CD4+ T-Lymphocytopenia — A Frequent Finding in Anti-SSA Antibody Seropositive Patients with Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** Case reports have described an association between idiopathic CD4+ T-lymphocytopenia (ICL) and non-Hodgkin's malignant lymphoma (NHML), and both entities have an increased prevalence in patients with primary Sjögren's syndrome (SS). We investigated lymphocyte subset counts in patients with primary SS to determine if presence of different autoantibodies is associated with ICL and hence may represent an increased risk for development of NHML.

Methods. A total of 80 patients with primary SS according to the American-European Consensus Classification Criteria (AECC) and 37 non-AECC sicca patients were studied for presence of different autoantibodies, and lymphocyte subsets were investigated by flow cytometry.

Results. Absolute CD4+ T-lymphocyte counts were significantly lower among anti-SSA antibody seropositive SS patients compared to correlating seronegatives and non-AECC sicca patients (601/µl vs 956/µl and 1087/µl; $p < 0.001$ and $p < 0.001$, respectively). ICL was found in 16% of anti-SSA seropositive patients.

Conclusion. ICL, a proposed risk factor for development of NHML, occurs frequently and presumably exclusively in patients with primary SS who are anti-SSA antibody seropositive. These findings support that this group comprises patients at risk for development of NHML. (J Rheumatol 2004;31:726–8)

Key Indexing Terms:
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Various immunological aberrations may be found in patients with primary Sjögren's syndrome (SS)[1]. Different autoantibodies may be present, and according to the US Centers for Disease Control criteria[2], idiopathic CD4+ T-lymphocytopenia (ICL) has been found in about 5% of patients with primary SS[3,4]. Case reports of non-Hodgkin's malignant lymphoma (NHML) in patients with ICL imply an association between development of NHML and ICL[3,5,6], and both entities have an increased prevalence in primary SS[1,3]. Since primary SS patients are not routinely investigated by flow cytometry but always for presence of different autoantibodies, we wished to determine the possible relationship between different autoantibodies and ICL in patients with primary SS.

MATERIALS AND METHODS

This retrospective study included 80 patients (72 women) fulfilling the American-European Consensus Classification Criteria (AECC) for primary SS[7] who had no immunodeficiency disorder or current/recent (previous year) treatment with glucocorticosteroids or immunomodulatory drugs, who were followed at our department between 1991 and 1996. Thirty-seven patients were found to be anti-SSA antibody seropositive, of which 30 were also anti-SSB antibody seropositive. Fifty-two were positive for antinuclear antibody (ANA) and 13 were positive for rheumatoid factor (RF).

Thirty-seven non-AECC sicca patients (32 women) were also investigated. These all had subjective and objective signs (pathological Schirmer-I test and unstimulated whole sialometry) of decreased lacrimal and salivary secretion, but had negative lower lip biopsies and were anti-SSA and SSB antibody seronegatives. Patients' characteristics are shown in Table 1.

Anti-SSA and SSB antibodies, ANA, and RF were analyzed as described[3]. Peripheral blood lymphocytes were analyzed using a flow cytometer as described[3]. ICL was defined as an absolute CD4+ T-lymphocyte count < 300 cells/µl and/or a relative CD4+ T-lymphocyte count < 20% of the total lymphocyte count, at more than one determination, with no evidence of human immunodeficiency virus-1 or -2 infection, and no other immunological disorder or immunomodulating therapy that might cause ICL[2].

When several flow cytometry analyses had been performed, means of the parameters were used in the statistical calculation. No patient with ICL was found to have anti-HIV-1 and -2 antibodies. Only 3 of 6 ICL patients were tested for presence of anti-HTLV-I and -II antibodies and in those examined, no antibodies were found.

Statistical analyses. One-way ANOVA, Student t test, chi-square tests, and Fisher exact test with Bonferroni adjustment within each variable were used when appropriate. P values < 0.05 were considered statistically significant. Results are given as mean ± standard deviation.

RESULTS

Anti-SSA antibody seropositive patients with primary SS

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Table 1. Characteristics of primary SS patients (n = 80) and non-AECC sicca patients (n = 37). Results are mean ± SD or percentage with abnormal results of tested individuals. Ophthalmological results are presented as sums of both eyes.

	pSS Patients, mean ± SD, Abnormal Results (%)	Non-AECC Sicca Patients, mean ± SD, Abnormal Results (%)	p
Age, yrs	60 ± 14	52 ± 15	< 0.01
Schirmer I-test, mm/5 min	5.9 ± 7.3	8.8 ± 8.3	NS
Van Bijsterveld score	9.9 ± 4.8	3.6 ± 3.1	< 0.001
Unstimulated whole sialometry, ml/15 min	0.6 ± 1.1	0.6 ± 0.9	NS
IgG, g/l	17.2 ± 7.1	10.8 ± 2.3	< 0.001
C3, g/l	0.94 ± 0.21	0.88 ± 0.20	NS
C4, g/l	0.28 ± 0.15	0.36 ± 0.20	< 0.05
Hb, g/l	131 ± 12	138 ± 11	< 0.01
Thrombocytes, 10^9/l	240 ± 62	245 ± 59	NS
Abnormal lip biopsy, focus score ≥ 1, %	93	0	< 0.001
Anti-SSA seropositive, %	49	0	< 0.001
Anti-SSB seropositive, %	40	0	< 0.001
ANA seropositive, %	67	0	< 0.001
IgM RF seropositive, %	19	0	< 0.01
Parotic swelling, %	21	14	NS
Purpura hypergammaglobulinemia, Waldenström, %	8	0	NS
Lymphadenopathy, %	6	5	NS
Glomerulonephritis, %	1	3	NS
Peripheral neuropathy symptoms, %	18	30	NS

NS: nonsignificant.

were found to have significantly decreased CD4+ T-lymphocyte counts compared to anti-SSA negative patients and non-AECC sicca patients. Sixteen percent of anti-SSA positive SS patients, 0% of anti-SSA negative SS patients, and 0% of non-AECC sicca patients were found to have ICL (Table 2). Similar but less consistent findings were found comparing anti-SSB, ANA, and RF seropositive to correlating seronegative SS patients and to non-AECC sicca patients (data not shown).

DISCUSSION

In this study anti-SSA positive patients with primary SS were found to have significantly lower CD4+ T-lymphocyte counts compared to anti-SSA seronegatives and to non-AECC sicca patients. ICL was found in 8% (6/80) of SS patients. It was notable that all 6 ICL patients were anti-SSA seropositives. Consequently, ICL was found in 16% (6/37) of anti-SSA seropositives, but in none of the anti-SSA seronegatives or the non-AECC sicca subjects. Hence an

Table 2. Lymphocyte subsets and ICL prevalence in anti-SSA antibody positive and negative SS patients and non-AECC sicca patients. Values are presented as mean ± SD.

Subsets	Anti-SSA Positive SS Patients, n = 37	Anti-SSA Negative SS Patients, n = 39	Non-AECC Sicca Patients, n = 37	ANOVA p
Total leukocytes, cells/µl	5340 ± 1320[b,d]	6730 ± 1760	7300 ± 2080	< 0.001
Total lymphocytes, cells/µl	1620 ± 480[c,d]	2100 ± 730	2390 ± 840	< 0.001
CD4+ T-lymphocytes, cells/µl	601 ± 269[a,d]	956 ± 382	1087 ± 399	< 0.001
CD4+ T-lymphocytes, %	38.0 ± 11.4[b,e]	45.8 ± 9.8	45.9 ± 7.9	< 0.01
CD8+ T-lymphocytes, cells/µl	539 ± 400	527 ± 337	663 ± 331	NS
CD8+ T-lymphocytes, %	31.9 ± 14.3[c]	25.0 ± 11.5	27.2 ± 8.5	< 0.05
CD4/CD8 ratio	1.52 ± 0.95[c]	2.36 ± 1.63	1.96 ± 1.00	< 0.05
CD19+ B-lymphocytes, cells/µl	248 ± 162	254 ± 123	277 ± 97	NS
CD19+ B-lymphocytes, %	15.1 ± 7.5[e]	12.3 ± 4.7	12.0 ± 4.0	< 0.05
CD4+CD45RA+ T-lymphocytes, cells/µl	207 ± 146	453 ± 827	382 ± 238	NS
CD3+DR+ T-lymphocytes, cells/µl	225 ± 189[c,f]	147 ± 121	131 ± 85	< 0.01
Patients fulfilling criteria for ICL, n	6[c]	0	0	< 0.01*

[a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$ vs anti-SSA seronegatives. [d] $p < 0.001$, [e] $p < 0.01$, [f] $p < 0.05$ vs non-AECC sicca patients. * Fisher exact test. NS: nonsignificant.

association between presence of anti-SSA antibody and ICL seems to exist.

It has also been reported that patients with primary SS are more prone to develop NHML[1], perhaps particularly those with ICL[3]. This is in accord with findings in patients with HIV[8] and in case reports of ICL patients without obvious concurrent disease[5,6]. ICL could thus be a predictor of development of NHML. ICL could be involved in lymphomagenesis due to a decreased level of immunosurveillance in combination with viral infections immortalizing lymphocytes[8]. As well, a lack of regulatory cytokines from CD4+ T-lymphocytes could impair immune control of B cell proliferation, thus enhancing malignant transformation[8].

We found that ICL, a proposed risk factor for development of NHML, was commonly and exclusively found in patients with primary SS who were seropositive for anti-SSA antibodies. These findings indicate that this group comprises patients at risk for development of NHML.

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