Whiteside *et al*[5] and Barrett *et al*[6] by using the indirect immunoflourescence method, reported that CD8+ supressor/cytotoxic T cells are decreased in SSc group, our findings differ from those of Whiteside and Barrett; we and Degiannis *et al*[7] have used the more sensitive flow cytometry method and could not find any difference between T lymphocyte subgroups of SSc patients whereas in the pathogenesis of SSc the role of CD4+ and CD8+ T lymphocytes is still obscure. Presence of autoantibodies and hypergammaglobulinaemia support the role of humoral immunity but B lymphocytes were rarely found in the skin biopsy specimens.[8] CD19+ is a cell surface marker of B lymphocytes and we could not observe any difference in the levels of CD19+ thus we can say that B lymphocytes might play only a minor part in the pathogenesis of SSc. CD25+ is one of the subunits of high affinity IL2R and known as the alpha chain of IL2R. Bruns *et al*[9] established a clear correlation between CD25+ and soluble IL2R in serum. T lymphocytes expressing CD25+ and T helper cell derived cytokines and growth factors stimulate matrix protein synthesis by fibroblasts, resulting in generalised fibrosis and sclerosis. In our study we found significant increases of CD25+ and this surface marker can be used in the follow up the inflammatory stage and activity of SSc. In further studies the investigation of CD25+ T cell subsets CD4 , CD8, TCR gamma–delta and other T cell activation markers HLA-DR, CD45RO/CD45RA will be useful to shed light on the pathogenesis of SSc. NK cell abnormalities have been described in a number of rheumatic diseases such as RA, Sjögren's syndrome, systemic lupus erythematosus. NK cells are large granular lymphocytes easily identified morphologically by the presence of azurophil granules in their cytoplasm and they commonly express certain cell surface markers such as CD16+ and CD56+; CD56+ is a homofilic adhesion molecule that belongs to the immunglobulin superfamily. NK cells are the main cellular effectors of antibody dependent cell cytotoxicity, they mediate antigen presentation and secrete immune modulator cytokines like interferon, IL2, colony stimulating factor, these functions suggested the involvement of NK cells in the pathophysiology of SSc.[6 10]

We found the percentage of CD56+ significantly higher in SSc patients (mean (SD) 22 (9)) than controls (mean (SD) 14 (5)). Although this finding suggested the role of CD56+ cells in the pathogenesis of SSc, various results in different investigations pointed out that further investigations on CD56+ and CD16+ NK cell percentage and activity are needed.

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## Lymphocyte populations and cytokine concentrations in pericardial fluid from a systemic lupus erythematosus patient with cardiac tamponade

Pericardial involvement is the most common cardiovascular complication in systemic lupus erythematosus (SLE).[1] The clinical picture varies from subclinical pericardial effusion and classic acute pericarditis to cardiac tamponade.[1 2] Immunological studies of pericardial fluid (PF) have been limited to determination of autoantibodies, complements and immune complexes.[3 4] To further study the pathogenic mechanisms involved in lupus pericarditis we examined the lymphocytic populations and cytokine concentration pattern in PF and peripheral blood (PB) from a SLE patient with cardiac tamponade.

We report a case of a 38 year old man with SLE diagnosed in December 1995 when he presented with polyarthritis, photosensitivity, oral ulcers, nephritis, non-hemolytic anaemia, positive ANA, increase of anti-dsDNA and hypocomplementaemia. The patient improved with corticosteroid and intravenous cyclophosphamide treatment. However, on 18 June 1997 he presented with syncope, hypotension (80/40 mm Hg), a tachycardia, jugular vein distension and cardiomegaly. The two dimensional echocardiogram showed a large pericardial effusion with right atria and ventricle collapse in diastole. Pericardiocenthesis was performed and 180 ml of an orange fluid was aspirated. Examination of PF showed white blood cell count of 5280/mm$^3$ (polymorphonuclear cells = 96%). The absolute number of lymphocytes was lower in PF than in PB (211 *v* 700/mm$^3$). PF level of protein was 4.1 g/dl (serum = 5.3 g/dl), glucose was 53 mg/dl (serum = 110 mg/dl) and LDH was 471 IU/l (serum = 110 IU/l). PF cultures were negative. No malignant cells were seen. He was treated with high dose corticosteroids and azathioprine. Prednisone was gradually decreased to 10 mg daily over a three month period. After a 22 month follow up, he remained clinically stable without recurrent pericardial involvement or SLE exacerbations.

*Table 1 Frequency of lymphocyte populations and cytokine concentrations in peripheral blood and pericardial fluid*

| *Parameter* | *Peripheral blood* | *Pericardial fluid* |
|---|---|---|
| Lymphocyte population (%) | | |
| Total T cells | 57.8 | 50.0 |
| CD4+ T cells | 17.6 | 25.0 |
| CD8+ T cells | 34.3 | 25.0 |
| B cells | 7.8 | 8.3 |
| NK cells | 34.3 | 41.7 |
| Cytokine concentration* (pg/ml) | | |
| IL1β | <3.0 | 240.0 |
| IL2 | 201.8 | <4.0 |
| IL4 | <6.0 | <6.0 |
| IL6 | 16.9 | 4714.0 |
| IL10 | <5.0 | 139.8 |
| TNFα | 3.8 | 15.4 |
| IFNγ | 1.5 | 32.8 |

*Manufacturer (Genzyme, Boston, MA) detection limits: 3 pg/ml for IL1β, INFγ and TNFα; 4 pg/ml for IL2; 6 pg/ml for IL4; 18 pg/ml for IL6; and 5 pg/ml for IL10.

Before starting immunosuppressive treatment, PF and PB were obtained simultaneously for immunological analysis. Mononuclear cells from both sources were isolated by gradient centrifugation and the frequency of lymphocyte populations was determined by flow cytometry. The cytokine concentrations from plasma and PF were determined by ELISA. Table 1 shows the results. Among lymphocytes, the percentage of CD4+ T cells and NK cells was higher in PF, while the frequency of CD8+ T cells was higher in PB. IL6 concentration was much higher in PF than plasma. Also, IL1β and IL10 concentrations were higher in PF. IL2 was detected in plasma but not in PF.

The considerable increase in pericardial IL6, with respect to plasma, is particularly interesting. PF concentrations of IL6 in our patient were substantially higher than those observed in PF from patients with inflammatory and non-inflammatory heart conditions.[5 6] IL6, not only can increase antibody production, but in SLE, B cells have increased reactivity to this cytokine.[7] As in our case, IL6 is usually expressed or increased in the affected organ or system rather than PB. IL6 has been found to be higher in cerebrospinal fluid and urine than in serum of SLE patients with CNS disease and active nephritis respectively.[8 9]

The decreased pericardial lymphocyte count and fluid characteristics observed here are in agreement with other studies.[10] The higher frequency of CD4+ T cells and NK cells in PF could be associated with the observed cytokine concentration pattern. For example, CD4+ memory T cells from SLE patients highly secrete IL10 compared with normal controls.[11]

In summary, different patterns of lymphocyte populations and cytokines were found in both sources, with type 2 cytokines predominating in PF and type 1 in PB. Further studies would be required to confirm the results presented here. In addition, immunocytochemical studies of pericardial

tissue are necessary as the composition of lymphocyte and cytokine profiles may differ between pericardial fluid and tissue.

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# HLA-B27+ anterior uveitis with or without associated spondyloarthritis: clinical and immunological features

Anterior uveitis (AU) is the most common form of uveitis,[1–3] and may be produced by different causes. An aetiological diagnosis is commonly established in approximately half of the patients with AU, being seronegative spondyloarthropathies (SA), and mainly ankylosing spondylitis, the most frequent cause of the disease. Approximately 50% of the patients with AU are HLA-B27 positive; half of them usually presenting with associated SA,[4–8] the other half are patients with HLA-B27+ but with no associated articular disease (HLA-B27+ AU). Several clinical features have been described to be common in patients with AU associated with HLA-B27, however, these features are similar either in patients with or without associated SA.[9 10] This is why we conducted this clinical and immunological study in patients with AU positive for HLA-B27 with the aim of discovering the differences between patients with and without associated SA.

*Table 1 Lymphocyte populations in AU patients and controls*

| | *AU patients (n=146)* | *Controls (n=31)* | *p Value* |
|---|---|---|---|
| Lymphocytes (no/mm$^3$) | 2425.60 (964.44) | 2567.74 (820.72) | NS |
| CD3 (no/mm$^3$) | 1734.20 (726.67) | 1835.64 (586.68) | NS |
| (%) | 71.96 (8.20) | 71.27 (4.28) | NS |
| CD4 (no/mm$^3$) | 1023.91 (489.16) | 1219.70 (427.56) | <0.05 |
| (%) | 42.56 (9.50) | 47.00 (6.13) | <0.05 |
| CD8 (no/mm$^3$) | 702.21 (359.67) | 675.90 (243.54) | NS |
| (%) | 29.25 (8.81) | 26.72 (6.81) | NS |
| CD4/CD8 | 1.70 (0.89) | 1.92 (0.78) | NS |
| CD19 (no/mm$^3$) | 266.87 (227.44) | 335.90 (142.35) | NS |
| (%) | 10.81 (5.65) | 13.64 (5.09) | <0.05 |
| CD4CD45R+ (no/mm$^3$) | 406.17 (304.18) | 657.70 (301.36) | <0.001 |
| (%) | 16.70 (9.99) | 25.20 (7.76) | <0.001 |
| CD4CD45R- (no/mm$^3$) | 661.53 (338.48) | 529.41 (219.04) | <0.05 |
| (%) | 27.70 (7.94) | 20.77 (6.40) | <0.001 |
| NK (no/mm$^3$) | 300.45 (179.63) | 323.80 (182.53) | NS |
| (%) | 13.30 (7.76) | 12.81 (5.86) | NS |

AU = anterior uveitis, NK = natural killer cells, NS = not significant. Data shown as mean (SEM).

A prospective study was conducted involving 146 patients with active AU seen between April 1988 and October 1995 referred from an ophthalmologist with the syndromic diagnosis of AU of unknown origin. Patients were classified in three aetiological groups: (1) Idiopathic anterior uveitis (IAU), all were HLA-B27−, (2) HLA-B27+ AU without associated SA, and (3) HLA-B27+ AU with associated SA.

Of the 146 patients with AU studied, 98 had IAU (67.1%) and 48 were positive for HLA-B27; of them, 19 (13%) had associated SA (HLA-B27+ AU with SA), and 29 (19.9%) did not (HLA-B27+ AU). No significant differences were found in clinical features of AU between the three study groups. Erythrocyte sedimentation rate, C reactive protein and IgA were found to be more increased in patients than in control, although without differences between the three groups of patients. With regard to lymphocyte populations, we found some differences between our AU patients and control group (table 1). Patients with IAU showed lower percentages (mean (SEM)) of CD4CD45R+ (15.47 (9.49)%) than controls (25.20 (7.76)%) and patients with SA (21.97 (10.16)%) (fig 1). Patients with IAU had higher percentages of CD4CD45R- (28.46 (7.89)%) than SA patients (23.23 (6.81)%) and the control group (20.77 (6.40)%) (fig 2).

Associated systemic pathology was demonstrated in 13% of the cases (19 patients with seronegative SA), 29 patients (19.9%) were HLA-B27+ without SA; not associated disease was found in the other 98 cases of AU (67.1%), which were classified as idiopathic. Seronegative SA are the most frequent entities found in uveitis patients, representing between 6%[2] and 13%[3] of all forms of uveitis, and 20 to 25% of the AU. HLA-B27+ AU without associated SA represents about 25% of the AUs; it has been considered by some authors a "frustrated" or monosymptomatic form of ankylosing spondylitis,[11] but today, it is still unclear as to whether or not it is the same clinical entity, or whether these patients will develop seronegative SA in future. We did not find differences in clinical features of AU between HLA-B27+ and HLA-B27− patients. A deficit of CD4CD45R+ (suppressor-inducer T lymphocytes) and an increase of CD4CD45R− (memory T lymphocytes), such as in our patients with IAU, have been described in certain autoimmune disease, suggesting that these disorders could be attributable to these changes.[12–15] In addition, differences found in the values of CD4CD45R cells between patiens with IAU and SA suggest a different physiopathogenetic mechanism in the development of both

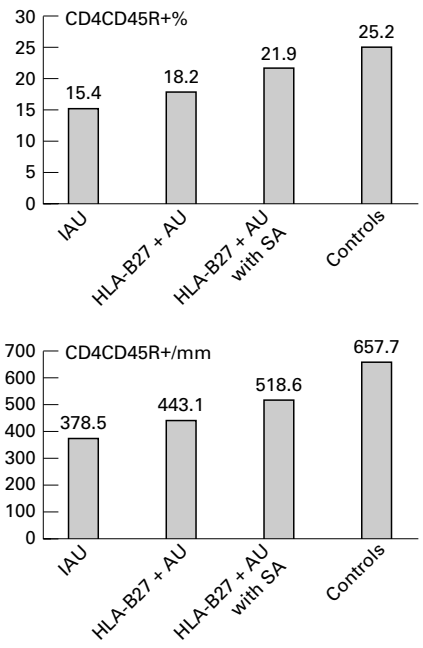


*Figure 1 Absolute values of CD4CD45R+ cells. Patients with IAU had absolute values lower than the control group, and percentages lower than those of SA patients (p<0.001). IAU= idiopathic anterior uveitis; AU= anterior uveitis; SA= spondyloarthritis.*

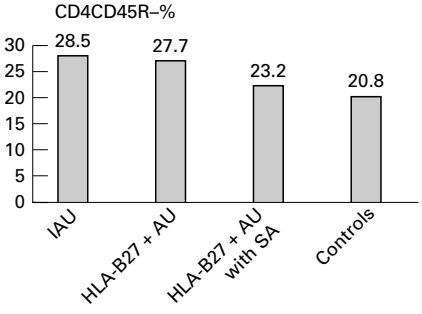


*Figure 2 Percentages of CD4CD45R− cells. Patients with IAU had higher percentages than the healthy subjects and SA patients (p<0.001). Abbreviations as in figure 1.*