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HIDDEN ANTIGLOBULINS IN RHEUMATIC DISORDERS

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SUMMARY

Sera from thirty-five patients with rheumatoid arthritis, fourteen patients with osteoarthritis, eleven patients with juvenile rheumatoid arthritis, ten patients with ankylosing spondylitis, twenty-two patients with arthritis of various etiologies and seven healthy subjects were studied for the presence of hidden IgM antiglobulins. The whole sera and the chromatographically separated IgM fractions were tested for antiglobulin activity against human and rabbit IgG. Significant antiglobulin activity was not detected in any of the whole sera, but was found in thirteen isolated IgM fractions. Of the thirteen patients with hidden antiglobulins, seven had rheumatoid arthritis, three had osteoarthritis, one had ankylosing spondylitis, one had subacute bacterial endocarditis and one was apparently healthy. All hidden antiglobulins reacted with human IgG, but only the rheumatoid sera contained antiglobulins directed against determinants on rabbit IgG. Antiglobulin titres were generally greater in those with rheumatoid arthritis as compared to the non-rheumatoid group. These data indicate that hidden IgM antiglobulins, particularly those with specificity for human and rabbit IgG, occur most frequently and in highest titre in patients with rheumatoid arthritis.

INTRODUCTION

Antiglobulins with specificity for human and rabbit γ-globulin have been detected in the sera of most patients with rheumatoid arthritis (RA); in approximately 20%, however, antiglobulins are absent (Ziff, 1957). The highest antiglobulin titres are usually found in those rheumatoid patients with severe disease and in those with extra-articular manifestations (Bywaters, 1968; Hill, 1968). Moreover, the interaction of antiglobulins with IgG within the synovial space has been implicated in the pathogenesis of RA (Restifo *et al.*, 1965; Rawson *et al.*, 1969). It has been suggested, therefore, that seropositive and seronegative RA may be fundamentally different disorders (Mongan *et al.*, 1969).

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In a preliminary communication, we described the presence of hidden rheumatoid factor in the sera of three subjects with seronegative nodular RA (Bluestone, Goldberg & Cracchiolo, 1969). Significant titres of antiglobulins were not present in whole sera, whereas the chromatographically separated IgM fractions contained antiglobulin activity in high titre. These findings suggested that antiglobulin activity can be masked by the presence of a serum factor, presumably IgG, and that certain presumed seronegative individuals are in fact seropositive. The present study was undertaken to further examine the frequency and character of hidden antiglobulins in seronegative RA and other rheumatic disorders.

MATERIALS AND METHODS

Sera were collected from thirty-five patients with seronegative RA, ten patients with ankylosing spondylitis, eleven patients with juvenile RA, fourteen patients with osteoarthritis, twenty-two patients with arthritis of various etiologies (psoriatic, Reiter's syndrome, traumatic synovitis, subacute bacterial endocarditis, Bechet's syndrome) and seven healthy subjects. Sera were heat-inactivated at 56°C for 30 min prior to testing.

IgM fractions were partially isolated by passing each serum, 5–7 ml, through Sephadex G-200 columns equilibrated with phosphate buffered saline, pH 7·35; and IgG fractions were prepared by diethylaminoethyl (DEAE) column chromatography using 0·01 M phosphate buffer, pH 7·5, as the eluant. Eluted peaks were measured spectrophotometrically at 280 mμ, concentrated by negative pressure dialysis at 4°C, and tested in agar gel double diffusion against rabbit antiserum monospecific for IgG, IgA and IgM (MacKenzie *et al.*, 1968). Immunoglobulin levels of the concentrated eluates and whole sera were determined by radial immunodiffusion in agar–antibody plates. The level of IgM in the void volume eluates was adjusted by saline-dilution or further concentration to match the IgM level in the respective whole serum.

Antiglobulin activity of each whole serum and its IgM fraction was measured simultaneously with latex particles coated with aggregated human IgG, human type O positive erythrocytes sensitized with an incomplete anti-Rh antibody (Ripley), and sheep red cells sensitized with rabbit γ-globulin. Heterophile antibody titres were determined in microtitre plates using a 2% solution of unsensitized sheep erythrocytes. Latex agglutination titres and Ripley coated erythrocyte titres of greater than 1:40 and differential agglutinin titres (sensitized sheep cell agglutination titre minus the heterophile antibody titre) of 1:4 or greater were consider abnormal. Hidden antiglobulins were said to be present when the antiglobulin titre of the IgM fraction exceeded the titre in whole serum by four-fold or more. In the sera containing hidden antiglobulins, euglobulin precipitates were prepared by dialysing 1 ml serum against distilled water for 24 hr at 4°C. The isolated, washed euglobulins were re-dissolved in saline and tested for immunoglobulin content and antiglobulin activity.

Antigenic specificity of the hidden antiglobulins was determined by inhibition studies. Four agglutinating units of each eluted antiglobulin were mixed with an equal volume of either saline, autologous native IgG (10 mg/ml), or heat aggregated human IgG (10 mg/ml). The mixture was incubated for 1 min at room temperature and tested against latex particles coated with aggregated IgG.

RESULTS

The void volume peaks obtained by G-200 gel filtration contained predominantly IgM with a small amount of IgG; only IgG was present in the peaks eluted by DEAE column chromatography. Hidden antiglobulins were detected in the IgM fractions isolated from the sera of seven patients with RA, three patients with osteoarthritis, one patient with ankylosing spondylitis, one individual with subacute bacterial endocarditis and one apparently healthy subject (Table 1). No significant antiglobulin activity was detected in any of the ninety-nine

TABLE 1. Antiglobulin activities in whole sera and isolated IgM fractions from patients with hidden antiglobulins

	Whole serum			IgM fraction		
Patient	Latex*	Ripley†	SSC‡	Latex	Ripley	SSC
Rheumatoid arthritis						
1	20§	20	1	1280	1280	4
2	40	40	0	2560	640	8
3	40	40	1	2560	2560	64
4	20	20	0	640	80	0
5	0	0	0	80	80	0
6	20	0	0	320	320	16
7	0	0	0	160	0	0
Osteoarthritis						
1	40	0	0	320	160	0
2	40	0	0	320	0	0
3	20	40	0	160	160	0
Ankylosing spondylitis						
1	0	0	0	160	160	0
Bacterial endocarditis						
1	0	0	0	80	160	0
Healthy subject						
1	0	0	0	0	0	8

* Latex agglutination test.
† Ripley-sensitized erythrocyte agglutination test.
‡ Sensitized sheep cell agglutination test.
§ Reciprocal of highest serum dilution giving positive reaction.

whole sera. All hidden antiglobulins gave positive reactions when tested with latex particles coated with aggregated human IgG and all but three reacted with Ripley-sensitized red cells; the IgM fractions from patient No. 2 with osteoarthritis and from patient No. 7 with RA reacted only in the latex test, and the IgM from one healthy subject reacted only in the sensitized sheep cell test (1:8).

The latex agglutination titre of the revealed antiglobulins in the six non-rheumatoid patients never exceeded 1:320; however, a titre of 1:640 or greater was found in the IgM fractions from four of the seven rheumatoid subjects. Of the twelve patients with rheumatoid diseases and hidden antiglobulins, only the antiglobulins from four patients with RA (Nos. 1, 2, 3, 6) also reacted with sheep cells sensitized with rabbit γ-globulin. The IgM

from one healthy subject contained an antibody which appeared to react only with determinants present on rabbit γ-globulin. Inhibition studies revealed that all hidden antiglobulins were inhibited by aggregated human IgG. The antiglobulin activities were also inhibited by autologous, native IgG in two patients, one with osteoarthritis (No. 2) and one with RA (No. 7). Euglobulin precipitates prepared from the sera with hidden antiglobulins contained IgM, IgG and a small amount of IgA. Antiglobulin activity was not detected in any of the re-dissolved euglobulins.

DISCUSSION

The results of this study indicate that hidden antiglobulins may occur in a variety of rheumatic diseases, including RA, osteoarthritis and ankylosing spondylitis. However, they were found with greatest frequency and in highest titre in the sera of patients with RA. Moreover, the hidden antiglobulins in RA, unlike those in the other rheumatic disorders, reacted with rabbit γ-globulin as well as with determinants on human IgG. These findings are remarkably similar to the earlier descriptions of antiglobulins (rheumatoid factor) which were detected in whole sera of patients with a variety of disorders (Ziff, 1957; Kunkel, Simon & Fudenberg, 1958; Howell, Malcolm & Pike, 1960), i.e. antiglobulins, particularly those with specificity for both rabbit and human γG, occur most frequently in patients with RA.

Antiglobulins may remain undetected in whole serum for several reasons. First, these antibodies to IgG may be bound to their specific antigen in an antigen–antibody complex and thus be undetectable by conventional methods. Indeed, Allen & Kunkel (1966) showed previously that acidic gel filtration of certain rheumatoid sera revealed antiglobulins reactive with native IgG; the whole sera of these patients, however, reacted with aggregated human IgG. It seems unlikely that tightly bound antiglobulin-IgG complexes existed in the sera of our patients since the IgM antiglobulins were readily separated by gel filtration at neutral pH. Furthermore, in earlier studies on the sera of three rheumatoid subjects with hidden antiglobulins (Bluestone *et al.*, 1969), acidic chromatography which would be expected to dissociate antigen–antibody complexes did not result in greater recovery of antiglobulin activity. A second explanation for the phenomenon of hidden antiglobulins might be the presence of a serum inhibitor, such as altered or denatured IgG. The altered IgG would not necessarily have to be bound to the antiglobulin to interfere with its *in vitro* detection. The failure to uncover antiglobulin activity in the euglobulin fractions was probably due to the presence of both IgM and IgG, thus duplicating the situation which exists in whole serum.

The major implication of this report is that certain patients, presumed to be seronegative, are in fact seropositive. Of the thirty-five patients with RA studied, 20% were shown to have hidden antiglobulins of the IgM class. Interestingly, Torrigiani *et al.* (1970) have described antiglobulins of the IgG class in the majority of patients with seronegative RA; such antibodies cannot be measured by conventional techniques and require the method of quantitative immunoabsorption for detection. We have also recently found several subjects with RA whose whole synovial fluid and serum were negative for antiglobulins; however, the synovial fluids from these patients formed cryoprecipitates which contained antiglobulins of either the IgM or IgG class (Cracchiolo, Bluestone & Goldberg, unpublished data, 1970). These studies would seem to indicate that seronegative RA may be a truly unusual entity.

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