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MOLECULAR ANALYSIS OF IgM RHEUMATOID FACTOR BINDING TO CHIMERIC IgG[1]

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To localize regions on IgG bound by rheumatoid factors (RF), we studied IgM RF binding to chimeric IgG antibodies consisting of murine V regions fused to human constant regions. Using a modified RF ELISA, we showed that polyclonal RF from rheumatoid arthritis patients bound IgG1, 2, and 4 strongly; IgG3 was also bound, although less well. The majority of 18 monoclonal RF from patients with Waldenstrom's macroglobulinemia bound IgG1, 2, and 4 only. In contrast to RF from RA, 14 of 18 monoclonal RF did not react with IgG3. Only 3 of 18 monoclonal RF bound IgG3 well. By shuffling C region domains between IgG3 and IgG4, we showed that sequence variation in the C_H3 domain is responsible for the differential binding of monoclonal RF to IgG3 and IgG4. Hybrid IgG3/IgG4 antibodies containing the C_H3 domain of IgG4 were bound by monoclonal RF, whereas those containing the C_H3 domain of IgG3 were not. To evaluate the contribution of the N-linked carbohydrate moiety at Asn-297 to RF binding sites on IgG, we measured RF binding to aglycosylated IgG antibodies produced by mutating Asn-297 to another amino acid. Glycosylated and aglycosylated IgG1, 2, and 4 were bound identically by monoclonal and polyclonal RF. Aglycosylated IgG3, however, was bound better than glycosylated IgG3 by polyclonal RF and by IgG3-reactive monoclonal RF.

RF[3] are autoantibodies that bind to the constant regions of IgG. Although their role in disease has not been fully elucidated, RF have been demonstrated in the sera of a majority of RA patients (1), and RF positivity has been shown to correlate with more active disease (2). IgM and IgG RF are abundantly produced in pannus tissue obtained from the joints of RF positive RA patients (3, 4), and these autoantibodies complexed with IgG may contribute to articular inflammation and destruction by fixing complement (5). However, the presence of polyclonal RF is not restricted to RA and, in fact, these RF have been detected in several other diseases of both rheumatic (6–8) and non-rheumatic origin (9). Monoclonal IgM RF are present in the sera of some patients with WMac, a lymphoproliferative disease without articular symptoms. Evidence that RF can be produced by normal individuals (10) and that humans (11) and mice (12) produce RF as a consequence of secondary protein immunization suggests that RF may serve a normal physiologic function.

To better understand the function of RF in disease and in the normal immune response, much attention has been focused on analysis of the specificities of RF from different diseases and on the characterization of human IgG determinants involved in RF binding to IgG. The initial IgG subclass binding pattern of polyclonal IgM RF to IgG1, 2, and 4 was termed the "Ga-specificity" by Allen and Kunkel (13, 14). More recent studies have generally substantiated a Ga specificity for polyclonal IgM RF from RA patients (15) but have also detected weak reactivity with IgG3 (16, 17). In contrast, monoclonal IgM RF from WMac patients were shown to bind strongly to IgG1 and variably to the remaining subclasses of IgG (17, 18). Immunochemical evidence indicates that both polyclonal and monclonal RF bind IgG near the interface of the C_H2 and C_H3 domains. Proteolytic digest experiments showed that only IgG fragments containing the intact C_H2-C_H3 interface were bound by RF (17, 19, 20). Furthermore, RF binding to IgG was shown to be inhibited by SPA, a protein known from x-ray crystallographic studies to bind IgG at the C_H2-C_H3 interface (17, 20, 21).

To localize RF binding sites on IgG more precisely, we have utilized a system in which chimeric mouse-human IgG genes are manipulated in vitro and expressed as mAb after transfection into lymphoid cells. Chimeric IgG antibodies were engineered by fusing the human H chain C region genes of each of the four subclasses of IgG to a murine anti-dansyl V_H gene, and by similarly joining the human κ C gene to a murine anti-dansyl V_K gene (22). The chimeric proteins consist of human C regions and murine V regions and therefore possess human effector functions (23) and specifically bind the hapten dansyl. A modified RF-ELISA was used to measure the interaction between RF and the four subclasses of monoclonal chi-

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[3] Abbreviations used in this paper: RF, rheumatoid factor; RA, rheumatoid arthritis; WMac, Waldenstrom's macroglobulinemia; SPA, Staph protein A; DNS-BSA, dansylated-BSA; RCRI, rheumatoid cross-reactive Id.

meric IgG antibodies as well as their genetically engineered variants.

We compared the specificities of monoclonal RF from WMac and polyclonal RF from RA and showed that these RF differ in their reactivity with IgG3. Although RF from both sources bind IgG1, 2, and 4, polyclonal RF bind IgG3 whereas monoclonal RF generally do not. Only 3 of 18 monoclonal RF react with IgG3 strongly. To localize the principle monoclonal IgM RF binding site, we created hybrid antibodies in which domains were exchanged between IgG subclasses. Our studies show that sequence variation in the C_H3 domain is responsible for the differential reactivity of monoclonal RF with IgG3 and IgG4.

Because galactosylation of serum IgG from RA patients is significantly reduced compared to that of normal individuals, it has been proposed that decreased galactosylation may expose or create new immunogenic determinants on IgG (24) that may be recognized by RF. IgG contains a conserved N-linked carbohydrate moiety interposed between the C_H2 domains of disulfide-linked H chains. To investigate the contribution of this carbohydrate moiety to RF binding sites on IgG, we have created a set of aglycosylated chimeric IgG antibodies in which the glycosylation site at Asn-297 was replaced using site-directed mutagenesis. Our results indicate that the absence of carbohydrate residues leads to increased reactivity of IgG3 with polyclonal RF and with monoclonal RF that bind IgG3.

MATERIALS AND METHODS

Chimeric antibody genes. Human C region genes for the four subclasses of IgG cloned from a genomic library were joined to the murine V_H gene cloned from the anti-dansyl-specific mouse hybridoma 27-44. The human κ L chain C region gene was similarly joined to the murine anti-dansyl V_K gene. The chimeric H chain and L chain genes were then subcloned into the pSV2 ΔH-gpt (25) and pSV184 ΔH-neo (26) expression vectors, respectively (Fig. 1*A*).

The IgG H chain C genes were sequenced by the dideoxy method (27) and the amino acid sequences deduced. The IgG1 H chain C gene contains the sequences associated with expression of the G1m(a) allotype (28). The IgG3 H chain C gene is identical in amino acid sequence to the IgG3 H chain OMM (29) and is expected to express the G3m(g) and G3m(b5) allotypes based on sequence analysis (28). The IgG4 clone is identical at the amino acid level to those IgG4 sequences previously reported (29).

Expression of chimeric IgG. Chimeric IgG was expressed by cotransfection of the H and L chain plasmids into P3X63AG8.653 cells, a non-Ig producing mouse myeloma cell line. Either electroporation (30) or protoplast fusion (31) was used as the method of gene transfer. After transfection, cells were plated at sufficiently low density in 96-well plates to ensure that resistant colonies would be monoclonal. Transfectants were selected and maintained in 1 mg/ml G418 (GIBCO, Grand Island, NY) for 3 to 4 wk. Antibody-producing clones were detected by screening 50 μl of culture supernatant from each well in an IgG ELISA using HPO-conjugated goat anti-human IgG antibody (Tago, Burlingame, CA).

Characterization of antibody-producing clones. To select transfectants with the highest rate of antibody production, 1×10^6 cells from several separate clones were grown for 24 h and IgG concentrations in the supernatant were assayed in a κ-chain specific ELISA (see below). Rates of antibody production for high producers ranged from 0.5 to 5 μg/10^6 cells/24 h. The assembly, secretion, and m.w. of chimeric IgG were determined by labeling cells with ^{35}S-methionine. The secreted IgG was immunoprecipitated with polyclonal rabbit anti-human IgG antiserum and fixed *Staphylococcus aureus* bacteria (Calbiochem, LaJolla, CA), and analyzed by SDS-PAGE under both reducing and non-reducing conditions.

Exon shuffling. To facilitate exon shuffling, the H chain C genes were subcloned into pBR322 as a *Sall-Bam*HI cassette. A *Pvu*I site, unique to the H chain gene, was inserted in the intron between two adjacent exons using *Pvu*I linkers. A set of constructs with *Pvu*I sites inserted between each pair of adjacent exons was created for IgG3 and IgG4 (Fig. 1*B*). Because pBR322 contains a single *Pvu*I site, restriction digest with *Pvu*I allowed the isolation of two DNA fragments each containing part of the H chain C gene from a single IgG subclass. These fragments, from either IgG3 or IgG4, were ligated to complementary fragments from IgG4 or IgG3, respectively, to create hybrid H chain genes composed of domains from different IgG subclasses.

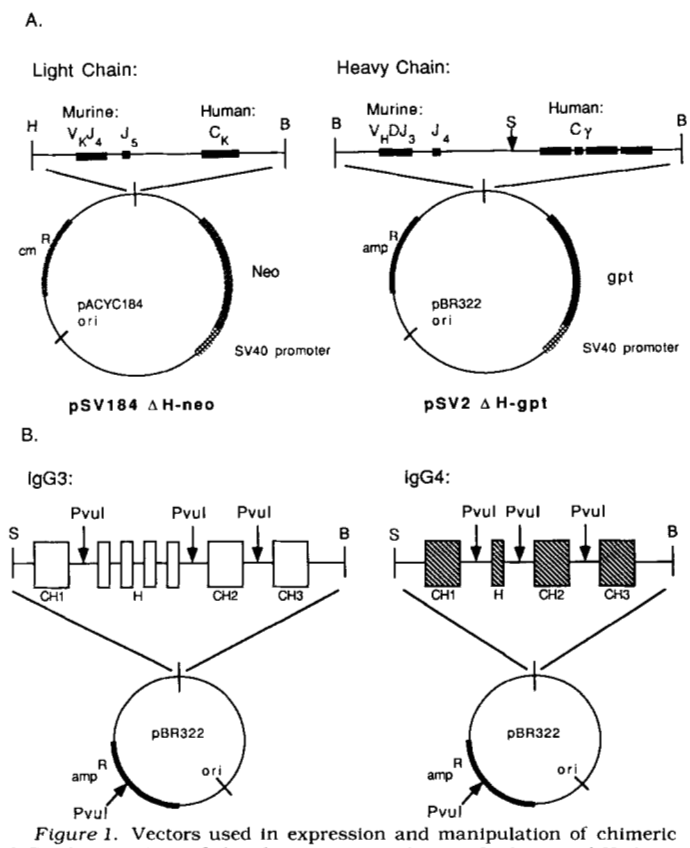

Figure 1. Vectors used in expression and manipulation of chimeric IgG. The structure of the chimeric mouse-human L chain and H chain genes and their respective expression vectors are shown (*A*). To facilitate exon shuffling, H chain C genes were subcloned in pBR322 as a *Sall-Bam*HI cassette and a unique *Pvu*I site was inserted between exons. A composite of three constructs, each containing a single *Pvu*I site in the H chain gene, is shown for both IgG3 and for IgG4 (*B*).

Oligonucleotide site-directed mutagenesis. The H chain C region gene of each IgG subclass was subcloned into the *Sall* and *Bam*HI sites of the M13mp19 polylinker. The two primer method (32) was used to introduce specific mutations in the codon for Asn-297. Mutations were verified by dideoxy sequencing (27).

Cell culture supernatants containing chimeric IgG. Secreting clones were grown in culture for 3 to 4 days in IMDM (GIBCO) and 5 or 10% FCS (HyClone, Logan, UT). After pelleting the cells, supernatants were collected and sodium azide was added to 0.02% (w/v) to inhibit microbial growth. Supernatant IgG concentrations were measured in an IgG ELISA. The 96-well microtiter plates (Immulon II, Dynatech, Chantilly, VA) were coated with 1.0 μg/well DNS-BSA for at least 12 h at 4°C. The plates were washed in PBS-Tween, blocked with 2.5% BSA at 37°C for 1 h, incubated with culture supernatant for 2 to 3 h, washed, and incubated with HPO-conjugated goat anti-human kappa chain antibody (Tago) at 1/10,000 dilution for 1 h. After washing and developing, the OD (490 nm) of individual wells were measured using an ELISA reader (Dynatech). IgG concentrations were interpreted from a standard curve of chimeric IgG of known concentration. Polyclonal anti-κ HPO-conjugated antibodies were used to ensure that all four subclasses of IgG would be identified equally.

Rheumatoid factor binding assay. To quantitate RF binding to IgG, a direct binding ELISA was developed in which 96-well plates were coated with DNS-BSA as stated above. After washing with PBS-Tween, the plate was blocked as above and incubated with 0.22 μg/well chimeric IgG for 12 to 16 h at 4°C. Serum or purified IgM RF was added to the plate for 3 h at room temperature and bound IgM was detected with HPO conjugated goat anti-human IgM specific antibody (Tago) at 1/20,000 dilution. After a 1 h incubation at room temperature, the plate was washed, developed, and the OD determined as stated above. Because all IgM RF bound strongly to IgG4, RF binding to each chimeric IgG protein is expressed as a percentage

of IgM RF binding to IgG4. RF⁻ WMac serum added to DNS-BSA-coated plates blocked with BSA yielded background OD units, comparable to those detected by adding the anti-IgM HPO conjugate to wells containing IgG, but no serum. Sera without RF activity from normal individuals yielded similar results. All four subclasses of chimeric IgG bound comparably to DNS-BSA-coated plates after an overnight incubation at 4°C as determined by measuring the concentration of unbound chimeric IgG by ELISA.

The concentration of chimeric IgG in the culture supernatants used above was chosen to ensure that the RF ELISA was performed under conditions of Ag excess. RF ELISA studies using varying amounts of immobilized IgG showed that RF binding for monoclonal RF-Tabb peaked and plateaued at 0.1 to 0.2 μg IgG/well over a wide range of RF concentrations.

SPA binding. SPA (Pharmacia Fine Chemicals, Piscataway, NJ) at a concentration of 0.5 μg/well was incubated overnight at 4°C in 96-well plates (Immulon II, Dynatech). Wells were then washed and blocked as stated above. Culture supernatants containing chimeric IgG were diluted to 0.02 μg/well and were incubated for 2 h at room temperature. The wells were then washed, incubated with HPO conjugated goat anti-human κ antibodies, developed, and read as stated above.

Immunoprecipitations of chimeric IgG with fixed *Staphylococcus aureus* bacteria were performed as above, except that rabbit anti-human IgG antiserum was omited.

RF⁺ sera. Whole sera from RA or WMac patients were used to study RF binding to IgG, except for monoclonal RF Ea, Tabb, and Wa, in which case purified 19S IgM RF was used. All RA sera had high titer RF activity measured by latex fixation (33) or Rose-Waaler hemagglutination assay (34). All RA patients met the ARA criteria for this diagnosis (35) and had active sustained disease for a minimum of 2 y. All WMac patients received this diagnosis based on clinical parameters and on serum electrophoresis data that indicated the presence of a monoclonal IgM spike. In all WMac patients, the monoclonal IgM antibody contained κ L chains.

Before use in the RF ELISA, RF⁺ sera were incubated at 37°C for 1 h to resuspend cryoprecipitates. Insoluble material was then pelleted by centrifugation for 5 min at 12,000 × *g*. Dilutions of sera were made in PBS-Tween containing 0.125% BSA. Working dilutions ranged from 1/5000 to 1/100,000 for RA sera and from 1/10,000 to 1/500,000 for WMac sera.

Purification of monoclonal IgM RF. Whole sera from WMac patients Ea, Tabb, and Wa were dialized in 0.1 M acetate buffer, pH 4.1, at 37°C. Sera were then fractionated by ACA 34 (Pharmacia) m.w. sieve chromatography in 0.1 M acetate buffer pH 4.1. The excluded volume peak contained purified 19S IgM RF.

Wa RCRI. All monoclonal RF were assayed for expression of the Wa RCRI using polyclonal rabbit $F(ab')_2$ anti-RCRI antibodies in an inhibition ELISA as previously described (36).

RESULTS

RF binding to subclasses of IgG. The IgG subclass binding patterns of 12 polyclonal IgM RF and 18 monoclonal IgM RF were determined using chimeric IgG antibodies in a RF-ELISA. Polyclonal IgM RF from the sera of patients with RA bound strongly to IgG1, 2, and 4 and generally had lower reactivity with IgG3 (Table I). Of 12 polyclonal RF examined, 10 RF had IgG3 reactivity ranging from 20 to 49% of binding to IgG4. Two RF, Bre and Ger, exhibited IgG3 reactivity of 76 and 96%, respectively, the highest in our panel of polyclonal RF. Polyclonal RF binding to IgG1 was equivalent to, or greater than, binding to IgG4 for all 12 RF tested. A total of 11 of 12 polyclonal RF bound IgG2 as well as, or better than, IgG4.

All 18 monoclonal RF from the sera of WMac patients bound IgG4 strongly. In contrast to polyclonal RF, 14 of 18 monoclonal RF had no reactivity with IgG3 (Table I). The monoclonal RF were divided into two groups based on their subclass binding patterns. Group I consists of 15 RF that bound IgG4 but had low or undetectable reactivity with IgG3. The binding patterns in group I fell into two subgroups. Group Ia consists of 12 RF that bound IgG1, 2, and 4. A total of 11 of these 12 did not react with IgG3, whereas one RF, Bla, demonstrated low but detectable binding to IgG3 (17%). The three RF in group Ib reacted with IgG2 and 4 but bound poorly or undetectably to IgG1 and IgG3. Group II contains the only three RF with strong binding to IgG3 (48 to 74%). These three RF each exhibited a different overall subclass binding pattern, suggesting that they possess different specificities despite their common interaction with IgG3. In addition to WMac, one of these patients, B13, also had longstanding RA and therefore the possibility of contamination with polyclonal RF cannot be excluded. In fact, the overall IgG subclass binding pattern for B13 is similar to that of the polyclonal RF from RA patients.

Nine monoclonal RF expressed the Wa RCRI previously demonstrated on approximately 60% of monoclonal RF (37). There was no correlation between the presence of the Wa RCRI and a particular subclass binding pattern.

Monoclonal RF binding to domain shuffled IgG. Our data indicate that 14 of 18 monoclonal IgM RF bind IgG4 but not IgG3. To localize the structures responsible for the difference in RF binding properties of these two proteins, a series of hybrid IgG3/IgG4 antibodies was produced by shuffling exons between the IgG3 and IgG4 H chain constant region genes (Fig. 1*B*).

Monoclonal RF in group I that reacted with IgG4 but had low or undetectable reactivity with IgG3 were assayed for binding to a set of six domain shuffled chimeric IgG proteins (Table II, summarized in Fig. 2). Constructs 2203 and 2205 represent reciprocal exchanges of C_H1 between IgG4 and IgG3. Hybrid protein 2203 was recognized by all 15 monoclonal RF, whereas antibody 2205, containing the C_H1 domain of IgG4, showed the same reactivity as IgG3. This implies that the C_H1 domain of IgG4 does not constitute the monoclonal RF binding site. Hybrid antibody 2206, consisting of IgG3 C_H1 and IgG3 hinge joined to the C_H2 and C_H3 domains of IgG4, was bound by all monoclonal RF studied. The reciprocal hybrid 2207, with the C_H2-C_H3 domains of IgG3, was not bound by RF. These results exclude the hinge region as a significant contributor to the RF binding site and localize monoclonal RF binding to the C_H2-C_H3 portion of IgG.

To ascertain which domain contributes the determinants responsible for differential binding to IgG3 and IgG4, the C_H3 domains were exchanged between these subclasses. Hybrid protein 2204 with C_H1-hinge-C_H2 from IgG4 and C_H3 from IgG3 was not bound by any members of our panel of monoclonal RF. However, antibody 2208 with C_H1-hinge-C_H2 from IgG3 and C_H3 from IgG4 was bound by all monoclonal RF. These data indicate that the sequences responsible for the differential binding of monoclonal IgM RF to IgG3 and IgG4 lie in the C_H3 domain. A subset of monoclonal RF including B3, B8, and B11 bound less well to domain shuffled antibodies containing the CH_3 domain of IgG4 than to wild type IgG4 (Table II). Binding of these monoclonal RF was shown to increase to near wild type levels by increasing the amount of immobilized hybrid IgG3/IgG4 antibodies (data not shown).

Because of the evidence indicating that SPA and RF may bind similar or identical sites, we measured SPA binding to our domain shuffled IgG antibodies. Immunoprecipitation of ^{35}S-methionine-labeled IgG with whole fixed *S. aureus* bacteria and direct binding of chimeric IgG antibodies to SPA by ELISA were used. Both techniques showed that SPA and group I monoclonal RF have

TABLE I

IgM RF and SPA binding to subclasses of chimeric IgG[a]

RF	WA RCRI	IgG1	IgG2	IgG3	IgG4
A. Monoclonal RF					
Group I. Reactivity with IgG4, but not IgG3:					
Ia. IgG1, 2 and 4 binding pattern					
BRO	+	73 ± 1.7	55 ± 1.2	2 ± 1.1	100
TABB		99 ± 5.9	97 ± 3.7	2 ± 1.3	100
B2	+	79 ± 4.9	89 ± 4.2	3 ± 1.9	100
B3		68 ± 9.5	98 ± 4.3	0 ± 0.1	100
B4	+	107 ± 1.4	82 ± 5.2	2 ± 1.2	100
B5		86 ± 6.9	81 ± 5.0	0 ± 0.4	100
B6	+	48 ± 3.5	61 ± 1.3	0 ± 0.1	100
B8		51 ± 8.3	106 ± 1.7	0 ± 0.2	100
B9		105 ± 4.0	69 ± 6.6	5 ± 1.7	100
B11		86 ± 7.1	61 ± 3.2	0 ± 0.2	100
B17	+	32 ± 9.6	49 ± 3.9	0 ± 0.9	100
BLA		106 ± 1.6	92 ± 3.2	17 ± 1.4	100
Ib. IgG2 and IgG4 binding pattern					
EA	+	6 ± 0.4	30 ± 1.6	0 ± 0.6	100
WA	+	13 ± 0.8	78 ± 1.7	6 ± 1.5	100
B18	+	9 ± 3.3	23 ± 4.6	0 ± 1.5	100
Group II. Reactivity with IgG3					
B12	+	6 ± 4.0	76 ± 4.2	58 ± 3.2	100
B13		69 ± 16.1	77 ± 5.5	48 ± 1.5	100
B15		15 ± 5.8	9 ± 1.1	74 ± 1.0	100

RF	IgG1	IgG2	IgG3	IgG4
B. Polyclonal RF				
AYE	89 ± 7.2	95 ± 2.9	46 ± 1.5	100
BAR	188 ± 9.2	147 ± 10.8	43 ± 1.8	100
BON	147 ± 4.3	117 ± 7.7	47 ± 3.2	100
BRE	152 ± 5.7	161 ± 10.3	76 ± 7.1	100
FEL	105 ± 2.0	99 ± 1.2	49 ± 2.0	100
FIA	110 ± 4.0	136 ± 6.4	28 ± 3.2	100
GER	113 ± 3.2	143 ± 1.0	96 ± 1.2	100
IMP	99 ± 2.9	102 ± 3.0	49 ± 1.8	100
MER	103 ± 0.7	96 ± 2.2	32 ± 2.5	100
QUI	91 ± 2.3	79 ± 0.9	20 ± 2.2	100
SIN	100 ± 2.9	101 ± 3.0	23 ± 1.6	100
WEI	142 ± 4.3	119 ± 2.8	30 ± 3.9	100

	IgG1	IgG2	IgG3	IgG4
C. Staph protein A				
SPA	88 ± 3.3	94 ± 0.6	1 ± 0.6	100

[a] IgM RF binding to the four subclasses of chimeric IgG was measured using a RF ELISA. Chimeric antibodies were first bound to Ag-coated plates and then RF binding to the immobilized chimeric antibody was detected using an enzyme conjugated anti-μ chain specific antiserum. The results for each RF are expressed as a percentage of binding to IgG4, which is defined as 100%. For the purpose of grouping monoclonal RF, RF binding was considered to be positive if reactivity was more than 20% of binding to IgG4. The data are presented as the mean of three determinations ± SEM. All monoclonal RF were assayed for the presence of the Wa rheumatoid cross-reactive idiotype (RCRI) and those RF that express the Wa RCRI are indicated with +. SPA binding was measured by adding chimeric IgG to SPA-coated plates and using an enzyme conjugated anti-κ chain antiserum to detect bound IgG.

similar specificities. SPA bound wild type chimeric IgG 1, 2, and 4 but not IgG3 (Table I). SPA and monoclonal RF bound to the same domain shuffled IgG antibodies (Table II). The differential binding of SPA to IgG3 and IgG4 is therefore also attributable to structural differences in the C_H3 domain, consistent with x-ray crystallographic studies (21).

Role of IgG carbohydrate in RF binding. Chimeric IgG antibodies free of carbohydrate at residue 297 were created by substituting an alternate amino acid for Asn at this position by site-directed mutagenesis. Two mutations were introduced at this position for each subclass: a conservative change of Asn to Gln and non-conservative substitutions to His for IgG1, 2, and 4, or Lys for IgG3. These mutants were shown to be free of N-linked carbohydrate by comparing the electrophoretic mobility of these proteins with that of wild type antibodies produced in the presence or absence of tunicamycin (38) (M.-H. Tao and S. L. Morrison, unpublished observations). To evaluate the role of IgG carbohydrate in RF binding, we compared the ability of several monoclonal and polyclonal IgM RF to bind glycosylated and aglycosylated IgG.

Monoclonal and polyclonal RF binding to aglycosylated IgG1, 2, and 4 was equivalent to binding to glycosylated chimeric IgG1, 2, and 4 (Table III). Monoclonal RF that do not bind IgG3 were similarly unable to bind aglycosylated IgG3. However, all four polyclonal RF examined bound aglycosylated IgG3 significantly better than wild type IgG3. A monoclonal RF, Bla, which reacted weakly with IgG3, also exhibited increased reactivity with aglycosylated IgG3. For the polyclonal RF and monoclonal RF-Bla, binding to aglycosylated IgG3 was 38 to 69% greater than binding to wild type IgG3. All three monoclonal RF in group II also showed significantly increased reactivity with aglycosylated IgG3 (data not shown). No significant difference in RF binding was seen between conservative and non-conservative substitutions at position 297 for any of the aglycosylated antibodies.

We explored the possibility that this difference in RF binding to glycosylated and aglycosylated IgG may vary with IgG concentration. We bound increasing amounts of glycosylated and aglycosylated IgG1 and IgG3 to DNS-BSA coated microtiter plates and measured RF binding using a fixed concentration of RF. IgG1 and aglycosylated

TABLE II

Monoclonal IgM RF and SPA binding to domain shuffled IgG[a]

RF	IgG3	IgG4	C_H1 Exchanges		C_H2-C_H3 Exchanges		C_H3 Exchanges	
			2205 4-3-3-3	2203 3-4-4-4	2207 4-4-3-3	2206 3-3-4-4	2204 4-4-4-3	2208 3-3-3-4
BRO	1 ± 0.3	100	3 ± 2.0	95 ± 2.2	2 ± 1.2	91 ± 3.0	2 ± 1.5	84 ± 2.9
TABB	0 ± 0.3	100	5 ± 2.5	87 ± 2.4	1 ± 0.7	95 ± 4.2	2 ± 1.5	83 ± 5.0
B2	0	100	6 ± 1.3	95 ± 1.5	1 ± 0.3	96 ± 1.2	2 ± 0.6	97 ± 1.0
B3	0 ± 0.3	100	0 ± 0.3	49 ± 7.3	0	60 ± 2.4	0	61 ± 8.0
B4	2 ± 0.9	100	4 ± 1.2	101 ± 1.5	2 ± 0.9	100 ± 2.3	3 ± 1.2	94 ± 1.3
B5	0 ± 0.3	100	0 ± 0.3	65 ± 4.6	0 ± 0.3	75 ± 2.8	1 ± 0.6	65 ± 2.5
B6	0	100	0 ± 0.3	95 ± 1.5	0 ± 0.3	93 ± 1.8	0 ± 0.3	91 ± 2.5
B8	1 ± 0.3	100	3 ± 0.9	34 ± 10.1	0	23 ± 5.5	2 ± 0.9	37 ± 5.8
B9	4 ± 2.0	100	13 ± 1.9	89 ± 0.9	2 ± 0.9	96 ± 1.2	7 ± 1.2	87 ± 2.6
B11	0	100	0 ± 0.3	53 ± 4.4	0 ± 0.3	57 ± 5.5	1 ± 1.0	53 ± 2.3
B17	1 ± 0.7	100	2 ± 0.9	88 ± 3.5	1 ± 0.7	85 ± 4.7	3 ± 1.5	86 ± 4.4
BLA	15 ± 1.2	100	23 ± 3.2	97 ± 2.9	16 ± 2.6	97 ± 2.6	19 ± 2.9	88 ± 2.9
EA	0	100	0	64 ± 5.7	0	47 ± 3.1	0	45 ± 2.1
WA	2 ± 0.3	100	16 ± 5.4	95 ± 3.8	8 ± 2.7	88 ± 0.9	11 ± 2.6	81 ± 2.3
B18	2 ± 0.9	100	0 ± 0.3	72 ± 4.6	1 ± 0.9	65 ± 3.7	1 ± 0.9	54 ± 2.4
SPA[b]	1 ± 0.6	100	3 ± 0.6	81 ± 1.3	2 ± 0.6	101 ± 0.3	1 ± 0.3	100 ± 0.3

[a] H chain C gene exons were shuffled between IgG3 and IgG4, generating six hybrid antibodies. The domain structure of each protein is represented schematically at the top of each column in the order C_H1-hinge-C_H2-C_H3. The number 3 or 4 indicates whether the exon originated from the IgG3 or IgG4 H chain gene. Monoclonal IgM RF that reacted strongly with IgG4 and weakly or undetectably with IgG3 (group I) were assayed for binding to these domain shuffled antibodies in a RF ELISA and the results for each RF are expressed as a percentage of binding to IgG4, defined as 100%. Results are presented as the mean of three determinations ± SEM.

[b] SPA binding to these hybrid antibodies is included for comparison.

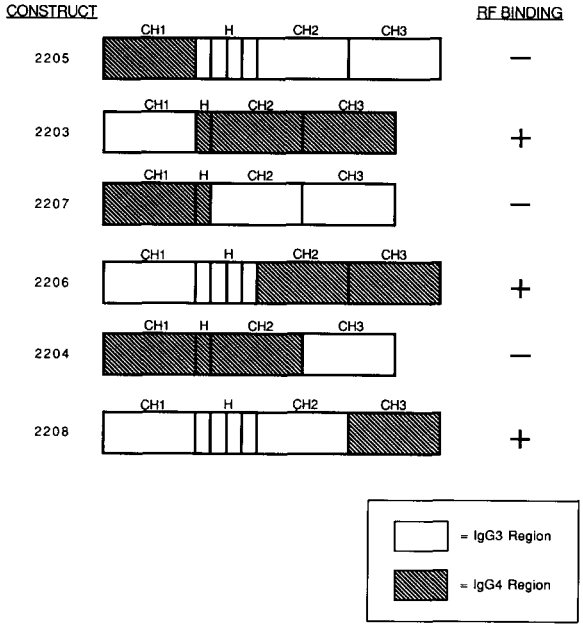

Figure 2. Group I monoclonal RF binding to domain shuffled IgG. Results of monoclonal RF binding to hybrid IgG3/IgG4 antibodies from Table II are summarized above. The structure of the hybrid antibodies are represented schematically. *Open boxes* indicate IgG3 sequences and hatched boxes represent IgG4 sequences. The ability of these hybrid proteins to be bound by monoclonal RF is indicated by + or −.

IgG1 were bound identically by both polyclonal RF-Sin and monoclonal RF-Bla (Fig. 3). However, the difference in binding of polyclonal RF-Sin to the glycosylated and aglycosylated forms of IgG3 increased with increasing amounts of immobilized IgG. This effect was also seen for polyclonal RF-Ger (data not shown). Although monoclonal RF-Bla binds significantly better to aglycosylated IgG3 than to wild type IgG3, the dependence of this effect on Ag concentration is not as dramatic. Control experiments designed to measure the proportion of IgG that actually binds to the Ag-coated plate indicate that the glycosylated and aglycosylated forms of IgG3 bind with similar efficiency.

DISCUSSION

The precise role of IgM RF in the development of the chronic articular disease characteristic of RA is unknown. Although these autoantibodies are synthesized in pannus tissue (3, 4), are found complexed with autologous IgG and can fix complement (5), the association of specific RF with the initiation or development of chronic arthritis has not been established. The characterization of RF binding sites on IgG is an important step in understanding the functions of RF and the mechanisms of RF induction.

The nature of these epitopes on IgG bound by IgM RF autoantibodies has been unclear in part because the methods previously available for localizing RF binding sites relied on myeloma IgG antibodies and antibody fragments produced by enzymatic digestion as RF binding substrates. Myeloma reagents can be contaminated by antibodies of other IgG subclasses (17); furthermore, conformational changes in IgG fragments may occur as a consequence of proteolytic digestion. The use of IgG antibodies expressed from cloned genes represents an alternative approach for precisely identifying RF binding epitopes within the C region of human IgG. Monoclonal chimeric IgG antibodies are expressed in culture free from contamination with other IgG subclasses, and they do not require purification prior to their use as reagents in our RF binding assay.

To determine the IgG subclass specificity of IgM RF, we developed a modified RF ELISA using chimeric IgG antibodies as binding substrates. All polyclonal IgM RF studied bound IgG1, 2, and 4 strongly and showed reactivity with IgG3 ranging from 20 to 96% of the binding to IgG4. Reactivity with IgG3 is not characteristic of the Ga specificity initially described for a majority of polyclonal IgM RF (13), but is more consistent with recent studies (16, 17). Of the 18 monoclonal IgM RF studied, 12 selectively bound IgG1, 2, and 4 and 14 did not react with IgG3.

Because RF can occur in normal individuals and in WMac patients without pathogenic consequences, it is

TABLE III

Comparison of IgM RF binding to glycosylated and aglycosylated chimeric IgG[a]

IGG	POS.297	BRO	TABB	B2	BLA
A. Monoclonal RF					
IgG1	ASN	69 ± 0.5	104 ± 6.7	73 ± 7.2	100 ± 4.1
IgG1-H	HIS	71 ± 3.0	102 ± 8.5	77 ± 5.8	99 ± 5.8
IgG1-Q	GLN	73 ± 2.4	102 ± 12.0	80 ± 4.4	99 ± 5.0
IgG2	ASN	54 ± 3.3	92 ± 5.5	84 ± 4.0	96 ± 7.7
IgG2-H	HIS	66 ± 4.0	99 ± 3.2	93 ± 1.1	98 ± 3.5
IgG2-Q	GLN	85	108	100	106
IgG3	ASN	2 ± 0.2	1 ± 0.2	2 ± 0.3	15 ± 3.2
IgG3-K	LYS	4 ± 0.3	6 ± 2.0	4 ± 0.9	25 ± 2.0
IgG3-Q	GLN	4 ± 1.1	8 ± 2.6	4 ± 0.9	26 ± 3.5
IgG4	ASN	100	100	100	100
IgG4-H	HIS	92 ± 5.2	94 ± 5.9	94 ± 5.6	98 ± 4.0
IgG4-Q	GLN	96 ± 2.8	100 ± 4.7	99 ± 1.1	99 ± 5.5
IGG	POS.297	AYE	BAR	GER	SIN
B. Polyclonal RF					
IgG1	ASN	92 ± 6.2	189 ± 21.0	113 ± 7.9	99 ± 8.2
IgG1-H	HIS	92 ± 4.5	185 ± 22.6	110 ± 8.3	100 ± 7.7
IgG1-Q	GLN	96 ± 5.5	190 ± 18.5	114 ± 4.4	102 ± 8.2
IgG2	ASN	100 ± 10.1	155 ± 20.4	154 ± 9.8	105 ± 9.4
IgG2-H	HIS	107 ± 5.6	160 ± 11.2	153 ± 8.7	107 ± 5.3
IgG2-Q	GLN	105	171	158	107
IgG3	ASN	51 ± 1.6	46 ± 3.8	103 ± 6.8	23 ± 1.2
IgG3-K	LYS	74 ± 0.9	69 ± 4.0	137 ± 3.1	40 ± 5.1
IgG3-Q	GLN	74 ± 2.0	66 ± 5.8	136 ± 1.6	37 ± 3.7
IgG4	ASN	100	100	100	100
IgG4-H	HIS	86 ± 8.1	87 ± 1.3	83 ± 2.3	79 ± 7.0
IgG4-Q	GLN	99 ± 1.3	101 ± 1.4	98 ± 1.2	99 ± 1.9

[a] Aglycosylated IgG antibodies were engineered by replacing the Asn glycosylation site at position 297 by site-directed mutagenesis. Two substitutions at residue 297 were made for each subclass and in the table these aglycosylated antibodies are symbolized by the name of the IgG subclass followed by the one letter amino acid code representing the substitution at position 297. Glycosylated antibodies are represented simply by the name of the IgG subclass. IgM RF binding to glycosylated chimeric IgG and to aglycosylated chimeric IgG was measured using a RF ELISA and the results are expressed as a percentage of binding to IgG4, defined as 100%. The data are presented as the mean of three determinations ± SEM, except for IgG2-Q which is only a single determination.

possible that a subpopulation of RF in RA have unique specificities associated with disease, and that these specificities are absent in WMac (39). We have shown that polyclonal RF from RA patients bind IgG3 whereas 14 of 18 monoclonal RF from WMac do not bind IgG3. These data suggest that IgG3 reactive RF may be important in RA and, in fact, the significance of anti-IgG3 antibodies in RA has been suggested previously. B cells from rheumatoid synovia have been shown to produce IgM RF that have higher avidity for IgG3 than do serum RF (16). Because rheumatoid synovial cells produce increased amounts of IgG3 antibody (40), it has been postulated that anti-IgG3 RFs are produced in the joint, precipitate as immune complexes after interaction with locally produced IgG3, and fix complement thereby amplifying articular inflammation (15). Whether these RF react solely with IgG3 or also with other subclasses is unclear. However, IgG3-reactive monoclonal RF produced by cells isolated from rheumatoid synovia have been shown to possess both types of specificities: IgG3 binding only and reactivity with all four IgG subclasses (41).

In a recent report, a predominant IgG subclass binding pattern was not demonstrated for monoclonal IgM RF (17). These monoclonal RF bound principly to IgG1 and, in contrast to our findings, more than 50% of the monoclonal RF bound IgG3 strongly (17). These results may differ from our findings because of impurities in myeloma IgG antibody preparations or because of possible differences in patient populations used in the two studies.

In addition to the potential disadvantages of using myeloma antibodies, differences between previously published polyclonal RF binding patterns and those presented here may be attributable to purification of RF from serum. Isolation of RF under acidic conditions may alter subclass binding patterns by selectively destroying important subpopulations of polyclonal RF (42). Purification of polyclonal RF by affinity chromatography on polyclonal IgG immunoabsorbants, which consist predominantly of IgG1 and IgG2, may cause selective loss of IgG3 binding polyclonal RF. Finally, because polyclonal RF from RA patients have been shown to contain anti-allotypic specificities (43–45), allotypic differences among IgG antibodies used in RF binding assays may contribute to observed differences in IgG subclass binding patterns. The use of IgG expressed from cloned genes offers the advantage of altering allotypes at desired positions by site-directed mutagenesis to evaluate the contribution of anti-allotypic RF.

We have shown using domain shuffled IgG that sequences lying within the C_H3 domain are responsible for the differential reactivity of group I monoclonal IgM RF with IgG3 and IgG4. A hybrid protein containing the C_H3 domain of IgG4 fused to the remainder of the IgG3 molecule is bound by monoclonal RF. Conversely, replacing the C_H3 domain of IgG4 with the C_H3 domain of IgG3 creates a hybrid antibody which is not bound by monoclonal RF (Fig. 2).

Although the C_H3 domain contains a critical determi-

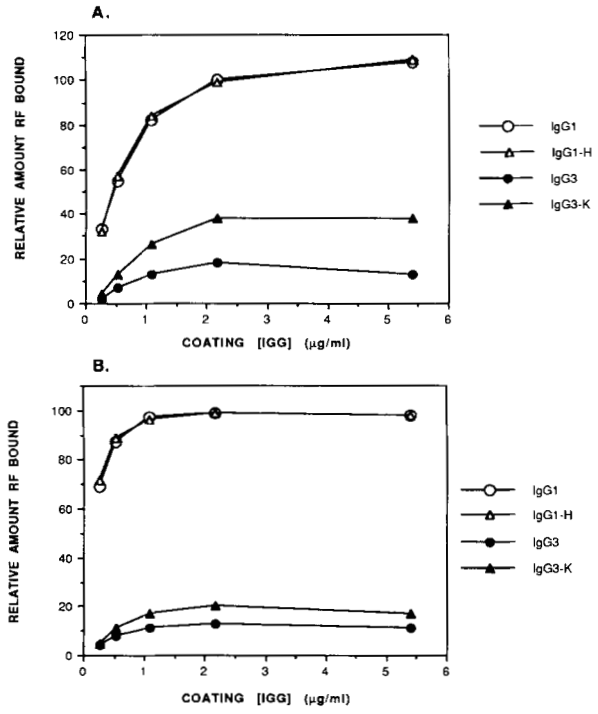

Figure 3. RF binding to increasing amounts of immobilized IgG. Binding to glycosylated IgG1, aglycosylated IgG1 (IgG1-H), glycosylated IgG3, and aglycosylated IgG3 (IgG3-K) were compared. Ag-coated microtiter plates were incubated with increasing concentrations of each IgG. RF binding was measured for polyclonal RF-Sin (*A*) and monoclonal RF-Bla (*B*). Results are means of triplicates and are expressed as a percentage of RF binding to a constant amount of IgG4 (2.2 μg/ml). SE for IgG1 points were less than 5% of binding to IgG4 and for IgG3 points were less than 2.8% of binding to IgG4.

nant of the monoclonal RF binding site, the existence of additional RF contact residues in C_H2, conserved between IgG3 and IgG4, is not precluded by our results. In fact, previous studies showed that only proteolytic fragments of polyclonal IgG containing the intact C_H2-C_H3 interface of the Fc fragment were capable of being bound by RF (17, 19, 20). SPA competition experiments (17, 20) suggested that, like SPA, RF may contact residues in both the C_H2 and C_H3 domains (21). It remains to be determined if group II monoclonal RF that bind IgG3 are directed against this same interface or a very different epitope.

Chemical modification of IgG and pH titration studies on the RF-IgG interaction have implicated His and Tyr residues as important RF contact points on IgG (17, 20). At positions 435 and 436, IgG1, 2, and 4 contain the sequence His-Tyr, whereas most IgG3 proteins contain Arg-Phe. Because His-435 and Tyr-436 lie in the C_H3 domain at the C_H2-C_H3 interface and are SPA contact residues (21), it has been suggested that these amino acids are critical RF contact residues and that substitution of Arg and Phe for His and Tyr at 435-436 is responsible for RF binding to IgG1, 2, and 4, but not IgG3 (17, 20, 46). However, positions 435 and 436 in IgG3 are sites of allotypic variation (47) and the sequence of our IgG3 clone at 435-436 is Arg-Tyr. Therefore, the single amino acid change of His to Arg at 435 may account for the different RF binding properties of the IgG3 and IgG4 C_H3 domains. There are six other amino acid differences between IgG3 and IgG4 in the C_H3 domain, but none of these are located at the C_H2-C_H3 interface. We are conducting site-directed mutagenesis studies to determine if His at 435 is essential for monoclonal RF binding and if substitution of Tyr-436 by the more common Phe residue alters the monoclonal or polyclonal RF binding properties of IgG3.

Aglycosylated chimeric IgG, produced by replacing the glycosylation site at Asn-297 by site-directed mutagenesis, was used to study the role of the conserved carbohydrate moiety in RF binding. Aglycosylated and glycosylated IgG1, 2, and 4 possess identical monoclonal and polyclonal RF binding properties. SPA also binds equally well to each form of IgG1, 2, and 4 (38) (M.-H. Tao and S. L. Morrison, unpublished observations). Although aglycosylation perturbs the structure of the C_H2 domain and alters effector functions dependent upon sequences in C_H2 (38), lack of carbohydrate does not seem to affect the conformation of the C_H2-C_H3 interface for IgG1, 2, and 4. However, because polyclonal RF as well as IgG3 reactive monoclonal RF bind more strongly to aglycosylated IgG3 than to glycosylated IgG3, it appears that lack of glycosylation does alter the structure of IgG3 in a region bound by these RF (Table III).

The difference between RF binding to glycosylated and aglycosylated IgG3 increases with increasing amount of immobilized IgG (Fig. 3). This effect is much more dramatic for polyclonal RF than for monoclonal RF-Bla (Fig. 3) (data not shown). A low concentration of immobilized antigen favors binding by high affinity antibody subpopulations, whereas a high concentration of immobilized Ag permits binding of low and high affinity antibodies in a polyclonal mixture (48). Therefore, at high concentrations of immobilized IgG, increased polyclonal RF binding to aglycosylated IgG3 compared to wild type IgG3 may be attributable to the presence of RF antibodies with low affinity for aglycosylated IgG3. This subpopulation of aglycosylated IgG3-specific RF may bind either to the C_H2-C_H3 interface or to a different epitope altered by the absence of carbohydrate.

The identification of a subpopulation of RF with improved binding to aglycosylated IgG3 in the sera of RA patients is of particular interest because of the observation that terminal galactosylation of IgG is reduced in RA (24). However, it is not clear that the absence of terminal galactose residues and the complete absence of the carbohydrate moiety will result in similar perturbation of the C_H2 domain or similar increases in polyclonal RF binding to IgG3. A recent study found no differences in monoclonal or polyclonal RF binding to normal polyclonal IgG and to polyclonal IgG with decreased galactosylation from RA patients, although these IgG preparations lacked IgG3 (49). This study also showed that partial enzymatic removal of carbohydrate from the Fc fragment of IgG1 did not affect RF binding, consistent with our results. The effect of the described defect in terminal galactosylation of IgG in RA on RF binding remains to be determined.

The determination of the antigenic sequences on IgG bound by IgM RF is important in that it may help identify other Ag responsible for the induction of RF autoantibodies. Antibodies to other molecules bearing "IgG like" determinants, such as members of the growing Ig superfamily, may cross-react with IgG and therefore have RF activity. The novel application of chimeric IgG antibodies and their genetically engineered variants as RF binding substrates will allow a precise identification of RF bind-

ing sites on IgG and may further our understanding of the role of RF in disease.

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REFERENCES

1. **Hay, C., L. J. Nineham, and I. M. Roitt.** 1975. Routine assay for detection of IgG and IgM anti-globulins in seronegative and seropositive rheumatoid arthritis. *Br. Med. J. 3:203.*
2. **Masi, A. T., J. A. Maldonado-Cocco, S. B. Kaplan, S. L. Geigenbaum, and R. W. Chandler.** 1976. Prospective study of the early course of rheumatoid arthritis in young adults. *Semin. Arthritis Rheum. 5:299.*
3. **Mellors, R. C., R. Heimer, J. Corcos, and L. Korngold.** 1960. Cellular origin of rheumatoid factors. *J. Exp. Med. 110:875.*
4. **Pernis, B., V. R. Bonagura, D. Posnett, and H. G. Kunkel.** 1984. Idiotype expression in rheumatoid synovial plasma cells. *Rheumatol. Int. 4(Suppl.):39.*
5. **Winchester, R. J., V. Agnello, and H. G. Kunkel.** 1970. Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterization and relationship to lowered complement levels. *Clin. Exp. Immunol. 6:689.*
6. **Estes, D., and C. L. Christian.** 1971. The natural history of systemic lupus erythematosus by prospective analysis. *Medicine 50:85.*
7. **Carson, D. A., A. S. Bayer, R. A. Eisenberg, S. Lawrence, and A. Theofilopoulos.** 1978. IgG rheumatoid factor in subacute bacterial endocarditis: relationship to IgM rheumatoid factor and circulating immune complexes. *Clin. Exp. Immunol. 31:100.*
8. **Bunim, J. J., W. W. Buchanan, P. T. Wertlake, L. Sokoloff, K. J. Bloch, J. S. Beck, and F. P. Alepa.** 1964. Clinical, pathologic, and serologic studies in Sjogren's syndrome. *Ann. Intern. Med. 61:509.*
9. **Dresner, E., and P. Trombly.** 1959. The latex-fixation reaction in nonrheumatic diseases. *N. Engl. J. Med. 261:981.*
10. **Fong, S., P. P. Chen, J. H. Vaughan, and D. A. Carson.** 1985. Origin and age-associated changes in the expression of a physiologic autoantibody. *Gerontology 31:236.*
11. **Levine, P. R., and D. A. Axelrod.** 1985. Rheumatoid factor isotypes following immunization. *Clin. Exp. Immunol. 3:147.*
12. **Nemazee, D. A., and V. L. Sato.** 1983. Induction of rheumatoid antibodies in the mouse. Regulated production of autoantibody in the secondary immune response. *J. Exp. Med. 158:529.*
13. **Allen, J. C., and H. G. Kunkel.** 1966. Hidden rheumatoid factors with specificity for native gamma globulins. *Arthritis Rheum. 9:758.*
14. **Normansell, D. E., and C. W. Young.** 1975. The IgG subclass specificity of anti IgG immunoglobulin rheumatoid factors. *Immunochem. 12:187.*
15. **Robbins, D. L., J. Skilling, W. F. Benisek, and R. Wistar.** 1986. Estimation of the relative avidity of 19S IgM rheumatoid factor secreted by rheumatoid synovial cells for human IgG subclasses. *Arthritis Rheum. 29:722.*
16. **Robbins, D. L., W. F. Benisek, E. Benjamini, and R. Wistar.** 1987. Differential reactivity of rheumatoid synovial cells and serum rheumatoid factors to human immunoglobulins G subclasses 1 and 3 and their CH3 domains in rheumatoid arthritis. *Arthritis Rheum. 30:489.*
17. **Sasso, E. H., C. V. Barber, F. A. Nardella, W. J. Yount, and M. Mannik.** 1988. Antigenic specificities of human monoclonal and polyclonal IgM rheumatoid factors. *J. Immunol. 140:3098.*
18. **Capra, J. D., J. M. Kehoe, R. J. Winchester, and H. G. Kunkel.** 1971. Structure-function relationships among anti-gamma globulin antibodies. *Ann. N.Y. Acad. Sci. 190:371.*
19. **Nardella, F. A., D. C. Teller, and M. Mannik.** 1981. Studies on the antigenic determinants in the self-association of IgG rheumatoid factor. *J. Exp. Med. 154:112.*
20. **Nardella, F. A., D. C. Teller, C. V. Barber, and M. Mannik.** 1985. IgG Rheumatoid factors and staphylococcal protein A bind to a common molecular site on IgG. *J. Exp. Med. 162:1811.*
21. **Deisenhofer, J.** 1981. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9 and 2.8-angstrom resolution. *Biochemistry 20:2360.*
22. **Morrison, S. L., M. J. Johnson, L. A. Herzenberg, and V. T. Oi.** 1984. Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. USA 81:6851.*
23. **Bruggemann, M., G. T. Williams, C. I. Bindon, M. R. Clark, M. R. Walker, R. Jeffris, H. Waldmann, and M. S. Neuberger.** 1987. Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. *J. Exp. Med. 166:1351.*
24. **Parekh, R. B., R. A. Dwek, B. J. Sutton, D. L. Fernandes, A. Leuag, D. Stanworth, T. W. Rademacher, T. Mizuochi, T. Taniguchi, K. Matsuta, F. Takeuchi, Y. Nagano, T. Miyamoto, and A. Kobata.** 1985. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature 316:452.*
25. **Mulligan, R. C., and P. Berg.** 1981. Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase. *Proc. Natl. Acad. Sci. USA 78:2072.*
26. **Oi, V. T., and S. L. Morrison.** 1986. Chimeric antibodies. *Biotechniques 4:214.*
27. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA 74:5463.*
28. **Schanfield, M. S., and E. Van Loghem.** 1986. Human Immunoglobulin allotypes. In *Handbook of Experimental Immunology, Vol. 3.* D. M. Weir, ed. Blackwell Scientific Publications, Oxford, p. 94.1.
29. **Kabat, E. A., T. T. Wu, M. Reid-Miller, H. M. Perry, and K. S. Gottesman.** 1987. *Sequences of Proteins of Immunological Interest.* United States Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD, p. 307.
30. **Morrison, S. L., L. Wims, S. Wallick, L. Tan, and V. T. Oi.** 1987. Genetically engineered antibody molecules and their application. *Ann. N.Y. Acad. Sci. 507:187.*
31. **Oi, V. T., S. L. Morrison, L. A. Herzenberg, and P. Berg.** 1983. Immunoglobulin gene expression in transformed lymphoid cells. *Proc. Natl. Acad. Sci. USA 80:825.*
32. **Zoller, M. J., and M. Smith.** 1984. Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template. *DNA 3:479.*
33. **Singer, J. M., and C. M. Plotz.** 1956. The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med. 21:888.*
34. **Rose, H. M., C. Ragan, E. Pearce, and M. O. Lipman.** 1948. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc. Soc. Exp. Biol. Med. 68:a1.*
35. **Bennett, P. H., and T. A. Burch.** 1967. New York symposium on population studies in the rheumatic diseases: new diagnostic criteria. *Bull. Rheum. Dis. 17:453.*
36. **Bonagura, V. R., J. F. Wedgwood, N. Agostino, L. Hatam, L. Mendez, I. Jaffe, and B. Pernis.** 1989. Seronegative rheumatoid arthritis, rheumatoid factor cross-reactive idiotype expression and hidden rheumatoid factors. *Ann. Rheum. Dis. 48:488.*
37. **Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra.** 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-gamma globulin activity. *J. Exp. Med. 137:331.*
38. **Tao, M.-H., and S. L. Morrison.** 1989. Studies of aglycosylated chimeric mouse-human IgG: role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J. Immunol. 143:2595.*
39. **Monestier, M., B. Bellon, A. J. Manheimer, and C. A. Bona.** 1986. Rheumatoid factors. Immunochemical, molecular, and regulatory properties. *Ann. N.Y. Acad. Sci. 475:106.*
40. **Hoffman, W. L., M. S. Goldberg, and J. D. Smiley.** 1982. Immunoglobulin G3 subclass production by rheumatoid synovial tissue cultures. *J. Clin. Invest. 69:136.*
41. **Randen, I., K. M. Thompson, J. B. Natvig, O. Forre, and K. Waalen.** 1989. Human monoclonal rheumatoid factors derived from the polyclonal repertoire of rheumatoid synovial tissue: production and characterization. *Clin. Exp. Immunol. 78:13.*
42. **Agnello, V., and J. L. Barnes.** 1986. Human rheumatoid factor crossidiotypes I. *J. Exp. Med. 164:1809.*
43. **Gaarder, P. I., and J. B. Natvig.** 1970. Hidden rheumatoid factors reacting with "non a" and other antigens of native autologous IgG. *J. Immunol. 105:928.*
44. **Natvig, J. B., M. W. Turner, and P. I. Gaarder.** 1972. IgG antigens of the C-gamma-2 and C-gamma-3 homology regions interacting with rheumatoid factors. *Clin. Exp. Immunol. 12:177.*
45. **Gaarder, P. I., and J. B. Natvig.** 1972. Two new antigens of human IgG "non b1" and "non b2" related to the Gm system. *J. Immunol. 108:617.*
46. **Jefferis, R., M. I. NikJaafar, and M. Steinitz.** 1984. Immunogenic and antigenic epitopes of immunoglobulins. VIII. A human monoclonal rheumatoid factor having specificity for a discontinuous epitope determined by histidine/arginine interchange as residue 435 of immunoglobulin G. *Immunol. Lett. 7:191.*
47. **Huck, S., P. Fort, D. H. Crawford, M.-P. Lefranc, and G. Lefranc.** 1986. Sequence of a human immunoglobulin gamma 3 heavy chain constant region gene: comparison with the other human C genes. *Nucl. Acids Res. 14:1779.*
48. **Nieto, A., A. Gaya, M. Jansa, C. Moreno, and J. Vives.** 1984. Direct measurement of antibody affinity distribution by hapten-inhibition enzyme immunoassay. *Mol. Immunol. 21:537.*
49. **Newkirk, M. M., A. Lemmo, and J. Rauch.** 1990. Importance of the IgG isotype, not the state of glycosylation, in determining human rheumatoid factor binding. *Arthritis Rheum. 33:800.*