Lymphocyte Phenotype and Function in Pseudolymphoma Associated with Sjögren's Syndrome

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ABSTRACT Lymph node (LNL) and salivary gland lymphocytes (SGL) from three patients with pseudolymphoma and primary Sjögren's syndrome (1^0SS) were characterized with monoclonal antibodies to demonstrate (*a*) a predominance of T cells (>80%) reactive with anti-T cell antibodies OKT4 (>70%) and OKT8 (<20%); (*b*) a high prevalence of activation antigens (>50% of cells reactive with antibody OKT10 and anti-Ia antibody); (*c*) polyclonal B cells (8–15% of all cells expressing kappa or lambda); and (*d*) a specific B cell subset defined by reactivity with antibody B532 that was not present in their peripheral blood. In vitro functional studies showed that both SGL and LNL provided T helper activity for immunoglobulin synthesis and that this activity could be abolished by treatment with antibody OKT4 plus complement. The SGL and LNL exhibited little natural killer, antibody-dependent cellular cytotoxicity, or cytotoxic T cell activity. Normal karyotype was observed in SGL, LNL, and peripheral blood lymphocytes (PBL) from these patients. These findings indicate that pseudolymphoma in 1^0SS results from the infiltration of salivary glands and extraglandular tissues by nonneoplastic T helper cells. Monoclonal antibodies provide an important tool to distinguish pseudolymphoma from non-Hodgkins (B cell) lymphomas that have a markedly elevated incidence in 1^0SS patients. Our finding of T helper cells in pseudolymphoma tissues supports the hypothesis that chronic stimulation of B cells by helper T cells leads to eventual escape of a malignant B cell clone.

INTRODUCTION

Epidemiologic evidence suggests that the frequency of non-Hodgkin's lymphoma is increased in patients with autoimmune disease as a result of chronic antigenic stimulation and lymphoproliferation (reviewed by Berard et al. [1]). A particularly high prevalence of non-Hodgkin's lymphoma has been noted in patients with Sjögren's syndrome (SS)[1] (1–3), a disease characterized by lymphocytic infiltration of salivary and lacrimal glands (4) leading to symptomatic dry eyes and mouth. Although the majority of SS patients have benign infiltrates with minimal glandular enlargement, a small number develop massive swelling of salivary glands (5, 6) and cervical lymphadenopathy (3–5) clinically suggestive of lymphoma. However, biopsy samples from these patients do not meet histologic criteria for frank malignancy and these infiltrates have been termed pseudolymphomas (3–6); these patients appear to be at particularly high risk for subsequent development of malignant lymphoma (1–6). The distinction between pseudolymphoma and lymphoma using routine histologic stains is often extremely difficult (2, 3). We have found that monoclonal antibodies aid in differentiating these tissue lesions.

The etiopathogenesis of SS remains unknown but the presence of polyclonal hypergammaglobulinemia, au-

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[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cellular cytotoxicity; ANA, antinuclear antibodies; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocytes sedimentation rate; FCS, fetal calf serum; PBL, peripheral blood lymphocytes; PFC, plaque-forming cells; PWM, pokeweed mitogen; RA, rheumatoid arthritis; SCRF, Scripps Clinic and Research Foundation; SGL, salivary gland lymphocytes; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; primary, 1^0SS; WBC, white blood cell count.

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toantibodies such as rheumatoid factor or antinuclear antibodies (ANA), and the association of SS with other autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or scleroderma (2–6) suggest an autoimmune process. The cells infiltrating the minor salivary glands of primary Sjögren's syndrome (1^0SS) patients are predominantly T cells (7–9), while the non-Hodgkin's lymphomas that develop in these patients are predominantly B cells (9–15). Therefore, the study of tissue from patients with pseudolymphoma is particularly important since it allows characterization of function and karyotype of lymphocytes in an autoimmune disease that appears to represent a transitional stage in the progression from benign lymphocytic infiltrates to lymphoma (2–5).

METHODS

Patients

All patients with 1^0SS were seen at Scripps Clinic and Research Foundation (SCRF) and fulfilled the following criteria: grade IV lip biopsy on a scale of I-IV by the Chisholm-Mason Scale (16); a Schirmer's test of ≤5 mm of wetting in each eye after 5 min; typical filamentary keratitis after rose-Bengal staining; and absence of criteria sufficient for a diagnosis of RA, SLE, or scleroderma (4). None of these patients had received corticosteroids or other immunosuppressive agents for at least 3 mo before evaluation of their lymphocyte subsets.

Salivary glands were surgically removed in three patients with 1^0SS because of recurrent salivary gland infection. In each of these cases, the diagnosis of malignant lymphoma was considered unlikely based on their clinical features. The extent of lymphocytic infiltration was much less marked than in the pseudolymphoma cases. Only 25–50% of the lobules were infiltrated with lymphocytes in contrast to the pseudolymphoma patients where all lobules were extensively involved. Histologically normal lymph nodes and parotid tissues were obtained from three patients without neoplastic or autoimmune diseases for comparison.

Case reports of pseudolymphoma patients

The term "pseudolymphoma" in SS patients refers to extensive lymphocytic infiltration with disruption of normal architecture in glandular tissues and/or extraglandular sites such as cervical lymph nodes or lung (3–5). The enlarged salivary glands and lymph nodes or pulmonary infiltrates fluctuate over the course of years and are not characterized by the progressive course expected of malignant lymphoma (3–6). Three cases that were clinically felt to represent pseudolymphoma and SS were seen at SCRF in the past 5 yr from a patient population of >70 patients with 1^0SS:

Case 1. This 54-yr-old white woman was first seen at SCRF in 1975 with massive bilateral parotid gland swelling (Fig. 1). She had a 30-yr history of keratoconjunctivitis sicca, xerostomia, and Raynaud's phenomenon. In 1960 she noted intermittent swelling of the left parotid and in 1973 persistent left parotid swelling and onset right parotid swelling. There was no evidence of RA, SLE, scleroderma, sarcoidosis, or malignancy. She had received prednisone (5–20 mg/d from 1974–1978) but no other immunosuppressive medications or

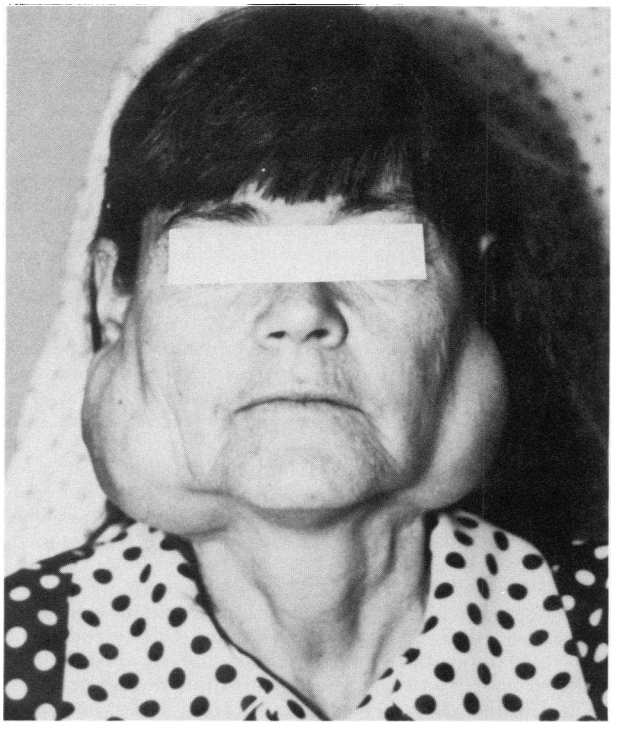

FIGURE 1 Massive bilateral parotid gland enlargement in patient I.R. with pseudolymphoma and Sjögren's syndrome.

radiation. Her physical examination revealed extreme drying of the oral and ocular tissues and small, mobile lymph nodes in the cervical and axillary regions. Laboratory studies in 1975 included hemoglobin 12.4 g%, white blood cell count (WBC) 6.4 (15% lymphs), erythrocytes sedimentation rate (ESR) 117 mm/h, IgG 1,267 mg%, IgA 253 mg%, IgM 238 mg%, a normal protein electrophoresis, and a normal urinalysis. Rheumatoid factor was positive (1:640) and ANA was 1:64 (speckled) with negative SS-A, SS-B, and anti-DNA antibodies (17). A chest x ray obtained in 1978 showed a nodular lesion in the right middle lobe that was evaluated by bronchoscopy to demonstrate lymphocytic infiltrates consistent with pseudolymphoma. Progressive enlargement of the parotid glands, cervical, and axillary lymph nodes occurred from 1978 to 1981, resulting in the removal of left parotid gland (1978), right parotid gland, and right axillary lymph node (in June 1981) and repeat axillary lymph node biopsy (in November 1981). The tissues removed in 1981 were evaluated using monoclonal antibodies. Laboratory studies in 1981 revealed a fall in her serum IgG level to 420 mg%, IgM 140 mg%, IgA 170 mg% (with no monoclonal spike on electrophoresis), hemoglobin to 8 g% and WBC to 2.9 (10% lymphocytes). Bone marrow aspiration on two separate occasions showed no evidence of lymphoma, and an extensive search for occult infection including *Mycobacterium tuberculosis*, and malignancy was negative. In October 1982, she was begun on prednisone (100 mg/m^2) and cytoxan (400 mg/m^2 for days 1–5 every 4 wk for six courses) because of continued weight loss, fever, and the development of pleural effusions and vasculitic skin lesions unresponsive to corticosteroids alone. These symptoms initially improved but in February 1983 a new mass in the nasal antrum was biopsied and showed

diffuse infiltration of monoclonal IgM kappa immunoblasts leading to diagnosis of non-Hodgkin's lymphoma.

Case 2. A 76-yr-old white woman presented to our clinic in 1981 because of recurrent parotid and submandibular gland swelling of 10-yr duration. She had undergone surgical removal of massively enlarged parotid and submandibular glands on three previous occasions. A review of the histologic specimens showed pseudolymphoma associated with Sjögren's syndrome but no evidence of lymphoma. She had no history suggestive of RA, SLE, scleroderma, or malignancy. There was no prior use of corticosteroids, immunosuppressive agents, or radiation therapy. Laboratory studies included hemoglobin 11.1 g%; WBC 3.6 (10% lymphs), ESR 62 mm/h, IgG 2,181 mg%, IgM 120 mg%, IgA 540 mg% with normal serum electrophoresis. Her rheumatoid factor was positive (1:640) with ANA 1:80 (speckled), a positive SS-A and negative SS-B antibody. Physical examination revealed scars from prior left parotid surgery with swelling of the right parotid (4 × 4 cm), left submandibular gland (3 × 3 cm), and bilaterally enlarged cervical lymph nodes. Biopsies of a left anterior cervical lymph node, left submandibular gland, and right parotid gland were obtained to exclude malignancy, and the lymphocytes from these tissues were characterized as described below.

Case 3. This 72-yr-old female, had increasingly dry eyes and dry mouth for 6 yr. 1 yr before admission, she developed tender and swollen parotid and submandibular glands bilaterally. Subsequently, a 2 × 2-cm right anterior cervical lymph node was noted and biopsied. At this time she had WBC 6.6 (40% lymphocytes), hemoglobin 13.3 g%, and quantitative immunoglobulin levels IgG 478 mg%, IgM 52 mg%, and IgM 57 mg%. Tests for rheumatoid factor and ANA were negative. 6 mo later she developed pain and swelling of the left eye that was found to result from a 2 × 2-cm mass within the orbit arising from the lacrimal gland. The lymph node and lacrimal gland mass were excised and examined with monoclonal antibodies.

Monoclonal antibodies

We utilized antibodies to lymphocyte cell surface antigens of the OKT series (OKT3, OKT4, OKT6, OKT10, OKT11, OKM1) provided by Ortho Immunobiologic Research Division, Raritan, NJ, and the Leu series (Leu 2a, Leu 3a, Leu 4, and Leu 7) provided by the Becton, Dickinson & Co., Research Division, Mt. View, CA. The preparation and properties of these antibodies have been described previously (18–20); briefly, total T cells are detected by Leu 1 and Leu 4, while T helper cells are included in the Leu $3a^+$ subset, T suppressor cells in the Leu $2a^+$ subset, and natural killer (NK) cells in the Leu $7a^+$ subset in normal blood. However, when lymphocytes have been activated in vitro by mitogens, T cells of the Leu 3a subset can perform suppression, NK, or cytotoxic functions (21, 22).

In staining cell suspensions for cytofluorographic analysis, the OKT and Leu reagents gave similar results (OKT3 = Leu 4; OKT4 = Leu 3a; OKT8 = Leu 2a); however, the Leu series of antibodies gave better staining of frozen tissue sections (23) and were used for this purpose. Antibody 3A1 reacts with normal T cells in thymus and blood (24). Antibody SC1 (25) detects the same 67,000-D antigen on mature T cells as antibody OKT1 or Leu 1 (18, 19), and antibody SC2 reacts with an Ia-like molecule (23). Antidelta, -kappa, -lambda, -gamma, and -mu antibodies were purchased from BRL Laboratories, Bethesda, MD. Antibody B532 reacts with a 45,000-D antigen present on B cells from germinal centers of normal lymph nodes but not on circulating PBL (9, 26). Antibody L22 reacts with the transferrin receptor since it immunoprecipitates the same 90,000-D molecule[2] detected by monoclonal antibody B3/25 that binds to this receptor (27). Previous studies have demonstrated that expression of Ia-like antigen, OKT10 or transferrin receptor is not limited to a particular lineage since they may be found on T cells, B cells and/or monocytes in certain circumstances (18, 23, 27, 18). A low percentage of T cells in normal peripheral blood or thymus express these antigens; however, mitogen stimulation in vitro leads to expression of these markers on the majority of T cell blasts and thus they can be termed activation antigens (27, 28). Myeloma proteins with no known anticell activity, MOPC 21 (IgG1) and GPC 7 (IgG2), were used as negative controls.

Quantitation and separation of lymphocyte subsets

Single cell suspensions of lymph node lymphocytes (LNL) or major salivary gland lymphocytes (SGL) were made by passing the minced tissue through a steel mesh screen followed by Ficoll-Hypaque gradient centrifugation. These lymphocytes were stained by an indirect immunofluorescence technique by incubating 10^6 lymphocytes with 50 μl of monoclonal antibody, washing through fetal calf serum (FCS) and resuspending in fluoresceine-conjugated $F(ab')_2$ goat anti-mouse immunoglobulin (8, 9). The cells were then analyzed cytofluorometrically using a FACS IV fluorescence-activated cell sorter (Becton, Dickinson & Co.) (29). Peripheral blood lymphocytes (PBL) from the pseudolymphoma patients, 14 1^0SS patients without pseudolymphoma, and 10 age-, sex-matched controls were prepared by Ficoll-Hypaque centrifugation after monocyte depletion by carbonyl iron and a magnet (30) and stained in a similar manner as the SGL. T cells were prepared from PBL by rosetting with neuraminidase-treated sheep erythrocytes (30).

To remove particular subsets by complement-mediated lysis, 10^7 cells were incubated with monoclonal antibody OKT4, OKT8, or control myeloma protein in 1 ml phosphate-buffered saline, containing a 1:100 dilution of ascitic fluid for 0.5 h at 5°C, then washed, and 200 μl of rabbit complement was added for 0.5 h at 5°C followed by 0.5 h at 37°C. Complement-mediated lysis was determined to be >94% complete based on subsequent staining with FL-$F(ab')_2$ anti-mouse immunoglobulin (Ig) and cytofluorographic analysis to detect cells stained but not lysed. Less than 10% cell lysis occurred with the control myeloma protein and complement.

Staining of frozen tissue sections by biotin-avidin immunoperoxidase technique

Tissue was obtained at the time of surgery, cut into 1-mm cross-sections, placed in embedding medium (O.C.T. compound, Tissue Tek II; Lab Tek Products, Miles Laboratories, Naperville, IL) in airtight plastic capsules (No. 130 Beem, size 00, Palco, Tustin, CA) and frozen in isopentane/dry ice. Cryostat sections (4–6 μm) were fixed by a brief immersion in acetone and stored at −20°C until staining by monoclonal antibody, biotin-conjugated $F(ab')_2$ goat anti-mouse Ig and

[2] Fox, R. I., and R. Dillman. Unpublished observations.

avidin-conjugated horseradish peroxidase as previously described (8). The slides were counterstained in 1% methylene blue for 5 min.

Karyotype analysis

Suspensions of lymphocytes from peripheral blood, lymph node, and salivary glands were examined before and after 72 h stimulation with phytohemagglutinin (5 mg/ml; Sigma Chemical Co., St. Louis, MO) in the laboratory of Dr. O. W. Jones, Department of Medical Genetics, University of California, San Diego. Briefly, 10^6 lymphocytes/ml were treated with colcemid 1 μg/ml and the culture was incubated at 37°C for 90 min in a 10% CO_2 atmosphere. Chromosomes were prepared for trypsin-Giemsa banding as previously described (31).

In vitro functional analysis

In vitro synthesis of Ig was determined by incubating lymphocytes (10^6/ml) in round bottom tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) in a humid atmosphere with 5% CO_2 in RPMI 1640 supplemented with 10% heat-inactivated FCS and pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island, NY) at a final concentration of 0.1 μg/ml. Cultures were terminated on day 7 and

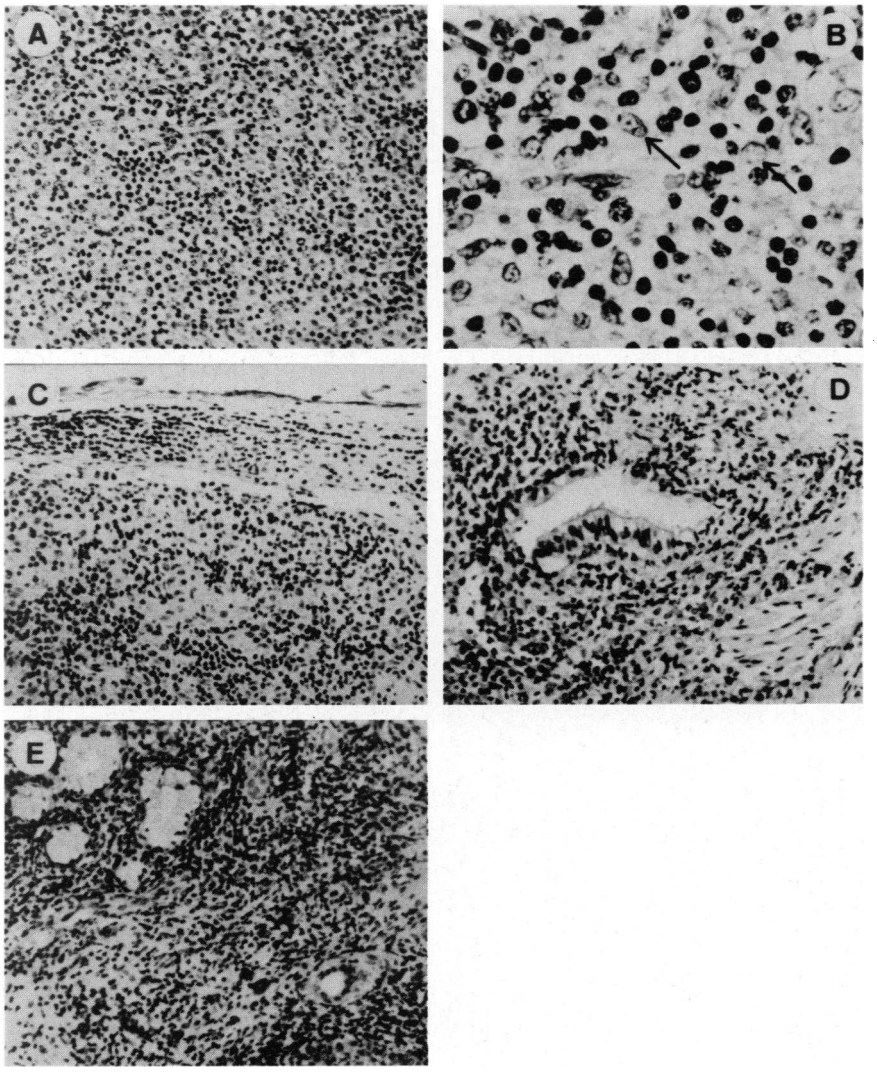

FIGURE 2 Biopsy samples from Sjögren's syndrome patients stained with hematoxylin and eosin. Submandibular gland biopsy from case 1 is shown in panel *A* (×100) and *B* (×400), her lymph node in panels *C*, lung nodule biopsy in frame *D*, and minor salivary gland biopsy in frame *E*. The arrows indicate large lymphocyte with open vesicular nuclei and pale, eosinophilic cytoplasm. The submandibular and cervical lymph node tissue from cases 2 and 3 were virtually identical to those noted in case 1.

supernatants examined for total IgG and IgM and for rheumatoid factor (IgM anti-IgG) using a solid-phase enzyme-linked immunosorbent assay (ELISA). A separate aliquot was analyzed using monoclonal antibodies to ascertain the proportion of T cells, B cells, and monocytes in the cultures on day 0. In certain experiments, non-T cells (0.5×10^6/ml) were mixed with T cells (0.5×10^6/ml) and incubated in round-bottom tubes as described above.

To determine the kinetics of antibody production, SGL or LNL (1.5×10^6 cells/ml) were placed in flat-bottom microtiter plates (3040 Microtest II, Falcon Labware) in RPMI 1640 containing 10% FCS. Polyclonal antibody production, was stimulated by addition of Fc fragments of a human IgG1 myeloma protein (final concentration 0.1 or 1 μg/ml) and the number of plaque-forming cells (PFC) was determined 2, 4, and 6 d later (32).

NK activity was determined using ^{51}Cr-labeled K562 target cells and antibody-dependent cellular cytotoxicity (ADCC) using ^{51}Cr-labeled Chang liver cells coated with rabbit anti-Chang cell antibody (33). Cytotoxic T cell (CTL) activity was measured by the lysis of ^{51}Cr-labeled Epstein-Barr virus (EBV)-transformed B cells (34). Lymphoblastoid cell lines from 1°SS patients and from normal donors were established by infecting PBL with EBV (strain B95-8) and maintaining the cell lines in RPMI 1640 with 10% FCS (34).

RESULTS

Lymphocyte infiltrates in SS patients with pseudolymphoma. The parotid and submandibular glands obtained from these patients showed almost complete replacement of the salivary gland tissue by a diffuse infiltration of lymphocytes (Fig. 2, frames *A* and *B*). The architecture of the cervical lymph node (frame *C*), lung nodule (frame *D*), and minor salivary gland (frame *E*) also was distorted by lymphocytic infiltrates. In each of these tissues, small, normal-appearing lymphocytes and a subpopulation of large lymphoid cells with immunoblastic or plasmacytoid appearance (arrows in frame *B*) were present. There was preservation of the salivary gland lobular architecture and no infiltration

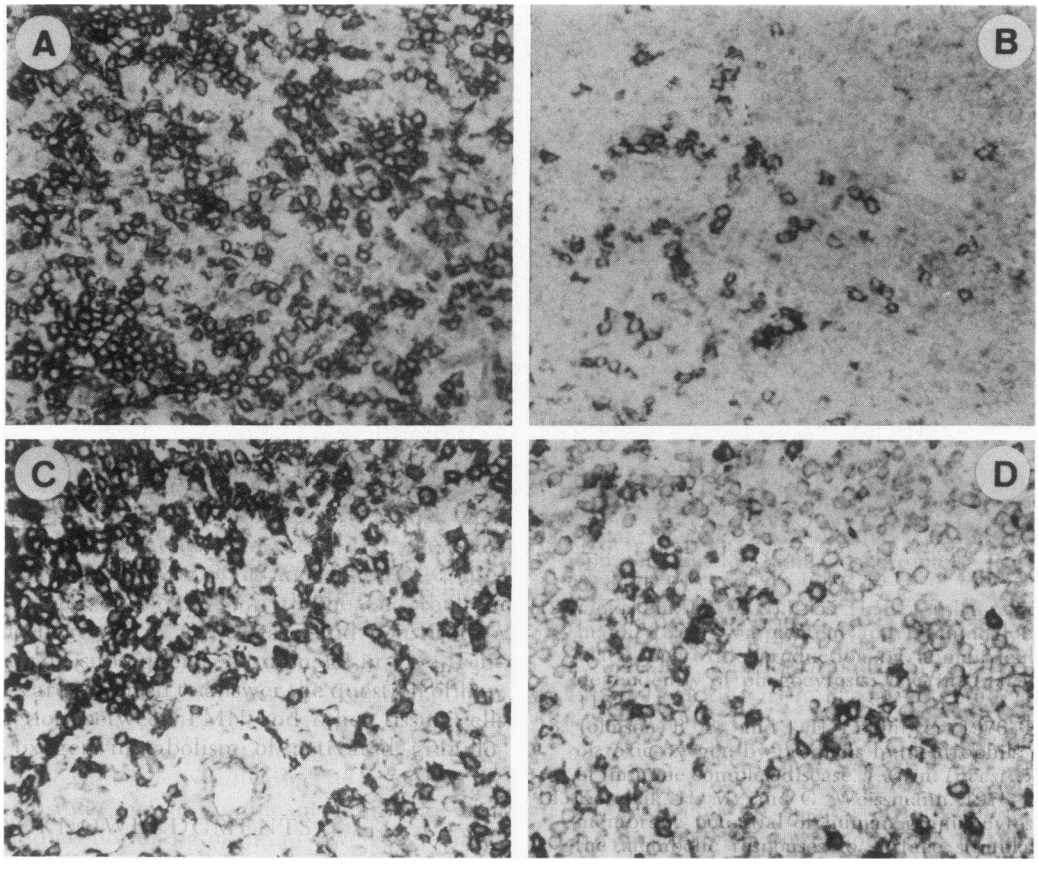

FIGURE 3 Frozen tissue sections of parotid gland biopsies stained with monoclonal antibodies directed against T cells. A positively stained cell is shown by a black ring around the cell membrane. All slides were counterstained with methylene blue. Tissue sections were stained with antibody Leu 4 (mature T cell) in frame *A;* monoclonal anti-IgD in frame *B;* antibody Leu 3a (helper/inducer phenotype) in frame *C;* and antibody Leu 2a (suppressor/cytotoxic phenotype) in frame *D*.

beyond the lymph node capsule; these features help distinguish pseudolymphoma from malignant lymphoma, which generally disrupts the lobular architecture of salivary glands and invades the extracapsular adipose tissue surrounding lymph nodes (2, 3, 5).

Monoclonal antibody staining of the salivary gland biopsy from each patient showed that the majority of lymphocytes were T cells (reactive with antibody Leu 4), although some B cells (reactive with antidelta antibody) were also present (Fig. 3, frames *A* and *B*). Similar results were obtained when total T cells were detected with antibodies SC1 (25) or 3A1 (23). The T cells reacted primarily with antibody Leu 3a (helper/inducer phenotype) (frame 3 *C*); significantly fewer cells reacted with antibody Leu 2a (suppressor/cytotoxic phenotype) (frame 3 *D*).

Activation antigens OKT10 and Ia were detected in the tissue infiltrates (Fig. 4 *A* and *B*). Antibody OKT10 showed cytoplasmic staining of immunoblastic and plasmacytoid cells as well as peripheral staining of small lymphocytes. Anti-Ia antibody showed very intense staining of the entire gland, including cytoplasmic staining of the large lymphoid cells. Antibody B532, which detects a subset of B cells (9), was found to react with scattered cells (frame *C*). Detection of cell surface Ig (i.e., B cells) using antikappa or antilambda antibodies was difficult due to the large amount of interstitial Ig present. Plasma cells, detected by their cytoplasmic Ig were relatively rare (<10%) in these tissues. Analysis of lymph node biopsies from each patient and the lacrimal gland mass in patient 3 gave similar results.

Cytofluorographic analysis of lymphocyte subsets in salivary gland and peripheral blood. Cytofluorographic analysis of SGL was performed to confirm the results of staining frozen sections and to obtain a more precise quantitation of the percent stained cells. As shown in Table I, the majority of SGL were T cells

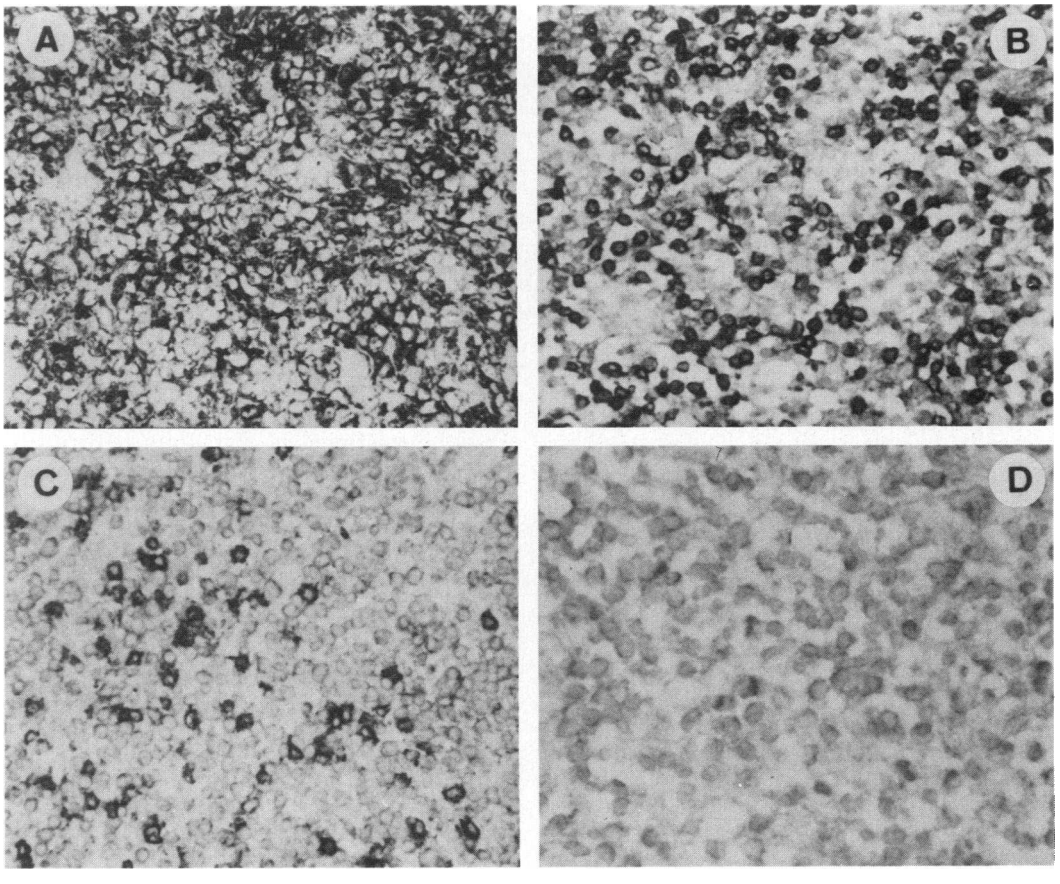

FIGURE 4 The cervical lymph of case 1 was stained with the following monoclonal antibodies: antibody SC2 that detects on Ia-like antigen in frame *A*; antibody OKT10 in frame *B*; antibody B532 in frame *C*; myeloma protein MOPC-21 that contains no anti-cell activity and thus serves as a control for nonspecific binding in frame *D*.

TABLE I

Percent Cells Reactive with Monoclonal Antibodies

Monoclonal antibody	Case 1		Case 2		Case 3	
	PBL*	SGL	PBL*	SGL	PBL*	SGL
T cell markers						
OKT11 (79±6)‡	81	92	80	78	76	83
OKT3 (75±5)	70	91	62	72	71	76
OKT4 (51±6)	60	70	54	59	41	71
OKT8 (30±4)	12	15	8	17	32	14
Ratio (OKT4/OKT8) (1.8 + 0.3)	5	4.6	6	3.5	1.2	5.1
OKT6 (<4)	<4	<4	<4	<4	<4	<4
3A1 (73±7)	74	83	74	70	61	69
B cell markers						
Kappa (7±3)‡	14	12	13	15	5	40
Lambda (5±3)	5	4	5	4	7	9
Delta (11±3)	12	7	5	12	6	12
IgM (10±4)	11	10	11	10	7	8
B532 (<4)	<3	10	<3	11	<4	14
Other markers						
SC2 (14±4)	18	63	15	55	11	44
OKT10 (12±5)	20	55	17	51	18	35
L22 (<4)	<3	12	<3	9	<3	12
OKM-1 (15±6)	18	7	14	4	15	13
Leu 7 (17±7)	11	7	12	8	28	7

Prior to Ficoll-Hypaque centrifugation.

* PBL were monocyte depleted; the number of PBL/mm^3 was 1,410±160 for pseudolymphoma patients, 1,603±795/mm^3 for 14 1°SS patients without pseudolymphoma, and 2,150±375 for age-matched normal individuals.

‡ For each antibody, the mean±SD for normal PBL is given in parentheses.

TABLE II

Characterization of Lymphocyte Subsets in Salivary Gland of SS Patients with and without Pseudolymphoma

	1°SS with pseudolymphoma	1°SS without pseudolymphoma	Adult tonsil
OKT3	80±10	78±7	72±9
Ratio OKT4/OKT8	4.4±0.8	5.1±0.7	4.2±0.6
B532	12±2	12±5	15±4
Ia	54±6	43±9	29±8
OKT10	47±11	33±3	22±11
L22	11±2	6±2	5±3
MOPC-21	<4	<4	<4

(reactive with OKT3, 3A1, and OKT11) of the OKT4$^+$ subset. A small number of B cells (detected by surface IgM, IgD, kappa, and lambda) also were present. Based on the data in Table I, we wish to emphasize that (*a*) the cytofluorographic results are in good general agreement with our frozen tissue section results; (*b*) SGL differ from the same patient's PBL, especially in the frequency of activation antigens (OKT10, transferrin receptor L22, and anti-Ia SC2) and the B cell subset reactive with antibody B532; and (*c*) antigen OKT6, a marker found on immature T cells, was not present in the SGL or LNL.

The increased frequency of Ia$^+$ antigens on SGL was due to Ia$^+$ T cells as shown by two color staining methods (FL-anti-T cell antibody SC1 and rhodamine anti-Ia antibody SC2) which revealed 46±7% double-stained cells. This Ia$^+$ antigen was synthesized, at least in part, by the SGL T cells since T cells purified by rosetting methods (<2% contamination by surface Ig or esterase-positive cells) were able to incorporate [^{35}S]methionine into Ia-antigen detectable by immunoprecipitation with antibody SC2 and gel electrophoresis.[3]

Cell suspensions of additional lymph node biopsies obtained from patient 1 in 1981 and 1982 demonstrated virtually identical cell surface markers as her SGL listed in Table I. Similarly, the lacrimal gland mass in patient 3 had a similar phenotype to her SGL. Thus, we found similarity in each of the tissue biopsies analyzed from the pseudolymphoma patients.

Finally, we have compared the phenotype of SGL in pseudolymphoma patients to SGL obtained from patients without pseudolymphoma (three cases) and to LNL from tonsils of otherwise normal adults. As shown in Table II, the phenotypes of lymphocytes from these sources were similar; a slightly lower percentage of cells reactive with activation antigens was noted in the LNL.

In vitro functional analysis of lymphocyte subsets for T helper activity. SGL from these patients can synthesize Ig in vitro (Fig. 5). This activity was abolished by treatment of SGL from patient 2 or 3 or of normal LNL by antibody OKT4 plus complement (Fig. 5). However, SGL from patients synthesized Ig even after this treatment, suggesting that the B cells had been previously stimulated in vivo. To determine whether these T cells possessed helper activity, we separated her SGL into T and non-T cell subpopulations. The T cells possessed helper activity based on their ability to stimulate normal B cells to secrete Ig (Fig. 6). Her non-T cells spontaneously (i.e., in the absence of PWM) secreted Ig (data not shown), thus confirming our observation that OKT4 and complement did not abolish Ig production. Taken together, these results demonstrate that salivary gland T cells of each patient possess helper activity. Kinetic studies, shown in Fig. 7, demonstrate a small increase in the number of cells

[3] Fox, R. I., and L. Walker. Unpublished observations.

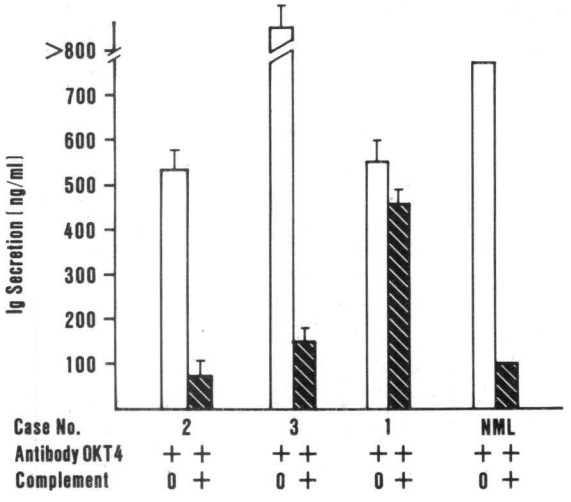

FIGURE 5 Induction of Ig secretion by salivary gland lymphocytes or normal tonsil. These lymphocytes were first treated with antibody OKT4; in some cases rabbit complement was also added to allow complement-mediated lysis. After washing, the cells were placed in culture for 7 d in the presence of PWM (0.1 μg/ml). The amount of Ig synthesized (IgM plus IgG) was detected using an ELISA assay.

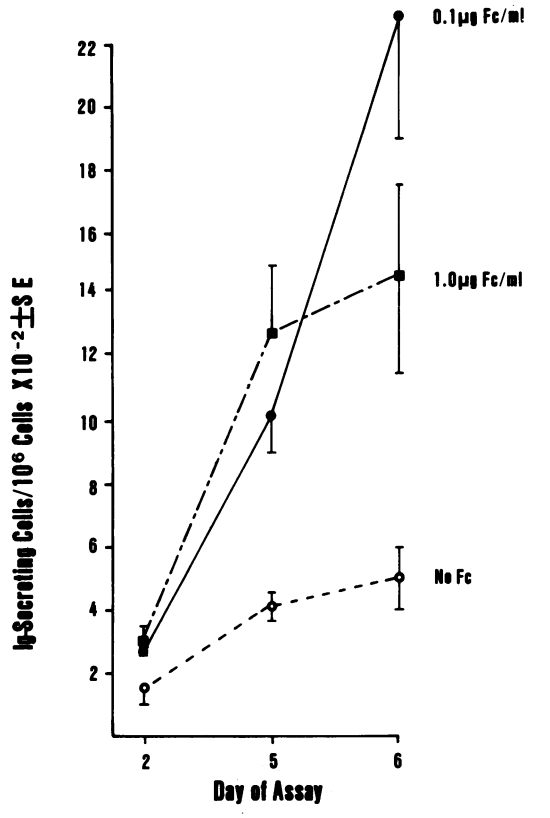

FIGURE 7 SGL from case 3 were incubated with 0, 0.1, or 1 μg/ml Fc fragments derived from human IgG1 myeloma protein. At various time intervals, the number of PFC was determined using a protein A plaque assay. Amplifying sera in this assay recognize human IgG + IgM + IgA (32).

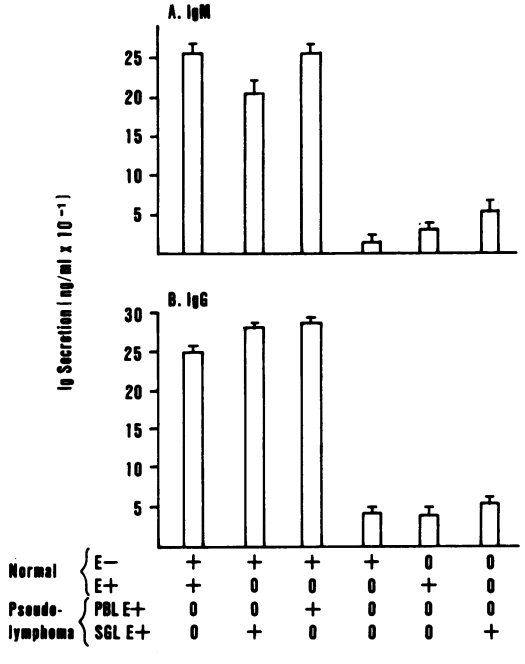

FIGURE 6 SGL and PBL from case 1 were fractionated into T cells (E^+) and non-T cells (E^-) by rosetting with neuraminidase-treated sheep erythrocytes. These fractions were then mixed with T cells and non-T cells from a normal donor in the presence of PWM (0.1 μg/ml). After 7 d, the culture supernatants were examined for IgG or IgM by ELISA assay.

synthesizing antibody (PFC) after 6 d in the absence of polyclonal activator but a larger rise in the presence of Fc fragments.

Absence of NK, ADCC, and CTL effector function in SGL. Since T cells of the $OKT4^+$ subset derived from normal donors can be induced to express NK and ADCC activity in vitro, (21, 22), we studied our patients' cells for these functions. The NK and ADCC activities found in SGL were lower than in their PBL (data shown for patient 1 in Table III). This result is consistent with the diminished number of cells staining with antibody Leu 7 (Table I), which detects the subset containing NK and ADCC cells in normal blood (20).

$OKT4^+$ T cells derived from normal controls also may possess CTL function (22), apparently directed against target cells bearing Ia-like antigens (36, 37). SGL were examined for their ability to lyse four different Ia-bearing EBV-transformed B cell lines, including an autologous transformed line; no CTL activity (<2 lytic units) (33, 34) was noted. Similar to normal PBL, SGL could be activated to express CTL activity

TABLE III
NK and ADCC Activity in Case 1 with SS and Pseudolymphoma

	Percent lysis of target cells		
I. NK activity			
Effector target ratio	20:1	40:1	60:1
Normal PBL (range)	(7–33)	(15–44)	(19–53)
1°SS-SGL	8	8	5
1°SS-PBL	14	15	20
II. ADCC activity			
Effector target ratio	20:1	40:1	60:1
Normal PBL (range)	(44–75)	(46–88)	(47–86)
1°SS-SGL	6	15	20
1°SS-PBL	55	63	65

(>30 lytic units) by exposing the SGL to mitomycin-C-treated autologous EBV-transformed B cells for 6 d before assay (33, 34).

Normal karyotype of PBL and SGL in pseudolymphoma. Chromosomal preparations of SGL and PBL from case 1 examined before and after phytohemagglutinin stimulation showed normal 46, XX karyotypes. PBL from case 2 had normal karyotypes in 15/17 metaphases examined; one metaphase showed 47, XXX and one was 47, XX, +20 but these abnormalities were not considered to be significant; SGL from patient C.W. were 46, XX karyotype.

DISCUSSION

Pseudolymphoma in three patients with 1°SS was due to infiltration of glandular and extraglandular tissues by T lymphocytes that had a normal karyotype and performed helper function in vitro for Ig synthesis. The predominant phenotype in the involved major salivary gland and lymph nodes was $OKT3^+$, $OKT4^+$, $OKT10^+$, Ia^+. We previously reported that minor salivary gland biopsies in patients with 1°SS contain cells with a similar phenotype (19). However, the small amount of tissue derived from a lip biopsy did not allow isolation of lymphocytes for functional or karyotypic studies. These patients with pseudolymphoma provided a unique opportunity to obtain sufficient numbers of lymphocytes for such studies and allowed us to perform cytofluorimetric analysis of lymphocyte subsets stained with monoclonal antibodies to confirm the results of our stained tissue sections. Since the phenotype of SGL may differ significantly from that in the same patient's blood (9, 35), our results emphasize the need to study the cells in the affected tissues.

Characterization of lymphocytes at the site of tissue damage/injury in 1°SS patients may provide insight into other autoimmune diseases where the target organs are not easily accessible to biopsy. Previous studies have shown that patients with juvenile onset diabetes, thyroiditis, and Addison's disease have a high prevalence of HLA-DR3, lymphoid infiltrates of target organs, and organ-specific autoantibodies (39). The high frequency of autoimmune disease in 1°SS patients, including thyroiditis, supports the hypothesis that similar pathogenetic mechanisms are operative in each disease.

The prevalence of non-Hodgkins lymphoma is increased in patients with SS (1–3). These lymphomas are most commonly classified as histiocytic lymphoma or reticulum cell sarcoma (2–4), although other histologic types including myeloma, Waldenstrom's macroglobulinemia, and Franklin's heavy chain disease have been observed (11–14). Immunologic markers have indicated that lymphomas in SS patients are of B cell origin (1, 11–15, 40). Actual progression from pseudolymphoma to frank malignancy was observed in one of our patients (case 1) and has been reported previously (5–7, 10, 13). In our patients with pseudolymphoma, the majority of cells were polyclonal T cells (both $OKT4^+$ and $OKT8^+$ subsets present) although $OKT4^+$ cells predominated. These T cells differed from those present in Sezary syndrome, a T helper lymphoma, in their ability to react with antibody 3A1 (24). Based on these findings, the patient's age of onset of disease and their long clinical course, it is unlikely that these patients had occult T cell lymphoma. In addition, the pseudolymphoma cells had normal karyotype, in contrast to the chromosomal translocation noted in the majority of patients with malignant lymphoma (41). Since distinction between pseudolymphoma and frank lymphoma is often difficult using routine histologic stains (2–4), monoclonal antibodies provide a tool to aid in distinguishing these conditions.

Functional studies revealed that pseudolymphoma T cells provided helper function for antibody production. Relatively little NK or ADCC activity was noted in the SGL even though these activities may be found in $OKT4^+$ T-cells from normal donors after in vitro activation by mitogens (21, 22). Similar to normal PBL or LNL, the SGL did not exhibit CTL activity against autologous EBV-transformed B cells. However, the SGL could be activated in vitro to lyse these target cells. Since the induction of CTL activity requires T inducer cells (34, 38), we conclude that SGL must contain this activity.

Our findings suggest the following sequence of events in the pathogenesis of SS. Initially, $OKT4^+$ T cells home to salivary gland tissue as a result from tissue-specific localization of antigen (42) and/or specific accessory cells (43). This is followed by clonal expan-

sion of these T cells with concomitant expression of activation antigens (OKT10, Ia, L22) associated with cell replication (23, 28). As the foci of T lymphocytes become larger, B cells bearing the B532 antigen (found on normal germinal center B cells but not on circulating B cells) are detected (9) and synthesize Ig and autoantibodies (44). In certain patients, proliferation of $OKT4^+$ T cell occurs leading to glandular enlargement. This is the pseudolymphoma stage. Under the influence of chronic T helper cell stimulation, B cells may eventually undergo certain changes, e.g., a chromosomal translocation that confers a selective growth advantage, leading to non-Hodgkin's lymphoma (41, 45). The development of the lymphoma in SS may depend on environmental factors such as radiation or cytotoxic agents that decrease the T cell regulatory controls or increase the chance of chromosomal alterations (1–3, 45). This hypothesis is supported in part by a recent study of patients with non-Hodgkin's lymphoma (but without reported SS) demonstrating increased T helper activity in their peripheral blood (46). Therefore, patients with pseudolymphoma in SS may occupy an intermediate position in the spectrum ranging from "benign" T cell salivary gland infiltrates to the B cell neoplasms of non-Hodgkin's lymphoma.

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REFERENCES

1. Berard, C. M., E. Greene, I. Jaffe, MacGrath, and J. Ziegler. 1981. National Institutes of Health Conference: A multidisciplinary approach to non-Hodgkin's lymphoma. *Ann. Intern. Med.* 94:218–235.
2. Talal, N., and J. Bunim. 1964. The development of malignant lymphoma in the course of Sjögren's syndrome. *Am. J. Med.* 36:529–540.
3. Talal, N., N. Sokoloff, and W. Barth. 1967. Extrasalivary lymphoid abnormalities in Sjögren's syndrome (reticulum cell sarcoma, pseudolymphoma, macroglobulinemia). *Am. J. Med.* 43:50–65.
4. Block, K., W. Buchanan, M. Wohl, and J. Bunim. 1965. Sjögren's syndrome. A clinical, pathological, and serological study of sixty-two cases. *Medicine (Baltimore).* 44:187–226.
5. Anderson, L., and N. Talal. 1971. The spectrum of benign to malignant lymphoproliferation in Sjögren's syndrome. *Clin. Exp. Immunol.* 9:199–221.
6. Whaley, K., J. Williamson, D. Chisholm, J. Webb, D. Mason, and W. Buchanan. 1973. Sjögren's syndrome. I. Sicca components. *Q. J. Med.* 42:279–304.
7. Talal, N., R. Sylvester, T. Daniels, J. Greenspan, and R. Williams. 1974. T and B lymphocytes in peripheral blood and tissue lesions in Sjögren's syndrome. *J. Clin. Invest.* 53:180–189.
8. Fox, R. I., S. A. Carstens, S. Fong, C. A. Robinson, F. V. Howell, and J. H. Vaughan. 1982. Use of monoclonal antibodies to analyze peripheral blood and salivary gland lymphocyte subsets in Sjögren's syndrome. *Arthritis Rheum.* 25:419–426.
9. Adamson, T., R. I. Fox, D. M. Frisman, and F. V. Howell. 1982. Immunohistologic analysis of lymphoid infiltrates in primary Sjögren's syndrome using monoclonal antibodies. *J. Immunol.* 130: 203–208.
10. Zulman, M., R. Jaffe, and N. Talal. 1979. Evidence that malignant lymphoma of Sjögren's syndrome is a monoclonal B-cell neoplasm. *N. Engl. J. Med.* 299:1215–1218.
11. Faguet, G., H. Webb, J. Agee, W. Ricks, and A. Sharbaugh. 1978. Immunologically diagnosed malignancy in Sjögren's pseudolymphoma. *Am. J. Med.* 65:424–429.
12. Rosenthal, D. 1981. Case records of the Massachusetts General Hospital: Weekly CPC. *N. Engl. J. Med.* 30S:153–160.
13. Diaz-Jovan, E., G. Ruiz-Arguells, J. Vega-Ortiz, G. Villareal, and D. Alarcon-Segovia. 1981. From benign polyclonal to malignant monoclonal lymphoproliferation in a patient with primary Sjögren's syndrome. *Arthritis Rheum.* 24:850–853.
14. Shearn, M., H. Moutsopoulos, S. Sawada, and C. Gak. 1975. Sjögren's syndrome with light chain myeloma. *West. J. Med.* 123:496–497.
15. Pierce, D., R. Stern, R. Jaffe, J. Zulman, and N. Talal. 1979. Immunoblastic sarcoma with features of Sjögren's syndrome and systemic lupus erythematosus in a patient with immunoblastic lymphadenopathy. *Arthritis Rheum.* 22:911–915.
16. Greenspan, J., T. Daniels, N. Talal, and R. Sylvester. 1974. The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Pathol.* 37:217–228.
17. Martinez-Lavin, M., J. H. Vaughan, and E. Tan. 1979. Autoantibodies and the spectrum of Sjögren's syndrome. *Ann. Intern. Med.* 91:185–190.
18. Kung, P., and G. Goldstein. 1981. Strategies for generation of monoclonal antibodies to human T-cell differentiation antigens. *Transplant. Proc.* 12:141–151.
19. Ledbetter, J., R. Evans, M. Lipinski, C. Cunningham-Rundles, R. Good, and L. Herzenberg. 1981. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and T cytotoxic/suppressor subpopulations with mouse and man. *J. Exp. Med.* 153:310–323.
20. Abo, T., M. Cooper, and C. Balch. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* 127:1024–1029.

21. Rimm, I., S. Schlossman, and E. Reinherz. 1981. Antibody-dependent cellular cytotoxicity and natural killer-like activity are mediated by subsets of activated T-cells. *Clin. Immunol. Immunopathol.* 21:134–141.
22. Moretta, C., M. Cristina, C. Mingari, P. Sekaly, A. Moretta, B. Chapuis, and J. Cerottini. 1981. Surface markers of cloned human T cells with various cytolytic activities. *J. Exp. Med.* 154:569–574.
23. Fox, R., and T. Adamson. 1982. Human lymphocyte cell surface antigens defined by monoclonal antibodies. *In* Immunologic Analysis II: New Directions in Clinical Laboratory Assays. R. Nakamura, W. Dito, and E. Tucker, editors. Masson Press, New York. In press.
24. Haynes, B., P. Bunn, D. Mann, C. Thomas, G. Eisenbarth, J. Minna, and A. Fauci. 1981. Cell surface differentiation antigens of the malignant cell in Sezary syndrome and mycosis fungoides. *J. Clin. Invest.* 67:523–530.
25. Fox, R. I., D. Harlow, I. Royston, and J. Elder. 1982. Structural characterization of the human T cell surface antigen (p67) isolated from normal and neoplastic lymphocytes. *J. Immunol.* 129:401–405.
26. Frisman, D., S. Slovin. I. Royston, and S. Baird. 1982. Characterization of a monoclonal antibody which reacts with an activation antigen on human B cells: reactions on cell lines, blood lymphocytes, and cells of normal and malignant lymph nodes. *Blood.* In press.
27. Trowbridge, I., and B. Omary. 1981. Monoclonal antibody B 3/25 detects the transferrin receptor. *Proc. Natl. Acad. Sci. USA.* 78:3039–3043.
28. Terhorst, C., A. Van Agthoven, K. LeClair, P. Snow, E. Reinherz, and S. Schlossman. 1981. Biochemical studies of the human thymocyte cell surface antigens T6, T9, and T10. *Cell.* 23:771–781.
29. Herzenberg, L., and L. Herzenberg. 1978. Analysis and separation using the fluorescence-activated cell sorter. *In* Handbook of Experimental Immunology. D. Wier, editor. Blackwell Scientific Publications, Oxford.
30. Fox, R. I., L. Thompson, and J. Huddlestone. 1981. Te cells express other T-cell markers. *J. Immunol.* 126:2062–2063.
31. Fukohara, S., S. Shirakama, and H. Uchino. 1976. Specific marker chromosome 14 in malignant lymphoma. *Nature (Lond.).* 259:210–213.
32. Morgan, E., and W. Weigle. 1981. Polyclonal activation of human B lymphocytes by Fc fragments. *J. Exp. Med.* 154:778–790.
33. Chisari, F., M. Bieber, C. Josephro, and D. Anderson. 1981. Functional properties of lymphocyte populations in hepatitis B virus infection. *J. Immunol.* 126:45–49.
34. Tsoukas, C. D., R. I. Fox, D. A. Carson, S. Fong, P. Kung, and J. H. Vaughan. 1981. T lymhocyte-mediated cytotoxicity against autologous EBV genome-bearing B-cells. *J. Immunol.* 126:1742–1746.
35. Morimoto, C., E. Reinherz, L. Hadler, J. Distaso, A. Steinberg, and S. Schlossman. 1982. Comparison of T- and B-cell markers in patients with Sjögren's syndrome and systemic lupus. *Clin. Immunol. Immunopathol.* 22:270–278.
36. Meuer, S., S. Schlossman, and E. Reinherz. 1982. Clonal analysis of human cytotoxic T lymphocytes: $T4^+$ and $T8^+$ effector T-cells recognize products of different major histocompatibility complex regions. *Proc. Natl. Acad. Sci. USA.* 79:4395–4399.
37. Krensky, A., C. Reiss, J. Mier, J. Strominger, and S. Burakoff. 1982. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 are $OKT4^+$. *Proc. Natl. Acad. Sci. USA.* 79:2365–2369.
38. Reinherz, E., P. Kung, G. Goldstein, and S. Schlossman. 1979. Further characterization of the human inducer T cell subset defined by monoclonal antibody. *J. Immunol.* 123:2894–2896.
39. Montsopoulos, H., D. Mann, A. Johnson, and T. Chused. 1979. Genetic differences between primary and secondary sicca syndrome. *N. Engl. J. Med.* 301:761–763.
40. Levy, R., R. Warnke, R. Dorfman, and J. Haimovich. 1977. The monoclonality of human B cell lymphomas. *J. Exp. Med.* 145:1014–1020.
41. Fukuhara, S., and J. Rowley. 1978. Chromosome 14 translocations in non-Burkitt's lymphoma. *Int. J. Cancer.* 22:14–18.
42. Sprent, J., J. Miller, and G. Miller. 1971. Antigen-selective recruitment of circulating lymphocytes. *Cell. Immunol.* 2:171–180.
43. Dailey, M., G. Fathman, E. Butcher, E. Pillemer, and I. Weissman. 1982. Abnormal migration of T lymphocyte clones. *J. Immunol.* 128:2134–2136.
44. Talal, N., R. Asofsky, and P. Lightbody. 1970. Immunoglobulin synthesis by salivary gland lymphoid cells in Sjögren's syndrome. *J. Clin. Invest.* 49:49–54.
45. Klein, G. 1980. Immune and non-immune control of neoplastic development: contrasting effects of host and tumor evolution. *Cancer.* 45:2492–2512.
46. Gaje-Peczalska, K., S. Chartrand, and C. Bloomfield. 1982. Abnormal immunoregulation in patients with non-Hodgkin's malignant lymphomas. *Clin. Immunol. Immunopathol.* 23:366–378.