Monomeric (7S) IgM Found in the Serum of Rheumatoid Arthritis Patients Share Idiotypes with Pentameric (19S) Monoclonal Rheumatoid Factors

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Abstract

Serum from some seropositive (RF^+) rheumatoid arthritis (RA) patients contains relatively high concentrations of monomeric (7S) IgM molecules. Seven S IgM molecules fail to bind the Fc portion of IgG, unlike 19S IgM RFs that bind aggregated IgG in classical RF assays. Some pentameric IgM RFs are marked by crossreactive idiotypes (RCRI) defined by prototypic monoclonal RFs. In previous studies, we observed that a proportion of pokeweed mitogen (PWM) induced plasma cells from RA patients' blood lymphocytes express the major RCRI as assayed by indirect immunofluorescence with polyclonal anti-RCRI antibodies. In this study, 7S IgM obtained from three different RF^+ RA patients inhibits specific anti-RCRI intracytoplasmic staining of PWM induced RF^+ RA-derived plasma cells. These 7S molecules also block polyclonal anti-RCRI antibodies from reacting with red blood cells bearing 7S IgM molecules from RF^+ patients with RA or Waldenstrom's macroglobulinemia. We conclude that some 7S IgM molecules in the serum of RF^+ RA patients are marked by the major RCRI idiotype and are related to 19S monoclonal and polyclonal RFs.

Introduction

Previous reports document the occurrence of polyclonal, low molecular weight IgM (7S, IgM) in various patients with autoimmune diseases (1–8) and occasionally in apparently normal individuals (9, 10). In rheumatoid arthritis (RA)[1] patients, seropositive for rheumatoid factor (RF^+), the occurrence of 7S IgM is associated with disease severity and frequently with vasculitis, (11, 12). In immunodiffusion studies using specific anti-IgM antibodies reacting with 19S or 7S IgM the characteristics of 7S IgM appear identical to those of pentameric 19S IgM (4, 13, 14). These studies failed to identify unique determinants on 7S IgM as opposed to the 19S species (14). Antinuclear reactivity of 7S IgM, derived from lupus erythematosus patients has previously been described (1, 3). In addition, some 7S IgM molecules from RA patients show low efficiency binding to aggregated human immunoglobulin by a double antibody binding radioimmunoassay (14).

In the present study, we report the presence of the major rheumatoid, crossreactive idiotype (RCRI) among 7S IgM molecules obtained from the sera of the three RF^+ RA patients, as defined by the prototypic monoclonal 19S IgM molecules originally described by Kunkel (15, 16). Utilizing indirect immunofluorescence (17) and hemagglutination inhibition assays (15, 16), we observed that 7S IgM molecules obtained from RF^+ patients' sera inhibit polyclonal anti-major RCRI antibodies from binding $RCRI^+$ molecules in the cytoplasm of peripheral blood lymphocyte (PBL)-derived, pokeweed mitogen (PWM)-induced plasma cells from RA patients, or from reacting with prototypic reduced and alkylated 19S IgM RFs placed on the surface of sheep red blood cells (SRBC). The reaction of anti-RCRI antibodies with $RCRI^+$ monoclonal IgM RF was not inhibited in either assay by seronegative (RF^-) 7S IgM obtained from one "RA" patient, or by reduced and alkylated polyclonal normal IgM. These observations imply that RA 7S IgM include molecules that belong to the major RCRI group and therefore are closely related to their 19S IgM, RF counterparts.

Methods

Human cells. Nine active rheumatoid arthritis patients with high titer RF, measured by IgG-coated latex particle agglutination, were studied for the production of RCRI in PWM cultures. The serological data of these patients, as well as that of seven normal volunteers used as controls, are given in Table I.

Production, screening, and preparation of anti-RCRI antibodies. Anti-RCRI antibodies raised in Flanders Giant rabbits were obtained as previously described in detail (17, 18). In short, Ea monoclonal pentameric IgM (RF^+, member of the major RCRI group) was injected subcutaneously in complete Freund's adjuvant into rabbits. Sequential postimmunization bleedings were performed and the anti-RCRI activity assayed as previously described (17). Aliquots of sera were tested with two members of the major RCRI group (Ea, Wa) by hemagglutination inhibition after subjecting the anti-RCRI antibodies to exhaustive absorption on solid phase, Sepharose coupled immunoabsorbents, including cord serum, pooled polyclonal IgG, and four monoclonal IgM, kappa, RF^- immunoglobulins (17, 18). Nonadherent antibodies were then absorbed on a solid-phase column of Wa IgM protein, (member of the major RCRI group), to enrich for anti-RCRI antibody activity. Anti-RCRI antibodies, obtained after cross-absorption on Sepharose coupled Wa IgM proteins, did not agglutinate SRBC coated with RF^- IgM kappa monoclonal proteins or SRBC coated with polyclonal IgM or IgG in a titer greater than 2^{-1}. These affinity purified anti-RCRI antibodies reacted with SRBC coated with 7S Ea and Wa in high titer $\geq 2^{10}$ after absorption. Affinity purified antibodies obtained from this final absorption were then further absorbed with 12% normal human serum in the fluid phase before use in indirect immunofluorescence as described (17, 18).

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Received for publication 25 June 1986 and in revised form 6 November 1986.

1. *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; PBL, peripheral blood lymphocyte; PWM, pokeweed mitogen; RA, rheumatoid arthritis; RCRI, rheumatoid crossreactive idiotype; RF, rheumatoid factor; SRBC, sheep red blood cells; TRITC, tetramethyl rhodamine isothiocyanate.

J. Clin. Invest.

0021-9738/87/03/0813/06 $1.00
Volume 79, March 1987, 813–818

Table I. Summary of Subjects

Patients	Gender	Age	Latex titer $\times 10^{-1}$	Waaler-Rose titer $\times 10^{-1}$
De	F	24	640	1,024
To	M	55	1,280	>4,096
Du	F	68	2,560	>4,096
Ty	M	60	1,280	>4,096
Br	F	51	640	512
Fi	F	32	2,560	512
Bo	F	35	2,560	>4,096
Ke	F	60	640	2,040
Ge	F	58	5,120	>4,096
Controls				
Ag	M	30	Negative	4
Bo	M	36	Negative	2
Le	F	29	Negative	0
Cl	F	29	Negative	2
To	M	45	Negative	0
Ml	F	40	Negative	2
Ed	M	33	Negative	2

Preparation of anti-human F(ab')$_2$, and anti-rabbit IgG antibodies conjugated with tetramethyl rhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC). Goat antisera to human IgM were absorbed on a Sepharose coupled column of human IgG. The eluted, affinity purified anti-human F(ab')$_2$ antibodies, were then directly conjugated with TRITC as described (17). All anti-human immunoglobulin reagents were absorbed with Sepharose coupled rabbit IgG before use in indirect fluorescence double staining.

In addition, a sheep anti-rabbit IgG reagent was prepared and directly conjugated with FITC (17), to stain indirectly, the RCRI$^+$ plasma cells that bound rabbit anti-RCRI antibodies.

Preparation of PWM-induced plasma cells from rheumatoid patients and normal PBL donors. Heparinized peripheral blood was obtained from RA patients and normal volunteers and separated on Ficoll-Hypaque density gradients to obtain PBL. Cells were further prepared for tissue culture as described previously (17). In short, 1×10^6 PBL were incubated with PWM for 6 d at 37°C, 5% CO_2 air atmosphere in RPMI 1640 with 10% fetal bovine serum (FBS), and harvested at day 6. PWM-induced plasma cells were made ready for immunofluorescent staining by preparing cytocentrifuge slides and fixing these mounted cells in acid alcohol (5% glacial acetic acid, 95% ethanol) for 30 min 0°C followed by two washes in ice-cold phosphate-buffered saline (PBS) 0.01 M, pH 7.2.

Staining of plasma cells. Plasma cells were stained by indirect immunofluorescence assay with rabbit anti-RCRI antibodies, 150 μg/ml, 20 μl per slide, followed by sheep anti-rabbit IgG-FITC as described (17). After exhaustive washing in PBS, all slides were counter stained, to enumerate total number of plasma cells, with goat anti-human F(ab')$_2$-TRITC, washed again and then mounted in polyvinyl alcohol before counting. In all patients and control individuals studied, 100 plasma cells were counted on each of two or more separate slides. These data reflect a total of ≥ 200 PWM-induced plasma cells counted in each experimental condition described and RCRI$^+$ plasma cells are expressed as the percentage, i.e., RCRI$^+$ plasma cells of total PWM-induced plasma cells counted.

Waaler-Rose hemagglutination assay for rheumatoid factor activity. Washed SRBC were coupled with purified human IgG by the chromium chloride ($CrCl_3$) method as previously described (19). Aliquots of sera, 20 μl in each well were diluted 2^{12} (1:4,096), in PBS 0.01 M, pH 7.2, with 1% FBS, (preabsorbed with SRBC), before adding the human IgG-coated SRBC.

Latex fixation by rheumatoid sera. Latex particles coated with purified human IgG (Hyland Laboratories, Costa Mesa, CA) were used to measure the RF IgG binding titer of the various patient and control sera as previously described (20).

Detection of monomeric 7S IgM from rheumatoid patients' sera. A solution of 4% acrylamide was poured in small petri dishes, allowed to polymerize for 20 min and then prepared for immunodiffusion in two directions with an Ouchterlony template hole punch. A goat antiserum, specific for human μ-chains, was added to the center well and patient's sera added to the surrounding wells as described (4). Low molecular weight 7S IgM, as opposed to 19S IgM, was specifically identified in rheumatoid sera by the exclusion capacity of 4% polyacrylamide gel matrix for the 19S species.

Preparation of low molecular weight 7S from rheumatoid patients' sera. Four different polyclonal monomeric IgM reagents were prepared after their initial identification. 20-ml aliquots of serum from each patient Ty, Ha, Sin (RF$^+$ sera), and Ho, (RF$^-$ serum), were made 40% vol/vol with a saturated $(NH_4)_2SO_4$ solution. The resulting precipitate was solubilized and dialyzed into borate saline pH 8.0. The mixture of immunoglobulins was separated by AcA 34 (LKB, Bromma, Sweden) molecular weight chromatography into 19S and 7S species. The 7S peak was pooled, concentrated and then applied to a Sepharose-coupled rabbit anti-human μ-affinity chromatography column. The affinity pure 7S IgM species was eluted by 0.05 M glycine HCl pH 2.8 and then neutralized to pH 8.0, concentrated, and tested for purity in Ouchterlony agar gel diffusion for reactivity with anti-μ- or anti-gamma specific goat antisera. None of the eluted 7S IgM species reacted with anti-gamma antibodies (14). All species were anti-human μ-reactive. 7% polyacrylamide gel electrophoresis was performed to check the molecular weight of all species of 7S IgM recovered (14).

Reduction and alkylation of 19S IgM obtained from Ea, (monoclonal IgM), and control sera (polyclonal IgM). Reduction and alkylation of monoclonal IgM from patient Ea, (member of the major RCRI group, immunogen for the polyclonal anti-RCRI antibodies) and polyclonal IgM obtained from control sera was performed as described previously (21). IgM preparations were reduced by dialysis against 0.1 M mercaptoethanol (Eastman Kodak Co., Rochester, NY) in sodium phosphate buffer 0.2 M, pH 7.5. Dissociated samples were alkylated to prevent spontaneous reassociation by dialysis against large volumes of 0.2 M monoiodoacetamide (K and K Laboratories, Plainview, NY) prepared in the same buffer. The reduced and alkylated samples were finally dialyzed against 0.15 M NaCl and run on 7% polyacrylamide gels to determine molecular weight.

In addition, aliquots of Ea and control polyclonal 19S IgM, were reduced by dialysis against 0.02 M cysteine HCl in 0.2 M Tris, pH 8.6 for 22 h at 22°C, as described (14), to prevent intrasubunit disulfide bond disruption that might change the antigenic tertiary structure of these IgM molecules and prevent anti-RCRI antibody antigen binding. Alkylation of cysteine HCl reduced IgM molecules was performed as described above.

Absorption of anti-RCRI antibody by low molecular weight 7S IgM. Aliquots of anti-RCRI antibodies absorbed with 12% normal human serum were further absorbed with 7S IgM. 20 μl of anti-RCRI antibodies at 150 μg/ml were mixed with 20 μl of 7S IgM at 300 μg/ml and incubated 30 min at 4°C and then spun 9,000 *g* for 2 min at 25°C. The resulting supernatant was used to stain PWM-induced plasma cells from patients or controls. Aliquots of anti-RCRI antibodies were also absorbed with reduced and alkylated 19S IgM from control sera. After incubation, these absorbed anti-RCRI antibodies were used in parallel experiments to stain PWM-induced plasma cells as previously stated (16, 17).

Hemagglutination inhibition of anti-RCRI antibodies reacting with monomeric IgM coated SRBC by 7S IgM obtained from rheumatoid patients. Washed SRBC were coupled with monomeric IgM, Ty, Sin, and reduced and alkylated Ea with various concentrations of $CrCl_3$ as described (19). 50 μl of anti-human F(ab')$_2$ antibodies at a concentration of 1 mg/ml were serially diluted 2^{12} (1:4,096), in 1% FBS, (preabsorbed with SRBC), in PBS.

The various coated SRBC (7S, Ty, Sin, or Ea) were then added to

the hemagglutination titer plates, 25 μl per well of a 1% SRBC suspension, to determine the efficiency of $CrCl_3$ induced SRBC coating as measured by the agglutination titer. Bovine serum albumin coupled SRBC were used as a control for each antiserum assayed. 50 μl of anti-RCRI antibodies at 300 μg/ml were added to the first well of each row of a 96-well microtiter plate. The anti-RCRI antibodies were then serially diluted 2^{12} (1:4,096) in PBS, 1% FBS preabsorbed with SRBC. Monomeric IgM from Ty, Ha, Sin, Ho, or Ea was added, 50 μl/well of a 300 μg/ml stock solution for 30 min at 25°C to each microtiter well in which the anti-RCRI antibodies had been serially diluted. The different 7S IgM coated SRBC were then added to separate rows of the microtiter plate. Titers are expressed as the final positive agglutination well observed $\times 10^{-1}$.

Results

Appearance of RCRI$^+$ plasma cells, induced from RA patients' PBL by PWM. Table I represents the clinical and serologic, RF binding assay data of the RA patients and the controls studied. All RA patients studied (Table I) showed significant latex fixation titers, ranging 640–5120, and elevated Waaler-Rose hemagglutination titers as opposed to controls. RA patients', Waaler-Rose hemagglutination titers ranged from 512 → 4096, control 0–4 (Table I).

RA patients' or control PBL cultivated for 6 d with PWM, induced B lymphocyte maturation to plasma cell formation. These plasma cells contained intracytoplasmic immunoglobulin demonstrable by direct immunofluorescence. The frequency of these cells, shown in Table II, varied between 3 and 16% of all cells recovered after 6 d PWM incubation with PWM. Considerable variation among individual patients and controls was seen, although no significant difference between these groups was noted.

A marked difference between RA patients and controls was found in the frequency of RCRI$^+$ plasma cells obtained after 6 d PWM stimulation. Seven RA patients generated 20–40% RCRI$^+$ cells of total plasma cells, compared with two others (Ke, Ge) expressing 4–10% RCRI$^+$ plasma cells of total plasma cells (Table II). One control expressed 12% RCRI$^+$ plasma cells, of all plasma cells stained, by anti-RCRI antibodies (Table III). The remaining six ranged between 4.2 and 8.2% RCRI$^+$ plasma cells of total plasma cells.

These data are approximately twofold greater than those previously reported (17, 18) and were obtained by utilizing a different source of rabbit antiidiotypic (RCRI), anti-serum than the original one described (17). The difference in percent RCRI expression between the RA patients and the controls previously reported is comparable.

Plasma cells from patients Ty (reported here) and Al (not reported in this communication), were stained in parallel experiment with another polyclonal anti-RCRI antibody source (V. Bonagura, P. G. Chen, and B. Pernis, unpublished). Anti-RCRI antibodies (PSL-2) generated by immunizing rabbits with peptide fragments of RCRI$^+$ light chains from monoclonal IgM proteins, stained comparable percentages of total plasma cells generated in PWM cultures as did our anti-RCRI antibody reagent although direct comparison of these two different reagents by two-color fluorescence was not performed.

Detection of the RCRI among low molecular weight 7S IgM obtained from RF$^+$ RA patients. Low molecular weight IgM obtained from three active RF$^+$ RA patients, inhibited anti-RCRI cytoplasmic fluorescence staining of PWM-induced RA patient plasma cells by 30–83% in six of seven high RCRI expressors (Table II). One RA patient's plasma cells (Du) were identified by anti-RCRI antibodies equally well with or without prior 7S IgM absorption of the antiidiotypic antibodies. Patient Ty's 7S IgM inhibited the anti-RCRI antibody staining of his own PWM-induced plasma cells by 50%. Two other RA patients Ke and Ge, both RF$^+$, did not express as high a RCRI percentage of total plasma cells as the seven other RA patients (Table II). These RCRI$^+$ plasma cells were stained equally well in the presence or absence of low molecular weight IgM. These findings were also seen in all seven controls (Table III), including one control (Ag) who expressed a relatively high percentage (12%) RCRI$^+$ cells of total plasma cells.

In all cases, 7S IgM obtained from a RF$^-$ RA patient, as well as reduced and alkylated polyclonal normal 19S IgM, by

Table II. Cytoplasmic Immunoglobulins and RCRI Detection in RA Plasma Cells in the Presence or Absence of RF$^+$ and RF$^-$ 7S IgM, after 6 d Culture of PBL with PWM

Patients	%PC/Total cells*	RCRI$^+$ PC/Total PC‡	Anti-RCI absorbed with 7S IgM from§				R/A normal‖ IgM
			TY(RF$^+$)	HA(RF$^+$)	SIN(RF$^+$)	HO(RF$^-$)	
De	15.5	20.2	11.4	10.9	ND	25.0	20.0
To	6.9	22.0	14.3	12.8	16.5	21.4	20.1
Du	7.9	40.3	36.2	35.2	ND	ND	ND
Ty	7.0	25.5	12.1	18.8	ND	ND	ND
Br	9.5	23.5	4.0	ND	10.1	23.5	22.5
Fi	5.0	24.5	7.5	18.2	12.0	24.0	ND
Bo	6.9	28.8	15.5	ND	10.8	ND	25.4
Ke	4.0	9.9	8.2	9.5	8.3	11.8	10.2
Ge	3.1	4.1	2.9	3.8	4.0	ND	ND

* Data indicate the percentage of cells showing cytoplasmic staining with fluorescent anti-human F(ab')$_2$ antibodies (see Methods), as counted among ≥ 100 total cells identified by phase contrast. ‡ Data indicate the percentage of cells stained with anti-RCRI antibodies absorbed with 12% NHS as counted among ≥ 100 immunoglobulin-containing cells for each of two slides or more counted, identified by staining with rhodamine-conjugated anti-human F(ab')$_2$ antibodies (see Methods). Two or more slides were counted for each experimental condition described. A difference of ≤ 5% RCRI$^+$ cells of total plasma cells was noted between the separate slides counted for each individual experiment. § Anti-RCRI antibodies were preabsorbed with 7S IgM from RF$^+$ and RF$^-$ patients (see Methods) and then used to stain 6d PWM induced plasma cells from RA patients and controls. ‖ Polyclonal 19S IgM from control individuals was reduced and alkylated (7S) and used to preabsorb anti-RCRI antibodies (see Methods).

Table III. Cytoplasmic Immunoglobulins and RCRI Detection in Control Cells in the Presence or Absence of RF⁺ and RF⁻ 7S IgM, after 6 d Culture of PBL with PWM

Controls	%PC/Total cells*	RCRI⁺ PC/Total PC‡	Anti-RCI absorbed with 7S IgM from§ TY(RF⁺)	HA(RF⁺)	SIN(RF⁺)	HO(RF⁻)	R/A normal‖ IgM
Ag	9.5	12.0	12.2	11.8	ND	10.1	9.6
Bo	12.7	5.6	6.3	6.0	7.0	5.3	4.6
Le	10.4	5.5	5.0	4.6	7.0	ND	ND
Cl	8.2	5.1	6.0	5.4	4.9	ND	ND
To	12.2	5.1	6.5	ND	5.0	ND	5.5
Ml	14.3	4.2	5.3	ND	5.9	5.0	4.0
Ed	15.6	8.2	9.5	ND	10.2	ND	ND

* Data indicate the percentage of cells showing cytoplasmic staining with fluorescent anti-human $F(ab')_2$ antibodies (see Methods), as counted among ≥ 100 total cells identified by phase contrast. ‡ Data indicate the percentage of cells stained with anti-RCRI antibodies absorbed with 12% NHS as counted among ≥ 100 immunoglobulin-containing cells for each of two or more slides counted, identified by staining with rhodamine-conjugated anti-human $F(ab')_2$ antibodies (see Methods). Two or more slides were counted for each experimental condition described. A difference of ≤ 5% RCRI⁺ cells of total plasma cells was noted between the separate slides counted for each individual experiment. § Anti-RCRI antibodies were preabsorbed with 7S IgM from RF⁺ and RF⁻ patients (see Methods) and then used to stain 6 d PWM-induced plasma cells from RA patients and controls. ‖ Polyclonal 19S IgM from control individuals was reduced and alkylated (7S) and used to preabsorb anti-RCRI antibodies (see Methods).

either mercaptoethanol or cysteine HCl reduction, did not inhibit anti-RCRI antibody staining of PWM-induced plasma cells from RA patients or controls.

Hemagglutination inhibition of anti-RCRI antibody recognition of RF⁺ IgM coated SRBC by RA derived 7S IgM. SRBC coated with 7S IgM obtained from a RF⁺ RA patient Ty were agglutinated by anti-RCRI antibodies as shown in Table IV. Ty's low molecular weight IgM at 100, and 50 μg/ml final concentration, mixed with 50 μl of a 300-μg stock solution of anti-RCRI antibodies inhibited the observed hemagglutination titer of 64, obtained with Ty-coated SRBC reacting with anti-RCRI antibodies. Ty, 7S IgM absorbed RCRI antibodies demonstrated titers of 0 and 4, respectively (Table IV). RF⁺ RA patient Ha's 7S IgM at 100 and 50 μg/ml final concentration cross-inhibited the same anti-RCRI titer to 4 and 8, respectively. A similar experiment, utilizing a different Ty 7S IgM SRBC coat, showed even more pronounced hemagglutination inhibition; a 7S unabsorbed titer of 512 was reduced by Ty, 7S absorption of anti-RCRI antibodies to 4 and 16, respectively (Table IV). In both cases, neither 7S IgM from RF⁻ RA patient Ho or reduced and alkylated normal 19S IgM inhibited hemagglutination of Ty coated SRBC by anti-RCRI antibodies. Seven S IgM from patient Sin, on the surface of SRBC was also recognized by anti-RCRI antibodies. This reaction was blocked by the addition of 7S Ea IgM, Table IV. In these experiments 50 μl of rabbit IgG (RIgG), at a concentration of 300 μg/ml agglutinated Ty or Sin coated SRBC at a titer of 2, 4, and 2, respectively.

Table V expresses hemagglutination inhibition data in which Waldenstrom macroglobulinemia patient Ea, (member of the major RCRI group, initial immunogen for the anti-RCRI antibodies), or normal reduced and alkylated IgM, were used as 7S SRBC coats. As expected, Ea 7S IgM was a more potent hemagglutination inhibitor of anti-RCRI antibodies than polyclonal 7S IgM from an RF⁺ RA patient, Sin, Ha, or Ty (Table V). Neither 7S IgM from RF⁻ RA patient Ho or reduced and alkylated normal 7S IgM inhibited specific hemagglutination by anti-RCRI antibodies. Again, 50 μl of RIgG at a concentration of 300 μg/ml failed to agglutinate 7S Ea or Sin coated SRBC at a titer > 2. Normal 7S IgM coated SRBC were not agglutinated by anti-RCRI antibodies. These SRBC were well coated with IgM as shown by agglutination with anti-human $F(ab')_2$ antibodies (Table V).

Discussion

The presence of 7S, IgM in sera obtained from patients with RA, is associated with disease severity, articular, and extraarticular manifestations of RA, and is indicative of rheumatoid vasculitis (11, 12). Although, 7S, IgM may therefore be viewed as a serologic marker for RA activity, its pathophysiologic role in the disease process remains unclear (14). 7S IgM has been shown to bind complement in an anti-I, complement mediated hemolysis assay (22) and complement binding aggregate assay (14). In addition some 7S IgM molecules can form soluble immune complexes with aggregated IgG (14) and react with DNA (1, 3).

The major RF, RCRI, originally described by Kunkel (15, 16) defines a set of monoclonal 19S, IgM RF by their shared idiotopes (18) independent of their IgG, Fc binding potential. This major RCRI includes 60% of all monoclonal 19S, IgM RFs as defined by polyclonal anti-major RCRI antibodies. Polyclonal anti-RCRI antibodies identify RFs and related molecules free of the problems found in low affinity for "antigen" interactions characteristic of RFs in the classic IgG, Fc binding assays (23, 24). Typical association constants for IgM or IgG, RFs average between 1×10^4, 5×10^5 L/M (23, 24), which is 10- to 100-fold less than the affinities of heteroantibodies produced following exogenous immunization. In addition, we (17) and others (25), have observed the major RCRI in high frequency in RA both at the cellular and serologic level employing polyclonal anti-major RCRI antibodies (17, 18).

Utilizing the immunofluorescence RCRI system, two populations of RCRI expressing plasma cells in RF⁺ RA patients are clearly seen (Table II). This observation is independent of plasma cell surface IgM expression since plasma cells are not surface stained by anti-RCRI antibodies in the staining methodology employed and is only representative of PWM-induced cytoplasmic collections of RCRI⁺ immunoglobulins.

Table IV. Hemagglutination Inhibition of Anti-RCRI AB Binding to SRBC Coated with Polyclonal 7S IgM Derived from RF⁺ Arthritis Patients Ty, Sin

SRBC coat	Antiserum	7S Absorption* reagent	Titer
		μg/ml	
Ty‡	R anti-H F(ab')$_2$	0	>4,096
	R anti-RCRI	0	64
		Ty 100‡	0
		Ty 25	4
		Ha 100‡	4
		Ha 50	8
		Ho 100§	64
	RIgG‖	0	2
Ty‡	R anti-H F(ab')$_2$	0	>4,096
	R anti-RCRI	0	512
		Ty 100‡	4
		Ty 50	16
		Ha 150‡	2
		Ho 100§	512
		Ho 50	512
		Control R/A¶ IgM 100	256
	RIgG‖	0	4
Sin**	R anti-H F(ab')$_2$	0	>4,096
	R anti-RCRI	0	128
		Ea 100 (R/A)	2
	RIgG	0	2

* A fixed concentration of 7S IgM from RF⁺ patients Ty or Ha, RF⁻ patient Ho or reduced and alkylated 19S IgM from control individuals was reacted with serially diluted anti-RCRI antibodies in microtiter wells before adding SRBC coated with 7S IgM from patient Ty.
‡ Seven S IgM obtained from a RF⁺ RA patient.
§ Seven S IgM obtained from a RF⁻ RA patient.
‖ Rabbit IgG.
¶ Polyclonal 19S IgM from control individuals was reduced and alkylated (7S) and used to preabsorb anti-RCRI antibodies (see Methods).
** SIN RF⁺ RA patients' 7S IgM coated SRBC were reacted with rabbit anti-human F(ab')$_2$ [R anti-HF(ab')$_2$], or rabbit anti-RCRI (R anti-RCRI) antibodies. Serially diluted rabbit anti-RCRI antibodies were reacted with reduced and alkylated monoclonal 19S IgM from Ea before SIN, 7S IgM coated SRBC were added to microtiter wells.

Table V. Hemagglutination Inhibition of Anti-RCRI AB Binding to SRBC Coated with 7S IgM Derived from the Immunogen, (19S) Ea Monoclonal IgM RF⁺

SRBC coat	Antiserum	7S Absorption* reagent	Titer
		μg/ml	
7S Ea IgM (R/A)	R anti-H F(ab')$_2$	0	>4,096
	R anti-RCRI	0	256
		Ea 100 (R/A)	2
		Sin 100‡	32
		Sin 150	8
		Ha 150‡	32
		Ty 150‡	16
		Ho 100§	128
		Control (R/A)‖ IgM 100	128
	RIgG¶	0	2
7S Control IgM (R/A)**	R anti-H F(ab')$_2$	0	>4,096
	R anti-RCRI	0	2

* A fixed concentration of reduced and alkylated monoclonal RF⁺ 19S IgM from Ea or polyclonal 7S IgM from RF⁺ patient Sin, Ty or Ha, RF⁻ patient Ho or reduced and alkylated 19S IgM from control individuals, was reacted with serially diluted rabbit anti-RCRI antibodies in microtiter wells before adding SRBC coated with reduced and alkylated 19S IgM from Ea (see Methods).
‡ Seven S IgM obtained from a RF⁺ RA patient.
§ Seven S IgM obtained from a RF⁻ RA patient.
‖ Polyclonal 19S IgM from control individuals was reduced and alkylated (7S) and used to preabsorb rabbit anti-RCRI (R anti-RCRI) antibodies (see Methods).
¶ Rabbit IgG.
** Reduced and alkylated 19s IgM from control individuals was coated on SRBC and reacted with specific rabbit anti-human F(ab')$_2$ [R anti-H F(ab')$_2$] or rabbit anti-RCRI (R anti-RCRI) antibodies.

Seven of nine RF⁺ RA patients studied expressed high proportions of RCRI⁺ plasma cells, of total plasma cells, obtained by PBL incubation with PWM. Two patients, (Ke, Ge), were low expressors of the major RCRI and are comparable to that observed in the controls (Table II, III). Ke and Ge may express the Po, minor crossreactive idiotype, as originally described by Kunkel (15, 16), or private RF idiotypes with unique, unshared idiotopes in preference to the major RCRI.

By preabsorption of anti-RCRI antibodies with 7S, IgM before indirect immunofluorescence (Table II), the plasma cells reacting with anti-major RCRI are divided into two subpopulations, one 7S IgM absorbable and the other resistant to absorption. 7S IgM from three different RF⁺ RA patients absorbed 30–83% of the anti-RCRI binding activity toward RCRI⁺ plasma cells in six of the seven RF⁺ RA "high" RCRI expressor patients, and had no effect on two other RF⁺ RA patient or control cells. The remaining "background," RCRI⁺ plasma cells may represent immunoglobulin molecules produced by a set of separate non-RF related B-cell clones that share some determinants with the immunogen but are not related to the immunologic abnormality of RA. In this respect, it is noteworthy that the frequency of 7S, IgM nonabsorbable RCRI⁺ plasma cells from Ke and Ge, RF⁺ RA patients, is comparable to that observed in PWM induced plasma cells in non-RA individuals. Accordingly, the 7S IgM obtained from RF⁺ RA patients, which absorbs precisely that proportion of anti-RCRI antibodies reacting with the proportion of PWM induced plasma cells differentiating some RA patients from normal individuals, may represent a set of B-cell clones more closely related to the idiotypic dynamics observed in RA.

Patient Du differs from the remaining six RF⁺ RA high expressors of RCRI⁺ plasma cells in that the majority of the RCRI⁺ positive plasma cells are non-7S, IgM absorbable.

7S, IgM obtained from an RF⁻ "RA" patient (Ho), and reduced and alkylated normal polyclonal IgM, both failed to absorb any activity of specific anti-RCRI antibodies (Table II). These observations in conjunction with those seen in hemagglutination inhibition assay (Tables IV and V) show a lack of idiotypic expression by B-cell clones producing normal polyclonal 19S, IgM, and 7S IgM from a RF⁻ RA patient. Tables IV and V also reiterate the idiotypic relatedness of some 7S, IgM molecules obtained from three RF⁺ RA patients to each other and with the immunogen, 19S monoclonal IgM.

Control Ag, expresses a high proportion of RCRI⁺ plasma cells of total plasma cells as compared to the remaining six. Although the frequency of RCRI⁺ plasma cells is relatively higher

than the others and similar to one of the two RF^+ RA low expressors of RF idiotype, these plasma cells are of the "background" nonabsorbable subgroup representing B cell clones producing RCRI immunoglobulins that are identified by anti-RCRI antibodies in the presence of RF^+ RA derived 7S, IgM. Ag does not show significant levels of RF or signs of disease.

Finally, 7S, IgM appear to be independent biosynthetic IgM molecules and not degradation products of pentameric IgM (3, 5, 14, 26). Except for its low molecular weight as compared to the pentamer, and the difference in binding avidity for the Fc portion of IgG, 7S, IgM molecules are similar to their 19S counterparts (3–6, 13). Our observations idiotypically link 7S, IgM with 19S, IgM RFs both monoclonal and polyclonal, and are consistent with those observations in which weak IgG, Fc binding activity by some 7S, IgM species was noted (14). Idiotypic determination of RF anti-immunoglobulins by the polyclonal anti-RCRI system circumvents "antigen" binding problems encountered in 7S IgM, RF assays (14) and easily demonstrates their association with RF.

Acknowledgments

This work was supported by National Institutes of Health grant AM-3193604. Dr. Bonagura is the recipient of a National Arthritis Investigator Award.

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