

Immunological reactivity in ankylosing spondylitis

Circulating immunoblasts, autoantibodies, and immunoglobulins

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Eghtedari, A. A., Davis, P., and Bacon, P. A. (1976). *Annals of the Rheumatic Diseases*, 35, 155–157. Immunological reactivity in ankylosing spondylitis. Circulating immunoblasts, autoantibodies, and immunoglobulins. Circulating immunoblasts were studied in 39 cases of ankylosing spondylitis. The results were compared with 20 normal subjects and a group of 39 patients with rheumatoid arthritis. Immunoblasts were found to be increased in 11 patients with ankylosing spondylitis and in 22 patients with rheumatoid arthritis in contrast to the controls who were found to have a normal lymphoid cell population in the peripheral blood. Fifteen patients showed raised levels of one or more class of immunoglobulin. Autoantibodies, including antinuclear factors, were negative in all cases. There was a correlation between raised immunoblasts and plasma viscosity but not with clinical assessment of activity. The increase of immunoblasts in the peripheral blood, together with the raised immunoglobulins supports the suggestion of an immunological basis for ankylosing spondylitis.

The presence of large lymphoid cells in the peripheral circulating blood indicates that an active immunological reaction is in progress. Reactive lymphoid cells, or immunoblasts, are found to be increased after immunization and in bacterial or viral infection (Crowther, Fairley, and Sewell, 1969a), in Hodgkin's disease (Crowther, Fairley, and Sewell, 1969b), and in autoimmune disease, including systemic lupus erythematosus and rheumatoid arthritis (RA) (Bacon, Sewell, and Crowther, 1975).

Recent observations have suggested that in ankylosing spondylitis (AS) the immune response may play an important part in the aetiology and pathogenesis of this disease. This study investigates the incidence of circulating immunoblasts in a group of patients with AS attending a routine rheumatology clinic. This has been correlated with a number of clinical features and also with levels of serum immunoglobulins and a range of autoantibodies.

Patients and methods

Thirty-nine patients with definite AS according to the New York criteria were investigated. Findings were com-

pared with 39 cases of definite or classical RA and 20 normal healthy adult controls. Patients with AS were examined for the presence of peripheral joint involvement, the presence of ocular involvement, and were assessed for the overall degree of functional capacity according to the Steinbrocker scale. They were also asked to give their own assessment of whether the disease was active or inactive at the time.

Routine haematological studies included haemoglobin and plasma viscosity. Serum immunoglobulins were measured by automated immunoprecipitation technique in the regional protein reference laboratory (Cardiff). Rheumatoid factors were examined by a commercial latex fixation test (Hoechst). Autoantibodies were sought by routine immunofluorescence using a standard autoantibody-profile, scanning for antibodies to nuclei, thyroid, gastric parietal cells, and to smooth muscle. DNA binding was also assessed using a modified Farr technique (Cohen and others, 1971). Lymphocyte separation was performed using defibrinated blood and carbonyl iron by a technique modified from Coulson and Chalmers (1967). In addition, in order to obtain further purification, the lymphocyte-rich supernate was further separated on a Ficoll-Hypaque gradient. The resultant washed deposit, containing virtually pure lymphoid cells, was stained with Wright's stain. At least 500 cells were counted independently by two observers who recorded the average number of immunoblasts (large lymphoid cells 15–25 μm in diameter with prominent nucleolus and hyperbasophilic cytoplasm).

Accepted for publication September 8, 1975.

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Results

Increased numbers of circulating immunoblasts were seen in 11 of 39 cases of AS (Fig. 1), the remainder having <0.5% of such cells, as did all of the controls in agreement with previous findings. This contrasts with the raised level of immunoblasts in 22 of 39 cases of RA randomly selected from the clinic. Table I

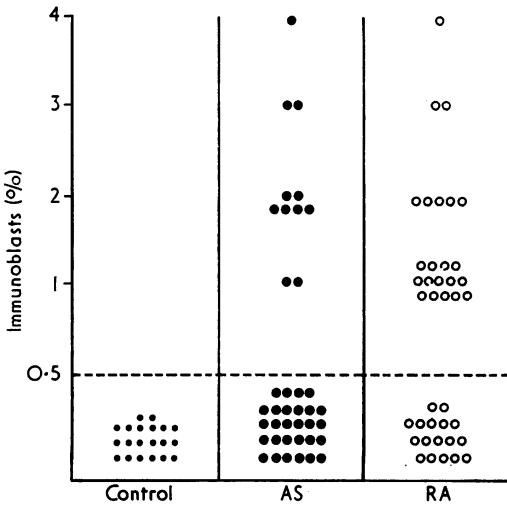


FIG. 1 Incidence of immunoblasts in patients with ankylosing spondylitis (AS), compared to patients with rheumatoid arthritis (RA) and normal controls

Table I Clinical data in the 39 patients with ankylosing spondylitis, comparing those with and those without circulating immunoblasts

Immuno- blasts	Case no. and sex	Age (years) (mean)	Age at diagnosis (years) (mean)	Duration (years) (mean)
+	9 M	35	24	11
	2 F			
-	21 M	39	25	12
	7 F			

Table II Serological studies in 39 patients with ankylosing spondylitis

Immuno- blasts	Total no. of cases	RF +ve	DNA binding >30%	Auto- antibodies	Serum immunoglobulins		
					IgA > 480	IgM > 200	IgG > 1700
+ve	11	0	0	0	3	4	2
-ve	28	0	0	0	0	7	1

shows that there is no difference between those cases of AS with or without peripheral circulating immunoblasts in respect to sex, age, duration of disease, or age at onset of disease. Similarly, there was no correlation between circulating immunoblasts and the patients' assessment of activity of their disease or the overall functional capacity, as rated by an independent physician. The presence of iritis and of a history of peripheral joint involvement did not correlate with the finding of circulating immunoblasts either. However, the 4 patients who showed peripheral joint involvement at the time of examination all had raised numbers of immunoblasts.

The relationship between the plasma viscosity at the time and the circulating immunoblasts is shown in Fig. 2. With the exception of one case, all patients with raised immunoblasts had a plasma viscosity >1.85, a significant finding (Fisher's exact test, $P < 0.001$). The relationship between raised immunoglobulins and circulating immunoblasts is shown in Table II. Seven of the 11 cases with positive immunoblasts had immunoglobulin levels above the normal range for this laboratory, in contrast to 8 of the 28 cases with no circulating immunoblasts. The incidence of raised immunoglobulins was thus higher in the cases with abnormal immunoblasts but was not significant. Latex tests for rheumatoid factor and search for autoantibodies were negative in all cases. DNA binding was less than 30% in all cases.

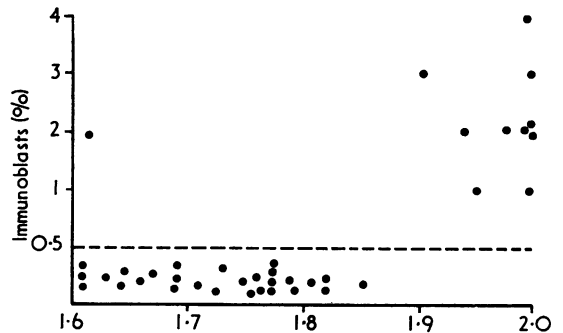


FIG. 2 Relationship between plasma viscosity and circulating immunoblasts in the patients with ankylosing spondylitis. High levels of immunoblasts show a significant correlation with a viscosity >1.85

Discussion

There is increasing evidence that there is an immunological basis for ankylosing spondylitis. Synovial follicles are found which have histological features suggestive of autoimmune disease (Julkunen, 1966). Raised immunoglobulins have been found in serum (Veys and Van Laere, 1973) and in synovial fluid (Kendall and others, 1973). Synovial fluid studies have shown the lymphocyte count to be higher in AS than in RA. The evidence from this study that there are increased numbers of circulating immunoblasts in patients with AS, supports the concept of an immunological pathogenesis for this disease. Similar reactive changes in the circulating lymphoid cells are found in the peripheral blood of patients with other autoimmune diseases, including RA and systemic lupus erythematosus (Bacon and others, 1975).

There was no correlation between any clinical feature studied and the presence of circulating immunoblasts, probably owing to the difficulty in assessing the degree of disease activity in AS. Most of the patients were not clinically ill. However, the few patients whom all observers agreed were ill had positive immunoblasts. Plasma viscosity is a good index of inflammatory activity (Lawrence, 1961), depending on similar parameters to that of the erythrocyte sedimentation rate. It is therefore not surprising that the one strong correlation was between raised immunoblasts and raised plasma viscosity.

The incidence of abnormal immunoglobulins in the study was lower than that found by Veys and Van Laere (1973). However, the limits of normal which we used were not standardized for age and sex, so that it would have detected fewer raised levels.

Nevertheless, there was some association between raised immunoglobulins and the presence of circulating immunoblasts, suggesting that both reflect current immunological reactivity. In spite of the raised immunoglobulins, there was no evidence of an increased incidence of any of the autoantibodies commonly found in other connective tissue disease. The absence of rheumatoid factor is accepted in AS, although IgG antiglobulin factors have been reported (Torrighiani and Roitt, 1967). Other autoantibody studies have not been presented in this disease. Antibodies to nuclear antigens were negative by both fluorescence and by DNA binding in this study.

The association of the histocompatibility antigen, HLA-B 27, with ankylosing spondylitis has recently been extensively documented (Schlosstein and others, 1973; Brewerton and others, 1973). It has been suggested that this gene is closely linked with genes controlling the immune response, thus accounting for the high incidence of spondylitis in patients with this antigen. If that were the case, an increased incidence of autoantibodies and abnormal immune responses would be expected in patients with AS. In this study there was a very low incidence of autoantibodies and the incidence of circulating immunoblasts was lower than that seen in a population with RA. This does not suggest a specific link with abnormal immune response genes, but fits with a lower degree of immunological reactivity in AS as compared with other chronic inflammatory arthritides.

We are grateful to the Arthritis and Rheumatism Council and to the South West Regional Hospital Board for their generous support. We thank Mrs. Elizabeth Collins, B.Sc., for help with statistics.

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