Functional affinity of IgM rheumatoid factor in patients with rheumatoid arthritis and other autoimmune diseases

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SUMMARY The functional affinity of IgM rheumatoid factors (RF) was measured in 31 patients with rheumatoid arthritis (RA), 24 with systemic lupus erythematosus (SLE), 13 with Sjögren's syndrome (SS), and in 13 seropositive healthy individuals. The functional affinity of IgM RF from patients with RA was significantly lower than in the other clinical groups studied. In addition, there was a significant inverse correlation between functional affinity and titre of IgM RF in all the groups. These results suggest that the usual mechanisms of affinity based selective pressure (somatic diversification and antigen selection) may operate differently for autoantibodies to serum antigens such as IgG.

Key words: antibody affinity, systemic lupus erythematosus, Sjögren's syndrome.

The mechanisms and consequences of autoantibody production are of interest from both a clinical and scientific point of view. Rheumatoid factors (RF)—autoantibodies that bind to constant regions of immunoglobulins—are unusual in this group in being regularly produced in detectable amounts during the normal immune response to exogenous antigens in both animals and man.^{1–4} It is clearly relevant to the understanding of the genetic basis of autoantibody formation to determine whether IgM RF produced in normal individuals differ from those produced in autoimmune diseases, such as rheumatoid arthritis. A structural approach to this question has been used by Carson and coworkers, who suggested that IgM RF occurring 'normally' in the elderly and in patients with Sjögren's syndrome have an idiotypic homogeneity with respect to germline idiotopes compared with IgM RF from patients with rheumatoid arthritis.⁵

The affinity of IgM RF for autologous IgG in the sera of patients with rheumatoid arthritis has been found to be relatively low compared with that of conventional antibodies.^{6,7} We have attempted to compare IgM RF from various clinical groups in terms of functional affinity, and we report here data

showing significant differences between these groups that parallel the structural differences that have been shown previously.

Materials and methods

SERUM SAMPLES

Serum samples were obtained from 13 seropositive healthy individuals; 13 patients with Sjögren's syndrome (SS) diagnosed according to the criteria of Isenberg *et al*⁸; 24 patients with systemic lupus erythematosus (SLE), each of whom met four or more of the American Rheumatism Association's revised criteria for the classification of the disease⁹; and 31 patients with definite or classical rheumatoid arthritis (RA) according to the criteria of the American Rheumatism Association.¹⁰ Serial samples were also obtained from some of these individuals after intramuscular immunisation with 40 IU alum adsorbed tetanus toxoid (Wellcome).

ENZYME LINKED IMMUNOSORBENT ASSAYS (ELISA)

Polystyrene microtitre plates (Alpha, UK) were coated with human IgG at a concentration of 10 µg/ml in carbonate-bicarbonate buffer (0.1 M, pH 9.6) by overnight incubation at 4°C. The plates were blocked with 1% gelatin in phosphate buffered saline (PBS, pH 7.4) for two hours at 37°C, washed, dried, and stored at 4°C until used. For detection of

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IgM RF, serum samples were serially diluted in PBS/0.025% gelatin/0.05% Tween 20 (PBS/G/T) followed by 1/500 dilution of goat antihuman IgM (μ chain specific) conjugated to peroxidase (Sigma). All incubations were for one hour at 37°C, and the plates were washed with PBS/G/T between steps. Colour was developed with H_2O_2 and *o*-phenylenediamine and absorption measured at 492 nm on a Titertek Multiskan (Flow Labs, UK). End point titres were measured at an absorbance of 0.2.

Functional affinity was measured by a modification of the above ELISA, using the chaotropic agent diethylamine to inhibit low affinity antibody interactions with the solid phase antigen as described previously.¹¹ Briefly, serum samples were serially diluted on human IgG coated plates in the presence or absence of 10 mM diethylamine, the plates were sealed to prevent evaporation and incubated at 37°C. After aspiration of well contents and thorough washing the remaining steps of the assay were performed as described above. Dose-response curves were plotted and the fall in log titre due to diethylamine measured at half maximal absorbance. The extent of this fall in titre (inhibition index) was taken as an estimate of functional affinity. Thus high affinity interactions between RF and human IgG were reflected by a low inhibition index, whereas low affinity interactions gave high inhibition index values.

STATISTICS

Student's *t* test and linear regression analysis were used where indicated.

Results

AMOUNT AND FUNCTIONAL AFFINITIES OF IgM RF FROM VARIOUS CLINICAL GROUPS
The Table shows the amount (expressed as a $\log_{10}$ end point titre) and the functional affinity of IgM RF from the various clinical groups. As expected, patients with RA had significantly higher titres of IgM RF than the other groups. Although the mean functional affinity of IgM RF was very similar in patients with SLE and SS and in normal individuals, patients with RA had IgM RF of lower affinity, as shown by a significantly higher mean inhibition index.

CORRELATION BETWEEN THE AMOUNT AND AFFINITY OF IgM RF

There was a significant correlation between the titre and inhibition index of IgM RF in each group (Fig. 1), suggesting that an increase in the amount of IgM

Table Titre and functional affinity of IgM RF in the different clinical groups

Group	n	Mean titre ($\log_{10}$) (SD)	Mean inhibition index ($\log_{10}$) (SD)
RA	31	3.98 (0.92)	1.35 (0.30)
SLE	24	3.19 (0.92)**	1.04 (0.47)***
SS	13	2.82 (0.50)*	1.04 (0.35)***
Normals	13	2.70 (0.44)*	0.85 (0.29)*

Significant difference by Student's *t* test compared with RA: * $p < 0.001$; ** $p < 0.005$; *** $p < 0.01$.

Fig. 1 Correlation between functional affinity (inhibition index) and amount of IgM RF in Sjögren's syndrome (SS), $t = 3.975$, $p < 0.002$; rheumatoid arthritis (RA), $t = 3.783$, $p < 0.001$; systemic lupus erythematosus (SLE), $t = 2.994$, $p < 0.01$; and controls, $t = 3.783$, $p > 0.005$.

Fig. 2 Functional affinity and amount of IgM RF in sequential bleeds after immunisation with tetanus toxoid in controls (O), SLE (Δ), and RA ($\bullet$).

RF correlated with a fall in its functional affinity, irrespective of clinical diagnosis. This relation was further examined by measuring the titres and functional affinity of IgM RF in sequential serum samples taken from one control, two patients with SLE, and four patients with RA at intervals after immunisation with tetanus toxoid. The results showed that an increase in the titre of IgM RF occurring in sequential bleeds from the same individual was invariably accompanied by a fall in its functional affinity (Fig. 2).

Discussion

Two major points emerge from this study. Firstly, the functional affinity of IgM RF from patients with RA is significantly lower than that in the other clinical groups studied. Secondly, this fall in functional affinity correlates significantly with an increase in titre. Indeed, it appears that an increase in the amount of IgM RF from any clinical source is accompanied by a decrease in affinity.

The implications of this finding need to be considered in the light of known structural data on IgM RF. It has been shown that certain idiotopes, common among IgM RF from seropositive normal elderly subjects and from patients with SS, are absent in IgM RF from patients with RA.⁵ These idiotopes appear to be related to germline V region genes, and hence it has been suggested that IgM RF in seropositive normal people and in SS are closer to the germline and more idiotypically homogeneous than are IgM RF in RA.⁵

It is now generally accepted that the preimmune repertoire contains relatively few germline V region genes for a particular antigen.¹² After antigenic

stimulation these genes undergo somatic diversification, and an overall selective pressure for complementarity to the antigen generates antibodies of increasing affinity.^{12 13} There is evidence that this also occurs for IgM antibody responses as well as for IgG, though less efficiently for the former.¹⁴ Therefore, it is likely that if somatic mutation occurs during expansion of IgM RF producing clones the germline idiotypes prevalent in normal sera (or in conditions such as SS and SLE characterised by relatively low levels of RF) will be lost as somatic mutants develop. This should result in an increasing amount of idiotypically heterogeneous IgM RF, as found in patients with RA,⁵ and, in addition, the affinity of these RF would be expected to be greater than that found in normal individuals.

Our results, however, appear to show the opposite as IgM RF from RA were of significantly lower affinity than those from other clinical groups. This may seem surprising but if the possible mechanisms of affinity based selective pressure are considered an explanation can be suggested. It is known that affinity based selection operates by competition for available antigen^{12 15} and that in the presence of decreasing amounts of antigen only high affinity clones can successfully bind to antigen and be triggered. This mechanism, however, presupposes a limiting amount of antigen and presumably will not operate when there is excess antigen, as with a serum autoantigen such as IgG. Therefore, if high affinity clones are not selected the hypermutational mechanisms of proliferating B cells will generate random mutants and the affinity of the antibody produced by these will be always lower than that of the original clones. Therefore, once the normal restraints on RF production are removed, as in RA, it is likely that the larger the amount of RF produced, the lower its affinity will be. It is also of possible relevance that patients with RA appear to be unable to show affinity maturation of their antibody responses to exogenous antigens.¹⁶

Additional confirmation for this hypothesis would be obtained if these low affinity IgM RF in RA displayed a greater idiotypic heterogeneity than the higher affinity RF in the other groups. Although RF may be of physiological importance in the secondary immune response, for instance by its ability to interact with circulating immune complexes,¹⁷ the clinical relevance of low affinity IgM RF in RA has yet to be determined.

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