Studies on blood T and B lymphocytes in rheumatoid arthritis

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Lymphocytes, important in immune mechanisms, have been studied in several human clinical states where these mechanisms are thought to be involved (Pincus, Bianco, and Nussenzweig, 1972; Stjernswärd, Jondal, Vánky, Wigzell, and Sealy, 1972; Wybran and Fudenberg, 1973), particularly in rheumatic diseases (Frøland, Natvig, and Husby, 1973; Keith and Currey, 1973; Papamichail, Brown, and Holborow, 1971; Williams, DeBord, Mellbye, Messner, and Lindström, 1973). In the human, lymphocytes of T and B type have distinct markers. T-cells form rosettes after incubation with fresh, unsensitized sheep red blood cells (SRBC-rosettes; Bach, Dormont, Dardenne, and Balner, 1969; Coombs, Gurner, Wilson, Holm, and Lindgren, 1970; Jondal, Holm, and Wigzell, 1972), whereas B-cells are usually recognized either by surface immunoglobulins (Papamichail and others, 1971) or by the presence of a receptor for complement (Bianco, Patrick, and Nussenzweig, 1970).

Reports have been published, sometimes contradictory, about the ratios of T and B lymphocytes in circulating blood, and in synovial fluid (Williams and others, 1973; Frøland and others, 1973; Keith and Currey, 1973; Papamichail and others, 1971).

In the present paper, the relative number of T and B lymphocytes was studied in the peripheral blood of three groups: (1) patients suffering from rheumatoid arthritis (RA), (2) patients with other arthropathies, and (3) normal subjects.

T-lymphocytes were characterized by formation of SRBC-rosettes, and B-lymphocytes by formation of 'complement-rosettes'.

Materials and methods

In the first of the three groups of subjects studied, all 17 suffered from classical or definite RA (A.R.A. criteria); the second group included 19 patients with various, non-inflammatory arthropathies; and in the third group were 17 normal subjects (medical and paramedical personnel).

Circulating lymphocytes were isolated by the method of Boyüm (Boyüm, 1968). Repeated controls have shown that 90 to 95% of the cells prepared by this technique had the morphology of lymphocytes.

For the SRBC-rosette test, lymphocytes (Ly) were incubated with washed sheep red cells in a ratio of 10^6 Ly: 10^7 SRBC in a volume of 0.5 ml (Stjernswärd and others, 1972). After incubation for 15 mins at 37°C, the mixture was centrifuged at 200 *g* for 5 mins and the tubes kept cold overnight. The cells were resuspended by gentle agitation (by hand) for 5 mins, and the ratio of rosettes to the total number of lymphocytes was determined.

The complement-rosette test was done according to the method of Stjernswärd and others (1972). Briefly, this consisted of sensitizing washed SRBC with a rabbit antibody to SRBC at a dilution of 1:2000. After washing the sensitized SRBC, human complement was added as whole fresh serum diluted 1:20. After 30 mins at 37°C, three more washings were done in Hanks's balanced salt solution (HBSS) before the cells were mixed (10^6 Ly + 10^7 SRBC) and centrifuged at 200 *g* for 2 mins and then left at room temperature for 30 mins. Most of the supernatant was aspirated and the cells resuspended on a rotator. The rosettes were then counted under low magnification, and related to the lymphocyte count.

To test for the specificity of the complement-rosette (Pincus and others, 1972). SRBC were incubated, after treatment with antibody and with complement, with a Fab fragment of a rabbit IgG antihuman C3 (β_1c/β_1A). Inhibition of the rosettes by this antibody fragment was considered as indicative of the specificity of the complement-rosette, because in our hands incubation with normal rabbit Fab fragment produced no inhibition. Furthermore, incubation of lymphocytes with SRBC sensitized only with amboceptor, without complement, repeatedly failed to give more than 3 to 5 rosettes/100 lymphocytes.

Results

The Table shows the results obtained in the two types of rosette tests for the three groups investigated. No statistically significant differences were found among groups for either SRBC-rosettes or complement-rosettes (T- and B-lymphocytes, respectively).

Discussion

The results show that, with the techniques used for marking B-cells and T-cells, respectively, no statistically significant difference was found among groups of RA patients, normals, or patients suffering from

Table *Results of rosette tests in 3 groups*

Group	No. individuals	Spontaneous-rosettes (T-cells)		Complement-rosettes (B-cells)	
		No. tests	$\bar{X} \pm s$ (%)	No. tests	$\bar{X} \pm s$ (%)
Normals	17	18	57.6 $\pm$ 12.0	19	26.7 $\pm$ 11.2
RA	17	31	63.1 $\pm$ 11.5	33	29.8 $\pm$ 11.1
Other diseases	19	30	64.0 $\pm$ 11.5	30	25.1 $\pm$ 12.6

noninflammatory arthropathies. This conforms with the results of Keith and Currey (1973), but contradicts those of Mellbye, Messner, DeBord, and Williams (1972) and those of Papamichail and others (1971). Mellbye and others found fewer B-cells (C3-receptor lymphocytes) in RA patients, as compared to normals, whereas Papamichail and others found an increased number of B-cells (surface-Ig), detected by immunofluorescence, among circulating lymphocytes of patients with active RA. Frøland and others (1973), using a different technique, found an increased number of T-cells (SRBC-rosettes) in peripheral blood of RA patients as compared to normals. These authors, however, found 14.8% of T-cells in normal human subjects as compared to our figure of 57.6%. These discrepancies may be explained either by differences of technique or by a difference in the

selection of subjects. More studies will be needed to clarify the question, as well as to develop better standardization of the techniques for marking T- and B-cells.

Summary

Lymphocytes were isolated from the blood of subjects forming three groups: 17 patients with rheumatoid arthritis, 19 patients with other arthropathies, and 17 normal subjects. These lymphocytes were characterized as T-cells (sheep red blood cell rosettes) or B-cells (complement-receptor) and the percentage of T- and B-lymphocytes were counted. No statistically significant difference was found among the three groups as to the percentage of T- and B-cells.

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