Lymphocyte subpopulations in rheumatoid synovial tissue

A. WANGEL AND M. KLOCKARS

From the Fourth Department of Medicine, University of Helsinki, and Rheumatism Foundation Hospital, Heinola, Finland

SUMMARY Synovial tissue obtained at synovectomy of the knee joint in 21 patients with rheumatoid arthritis contained a significantly lower proportion of T lymphocytes as measured by spontaneous rosette formation with nonsensitized sheep red blood cells than did synovial fluid or blood from the same patients. There was no concomitant increase in synovial tissue lymphocytes with B-cell markers such as surface immunoglobulin or Fc fragment receptors. Removal of lymphocyte receptors with trypsin followed by culture to allow new receptors to form, led to an increase in rosette forming cells, suggesting that part of the synovial cells without B- or T-cell markers may be T lymphocytes with blocked receptors.

Interest in immunological mechanisms in the pathogenesis of rheumatoid arthritis has largely concerned serological markers such as rheumatoid factor (Franklin *et al.*, 1957) and immune complexes (Rodman *et al.*, 1967; Bauman and Broder, 1968; Kinsella *et al.*, 1969). More recent evidence has suggested cell mediated immunity to synovial antigens (Bacon *et al.*, 1973) and there have been studies of lymphocyte subpopulations in the blood (Papamichail *et al.*, 1971; Mellbye *et al.*, 1972; Williams *et al.*, 1973; Keith and Currey, 1973; Yu *et al.*, 1974; Clements *et al.*, 1974; Micheli and Bron, 1974; Vernon-Roberts *et al.*, 1974) and synovial fluid of rheumatoid patients (Mellbye *et al.*, 1972; Froland *et al.*, 1973; Winchester *et al.*, 1973; Vernon-Roberts *et al.*, 1974). However, there are few published reports on the proportion of T and B lymphocytes in rheumatoid synovium (Abrahamsen *et al.*, 1975; van Boxel and Paget, 1975; Loewi *et al.*, 1975). We describe the results of such a study which differs from the previous ones in that T and B markers were sought immediately the lymphocytes had been harvested from the synovial tissue by mechanical mincing and gradient centrifugation. In 3 patients the proportion of synovial T-cells was again determined after the lymphocytes had been subjected to trypsinization and 30-hour culture.

Patients

The 21 patients with rheumatoid arthritis whose blood and synovial tissue lymphocytes were studied were under treatment at the Rheumatism Foundation Hospital, Heinola, Finland. 4 were receiving prednisolone at the time but in none did the daily dose exceed 10 mg. Clinical details are given in Table 1. Blood lymphocytes only were studied in a further 6 patients, none of whom were receiving corticosteroid drugs. The control group consisted of healthy laboratory staff and patients attending the casualty clinic with minor complaints (mean age $\pm$ SD, 37.2 ± 11.8 years).

Table 1 Clinical details of 21 patients with rheumatoid arthritis

Sex		
Male	6	
Female	15	
Age (years)		
Mean	43.5	
Range	13-59	
Mean duration of RA	15 years	
Rheumatoid factor		
Present	16	
Absent	5	
Gold treatment within preceding 6 months		5
Intra-articular steroids		2
Intra-articular osmium		0

Materials and methods

Synovial tissue, synovial fluid, and blood were obtained at synovectomy of the knee joint. Tissue

specimens were minced with scissors and incubated with periodic shaking for 2 hours at room temperature in Dulbecco phosphate buffered saline (PBS), containing 500 IU heparin and one drop of a latex suspension (Bacto-Latex 0.81 u, Difco) in each 10 ml. Blood and synovial fluid samples were collected into heparin tubes and incubated as above before dilution with two volumes of PBS before lymphocyte separation.

LYMPHOCYTE SEPARATION

Ficoll-Hypaque gradient centrifugation according to the method of Boyum (1968) was used for lymphocyte separation.

ROSETTE FORMING CELLS

Lymphocytes were tested for their ability to form rosettes with sheep red blood cells (SRBC) as described by Jondal *et al.* (1972), except that the initial incubation of lymphocytes with SRBC was done at +4°C, that fetal calf serum was used in a final concentration of 40% to stabilize the rosettes, and that one drop of 0.5% toluidine blue in PBS was added to the tubes immediately before resuspension and counting.

SURFACE IG BEARING CELLS

These cells were detected by staining with FITC conjugated antihuman gammaglobulin (Behringwerke, Germany). Incubation for 30 minutes at +4°C was followed by three washes in PBS. The percentage of labelled cells was counted in a Leitz Dialux Incident Light UV microscope. Cells containing two or more latex beads were regarded as phagocytic and excluded from the calculations.

FC RECEPTOR BEARING CELLS

These cells were determined by the method of Dickler and Kunkel (1972). Human gammaglobulin (16.5% human normal immunoglobulin, Kabi, Stockholm) was diluted to a concentration of 50 mg/ml, incubated at 56°C for 30 minutes, and heat aggregated at 66°C for 15 minutes. It was then centrifuged to obtain a pellet which was homogenized in PBS at pH 8.0. After centrifugation at 3000 g for 30 minutes the protein concentration of the supernatant was adjusted to 2 mg/ml. One drop of this solution and one drop of PBS containing 2% bovine serum albumin (BSA, Armour) and 0.02% Na azide were added to one drop of the lymphocytes to be tested. After incubation for 30 minutes at +4°C the cells were washed three times in the BSA-azide buffer and then incubated for 30 minutes at 4°C with one drop of FITC conjugated antihuman IgG (Behringwerke, Germany). After three further washes in BSA-azide buffer the percentage of fluorescein-labelled cells was determined as above.

WHITE CELL COUNTS

Total leucocyte and differential white cell counts were made from blood taken at operation into EDTA.

Results

Patients with rheumatoid arthritis had a leucocytosis compared with control subjects, but absolute lymphocyte counts were not significantly different in the two groups (Table 2). The percentages and absolute numbers in the peripheral blood of rosette forming cells, surface Ig bearing cells, and Fc receptor bearing cells were also similar in the two groups (Table 3).

Rheumatoid synovial tissue contained a significantly lower proportion of rosette forming cells than either synovial fluid or blood (Table 4). There was no significant difference between serologically positive and serologically negative patients, nor between patients who had received gold therapy within the past 6 months and those who had not (Table 5).

The low proportion of synovial tissue rosette forming cells was not accompanied by an increase in the percentage of cells bearing B-cell markers and thus more than half of the lymphocytes harvested from rheumatoid synovial tissue appeared to carry neither T nor B lymphocyte markers. Further experiments were carried out to try to determine whether some of these cells might be T lymphocytes which were incapable of forming rosettes because the receptors for SRBC were blocked.

Table 2 Total blood leucocytes and lymphocytes (mean $\pm$ SD)

	n	Leucocytes/mm ³ ($\times 10^9/l$)	Lymphocytes/mm ³ ($\times 10^9/l$)
Control group	24	6216 $\pm$ 2035 (6.2 $\pm$ 2.0)	2020 $\pm$ 881 (2.0 $\pm$ 0.9)
RA	27	7692 $\pm$ 2055 (7.7 $\pm$ 2.0)	1596 $\pm$ 679 (1.6 $\pm$ 0.7)

Statistical comparison by Students 't' test.

Table 3 Percentages and absolute numbers of rosette forming (RFC), surface Ig bearing (SIg), and Fc receptor bearing (Fc) lymphocytes in peripheral blood from patients with RA and control subjects (mean $\pm$ SD)

	n	RFC	SIg cells	Fc cells
Control group	24	66 $\pm$ 8.0% 1309 $\pm$ 538/mm ³ NS	19 $\pm$ 5% 380 $\pm$ 180/mm ³ NS	21 $\pm$ 7% 401 $\pm$ 182/mm ³ NS
RA	27	64 $\pm$ 12% 1039 $\pm$ 486/mm ³	26 $\pm$ 13% 428 $\pm$ 288/mm ³	25 $\pm$ 10% 424 $\pm$ 243/mm ³

Table 4 Percentages of lymphocytes with T- and B-cell markers in blood, synovial fluid, and synovial tissue from patients with RA (mean $\pm$ SD)

	RFC (%)		Slg cells (%)	Fc cells (%)
RA blood	63.9 $\pm$ 11.8 (27)		26.5 $\pm$ 12.7 (27)	24.6 $\pm$ 10.5 (27)
RA synovial fluid	57.4 $\pm$ 12.9 (7)	P < 0.001	19.1 $\pm$ 14.8 (7)	20.4 $\pm$ 10.5 (7)
RA synovial membrane	23.1 $\pm$ 15.3 (21)		NS 16.0 $\pm$ 6.7 (14)	NS 20.9 $\pm$ 7.3 (14)

Number of patients studied in parentheses.

Table 5 Comparison of synovial tissue RFC in RA patients with regard to serological status and recent gold treatment (mean $\pm$ SD)

	n	Synovial tissue RFC (%)	Student's 't' test
Seropositive	16	20.7 $\pm$ 15.0	t = 1.53, NS
Seronegative	5	32.2 $\pm$ 13.5	
Recent gold treatment	5	31.8 $\pm$ 20.4	t = 1.58, NS
No gold last 6 months	16	19.8 $\pm$ 12.6	

SRBC ROSETTE FORMATION BY TRYPSIN TREATED LYMPHOCYTES

Lymphocytes were isolated from peripheral blood and synovial tissue of patients with rheumatoid arthritis as described above and SRBC rosettes were prepared from an aliquot (native cells). The remainder of the lymphocytes were incubated for 30 minutes at room temperature with a 0.15% solution of trypsin (Worthington Biochemicals, 2 x crystallized) in Ca- and Mg-free PBS. The cells were washed three times in Dulbecco's PBS and an aliquot was tested for SRBC rosette formation (trypsin treated cells). The remainder of the cells were incubated in BHK-21 medium supplemented with 10% fetal calf serum, glutamine, penicillin, and streptomycin for 30 hours at 37°C and, after the culture medium had been washed away, tested for rosette forming activity (trypsin treated and cultured cells).

In the 3 patients studied trypsin treatment virtually eliminated the rosette forming ability of synovial

tissue lymphocytes (Table 6). However, trypsinized cells which had been cultured for 30 hours had regained this ability so that the proportion capable of rosette formation was now higher than that of native cells and of a similar order to that seen in peripheral blood and synovial fluid. In the 2 patients studied the proportion of rosette forming cells in peripheral blood lymphocytes was not increased above initial values by trypsin treatment and culture.

Discussion

The main finding of this study was the unexpectedly low proportion of rosette forming cells in rheumatoid synovial tissue. The relative proportion of lymphocytes bearing T- or B-cell markers in normal synovial tissue is not known; hence it cannot be stated with certainty that the low proportion of T-cells in rheumatoid synovium is abnormal. It is, however, significantly lower than the proportion in rheumatoid synovial fluid.

The most obvious explanation for this finding is that T-cell damage or depletion could occur during harvesting of lymphocytes from synovial tissue, particularly during mincing. However, lymphocytes derived from other synovial tissue specimens treated in an identical manner were more than 95% viable by Trypan blue exclusion.

Another possibility is that T-cells might be 'fettered' in the synovial tissues through attachment to cell-bound antigens and therefore not readily released by mincing. If this were the case B-cells might have been expected to be proportionally increased. However, this was not so, instead the low proportion of T-cells was accompanied by an increase in cells with neither T nor B markers.

Thirdly, it is possible that some of the synovial tissue cells without T or B markers are T-cells which are unable to form rosettes for immunological or other reasons. Incubation of normal peripheral blood lymphocytes with rheumatoid synovial fluid blocks the C3 receptor (a B-cell marker) while incubation with normal synovial fluid has a similar though less marked effect (Mellbye *et al.*, 1972). It is

Table 6 Effect of trypsin treatment and culture on rosette forming ability of synovial tissue and peripheral blood lymphocytes from RA patients

Source of lymphocytes	Native cells	%RFC trypsin treated cells	Trypsin treated and cultured cells
Synovial tissue			
Case 1	36	0	72
2	64	6	86
3	48	9	90
Peripheral blood			
4	55	5	52
5	79	0	44

conceivable that some of the cells harvested from rheumatoid synovial tissue were T-cells whose SRBC receptors were made unavailable through attachment of a synovial tissue antigen or antigens to adjacent receptors or in some other but less specific way. T-cell specific antilymphocyte antibodies (Terasaki *et al.*, 1970; Mittal *et al.*, 1970; Lies *et al.*, 1973) should be detected by staining for SIg and are thus unlikely to be the reason.

SRBC receptors can be removed from the cell surface by treatment with trypsin but regenerate when the cells are washed and cultured (Jondal *et al.*, 1972). In our study the synovial tissue lymphocytes of only 3 patients were treated in this manner but the increase in T lymphocytes suggested that most of the cells which initially had neither T nor B characteristics were in fact T lymphocytes. This accords with the recent report of Loewi *et al.* (1975) who found that, after trypsin treatment and 4-day culture, up to 94% of rheumatoid synovial membrane lymphocytes formed SRBC rosettes. Two other studies (Abrahamsen *et al.*, 1975; van Boxel and Paget, 1975) also reported that the majority of lymphocytes studied after enzyme treatment and culture formed SRBC rosettes. There is thus good agreement between these studies and ours that most of the lymphocytes were harvested from the rheumatoid synovial membrane by mechanical means with or without enzyme digestion form SRBC rosettes when tested some hours later. In addition, our study shows that most of these cells are unable to manifest this T-cell marker at the time of their release from the synovial tissue. If this phenomenon were due to blocking by synovial antigen(s), later to be released into the culture medium, attempts at isolation of such antigen(s) might be of interest.

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