Lymphocyte abnormalities in ankylosing spondylitis

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SUMMARY Peripheral blood T (SRBC rosette) and B (AgG- and C-receptor) lymphocyte sub-populations and responsiveness to phytohaemagglutinin (PHA) were assayed in 40 patients with ankylosing spondylitis and in 55 normal subjects. There was no significant difference in the lymphocyte concentrations or responsiveness to PHA between the two groups. However, the percentages of T lymphocytes were significantly lower in the patients irrespective of their HLA typing. This was probably due to an increase in the 'null' population since the percentages of both the AgG- and C-receptor cells were normal.

Recent studies have shown that one histocompatibility antigen of the second segregant series, HLA B27, is highly associated with a whole group of rheumatic diseases, the seronegative spondyloarthropathies (Brewerton and James, 1975; Goldin and Bluestone, 1976). Ankylosing spondylitis (AS) in particular provides the strongest evidence we have of disease susceptibility being related to the possession of a specific gene (Schlosstein *et al.*, 1973). A class of immune response genes has been discovered in experimental animals which govern the expression of specific immune responses and are closely linked to histocompatibility determinants (Benacerraf and McDevitt, 1972). By analogy, the probability of the existence of histocompatibility-linked immune response genes in humans is high. We therefore studied various parameters of the cellular immune response in AS to look for immunological abnormalities.

Materials and methods

Forty patients with AS were studied. The diagnostic criteria used have been described previously (Schlosstein *et al.*, 1973). 55 healthy volunteers served as normal controls. Total lymphocyte counts were determined from the white cell count using white-cell pipettes and haemocytometers, and the differential count from Wright-stained peripheral blood smears. Mononuclear cells were separated from peripheral blood by Ficoll-Hypaque gradient

(Yu *et al.*, 1974). Monocytes were identified by latex-bead ingestion and by light microscope appearance (Clements *et al.*, 1974). B (bone-marrow derived) lymphocytes were identified by surface receptors for aggregated gammaglobulin and complement as described previously (Yu and Clements, 1976).

In the first technique lymphocytes were incubated with heat-aggregated human gammaglobulin (Pentex, Kankakee, Ill.) followed by washing and then incubation with fluorescein-conjugated polyvalent goat antihuman immunoglobulin (Meloy Lab, Springfield, Va.). The percentage of fluorescent lymphocytes was determined by epifluorescent and phase contrast microscopy (Zeiss, Ultraphot). This was an indirect technique which identified without discrimination both the Fc-receptor bearing and surface immunoglobulin bearing lymphocytes. In the second method zymosan beads were incubated with fresh human serum. Complement (C) receptor bearing B lymphocytes were identified by their ability to form rosettes with the complement-coated zymosan granules upon incubation (Yu and Clements, 1976).

T (thymus derived) lymphocytes were quantitated using a spontaneous sheep erythrocyte rosette assay previously described (Yu *et al.*, 1974). Lymphocytes were incubated overnight at 4° with sheep red blood cells in a 1:16 ratio and resuspended by a mechanical rotator. Sheep erythrocyte (SRBC) rosettes were counted in a haemocytometer. Lymphocyte function was assessed by the response to optimal phytohaemagglutinin (PHA) stimulation (Sengar and Terasaki, 1971). 10⁵ lymphocytes in 0.1 ml volume

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were incubated at 37° C for 72 hours with 25 µg PHA (PHA-M; Difco, Detroit, Mich.). Tritiated thymidine (0.8 µCi; sp. act 22 Ci/MM; Schwartz/Mann Div. Becton, Dickinson, Orangeburg, N.Y.) was then added and the culture was harvested after an additional overnight incubation. All cultures were made in triplicate and the stimulation ratios calculated by dividing the counts per minute of stimulated to that of the control cultures.

HL-A typing was performed on 30 of the 40 patients using the microdroplet lymphocyte cytotoxicity test (Mittal *et al.*, 1968).

Results

The average ($\pm$ SE) lymphocyte concentration of 55 normal subjects was 2770 ± 138 per mm^3 ($27.7 \pm 0.138 \times 10^9/\text{l}$), the values being the same for the 40 AS patients (Table 1). In 6 normal subjects peripheral white cell counts and differentials were determined daily for 5 consecutive days. There were wide variations in the daily lymphocyte concentrations between individuals and in the day-to-day determinations within the same individual. The highest and lowest lymphocyte concentrations in the 30 samples were 4290 and $1970/\text{mm}^3$ (42.9 and $19.7 \times 10^9/\text{l}$) respectively. In none of the AS patients did the lymphocyte concentration fall outside this range. This wide variation in peripheral lymphocyte concentrations agreed with those reported by others (Davidsohn and Nelson, 1974), and no further analyses of the differences in absolute concentrations of individual subpopulations were made.

The average ($\pm$ SE) T cell percentages in 36 AS patients was 61.9 ± 1.6 , being significantly lower than that of normal subjects (72.3 ± 1.0 ; $P < 0.001$; Table 1). In 6 normal subjects T lymphocyte percentages were assayed over 5 consecutive days and the variation was much less than that of the absolute lymphocyte concentrations (results not shown). The highest and lowest values in the 30 determinations were 81 and 61% respectively. In 18 of the 36 AS patients T lymphocyte percentages were less than 61% (Fig.). Both HLA B27-positive and negative

Fig. Percentage of SRBC rosette in AS patients and in normal subjects.

AS patients had significantly lower T cell percentages than normal ($P < 0.001$, $P < 0.01$ respectively; Table 1) and there was no significant difference between the two groups of AS patients (60.2 ± 2.5 vs. 63.5 ± 2.5 ; $P > 0.05$).

There were no statistically significant differences in the percentages of AgG and C-receptor cells between AS patients and normal subjects (Table 1). PHA stimulation performed on 27 AS patients (23 HLA B27 positive, 4 HLA B27 negative) and 19 normal subjects showed no significant difference in response in the three populations (Table 2).

Table 2 Response to PHA stimulation

Subject	No. tested	Mean stimulation ratio $\pm$ SE
Normals	19	74 ± 22
AS positive HLA B27	23	89 ± 14
AS negative HLA B27	4	72 ± 19

Table 1 Mean lymphocyte counts, T and B cells in patients with AS and in normal subjects

Subjects	Lymphocytes ($\text{mm}^3 \pm \text{SE}$)	SRBC rosette (% $\pm$ SE)	AgG-receptor (% $\pm$ SE)	C-receptor (% $\pm$ SE)
Normals	$2770 \pm 138(55)$	$72.3 \pm 1.0(46)$	$22.5 \pm 0.9(55)$	$14.5 \pm 0.9(19)$
AS	$2799 \pm 243(40)$	$61.9 \pm 1.6(36)$	$20.8 \pm 1.4(21)$	$13.0 \pm 0.9(32)$
B27 positive		$60.2 \pm 2.5(19)$		
B27 negative		$63.5 \pm 2.5(8)$		

Number of subjects studied in parentheses. Conversion: Traditional to SI units—Lymphocytes: $1000/\text{mm}^3 = 1.0 \times 10^9/\text{l}$.

Discussion

We have shown that the percentage of T cells in the peripheral blood is decreased both in HLA B27-positive and negative patients with AS. This abnormality is probably accounted for by an increase in the 'null' cell population since the percentage of surface immunoglobulin, Fc-receptor, and complement-receptor bearing lymphocytes were not significantly different from normal. Because of the wide variation in the total lymphocyte concentrations both in normal subjects and AS patients, valid comparisons of absolute concentrations in the lymphocyte subpopulations could not be made. The functional significance of a decreased T cell percentage in AS patients is uncertain since the response of the cells to stimulation by optimal concentrations of PHA was normal. It has been shown that the response of lymphocytes from AS patients to a suboptimal dose of PHA was significantly depressed (Sturrock *et al.*, 1975). That and the present data heighten the suspicion that immunological abnormalities may play a role in AS. Further investigations of immune function in this disease may be fruitful.

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