Immunoregulation in Sjögren's Syndrome

INFLUENCE OF SERUM FACTORS ON T-CELL SUBPOPULATIONS

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A B S T R A C T   21 patients with Sjögren's syndrome (sicca syndrome) with either glandular or extraglandular involvement, but without other connective tissue diseases, were studied with regard to immunoregulatory T-cell subpopulations, B-cell function, and suppressor cell capabilities. Patients with isolated glandular disease as well as patients with extraglandular disease had normal absolute numbers of total lymphocytes, T cells, and B cells. However, 9 of 11 patients with extraglandular disease and only 3 of 10 patients with glandular disease had decreased relative proportions of T cells bearing receptors for the Fc portion of immunoglobulin (Ig)G (T_G) which was explained by a factor that blocked the expression of the IgG Fc receptor on T_G cells. This blockage was reversible since the factor could be removed by trypsinizing the T cells before T_G determination. Serum from patients with abnormal proportions of T_G cells, but not serum from patients with normal proportions of T_G cells, blocked the expression of the IgG Fc receptor on normal T cells. The serum factor upon fractionation over Bio-Gel A 1.5 columns as well as over staphylococcal protein A-Sepharose 4B columns was found diffusely within the IgG fraction, and not in the IgM fraction.

Neither patients with glandular nor patients with extraglandular disease manifested increased numbers of in vivo-activated circulating lymphocytes as determined by spontaneous anti-trinitrophenyl (TNP) plaque-forming cells (PFC). However, patients with glandular disease had reduced numbers of pokeweed mitogen-induced anti-sheep erythrocyte PFC (P < 0.01) as compared with normals and patients with glandular disease. Of note was the fact that despite the modulation of T_G subpopulation by the serum factor in patients with extra-glandular disease, these patients manifested normal concanavalin A-generated suppressor cells of pokeweed mitogen-induced PFC responses in allogeneic co-cultures. This was unlike the suppressor cell defect previously described in this system with systemic lupus erythematosus patients. The discrepancy was attributed both to the fact that the T_G defect was reversible and to the fact that concanavalin A-generated suppressor cells are not limited to the T_G subset. Thus, these studies have demonstrated reversible abnormalities in T_G cells in patients with extraglandular Sjögren's syndrome which are not associated with suppressor cell defects. The discrepancy between these findings and the immunoregulatory defects demonstrated in systemic lupus erythematosus may explain the difference in severity of the autoimmune expression in these diseases.

INTRODUCTION

Sjögren's syndrome (sicca syndrome) is a chronic autoimmune disease characterized by T- and B-lymphocyte infiltration of exocrine glands, particularly the lacrimal and salivary glands, resulting in xerostomia and xerophthalmia (1, 2). Extraglandular lymphocytic infiltration occurs in one-fourth of patients and usually presents as interstitial pneumonitis, nephritis, myositis, or pseudolymphoma. Rarely, malignant lymphoma occurs, and recently it was shown that sicca syndrome patients are 40 times more prone to develop lymphoma compared with controls (3–7). Sjögren's syndrome can exist as a primary disorder with disease limited to the exocrine glands or with additional extraglandular disease (primary Sjögren's syndrome), or as a component of another connective tissue disease such as rheumatoid arthritis or systemic lupus erythematosus (secondary Sjögren's syndrome) (1, 8).

A number of findings suggestive of B-cell hyperreactivity and/or abnormalities of immunoregulation occur in Sjögren's syndrome including hypergammaglobulinemia, nonorgan-specific autoantibodies such as

rheumatoid factor, and antibodies to extractable nuclear antigen(s) (1, 7–10). In addition, nonrheumatoid factor-circulating IgG immune complexes have been demonstrated in certain patients with this disease (11).

The precise etiology of these abnormalities is unknown. Hyperreactivity of B-cell function may be a primary phenomenon or may be directly or indirectly related to alterations in immunoregulatory mononuclear cells such as thymus-derived (T) cell subpopulations (12). The present study was undertaken to determine if any abnormalities in immunoregulatory T-cell subpopulations as well as B-cell function occur in Sjögren's syndrome. The study was conducted on patients with Sjögren's (either glandular or extraglandular) without other connective tissue diseases. None of the patients were receiving or had received any form of therapy for their disease.

METHODS

Patient population. 21 patients with Sjögren's syndrome were studied. There were 1 male and 20 females, ranging in age from 34 to 75 yr. The diagnosis was based on the presence of the following findings: xerostomia (decreased parotid flow rate, abnormal parotid scan), keratoconjunctivitis sicca demonstrated on slit-lamp examination, and a focal lymphocyte infiltrate on minor salivary gland biopsy. All patients had Sjögren's syndrome alone (primary Sjögren's syndrome) without other connective tissue diseases (1). 10 of the patients had disease limited to exocrine glands, and the remaining 11 had the following extraglandular manifestations: hypergammaglobulinemic purpura, pseudolymphoma, biopsy-proven vasculitis, renal involvement (urine pH higher than 7, serum bicarbonate concentration lower than 19 meq/dl, decreased creatinine clearance, and interstitial nephritis on renal biopsy), and interstitial pneumonitis demonstrable on chest roentgenogram and pulmonary function tests (Table I). None of the patients studied were receiving any form of therapy, nor had they ever received any form of therapy for their disease.

The normal control group consisted of 11 age-, sex-, and race-matched individuals.

Cell suspensions. Mononuclear cell suspensions were obtained from heparinized venous blood of patients and normal controls by standard Hypaque-Ficoll gradient centrifugation. Cells were counted in a Coulter counter (model Fn, Coulter Electronics Inc., Hialeah, Fla.), and mononuclear cells were examined in cytocentrifuge preparations stained with Wright-Giemsa stain. T cells were identified by the presence of a sur-

TABLE I

Patients with Sjögren's Syndrome Studied at the National Institutes of Health

Patient	Sex	Age	Time since diagnosis of Sjögren's syndrome	Extraglandular manifestations
		yr	*yr*	
1	F	51	3	Hypergammaglobulinemic purpura; renal involvement
2	F	56	1	None
3	F	68	1	Pseudolymphoma
4	F	48	1	None
5	F	45	2	None
6	F	34	2	None
7	F	55	6	Pseudolymphoma; vasculitis
8	F	57	3	Pulmonary involvement
9	F	54	2	Vasculitis
10	F	48	2	None
11	M	64	1	Vasculitis
12	F	62	7	Renal involvement
13	F	60	8	Hypergammaglobulinemic purpura
14	F	67	1	None
15	F	62	1	None
16	F	56	2	Hypergammaglobulinemic purpura; lymphadenopathy; Waldenstrom's macroglobulinemia
17	F	63	5	Hypergammaglobulinemic purpura; chronic persistent hepatitis; interstitial pneumonitis
18	F	60	1	None
19	F	36	1½	None
20	F	44	3	None
21	F	75	1	Hypergammaglobulinemic purpura

face receptor for sheep erythrocytes (E),[1] and bone marrow-derived (B) cells were identified by the presence of surface immunoglobulin (sIg) determined by a fluoresceinated F(ab)₂ goat anti-human Ig reagent as previously described (13).

Fractionation of mononuclear cell suspensions. T cell-enriched suspensions were obtained by E-rosetting of lymphocytes with sheep erythrocytes (SRBC) followed by centrifugation of rosetted cells over Hypaque-Ficoll gradients as previously described in detail (14). E-rosetted cells (T cell-enriched) separated into the pellet, leaving nonrosetted cells (T cell-depleted) at the interface. T cell-enriched suspensions contained greater than 98% T cells, and T cell-depleted suspensions contained less than 1% T cells. Greater than 90% of the cells were recovered after the fractionation procedure.

Identification of T-cell subpopulations. T-cell subpopulations were determined as previously described in detail (15, 16). Briefly, to identify T cells bearing receptors for the Fc portion of IgG (T_G), 0.1 ml of purified T cells at a concentration of 10×10^6 cells/ml was mixed with 0.1 ml of a 2% suspension of bovine E coated with highly purified rabbit antibovine E IgG antibody (7S EA). This mixture was spun into a pellet and incubated at 37° C for 30 min. To identify T cells bearing receptors for the Fc portion of IgM (T_M cells), purified T cells were incubated overnight at a concentration of 10^6 cells/ml in TC 199 media (Grand Island Biological Co., Grand Island, N. Y.) and 20% fetal calf serum. After overnight incubation, the T cells were suspended in TC 199 at 3×10^6 cells/ml, and 0.1 ml was added to 0.1 ml of a 0.5% suspension of bovine E coated with purified rabbit antibovine E IgM (19S EA). The T-cell-19S EA mixture was spun into a pellet and incubated on ice for 45 min.

After incubation of T cells with either IgG or IgM EA reagents, the buttons were gently resuspended, and the percentage of rosette-positive cells was counted under phase-contrast microscopy. On every sample, 200 cells were counted by the same observer throughout the study. Lymphocytes binding three or more E were classified as rosettes. The percentages of T cells bearing Fc receptors for IgG (T_G) or IgM (T_M) were thus determined. The percentage of T-cells having neither receptor ($T_{non-G, non-M}$, or T_{null}) was determined by subtracting the sum of the percentage of T_G cells and T_M cells from 100.

Trypsin treatment of T cells. To determine if a factor which was trypsin sensitive might be influencing the expression of the Fc receptor for IgG in the patients studied, in certain experiments purified T cells from normal subjects and patients were examined for the proportion of T_G cells both before and after treatment with trypsin. The trypsin treatment protocol consisted of determining the proportion of T_G in separate aliquots of purified T cells which had been incubated with either RPMI-1640 media (Microbiological Associates, Walkersville, Md.) or trypsin (Sigma Chemical Co., St. Louis, Mo.) 2 mg/ml for 30 min at 37°C followed by three washes in RPMI-1640.

Preincubation of T cells with whole serum and serum fractions. To determine the effect of whole serum from Sjögren's syndrome patients on the expression of the Fc receptor for IgG found on normal T cells, the following experiments were performed. A determination of the proportion of T_G cells was made on aliquots of T-cell suspensions from normal individuals. Separate aliquots of T cells were then preincubated for 45 min at 37°C with 1:10 dilution of heat-inactivated sera from normal individuals or from patients with Sjögren's syndrome. Cells were then washed twice and T_G determinations were repeated.

For the experiments using serum fractions, the following procedures were carried out: sera from three patients and one normal individual were heat-inactivated at 56°C for 30 min and were fractionated on a Bio-Gel A 1.5 column (1 × 90 cm) as previously described (17). 1-ml fractions were collected from the column and localization of the 19S (IgM) and 7S (IgG) peaks was performed by radial immunodiffusion (Hyland Diagnostics Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). The fractions were then dialyzed against phosphate-buffered saline (pH 7.4), concentrated to the original volume and airfused before use (Airfuse; Beckman Instruments, Inc., Fullerton, Calif.).

In a separate series of experiments the serum fractions from a patient with extraglandular Sjögren's syndrome and a low proportion of T_G (2%), serum from a patient with glandular Sjögren's syndrome and a normal proportion of T_G (12%), and serum from a normal individual (15% T_G) were fractionated by multiple passages over a column of Sepharose 4B with bound staphylococcal protein A (Pharmacia Fine Chemicals, Div. Pharmacia Inc., Piscataway, N. J.) according to previously described methods (18). Pooled serum fractions either devoid of detectable IgG or enriched in IgG were obtained by this fractionation procedure. Preincubation of T cells with the serum fractions was carried out in the same manner as described above for the unfractionated sera.

Blastogenic responses. Lymphocyte blastogenic responses to stimulation with the mitogens phytohemagglutinin (PHA) (concentration range from 0.5 to 10 μg/ml of culture), concanavalin A (Con A) (10-250 μg/ml of culture), and pokeweed mitogen (PWM) (1:20 through 1:1,000 dilution of stock solution per milliliter of culture) were determined by the incorporation of tritiated thymidine in microcultures as previously described in detail (13). Cultures stimulated with PHA and Con A were incubated for 3 d, and cultures stimulated with PWM were incubated for 5 d.

Determination of plaque-forming cell (PFC) responses. Determination of B cells spontaneously producing Ig was made by immediately assaying freshly drawn Hypaque-Ficoll-separated mononuclear cells in a hemolysis-in-gel PFC assay as previously described (19), with the modification that the SRBC targets were haptenated with the trinitrophenyl hapten (20).

The polyclonally induced PFC responses against SRBC of lymphocytes from Sjögren's syndrome patients and normal subjects after 6–7 d of culture with PWM were also determined as previously described (19). Briefly, cells were cultured in RPMI-1640 media (Grand Island Biological Co.) containing 0.3% trypticase soy broth, 2 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin sulfate, and supplemented with 10% pooled human A serum absorbed twice with SRBC. Cultures were performed in 12 × 75-mm plastic tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at a density of 2×10^6 cells in 1 ml, on a rocker platform (7 cycles/min) for 6–7 d at 37°C in 5% CO_2 in air at 100% humidity. Cultures were stimulated either with PWM in a wide concentration range (1:20 through 1:10,000 final dilution) or media alone as control (background PFC).

In allogeneic co-cultures of cell suspensions from a Sjögren's syndrome patient and a normal individual, 1×10^6 cells of one suspension were co-cultured with 1×10^6 cells of another suspension to keep the cell density constant at 2×10^6 cells in 1 ml.

At the end of the culture period (6–7 d), cells were harvested and assayed for direct PFC against SRBC by an ultrathin layer

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[1] *Abbreviations used in this paper:* A, antibody; Con A, concanavalin A; E, erythrocyte; PFC, plaque-forming cells; PHA, phytohemagglutinin; PWM, pokeweed mitogen; sIg, surface immunoglobulin; SLE, systemic lupus erythematosus; SRBC, sheep erythrocyte; T_G, T cells bearing receptors for the Fc portion of IgG; T_M, T cells bearing receptors for the Fc portion of IgM.

hemolysis-in-gel technique as previously described in detail (19). Data are expressed as PFC per 10^6 cells.

Con A-generated suppressor cells. Suppressor cells were activated by incubation with Con A (grade IV, Sigma Chemical Co.) as previously described in detail (21). Briefly, lymphocytes at a density of 5×10^6 cells/2 ml of RPMI-1640 were incubated in flat-bottomed Linbro plates (Linbro Chemical Co., Hamden, Conn.) either with 10 μg/ml Con A, a dose previously shown to be optimal for generating suppressor cells (21), or with no Con A as a control. At 48 h, cells were harvested, washed three times with 0.2 M α-methyl mannoside (Cal-biochem-Behring Corp., American Hoechst Corp., San Diego, Calif.), and once with RPMI-1640 media. Control cells were not exposed to Con A on initiation of culture, but were cultured alone for 48 h and washed with α-methyl mannoside in the same manner as the cells that were cultured with Con A. Con A-activated or control cells (unstimulated) from normals or patients were co-cultured with freshly drawn allogeneic normal lymphocytes in the presence of PWM for an additional 6-7 d to determine the effect of Con A-activated suppressor cells on the PWM-induced PFC response against SRBC.

RESULTS

Lymphocyte subpopulations. The absolute numbers of lymphocytes, total T cells, and B cells in normal individuals and patients with either glandular Sjögren's syndrome or extraglandular Sjögren's syndrome are shown in Fig. 1. There were no significant differences between normal individuals and patients with glandular Sjögren's syndrome in absolute numbers of lymphocytes, T cells, or B cells. Patients with extraglandular Sjögren's syndrome had slightly lower numbers of total lymphocytes and T cells than normal individuals, but these differences were not significant. When relative proportions of T-cell subsets were determined, again there were no significant differences among normals, glandular, and extraglandular Sjögren's syndrome patients (Fig. 2). However, when proportions of T_G cells (by 7S EA-rosetting) were examined in individual patients with glandular and extraglandular Sjögren's syndrome, an obvious difference was noted (Fig. 3). 7 of 10

FIGURE 2   Proportions of T-cell subsets in normals, patients with glandular Sjögren's syndrome, and patients with extraglandular Sjögren's syndrome. There were no significant different in T-cell subsets or B cells among the three groups ($P > 0.2$).

patients with glandular Sjögren's syndrome had proportions of T_G cells which were within the range of concomitantly determined normal values. On the contrary, 9 of 11 patients with extraglandular Sjögren's syndrome had proportions of T_G cells which were decreased below the normal range. Incubation of the T cells for
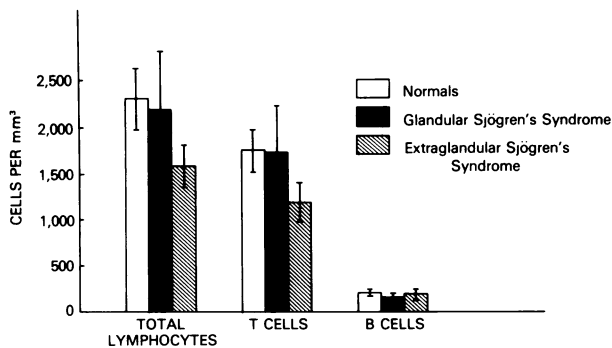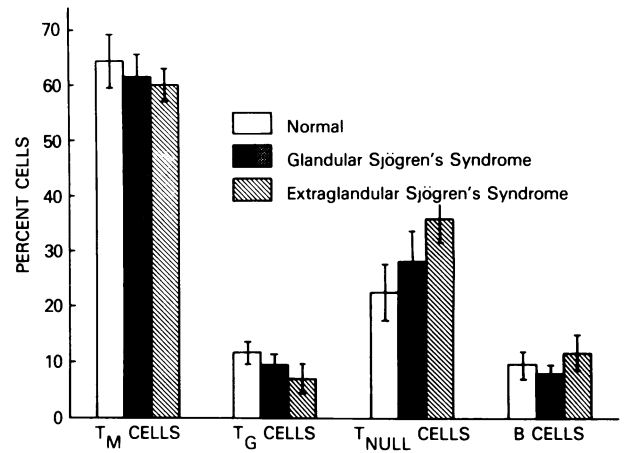

FIGURE 1   Absolute numbers of lymphocytes, T cells, and B cells in normals, patients with glandular Sjögren's syndrome, and patients with extraglandular Sjögren's syndrome. There were no significant differences in lymphocytes, T, or B cells among the three groups.
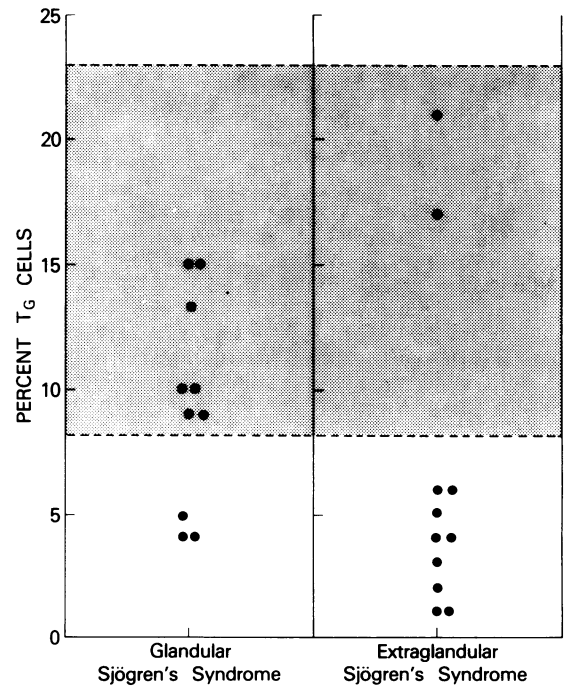

FIGURE 3   Proportion of T_G cells in patients with glandular and extraglandular Sjögren's syndrome. Each dot represents a patient and shaded area signifies the normal range of concomitantly determined T_G proportions among normal individuals.

1 h at 37°C followed by washing in RPMl-1640 failed to result in normalization of the expression of IgG Fc receptors in those cell suspensions with low levels of detectable T_G cells. Determinations were repeated at least once and sometimes twice over a period of 1–4 mo in seven patients (five patients with low T_G and two with normal T_G). Values were within 2% rosettes on these repeat determinations.

Effect of trypsin treatment of T cells upon expression of IgG Fc receptor. The effect of trypsin treatment of T cells from extraglandular Sjögren's syndrome patients who manifested low proportions of T_G cells is shown in Table II. In four of the five individuals tested, trypsin treatment resulted in an increase in detectable IgG Fc receptor-bearing T cells to near normal or normal levels suggesting that a trypsin-sensitive factor was reversibly blocking the detection of the IgG Fc receptor on a proportion of these cells. In addition, trypsin treatment of T cells from normal individuals and Sjögren's syndrome patients with normal proportions of T_G cells did not affect the proportion of cells with detectable IgG Fc receptors. Trypsin treatment of the T cells from one patient with glandular Sjögren's syndrome and low T_G resulted in normalization of the proportion of T_G.

Effect of sera from patients with Sjögren's syndrome on the expression of IgG Fc receptors on normal T cells. The effect of preincubation of normal T cells with sera from normal individuals, from glandular

Sjögren's syndrome patients with normal T_G-cell proportions, and from extraglandular Sjögren's syndrome patients with low T_G-cell proportions is shown in Fig. 4. Preincubation of T cells with normal sera or sera from patients with glandular Sjögren's syndrome and normal T_G-cell levels had very little effect on the detection of IgG Fc receptors on these normal T cells. In contrast, sera from each of six patients tested with extraglandular Sjögren's syndrome who had low T_G cell proportions blocked the expression of the IgG Fc receptor on normal T cells.

In additional studies in which normal T cells were incubated with sera at 27°C or 4°C, sera from normals or patients with glandular Sjögren's syndrome and normal proportions of T_G still did not block the expression of the IgG Fc receptor on the normal T cells. This suggests that low titer-blocking factors which might bind detectably only at lower temperatures were not present in the sera of normal individuals and patients with normal T_G.

Effect of serum fractions. Sera from normal individuals and patients with extraglandular Sjögren's syndrome whose whole serum had blocked the expression of the IgG Fc receptor on normal T cells were fractionated over Bio-gel A 1.5 columns. The Fc receptor-blocking activity was found in the fractions containing IgG and in fractions containing IgM. Fig. 5 illustrates a typical fraction curve containing the Fc receptor-blocking activity. None of the fractions from normal serum blocked Fc receptor expression. The inhibitory fractions illustrated in Fig. 5 upon incubation with normal T cells decreased the percentage of Fc receptor expressing cells from 13% to a range of from 2 to 5%.

TABLE II
Effect of Trypsinization of T Cells on the Expression of the IgG Fc Receptor in Sjögren's Syndrome and in Normal Individuals

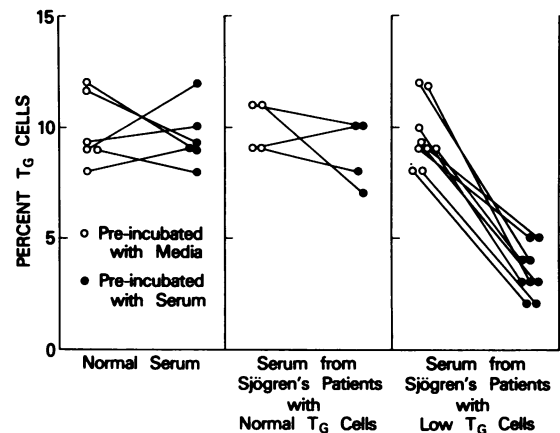|	Percent T_G	
Subject	Before trypsinization	After trypsinization
Extraglandular Sjögren's syndrome		
A	4	19
B	5	11
C	4	8
D	2	1
E	2	12
Glandular Sjögren's syndrome		
A	15	14
B	14	15
C	10	12
D	10	11
Normals		
A	12	13
B	11	13
C	15	12
D	11	13

FIGURE 4 Effect of incubation of normal T cells with sera from Sjögren's syndrome patients on the expression of IgG Fc receptor. Serum from six patients with Sjögren's syndrome who had low levels of T_G blocked the expression of the IgG Fc receptor on normal T cells. In this figure, sera from three of the six patients with low T_G were assayed against two separate normals, thus giving a total of nine determinations (right panel).

BIOGEL A 1.5 COLUMN
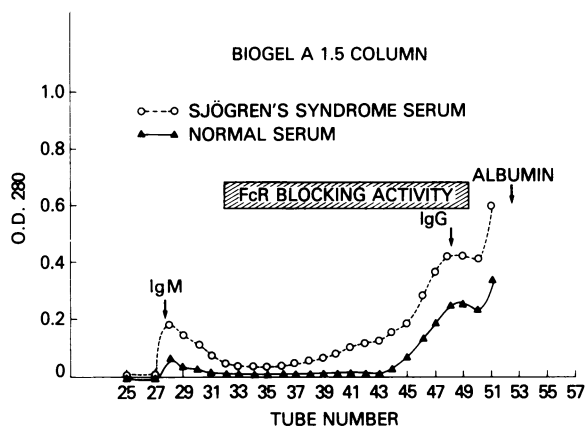
o---o SJÖGREN'S SYNDROME SERUM
▲——▲ NORMAL SERUM

FIGURE 5 Fractionation of Sjögren's syndrome serum. This figure represents a typical fractionation profile of the serum from a patient with low T_G proportion whose whole serum blocked the expression of the IgG Fc receptor on normal T cells. The blocking activity was diffusely distributed (represented by shaded bar) but excluded the fractions containing IgM.

In a separate series of experiments in which various sera were fractionated into IgG-containing and IgG-depleted fractions by passage over columns of staphylococcal protein A bound to Sepharose 4B, only the IgG-containing fraction of the patient with extraglandular Sjögren's syndrome and low T_G proportion blocked the expression of the IgG Fc receptor on normal T cells (Table III). This finding was reproducible in three separate experiments.

Lymphocyte blastogenic responses. The peak blastogenic responses of lymphocytes from normal individuals and patients with glandular and extraglandular Sjögren's syndrome to stimulation with various mitogens are shown in Fig. 6. Although there appeared to be a trend towards slightly decreased responses to all mitogens tested in patients with glandular Sjögren's syndrome and even more so in patients with extraglandular Sjögren's syndrome, these decreases were not statistically significant ($P > 0.2$, Student's t test). When suboptimal concentrations of mitogens were used, responses were still not statistically different among the three groups.

PFC responses. There was no difference between normal individuals and any patients with Sjögren's syndrome in the presence of spontaneously occurring PFC responses against TNP-coated sheep cells. There were virtually no responses (0–1 PFC/10⁶ cells) in any of the groups studied.

The PWM-induced PFC responses after 6–7 d in culture of lymphocytes from normal individuals and patients with Sjögren's syndrome are shown in Fig. 7. Patients with glandular Sjögren's syndrome gave PFC responses which were low but which overlapped those of normals. However, PFC responses of patients with

TABLE III
Effect of Serum and Serum Fractions on Expression of Fc Receptor on Normal T Cells*

Source of serum	Percentage of Fc receptor expression on normal T cells‡
Extraglandular Sjögren's syndrome	
Media	19
Whole serum	8
IgG-enriched fraction	9
IgG-depleted fraction	20
Glandular Sjögren's syndrome	
Media	15
Whole serum	15
IgG-enriched fraction	17
IgG-depleted fraction	15
Normal	
Media	15
Whole serum	16
IgG-enriched fraction	16
IgG-depleted fraction	14

* Serum fractions were obtained by passage over columns of staphylococcal protein A bound to Sepharose 4B (Methods).
‡ Normal T cells were preincubated with either media, whole serum, or serum fractions before determination of the proportion of T_G (Methods).

extraglandular Sjögren's syndrome were markedly decreased below normal ($P < 0.001$).

The low PFC responses of the extraglandular Sjögren's syndrome patients were not due to the exces-
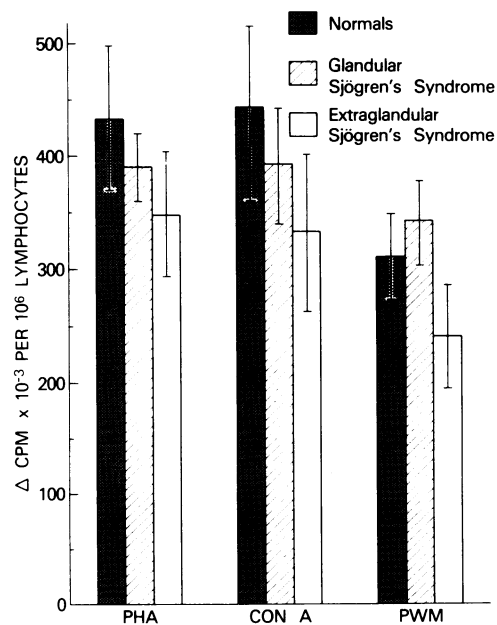

FIGURE 6 Peak blastogenic responses of normals and Sjögren's syndrome patients to mitogenic stimulation. There were no significant differences ($P > 0.2$) among the three groups in blastogenic responses to PHA, Con A, or PWM.
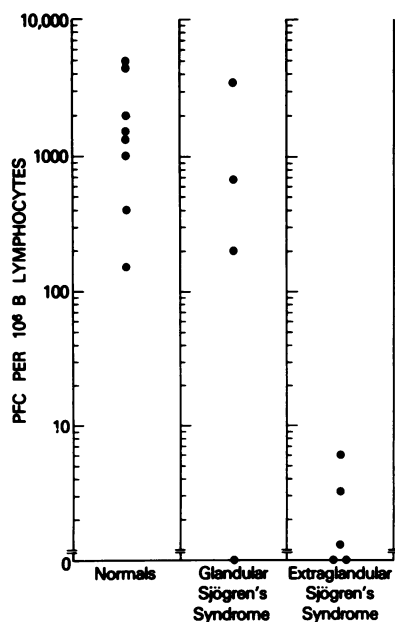
FIGURE 7 PWM-induced anti-SRBC PFC responses in normal individuals and in patients with Sjögren's syndrome. Patients with glandular disease only have PFC responses within the normal range while patients with extraglandular disease have markedly reduced PFC responses. Each dot represents an individual subject and data are plotted on a logarithmic scale as PFC per 10^6 lymphocytes.

sive activity of suppressor cells since co-culture of normal lymphocytes with low responder Sjögren's syndrome lymphocytes, a procedure which detects active suppressor cells (22), did not decrease the expected PFC responses of the normal lymphocytes.

Con A-generated suppressor cells. Lymphocytes from patients with Sjögren's syndrome (either glandular or extraglandular) generated Con A-induced suppressor cells normally. In particular, mononuclear cells from patients, when cultured for 48 h with Con A, subsequently suppressed the anti-SRBC PFC responses when added to PWM-stimulated allogeneic co-cultures. The suppression was no different from that

TABLE IV
Effect of Con A-Generated Suppressor Cells from Normal Individuals and Patients with Sjögren's Syndrome on the PWM-Induced PFC Responses of Normal Allogeneic Lymphocytes

Subjects	Percentage of suppression
Normals (n = 10)*	79 (±10.5)‡
Glandular Sjögren's syndrome (n = 4)	78 (±9)
Extraglandular Sjögren's syndrome (n = 5)	74 (±8)

* n represents the number of subjects studied.
‡ Data are given as the mean (±SEM).

mediated by Con A-induced suppressor cells from normal individuals ($P > 0.2$) (Table IV).

DISCUSSION

This study demonstrates that patients with Sjögren's syndrome have an abnormality in the expression of the IgG Fc receptor found on a subpopulation of circulating T cells (T_G cells). This defect could be normalized by trypsinizing the T cells, after which a normal percentage of these cells expressed IgG Fc receptors. This, together with the finding that sera from patients with low percentages of T_G cells when incubated with T cells from normal individuals, blocked the expression of the IgG Fc receptor on the normal T cells. The blocking factor was found in the IgG fraction of serum as well as in the fractions between IgG and IgM, but not including IgM. Absorption out of IgG from the serum removed the blocking factor, a finding which suggests that the blocking factor is either an IgG molecule itself or an IgG-containing immune complex. The low percentages of T_G as well as the serum factors which blocked the expression of the IgG Fc receptor were found in the patients with extraglandular Sjögren's syndrome and were not found in patients with Sjögren's syndrome limited to the exocrine glands. These findings are of particular interest and potential importance for several reasons. The patients in this study did not have an absolute lymphocytopenia, nor did they have a T lymphocytopenia. Furthermore, although patients with extraglandular Sjögren's syndrome had slightly lower absolute numbers of T cells and all T-cell subpopulations (T_G, T_M, and T_{non-G}, T_{non-M}) than normals, these differences were not statistically significant. This agrees with the findings that the expression of receptors of a given T-cell subpopulation were blocked and that the subpopulation itself was not actually depleted. This contrasts with the findings in patients with systemic lupus erythematosus (SLE) about whom several studies have reported a selective depletion of circulating T_G cells (23, 24) as well as T-cell subsets determined by a number of other methodologies (25–29). This is of particular relevance in light of the fact that in several in vitro systems, SLE has been demonstrated to manifest a deficiency of suppressor cell function (23, 30–35). Although the actual suppressor cell in most of these systems has not been conclusively identified, at least in some systems of in vitro B-cell function, the T_G cell has been demonstrated to be the suppressor cell (16). With regard to the relationship between deficiency of T_G cells and lack of suppressor cell function, it should be pointed out that patients with SLE in addition to the deficiency of T_G cells (23, 24) also manifest a defective ability to generate, after triggering with Con A, suppressor cells of PWM-induced PFC responses (23). In the present study,

using that same PFC assay of B cell function, patients with Sjögren's syndrome were able to generate Con A-suppressor cells normally. This was true even of those patients with extraglandular disease who had "blocked" T_G Fc receptors.

The modulation of Fc receptors by immune complexes and other factors as well as the effect of such modulation of the Fc receptor upon the functional capability of the cell is currently a topic of great interest (36–39). With regard to the relationship between Fc receptor modulation and the ability to be subsequently triggered by Con A to generate into a suppressor cell, we have demonstrated that T_G cells which are positively selected during purification, i.e., by interaction with 7S EA to form rosettes for fractionation over Hypaque-Ficoll gradients, are unable to be generated by Con A to express suppressor cell function in the PWM-induced PFC assay (40). It was this finding of inhibition of T_G function by modulation of Fc receptors that led to the hypothesis that at least one of the potential mechanisms of the defect in Con A-induced suppressor cell function in SLE was the in vivo modulation of T_G by circulating immune complexes (23). This however, could not be the entire explanation of the defect in Con A-induced suppressor cell function in SLE, since we (40) and others (41) have demonstrated that the Con A-induced suppressor cell of human B-cell function can be found in multiple subpopulations of T cells and clearly is not confined to the T_G population. It is noteworthy, however, that the deficiency of the number of T_G cells in SLE is apparently irreversible and cannot be normalized by trypsinizing the T cells (unpublished observations)[2], whereas patients with Sjögren's syndrome who have normal Con A-induced suppressor cell function have a reversible deficiency of T_G. A similar reversible effect of serum factors on Fc receptor function in Sjögren's syndrome was previously demonstrated in a study which showed that a diffusely distributed serum factor in SLE and polyarteritis nodosa reversibly blocked Fc receptor-mediated antibody-dependent cellular cytotoxicity (42).

Since ~ 80% of patients with Sjögren's syndrome have circulating immune complexes (11), the role of immune complexes and particularly the probable differences in the effects of immune complexes upon immunoregulatory lymphocyte subpopulations in Sjögren's syndrome as compared with SLE assumes potential importance in our understanding of the modulation of immunoregulatory mechanisms by different types of circulating immune complexes. The complexity of the situation cannot be minimized, however, since other factors such as antilymphocyte antibody may play a major role in the inhibition, if not elimination of suppressor cell populations in SLE (28, 29).

Another important difference between Sjögren's syn-drome and SLE as demonstrated in the present study is the absence in Sjögren's syndrome of increased numbers of spontaneous antibody-producing cells as measured by the the presence in the circulation of spontaneous IgM anti-TNP PFC. Increased numbers of such "preactivated" B cells have been demonstrated in SLE (43-45). We have previously hypothesized that the preactivation of circulating IgM-producing B cells in SLE was likely due to an in vivo polyclonal triggering (23). This was accompanied by a decrease in ability to be triggered in vitro by a de novo polyclonal stimulus such as PWM and hence a decreased PWM-induced anti-SRBC PFC response (23) as was demonstrated in the present study with patients with the more severe extraglandular disease. This same theory was hypothesized by others for the simultaneous in vivo hyperreactivity of B cells and decreased ability of B cells to be polyclonally triggered in vitro as demonstrated in the NZB/NZW mouse model of SLE (46–48).

Since Sjögren's syndrome is a disease of hyperreactivity of B cells, the in vivo preactivation theory is still a likely explanation. Cells that have been preactivated to the point of appearing as spontaneous IgM-producing PFC might not have been found in the circulation in Sjögren's syndrome because they had already been compartmentalized in other lymphoid organs.

Previous studies have demonstrated a T lymphocytopenia in Sjögren's syndrome (49, 50), whereas in this study absolute numbers of T-cell subpopulations in patients with Sjögren's syndrome were found to be within normal limits. It should be pointed out that these patients differ from the Sjögren's syndrome patients in these other studies inasmuch as our patients had Sjögren's syndrome alone without any other concomitant connective tissue diseases such as rheumatoid arthritis or SLE. Also, and perhaps most importantly, none of the patients in the present study were receiving any form of therapy nor had they ever received any form of therapy for their Sjögren's syndrome. In this regard, the profound and selective effects of agents such as corticosteroids on T cells and T-cell subpopulations has been previously described (51, 52).

Thus the present study has demonstrated that certain patients with Sjögren's syndrome, particularly of the extraglandular type without coexisting additional connective tissue diseases, have a deficiency of expression of the Fc receptor for IgG on a population of their T cells (T_G cells). The defective expression is caused by the reversible blocking by a serum factor. This reversibility of effect may explain the lack of detectable abnormalities of modulation of immunoregulatory lymphocyte subpopulations in Sjögren's syndrome as compared with SLE in which irreversible deficiencies of Fc receptor-bearing T cells are accompanied by profound immunoregulatory abnormalities.

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