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Quantification of cross-reactive idiotype-positive rheumatoid factor produced in autoimmune rheumatic diseases. An indicator of clonality and B cell proliferative mechanisms

F. SHOKRI*, R. A. K. MAGEED*, G. D. KITAS†, P. KATSIKIS‡, H. M. MOUTSOPOULOS‡ & R. JEFFERIS* *Departments of *Immunology and †Rheumatology, University of Birmingham Medical School, Birmingham, England, and ‡Department of Internal Medicine, University of Ioannina, Ioannina, Greece*

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SUMMARY

The aetiology of sustained autoantibody production in human autoimmune diseases is unknown. Evidence for structural similarities and common clonal origin among autoantibodies have been demonstrated through the expression of cross-reactive idiotype (CRI). In the present study we use four monoclonal antibodies (MoAbs) with specificity for non-overlapping CRI on human rheumatoid factor (RF) autoantibodies to define the structural features of polyclonal RF characteristic of patients with autoimmune rheumatic diseases. The pattern of CRI expression in the serum of 12 patients with rheumatoid arthritis (RA), eight with systemic lupus erythematosus (SLE) and 20 with primary Sjögren's syndrome and 34 normal individuals were determined in parallel with the level of IgM RF, IgA RF and autoantibodies to the cellular antigens SS-A, SS-B, Sm, nRNP and dsDNA and cryoglobulins. The results demonstrate significant elevation in the level of IgM and IgA expressing V_HI (G6 and G8) and V_HIII (B6 and D12) associated CRI in the serum of patients with autoimmune rheumatic diseases compared with normal individuals. These increases paralleled, but did not equal the increase in the level of immunoglobulins and RF. However, when expressed as proportion of immunoglobulin, only the V_HI-associated CRI were significantly elevated in patients compared with normal individuals. The proportion of IgM RF expressing the V_HI-associated CRI was higher in patients with Sjögren's syndrome compared with SLE and RA. Furthermore, the proportion of IgA RF expressing the G6 CRI was higher than $G6^+$ IgM RF. These findings imply that different mechanisms contribute to RF production in autoimmune diseases. It is suggested that polyconal B cell activation is likely to be a contributing mechanism. However, such polyclonal activation is unlikely to be random since a selective elevation in the level of specific autoantibodies and V_HI-associated CRI is observed. Furthermore, the data demonstrate that a proportion of autoantibodies in autoimmune diseases are immunoglobulin germline gene encoded. This is more evident in some patients with primary Sjögren's syndrome, where RF is likely to be oligoclonal or monoclonal in individuals with lymphoproliferation.

Keywords cross-reactive idiotype autoimmune diseases

INTRODUCTION

Autoimmune rheumatic diseases are characterized by B lymphocyte hyperactivation and elevated levels of serum autoantibodies (Theofilopoulos & Dixon, 1985). Although the aetiology of most autoimmune diseases is unknown, two alternative hypotheses have been proposed to account for sustained autoantibody production. One hypothesis suggests that chronic polyclonal B cell activation (Izui, McConahey & Dixon, 1978; Miyasaka *et al.*, 1985), due to overproduction of B cell stimulating factors (Hirano *et al.*, 1988), intrinsic factors (Theofilopoulos & Dixon, 1985) or inadequate suppressor mechanisms (Bresnihan & Jasin, 1977), result in the production of polyconal antibodies that include the germline immunoglobulin repertoire (Klinman & Steinberg, 1987). The second hypothesis suggests that autoantigen-driven mechanisms result in specific autoantibody production with the induction of somatic mutation of immunoglobulin V genes (Hardin, 1986; Schlomchick *et al.*, 1987; Gharavi, Chu & Elkon, 1988).

Evidence in favour of either mechanism has been sought by analysing the genetic basis of autoantibody production in

Correspondence: Dr R. A. K. Mageed, Department of Immunology, School of Medical Science, University of Birmingham, Birmingham B15 2TT, UK.

humans, using serological and molecular techniques (Dersimonian *et al.*, 1987; Sanz *et al.*, 1989; Davidson *et al.*, 1990).

The expression of cross-reactive idiotype (CRI) has been demonstrated to reflect restricted immunoglobulin V germline gene utilization with the generation of clonally related autoantibodies (Schiff *et al.*, 1986; Goldfien *et al.*, 1987). Polyclonal and monoclonal anti-CRI have been used as serological probes to investigate the degree of structural heterogeneity among autoantibodies in human autoimmune and lymphoproliferative diseases (Kunkel *et al.*, 1973; Shoenfeld *et al.*, 1983; Solomon *et al.*, 1983; Isenberg *et al.*, 1985). One of the most extensively studied autoantibodies is rheumatoid factor (RF) (Krunkel *et al.*, 1974; Newkirk *et al.*, 1987; Fong *et al.*, 1987; Crowley *et al.*, 1988; Shokri *et al.*, 1990). High titre of RF is a characteristic serological feature in a majority of patients with rheumatoid arthritis (RA) and is frequently present in the serum of patients with primary Sjögren's syndrome and patients with B cell lymphoproliferative disorders (Bunim *et al.*, 1964; Preud'homme & Seligmann, 1972; Carson, 1985). Although autoantibodies to SS-A and SS-B antigens, and Sm and DNA antigens are prominent in patients with primary Sjögren's syndrome and systemic lupus erythematosus (SLE), respectively, many of these patients also produce high titres of RF (Alexander *et al.*, 1982; Carson, 1985; Hansen & Manthorpe, 1986). Furthermore, several patients with primary Sjögren's syndrome develop non-Hodgkin's lymphoma and circulating RF paraproteins (Kassan *et al.*, 1978; Sugai, Shimizu & Konda, 1986). While monoclonal RF associated with lymphoproliferative diseases have been well characterized, the structure and clonality of polyclonal RF characteristic of systemic autoimmune diseases are unknown.

Here we used a panel of murine monoclonal antibodies specific for RF-associated CRI from known V_H subgroups, to quantify CRI within IgM RF and IgA RF from patients with systemic rheumatic diseases in order to investigate the contribution of defined germline gene products to RF in these conditions. We also measured, in parallel, the level of antibodies to a number of autoantigens to address the issue of polyclonal B cell activation as a factor in sustained autoantibody production.

SUBJECTS AND METHODS

Patients and controls

Sera were collected from 12 RA patients (eight men and four women, aged 58 ± 13 years) with classical disease according to the American Rheumatism Association (ARA) classification (Ropes *et al.*, 1958), eight women with SLE (aged 32 ± 12 years) and 20 women with primary Sjögren's syndrome (aged 52 ± 13 years). Diagnosis of primary Sjögren's syndrome was based on the presence of at least two of the following parameters: characteristic eye symptoms or keratoconjunctivitis sicca (positive Rose-Bengal staining); decreased parotid flow rate (less than 1 ml/5 min), or xerostomia; and parotid gland enlargement.

All patients with primary Sjögren's syndrome had lymphocytic infiltration of minor gland biopsies (Bunim *et al.*, 1964).

The control groups included 24 elderly normal individuals (14 women and 10 men, aged 82 ± 6 years) and 10 young normals (five men and five women, aged 35 ± 10 years). All sera were collected from individuals attending Ioannina General Hospital (Ioannina, Greece) and tested with no prior knowledge of their clinical status.

Protein purification

IgM paraproteins having RF activity, Ko, He and Fr were isolated from the plasma of patients with essential mixed cryoglobulinaemia (EMC) by affinity chromatography on IgG-Sepharose 4B column (Pharmacia, Uppsala, Sweden). Polyclonal human IgG was purified from a normal human immunoglobulin preparation (Lister Institute, UK) as the 'breakthrough' fraction from a DE-52 column (Whatman, Maidstone, UK) in 0·01 M phosphate buffer, pH 7·0. The heavy chain disease protein Per (Albutt *et al.*, 1981), characterized as IgG1 Fc, was purified as described elsewhere (Nik Jaafar *et al.*, 1983). Polyclonal IgA RF and $G6^+$IgA were purified from the serum of a RA patient (Gi) with high titre of both proteins using a combination of gel filtration and affinity chromatography. Immunoglobulins were first semi-purified by precipitation at 40% ammonium sulphate. The precipitate was redissolved in saline and dialysed against 0·2 M acetate buffer, pH 4·4. The preparation was then passed over a Sephacryl S-200 (2×100 cm; Pharmacia), equilibrated with 0·2 M acetate buffer, pH 4·4. IgA-enriched fractions were pooled and dialysed against phosphate-buffered saline (PBS) at pH 7·4 prior to affinity chromatography on a column of G6 $F(ab)_2$ coupled to Sepharose-4B. The unbound fraction was immediately passed over IgG-Sepharose 4B column to isolate polyclonal $G6^-$IgA-RF. Bound proteins from both columns were eluted with a solution of 3 M potassium thiocyanate (KSCN) in double-strength PBS, pH 7·4, dialysed against PBS and used as enriched $G6^+$IgA and IgA RF. Both preparations were purified further by affinity chromatography over monoclonal anti-IgA antibody 2D7-Sepharose 4B column as described above.

Monoclonal anti-idiotype production and purification

Immunization protocols, using the murine myeloma cell line NS0 as a fusion partner are described in detail elsewhere (Mageed *et al.*, 1986). Monoclonal antibodies to V_HI-associated idiotopes (G6 and G8) were produced to Ko IgM RF (V_HI–V_KIIIb) and to V_HIII-associated idiotopes (B6 and D12) using He IgM RF (V_HIII–V_KIII) as immunogen (Mageed *et al.*, 1986; Mageed, Goodal & Jefferis, 1990; Crowley *et al.*, 1990). The clones were expanded as ascities and antibodies purified by affinity chromatography on Staphylococcal protein A-Sepharose 4B (SPA-Seph 4B) column in 0·1 M Tris/HCl, pH 8·0, at 4°C.

Preparation of $F(ab)_2$ fragments

The $F(ab)_2$ fragments of monoclonal antibodies were prepared by pepsin digestion of the purified IgG in 0·1 M acetate buffer, pH 4·1, using an enzyme : protein ratio of 1 : 40 (w/w) at 37°C for 8 h. Undigested IgG was removed by affinity chromatography using SPA-Seph 4B column equilibrated in 0·1 M Tris/HCl, pH 8·0, at 4°C. The purity of the $F(ab)_2$ preparations was assessed by SDS–PAGE (Lamoyi & Nisonoff, 1983).

Characterization of cryoglobulins

Cryoglobulins were detected following incubation of serum samples at 4°C for 72 h. Homogeneity of the cryoglobulins was investigated using a combination of immunodiffusion in 1% agarose, high-resolution agarose gel electrophoresis and immunofixation, as described in detail elsewhere (Papadopoulos & Kintzios, 1967; Pascali, Pezzoli & Chiarandini, 1982).

Table 1. Concentration of serum IgM, IgA, IgM RF and IgA RF in the groups included in the study

Group	IgM (mg/ml)	IgA (mg/ml)	IgM RF (μg/ml)	IgA RF (μg/ml)
Primary Sjögren's syndrome ($n=19$)	1·3 (0·3)*	1·4 (0·2)	133 (48)*	16 (8)
SLE ($n=8$)	0·7 (0·2)	1·3 (0·3)	32 (23)	19 (13)
RA ($n=12$)	0·8 (0·1)	1·4 (0·2)	207 (57)	41 (19)
Elderly controls ($n=24$)	0·7 (0·2)	1·4 (0·3)	7 (1.6)	4 (1·4)
Young controls ($n=10$)	0·4 (0·1)	1·2 (0·3)	2 (0.5)	0·6 (0·2)

Results are presented as the mean (s.e.m.) for each group.

* Values for one patient having a monoclonal IgM RF paraprotein were omitted from calculations.

Detection of autoantibodies

Sera from patients with primary Sjögren's syndrome, SLE and RA were tested for autoantibodies to the following antigens: SS-A, SS-B, Sm, nRNP and dsDNA. Immunodiffusion in 1% agarose was used to detect antibodies to SS-A, SS-B, Sm and nRNP antibodies as previously described (Moutsopoulos *et al.*, 1984); anti-dsDNA were detected by ELISA (Tziofas *et al.*, 1987).

Quantification of total IgM and IgA

Polystyrene microtitre ELISA plates (Flow, UK) were sensitized with 10 μg/ml of purified monoclonal anti-IgM (AF6) or anti-IgA (2D7) antibodies (Oxoid-Unipath, Bedford, UK) in PBS, pH 7·4, at 37°C for 2 h. Plates were then washed three times with PBS containing Tween 20 (PBS/T) and serum samples diluted to 1/5000, 1/10 000, 1/20 000 and 1/40 000 in PBS/T added to the wells and further incubated for 2 h at 37°C. Dilutions of monoclonal Ko IgM-RF or a mixture of IgA1 and IgA2 paraproteins (5/1) were used to construct standard curves. Bound IgM or IgA was revealed using horseradish-peroxidase-conjugated sheep anti-human μ or α chains, respectively (The Binding Site, Birmingham, UK). ELISA plates were developed with OPD substrate and optical density (OD) values measured at 492 nm using a Titertek Multiscan ELISA reader (Flow). The concentration of IgM or IgA in each sample was determined by extrapolation from the standard curves.

Quantification of IgM RF and IgA RF

ELISA plates were sensitized with 100 μl of Fc-Per (IgG1 Fc) at 20 μg/ml and serum samples at dilutions of 1/200, 1/500 and 1/2000 in PBS/T added in duplicates. The assay protocol was as described above. IgM RF and IgA RF concentrations were extrapolated from standard curves constructed from OD values for known inputs of monoclonal Fr IgM RF or polyclonal IgA RF, respectively.

Quantification of CRI

A capture ELISA using microtitre plates sensitized with the $F(ab)_2$ fragment of monoclonal antibodies G6, G8, B6 and D12, was used to quantify the level of CRI in the serum samples (Shokri *et al.*, 1990). Plates sensitized with the $F(ab)_2$ fragment of monoclonal antibody G4 (mouse IgG1 isotype), with specificity for a private idiotype on a human IgG2 paraprotein (Cam), were used in parallel as negative controls. Serum samples were tested in duplicates of three dilutions (1/100, 1/250 and 1/1000) and bound IgM or IgA revealed with horseradish-peroxidase-conjugated sheep anti-μ or anti-α chains, respectively. Quantification was achieved, after subtracting any non-specific binding to mouse IgG1 $F(ab)_2$, by extrapolation from standard curves constructed for known inputs of the immunogens Ko IgM RF (G6 and G8) or He IgM RF (B6 and D12). $G6^+$IgA levels were determined using a standard curve constructed for known inputs of affinity-purified polyclonal $G6^+$IgA.

Inhibition of polyclonal RF binding to IgG Fc

The ability of G6 to inhibit the binding of polyclonal IgM RF from patients' sera to IgG Fc was tested in ELISA using microtitre plates sensitized with IgG1 Fc (Fc-Per) at 20 μg/ml.

Sera were diluted to give 50% of their maximum binding to IgG before reaching a plateau, as measured from their binding curves, and mixed with a doubling dilution series of G6 starting from 50 μg/ml. After incubation for 2 h at 37°C the mixtures were added to IgG1-Fc sensitized plates and incubated for a further 2 h at 37°C. Bound IgM revealed with horseradish-peroxidase-conjugated sheep anti-human μ chain, as described above. Percentage of inhibition of RF binding was calculated according to the formula:

$$\% \text{ inhibition} = \frac{OD_c - OD_i}{OD_c} \times 100$$

Where OD_c = optical density at 492 nm for RF binding with no added inhibitor, and OD_i = optical density at 492 nm for RF binding with added inhibitor.

Statistical analysis

Results were analysed using the Mann–Whitney *U*-test and probability values less than 0·05 were considered significant.

RESULTS

Immunoglobulin and RF levels

Total IgM and IgA were measured using monoclonal antibodies to immunoglobulin isotype in an ELISA system. Although within normal range, the level of IgM in young normal individuals (YNI) was lower than the other groups. A significant difference in IgM levels was seen between primary Sjögren's syndrome patients and YNI ($P<0·001$). No significant differences were noted in the level of IgA (Table 1).

Table 2. Frequency of patients with autoantibodies and cryoglobulins

Group	Positive (%) for autoantibodies to					(%) Positive for cryoglobulins
	dsDNA	SS-A	SS-B	Sm	nRNP	
Primary Sjögren's syndrome	0	70	40	0	0	35
SLE	14	28	0	0	14	NT
RA	0	17	0	0	0	NT

NT, not tested.

Table 3. Prevalence of CRI ± IgM in the subject groups

CRI	Primary Sjögren's syndrome	SLE	RA	Elderly controls	Young controls
G6					
Concentration (μg/ml)	16·5 (5·7)	4 (2)	10·7 (6·5)	2·1 (0·8)	0·4 (0·1)
IgM (%)	1·3*	0·6	1·4	0·3	0·1
IgM RF (%)	12·5†	12·5	5·2	29·8	25·7
G8					
Concentration (μg/ml)	31·8 (9·8)	6·2 (2·1)	27·2 (13·7)	2·5 (0·6)	1·5 (0·3)
IgM (%)	2·5	0·9	3·5	0·3	0·4
IgM RF (%)	24·0	19·5	13·0	36·0	89·0
B6					
Concentration (μg/ml)	44·6 (11·1)	20 (5·8)	27·1 (9·4)	12·8 (3·2)	11·8 (2·4)
IgM (%)	3·6	2·9	3·5	1·8	2·9
IgM RF (%)	NA	NA	NA	NA	NA
D12					
Concentration (μg/ml)	118 (19·4)	63 (19)	110 (29·6)	76 (12·1)	69 (12·1)
IgM (%)	9·5	9·1	13·3	10·5	17·3
IgM RF (%)	NA	NA	NA	NA	NA

Results are presented as the mean (s.e.m.) of concentration in μg/ml as determined in ELISA.

* Values are determined relative to the concentration of IgM for each individual.

† Values are determined relative to the concentration of IgM RF for each individual.

NA, not applicable; the proportion of G6 and G8 to IgM RF are given due to the strong association of these CRI with RF activity (see text). Due to the weaker association between B6/D12 and RF activity no proportions are given.

RF levels were generally higher in the patient groups compared with normals (Table 1); thus IgMRF levels were significantly higher in patients with RA and primary Sjögren's syndrome ($P < 0.01$ and $P < 0.05$, respectively) and to a lesser extent in patients with SLE compared with the normal groups. IgA RF levels were higher in the patient groups compared with normals, although less evidently so than IgM RF levels.

Detection of autoantibodies to cellular antigens

Most patients with primary Sjögren's syndrome had detectable levels of autoantibodies to the cellular antigens SS-A (anti-Ro) and SS-B (anti-La) (Table 2). In contrast, anti-Ro antibodies were detectable in the serum of only 28% and 17% of patients with SLE and RA, respectively. Anti-La antibodies were not found in the serum of any patient with SLE. Cryoglobulins were detectable in the serum of 35% of patients with primary Sjögren's syndrome. Interestingly, 87% of primary Sjögren's syndrome patients with cryoglobulins were positive for anti-Ro antibodies. Furthermore, the presence of cryogobulins was associated with systemic manifestations (87%).

CRI expression in polyclonal IgM

The mean level of IgM expressing G6, G8 and B6 were higher in the patient groups and elderly normal individuals, compared with young normals (Table 3). The level of D12$^+$IgM was higher in patients with RA, primary Sjögren's syndrome and elderly compared with young normal individuals. The general pattern of increase in the level of CRI was more evident for the V_HI- than the V_HIII-associated CRI. However, when CRI$^+$IgM was determined as a percentage of total IgM or IgM RF, no significant differences were observed between the groups in the proportion of the V_HIII-associated CRI (Table 3). Indeed, the

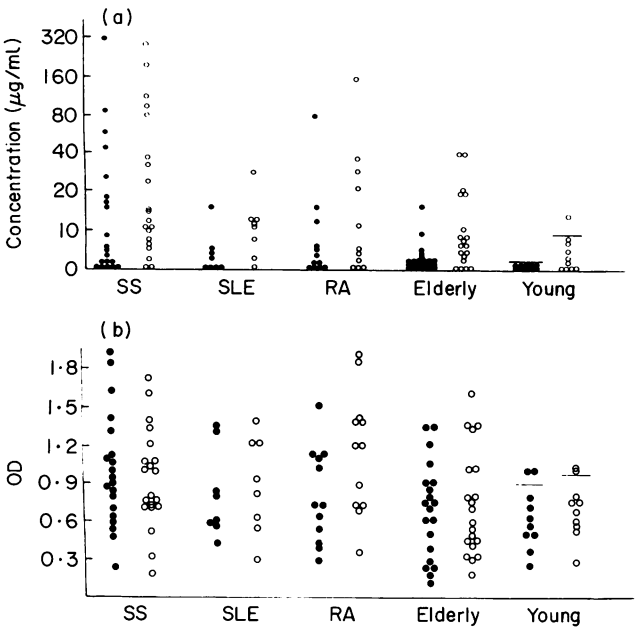

Fig. 1. (a) Concentration (μg/ml) of G6$^+$ IgM (●) and G6$^+$IgA (○) in the serum of patients with primary Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and elderly and young control individuals; (b) level of D12$^+$IgM (●) and D12$^+$IgA (○) in the subject groups. The data are expressed in optical density (OD) units at 492 nm for each CRI at serum dilutions of 1/1000. The horizontal lines represent the mean + 1 s.d. for the level of G6 or D12 in young control individuals.

data demonstrate a higher percentage of D12 to total IgM in YNI compared to the other groups. However, the percentage of the V_HI-CRI were still higher in RA and primary Sjögren's syndrome patients compared with the other groups.

Since our previous studies have demonstrated a significant association between the expression of V_HI-associated CRI and RF activity (Crowley *et al.*, 1988; Shokri *et al.*, 1990), the percentage of G6 and G8 in IgM RF was estimated. Substantially lower proportions of IgM RF expressing V_HI-associated CRI were detectable in the patient groups compared with the controls (Table 3). This was more evident for the proportion of G8$^+$IgMRF. The mean level and the percentage of both V_HI-associated idiotypes were higher in primary Sjögren's syndrome compared with RA patients.

CRI expression in polyclonal IgA

Although the level of G6$^+$IgA was higher than G6$^+$IgM, the general pattern of representation of IgA expressing V_HI-associated CRI was similar to that of IgM. The highest level of G6$^+$IgA was observed in primary Sjögren's syndrome patients followed by RA, while young controls had the lowest levels (Fig. 1a). The percentage of G6$^+$IgA to total IgA was also higher than G6$^+$IgM to total IgM. This increase was more evident in patients with primary Sjögren's syndrome (two-fold) than SLE (1·6-fold) or RA (1·5-fold).

The levels of D12$^+$ IgA were measured as described for IgM, while the results were expressed as OD values for 1/1000 dilution of the samples tested. Elevated levels of D12$^+$IgA were apparent in the patient groups compared with controls (Fig. 1b).

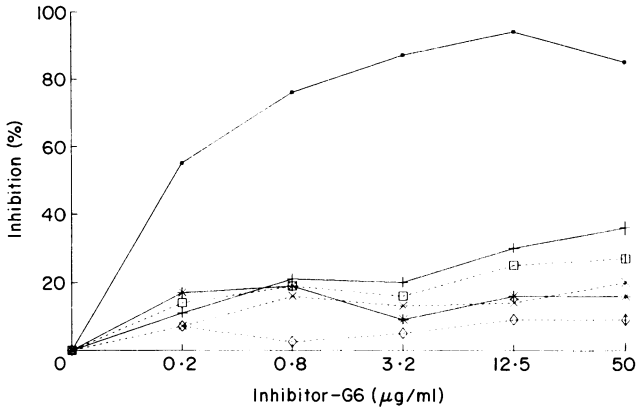

Fig. 2. Inhibition of polyclonal IgM RF binding to IgG1 Fc in ELISA using the F(ab)$_2$ fragment of monoclonal antibody G6. Broken lines, rheumatoid arthritis; solid lines, primary Sjögren's syndrome.

Heterogeneity of polyclonal IgM RF in primary Sjögren's syndrome and RA

G6 CRI expression was used to assess the degree of IgMRF heterogeneity in primary Sjögren's syndrome and RA using ELISA inhibition of RF binding to IgG Fc. In earlier studies we demonstrated that G6 monoclonal antibody completely inhibited the binding of monoclonal IgM RF paraprotein to IgG Fc (Mageed *et al.*, 1986). To determine the relative proportion of G6$^+$ IgM RF in polyclonal IgM RF, six serum samples from patients with primary Sjögren's syndrome (three patients) and RA with elevated G6$^+$IgM and IgM-RF levels were tested in an antigen binding inhibition assay, using G6 F(ab)$_2$.

Sera at predetermined dilutions were mixed with different concentrations of G6 F(ab)$_2$ and tested for IgM RF binding to Fc-Per. Under these conditions, IgM RF from three RA patients with high G6 levels were inhibited by less than 20% (Fig. 2). In marked contrast, G6 inhibited the binding of IgM RF in one primary Sjögren's syndrome patient by more than 90% and by about 30% in a second patient.

Association between CRI expression and autoantibodies to cellular antigens and to cryoglobulins in primary Sjögren's syndrome

The complied data demonstrate an apparent correlation between CRI expression and autoantibody levels in patients with primary Sjögren's syndrome. Serum samples positive for anti-Ro and anti-La antibodies were associated with elevated levels of total IgM, IgM RF, IgA RF, CRI$^+$IgM and G6$^+$IgA. G6$^+$IgM, G8$^+$IgM and G6$^+$IgA were three-fold, nine-fold and seven-fold higher, respectively, in seropositive patients compared with seronegatives, whereas B6$^+$IgM and D12$^+$IgM were higher by less than two-fold in seropositive patients (Fig. 3). In marked contrast, no significant difference in the level of B6$^+$IgA and D12$^+$IgA and total IgA was seen between seropositive and seronegative patients.

When cryoglobulin positive samples were compared with negative ones, a substantial increase in the level of CRI$^+$IgM but not CRI$^+$IgA was observed. Patients positive for cryoglobulins had an increase of nine-fold in the level of G6$^+$IgM ($P < 0{\cdot}01$) and two-fold of B6$^+$IgM and D12$^+$IgM compared with cryoglobulin-negative patients (Fig. 3). The level of IgM RF was substantially higher in the serum of patients with

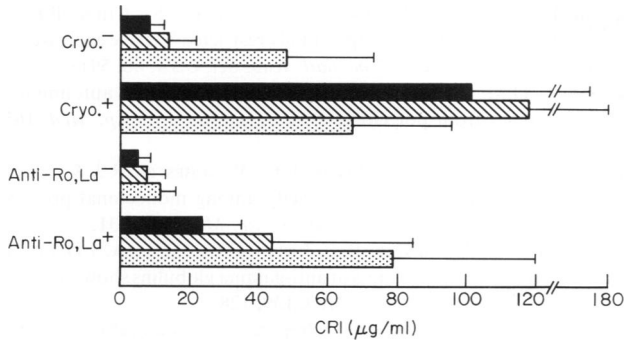

Fig. 3. Correlation between the concentration of G6⁺IgM (■), G8⁺IgM (▧) and G6⁺IgA (▩) in μg/ml and the presence of cryoglobulins, anti-Ro and anti-La antibodies. CRI, cross-reactive idiotype.

cryoglobulins. In contrast, IgA RF level was higher in patients with no cryoglobulins.

DISCUSSION

The pattern of expression of four RF-associated CRI in serum was used to probe for the nature of specific B cell responses in autoimmune rheumatic diseases. ELISA assays developed to quantify the level of V_HI (G6 and G8) and V_HIII (B6 and D12) associated CRI characteristic of RF were used to identify unique patterns of CRI expression in individual patients.

Significant elevation in the level of CRI⁺IgM was observed in the serum of patients with autoimmune rheumatic diseases compared with normal individuals. The increases were more evident for the V_HI-associated CRI (8–35-fold) than the V_HIII-associated CRI (1·5–4-fold). This was paralleled by an increase in the level of total IgM in the patient groups and elderly normal individuals compared with young normal individuals, suggesting a polyclonal expansion of B cells. However, when expressed as percentage of total IgM only the V_HI-associated CRI were found to be significantly elevated (5–13-fold), indicating a preferential expansion of B cell clones expressing V_HI-associated CRI relative to those expressing V_HIII-associated CRI.

Quantification of CRI⁺IgA revealed similar patterns as CRI⁺IgM, in that elevated levels of CRI, and more evidently of the V_HI-associated CRI, were found in the patient groups compared with normals. Furthermore, G6⁺IgA was proportionally higher than G6⁺IgM. In contrast, the level of the V_HIII-associated CRI were lower in IgA than in IgM. The levels of B6⁺IgA and D12⁺IgA in each group reflected the concentration of total IgA. However, the level of G6⁺IgA paralleled, though did not equal, the level of IgA RF and was significantly elevated in patients with primary Sjögren's syndrome ($P < 0·05$). Furthermore, high levels of G6⁺IgA related to the presence of anti-Ro and anti-La antibodies.

The general pattern of expression of CRI, that are characteristic of a specific autoantibody response (RF), in polyclonal IgM and IgA suggest that different mechanisms may lead to autoantibody production in different autoimmune conditions. In contrast to the relative restriction in the structure of RF in normal individuals, pathological mechanisms unique to each autoimmune disease may result in polyclonal expansion of the available B cell repertoire and the recruitment of different immunoglobulin V genes coding for RF specificity which do not express CRI. In RA, IgG-induced maturation of the immune response may lead to somatic diversification of the germline genes encoding RF found in the normal population. In primary Sjögren's syndrome however, polyclonal expansion of a limited number of B cell clones in association with malignant or benign gammopathies result in a more restricted RF repertoire than is found in RA.

A high proportion of IgM RF expressing the V_HI-associated CRI was observed in primary Sjögren's syndrome patients together with, in certain cases, limited heterogeneity. Thus, the binding of RF from the serum of one patient with primary Sjögren's syndrome with high RF and G6⁺IgM levels (IgM RF > 500 μg/ml and G6 > 300 μg/ml) was inhibited by more than 90% with the G6 $F(ab)_2$ suggesting monoclonality, or oligoclonality of IgM RF in the serum of that patient. This prompted us to suspect lymphoma in the patient concerned. Access to clinical details of the patient confirmed our suspicion. This finding, and those of others (Fox *et al.*, 1986), highlights the possibility of using monoclonal anti-CRI as serological probes to monitor lymphoproliferation in primary Sjögren's syndrome. Such probes would be valuable since an increased incidence of monoclonal gammopathies (Walters *et al.*, 1986; Sugai *et al.*, 1986) and non-Hodgkin's lymphoma are known to occur in primary Sjögren's syndrome. The risk of lymphoma is 44 times greater in patients with Sjögren's syndrome compared with the normal population (Kassan *et al.*, 1978). Interestingly, monoclonal gammopathies in patients with primary Sjögren's syndrome involve predominantly RF-producing B cell clones (Sugai *et al.*, 1988). Follow-up studies of patients with primary Sjögren's syndrome are currently underway in our laboratory to assess the value of these anti-CRI in monitoring lymphoma in primary Sjögren's syndrome. If G6 and G8 monoclonal antibodies prove to be useful markers for lymphoproliferation in primary Sjögren's syndrome, this would suggest that B cells bearing the G6 and G8 CRI have an increased frequency of neoplastic transformation.

Our previous studies have demonstrated that the V_HI-associated CRI are highly associated with monoclonal RF present in the serum of patients with essential mixed cryoglobulinaemia (Crowley *et al.*, 1988; Mageed *et al.*, 1990) and polyclonal RF in RA patients (Shokri *et al.*, 1990). Furthermore, the V_H gene coding for the G6 CRI was recently cloned from B cells of a patient with chronic lymphocytic leukaemia and demonstrated to be 98% homologous to the germline Hv51p1 gene (Kipps *et al.*, 1989). The results reported in our study are in agreement with those of others regarding V_L expression (Fong *et al.*, 1986) and imply that a proportion of polyclonal IgM RF in autoimmune diseases are encoded by germline gene segments of the V_HI and V_KIII families. Furthermore, the study suggests that at least two mechanisms may account for RF production in RA and primary Sjögren's syndrome. One mechanism is polyclonal activation of B cells suggested by the concomitant increase in the level of IgA and IgM positive for all four CRI investigated. The second mechanism is likely to be selective inasmuch as a selective expansion of B cell clones expressing the V_HI-associated CRI or producing specific autoantibodies, was demonstrated. Therefore, the expression of CRI can be used as an indicator of a specific antibody response and a marker for clonal expansion and proliferation of a subset of B cells.

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