Lack of hidden complement fixing IgM rheumatoid factor in adult seronegative rheumatoid arthritis

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SUMMARY IgM rheumatoid factors capable of complement fixation and activation are commonly present in the sera of adults with rheumatoid arthritis. Hidden complement fixing IgM rheumatoid factor has been demonstrated in the majority of patients with juvenile RA and hidden agglutinating IgM rheumatoid factors have been demonstrated in the serum of adults with seronegative rheumatoid arthritis. We studied 27 adults with seronegative rheumatoid arthritis and were unable to demonstrate hidden complement fixing IgM rheumatoid factor in their sera.

The majority of adult patients with rheumatoid arthritis (RA) have rheumatoid factor (RF), that is, a 19S IgM globulin directed against the Fc piece of IgG. RFs are capable of agglutinating latex particles (LFT) and sheep erythrocytes (SCAT) coated with IgG. About 20% of patients with RA do not contain RF by the standard tests, that is, their serum does not contain agglutinating IgM-RF. However, 'hidden' IgM-RFs have been demonstrated in the serum of adults with seronegative RA, but their significance is yet to be defined (Allen and Kunkel, 1966; Bluestone *et al.*, 1969; Cracchiolo *et al.*, 1970). In addition, IgG-RFs existing as 'intermediate complexes' have been demonstrated in the sera of adult RA patients (Pope *et al.*, 1974, 1975). Although both IgM and IgG-RFs are thought to be intimately involved in the inflammatory process leading to tissue damage in RA, their precise role is still unclear.

Complement fixation by IgM-RF has been shown to occur predominantly through classical pathway, thus enabling it to participate in the inflammatory reaction (Tanimoto *et al.*, 1975; Nydegger *et al.*, 1977; Kaplan *et al.*, 1978). We have recently shown that qualitative differences in IgM-RF complement fixation exist in seropositive RA patients which may act independently from the concentration of IgM-RF in the serum (unpublished results). More recently, the presence of hidden complement fixing IgM-RF

has been demonstrated in the IgM fractions of the majority of patients with juvenile RA (especially the polyarticular type) when these same IgM-RFs remained undetectable by the standard agglutination tests in the serum. Furthermore, the haemolytic titre correlated with disease activity (Moore *et al.*, 1978). This study was undertaken to determine the possible significance and presence or absence of hidden complement fixing IgM-RFs in adults with seronegative RA.

Materials and methods

SOURCE OF SERA

Sera were obtained from 27 patients with definite or classical RA (American Rheumatism Association criteria) all of whom were agglutinin negative by the standard LFT or SCAT. (Several sera were kindly provided by Drs Robert Shapiro and Kenneth Weisner). Additionally, sera were obtained from 13 RA patients (ARA criteria) who were agglutinin positive; 10 JRA patients with agglutinin-negative sera; 7 patients who had oligoarticular asymmetrical peripheral arthritis and were agglutinin negative but positive for the HLA B27; and 6 normal volunteers. All sera were heat inactivated at 56°C for 30 minutes and incubated with 1:5 dilution washed packed sheep erythrocytes (SRBC) at 37°C for 90 minutes to remove natural antibody to SRBC.

MEASUREMENT OF COMPLEMENT—INDUCED HAEMOLYSIS

The IgG fraction of rabbit anti-SRBC haemolysin

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(Cappel Laboratories, Downingtown, PA) was reduced and alkylated so as to abolish its ability to fix complement (Tanimoto *et al.*, 1975). The haemolysin was further treated with 0.15 M glycine-HCl at pH 2.5 for 90 minutes at 22°C and dialysed back to neutrality with phosphate buffered saline (PBS) pH 7.32. The latter allowed for hybridisation of the IgG haemolysin, which in turn allows for a higher sensitisation of the SRBC without attendant agglutination (Nisonoff and Palmer, 1964).

Complement-induced haemolysis by IgM-RF was quantified in tube assays. The patient's serum was diluted serially in 0.15 M barbitone buffered saline containing 0.1% gelatin and optimal concentrations of Ca^{++} and Mg^{++} (GVB $^{++}$). To each dilution a standard amount of 1% sensitised SRBC was added. After incubation at 37°C for 60 minutes and 4°C for 90 minutes the cells were washed once, and 1:40 diluted SRBC-absorbed guinea-pig serum (Pel-Freez, Rogers, Arkansas) was added as a source of complement. After another 60 minutes at 37°C haemoglobin released into the supernatant was assayed at 412 nm.

PREPARATION OF IGM AND IGM-RF

Companion sera after heat inactivation and absorption with SRBC obtained from all the patients as mentioned above were then applied to a Sephadex G-200 (Pharmacia Fine Chemicals Inc., Piscataway, NJ) gel filtration column, equilibrated with 0.1 N pH 4.0 acetate buffer. The first peak eluted in the void volume contained IgM. It was dialysed against 0.15 M NaCl buffered to pH 7.32 with sodium phosphate (PBS) and concentrated by pervaporation to a volume equal to or less than the serum specimen originally applied to the column. These preparations were examined by double immunodiffusion in agar at a concentration of 10 mg/ml by means of antisera specific for IgM, IgG, and IgA. Only IgM was detected. The IgM fractions were then used in the complement dependent haemolytic assay exactly as described for the sera.

Results

The results of IgM-RF complement fixation present in both the whole sera and isolated IgM fractions at 1:2 dilution are displayed in Fig. 1. The optical density is a measurement of haemoglobin release mediated by complement and is therefore directly proportional to the degree of complement fixation mediated by IgM-RF. We could not detect any complement fixing IgM-RF in the 27 seronegative adult RA patients studied either in the whole serum or in the isolated IgM fraction. Although there are occasional seronegative sera and isolated IgMs that

Fig. 1 Comparison of haemolytic activity of whole serum and IgM fractions from all patients and groups studied at a dilution of 1:2. The optical density is directly proportional to the amount of complement fixation mediated by IgM-RF. RA+; seropositive rheumatoid arthritis. RA-; seronegative rheumatoid arthritis. JRA; juvenile rheumatoid arthritis.

showed low-grade haemolysis at dilutions of 1:2, they were all completely negative when diluted to 1:16 or greater, which is not statistically different from the normal and negative control groups. This is in contrast to the seropositive adult RA group, whose IgM-RF showed good complement fixation in both the whole serum and isolated IgM, and with the polyarticular juvenile RA group, whose serum showed no haemolytic capability but whose isolated IgM had good haemolytic capability. Thus, our results show the absence in the serum of 'hidden' complement fixing IgM-RF in the 27 adult seronegative RA patients studied here. There was no evidence for complement fixing IgM-RF in the serum or separated IgM fraction in either of the negative control groups, the HLA B27+ group, or the normal adults.

Fig. 2 is a composite linear representation of the complement fixing properties of the sera and IgMs in each patient group studied. It is clearly seen that the serum and isolated IgM-RF from the seropositive RA patients show good complement fixation, as does the isolated 'hidden' IgM-RF from the polyarticular juvenile RA group. The serum and isolated IgM from the adult seronegative patients as well as the normal controls and HLA B27 patients do not show haemolytic capability significantly different from zero.

Discussion

The pathogenic role for IgM-RF in RA has not been clearly defined, though most investigators believe it is intimately involved in the inflammatory

Fig. 2 Composite linear representation of the complement fixing properties of the sera and IgMs in each patient group studied.

reaction leading to tissue damage in this disease (Weissman, 1972). Patients with high titres of circulating IgM-RF have more aggressive joint disease and a higher incidence of extra articular manifestations (Mongan *et al.*, 1969). Furthermore, the rheumatoid synovium has been shown to produce IgM-RF and IgG and to contain immune complexes consisting of IgM, IgG, and C_3 (Schurr *et al.*, 1975; Taylor-Upsahl *et al.*, 1977). Also, synovial fluid complement is decreased in RA, which has been correlated with more severe joint damage, and complexes of IgM, IgG, and C_3 have been found in phagocytic cells within the joint fluid (Schur *et al.*,

1975; Zubler *et al.*, 1976; Carson *et al.*, 1977). IgM-RF has been shown to fix complement predominantly by the classical pathway, which enables it to participate in the inflammatory reaction (Tanimoto *et al.*, 1975; Nydegger *et al.*, 1977; Kaplan *et al.*, 1978).

Recently 'hidden' complement fixing IgM-RF has been demonstrated in patients with juvenile RA, especially the polyarticular group. This was present in the isolated IgM fraction separated under acid conditions. Moreover, the degree of IgM-RF complement fixation in these patients correlated with disease activity, a correlation which was not present by the standard SCAT or LFT. Thus it was postulated that the haemolytic assay might be detecting a group of IgM-RF molecules different from those producing agglutination and that these complement fixing IgM-RF molecules could have significant pathogenic effects by means of their ability to participate in the inflammatory reaction. The whole sera of these patients did not show agglutinating or complement fixing haemolytic capability (Moore *et al.*, 1978).

'Hidden' IgM-RF possessing agglutinin activity has been detected in the serum of adults with seronegative RA, suggesting a pathogenic role for IgM-RF in that patient population (Allen and Kunkel, 1966). Furthermore, IgM-RF separated under acid conditions showed a greater avidity for monomeric IgG than that separated under neutral conditions. This difference in avidity has been shown to be due to the multivalency of the acid-separated IgM-RF molecules (Dissanayake *et al.*, 1977). Presumably this IgM-RF exists in serum complexed to IgG, thus rendering it unable to participate in agglutination or haemolytic reactions when studied as whole sera. However, we could not demonstrate significant complement fixation by IgM-RF either in the whole serum or isolated IgM fraction in any of the 27 adult patients with seronegative RA whom we studied. These data militate against a pathogenic role for IgM-RF in this group of patients. However, IgM-RF has been shown to be present in the synovial fluids of adults with seronegative RA (Dissanayake *et al.*, 1977) and it is possible that IgM-RF may have pathogenic significance within the joint space.

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