcloned into the pSV2 ΔH-gpt vector downstream of a murine anti-dansyl V heavy-chain gene. Each of these heavy-chain expression vectors was cotransfected by electroporation along with pACYC184 ΔH-neo, an expression vector containing a murine anti-dansyl V κ gene joined to the human C κ gene, into a non-immunoglobulin-producing mouse myeloma cell line, P3X63AG8.653. Transfectants were selected in G418 (GIBCO) at 1 mg/ml.

Binding Assays. A RF ELISA was developed for measuring RF binding to chimeric IgG (11). Ninety-six-well microtiter plates were coated with dansylated bovine serum albumin for at least 12 hr at 4°C. Plates were blocked with 2.5% bovine serum albumin for 1 hr at 37°C and then incubated with chimeric IgG at 0.22 μg per well for 12–16 hr at 4°C. Serum or purified IgM RF was added to the plate for 3 hr at room temperature, and bound IgM was detected with goat anti-human IgM-specific antibody conjugated to horseradish per-

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Abbreviations: RF, rheumatoid factor; RA, rheumatoid arthritis; SPA, staphylococcal protein A; C and V, constant and variable, respectively; C_H, heavy-chain C-region domain(s); mAb, monoclonal antibody.

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oxidase (Tago) at 1:20,000 dilution for 1 hr at room temperature. The plates were developed, and the ODs were measured. SPA binding to chimeric IgG was measured by ELISA as described (11).

RESULTS

We previously showed that a majority of monoclonal RFs bound IgG4 and did not bind IgG3 (group I RFs) and that sequence variation in the C_H3 domain could destroy the epitope recognized by the RFs (11). To determine the responsible amino acids in the C_H3 domain, site-directed mutagenesis was used to change residues 435 and 436, positions at which IgG3 differs from IgG1, -2, and -4 (Fig. 1). The amino acid sequence at positions 435 and 436 in IgG1, -2, and -4 is His-Tyr; IgG3 can contain Arg-Phe or Arg-Tyr (34), and the sequence of our IgG3 clone is Arg-Tyr at these positions. These amino acids have been implicated in RF binding (12, 14, 20) because (*i*) they are located at the C_H2–C_H3 interface (Fig. 1), (*ii*) they are SPA contact residues, and (*iii*) pH titration and chemical modification studies suggested a role for histidine and tyrosine residues in RF binding.

Variation at Position 435 in C_H3 Contributes to, but Is Not Solely Responsible for, the Differential Binding of Group I RFs. Residue 435 was first mutated in a hybrid antibody containing the C_H1, hinge, and C_H2 of IgG4 fused to the C_H3 of IgG3 (abbreviated 4-4-4-3). This hybrid, unmutated antibody was not bound by group I monoclonal RFs (Table 1). Replacing Arg-435 with histidine in the C_H3 of this hybrid antibody resulted in strong binding by 14 of 15 group I monoclonal RFs (Table 1). To determine whether the presence of Arg-435 was

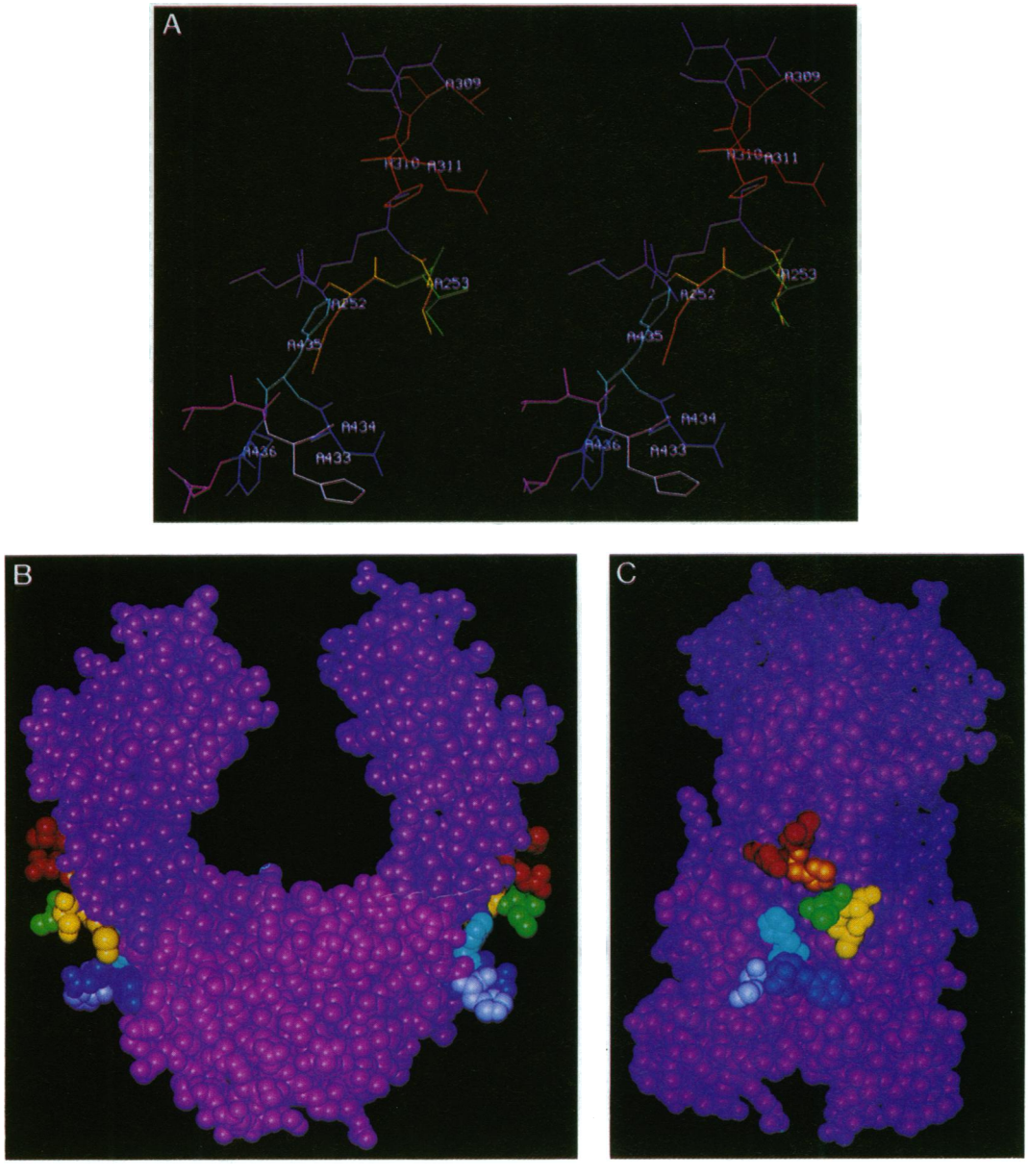

FIG. 1. Graphic representation of the Fc fragment of IgG1 (15). Residues in three loops contributing to the monoclonal RF or SPA-binding site are highlighted. The remaining residues in C_H2 are colored violet, whereas the remaining residues in C_H3 are colored magenta. Highlighted residues are colored as follows: the C_H3 loop—His-433 (gray), Asn-434 (blue), His-435 (aqua), and Tyr-436 (blue); the proximal C_H2 loop—Met-252 (yellow), Ile-253 (green), and Ser-254 (yellow) (see below); the distal C_H2 loop—Leu-309 (red), His-310 (orange), and Gln-311 (red). (*A*) Close-up view in stereo of the three loops contributing to the binding site. An additional residue in backbone representation is shown at the carboxyl and amino termini of each loop for orientation. Relevant residues are numbered, except for Ser-254, the label of which was omitted for clarity. (*B*) Space-filling model of the entire Fc fragment in the same orientation as *A*. (*C*) Space-filling model of the Fc rotated 90° from the view shown in *A* and *B*. Graphics were made using INSIGHT II (Biosym Technologies, San Diego) on a Personal Iris workstation.

solely responsible for the RF-binding phenotype of IgG3, we expressed IgG3 with the C_H3 exon containing His-435. Surprisingly, IgG3 mutants containing His-435 were bound poorly, or not at all, by most monoclonal RFs (Table 1). Thus, a single amino acid change of arginine to histidine in C_H3 of IgG3 is sufficient to generate a monoclonal RF binding site when this mutated domain is combined with C_H2 of IgG4 but not when it is combined with C_H2 of IgG3. This result suggests that C_H2 contributes to the RF-binding site on IgG and that C_H2 of IgG3 differs from C_H2 of IgG4 in its ability to generate a functional binding site. In addition, the His-435-containing C_H3 of IgG3 is not equivalent to C_H3 of IgG4, which can form an epitope in association with C_H2 of IgG3 (11). Therefore, the C_H3 of IgG3 must contain amino acid differences, in addition to the His → Arg replacement, which reduce its potential for forming an RF epitope. Conversely, when arginine was substituted at position 435 in IgG4 (Table 1), binding for 10 of 15 RFs was destroyed, but the remaining 5 RFs exhibited reduced, but significant, binding to this mutant. Therefore, although residue 435 plays a major role in the epitope recognized by RFs, other residues in C_H2 and C_H3 must also contribute to the differential binding of monoclonal RFs to IgG3 and IgG4.

To determine whether allotypic variation at position 436 affects RF binding, both phenylalanine and tyrosine were expressed at that position in various IgG antibodies (Table 1). Monoclonal RF binding was not significantly altered by the Phe-436 → Tyr substitution although three RFs unable to bind the tyrosine-containing allotype bound the phenylalanine allotype of IgG3 at low levels (14–24%). SPA binding was not affected by this substitution.

Two RFs in our panel of 17 bound both IgG3 and IgG4 (group II) but had different overall IgG subclass binding patterns (11). Group II RFs bound well to IgG4/IgG3 hybrid antibodies with His-435 but did not react with the intact IgG3 antibodies containing His-435. This result was surprising because these RFs bound strongly to IgG4 containing Arg-435 and suggests that the His-435 substitution in IgG3 alters the C_H2–C_H3 interface in such a manner that it is neither "IgG3-like" nor "IgG4-like" for RF binding.

Two Amino Acid Loops in C_H2 Contribute to the RF Binding Site. To identify sequences in C_H2 that contribute to the epitope on IgG recognized by monoclonal RFs, we introduced site-directed mutations into two amino acid loops connecting β-strands in C_H2. These residues, Met-Ile-Ser at 252–254 and Leu-His-Gln at 309–311, identical in IgG3 and IgG4, are located at the C_H2–C_H3 interface and are SPA contact residues (Fig. 1). We replaced these residues either with glycines, which removed the existing amino acid side chains, or with proline residues. Substitution of the residues at positions 252–254 of IgG4 with either three glycine or three proline residues eliminated binding for 14 of 15 group I RFs and for both group II RFs (Table 2).

The loop containing residues 309–311 is further away from the C_H2–C_H3 interface than the loop containing residues 252–254. Replacing the amino acids in this more distal loop with three glycine residues in IgG4 eliminated binding for 12 of 15 group I monoclonal RFs and for both group II RFs (Table 2). However, substitution of three proline residues at these positions resulted in detectable binding (>20%) for 10 of 15 group I RFs and for one group II RF. Surprisingly, seven group I RFs and one group II RF unable to bind to the mutant

Table 1. RF and SPA binding to chimeric IgG containing mutations at positions 435 and 436 in the C_H3 domain

	IgG3 Arg-Tyr	4-4-4-3 Arg-Tyr	4-4-4-3 His-Tyr	4-4-4-3 His-Phe	IgG3 His-Tyr	IgG3 His-Phe	IgG4 Arg-Tyr	IgG3 Arg-Phe
				Group I: Reactivity with IgG4 but not with IgG3				
Monoclonal RF								
Bro	2 ± 1.7	2 ± 1.5	89 ± 3.2	119 ± 6.6	11 ± 1.5	22 ± 2.6	9 ± 2.5	4 ± 2.3
Tabb	3 ± 1.5	2 ± 1.5	98 ± 0.9	94 ± 1.5	16 ± 2.8	16 ± 3.3	30 ± 7.1	1 ± 0.9
B2	3 ± 2.9	2 ± 0.6	92 ± 3.6	100 ± 1.2	35 ± 3.9	67 ± 7.5	41 ± 6.5	21 ± 6.3
B3	0 ± 0.2	0	93 ± 1.4	53 ± 7.0	8 ± 1.4	5 ± 0.8	0 ± 0.3	0 ± 0.1
B4	2 ± 2.0	3 ± 1.2	99 ± 0.5	101 ± 1.1	37 ± 1.9	40 ± 6.8	12 ± 4.9	14 ± 4.3
B5	0	1 ± 0.6	107 ± 8.0	89 ± 6.7	9 ± 4.1	11 ± 3.7	1 ± 0.6	0
B6	0	0 ± 0.3	90 ± 2.3	101 ± 1.8	17 ± 2.6	35 ± 4.6	0 ± 0.3	0
B8	0 ± 0.4	2 ± 0.9	100 ± 9.5	68 ± 3.6	4 ± 0.2	6 ± 0.7	0	0
B9	6 ± 2.6	7 ± 1.2	92 ± 5.6	88 ± 3.2	27 ± 4.2	25 ± 3.9	28 ± 6.1	3 ± 2.0
B11	0	1 ± 1.0	93 ± 3.6	76 ± 0.8	3 ± 0.5	5 ± 0.8	0	0
B17	0 ± 0.8	3 ± 1.5	67 ± 2.0	109 ± 2.0	2 ± 0.8	33 ± 0.9	3 ± 1.6	5 ± 1.1
Bla	16 ± 3.3	19 ± 2.9	99 ± 5.0	99 ± 2.9	45 ± 7.8	48 ± 5.0	33 ± 3.8	14 ± 2.6
Ea	0 ± 0.7	0	1 ± 1.5	16 ± 4.8	0	0 ± 0.2	0 ± 0.5	0 ± 0.5
Wa	7 ± 3.1	11 ± 2.6	96 ± 2.3	113 ± 3.1	7 ± 0.6	17 ± 1.7	58 ± 14.3	24 ± 2.6
B18	0	1 ± 0.9	73 ± 3.4	114 ± 5.0	0	15 ± 1.5	0	0
				Group II: Reactivity with IgG3 and IgG4				
B12	61 ± 4.1	111*	81 ± 4.4	100 ± 1.5	1 ± 0.7	19 ± 2.4	102 ± 3.5	55 ± 2.8
B15	75 ± 3.0	118*	68 ± 1.1	98 ± 3.5	5 ± 1.8	13 ± 2.5	151 ± 10.0	103 ± 8.0
Polyclonal RF								
Aye	46 ± 2.3	63*	99 ± 2.4	96 ± 2.9	21 ± 5.0	23 ± 4.8	76 ± 0.7	33 ± 4.8
Bar	44 ± 7.1	68*	124 ± 11.2	123 ± 6.7	34 ± 4.2	37 ± 3.3	38 ± 2.3	32 ± 6.9
Ger	97 ± 3.0	120*	136 ± 3.9	134 ± 1.2	48 ± 3.3	53 ± 3.3	89 ± 7.6	82 ± 7.0
Sin	20 ± 5.9	30*	98 ± 0.7	98 ± 1.6	30 ± 4.7	39 ± 4.7	40 ± 5.1	22 ± 3.9
SPA								
SPA	1 ± 0.6	1 ± 0.3	97 ± 0.6	87 ± 0.3	105 ± 0.3	101 ± 2.3	1 ± 0.6	1 ± 0.6

RF binding to mutants of chimeric IgG was measured by ELISA and is presented as % of binding to IgG4, which is defined as 100% for each RF. Values represent the mean of three determinations ± SEM. IgG3 or IgG4 indicates that the mutation was introduced in the context of the wild-type chimeric IgG3 or IgG4 antibody. 4-4-4-3 indicates that the mutation was introduced into an antibody with C_H1, hinge, and C_H2 derived from IgG4 and the C_H3 domain from IgG3. The identity of residues at positions 435 and 436 for each mutant is indicated beneath its number code. As an example of ODs from a single experiment, RF binding to IgG4 yielded 0.542 unit for Tabb, 1.216 for B2, and 0.776 for Sin, whereas background readings were 0.022, 0.026, and 0.031, respectively.

*Single determinations only.

containing glycine substitutions bound the mutant containing proline substitutions at levels from 20% to 83%. All eight RFs exhibiting this differential binding to glycine- and proline-containing mutants at 309–311 (Bro, Ea, Wa, B2, B6, B12, B17, and B18) express the Wa cross-reactive idiotype (11, 19). Only one Wa cross-reactive idiotype-positive RF in our panel did not exhibit this behavior (B4).

Binding Specificities of Monoclonal RFs and SPA Are Not Identical. SPA did not react with IgG4 containing an arginine substitution at position 435, but unlike monoclonal RFs, SPA bound equally well to all mutant antibodies containing His-435 (Table 1). This indicates that differential binding of SPA to IgG3 and IgG4, in contrast to monoclonal RFs, is attributable simply to the interchange of arginine and histidine at position 435 in IgG3 and IgG4.

SPA did not bind to any of the IgG4 antibodies with substitutions in the C_H2 loops. Only five monoclonal RFs, four from group I and one from group II, were similar to SPA in this respect (Table 2), further indicating that the binding specificities of monoclonal RFs and SPA are not identical.

A Subpopulation of Polyclonal RFs Bind IgG at the Epitope Recognized by Monoclonal RFs. Like monoclonal RFs, polyclonal RFs from RA patients bound significantly better to the His-435-containing IgG4/IgG3 hybrid antibodies than to the IgG3 mutants containing the His-435 substitution (Table 1). Polyclonal RF binding to the IgG4 antibodies containing mutations in C_H2 indicated that mutations in the proximal loop, 252–254, were more deleterious than mutations in the distal loop, 309–311 (Table 2). These data suggest that polyclonal RFs contain a subpopulation of RF antibodies with a binding specificity similar to that of the monoclonal RFs but also contain autoantibodies directed at other epitopes on IgG.

Table 2. RF and SPA binding to IgG4 containing mutations in two C_H2 amino acid loops

	Proximal loop		Distal loop	
	252–254 G-G-G	252–254 P-P-P	309–311 G-G-G	309–311 P-P-P
Group I: Reactivity with IgG4 but not IgG3				
Monoclonal RF				
Bro	1 ± 0.9	1 ± 0.4	3 ± 1.8	24 ± 2.7
Tabb	0 ± 0.3	0 ± 0.4	61 ± 4.9	90 ± 3.9
B2	0 ± 0.2	0 ± 0.6	9 ± 2.8	86 ± 1.8
B3	0 ± 0.2	0 ± 0.5	0 ± 0.2	0 ± 0.4
B4	1 ± 0.4	1 ± 0.5	57 ± 16.3	91 ± 4.2
B5	0 ± 0.3	1 ± 0.2	10 ± 3.8	19 ± 4.5
B6	0 ± 0.2	0 ± 0.1	2 ± 2.1	58 ± 6.5
B8	0 ± 0.2	0 ± 0.8	2 ± 0.4	1 ± 0.7
B9	0 ± 0.7	0 ± 0.8	11 ± 4.5	12 ± 5.8
B11	0 ± 0.2	0 ± 0.3	1 ± 0.6	1 ± 0.4
B17	0 ± 0.2	1 ± 0.7	1 ± 0.7	55 ± 1.9
Bla	33 ± 9.4	22 ± 3.3	92 ± 4.7	76 ± 3.3
Ea	0	0 ± 0.8	0 ± 0.8	28 ± 7.3
Wa	0 ± 0.2	2 ± 1.9	2 ± 1.8	83 ± 0.4
B18	0 ± 0.5	0 ± 1.3	0 ± 3.8	20 ± 11.2
Group II: Reactivity with IgG3 and IgG4				
B12	0 ± 0.5	1 ± 1.3	6 ± 2.4	69 ± 3.0
B15	0 ± 0.4	1 ± 0.5	1 ± 1.9	2 ± 1.0
Polyclonal RF				
Aye	55 ± 5.6	48 ± 5.1	90 ± 4.4	82 ± 1.2
Bar	18 ± 4.2	13 ± 0.8	67 ± 2.8	54 ± 1.0
Ger	43 ± 8.1	28 ± 6.8	97 ± 11.2	80 ± 8.3
Sin	74 ± 12.1	13 ± 6.4	78 ± 5.9	63 ± 7.0
SPA				
SPA	1 ± 0.7	1 ± 0.3	2 ± 0.6	0 ± 0.3

RF binding to chimeric IgG4 containing mutations at residues 252–254 or 309–311 was measured and presented as in Table 1. These residues were replaced either with three glycines (G-G-G) or with three prolines (P-P-P). Binding to wild-type IgG4 is defined as 100%.

DISCUSSION

Site-directed mutagenesis coupled with exon exchange has now been used to investigate the binding specificity of monoclonal RFs. Three regions of exposed loops make a major contribution to the epitope recognized by the RFs: residues 252–254 and 309–311 in C_H2 and residue 435 in C_H3 (Fig. 1). Therefore three regions, noncontiguous in linear sequence, fold into close proximity to make an epitope similar to other protein epitopes recognized by monoclonal antibodies (mAbs) (21).

Residue 435 is polymorphic in human IgG, being histidine in IgG1, -2, and -4 and arginine in IgG3. This arginine substitution in IgG3 is largely responsible for disrupting the epitope recognized by group I monoclonal RFs, but additional amino acid differences in C_H2 and C_H3 must contribute to the loss of the epitope in IgG3. The identity of the contributing polymorphic residues is not obvious because none of the nine amino acid differences between IgG3 and IgG4 in C_H2 or the six differences in C_H3, excluding residue 435, are located at the C_H2–C_H3 interface or contact SPA. Instead, it is more likely that these residues modify the folding of the domain leading to subtle conformational changes. It remains formally possible that the conformational change in the C_H2 domain of IgG3 is actually mediated by more amino-terminal sequences in the hinge or C_H1 domain.

His-435 (aqua in Fig. 1) lies in a loop projecting into the C_H2–C_H3 interface. Modeling studies indicate that arginine at this position cannot be accommodated in the space occupied by histidine (15). In addition to stabilizing the C_H2–C_H3 interface, His-435 may also directly contact RFs as it does SPA (15). Additional residues in the C_H3 loop, including His-433, Asn-434, and Tyr-436, protrude from the surface of the protein at the C_H2–C_H3 interface (see Fig. 1) and contact SPA; some of these residues probably also contact RFs. Replacing Tyr-436 with phenylalanine did not affect monoclonal or polyclonal RF binding, indicating that this allotypic polymorphism does not functionally alter the RF epitope.

To determine whether two amino acid loops in the C_H2 domain involved in SPA binding similarly contribute to RF binding, we replaced these amino acids with either glycine or proline residues. The proximal loop containing residues 252–254 is critical for RF binding, and substitution of either glycine or proline residues at these positions eliminates RF binding for all but one group I RF. This loop contains a residue, Ile-253 (green in Fig. 1), the side chain of which is extensively covered by side chains from SPA in the SPA–IgG complex (15) and is reminiscent in this sense of the lysozyme residue Gln-121 in the lysozyme–mAb D1.3 complex (22). The functional importance of Gln-121 in mAb D1.3 binding to lysozyme (22) and its contribution to the "energetic epitope" recognized by mAb D1.3 (23) suggests that Ile-253 may be similarly important in SPA and RF binding to IgG. The effects on RF binding observed for mutation of residues 252–254 may be mediated largely by replacement of Ile-253.

The dependence of RF binding on the distal C_H2 loop containing residues 309–311 is more variable. The distal loop is critical for IgG binding for six RFs and dispensable for three other RFs. However, the remaining eight RFs exhibit differential binding to IgG4 containing glycine and proline substitutions in this loop, suggesting that the residues in this loop contribute to the epitope and that the side chains of proline are sufficient to stabilize the RF–IgG interaction. In addition, the presence of three glycines, with their variety of permissible torsion angles, may significantly alter the conformation of this region and thereby disrupt RF binding. The correlation between expression of the Wa cross-reactive idiotype and differential binding to these glycine- and proline-

containing mutants suggests that the Wa cross-reactive idiotype may be associated with a structure on RF V regions involved in interaction with the distal C_H2 loop of IgG.

Although they bind IgG at the same region, SPA and monoclonal RFs clearly contact the residues at this site differently. It has been previously hypothesized that RFs are generated by an autoantiidiotypic mechanism in which RFs are produced as antiidiotypic antibodies to antibodies against SPA (12, 14). Although none of the binding specificities of the 17 monoclonal RFs studied here are identical to that of SPA, an antiidiotypic mechanism that copies the binding specificity of SPA imprecisely cannot be excluded as a means for generating these RFs. In fact, imprecision in such a mechanism is consistent with studies showing differences in the contacts between a mAb and either its cognate antigen or a specific antiidiotypic mAb (24).

Except for a conservative Leu-309 → Val change in IgG2, the residues we have identified as contributing to the epitope recognized by monoclonal RFs are conserved among IgG1, -2, and -4 and probably comprise the "Ga antigen" described by Allen and Kunkel 25 years ago as a prominent target for polyclonal RFs from RA patients (25). Despite their interaction with both IgG3 and IgG4, group II RFs appear to bind IgG in the same region because binding depends on one or both of the C_H2 loops, and binding to IgG3 containing His-435 is sharply reduced. Furthermore, binding of both group II RFs to IgG4 can be completely inhibited by SPA (unpublished data). Therefore, although there is variability among RFs in IgG subclass binding patterns and in the importance of various contact residues, all 17 monoclonal RFs studied bind IgG at the same region: the C_H2–C_H3 interface.

Extensive analysis of monoclonal RF V genes and idiotypes indicates that monoclonal RFs predominantly use two light-chain genes, humvk325 and humvk328 (26), in conjunction with a restricted set of V heavy-chain genes (27). This restricted V gene usage may simply be a reflection of the highly restricted specificities of RF autoantibodies for the epitope at the C_H2–C_H3 interface of IgG. It seems unlikely that the hydrophobic nature of the epitope alone could account for monoclonal RF binding exclusively to this site because there are several other hydrophobic patches on IgG (28) and because heterologous anti-IgG mAbs can be easily generated against other parts of the IgG molecule (29). Therefore, reactivity of all monoclonal RFs with this region strongly suggests that selection for this specificity is involved in the production of these antibodies.

RF mAbs with this binding specificity may be selected during development of the immune system because they have a beneficial function such as: (*i*) facilitating clearance of immune complexes (30), (*ii*) involvement in an important regulatory immune network during development (31), or (*iii*) serving as precursors of antibodies with other specificities (32). Low-affinity autoantibodies like RFs, so called "natural" antibodies, may therefore be selected by "natural" antigens like IgG or structurally similar molecules during development of the immune system.

Because polyclonal RFs from RA patients can express idiotypes other than those present on monoclonal RFs from Waldenstrom macroglobulinemia, polyclonal RFs may either use different combinations of variable elements or have somatically diverged from germ-line-encoded physiologic RFs similar to monoclonal RFs (19, 33). This divergence may be responsible for the specific features of polyclonal RFs, including their binding to epitopes on IgG distinct from that of monoclonal RFs, their increased binding to IgG3 compared with monoclonal RFs (11), and the possible existence of pathogenic RFs in RA.

We thank Lynda Hatam for her help with ELISAs and John M. Hubbard, director of the Columbia University Molecular Modeling Facility for Molecular Biology (National Science Foundation Grant DIR-8720229), for his expert assistance with the generation of computer graphics images. This work was supported in part by grants from the Easter Seal Research Foundation, the New York Chapter of the Arthritis Foundation, Inc., and by National Institutes of Health Grants CA16858 and AI129470.

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