

34. Takagi S, Yoshida T, Tsubata K. An investigation of the materno-fetal hormonal milieu with special emphasis on the maternal influence. In: Nataka Y, Suzuki S, eds. Biological aspects of the fetus. Baltimore: University Park Press, 1987.
35. Simon M, Jouet JP, Demory JL, Pollet JP, Bauters F. Une cause meconnue de polyglobulie: le traitement anabolisant prolongé. Presse Med 1986;15:396.
36. Chappatte O, De Swiet M. Hereditary angioneurotic oedema and pregnancy: case reports and review of the literature. Br J Obstet Gynaecol 1988;95:938-42.
37. Cugno M, Cicardi M, Frangi D, et al. Plasma levels of C1 inhibitor complexes and modified inactive C1 inhibitor in hereditary angioedema [Abstract]. Complement 1988;5:181.

Altered T cell subpopulations and lymphocytes expressing natural killer cell phenotypes in patients with progressive systemic sclerosis

**Marianne Frieri, MD, PhD,* Channabasappa Angadi, PhD,*
Albert Paolano, MD,* Neill Oster, MD,* Sheldon P. Blau, MD,**
Sharon Yang, MS,* Carol Mele, BS,*** and Eugenia Hawrylko, MD*****
East Meadow and Brooklyn, N.Y.

Scleroderma (progressive systemic sclerosis [PSS]) is known to be associated with abnormal T cell immunoregulation. In the present study, we evaluated lymphocyte phenotypes in patients with PSS and normal control subjects by flow cytometry and monoclonal antibodies for total T (CD3), T suppressor (CD8), T helper (CD4), T helper-inducer (CDw29), T suppressor-inducer (CD45R), human leukocyte antigen, DR+B (CD19), DR+T, and natural killer subsets, HNK-1 (CD57) and NKH-1 (CD56) cells. Patients with PSS compared to normal subjects had significantly lower percentages of CD3+ (p < 0.005) and CD8+ (p < 0.05) (similar to several patients with rheumatoid arthritis also evaluated), as well as CD45R (p < 0.05), T+DR+ (p < 0.05), and NKH-1 (CD56) (p < 0.0005) cells. Patients with PSS with late-limited or generalized disease had lower percentages of CD8+, CD19, NKH-1+, and CDw29, but higher percentages of CD4+, HNK-1, and CD45R cells compared to patients with early stage disease, but these results were not statistically significant. These unique alterations in patients with PSS may prove to be useful in monitoring the stage of disease activity for therapy and further define immunologic defects. (J ALLERGY CLIN IMMUNOL 1991;87:773-9.)

From the Departments of *Pathology and **Medicine, Nassau County Medical Center of the State University of New York at Stony Brook, East Meadow, and ***Department of Allergy and Immunology, Long Island College Hospital, Brooklyn, N.Y.

Supported in part by a grant from the Scleroderma Society.

Presented in part at the Forty-fourth Annual Meeting of the American Academy of Allergy and Immunology, Anaheim, Calif., March 11-16, 1988.

Received for publication Jan. 15, 1990.

Revised Nov. 13, 1990.

Accepted for publication Nov. 21, 1990.

Reprint requests: Marianne Frieri, MD, Director of Clinical Immunopathology and the Allergy Immunology Training Program, Nassau County Medical Center, 2201 Hempstead Turnpike, East Meadow, NY 11554.

1/1/26920

Abbreviations used

Ia:	Immune response gene-associated
PSS:	Progressive systemic sclerosis
NK:	Natural killer
RA:	Rheumatoid arthritis
CREST:	Calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, and telangiectasia
HBSS:	Hanks' balanced salt solution
FITC:	Fluorescein isothiocyanate
MAb:	Monoclonal antibody
PBMC:	Peripheral blood mononuclear cell

TABLE I. Patient population

	CREST (N = 7)	PSS	
		Early (N = 12)	Late (N = 5)
Sex (M/F)	1/6	4/8	1/4
Mean age (range) (yr)	19-61	20-69	40-66
Organ involvement* (%)			
Skin†	100 (7)	83 (10)	80 (4)
Esophagus	100 (7)	42 (5)	80 (4)
Lung	57 (4)	42 (5)	60 (3)
Heart	14 (1)	8 (1)	20 (1)
Gastrointestinal	14 (1)	0 (0)	20 (1)

*Percent of patients with organ involvement; number of patients in parentheses.

†Sclerodactyly exclusive of Raynaud's phenomenon.

PSS, or scleroderma, is a clinically variable disease of the connective tissue characterized by cutaneous edema and inflammation with a predominant T-lymphocyte-mononuclear infiltrate¹ and excess collagen deposition that can progress to major organ sclerosis.

T cells involved in cellular immune reactions encompass an array of subsets that mediate a variety of immunoregulatory functions.^{2,3} They are directly involved in the production of cytokines, such as interleukins,⁴ which are signals for clonal expansion of T helper cells (CD4⁺), T suppressor cells (CD8⁺), the more recently identified T helper-inducer cells, CD4⁺4B4⁺ (CDw29), and T suppressor-inducer cells, CD4⁺2H4⁺ (CD45R⁺), and NK cells. NK cells are heterogeneous and include HNK-1 (CD57) cells identified as medium to large lymphocytes with azurophilic granules, NKH-1 (CD56) cells capable of suppressor and nonmajor histocompatibility complex cytotoxic activity in some immune responses, and CD8⁺ human large granular lymphocytes.⁵ An elevation in the number of peripheral blood T cells bearing Ia antigens has been described in patients with autoimmune diseases⁶ that may indicate a selective activation of the immune system. In addition to activated T-lymphocytes, antigen-presenting cells and B-lymphocytes express Ia antigens. Antigen presentation by Ia⁺ B cells can allow for interaction and subsequent activation of CD4 cells.⁷

Earlier studies of patients with PSS used Fc receptors for IgG and IgM to identify T-γ (suppressor) and T-μ (helper) cells, respectively, and produced conflicting results.^{8,9} Gupta et al.⁸ demonstrated an increase in T-γ (suppressor) cells but a decrease in T-μ (helper) cells, whereas Inoshita et al.⁹ reported a

reduction in T-γ cells. More recent studies of patients with PSS used a limited panel of MAbs and reported decreased percentages of CD8 cells and increased CD4/CD8.^{10,11} In this study, we evaluated lymphocyte phenotypes from patients with PSS and RA by flow cytometry with a broad panel of MAbs that included the more recently developed anti-CDw29 (4B4⁺, T helper-inducer cell) and anti-CD45R (2H4⁺, T suppressor-inducer cell), as well as MAbs for DR⁺T and DR⁺B cells and two NK subsets, HNK-1 (CD57) and NKH-1 (CD56).

MATERIAL AND METHODS

Patient and control population

Twenty-four patients with PSS and 11 with RA, as defined according to the criteria from the American Rheumatism Association,^{12,13} were referred by board-certified rheumatologists for inclusion in this study. The protocol was approved by the Nassau County Medical Center and Long Island College Hospital Institutional Review Board. Informed consent was obtained from all subjects. The demographics of the population are presented in Table I. The 24 patients with PSS included 18 female and six male patients, age range from 19 to 69 years. The spectrum of disease ranged from early disease, including PSS with the CREST variant or overlap syndrome, including mixed connective tissue disease to late diffuse disease with multiple organ involvement.

Patients with early disease were divided into patients with limited disease, less than 3 years of duration, and moderate or diffuse disease affecting the face, trunk, and proximal and distal portions of the extremities. Patients with late disease more than 5 years of duration with progressive systemic organ involvement were classified as having late-limited or late-generalized disease. Seven patients had the CREST variant or overlap syndrome, nine had early limited disease, three had early moderate or diffuse disease, and five patients had late-limited or generalized disease. One hundred percent of patients with CREST had skin and esophageal involvement with less involvement of other organs. Patients with late-stage disease had increased involvement of the lung, heart, and gastrointestinal tract. Pulmonary abnormalities included decreased diffusion capacity, pulmonary fibrosis, recurrent pneumonia, restrictive lung disease, or granulomatous disease. Cardiac involvement consisted of congestive heart failure, aortic insufficiency, and fibrocalcification. One patient with CREST had hiatal hernia, and one patient with late-phase disease had colonic sclerosis. No patient with PSS had renal involvement. Twenty patients were receiving medications, including 40 mg/day of prednisone (one patient with late-stage disease), nonsteroidal anti-inflammatory drugs (five patients with early stage disease), H₂ antagonists (three with early and three with late-stage disease), penicillamine (one with early and one with late-stage disease), colchicine (three with early-stage disease), and antihypertensive therapy (seven patients with early and one with late-stage disease). For comparison, 11 patients with RA were evaluated. Patients

ranged in age from 56 to 75 years (stage I to III) and included 10 female and one male patient. Two female patients had hypothyroidism, and one male patient had type II diabetes. None were receiving immunosuppressive therapy or corticosteroids. Age- and sex-matched healthy donors from the Nassau County Medical Center Blood Bank served as control subjects for all studies.

Separation of lymphocytes

PBMCs were isolated from heparinized blood by standard Ficoll-Hypaque gradient centrifugation.¹⁴ Briefly, 3 ml of whole blood was mixed with 3 ml of HBSS, layered onto 5 ml of Ficoll-Paque (Pharmacia-LKB Biotechnology, Inc., Piscataway, N.J.), and centrifuged at 400 g for 40 minutes at room temperature. The layer of PBMCs was transferred to another tube and washed two times with HBSS. Cells were counted and viability was assessed by trypan blue dye exclusion. The cell concentration was adjusted to 1×10^7 cells per milliliter before staining.

Staining and cell analysis

Washed cells (5×10^5) in 50 μ l of HBSS were incubated on ice in the dark for 30 minutes with saturated amounts of fluorescein and phycoerythrin-conjugated MAbs: Leu-3a FITC/Leu-2a PE (CD4/CD8), Leu-4 FITC/Leu-12 PE (CD3/CD19), Leu-4 FITC (CD3) HLA DR PE, Leu-7 FITC (CD57), Leu-19 (CD56) goat antimouse immunoglobulin FITC (Becton Dickinson Immunocytometry System, San Jose, Calif.), and T4 FITC/4B4 RD1 (CDw29⁺) and T4 FITC/2H4 RD1 (CD45R⁺) (Coulter Immunology, Hialeah, Fla.). CD56 was stained by an indirect method with goat antimouse immunoglobulin FITC as a second-step reagent. The 4B4 RD1 (CDw29⁺) and 2H4 RD1 (CD45R⁺) reagents were always in conjunction with T4 FITC and quantitated as double positives by dual parameter-correlated analysis. Cells were washed twice with phosphate-buffered saline containing 0.1% sodium azide. Stained cells were fixed in 100 μ l of 1% paraformaldehyde and stored on ice until analysis.¹⁵

Flow cytometric analysis of cells was performed within 24 hours of collection with the fluorescence-activated cell sorter analyzer (Becton Dickinson) connected to a Consort 30 data-management system. Approximately 10,000 cells were analyzed by dual color analysis for each pair of MAbs with appropriate instrument calibration and controls.

Statistics

Statistical analysis of the differences between groups was determined by the Student's *t* test; *p* values <0.05 were considered significant.

RESULTS

Phenotypes of lymphocytes from patients with PSS

Lymphocyte cell-surface markers in patients with PSS and normal control subjects are summarized in Tables IIA and IIB.

Patients with PSS had significantly decreased percentages of CD3 (total T cells), CD8 (suppressor T cells), T⁺DR⁺, CD56 (NKH-1), and CD45R (suppressor-inducer T cells) cells compared to that of control subjects (Table IIA). In contrast, patients with PSS had significantly increased percentages of CD4⁺, CD19, and CD57 (HNK-1) cells and ratios of CD4/CD8 cells. Results comparing *p* values for percentages and absolute cell numbers for CD8, T⁺DR⁺, B⁺DR⁺ (CD19), HNK-1 (CD57), and NKH-1 (CD56) did not differ statistically (Tables IIA and IIB). However, absolute numbers, but not percentages of CDw29 cells from patients with PSS, were significantly depressed compared to that of control subjects (*p* < 0.025), whereas the percentage, but not absolute values, for CD45R was significantly depressed (Tables IIA and IIB). Although loss of certain cell subpopulations can occur with Ficoll-Hypaque separation, we believe the supporting literature confirms our findings.

When patients with PSS were grouped into early (including the CREST) and late-stage disease and compared against each other, the latter demonstrated lower percentages of CD8⁺, CD19, CD56, and CDw29 (4B4) cells (Table III). In contrast, patients with late-stage disease tended to have elevated percentages of CD4⁺ and CD45R (2H4) phenotypes compared to patients with early stage disease. The number of patients with late-stage disease for T⁺DR⁺, CD19⁺, and HNK-1 cells was one less compared to Table IIA because of insufficient cell numbers. An additional patient with late-stage disease not analyzed in Table IIA was added for CD4/CDw29⁺ and CD4/CD45R⁺ cells in Table III. None of these comparisons were statistically significant in the small population evaluated.

Phenotypes of lymphocytes from patients with RA

Lymphocytes from patients with RA were evaluated with the same panel of MAbs used to study patients with PSS. Results for the group with RA as a whole, as well as results grouped by stages, were compared with normal control subjects (Table IV). Similar to patients with PSS, the percentage of CD3⁺ and CD8⁺ cells was significantly lower in patients with RA as a group compared to that of a normal group (*p* < 0.05). However, in contrast to results in patients with PSS (Table IIA), patients with RA as a group had significantly elevated HNK-1⁺ cells and especially at the earlier stage of disease (Table IV). Although the percentage of CD4⁺, T⁺DR⁺, CD45R⁺ (2H4) tended to increase with disease progression, this was not statistically significant compared to that of

TABLE IIA. Phenotypes of lymphocytes in PBMCs from patients with PSS and normal control subjects

Phenotype	No. of patients with PSS/C	PSS (%)*	Control subjects (%)*	<i>p</i> †
CD3 ⁺	17/15	63.3 ± 3.3	73.0 ± 1.6	0.005
CD8 ⁺	24/20	19.6 ± 2.1	30.1 ± 1.8	0.05
CD4 ⁺	24/20	47.6 ± 2.0	42.6 ± 1.8	0.05
CD4/CD8	24/20	3.1 ± 0.3	1.5 ± 0.1	0.01
T ⁺ DR ⁺	15/15	4.0 ± 0.4	6.2 ± 1.1	0.05
B ⁺ DR ⁺ (CD19 ⁺)	15/15	11.0 ± 1.6	8.4 ± 0.7	0.05
CD57 ⁺ (HNK-1)	14/15	10.5 ± 1.5	5.7 ± 0.5	0.025
CD56 ⁺ (NKH-1)	8/20	9.5 ± 1.5	21.0 ± 2.1	0.0005
CD4 ⁺ /CDw29 ⁺	8/20	21.0 ± 1.3	25.8 ± 1.8	0.25
CD4 ⁺ /CD45R ⁺	7/6	5.7 ± 0.8	7.0 ± 0.2	0.05

*Values are the mean ± SE.

†*p* Values by Student's *t* test.**TABLE IIB.** Phenotypes of lymphocytes in PBMCs from patients with PSS and normal control subjects

Phenotype	No. of patients with PSS/C	PSS (Abs. No.)*	Control subjects (Abs. No.)*	<i>p</i> †
CD3 ⁺	7/17	1209 ± 119	1170 ± 108	0.25
CD8 ⁺	13/20	412 ± 32	570 ± 54	0.025
CD4 ⁺	13/20	841 ± 86	850 ± 45	0.25
CD4/CD8	13/20	2.6 ± 0.4	1.6 ± 0.13	0.01
T ⁺ DR ⁺	7/7	122 ± 29	110 ± 25	0.25
B ⁺ DR ⁺ (CD19 ⁺)	7/7	210 ± 27	124 ± 17	0.05
CD57 ⁺ (HNK-1)	7/7	241 ± 63	148 ± 15	0.025
CD56 ⁺ (NKH-1)	6/9	179 ± 46	424 ± 50	0.0025
CD4 ⁺ /CDw29 ⁺	6/13	382 ± 31	848 ± 98	0.025
CD4 ⁺ /CD45R ⁺	6/10	176 ± 36	149 ± 21	0.25

Abs, Values are the absolute mean number ± standard error per cubic millimeter.

*Values are the mean ± SE.

†*p* Values by Student's *t* test.

the control subjects (*p* < 0.1) for the small samples studied.

DISCUSSION

Abnormalities in the immune response have been suspected in the pathogenesis of PSS because of the presence of autoantibodies,¹⁶ decreased percentage of CD8 with increased CD4 activity,¹⁷ altered cytokine production, and clinical and laboratory evidence of overlap with other autoimmune diseases.^{18, 19}

In this study we used an expanded panel of MAbs to identify the percentage according to disease stage and absolute numbers of lymphocyte phenotypes in patients with PSS. In addition to the standard T cell MAbs for CD3, CD4, and CD8 cells, we included

MAbs for DR⁺ T and B cells, two distinct NK phenotypes, HNK-1 and NKH-1, and for CDw29 (helper-inducer T cell) and CD45R (suppressor-inducer T cell) phenotypes. We not only confirmed that the percentage of CD8 cells are decreased in patients with PSS (Table II), as reported earlier,¹⁰ but also, in addition, demonstrated with recently developed MAbs that subdivide T cell phenotypes into CD45R (2H4⁺, naive, and suppressor-inducer T cells) and CDw29 (4B4⁺ memory, and helper-inducer T cells)²⁰ that the percentage of CD45R and absolute number of CDw29 cells were significantly decreased in patients with PSS (Tables IIA and IIB).

The decreased percentage of DR⁺ T cells and increased percentages of DR⁺ B cells (Table II) is of

TABLE III. Lymphocyte phenotypes from patients with early versus late-stage PSS

Phenotype	Stage			
	Early		Late	
	No. of patients	(%)*	No. of patients	(%)*
CD8 ⁺	19	21.3 ± 2.9	5	15.8 ± 2.4
CD4 ⁺	17	45.1 ± 2.5	7	51.8 ± 4.0
T ⁺ DR ⁺	10	4.1 ± 1.3	4†	4.0 ± 0.4
B ⁺ DR ⁺ (CD19 ⁺)	10	11.8 ± 2.3	4†	9.4 ± 2.4
CD57 ⁺ (HNK-1)	10	9.6 ± 1.7	3‡	10.1 ± 1.2
CD56 ⁺ (NKH-1)	5	9.7 ± 1.2	3	7.6 ± 2.8
CD4 ⁺ /CDw29 ⁺	6	21.3 ± 2.4	3‡	16.0 ± 3.5
CD4 ⁺ /CD45R ⁺	5	5.2 ± 0.4	3‡	10.6 ± 3.6

$p < 0.1$ for all results by Student's *t* test.

*Values are the mean ± SE.

†Number was one less compared to Table IIA.

‡Number was one more compared to Table IIA.

TABLE IV. Lymphocyte phenotypes from patients with RA and normal control subjects

Phenotype	Total (%)		Stage (%)		
	Control subjects (N = 6)	With RA (N = 11)	I Early (N = 2)	II Moderate (N = 3)	III Late (N = 6)
CD3 ⁺	73.1 ± 2.2	64.1 ± 2.9*	—	63.0 ± 3.0	64.8 ± 4.9
CD8 ⁺	31.4 ± 2.5	21.0 ± 2.6*	27.5 ± 6.0	19.7 ± 2.1	24.4 ± 3.2
CD4 ⁺	42.6 ± 2.4	45.1 ± 4.2	30.9 ± 0.5	42.0 ± 5.6	52.5 ± 4.3
T ⁺ DR ⁺	5.9 ± 1.2	6.0 ± 1.6	—	4.1 ± 1.1	7.3 ± 2.4
CD19 ⁺	6.4 ± 0.7	8.8 ± 1.8	—	5.5 ± 0.5	10.9 ± 2.2
CD4 ⁺ /CD45R	7.0 ± 0.2	8.6 ± 1.9	4.5	—	10.0 ± 2.0
CD57 ⁺ (HNK-1)	6.2 ± 2.0	9.5 ± 3.0	24.8	10.5 ± 5.2	9.3 ± 2.3

* $p < 0.05$.

interest in view of studies in patients with systemic lupus erythematosus revealing decreased percentages of DR⁺ T cells.²¹ Perhaps activated autologous-mixed lymphocyte reactions may allow T cell producing cytokines to induce differentiation of autoantigen-activated B cells.²²

When patients with PSS were grouped according to their disease stage, patients with late-stage disease tended to have fewer percentages of CD8, CD19, NKH-1, and CDw29 cells compared to that of patients with early stage disease (Table III), although these results were not significant for the small group studied. In contrast, patients with early stage disease tended to have a decrease in their CD45R cells (Table III).

RA is a multisystemic, inflammatory disease associated with excessive production of autoantibodies resulting from abnormal immunoregulatory T cell function and B cell hyperactivity.²³ We demonstrated that patients with RA as a group, like patients with

PSS, had significantly depressed percentages of CD8 cells compared to that of normal subjects but significantly elevated HNK-1 cells, especially in the early stage of the disease (Table IV). A trend toward elevated percentages of CD4, T⁺DR⁺, and CD45R cells was also noted with later stages of the disease. Some authors have reported a relative depletion of CD8⁺ cells in association with RA disease activity and immune complex formation²⁴ or in vivo activation of Ia bearing T cells.²⁵ Goto et al.²⁶ demonstrated a marked decrease in CD8⁺/Leu-15⁺ ("suppressor T cells") cells in the peripheral blood of patients with active RA. In our patients with early stage RA, we confirmed the results of Emery et al.²⁷ who reported that the CD4⁺/CD45R subpopulation in patients with RA is depleted.

T cells that express the CD4 antigen tend to induce or amplify other cells, such as B cells, CD8⁺ suppressor T cells, and NK cells.²³ We have confirmed

a decrease in CD8 cells in patients with PSS (Table II) and RA (Table IV),²⁴ which is most dramatic in patients with PSS in the late-stage disease (Table III). Some investigators have postulated that the loss of CD8⁺ cells in PSS might be due to lymphocytotoxic antibodies or antilymphocyte antibody-blocking determinants.¹⁰

NK activity has been reported to be impaired in patients with PSS with extensive visceral disease and normal in those patients with mild PSS,²⁸ but the percentages of HNK-1 were found to be reduced only in early diffuse disease.²⁹ In our study we used two phenotypic markers, HNK-1 (large granular lymphocytes)³⁰ and NKH-1 (suppressor-cytotoxic cells) that define different cell populations and found both the percentage and absolute number of HNK-1 cells were significantly elevated in patients with PSS ($p < 0.025$), but NKH-1 cells were significantly decreased compared to that of control subjects ($p < 0.0005$ to < 0.0025) both in early and late stages (Tables II and III). We believe that the NKH-1 phenotypic data are more meaningful, since the MAb is produced against an interleukin-2-dependent, cloned NK cell line.

The T cell subsets identified by CDw29 antibodies (4B4, memory, and helper-inducer T cells) and CD45R (2H4, naive, and suppressor-inducer T cells) are assuming increasing importance in clinical medicine.²⁰ CDw29 molecules are apparently part of a viruslike agent family of adhesion molecules, and activation of CDw29 cells leads to secretion of large amounts of interferon and interleukin-3.^{20, 21, 31} The CDw29 subset proliferates poorly on stimulation with concanavalin A and autologous cell antigens (autologous mixed lymphocyte reaction) but well on exposure to soluble antigens, and it provides a good helper signal for pokeweed mitogen-induced immunoglobulin synthesis.² Cells with high-density CDw29 antigen are the principal cell type found in normal subjects in gut mucosa, skin, and on lung epithelial surfaces^{32,34} and are considered to be memory cells at sites of exogenous antigen exposure and/or homing to areas required for host defense.²⁰

The CD45R subset has been demonstrated to play a crucial role in the activation of CD8⁺ suppressor cells that can lead to suppression of B cell immunoglobulin synthesis.³⁵ Perhaps the decreased CD45R is implicated in NK function and cell-mediated lympholysis.²⁰ Interestingly, NKH-1 is likewise distributed on all resting and activated NK cells and comprise a unique subset of cytotoxic T-lymphocytes that mediate nonmajor histocompatibility complex-restricted cytotoxicity.³⁶ Deficiency of CD45R cells has been postulated to contribute to the pathogenesis of several autoimmune diseases, such as multiple sclerosis,³⁷

RA,²⁷ systemic lupus erythematosus, and Sjögren's syndrome.³⁸ Although our preliminary studies were performed in a small number of patients with PSS without corresponding functional data, CD45R cell phenotypes were significantly depressed, especially in patients with early stage disease, although elevated in patients with late-stage disease (Tables II and III).

Thus, as observed with other autoimmune diseases, a quantitative defect of suppressor CD8 and suppressor-inducers or CD45R cells may play some role in defective immunoregulation in PSS. The phenotypic markers reported here may eventually be useful in clinically stratifying patients and in monitoring therapeutic intervention.

REFERENCES

1. Kondo H, Rabin BS, Rodnan GP. Cutaneous antigen stimulating lymphokine production by lymphocytes of patients with progressive systemic sclerosis (scleroderma). *J Clin Invest* 1976;58:1388-94.
2. Morimoto C, Letvin NL, Distaso JA, Aldrich WR, Schlossman SF. The isolation and characterization of the human suppressor-inducer T cell subset. *J Immunol* 1985;134:1508-15.
3. Frieri M, Angadi CV, Paolano A, Mele C, Hawrylyk E. Immunoregulatory defects in scleroderma [Abstract]. *J ALLERGY CLIN IMMUNOL* 1988;81:196.
4. Talal N. Interleukins, interferon, and rheumatic disease. In: Williams R Jr, ed. *Clinics in rheumatic diseases*. Philadelphia: WB Saunders, 1985:633.
5. Lanier LL, Le AM, Phillips JH, Walner NL, Babcock GF. Subpopulations of natural killer cells defined by expression of Leu-7 (HNK-1) and Leu-11 (NKP-15) antigens. *J Immunol* 1983;131:1789-96.
6. Pincus SH, Clegg DO, Ward JR. Characterization of T cells bearing HLA-DR antigens in rheumatoid arthritis. *Arthritis Rheum* 1985;28:8-15.
7. Unanue ER, Beller DI, Christopher YLU, Allen PM. Antigen presentation. *J Immunol* 1984;132:1-5.
8. Gupta S, Malaviga AN, Rajagopalan P, Good RA. Subpopulations of human T-lymphocytes. IX. Imbalance of T cell subpopulations in patients with progressive systemic sclerosis. *Clin Exp Immunol* 1979;38:342.
9. Inoshita R, Whiteside T, Rodnan G, Taylor FH. Abnormalities of T-lymphocyte subsets in patients with progressive systemic sclerosis (PSS, scleroderma). *J Lab Clin Med* 1981;97:264-277.
10. Keystone EC, Lau C, Gladman DD, Wilkinson S, Lee P, Shore A. Immunoregulatory T cell subpopulations in patients with scleroderma using monoclonal antibodies. *Clin Exp Immunol* 1982;48:443-8.
11. Umehara H, Kumagai S, Ishida H, Sugimoto T, Maeda M, Imura H. Enhanced production of interleukin-2 in patients with progressive systemic sclerosis. Hyperactivity of CD4 positive T cells? *Arthritis Rheum* 1988;31:401-7.
12. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581-90.
13. Popes MW, Bennett GA, Cobb S, Jacob R, Jessar RA. 1958 revision of diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 1958;9:175-6.

14. Fotino M, Masow EJ, Allen FH. Micromethod for rapid separation of lymphocytes from peripheral blood. *Ann Clin Lab Sci* 1971;1:131.
15. Lanier LL, Warner NL. Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis. *J Immunol Methods* 1981;47:25.
16. Tan EM, Rodnan GP, Garcia I, et al. Diversity of antinuclear antibodies in progressive systemic sclerosis: anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum* 1980;23:617-25.
17. Whiteside TL, Kumagi Y, Roumm AD, Almendinger R, Rodnan GP. Suppressor cell function and T-lymphocyte populations in peripheral blood of patients with progressive systemic sclerosis. *Arthritis Rheum* 1983;26:841-7.
18. Whiteside TL, Worrall JG, Prince RK, Buckingham RB, Rodnan GD. Soluble mediators from mononuclear cells increase the synthesis of glycosaminoglycan by dermal fibroblast cultures derived from normal subjects and progressive systemic sclerosis patients. *Arthritis Rheum* 1985;28:188-97.
19. Sharp GC, Singen BH. Mixed connective tissue disease, arthritis, and allied conditions. In: McCarty DJ, ed. *Arthritis and allied conditions*. 10th ed. Philadelphia: Lea and Febiger, 1985.
20. Sanders ME, Makgoba MW, Shaw S. Human naive and memory T cells. *Immunol Today* 1988;9:195-9.
21. Sanders ME, Makgoba MW, Sharrow SO, et al. Human memory T-lymphocytes express increased levels of three adhesion molecules (LFA-3, CD2, LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN- γ production. *J Immunol* 1988;140:1401-7.
22. Theofilopoulos AN. Autoimmunity. In: Stites DP, Stobo JD, Wells JV, eds. *Basic and clinical immunology*. Norwalk, Conn., Los Angeles: Appleton & Lange, chap 11. 1987:147.
23. Alpert SD, Koide J, Takada S, Engelman EG. T cell regulatory disturbances in the rheumatic diseases. *Rheum Dis Clin North Am* 1987;13:431-45.
24. Queiros MV, Rocha B. T cell subsets in patients with rheumatoid arthritis reduction of cells with Leu-2A phenotype in patients with serum IgG immunocomplex. *Clin Exp Immunol* 1983;54:509.
25. Kluin-Nelemans HC, Vander Linden JA, Gmeligin-Meyling FHJ, et al. HLA-Dr+T lymphocytes in blood and synovial fluid in rheumatoid arthritis. *J Rheumatol* 1984;11:272-6.
26. Goto M, Mixamoto T, Nishioka K, et al. T cytotoxic and helper cells are markedly increased and T-suppressor and inducer cells are markedly decreased in rheumatoid synovial fluids. *Arthritis Rheum* 1987;30:737-43.
27. Emery P, Gentry KC, Mackay IR, Rowley M. Deficiency of the suppressor-inducer subset of T-lymphocytes in rheumatoid arthritis. *Arthritis Rheum* 1987;30:849-56.
28. Wright JK, Hughes P, Rowell RR. Spontaneous lymphocyte-mediated (NK cell) cytotoxicity systemic sclerosis. *Ann Rheum Dis* 1982;41:409-13.
29. Miller EB, Histerodt JC, Hunt LE, Steen V, Medsger TA. Reduced natural killer cell activity in patients with systemic sclerosis. *Arthritis Rheum* 1988;31:1515-23.
30. Abo T, Balch CM. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J Immunol* 1981;127:1024-9.
31. Morimoto C, Letvin NL, Boyd AW, et al. The isolation and characterization of the human helper-inducer T cell subset. *J Immunol* 1985;134:3762-9.
32. James SP, Fiocchi C, Graeff AS, Strober W. Phenotypic analysis of lamina propria lymphocytes: predominance of helper-inducer and cytolytic T cell phenotypes and deficiency of suppressor-inducer phenotypes in Crohn's disease and controlled patients. *Gastroenterology* 1986;91:1483-9.
33. Bos JD, Zonneveld I, Das PK, et al. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *J Invest Dermatol* 1987;88:569-73.
34. Saltini C, Winestock K, Kirby M, Crystal RG. Antigen-primed T cells expressing 180 kd form of CD45/leukocyte common antigen. Compartmentalized to the lung epithelial surface [Abstract]. *Clin Res* 1988;36:446.
35. Morimoto C, Matsuyama T, Rudol CE, et al. Role of the 2H4 molecule in the activation of suppressor-inducer function. *Eur J Immunol* 1988;18:731-7.
36. Lanier LL, Le AM, Civin CI, Likem MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NK-1) antigen expression on human peripheral blood NK cells and cytotoxic T-lymphocytes. *J Immunol* 1986;136:4480-5.
37. Morimoto C, Hafler DA, Weiner H, et al. Selective loss of the suppressor-inducer T cell subset in progressive multiple sclerosis. *N Engl J Med* 1987;316:67-72.
38. Sato K, Miyosaka N, Yamaoka K, Okuda M, Yata J, Nishioka K. Quantitative defect of CD4+2H4+ cells in systemic lupus erythematosus and Sjögren's syndrome. *Arthritis Rheum* 1987;30:1407-11.