Immunoregulatory T cells in the peripheral blood of patients with Bechterew's syndrome

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SUMMARY Seventeen patients with ankylosing spondylitis (Bechterew's syndrome) were investigated. Only 3 of them had detectable autoantibodies, but the IgA and IgM concentrations in serum were increased ($p < 0.05$). The patients had a moderate reduction in con-A-induced suppressor cell activity of peripheral blood lymphocytes as detected in a con-A/MLC assay, compared with that of 15 controls ($41.0 \pm 8.6\%$ suppression compared with $59.4 \pm 5.2\%$, mean $\pm$ SEM; $0.05 < p < 0.1$ one-sided test). No differences were found in the percentages of $T\gamma$ cells (suppressor cells) and $T\mu$ cells (helper cells) between patients and controls. This is to our knowledge the first report of con-A-induced suppressor cell activity and T lymphocyte subpopulations in the peripheral blood of patients with ankylosing spondylitis.

Hypergammaglobulinaemia and the presence of autoantibodies are common immunological features of inflammatory rheumatic diseases. Patients with ankylosing spondylitis (AS, Bechterew's syndrome) also frequently have increased immunoglobulin levels,¹⁻⁴ but they usually lack detectable autoantibodies, although increased frequency of IgG rheumatoid factor (IgG RF) and granulocyte-reactive antinuclear antibodies have been detected.^{2 5 6} In contrast to the humoral immunity, cellular immune responses in AS patients are usually normal,^{3 7-10} even if reduced cellular responses to some antigens and to allogeneic cells have been observed.^{3 9 11}

Disorders characterised by hypergammaglobulinaemia and the presence of autoantibodies, as well as hypogammaglobulinaemic conditions, may have a complex aetiology, but they can in part be explained by dysfunction in the suppressor and helper cell systems (the $T\gamma$ and $T\mu$ cell compartments). Imbalances in these cells might therefore result in hyper- or hyporesponsiveness of the immunocompetent lymphocytes (B and T lymphocytes).

The present study was carried out to discover whether the immunological derangements in AS

patients (hypergammaglobulinaemia) were reflected in suppressor/helper cell dysfunction.

Materials and methods

Patients. Seventeen randomly chosen patients with AS diagnosed at Oslo Sanitetsforenings Rheumatism Hospital were examined. The 6 women and 11 men, all Norwegians, had an age distribution ranging from 20 to 48 years (mean 33 years). They were examined clinically as described elsewhere.¹² The clinical data, biochemical values, and radiographic findings are listed in Table 1. Fifteen blood donors served as controls in the cellular tests.

Isolation of mononuclear cells. Peripheral blood mononuclear cells were isolated from heparinised blood by Isopaque-Ficoll gradient centrifugation.¹³ T lymphocytes were enumerated and isolated by means of sheep red blood cells (SRBC) treated with 2-aminoethylisothiuronium bromide (AET, Sigma Chemical Co., St Louis, Mo).¹⁴

Detection of T lymphocyte subpopulations ($T\gamma$ and $T\mu$ cells). For the detection of T lymphocytes with receptors for the Fc part of IgG antibodies ($T\gamma$ cells), human O, Rh+ erythrocytes sensitised with anti-Rh Ripley antibodies were used.^{15 16} T lymphocytes with receptors for the Fc part of IgM antibodies ($T\mu$ cells) were detected by their ability to form

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Table 1 Clinical, biochemical, and radiographic data of 17 patients with ankylosing spondylitis¹

Patients							Biochemical values ⁵							Radiographic findings			
No.	Age	Sex	Dur. (yr.)	Psor. ²	AAU ³	Treat. ⁴	Hb	ESR	WBC	CRP	C3	C4	Bf	CH ₅₀	SI ⁶	Col. ⁷	Large Phal. ⁸
1	26	M	2-5	-	+	3	11.7	50	7.300	0.028	0.9	0.46	188	150	3	+	-
2	48	M	>10	?	+	3	12.4	83	8.400	0.032	0.9	0.34	193	150	5	+	+
3	47	M	>10	-	+	2	14.5	37	7.700	0.012	1.3	0.65	140	150	3	+	+
4	44	M	>10	-	+	3	14.6	33	11.800	0.011	0.9	0.29	152	120	5	+	+
5	39	M	>10	-	+	0	12.4	60	9.300	0.058	0.9	0.59	188	100	5	+	+
6	35	F	>10	-	-	2	13.2	35	9.200	<0.01	1.0	0.59	139	130	3	+	-
7	42	M	>10	+	-	4,5	15.0	13	8.700	0.016	0.8	0.36	98	100	4	+	+
8	34	M	>10	-	-	3	15.8	22	8.600	<0.01	1.0	0.48	152	160	3	+	-
9	35	F	2-5	-	-	2	15.2	14	8.900	>0.01	0.7	0.36	108	160	2B	-	-
10	24	M	5-10	-	+	2	16.2	3	9.600	<0.01	0.9	0.36	109	70	4	+	-
11	26	F	5-10	+	-	2	12.4	25	9.000	<0.01	0.8	0.46	131	165	5	+	+
12	23	M	2-5	-	-	3,4	14.0	82	15.000	0.030	1.3	0.37	210	110	2B	-	-
13	27	M	>10	-	+	2	13.8	45	8.500	0.027	0.9	0.47	165	120	5	+	+
14	20	M	5-10	-	-	3	10.8	76	9.000	0.120	1.3	0.60	177	120	4	-	+
15	42	F	>10	-	+	1	12.2	15	7.200	<0.01	0.6	0.29	91	60	2B	+	-
16	25	F	5-10	-	+	4	11.4	15	8.100	<0.01	0.6	0.21	58	120	4	-	+
17	32	F	5-10	-	-	3	13.4	10	Nd ⁹	<0.01	Nd	0.36	Nd	Nd	4	+	+

¹All patients were HLA B27 positive.

²Patients with dermatological findings typical of psoriasis.

³Patients giving a history of acute anterior uveitis (AAU).

⁴Treatment with anti-inflammatory drugs: 0 = no treatment; 1 = salicylates; 2 = naproxene; 3 = indomethacin; 4 = prednisone.

⁵Normal values and p values for the differences between patients and controls: white blood cell counts (WBC) $4-11 \times 10^9$ cells/l; C-reactive protein (CRP) = <0.01 g/l; $p < 0.001$; C3 = 0.73 ± 0.01 g/l, $p < 0.001$; C4 = 0.295 ± 0.02 g/l, $p < 0.001$; properdin factor B (Bf): $110 \pm 8\%$, $p < 0.01$; CH₅₀ = $111 \pm 4.0\%$, $p > 0.05$.

⁶Radiographic examination of sacroiliac (SI) joints, graded from 0 (normal joint) to 5 (bony ankylosis), according to Dahle³⁹; Grade 2B to 5 represents definite bilateral sacroiliitis.

⁷Radiographic inflammatory changes of the spine.

⁸The diagnosis of arthritis of large and phalangeal (Phal.) joints was also confirmed by clinical examination.

⁹Not determined.

rosettes with ox red blood cells (ORBC) sensitised with rabbit anti-ORBC IgM antibodies, according to methods described elsewhere.¹⁵⁻¹⁷ One drop of 0.01% acridine orange was added to the cell suspensions before rosettes were counted at $250 \times$ magnification under UV and ordinary light simultaneously. For all rosettes, cells binding 3 or more erythrocytes were considered positive. At least 200 lymphocytes were scored in each cell suspension.

Suppressor cell assay. Peripheral blood mononuclear cells (5×10^6 cells/ml) were incubated without or with concanavalin-A (con-A 20 μ g/ml) for 48 hr at 37°C in an atmosphere of 5% CO₂/95% air and 100% humidity.¹⁸⁻¹⁹ After incubation both unstimulated and con-A-activated cells from the patients and the controls were irradiated (3000 R), washed twice with 30 mM α -methyl D-mannoside in Hanks's balanced salt solution (HBSS) to remove con-A, then twice in RPMI (Roswell Park Memorial Institute) 1640+10% fetal calf serum (FCS) and antibiotics (complete medium), and resuspended in complete medium to a concentration of 1×10^6 viable cells/ml (stimulator cells). Freshly isolated mononuclear cells (responder cells) from a normal donor were isolated as described above, and adjusted to a concentration of 1×10^6 cells/ml in complete medium.

One of us served as source of responder cells in all the experiments in order to ensure a reproducible con-A-reactive responder cell population. In the 1-way mixed lymphocyte culture (MLC) experiments 0.1 ml of stimulator cells were incubated with 0.1 ml responder cells and con-A (6 μ g/ml) in an atmosphere of 5% CO₂/95% air and 100% humidity for 144 hr in microtitre plates (Costar 3596, Cambridge, Mass). 18 hr prior to harvesting on a Skatron semiautomatic harvesting machine (Skatron, Lierbyen, Norway) the cells were labelled with 0.5 mCi of ³H-thymidine (Amersham Radiochemicals, England). The incorporation of ³H-thymidine was measured by a liquid scintillation counter and the results expressed as mean cpm of triplicates $\pm$ standard error of the mean. The percentage suppression was calculated according to the formula:

$$\text{Percent. suppression} = \left[1 - \frac{\text{cpm}_{\text{s.con-A}} - \text{cpm}_{\text{s.medium}}}{\text{cpm}_{\text{c.con-A}} - \text{cpm}_{\text{c.medium}}} \right] \times 100$$

where cpm_{s.con-A} is mean cpm in cultures containing suppressor cells and con-A; cpm_{s.medium} is mean cpm in cultures containing suppressor cells without con-A; cpm_{c.con-A} is mean cpm in control cultures with con-A; and cpm_{c.medium} is mean cpm in cultures containing control cultures but no con-A.

HLA typing. The patients were HLA typed with a standardised 2-stage lymphocytotoxicity micro-method.

Quantitations of immunoglobulins and complement factors and detection of autoantibodies. Serum was frozen at -70°C and stored until all samples were collected. Quantitations of immunoglobulins, C-reactive protein (CRP), and complement factors, and detection of autoantibodies were done as described in detail elsewhere.^{20 21} Total haemolytic complement activity (CH_{50}) was measured by a haemolytic assay in agarose plates²² and the results expressed as percentage of that found in a standard pool of blood donors. Fifty healthy adults served as controls in the different quantitations.

Statistical methods. Laboratory values are given as mean $\pm$ standard error of the mean (SEM). Differences between groups were assessed by means of the Mann-Whitney U test. Significant probabilities equal to or less than 0.05 were considered significant. One-sided tests were used on 1-sided hypotheses.

Results

CLINICAL AND LABORATORY DATA

All patients fulfilled the criteria for ankylosing spondylitis^{23 24} and showed bilateral sacroiliitis on radiographs. Thirteen patients also had radiographic changes of the spine. All patients were HLA B27 positive. The majority of the patients had active disease as judged from ESR, CRP, and com-

plement factor levels. Detailed data are given in Table 1.

Serum IgA and IgM concentrations were increased (3.5 ± 0.5 g/l and 1.7 ± 0.2 g/l in the patients versus 2.3 ± 0.1 g/l and 1.4 ± 0.1 g/l in the controls, $p < 0.05$, 1-sided test). Serum IgG concentration was also slightly elevated in the patient group, but the increase was not significant (14.3 ± 1.2 g/l versus 12.4 ± 0.4 g/l; $0.05 < p < 0.1$, 1-sided test). Three patients had detectable autoantibodies. One patient (patient 2) had IgM RF in titre 128 (Waller-Rose reaction); another (patient 5) had anti-smooth-muscle antibodies in titre 32; and the third (patient 4) had low titred antinuclear antibodies (ANA) (titre 8) and antiparietal antibodies (titre 16).

CON-A-INDUCED SUPPRESSOR CELL ACTIVITY AND T LYMPHOCYTE SUBPOPULATIONS

The peripheral blood lymphocytes from patients and controls (stimulator cells) were incubated with con-A for 48 hr in order to generate maximal suppressor cell activity on to T lymphocytes from a constant donor (responder cells). In this assay a suppression of $41.0 \pm 8.6\%$ was detected in the patient group compared with $59.4 \pm 5.2\%$ in the controls ($0.05 < p < 0.1$, 1-sided test).

By means of different rosetting techniques the percentages of T lymphocytes (E-RFC), T_{γ} cells (suppressor cells), and T_{μ} cells (helper cells) were determined. No abnormalities were detected (Table 2).

Four patients (nos. 5, 6, 8, and 17) had low suppressor cell activity ($\leq 20\%$) (Table 3). Their

Table 2 Con-A-induced suppressor-cell activity, T lymphocyte subpopulations (T_{γ} and T_{μ} cells), and immunoglobulin concentrations in the peripheral blood of 17 patients with ankylosing spondylitis

Pat. no.	% Suppr.	T_{γ}	T_{μ}	IgG (g/l)	IgA (g/l)	IgM (g/l)
1	59	26	54	12.8	4.4	0.9
2	50	23	52	15.9	7.4	1.8
3	29	9	44	14.8	4.6	2.1
4	44	11	—	10.0	4.2	2.5
5	11	8	50	16.8	6.1	1.7
6	—8	13	46	12.8	2.5	1.1
7	88	8	77	9.8	3.0	1.5
8	20	7	43	13.6	1.2	1.2
9	77	6	53	7.1	1.0	2.3
10	—	6	—	10.9	2.4	1.7
11	—	11	—	11.3	4.6	1.3
12	—	7	51	21.4	2.7	2.2
13	67	13	55	15.9	4.9	3.1
14	—	7	45	24.8	5.3	1.2
15	—	7	54	17.9	0.8	3.1
16	48	6	41	7.5	0.8	1.6
17	7	22	—	19.6	2.9	0.3
Mean $\pm$ SEM of patients and of controls	41.0 ± 8.6	11.1 ± 1.6	51.2 ± 2.5	14.3 ± 1.2	3.5 ± 0.5	1.7 ± 0.2
	59.4 ± 5.2	13.0 ± 2.0	54.7 ± 2.0	12.4 ± 0.4	2.3 ± 0.1	1.4 ± 0.1

Three patients (nos. 2, 4, and 5) had autoantibodies in low titres).

Table 3 Con-A-induced suppressor cell activity, T γ and T μ cells, erythrocyte sedimentation rate (ESR), and immunoglobulin concentrations in different groups of patients with ankylosing spondylitis

Pat. no.	% Suppr.	T γ	T μ	ESR	IgG (g/l)	IgA (g/l)	IgM (g/l)
<i>Patients with low suppressor cell activity</i>							
5	11	8	50	60	16.8	6.1	1.7
6	—8	13	46	35	12.8	2.5	1.1
8	20	7	43	22	13.6	1.9	1.2
17	7	22	—	10	19.6	2.9	0.3
Mean $\pm$ SEM	7.5 $\pm$ 5.8	12.5 $\pm$ 3.4	46.3 $\pm$ 2.0	32 $\pm$ 11	15.7 $\pm$ 1.6	3.4 $\pm$ 0.9	1.1 $\pm$ 0.3
<i>Patients with high suppressor cell activity</i>							
1	59	26	54	50	12.8	4.4	0.9
7	88	8	77	13	9.8	3.0	1.5
9	77	6	53	14	7.1	1.0	2.3
13	67	13	55	45	15.9	4.9	3.1
Mean $\pm$ SEM	72.8 $\pm$ 6.3	13.3 $\pm$ 4.5	59.8 $\pm$ 5.8	31 $\pm$ 10	11.4 $\pm$ 1.9	3.3 $\pm$ 0.9	2.0 $\pm$ 0.5
<i>Patients with high disease activity</i>							
1	59	26	54	50	12.8	4.4	0.9
2	50	23	52	83	15.9	7.4	1.8
5	11	8	50	60	16.8	6.1	1.7
12	—	7	51	82	21.4	2.7	2.2
Mean $\pm$ SEM	40.0 $\pm$ 14.7	16.0 $\pm$ 4.9	51.8 $\pm$ 0.9	69 $\pm$ 8	16.7 $\pm$ 1.8	5.2 $\pm$ 1.0	1.7 $\pm$ 0.3
<i>Patients with high total immunoglobulin concentration</i>							
2	50	23	52	83	15.9	7.4	1.8
5	11	8	50	60	16.8	6.1	1.7
13	67	13	55	45	15.9	4.9	3.1
Mean $\pm$ SEM	42.7 $\pm$ 16.6	14.7 $\pm$ 4.4	52.3 $\pm$ 1.5	63 $\pm$ 11	16.2 $\pm$ 0.3	6.1 $\pm$ 0.7	2.2 $\pm$ 0.5
<i>Patients with low total immunoglobulin concentration</i>							
7	88	8	77	13	9.8	3.0	1.5
9	77	6	53	14	7.1	1.0	2.3
16	48	6	41	15	7.5	0.8	1.6
Mean $\pm$ SEM	71.0 $\pm$ 11.9	6.7 $\pm$ 0.7	57.0 $\pm$ 10.6	14.0 $\pm$ 0.6	8.1 $\pm$ 0.8	1.6 $\pm$ 0.7	1.8 $\pm$ 0.3

percentages of T γ and T μ cells did not differ from those in the rest of the patients or the controls. No deviations were found in IgG and IgA concentrations between these 4 patients and the rest of the patient group, but the IgM concentration was reduced (1.1 $\pm$ 0.3 g/l versus 1.9 $\pm$ 0.2 g/l; $p < 0.05$). Low suppressor cell activity was found both in patients with high and those with low disease activity as judged from ESR and CRP (Tables 1 and 3).

Four patients (nos. 1, 7, 9, 13) with *high* suppressor cell activity ($\geq 59\%$) had normal proportions of T lymphocyte subpopulations. The mean IgG concentration was slightly lower and the IgM concentration slightly higher than in the rest of the patients, but these variations did not reach statistical significance.

No differences in suppressor cell activity, or in the percentages of T γ and T μ cells were found between the 3 patients with *high* total immunoglobulin concentrations (> 23 g/l) and the other patients (Table 3).

Three patients with *low* total immunoglobulin levels (< 15 g/l) showed a moderately increased suppressor cell activity compared with the remaining patients (71 $\pm$ 11.9% versus 31 $\pm$ 8.5%, $0.05 < p <$

0.1). The percentage of T γ cells in these patients was lower (6.7 $\pm$ 0.7% versus 12.1 $\pm$ 1.9%, $p < 0.05$), while T μ cell percentages showed no significant deviation (Table 3).

No differences in suppressor cell activity or in T lymphocyte subpopulations were seen between patients with or without peripheral joint or spine involvement, or a history of acute anterior uveitis. One patient (no. 7) had severe psoriatic arthropathy. He also had the highest suppressor cell activity and the highest percentage of T μ cells in the total patient group (Table 1).

Discussion

A reduction in con-A-induced suppressor cell activity was detected in the peripheral blood of some patients with AS. T lymphocyte subpopulations with so-called suppressor and helper cell markers (T γ and T μ cells) were within the normal range. These observations may correspond with the moderately elevated humoral responses now detected, and with the normal cellular responses currently found in these patients.^{3 7-10} We have measured suppressor cell activity in a T cell system (con-A MLC), and, since it is not known whether the same or different

cells regulate B and T cell activities, the possibility that polyclonal B cell activators are responsible for the elevated immunoglobulin concentrations in some AS patients must also be considered. One possible B cell activator in AS patients is *Klebsiella pneumoniae*, a micro-organism which may cause chronic infections in AS patients because of similarities between bacterial and host antigens (HLA B27).²⁵ Owing to this antigenic similarity the micro-organism might be recognised as 'self' and thus escape normal defence mechanisms of the host's immune system.

Studies of patient subgroups characterised by significantly reduced or increased suppressor cell activity, or by reduced or elevated percentages of cells with suppressor and helper cell markers, respectively, revealed no clear association between markers and functions. This lack of association may be due to the heterogeneity of the T γ cell population, which comprise cells active in antibody-dependent cell-mediated cytotoxicity,^{26 27} as natural killer cells²⁷ as well as suppressor cells.²⁸ Functional heterogeneity may also be found in the T μ cell compartment. AS patients with the lowest total immunoglobulin levels had the most increased suppressor cell activity, indicating that detectable suppressor and helper cell disturbances may be found in AS patients with deviating activity in the immune system. However, no direct association was observed between suppressor cell activity, cells with suppressor and helper cell markers, and clinical parameters.

One of the patients (no. 5) was not on drug treatment. This patient had clinically a high disease activity, high levels of immunoglobulins, low suppressor cell activity, and a reduced percentage of cells with suppressor cell markers. The rest of the patients received various drugs (prednisone, naproxene, indomethacin, salicylates), and, as it is known that certain anti-inflammatory drugs—for example, prednisone, cyclophosphamide—have the capacity to normalise con-A-induced suppressor cell activity,^{29–31} the possibility exists that suppressor cell dysfunctions may be partly corrected in the treated patients.

This is to our knowledge the first report on suppressor cell activity and T cell subpopulations in patients with AS. Investigations of suppressor and helper cells in other rheumatic diseases, such as systemic lupus erythematosus (SLE), juvenile rheumatoid arthritis (JRA), and rheumatoid arthritis (RA), have shown a spectrum of results from a highly significant reduction of suppressor cell activity and cells with suppressor cell markers in patients with SLE,^{32 33} through normal suppressor cell activity but reduced percentages of cells with suppressor cell markers in patients with JRA,^{34 35}

to only minor deviations in RA patients³⁴ and now also in AS patients. These different findings clearly demonstrate the complexity of immunoregulation, and they also suggest that different mechanisms may operate in the different rheumatic diseases. Furthermore, the results from RA patients with a local suppressor cell defect in synovial tissue lymphocytes^{36 37} combined with normal suppressor cell activity in the peripheral blood lymphocytes³⁴ show that, in spite of normal findings in the peripheral blood, local derangements in immunoregulatory cells may be detected in the diseased joints. This may also be true of patients with AS.

The familial occurrence of ankylosing spondylitis is well known. The HLA B27 antigen, which is autosomally codominantly inherited, is present in most affected patients. But why some but not all persons born with this genetic marker contract the disease is not known. Certain infections (caused by, for example, salmonella strains and shigella strains) may induce arthritis in HLA B27 positive persons, indicating that AS may be a reactive disorder. One report also claims that decreased suppressor cell activity can be genetically determined.³⁸ Our study revealed only minor aberrations in the suppressor cell function in AS patients. Family studies are needed to clarify whether the suppressor cell dysfunction observed in some AS patients segregates as a genetic marker in their families.

The present study demonstrated almost normal suppressor cell activity in AS patients in an unspecific mitogen-induced suppressor cell assay (the con-A MLC assay). However, suppressor and helper cell systems measuring specific immune responses against possible aetiological antigens—for example, strains of *Klebsiella pneumoniae*, salmonella, and shigella—may be more useful for the detection of immunological defects in patients with AS.

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