Seronegative rheumatoid arthritis, rheumatoid factor cross reactive idiotype expression, and hidden rheumatoid factors

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SUMMARY The major rheumatoid factor cross reactive idiotype (RCRI), defined by prototypic monoclonal rheumatoid factors (RFs), is expressed as a dominant idiotype by pokeweed mitogen induced plasma cells obtained from seropositive (RF+) patients with rheumatoid arthritis (RA). Some patients who meet clinical diagnostic criteria for RA set by the American Rheumatism Association fail to express RFs at any time during their clinical course. To determine if seronegative (RF-) patients with RA, so designated by the latex fixation, Rose-Waaler classic binding assays, or a RF enzyme linked immunosorbent assay (ELISA), express the RCRI in the absence of detectable RFs we examined pokeweed mitogen plasma cells from these patients by indirect immunofluorescence. In addition, we used an inhibition ELISA to detect RCRI bearing molecules in the sera of RF- patients with RA. Five of 10 RF- patients with RA had a high prevalence of RCRI+ plasma cells (16-49% of total pokeweed mitogen plasma cells in culture). Six of 20 RF- patients with RA had high serum concentrations of molecules marked by the RCRI, equivalent to 21-110 µg/ml of RCRI+ reference monoclonal IgM RF. Four of five patients who expressed the RCRI in high prevalence in pokeweed mitogen plasma cells, also demonstrated high concentrations of RCRI in their sera detected by inhibition ELISA. There was significant concordance of RCRI expression determined by the two different assays. Four RF- patients with RA who expressed RCRI in their whole sera had hidden RFs detected in their 19S and, in one case, 7S serum fraction. Detection of RF related molecules in whole sera by the expression of RCRI in RF- patients with RA identifies a subgroup of RF- patients with RA who possess hidden RFs. Some RF- patients with RA can express the major RCRI in pokeweed mitogen plasma cells and in their sera and therefore are related to patients with prototypic Waldenström's macroglobulinaemia, who produce RCRI+ 19S IgM monoclonal RFs.

Previous studies have shown that some seropositive (RF+) patients with rheumatoid arthritis (RA) express the major rheumatoid factor cross reactive idiotype (RCRI), defined by prototypic human monoclonal IgM rheumatoid factors (RFs), as a

dominant idiotype among their pokeweed mitogen induced plasma cells derived from peripheral blood mononuclear cells.¹⁻³

Serum samples from patients with spondylarthritis and other heterogeneous arthritides are consistently negative for RFs.⁴ The term seronegative (RF-) RA, however, refers to patients with classic or definite RA meeting published clinical criteria for this diagnosis,^{5,6} who consistently lack evidence of serum RFs detected by IgG binding assays, such as latex fixation⁷ and the Rose-Waaler assay.^{8,9}

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We have identified a subgroup of patients with RA who meet the American Rheumatism Association criteria for this diagnosis, who are consistently RF- by classic IgG binding assays^{7,8} and RF enzyme linked immunosorbent assay (ELISA), yet express the RCRI in high concentration in their sera, and as a dominant idotype in their pokeweed mitogen plasma cells. Hidden 19S RFs, and in one instance 7S IgM hidden RFs, were detected by Rose-Waaler assay or RF ELISA, or both, after acid pH molecular weight separation of autologous IgG from these autoantibodies. These individuals are therefore idiotypically linked with RF+ patients with Waldenström's macroglobulinaemia belonging to the major RCRI group, originally described by Kunkel,^{10,11} and resemble some RF+ patients with RA previously reported.¹⁻³

We have recently reported that some low molecular weight (7S) IgM molecules obtained from RF+ adults with RA are marked by the RCRI¹² and are undetectable as 'functional' IgG binding RFs in classic RF assays.^{12,13} These RCRI+7S IgM molecules inhibited binding of affinity purified anti-RCRI polyclonal antibodies with some RCRI+ pokeweed mitogen plasma cells obtained from RF+ patients with RA expressing the RCRI as a dominant idotype in pokeweed mitogen culture.¹² Two populations of RCRI+ pokeweed mitogen plasma cells were therefore identified: one closely related to the monoclonal IgM RF dominant idotype defined by Waldenström's macroglobulinaemia prototypes, and another background population, found in similar prevalence both in patients with RA and in control individuals.¹² We now describe a similar observation in RF- patients with RA.

Patients and methods

PATIENTS AND CONTROLS

Twenty RF- patients with RA (16 female, four male) were studied. All had classic or definite RA by American Rheumatism Association criteria,^{5,6} had had active sustained disease for a minimum of two years, and had received or were currently receiving second line drug treatment. None of the patients had RF in their serum as demonstrated by latex fixation,⁷ Rose-Waaler haemagglutination assay using human or rabbit IgG,⁸ or by RF ELISA at any time in their clinical course. None was receiving D-penicillamine, cytotoxic drugs, or other immunosuppressive drugs at the time of diagnosis or when the initial or sequential blood specimens were obtained for this study. There was no obvious correlation between any second line drug treatment and RCRI expression. Their ages ranged from 24 to 81 years. The mean duration of disease activity for

the RF- patients with RA studied was four years, range two to six years.

Fifteen normal volunteers (five female, 10 male), aged 21-55, were studied in parallel with the RF- patients with RA. All controls lacked detectable RFs.

PRODUCTION, SCREENING, AND PREPARATION OF ANTIBODIES

Anti-RCRI antibodies raised in Flanders giant rabbits were obtained as previously described in detail.^{1,12} The immunogen Ea 19S monoclonal IgM (RF+ member of the major RCRI group) was injected subcutaneously into rabbits in complete Freund's adjuvant. Rabbit IgG anti-RCRI antibodies were digested with pepsin and the resulting F(ab')₂ fragments tested for specificity with two members of the major monoclonal RCRI group (Ea, Wa) by haemagglutination inhibition. Rabbit F(ab')₂ anti-RCRI fragments were absorbed on Sepharose coupled immunoabsorbents, including cord serum, pooled polyclonal IgG and IgM. The anti-RCRI antibody fragments were enriched for the RCRI 'public' specificity by cross absorption on Sepharose coupled Wa IgM.^{1,12} Affinity purified anti-RCRI antibody fragments, at 2 mg/ml, did not agglutinate sheep red blood cells coated with RF-IgMx monoclonal proteins, polyclonal IgM or IgG in a titre greater than 2, but did agglutinate sheep red blood cells coated with 19S Ea or Wa in high titre, ≥ 2 .¹⁰ Fluid phase absorption of anti-RCRI F(ab')₂ was performed as described previously.¹

Affinity purified goat IgG antihuman F(ab')₂ antibodies were directly conjugated with tetramethylrhodamine isothiocyanate as described previously,^{1,2,12} and absorbed with Sepharose coupled rabbit IgG before use. Sheep antirabbit IgG (cross absorbed on human IgG) was also directly conjugated with fluorescein isothiocyanate.^{1,2,12}

PREPARATION AND IMMUNOFLUORESCENCE STAINING OF POKEWEED MITOGEN PLASMA CELLS

Peripheral blood mononuclear cells were prepared for tissue culture as described previously in detail.¹ The cells (1×10^6) were incubated with pokeweed mitogen for six days at 37°C, 5% CO₂ air atmosphere in RPMI 1640 with 10% fetal bovine serum, and harvested on day 6. Cytochrome slides were prepared, fixed in acid alcohol (5% glacial acetic acid, 95% ethanol) for 30 minutes at 0°C, and washed twice in ice cold 0.01 M phosphate buffered saline pH 7.2.

RCRI+ pokeweed mitogen plasma cells were determined by indirect immunofluorescence using affinity purified, absorbed rabbit F(ab')₂ anti-RCRI

antibodies, 150 µg/ml, 20 µl per slide, followed by sheep antirabbit IgG-fluorescein isothiocyanate as described previously.¹ After exhaustive washing in phosphate buffered saline all slides were counter-stained with goat antihuman F(ab')₂-tetramethylrhodamine isothiocyanate to enumerate the total number of pokeweed mitogen plasma cells, washed again, and then mounted in polyvinyl alcohol before counting. More than 200 pokeweed mitogen plasma cells were counted in each experimental condition described. RCRI+ plasma cells are expressed as a percentage of the total pokeweed mitogen plasma cells counted. Replicate experiments with peripheral blood mononuclear cells of individual RF- patients with RA in pokeweed mitogen culture were performed within seven days of the original studies to detect RCRI expression. Variations of <5% were observed between sequential determinations of RCRI+ pokeweed mitogen plasma cells in RF- patients with RA.

ROSE-WAALER HAEMAGGLUTINATION ASSAY FOR RF ACTIVITY

Washed sheep red blood cells were coupled with either purified human IgG by the chromium chloride method¹³ (a modification of the classic Rose-Waaler assay) or coated with subagglutinating dilutions of rabbit IgG anti-sheep red blood cell antibodies as originally described.^{8,9}

Serial twofold dilutions (to 1:4096) were made in phosphate buffered saline with 1% fetal bovine serum (preabsorbed with sheep red blood cells) before adding either uncoated, human IgG coated,¹³ or rabbit IgG coated sheep red blood cells. Positive titres were considered to be obvious sheep red blood cell agglutination at titres $\geq 2^4$ (1/16).

DETECTION OF HIDDEN RFs IN ROSE-WAALER ASSAY

Serum fractions (19S and 7S) from seronegative patients Nos 1, 2, 3, and 5 were obtained by low pH, molecular weight ACA 34 gel chromatography, (LKB, Bromma, Sweden) in 0.1 M sodium acetate buffer pH 4.1. The 19S and 7S serum fractions obtained were neutralised and tested for hidden RF activity by diluting the 19S fraction to 0.1 mg/ml of IgM in phosphate buffered saline, and the 7S serum fraction to 0.5 mg/ml of IgG. These solutions were used neat and then serially titrated in a classic Rose-Waaler assay. Positive results were considered to be obvious sheep red blood cell agglutination at titres $\geq 1/8$, which represents a dilution $\geq 1:160$ of whole serum.

LATEX FIXATION BY RF- RA SERA

Latex particles coated with purified human IgG

(Hyland Laboratories, Costa Mesa, Ca) were used (commercial kit) to measure the RF IgG binding titre of the various patient and control sera as described by the manufacturer.

ELISA DETECTION OF RFs

IgM RF was determined by a modification of a previously published method.¹⁴ Aliquots of either 19S or 7S serum fractions were added to 96 well ELISA plates (Immulon II, Dynatech) coated with polyclonal IgG, and incubated for four hours at room temperature. Bound IgM RF was detected with horseradish peroxidase coupled goat anti-human IgM (µ chain specific) (Tago, Burlingame, Ca) and *o*-phenylenediamine/H₂O₂. A standard curve was prepared using varying dilutions of purified monoclonal IgM RF. Not all the monoclonal RF bound to ELISA wells and, therefore, the results are expressed as arbitrary RF binding units/mg of IgM added. All serum samples from RF- patients with RA were screened in this ELISA and again in parallel with pH 4.1 molecular weight separated serum fractions for the detection of hidden RFs.

PREPARATION OF LOW MOLECULAR WEIGHT (7S) IgM FROM SERA OF PATIENTS WITH RA
IgM (7S) was prepared from the sera of three RF- patients with RA (Nos 22, 23, 24) and one RF- patient with RA (No 21), who were found to have low molecular weight IgM detected by Ouchterlony diffusion in 4% acrylamide. The 7S fractions from these patients were prepared by molecular weight chromatography at pH 4.1, and the 7S IgM purified by affinity chromatography as described in detail previously.^{12,15}

Reduced and alkylated 7S IgM derived from polyclonal 19S IgM obtained from control sera was prepared and used in some experiments as described previously.^{12,13} The molecular weight of all affinity purified 7S IgM molecules was confirmed by sodium dodecylsulphate-polyacrylamide gel electrophoresis.

ABSORPTION OF ANTI-RCRI ANTIBODY WITH 7S IgM

Aliquots of absorbed, enriched anti-RCRI F(ab')₂ antibody fragments, 150 µg/ml, were further absorbed individually with equal volumes of 300 µg/ml of purified 7S IgM obtained from a patient with RA, or reduced and alkylated 19S IgM from the control. The 7S IgM absorbed anti-RCRI F(ab')₂ were then used to stain pokeweed mitogen plasma cells from patients or controls.

DETECTION OF RCRI IN WHOLE SERUM BY INHIBITION ELISA

Flat bottom, 96 well microtitre plates (Immulon II,

Dynatech) were coated with 0.1 µg/well of monoclonal Wa IgM (RF+ RCRI+) overnight at 4°C, and then washed three times with ELISA buffer. Residual binding sites were saturated with 2% bovine serum albumin for one hour at 37°C and then washed three more times. Anti-RCRI antibodies were mixed with diluted serum from patients or controls or with various concentrations of purified monoclonal Wa IgM. These mixtures were added to the microtitre plate, incubated for one hour at room temperature, and then washed with ELISA buffer. Bound rabbit F(ab')₂ anti-RCRI was detected with horseradish peroxidase coupled goat antirabbit IgG (Tago, Burlingame, Ca) and *o*-phenylenediamine/H₂O₂. A standard curve was prepared using known concentrations of purified Wa IgM.

Wells containing anti-RCRI F(ab')₂ antibody fragments only, or ELISA buffer alone, were used to determine 0.0 and 1.0 fractional inhibition. The fractional inhibition (Z) for each sample was then calculated. A straight line of log (Z/(1-Z)) and the log (Wa IgM) for the standard curve was calculated using the method of least squares. The amount of RCRI in each of the serum samples was then calculated. Results are expressed as µg Wa IgM equivalents/ml of serum. (A detailed description of this inhibition ELISA is given elsewhere, J Wedgwood, V R Bonagura, unpublished data.)

Results

DETECTION OF CLASSIC RF IgG BINDING IN SERUM SAMPLES

None of the patients or control subjects showed evidence of RFs in their sera by latex fixation⁷ or Rose-Waaler assay^{8 14} using human or rabbit IgG coated sheep red blood cells. By RF ELISA none of the RF- patients with RA had detectable RF binding activity greater than that observed in the control group (RF- patients (n=19): mean (SD) 142 (88) RF units; controls (n=12): 127 (71) RF units).

RCRI+ POKEWEE MITOGEN PLASMA CELLS INDUCED FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

Cultivation of peripheral blood mononuclear cells from RF- patients with RA or from controls with pokeweed mitogen for six days induced B lymphocyte maturation to plasma cells (Table 1). Considerable variation in pokeweed mitogen plasma cells as a percentage of the total cells obtained was seen among individual patients and controls (2-40%), though no significant difference between these groups was noted.

Ten of 20 RF- patients with RA produced

Table 1 *Rheumatoid factor cross reactive idiotype expression by pokeweed mitogen plasma cells from adult seronegative patients with rheumatoid arthritis*

Seronegative patient No	PWM-PCs*/total cells (%)	RCRI+* PWM-PCs†/total PWM-PCs (%)
1	40.0‡	48.8§
2	3.9	30.2
3	6.7	19.4
4	20.3	17.2
5	7.0	16.0
6	11.8	9.0
7	8.4	5.7
8	2.1	5.0
9	19.7	4.3
10	8.1	2.9
Control No		
1	9.5	12.0
2	15.6	8.2
3	7.3	7.3
4	12.7	5.6
5	10.4	5.5
6	8.2	5.1
7	12.2	5.1
8	14.3	4.2
9	18.0	2.2

*PWM-PCs=pokeweed mitogen plasma cells; RCRI=rheumatoid factor cross reactive idiotype.

†Experiments performed five to seven days after the initial experiments showed variation of <5% of RCRI expression by pokeweed mitogen plasma cells.

‡Data indicate the percentage of pokeweed mitogen induced plasma cells showing cytoplasmic staining with fluorescein conjugated antihuman F(ab')₂ antibodies (see 'Patients and methods') among 100 or more cells identified by phase contrast.

§Data indicate the percentage of cells stained with anti-RCRI antibodies among 200 or more pokeweed mitogen plasma cells. Two or more slides were counted for each experimental condition described. A difference of ≤5% RCRI+ cells of the total pokeweed mitogen plasma cells was noted between separate slides counted in each individual experiment.

sufficient numbers of pokeweed mitogen plasma cells for indirect cytoplasmic immunofluorescence RCRI detection to be performed (Table 1). RCRI expression by these 10 patients ranged from 2.9% to 48.8% (mean (SD) 15.8 (14.4)%). Nine controls ranged between 2.2% and 12.0% RCRI+ pokeweed mitogen plasma cells (mean (SD) 6.1 (2.8)%). The normal control who expressed 12% RCRI+ pokeweed mitogen plasma cells, had previously expressed a lower percentage.^{1 12} The difference in expression of RCRI+ pokeweed mitogen plasma cells between RF- patients with RA and controls approached significance (p=0.06, Wilcoxon rank sum). Of 10 RF- patients with RA studied, five had a prevalence of RCRI+ pokeweed mitogen plasma cells more than 2 SD above the mean for the controls (>12%) (Table 1) and comparable with those observed in some RF+ patients with RA.^{1 12} These five RF-

patients with RA generated 16–49% RCRI+ cells of total pokeweed mitogen plasma cells compared with 3–9% RCRI+ cells detected in the other RF– individuals with RA (Table 1). There were no unique clinical characteristics with respect to age, duration of disease, sex, or drug treatment between those patients who were RCRI+ and those who were RCRI–.

INHIBITION OF ANTI-RCRI STAINING BY 7S IgM OR REDUCED AND ALKYLATED 19S IgM
Low molecular weight (7S) IgM obtained from three active RF+ RCRI+ patients with RA (Nos 22, 23, 24) inhibited by 50–76% cytoplasmic RCRI staining of pokeweed mitogen plasma cells from three RF– patients with RA who showed high RCRI expression (Table 2). Three RF– patients with RA with low RCRI expression were equally stained by polyclonal anti-RCRI antibodies in the presence or absence of 7S IgM (Table 2). No inhibition was seen in control subjects, including control No 1, who had a comparatively raised expression of RCRI in pokeweed mitogen culture (Table 2).

In all cases 7S IgM obtained from a patient (No 21), who was weakly RF+ previously and persistently RF– by latex agglutination subsequently, did not inhibit anti-RCRI antibody binding RCRI+ pokeweed mitogen plasma cells or RCRI bearing sheep red blood cells. Patient No 21 is not included with the RF– patients described in this communication. In addition, reduced and alkylated polyclonal control 19S IgM did not inhibit anti-RCRI antibody staining of pokeweed mitogen plasma cells from RF– patients with RA or from controls. All three 7S IgM species obtained from the RF+ patients with RA (Nos 22, 23, 24) bound IgG only in RF ELISA, while 7S IgM from RF– patient No 21 and from the reduced and alkylated polyclonal control IgM did not (V R Bonagura, J F Wedgwood, S Artandi, unpublished data).

DETECTION OF RCRI IN WHOLE SERA BY AN ELISA

Twenty serum samples from RF– patients with RA and 11 samples from controls were studied for RCRI by inhibition ELISA. Serum RCRI expression by

Table 2 Cytoplasmic immunoglobulins and rheumatoid factor cross reactive idiotype detections in six day pokeweed mitogen plasma cells from seronegative patients with rheumatoid arthritis in the presence or absence of seropositive and seronegative monomeric (7S) IgM

Seronegative patient No	PWM-PCs*/total cells (%)	RCRI+* PWM-PCs†/total PWM-PCs (%)	Anti-RCRI absorbed with 7S IgM from patient No				Reduced and alkylated control IgM¶
			22 (RF+)*	23 (RF+)	24 (RF+)	21 (RF–)¶	
1	40.0‡	48.8§	11.9	14.9	ND	40.2	43.4
4	20.3	17.2	ND*	8.6	5.6	18.4	ND
5	7.0	16.0	5.2	6.0	ND	18.2	15.0
6	11.8	9.0	9.7	ND	8.2	10.8	ND
8**	10.4	5.3	5.5	6.0	4.0	4.8	5.0
9	19.7	4.3	ND	ND	4.1	3.5	ND
Control No							
1	9.5	12.0	12.2	11.8	ND	10.1	9.6
2	15.6	8.2	9.5	ND	10.2	ND	ND
4	12.7	5.6	6.3	6.0	7.0	5.3	4.6
5	10.4	5.5	5.0	4.6	7.0	ND	ND
6	8.2	5.1	6.0	5.4	4.9	ND	ND
7	12.2	5.1	6.5	ND	5.0	ND	5.5
8	14.3	4.2	5.3	ND	5.9	5.0	4.0

*PWM-PCs=pokeweed mitogen plasma cells; RCRI=rheumatoid factor cross reactive idiotype; RF+=seropositive; RF–=seronegative; ND=not determined.

†Experiments performed five to seven days after the initial experiments showed variation of <5% of RCRI expression by pokeweed mitogen plasma cells.

‡Data indicate the percentage of pokeweed mitogen induced plasma cells showing cytoplasmic staining with fluorescein conjugated antihuman F(ab')₂ antibodies (see 'Patients and methods') among 100 or more cells identified by phase contrast.

§Data indicate the percentage of cells stained with anti-RCRI antibodies among 200 or more pokeweed mitogen plasma cells. Two or more slides were counted for each experimental condition described. A difference of ≤5% RCRI+ cells of the total pokeweed mitogen plasma cells was noted between separate slides counted in each individual experiment.

||Anti-RCRI antibodies were preabsorbed with 7S IgM from RF+ and RF– patients (see 'Patients and methods') and then used to stain pokeweed mitogen plasma cells.

¶Polyclonal 19S IgM from control individuals was reduced and alkylated (7S IgM) and used to absorb anti-RCRI antibodies (see 'Patients and methods').

**The data presented for patient No 8 were obtained from a different pokeweed mitogen culture than the one assayed in Table 1.

these 20 patients ranged from 2 to 110 µg/ml, geometric mean 13.1 (SD 2.54). Serum from 11 controls contained 1–16 µg/ml of RCRI, geometric mean 3.9 (SD 2.31). The RCRI concentration in RF- RA sera was significantly raised compared with controls ($p=0.0003$, Wilcoxon rank sum, Fig. 1). Six of the 20 sera from RF- patients with RA

Fig. 1 Serum rheumatoid factor cross reactive idotype (RCRI) expression in seronegative (RF-) patients with rheumatoid arthritis (RA). Twenty RF- patients with RA and 11 normal individuals were studied for expression of the major RCRI in their sera by inhibition ELISA. Closed circles represent individual RCRI determinations, open circles represent the geometric mean RCRI expression, and bars indicate one standard deviation. RCRI expression by RF- patients with RA is significantly greater than that found in controls ($p=0.0003$, Wilcoxon rank sum).

had concentrations of RCRI more than two standard deviations above the geometric mean control value. These patients expressed molecules bearing the RCRI in concentrations equivalent to 21–110 µg/ml (Fig. 1).

ASSOCIATION OF RCRI EXPRESSION BY TWO INDEPENDENT ASSAYS

Correlation of RCRI expression in RF- patients with RA by the two different methods did not reach significance ($r=0.42$, $p=0.12$, Spearman rank correlation), but four of 10 RF- patients with RA had increased RCRI expression detected by both assays, one had an increased expression of RCRI in pokeweed mitogen plasma cells only, and five had RCRI expression similar to control in both assays. The level of agreement between the two assays was measured by the κ statistic which measures the level of agreement beyond chance. Concordance of RCRI expression in RF- patients with RA was significant ($\kappa=0.8$, $p\leq 0.01$).

Of seven controls assayed for RCRI expression by both methods, only one expressed a slightly raised percentage of RCRI+ pokeweed mitogen plasma cells, none of which was a 7S IgM absorbable RCRI+ pokeweed mitogen plasma cell. None of the controls expressed increased levels of RCRI in their sera.

DETECTION OF HIDDEN RFs IN RCRI+ RF- PATIENTS WITH RA

All four RF- patients with RA studied, who expressed high concentrations of RCRI in their sera, had hidden RFs in their 19S IgM fraction (Table 3). Patient No 5 showed hidden RFs in both 19S and 7S IgM fractions (Table 3). These hidden RFs reacted with human or rabbit IgG, or both, as demonstrated

Table 3 IgM hidden rheumatoid factors in seronegative patients with rheumatoid arthritis who expressed high concentrations of rheumatoid factor cross reactive idotype in their sera

Seronegative patient No	ELISA* human IgG	Rose-Waaler assay†	
		Human IgG	Rabbit IgG
2	19S IgM (52)	Negative	Negative
5	19S IgM (1700) 7S IgM (186)	19S IgM (1/32)	19S IgM (1/16)
1	19S IgM (46)	Negative	Negative
3	19S IgM (127)	19S IgM (1/8)	19S IgM (1/8)

*Serum fractions (19S and 7S) from RF- patients with rheumatoid arthritis obtained by molecular weight sieve chromatography pH 4.1 were assayed by ELISA using pooled human IgG coated plates developed with horseradish peroxidase conjugated goat antihuman IgM (μ chain specific) antibodies. Results are expressed as arbitrary rheumatoid factor (RF) units/ml of IgM used. Control values are less than 15 RF units/ml of IgM used.

†Aliquots of 19S (0.1 mg/ml) and 7S (0.5 mg/ml) serum fractions were serially diluted in microtitre plates to 2^{12} (1/4096) in 0.01 M phosphate buffered saline pH 7.2 before the addition of human or rabbit IgG coated sheep red blood cells as indicator of RF IgG binding. Positive results were considered to be obvious sheep red blood cell agglutination at titres $\geq 2^3$ (1/8). Control 19S and 7S serum fractions at similar concentrations did not agglutinate sensitised sheep red blood cells.

by classic Rose-Waaler haemagglutination assay or RF ELISA (human IgG).

Discussion

Rheumatoid arthritis is a clinical diagnosis based on specific criteria established by the American Rheumatism Association.⁵ Although different criteria and associated degrees of certainty in diagnosing RA have been published,^{5,6} those patients with RA and consistent RF+ disease are considered to be a relatively homogeneous disease group on genetic, epidemiological, and clinical grounds.¹⁶⁻¹⁹ In contrast, RF- arthritis represents a heterogeneous group of disorders,^{4,20} perhaps better defined as undifferentiated RF- arthritis, as recently suggested.⁴ Some RF- patients with RA become RF+ over time,²¹ while other patients with RA who are initially RF+ become RF- spontaneously or as a result of treatment.^{22,23} There are still others who have typical RA and are in fact always RF-. These patients represent a smaller group of patients than originally estimated²⁴ within the larger RF- arthritis patient group, which encompasses other diseases such as ankylosing spondylitis, Reiter's syndrome, and psoriatic arthropathy. The diagnosis of true RF- RA is, therefore, substantially less than 25% of all RF- arthritic patients as originally estimated.²⁴

We have previously shown that the major RCRI, defined by prototypic monoclonal IgM RFs, is expressed by RF+ patients with RA as a dominant idiotypic among pokeweed mitogen plasma cells derived from peripheral blood mononuclear cells.^{1,12} In addition, low molecular weight 7S IgM obtained from some RF+ adults with RA is marked by the major RCRI yet fails to bind human or rabbit IgG in classic 'functional' binding assays.^{12,15} In the present communication we have identified a group of RF- adults with RA who generate pokeweed mitogen plasma cells which express the major RCRI as a dominant idiotypic. We have previously described a similar finding in RF+ patients with RA.¹² These RF- patients with RA also express the RCRI in their sera. As these individuals meet criteria for the diagnosis of RA and express immunoglobulins bearing the RCRI they are at least idiotypically related to their RF+ adult RA counterparts and to prototypic patients expressing monoclonal 19S IgM RFs.^{10,11}

It is clear that the detection of 'functional' IgG binding RFs requires more than simple latex fixation or Rose-Waaler haemagglutination.^{7,8} Double antibody radioimmunoassay¹³ or ELISA can enhance the sensitivity of detection of IgG binding activity in some cases. The avidity and affinity of RF inter-

actions with IgG^{25,26} remain formidable problems in detecting RF autoantibodies. Recently, 19S IgM RF autoantibodies were identified in whole sera of some RF- patients with juvenile RA only by ELISA.²⁷ We have not observed such IgM RFs in any of the patients described in this communication.

Among the possible explanations for the detection of RCRI in patients who are RF- by classic IgG binding assays, three appear to be most likely: (a) saturation of pentameric IgM RF binding sites with autologous IgG, precluding further IgG binding in classic RF assays, but allowing anti-idiotypic heteroantibodies to bind idiotopes within the paratope, if the true valence of pentameric IgM is less than 10²⁸; (b) relative insensitivity of classic RF IgG binding assays,²⁹ which fail to detect some species of 7S IgM autoantibodies^{12,15}; (c) association of RCRI idiotopes on immunoglobulins belonging to the parallel set of antibodies generated following antigen challenge,^{30,31} which lack RF activity.

All of the RCRI+ RF- patients with RA tested had hidden RFs. Hidden RFs have been described in some RF- individuals with RA³² and are commonly found in all clinical subgroups of juvenile RA.^{15,28} The RCRI was found both in the 19S and the 7S IgM fractions in patient No 5, who also possessed hidden RFs in these fractions (V R Bonagura, J Wedgwood, unpublished data). It appeared that RF- patients with RA who have detectable RCRI in their serum and in the cytoplasm of pokeweed mitogen plasma cells may also have, as a rule, hidden RFs in their serum. Therefore, if one excludes the RA patients with hidden RFs, the truly RF- patients appear as non-producers not only of RFs but also of the group of immunoglobulins that are related to RF by virtue of their cross reacting idiotypic.

Although RFs have not been established as primary aetiological agents in the development of RA,^{33,34} they clearly can participate in the perpetuation of synovial inflammation.³⁵⁻³⁷ Some evidence does exist to suggest that RFs obtained from patients with RA may be disease specific autoantibodies.^{38,39} These observations imply a closer involvement of RFs in the development of articular disease in RA. Disease specific RFs may be generated in response to the initiating event, such as a virus or other agent,⁴⁰ which triggers the development of RA in the susceptible host.

Within the group of RF- arthritic patients who meet American Rheumatism Association clinical criteria for RA, and have no other underlying diagnosis, a subgroup of patients exists in whom the expression of the dominant RF idiotypic described in IgM monoclonal disease is reminiscent of RF+ RA, and in whom a simultaneous expression of hidden

RFs is present. Comparative clinical studies may show that RCRI expression by RF- and RF+ adults aids in determining the prognosis or response to treatment.

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