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Phenotype of peripheral blood lymphocytes in patients with progressive systemic sclerosis: activated T lymphocytes and the effect of D-penicillamine therapy

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SUMMARY

To understand better the role that immune mechanisms could play in the pathogenesis of progressive systemic sclerosis (PSS), the phenotypes of peripheral blood lymphocytes (PBL) from 24 PSS patients and normal controls were compared by a panel of monoclonal antibodies. PBL T cells of patients expressed an increased percentage of several activation markers as defined by monoclonal antibodies B33.1 (anti-Ia), TAC (anti-interleukin 2 receptor) and 5E9 (anti-transferrin receptor). These antigens were also found on PBL of patients with quiescent disease suggesting the persistence of an ongoing immune response despite the absence of clinically apparent disease activity. Patients treated with D-penicillamine had uniformly normal proportions of Ia^+ T cells ($< 5\%$) although the percentage of cells positive for Tac and transferrin receptor continued to be elevated. A second finding was that the percentage of natural killer (NK) cells studied with the monoclonal antibody against NK cells B73.1 (Leu 11c) and particularly an $OKT8^+$ NK cell subset was low in patients ($10{\cdot}4 \pm 4{\cdot}7$) compared with controls ($15{\cdot}9 \pm 5{\cdot}8$). Finally, both treated and untreated patients displayed increased $OKT4^+/OKT5^+$ ratios compared to controls. These data suggest that PBL from PSS patients are activated and have abnormal proportions of cell subpopulations. These abnormalities are also present in patients with quiescent disease. The observed effect of D-penicillamine on Ia expression in PSS PBL may reflect a mechanism of action of this drug in the control of autoimmune diseases.

Keywords scleroderma penicillamine lymphocytes phenotype

INTRODUCTION

Progressive systemic sclerosis (PSS) is a systemic disease characterized by excessive collagen deposition in the skin and internal organs associated with the expression of autoimmune phenomena. These phenomena which include circulating autoantibodies (Rothfield & Rodnan, 1968; Bernstein, Steigerwald & Tan, 1982) and a clinical overlap with diseases such as systemic lupus erythematosus (Kirkland, 1964) and polymyositis (Mimori *et al.*, 1981) have led to the characterization of PSS as an autoimmune disease. Although attention has been focused upon the serological abnormalities in PSS (Bernstein *et al.*, 1982; Mimori *et al.*, 1981; Moroi *et al.*, 1980) less is known about the cellular aspects of the immune system, and existing studies have been contradictory.

Defective immunoregulation may be important in the pathogenesis of PSS as manifested by inflammatory cell infiltrates in skin biopsies from PSS patients (Fisher & Rodnan, 1960; D'Angelo,

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Fries & Masi, 1969). These infiltrates consist of mononuclear cells (Fleishmajer, Perlish & Reeves, 1977) that are predominantly OKT4$^+$ and OKT8$^+$ lymphocytes (Roum *et al.*, 1984). The majority of these T cells appear to be activated, expressing both Ia antigen and transferrin receptor (Roum *et al.*, 1984).

Soluble products from mononuclear cells can regulate collagen synthesis by dermal fibroblasts *in vitro* (Johnson & Ziff, 1976; Jimenez, McArthur & Rosenbloom, 1979; Hibbs *et al.*, 1983; Postlethwaite *et al.*, 1984). In this context, gamma interferon (γ-IFN), appears to inhibit collagen synthesis through a reduction in the levels of collagen specific mRNA (Jimenez, Freundlich & Rosenbloom, 1984; Rosenbloom *et al.*, 1984). Lymphocytes may thus be important in the pathogenesis of PSS and information about the phenotype of circulating cells could be relevant. Studies of PBL phenotype in PSS patients have had conflicting results. T gamma lymphocytes which express the FcR for aggregated IgG, and appear to be mostly natural killer (NK) cells (Perussia *et al.*, 1984), were shown to be decreased in PSS patients (Inoshita *et al.*, 1981; Whiteside *et al.*, 1983), while another study found them to be increased (Gupta *et al.*, 1979). Recently, ratios of OKT4$^+$/OKT8$^+$ cells were found to be normal in untreated PSS patients except in a subgroup of patients (Keystone *et al.*, 1982). A second study, however, showed a significantly elevated ratio (Fleishmajer *et al.*, 1977). T cell activation markers on circulating PBL have not been studied in PSS patients, nor has the effect of D-penicillamine, one of the few accepted therapies for PSS (Steen, Medsger & Rodnan 1982), on lymphocyte subpopulations and activation markers been reported. We have characterized the phenotype of lymphocyte subpopulations and examined the expression of various T cell activation markers in a group of PSS patients. We have also examined the effect of disease activity and D-penicillamine therapy on the expression of these markers.

MATERIALS AND METHODS

Patients. Patients with PSS from the University of Pennsylvania Rheumatology Section were used for this study. PSS variants such as CREST, linear scleroderma, overlap syndromes, and morphea were excluded. The diagnosis of PSS was made by strict criteria (Subcommittee for Scleroderma Criteria of The American Rheumatism Association, 1980). Proximal skin thickening and biopsy-proven scleroderma were present in all patients. There were 24 patients (18 females) with an average age of 48 years. Twenty-three normal controls (16 female) average age 42 were studied. Controls were tested simultaneously with patients and all the procedures for patient and control samples were handled identically. The average duration of disease was 6·1 years (5·1 years if one patient with disease for 30 years was excluded). Patients were categorized as having either quiescent or stable disease (14 cases) or as having active rapidly advancing disease (10 cases). The stage of activity of disease was established based on clinical data obtained from serial evaluations. Patients were considered to have active disease when they had progression of sclerotic skin involvement (doubling of extent of body surface involved within a 6 month period), appearance of new visceral disease or documented deterioration of previously affected organs such as lungs or kidneys. Patients were considered to be in remission or in a quiescent phase when no detectable evidence of progression of skin or visceral involvement could be demonstrated in a 12 month follow up period. Nine patients were being treated with D-penicillamine at the time of study. Four of them had active disease (average dose 750 mg/day). The remaining 15 patients had either never taken D-penicillamine or had been off this drug for at least 6 months and six of these cases had active disease. The extent of sclerotic skin involvement varied from 6–40% of total body surface. Pulmonary involvement, as determined by a pulmonary diffusing capacity of 60% or less or a chest X-ray with evidence of pulmonary fibrosis, was present in 20/24 patients. Two patients had kidney involvement without renal failure and one had cardiac involvement. Seventeen patients fulfilling the revised American Rheumatism Association criteria for definite or classical rheumatoid arthritis (RA) were studied (Ropes *et al.*, 1958). Five were being treated with D-penicillamine while the remaining 12 were not on any remittive agents. None were on corticosteroids.

Antibodies. A panel of 11 monoclonal antibodies were tested on each PSS and control blood sample. A list of several of the monoclonal antibodies used in this study and their characterization is

Table 1. Monoclonal antibodies used in this study

Antibodies	Ig subclass	Antigen (mol. wt × 10^3)	Specificity
B33.1	G2a	28,32	HLA-DR non-polymorphic region
B73.1 (Leu IIc)	G1	50–72	NK/PMN-FcR
B36.1	G2b	59	Pan-T cells (N Leu 1)
TAC	G2a	50	IL-2 Receptor
5E9	G1	90	Transferrin Receptor
B67.1	G2a	45	SRBC-Receptor
B-1	G2	30	B cells

shown in Table 1. The anti-B cell antibody B1 was kindly provided by Dr L. Nadler (Dana Farber Cancer Inst., Boston, MA) (Stashenko *et al.*, 1980). The pan-T cell antibody B36.1 (Perussia *et al.*, 1983a), the anti-SRBC-R antibody B67.1 (Perussia *et al.*, 1983b), the anti-HLA-DR antibody B33.1, and the anti-NK FcR antibody B73.1 (Leu 11c) (Perussia *et al.*, 1983a) were generous gifts of Drs Bice Perussia and Giorgio Trinchieri (Wistar Inst., Philadelphia, PA). Antibody B73.1 (Leu 11c) detects only NK cells among mononuclear cells (Perussia *et al.*, 1983a). Anti-Tac which reacts with the IL-2 receptor was obtained from Dr T. Waldman, National Institutes of Health (Bethesda, MD) (Uchiyama, Broder & Waldman, 1981). The anti-transferin receptor antibody 5E9 (Haynes *et al.*, 1981) was produced from cells purchased from the American Tissue Type Collection (Rockville, MD) by Dr Perussia. The antibodies OKT4, OKT5 and OKT8 were purchased from Ortho Diagnostics (Raritan, NJ). The S26 antibody is directed against a non-polymorphic HLA determinant and was obtained from Dr Massimo Trucco (Wistar Inst., Philadelphia PA).

The proportion of Ia^+ T-cells was calculated by subtracting $B\text{-}1^+$ B cells from $B33.1^+$ Ia-bearing lymphocytes (by light scatter).

Preparation of PBL. Peripheral venous blood was anticoagulated in heparin and diluted 1:2 (vol/vol) with Ca^{2+}, Mg^{2+}-free phosphate buffered saline (PBS). Blood was sedimented on a Ficoll/Hypaque density gradient as previously described (Freundlich *et al.*, 1984). Mononuclear cells were recovered, washed and adhered to plastic flasks for 1 h. The non-adherent cells were then removed and this fraction was used for experiments. These cells were >95% non-specific α-naphtyl esterase stain negative (Platt, 1979).

Immunofluorescence analysis. Indirect immunofluorescence was performed as described previously in detail (Freundlich *et al.*, 1984). Cell surface-bound monoclonal antibodies were detected by adding FITC conjugated goat $F(ab')_2$ anti-mouse Ig (Cappel Labs). Antibodies were labelled in PBS with 5% pooled human plasma at 4°C to minimize non-specific FcR binding. Control cells were incubated with the FITC conjugated Ig alone and a threshold for fluorescence detection, at which 98% of cells were negative, was set by computer. The percentage of positive cells and their intensity of fluorescence were analysed on an Ortho 50H Cytofluorograf connected to a Data General MP/200 microprocessor (Ortho Instruments, Westwood, MA). A second threshold at which approximately 96% of cells were negative was set to study anti-TAC and 5E9 antibodies with stain with a lower intensity. A lymphocyte window was created based upon the light scattering properties of cells. This was used to ensure analysis of a cell population containing 99% esterase negative, granulocyte-free cells.

The proportion of cells positive for a given marker was determined after subtraction of background fluorescence obtained from control cells exposed to the FITC conjugated goat $F(ab')_2$ anti-mouse Ig alone. The percentage of HLA-positive cells (which represents WBC and is not expressed on RBC) was used as a positive control to standardize preparations.

Statistics. Statistical analysis was done by a two-tailed unpaired Student's *t*-test to compare the mean percent positive lymphocyte subpopulations between the PSS patient population and the

matched control population. Patient data was further analysed with regard to disease activity and D-penicillamine treatment. Comparisons were made between active and inactive, and treated and non-treated patients. The Mann-Whitney non-parametric test was used to analyse these data since group sizes were relatively small and were not necessarily in a normal distribution.

RESULTS

Lymphocyte phenotype from PSS patients and normal controls. Table 2 shows the results of PBL tested with a panel of monoclonal antibodies. The percentage of cells, positive by fluorescence, from controls was compared to positive cells from the total population of PSS patients. The proportion of T cells was equal in controls and patients regardless of patient disease activity level or use of D-penicillamine. This was shown with both the pan-T antibody B36.1 and the anti-SRBC-R directed antibody B67.1. When T cell subsets were examined, however, differences were discovered between patients and controls. The proportion of $OKT4^+$ cells was not different between PSS patients as a whole in relation to controls, but a trend towards elevated percentages was noted especially in D-penicillamine-treated patients. There was no statistical difference, however, between treated and untreated patients. Similarly $OKT5^+$ cells showed a trend towards lower percentages in the total PSS patients. In contrast, $OKT8^+$ cells were low in the total PSS group and this was not influenced by treatment or disease activity. The marker OKT8 is found on the same cells as OKT5 (suppressor/cytotoxic cells) but is also expressed on a subset of NK cells that are $B73.1^+$, $OKT3^-$ and $OKT5^-$ (Perussia, Fanning & Trinchieri, 1983c). The finding that the proportion of $OKT5^+$ cells was

Table 2. Peripheral blood lymphocyte phenotypes of patients with progressive systemic sclerosis (PSS)

		PSS Patients				
			D-penicillamine treatment		Activity	
Antibody	Control	Total	−	+	−	+
OKT3	74·9	74·1	71·0	77·3	74·2	73·5
	±8·8	±13·5	±20·7	±6·7	±15·1	±10·4
B67.1	78·9	76·9	74·1	82·4	77·8	76·4
	±10·2	±21·5	±15·4	±8·2	±14·1	±12·8
OKT4	48·0	53·6	50·5	58·3	53·4	53·6
	±10·0	±14·9	±17·1	±8·2	±14·5	±14·9
OKT5	22·7	18·7	18·4	17·0	18·4	18·8
	±6·6	±8·1	±9·9	±6·8	±9·7	±5·1
OKT8	29·6	22·8†	21·7	23·1	21·1	24·3
	±7·0	±7·7	±8·8	±5·4	±7·9	±0·9
B-1	9·6	13·3	12·1	14·2	13·4	14·2
	±3·9	±8·9	±10·9	±4·3	±10·1	±6·5
B33.1	13·5	23·2*	28·9	17·2*	21·3	27·2
	±3·7	±16·7	±19·3	±3·0	±11·1	±21·4

Numbers represent the mean percent ± standard deviation of positive fluorescing cells reacting with antibody standardized to an HLA positive control with background fluorescence subtracted out. Total PSS patients were compared with controls using a Student's *t*-test. Patients treated with D-penicillamine were compared with untreated patients and patients with active disease were compared to those with quiescent disease using a Mann-Whitney test.

* $P < 0{\cdot}05$.

† $= P < 0{\cdot}01$.

Table 3. NK cell markers from PSS peripheral blood lymphocytes

		PSS Patients				
			D-penicillamine treatment		Activity	
Antibody	Control	Total	−	+	−	+
B73.1*	15·9 ±5·8	10·4§ ±4·7	10·1 ±5·0	11·2 ±4·1	9·1 ±5·3	11·6 ±6·6
OKT8-OKT5†	5·8 ±3·5	3·1‡ ±2·7	1·9 ±1·3	4·4 ±4·2	2·7 ±2·4	3·4 ±3·3

* Numbers represent % positive fluorescing cells as in Table 3.

† Numbers represent the means of % OKT5(+) cells subtracted from OKT8(+) cells. Tests were performed on total patients and patient subgroups as in Table 3.

‡ $P<0·05$.

§ $P<0·01$.

Table 4. Ratios of OKT4 to OKT5 and OKT8 in patients with progressive systemic sclerosis (PSS)

		PSS patients				
			D-penicillamine treatment		Activity	
Antibody	Control	Total	−	+	−	+
OKT4/OKT8	1·66* ±0·5	2·74‡ ±1·3	2·78 ±1·6	2·70 ±0·8	2·93 ± ·6	2·38 ±1·0
OKT4/OKT5	2·12 ±0·6	3·59† ±2·2	3·70 ±2·4	3·35 ±1·6	3·95 ±2·5	3·13 ±1·3

* Numbers represent mean ratios of percent positive fluorescing cells as calculated in Table 3. Tests were performed on total patients and patient subgroups as in Table 3.

† $P<0·05$.

‡ $P<0·01$.

normal in patients (except those on D-penicillamine) whereas the proportion of $OKT8^+$ cells was decreased, might therefore indicate a decrease in NK cells. This was in fact confirmed using the NK-specific antibody B73.1, which showed that NK cells were decreased in all patient groups (Table 3). By subtracting $OKT5^+$ from $OKT8^+$ cells, the percentage of $OKT8^+$ NK cells can be calculated. As can be seen in Table 3, in normal controls the proportion of these cells was 5·8 whereas in PSS patients it was 3·1, a significant difference. Patients not on D-penicillamine treatment who had $OKT8^+$–$OKT5^+$ percentages of 1·9 probably accounted for this difference.

The percentage of B cells, as detected by the antibody B-1 (Table 2), demonstrated an upward trend in patients which was uninfluenced by D-penicillamine or disease activity. In contrast the anti-Ia antibody B33.1 demonstrated an increased proportion of Ia^+ cells in patients (Table 2). This increase was particularly marked in patients not receiving D-penicillamine and in fact there was a statistical difference between treated and untreated patients.

Ratios of OKT4$^+$ to OKT5$^+$ and OKT8$^+$ cells. Table 4 demonstrates the relationships between inducer and suppressor/cytotoxic cell subsets in PSS patients vs controls. The mean OKT4$^+$/OKT8$^+$ ratio was 1·66 in controls vs 2·74 in patients which was a significant difference. This difference was also found for OKT4$^+$/OKT5$^+$ with mean ratios of 2·12 for controls and 3·59 for total patients. Despite the lack of statistical significance for OKT4 and OKT5 staining cells among patients vs controls (although trends towards an increase in OKT4$^+$ and decrease in OKT5$^+$ were apparent) when the OKT4/OKT5 ratio was analysed an imbalance of inducer and suppressor/cytotoxic cells in PSS became apparent. This imbalance was present regardless of D-penicillamine use or disease activity.

Activation markers on PBL. As discussed above and shown in Table 2, although the proportion of B1$^+$ cells showed a trend to be increased in the total PSS population, Ia$^+$ cells were clearly elevated in patients as a whole. Since among lymphocytes the Ia marker is found both on B cells and activated T cells (Winchester & Kunkel, 1979), the number of Ia$^+$ T cells can be calculated by subtracting B-1$^+$ cells from B33.1$^+$ cells. In controls this value was below 5% as shown in Table 5. In contrast, PSS patients had a mean value of 10·3%. Of particular interest was the finding that D-penicillamine treated patients regardless of disease activity uniformly demonstrated normal levels of Ia$^+$ T cells (3·0%) and this was highly statistically different when compared to untreated patients (15·76). Anti-Tac labelling in contrast was elevated above normal in both treated (17·1%) and untreated patients (20·01%). Likewise 5E9 which is directed against transferrin receptor on activated T cells was high in patients (8·5%) compared with normals (1·1%) and did not differ in treated or untreated patients.

Activation markers in rheumatoid arthritis patients treated with D-penicillamine. Our finding that Ia expression on T cells was diminished in D-penicillamine-treated patients while Tac$^+$ and 5E9$^+$ cells remained unchanged prompted us to examine another group of D-penicillamine-treated patients to determine if this was a more generalized effect of D-penicillamine. The results of this analysis are shown in Table 6. The PBL of 17 patients with RA, five of whom were receiving D-penicillamine, were studied. Untreated RA patients similar to PSS patients also had elevated percentages of Ia$^+$, Tac$^+$, and 5E9$^+$ cells. In D-penicillamine-treated RA patients only the Ia activation marker was found to have significantly decreased expression while Tac and 5E9 expression remained elevated.

Table 5. Activation markers on peripheral blood lymphocytes of patients with progressive systemic sclerosis (PSS)

		PSS Patients				
			D-penicillamine treatment		Activity	
Antibody	Control	Total	−	+	−	+
B33.1-B-1	3·9*	10·3§	15·7	3·0¶	7·9	13·0
	±3·2	±7·3	±7·0	±2·9	±6·8	±9·1
TAC	6·6†	18·2‡	20·0	17·1	17·7	18·6
	±4·2	±11·7	±12·1	±10·8	±9·1	±12·5
5E9	1·1†	8·5§	9·4	6·0	7·9	11·4
	±1·2	±5·7	±7·2	±3·3	±4·1	±7·1

* Numbers in this row represent the means of % B1(+) positive cells subtracted from B33.1(+) cells. Tests were performed on total patients and patient subgroups as in Table 3.

† Numbers in these rows represent mean % positive fluorescing cells as in Table 3.

‡ $P<0·05$.

§ $P<0·01$.

¶ $P<0·001$.

Table 6. Phenotype of peripheral blood lymphocytes from rheumatoid arthritis patients

Patients	*n*	B-1	B33.1	B33.1-B-1	TAC	5E9
RA untreated	12	11·6* ±5·1	22·6 ±10·7	10·9 ±8·8	11·2 ±8·4	8·2 ±8·6
RA D-penicillamine treated	5	12·1 ±8·9	14·7 ±11·2	2·6† ±1·8	8·4 ±8·2	7·1 ±8·8

* Mean % positive fluorescing cells as in Table 3. B33.1-B-1 was calculated as in Table 6. Treated patients were compared to controls using a Student's *t*-test.

† $P < 0.05$ treated compared to untreated patients.

DISCUSSION

Activated PBL-T cells and abnormalities in lymphocyte subsets were found in PSS patients. HLA-DR expression was statistically increased in patients when compared to controls. This was only partially represented by a trend of increased B cells in patients. It was not due to monocyte contamination since adherent cells were removed and a lymphocyte window that excluded >98% of esterase positive cells was used to measure fluorescence. The cells in this window were >99% non-specific esterase stain negative. Also it is unlikely that this Ia elevation was due to the presence of dendritic cells since in individual patients the sum of B36.1 (T cell), B-1 (B cell) and B73.1 (NK cell) staining cells was frequently close to 100% (data not shown). In normals there was a small population of circulating Ia^+ T cells (<5%). The percentage of Ia^+ T cells was increased in PSS patients which was not statistically influenced by disease activity although active patients tended to have higher percentages. This finding of peripheral Ia^+ T cells was not completely surprising since Roum *et al.* (1984) reported Ia^+ T cells in skin lesions from PSS patients. An unexpected finding was that D-penicillamine-treated patients uniformly had percentages of Ia^+ T cells within normal range. Two other markers of T cell activation, Tac and 5E9 were elevated in all subgroups of patients and D-penicillamine did not affect the level of expression of these antigens. These antigens also elicit a low intensity fluorescence probably due to low levels of cell surface expression. Since the anti-Tac antibody is an IgG_{2a}, care was taken to label cells at 4°C in 5% plasma to prevent nonspecific FcR-mediated binding.

The finding of activated circulating T cells in PSS patients, even those with quiescent disease, further illustrates that PSS involves a systemic immune response. The fact that patients with quiescent disease express activation markers on their circulating T cells suggests the occurrence of an ongoing immune response to some antigens despite clinical stabilization of the sclerotic process. Thus, it can be postulated that although the abnormal regulatory mechanism of collagen production has apparently been modulated, a putative initiating process or event may still be present. Circulating activated mononuclear cells could produce mediators of collagen synthesis that affect susceptible fibroblasts even in the absence of local cellular infiltrates. In fact mononuclear cell infiltrates can only be demonstrated in 50% of PSS involved skin biopsies (Roum *et al.*, 1984; Hibbs *et al.*, 1983). In accordance with this possibility it has been shown that serum from PSS patients but not normals can enhance the proliferation of fibroblasts (Potter *et al.*, 1984).

The therapeutic efficacy of D-penicillamine in PSS (Steen *et al.*, 1982) and RA (Multicentre Trial Group, 1973) patients has been established. The exact mechanism of action of this drug is unknown. There is data which show that D-penicillamine inhibits the cross-linkage of Type I collagen *in vitro* (Nimni & Bavetta, 1965). This produces a less stable form of collagen and makes it more accessible to degradation. Lipsky (1984) has found *in vitro* that D-penicillamine interferes with T cell proliferation, possibly through the generation of hydrogen peroxide. This effect of T cells did not occur, however, using *ex vivo* lymphocytes from D-penicillamine-treated rats (Liyanag & Curry, 1972) in which proliferation to mitogenic stimuli actually increased after therapy. The effect of D-

penicillamine on humoral immunity does not appear to be striking, at least in RA, although rheumatoid factor may fall (Stanworth & Hunneyball, 1979).

Our data suggest another mechanism by which D-penicillamine might be effective in autoimmune diseases. In PSS we have shown that treated patients have a marked decline in Ia^+ T cells. This was unrelated to disease activity and appeared to be specific for the Ia antigen among activation markers since two other such markers, TAC and 5E9, were unaffected by therapy. The decrease in Ia^+ T cells caused by D-penicillamine was not unique for PSS patients, since we found that RA patients who also had a high percentage of circulating Ia^+ T cells compared with normals, similarly displayed decreased levels of Ia^+ T cells after treatment with D-penicillamine. This work is consistent with a previous study by Burmeister *et al.* (1981) which demonstrated a lack of Ia^+ T cells in peripheral blood of D-penicillamine treated RA patients. Human HLA-DR has been found on both mitogen and antigen stimulated T cells (Yu *et al.*, 1980). Although the function of the Ia molecule on T cells is uncertain, elimination of Ia^+ T cells can abrogate the ability of T cells to generate helper activity in mixed lymphocyte reactions (Winchester *et al.*, 1979). It has recently been reported that the L3T4 molecule on inducer T cells may stabilize the interaction of these cells with Ia^+ antigen-presenting accessory cells (Marrack *et al.*, 1983). It is possible therefore, that the Ia^+ molecule on T cells could also bind to L3T4 on other T cells and likewise enhance an otherwise low affinity reaction. D-penicillamine may therefore be able to modulate T cell–T cell interactions in which this binding is important.

Although the percentages of total circulating T cells in PSS patients was similar to controls, abnormalities were found in patient T cell subsets. Specifically, $OKT8^+$ cells were low and the $OKT4^+/OKT8^+$ and $OKT4^+/OKT5^+$ ratios which have been thought to roughly reflect an inducer to suppressor/cytotoxic index, were raised in patients. These differences were not a function of disease activity or D-penicillamine treatment as seen in Tables 3 and 5. The above findings agree with Whiteside *et al.* (1983) and Roum *et al.* (1984) who examined untreated patients only. We furthermore noted a decrease in NK cells (which can produce the collagen inhibitor γ-IFN; Trinchieri *et al.*, 1984; Jimenez *et al.*, 1984) in patients. The meaning of this finding will require further investigation.

In conclusion, an abnormally high percentage of peripheral blood T cells in PSS patients appear to be activated as shown by several surface markers. Also an abnormal distribution of PBL subpopulations was noted in patients including a low number of NK cells. These data reinforce the concept of an important immunological component in the pathogenesis of PSS. Furthermore, D-penicillamine may affect function in autoimmune diseases by modulating the expression of T cell Ia expression.

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