Subpopulations of T lymphocytes in patients with ankylosing spondylitis

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SUMMARY Subpopulations of human blood T lymphocytes were determined in 20 patients with ankylosing spondylitis and in 20 healthy individuals. The proportion of T lymphocytes with receptors for the Fc portion of IgG (T_G cells) was increased in 9 patients. The proportion of T lymphocytes bearing receptors for the Fc portion of IgM (T_M cells) was within the range found in healthy controls in all but 4 patients, who had a decreased level of T_M cells.

Subpopulations of human T lymphocytes have been identified on the basis of their ability to bind the Fc portion of either IgG (T_G cells) or IgM (T_M cells).^{1–5} T_G cells have been shown to have suppressor effect and T_M cells helper effect on pokeweed mitogen (PWM) induced responses and antigen-specific B cell responses.^{6–8} T_G cells and T_M cells also differ in their dose response to phytohemagglutinin in the stimulation test³ and show different morphological characteristics.⁹ Recently the demonstration of a small subset of T lymphocytes bearing receptors for the Fc portion of IgA has been reported.¹⁰

When studying T lymphocytes subpopulations in synovial fluid and blood of patients with various rheumatic diseases¹¹ we encountered a case of ankylosing spondylitis with a very high level of T_G cells and a low level of T_M cells in the blood. In the present study we have examined T lymphocyte subpopulations in the blood of patients with ankylosing spondylitis to determine if abundance of T_G cells is a common feature in this disease.

Materials and methods

Twenty patients with ankylosing spondylitis treated at the department of rheumatology at the Karolinska Hospital or St Erik's Hospital, Stockholm, were studied. Healthy subjects were examined as controls. Case reports of all patients were studied.

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CELL SEPARATION

Lymphocytes were purified from heparinised blood by centrifugation in a Ficoll-Isopaque (Ficoll-Paque, Uppsala, Sweden) gradient.¹² Phagocytic cells were removed with a magnet after treatment of the cell suspension with carbonyl iron (100 mg for 20 min at 37°C).

T lymphocytes were determined and separated by rosetting with neuraminidase-treated (Behringwerke, Germany) sheep red blood cells (SRBC=E)¹³ followed by gradient centrifugation as described in detail previously.¹¹ The T cell preparations contained 95–100% E rosette forming cells with the exception of 3 preparations which contained 93–94% E rosetting cells.

ANTIBODY-OXRBC

Anti-ox red blood cell antibodies were raised in rabbits by repeated injections intraperitoneally of 1–5 ml of 25% OxRBC. IgM and IgG fractions were prepared from rabbit antisera by Sephadex-G200 gel filtration. The haemagglutination (HA) titre of concentrated IgM fractions was 1/64 and of concentrated IgG fractions 1/128–1/512. Treatment with 2-mercaptoethanol completely abolished the haemagglutinating capacity of the IgM fractions but did not change the HA titre of the IgG-fractions.

PREPARATION OF OXRBC-ANTIBODY COMPLEXES

Equal volumes of washed OxRBC at a concentration of 2% and purified IgM antibody (1/4 HA unit) or IgG antibody (1/2–1/4 HA units) were mixed and

incubated at 37° for 30 min. The OxEAG and OxEAM complexes were then washed and resuspended to a concentration of 0.5%.

DETERMINATION OF T_G AND T_M CELLS

T_G and T_M cells were identified by rosette formation as described by Moretta and coworkers.³ 0.1 ml of a cell suspension containing 3×10^6 cells/ml were mixed with 0.1 ml of OxEAG or OxEAM and centrifuged at 200 g for 5 min followed by incubation at +4°C for 1 h. The pellet was gently resuspended, and 400 lymphocytes from each tube were counted for rosette formation. A rosette was defined as a lymphocyte with 3 or more red cells attached. All tests were performed in duplicate. T cells examined for OxEAM rosette formation had been incubated overnight at 37°C in RPMI-1640 medium supplemented with 20% FCS.³ Tests for T_G cells were done with freshly prepared T cells as well as T cells incubated overnight at 37°C.

ASSAY FOR DNA SYNTHESIS

Lymphocytes purified by Ficoll-Isopaque centrifugation and carbonyl iron treatment were cultured in RPMI 1640 medium (Biocult Laboratories, Paisley, Scotland) supplemented with 10% AB-serum pool, L-glutamin, penicillin, and streptomycin in microplates (3040 Microtest II, Falcon, USA). Each well contained 2×10^5 lymphocytes in 0.2 ml medium. Duplicate or triplicate cultures were set up with phytohaemagglutinin (PHA) at a final concentration of 2.5 µg/ml or with no additive. After 2 days of incubation at 37°C in humidified air with 5% CO₂ 0.05 µCi of ¹⁴C-thymidine (Radiochemical Centre, Amersham, England) was added to each well. After further incubation for 18 hours the cells were harvested with a Titertek cell harvester (Flow Laboratories, Scotland). Radioactivity was measured in a liquid scintillation counter and expressed in counts per minute (cpm) per culture.

Results

The percentages of total T lymphocytes, T_G cells, and T_M cells in the blood of 20 patients with ankylosing spondylitis and of 20 healthy controls are shown in Tables 1 and 2. The proportion of T_G cells among T lymphocytes cultured overnight was in the range 7–22% in all but 1 healthy individual, a 55-year-old woman, who had 26% T_G cells. Eight of the patients had an increase of T_G cells above 26%. The T_G cell level was considered to be slightly increased also in a 29-year-old patient (no. 3), who had 26% T_G cells, whereas the same percentage was not considered to represent an increase above normal

Table 1 Percentages of total T cells, T_G cells and T_M cells in the blood of 20 patients with ankylosing spondylitis

Patient	Age/sex	Total T cells	T _G cells		T _M cells
			Not cultured	Cultured overnight	
1	28/M	73	ND	6	73
2	28/M	84	14	15	54
3	29/F	ND*	ND	26	69
4	30/F	78	ND	13	63
5	31/M	75	37	ND	35
6	32/M	82	ND	9	53
7	33/M	74	28	27	60
8	34/M	76	20	18	ND
9	37/M	ND	20	18	69
10	38/M	74	19	18	52
11	39/M	63	27	30	63
12	40/M	70	19	13	69
13	44/F	79	28	34	63
14	49/M	ND	ND	30	63
15	49/F	75	ND	32	43
16	51/M	73	32	30	46
17	55/M	82	ND	26	57
18	57/M	ND	ND	10	34
19	59/M	72	78	81	15
20	72/M	75	28	23	35
Mean		73		25**	53
SD		5		16	15

*ND=not done. **In patient 5 the proportion of T_G cells was not determined with cultured T cells. In this case the T_G cell percentage obtained with fresh T cells (37%) has been included in the calculation of the mean of the proportion of T_G cells.

Table 2 Percentages of total T cells, T_G cells, and T_M cells in the blood of 20 healthy subjects

Subject	Age/sex	Total T cells	T _G cells		T _M cells
			Not cultured	Cultured overnight	
1	21/F	83	17	13	73
2	28/M	85	14	13	59
3	31/F	71	ND	21	61
4	32/F	87	15	10	50
5	34/F	78	14	13	62
6	35/M	69	8	8	56
7	35/M	81	13	12	73
8	35/M	76	17	17	ND
9	36/F	88	6	7	52
10	36/M	76	ND	20	43
11	37/F	85	ND	20	57
12	38/M	69	13	17	52
13	40/M	91	23	13	45
14	43/F	87	12	13	79
15	45/M	60	12	14	67
16	53/M	84	ND	19	60
17	53/M	73	16	17	52
18	54/M	60	20	22	50
19	55/F	73	29	26	68
20	58/M	79	19	20	45
Mean		78		16	58
SD		9	—	5	10

in a 55-year-old patient (no. 17). (The percentage of T_G cells has been shown to be increased in aging subjects when compared with young subjects.¹⁴) In 8 of the patients the increase of T_G cells was slight or moderate (26–37% T_G cells), whereas 1

patient, reported previously,¹¹ had an extremely high level of T_G cells (81%). The level of significance of the difference between the mean of the proportion of T_G cells in patients and controls was $P < 0.05$.

There were usually only small differences in the proportion of T_G cells between freshly prepared T cells and T cells incubated overnight (Tables 1 and 2). However, in 1 healthy subject (no. 13, Table 2) incubation of T cells overnight resulted in a considerably lower percentage of T_G cells. The proportion of T_M cells ranged from 43 to 79% in the healthy subjects (Table 2). In all but 4 patients the proportion of T_M cells was within the normal range (Table 1). Three of these patients had a slightly decreased level of T_M cells and the third patient a very low level of these cells.

There was no significant difference in the proportion of total T cells between patients and controls.

The group of patients with an increased level of T_G cells did not differ significantly from the group of patients with a normal level of T_G cells with regard to age, duration of the disease, clinical activity of the disease, occurrence of extra-articular symptoms (prostatitis, colitis, iritis), or medical treatment.

The lymphocyte stimulation response to an optimal dose of PHA was determined in 18 patients and in 11 healthy subjects. There was no significant difference in PHA responsiveness between patients (mean cpm $\pm$ SD = $17\,787 \pm 9834$) and controls ($13\,392 \pm 9447$).

Discussion

This study showed that almost 50% of patients with ankylosing spondylitis had a slight or moderate increase in the proportion of T_G cells. Only 1 patient had a very high proportion of T_G cells. Imbalances in T lymphocyte subpopulations have previously been demonstrated in some other diseases. An increase in the proportion of T_G cells has been found in certain immunodeficiency diseases,^{6,7,15} multiple sclerosis,¹⁶ sarcoidosis,¹⁷ and myasthenia gravis.¹⁸ A transient rise of T_G cells may occur in certain acute viral infections, for instance, influenza, acute hepatitis (Moretta, personal communication), and vaccine-induced rubella (Arneborn, Biberfeld, and Wasserman, in preparation). The cause of the T_G cell increase in various disorders is not clear. One possibility is that a strong antigen stimulation may trigger a selective increase of T_G cells.

A reduced level of T_G cells¹⁹⁻²¹ and impairment of the suppressor cell activity¹⁹⁻²⁵ have been demonstrated in patients with SLE. We have no data on the suppressor cell function in patients with ankylosing spondylitis. However, the finding of

raised immunoglobulins in patients with ankylosing spondylitis^{26,27} seems to speak against an enhanced suppressor effect on B cells in these patients.

A decrease in the percentage of T cells in patients with ankylosing spondylitis has been reported,²⁸ whereas in the present study no significant difference in T cell percentages between patients and healthy subjects was found. This discrepancy may be due to technical differences in the performance of the E rosette test.

The lymphocyte stimulation response to an optimal dose of PHA did not differ significantly between patients and healthy subjects in the present study and in a study by Fan *et al.*²⁸ However, it has been reported that patients with ankylosing spondylitis have a depressed lymphocyte stimulation response to a suboptimal dose of PHA.²⁹ Alteration of T lymphocyte subpopulations may influence the stimulation response to PHA.³

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