Failure to find C1q-binding material and anti-IgG antibodies in ankylosing spondylitis

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SUMMARY C1q-binding immune complexes (CIC), anti-IgG antibodies (anti-IgG Ab), and complement levels were investigated in the serum of 37 patients with ankylosing spondylitis (AS). In all these studies the mean levels observed in patients with AS were similar to those in 31 normal subjects. Moreover, no significant difference in either CIC or anti-IgG Ab levels was observed when the patients were classified in different clinical forms according to the localisation (peripheral and central) or to the gravity (mild and severe) of the AS. In a parallel study increased CIC and anti-IgG Ab levels were found in most of the 81 patients with seropositive or seronegative rheumatoid arthritis.

Most cases of inflammatory arthritis are thought to be associated with immunopathological disorders. Rheumatoid arthritis (RA) represents the classical example of this association, in which circulating immune complexes (CIC), rheumatoid factor (RF), and complement appear to play an important role in the pathogenesis of the disease.^{1–3} In ankylosing spondylitis (AS), despite some features in common with RA such as the occurrence of histologically nonspecific chronic inflammation, there is no clear evidence of immunopathological involvement. Howell and others⁴ showed the presence of anti-gammaglobulin antibodies in patients with AS. More recently Gabay and others,⁵ using the ¹²⁵I-C1q binding test, found a weak level of CIC in only a few patients with AS. Moreover, these authors were unable to detect any change in the complement catabolism of these patients. On the other hand Corrigan *et al.*,⁶ by using the inhibition of antibody-mediated lymphocyte-induced cytotoxicity, demonstrated CIC in more than 50% of AS patients. Finally, the association between AS and HLA B27 histocompatibility antigen⁷ is an other argument for a possible immunological origin of this disease.

The purpose of the present work was to investigate C1q-binding CIC, anti-IgG antibodies, and complement levels in patients with different clinical forms of AS.

Materials and methods

PATIENTS

Serum samples from 37 patients with AS, 81 patients with RA, and 31 healthy subjects were studied. The AS patients were classified by 2 criteria: (a) the localisation: 18 central and 19 peripheral forms, when the inflammation was confined to the sacroiliac and vertebral joints or affecting peripheral joints; (b) the illness: 29 mild and 8 severe, when they presented more than 2 attacks a year. The 81 RA patients had classical or definite RA (American Rheumatism Association criteria) and were separated into 47 seropositive (RA +) (rheumatoid factor titre in Waaler-Rose ≥ 64 or in latex >40) and 34 seronegative (RA –).

DETECTION OF CIRCULATING IMMUNE COMPLEXES

The CIC were investigated by the ¹²⁵I-C1q binding test.^{2,3} Briefly, ¹²⁵I-C1q was mixed with test serum, previously treated with 0.2 M EDTA. Free C1q was separated from C1q bound to CIC by precipitation with 3% polyethylene glycol (PEG). All tests were done in triplicate and the results were expressed as per cent ¹²⁵I-C1q precipitated compared to the radioactivity precipitated with trichloroacetic acid (TCA) in control tubes.

DETECTION OF ANTI-IgG ANTIBODIES

Anti-IgG Ab were investigated by 3 methods: classical Waaler-Rose and latex tests (reagents from

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the Pasteur Institute, Paris) and the radioimmuno-precipitation-PEG assay (RIPEGA).⁸ For the RIPEGA, ¹²⁵I-IgG was mixed with native test serum, then free IgG was separated from IgG bound to anti-IgG Ab by precipitation with 7% PEG. Results were expressed as per cent ¹²⁵I-IgG precipitated as compared with the protein-bound radioactivity precipitable with 20% TCA.

COMPLEMENT STUDIES

Levels of C1q, C4, C3, and properdin factor B were evaluated by radial immunodiffusion⁹ on plates supplied by the Behring Institute (Harburg-Lohn, West Germany). The 50% haemolytic complement (CH50) was measured according to a technique previously described.¹⁰

Statistical analysis was carried out by Student's *t* test.

Results

CIRCULATING IMMUNE COMPLEXES

No significant difference in the mean of C1q-binding activity was observed between the AS patients and the healthy subjects (Table 1). Only 4 patients with AS (Fig. 1) showed a C1q-binding activity discretely higher than the normal subjects (mean $\pm$ 2 SD). Among them 3 had a peripheral form and the last one a central form. As regards illness, 2 were mild and 2 severe. In contrast, most of the RA+ and RA- patients showed an increased C1q-binding activity (Fig. 1).

ANTI-IgG ANTIBODIES

Anti-IgG Ab were investigated by 3 different techniques. All the patients with AS gave negative results for rheumatoid factor by both the Waaler-Rose and latex tests. By using the RIPEGA the mean level of ¹²⁵I-IgG precipitation in AS patients was not different from that obtained with normal subjects (Table 1). Nevertheless, 3 patients with AS showed anti-IgG Ab levels higher than the mean $\pm$ 2 SD of normal subjects (Fig. 2). They had respectively 1 central and 2 peripheral forms and 2 severe and 1 mild form. These patients showed no C1q-binding

activity. Most of the patients with RA showed, moreover, higher levels of anti-IgG Ab.

COMPLEMENT LEVELS

The mean levels of the total haemolytic complement (CH50) and the complement components C1q, C4, C3, and factor B in patients with SA were within the normal range obtained with healthy persons (Table 2).

Fig. 1 C1q-binding activity in patients with ankylosing spondylitis (AS), seropositive (RA+) and seronegative rheumatoid arthritis (RA-), and in normal subjects (NHS)

Fig. 2 Anti-IgG antibodies in patients with ankylosing spondylitis (AS), seropositive (RA+) and seronegative rheumatoid arthritis (RA-), and in normal subjects (NHS)

Table 1 Levels of anti-IgG antibodies and circulating immune complexes (CIC) in patients with ankylosing spondylitis (AS), seropositive (RA+) and seronegative (RA-) rheumatoid arthritis, and in normal subjects

Subjects	Number of cases	CIC (¹²⁵ I-C1q binding)	Anti-IgG Ab (RIPEGA)
Normal	31	10.0 $\pm$ 1.5	9.7 $\pm$ 1.2
AS	37	11.4 $\pm$ 3.04	11.3 $\pm$ 1.8
RA+	47	19.2 $\pm$ 6.1	15.3 $\pm$ 3.7
RA-	34	18.0 $\pm$ 7.2	13.1 $\pm$ 3.0

Table 2 Levels of the whole haemolytic complement (CH50) and some of its components in patients with ankylosing spondylitis (AS)

	CH50 (U/ml)	C1q (mg/100 ml)	C4 (mg/100 ml)	Factor B (mg/100 ml)	C3 (mg/100 ml)
AS patients	86.3 ± 19.2	16.2 ± 4.6	42.4 ± 13.3	32.5 ± 3.0	111.1 ± 14.5
Normal range	80-108	10-25	20-50	10-45	80-140

SI: mg/l = mg/100 ml × 10.

Discussion

In the present study, by using highly sensitive techniques, we could not find significant departures from normal levels of CIC and anti-IgG Ab in serum from patients with AS. These observations do not allow us to consider that C1q-binding and rheumatoid-factor-like materials are involved in the clinical evolution of this disease, since no significant difference was observed when the AS patients were separated either according to the localisation (central and peripheral) or to the illness (mild and severe). In contrast, in the parallel investigation performed in serum from patients with RA highly significant levels of CIC and anti-IgG Ab were observed. Moreover, our results in RA are in agreement with several studies of CIC in this disease.^{3 11 12}

Gabay and others,⁵ by using the same method employed in our study, i.e., the ¹²⁵I-C1q binding test, found only slightly significant CIC levels in 14% of the tested patients with AS. The present of C1q-binding CIC in only 4 out of the 37 AS patients studied in this work, clearly confirms their results. Nevertheless, Corrigan and others,⁶ by using another technique, namely, the inhibition of antibody-mediated lymphocyte-induced cytotoxicity, detected CIC-like material in two-thirds of 18 patients with AS, but without clinical correlation. This discrepancy should probably be related to the difference in the methods used. Only a comparative study of these 2 techniques with the same AS patients could evaluate correctly the validity of these CIC studies.

The presence of anti-IgG Ab in AS patients was suggested by Howell and others⁴ and Arana and others.¹³ Moreover, Corrigan and others⁶ and Whaley and others¹⁴ considered anti-IgG Ab in AS might form immune complexes and initiate an inflammatory process similar to that observed in RA. This possibility was supported by the work of Gabay and others,⁵ in which CIC were found only in AS patients with Anti-IgG Ab. Nevertheless, by using the RIPEGA, a highly sensitive radioimmunoassay, we did not detect any difference in the

incidence of anti-IgG Ab between AS patients and normal subjects. In contrast, in seropositive or seronegative RA a significant correlation was observed between C1q-binding CIC and anti-IgG Ab evaluated by the RIPEGA.¹⁵ Our results do not support the involvement of CIC and anti-IgG Ab in AS.

The levels of complement and some of its components in AS patients did not differ from the normal range. These data are in agreement with most of the work in AS.^{14 16} All these observations weigh against the presence of CIC in AS patients. The failure to find CIC and anti-IgG Ab in AS suggests the pathology differs from that observed in RA.

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