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Research Article

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Immunoglobulin Synthesis by Salivary Gland Lymphoid Cells in Sjögren's Syndrome

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ABSTRACT The synthesis of immunoglobulins by cells infiltrating the labial salivary glands has been studied by radioimmunoelectrophoresis in 20 patients with Sjögren's syndrome (SS) and in 14 control patients with related disorders. The patients with SS were producing significantly greater quantities of IgG, IgM, and IgA. Synthesis of IgG and IgM correlated with the degree of lymphoid infiltration but not with serum immunoglobulin concentration. Patients with SS and rheumatoid arthritis (RA) showed greater synthesis of IgG and IgM than those with uncomplicated RA. The only extensive lymphoid infiltration was seen in patients with SS. One patient with SS and primary macroglobulinemia was synthesizing the paraprotein in the lip biopsy as well as in the bone marrow. These results establish the immunologic competence of the infiltrating lymphoid cells and suggest their origin from an extrasalivary source.

INTRODUCTION

Sjögren's syndrome (SS) is an autoimmune disease related to rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (1, 2). Patients with SS are prone to episodes of salivary and lacrimal gland enlargement. They eventually develop severe xerostomia and xerophthalmia due to insufficient production of glandular secretions. The sera of such patients often contain increased concentrations of γ-globulin, rheumatoid factor, and other autoantibodies.

Microscopic examination of salivary and lacrimal gland tissue in SS reveals diffuse infiltration with acinar degeneration and ductal proliferation. Similar lymphoid infiltrates are present in the salivary glands of NZB and NZB/NZW F_1 mice who develop an autoimmune disorder resembling SLE (3).

The infiltrating lymphoid cells in SS may be reacting immunologically against antigenic structures in the glands. The presence of antibodies directed against salivary duct epithelium in sera of many patients with SS supports this concept (4). For some time we wished to study the amount and nature of the immunoglobulins synthesized by these infiltrating lymphoid cells. However, because of the risks inherent in parotid gland surgery, we performed only four parotid gland biopsies over the last 4 yr. One year ago, we learned that the minor salivary glands in the lower lip became involved with an infiltrative process leading to parenchymal atrophy similar to that seen in the major salivary glands. We have since performed lower lip biopsies on 43 patients with SS and other connective tissue disorders. The study of immunoglobulin synthesis by 34 of these specimens is presented in this report.

METHODS

Subjects. The following adult subjects underwent lip biopsy: 13 patients with RA and SS, nine with sicca syndrome alone (uncomplicated SS), eight with RA alone, eight with SLE, and one each with discoid LE, scleroderma, sarcoidosis involving the parotid glands, keratoconjunctivitis sicca (KCS) without other features of SS, and idiopathic nephrotic syndrome. The latter patient and three with RA were the only males. The diagnosis of SS was based on previously published criteria (1, 2).

Lip biopsy. The lip biopsies were performed by one surgeon (P. L.). The patients were seated in a dental chair. After nerve block anesthesia of the inferior alveolar nerve with 2% xylocaine, an elliptical segment of tissue, 1 cm long, was removed with a scalpel. All tissue above the orbicularis oris was included in the specimen. Bleeding was easily controlled with the use of a chalazion forceps. The wounds were closed primarily with 4-0 black silk suture.

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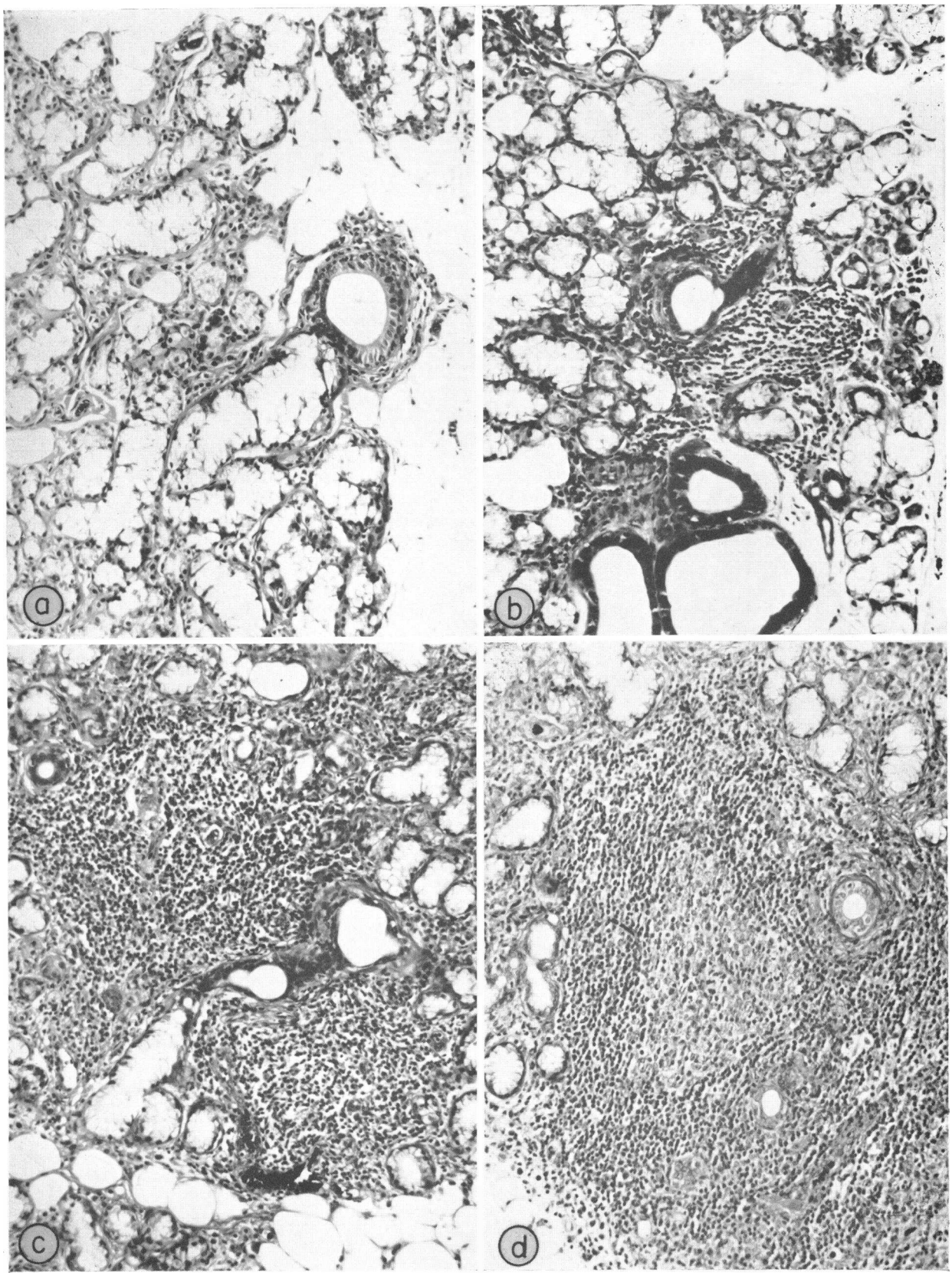

FIGURE 1 Representative lip biopsies graded 0 (*a*), one plus (*b*), two plus (*c*), and three plus (*d*). Hematoxylin and eosin. Magnification ×92.

TABLE I

Lymphoid Infiltration in Lip Biopsy Specimens

Diagnosis	No. of patients	Degree of infiltration				
		0	1+	2+	3+	4+
SS, RA	13	1	5	4	3	—
SS	9	1	—	4	3	1
RA	8	5	3	—	—	—
SLE	8	4	4	—	—	—
Other	5	4	1	—	—	—
Total	43	15	13	8	6	1

Abbreviations used: SS, Sjogren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; KCS, keratoconjunctivitis sicca.

Patients generally experienced mild discomfort for the next 1–2 days.

The tissue was divided for routine histologic examination and for study of immunoglobulin synthesis. Sections were stained with hematoxylin and eosin and examined by two observers who had no knowledge of the patient's diagnosis. The degree of lymphoid infiltration was graded on a scale ranging from 0 to 4 plus (Fig. 1). A score of 1 plus indicates no more than one submiliary aggregate of lymphoid cells, 2 plus, several small aggregates, and 3 and 4 plus, more extensive lymphoid infiltration with glandular atrophy.

Immunoglobulin synthesis. Studies of immunoglobulin synthesis were performed in 20 of the 22 patients with SS and in 14 of the 21 control patients. The tissue (5–10 mg) was minced in cold Hanks' balanced salt solution, using scalpels. Fragments about 1 mm^3 were disposed on the walls of a roller tube, then allowed to drain. The excess fluid was removed and 1 ml of a medium (5) containing 1 μc each of ^{14}C-labeled lysine and isoleucine/ml was added (SA 230 and 160 mc/mg, respectively). The tissues were incubated for 16–20 hr on a roller drum at 37°C.

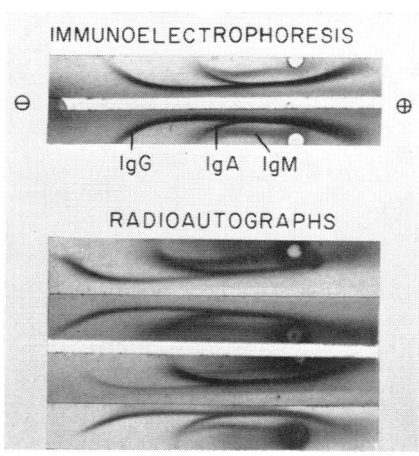

FIGURE 2 (Above) Immunoelectrophoretic analysis of the culture fluid developed with polyvalent anti-human serum. The precipitin lines are largely due to the added "carrier" human serum. (Below) Radioautographs of the culture fluids from four patients with rheumatoid arthritis and Sjögren's syndrome.

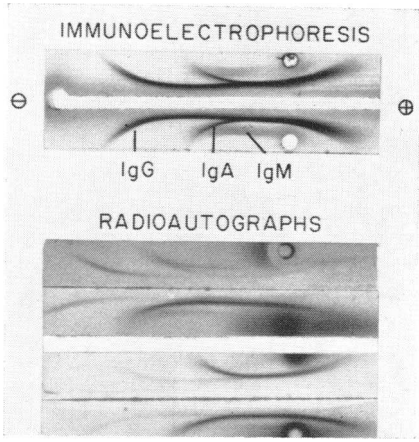

FIGURE 3 Same as in Fig. 2, except that the four patients have uncomplicated rheumatoid arthritis.

Analysis of culture fluids. Cultures were frozen and thawed once. The fluid was centrifuged (2500 *g*, 10 min) and the supernatant dialyzed against three changes of at least 10 volumes of 0.015 M NaCl. The culture fluids were then lyophilized and reconstituted to 0.1 ml with distilled water.

Antigen wells in plates of 1.5% agar were filled with a "carrier" human serum. When this had been absorbed by the agar, the well was filled twice with a culture fluid to be analyzed allowing sufficient time between filling for complete absorption. After electrophoresis (90 min, 5 v/cm) precipitin lines were developed with polyvalent anti-human serum, polyvalent antisera to immunoglobulins (Hyland Laboratories, Los Angeles, Calif.) and with antisera specific for IgG, IgM, and IgA. (These antisera were generously provided by Dr. William Terry, Immunology Branch, National Cancer Institute.) Coprecipitated radioactive protein in the washed-dried immunoelectrophoretic patterns was identified by placing Kodak Royal Pan film in contact with the washed-dried plates for 2 wk. The developed film was then matched to stained precipitin lines for identification of immunoglobulins. The intensity of labeling of each immunoglobulin class was scored on a scale ranging from 1 to 4 plus without knowledge of the patient's diagnosis. The final scores for synthesis were the highest scores recorded with polyvalent or specific antisera. Scores with the two polyvalent antisera were invariably identical. Occasionally the score obtained with specific antisera, especially anti-IgG, was somewhat lower. In a few cases, specific anti-IgM or anti-IgA gave a higher score than the polyvalent antisera.

RESULTS

Lymphoid infiltration. The only specimens containing a lymphoid infiltrate graded 2 plus or more were from patients with SS or SS and RA (Table I). Of 22 patients with SS, 15 showed this degree of infiltration. Occasional specimens from patients with SS contained large numbers of plasma cells and one or several germinal centers. Many acini were atrophic. The ducts showed a variable degree of ectasia and distortion. There were no myoepithelial islets.

TABLE II

Comparison of Lymphoid Infiltration with Immunoglobulin Synthesis and Serum Concentration

Patient	Age	Diagnosis	Lymphoid infiltrate (plus)	Synthesis (plus)			Serum concentration		
				IgG	IgM	IgA	IgG	IgM	IgA
	yr						*mg/ml*		
V. S.	60	SS, RA	3	4	4	3	13.0	2.2	5.4
L. H.	69	SS, RA	2	4	3	4	10.5	0.5	3.2
P. H.	44	SS, RA	2	4	3	2	24.0	4.0	5.0
M. S.	56	SS, RA	2	4	3	4	15.0	3.0	4.5
M. I.	43	SS, RA	1	4	2	4	24.0	1.0	3.5
H. R.	48	SS, RA	1	4	2	4	15.0	2.4	4.0
I. L.	42	SS, RA	3	3	4	2	17.0	9.0	5.0
N. M.	75	SS, RA	1	3	2	4	19.0	0.7	3.9
S. S.	45	SS, RA	1	2	1	4	9.0	1.4	2.0
M. H.	59	SS, RA	0	2	1	3	15.0	1.7	5.0
V. B.	55	SS, RA	1	2	4*	1	11.0	6.5	1.2
E. W.	64	SS	4	4	2	2	32.0	3.7	6.8
R. G.	56	SS	3	4	4	2	10.5	1.0	3.0
B. D.	52	SS	3	4	4	3	27.0	2.6	3.7
M. R.	70	SS	2	4	4	3	14.0	1.0	2.2
S. B.	52	SS	2	4	3	4	26.0	1.5	1.9
C. P.	49	SS	3	2	1	1	20.0	1.0	3.7
I. W.	49	SS	2	2	1	3	14.0	1.0	1.8
H. M.	34	SS	2	2	2	1	24.0	1.9	7.0
H. B.	75	SS	0	2	2	3	9.5	1.1	1.5
E. F.	39	RA	0	3	2	4	—	—	—
H. S.	50	RA	0	2	1	4	13.5	0.4	4.6
E. N.	71	RA	1	2	1	2	13.5	1.8	3.7
J. S.	51	RA	1	2	1	2	15.0	1.0	4.6
M. H.	46	RA	0	2	1	4	8.8	0.5	2.6
F. S.	57	RA	0	1	1	2	18.0	4.2	2.0
H. R.	37	SLE	0	3	1	3	17.5	1.1	2.7
T. B.	31	SLE	1	2	1	2	16.0	0.7	4.4
D. D.	35	SLE	1	2	1	1	49.0	2.7	3.7
D. B.	54	SLE	0	2	2	2	7.0	1.1	2.1
I. P.	23	Sarcoid	0	4	2	4	11.0	1.8	5.4
M. E.	64	Scleroderma	0	3	1	3	11.0	1.0	1.9
R. R.	35	Nephrosis	0	2	1	2	15.0	0.9	4.7
J. U.	74	KCS	1	2	1	2	—	—	—

* Characteristic of a paraprotein.

Immunoglobulin synthesis. The illustrations show characteristic radioautographs made from immunoelectrophoretic patterns of culture fluids from four patients with RA and SS (Fig. 2) and from four patients with uncomplicated RA (Fig. 3). The antiserum was a polyvalent anti-human immunoglobulin serum. Based on such radioautographic analyses, the synthesis of each immunoglobulin class was scored on a scale ranging from 1 to 4 plus. The scores for each patient are compared with the extent of lymphoid infiltration and serum immunoglobulin concentration in Table II. The results for immunoglobulin synthesis are combined for each diagnostic category in Table III and analyzed statistically in Table IV. The method employed for determining the amounts of immunoglobulin synthesis in the cultures is, at best, semiquantitative. Nevertheless, the distribution of scores obtained with the grading techniques employed may be analyzed statistically, using the mean score test described by Cochrane (6). This test gives a value for X^2 with 1° of freedom. It is clear that the two groups of patients with SS do not differ significantly. The patients with SS and RA, when compared with those with uncomplicated RA, showed significantly greater synthesis of IgG and IgM but not of IgA. When the 20 patients with SS were compared with the other 14 patients, all immunoglobulin classes were significantly increased. This difference was most striking for the IgM.

Using the Spearman rank correlation test, the synthesis of each immunoglobulin was compared with the degree of lymphoid infiltration, the serum concentration of that immunoglobulin, and the synthesis of the other two immunoglobulins. These comparisons were made for

TABLE IV

Statistical Analysis of Immunoglobulin Synthesis

Comparison*	IgG		IgM		IgA	
	X^2	*P*	X^2	*P*	X^2	*P*
SS, RA : SS	0.67	>0.30	0.36	>0.50	1.33	>0.20
SS, RA : RA	4.54	<0.05	7.85	<0.01	1.76	>0.20
Total SS : total other	5.49	<0.02	14.07	<0.001	4.57	<0.05

* In these comparisons, patients with the first diagnosis listed showed significantly greater synthesis whenever $P < 0.05$.

TABLE III

Immunoglobulin Synthesis by Lip Biopsy Specimens

Diagnosis	No. of patients	IgG				IgM				IgA			
		1+	2+	3+	4+	1+	2+	3+	4+	1+	2+	3+	4+
SS, RA	11	—	3	2	6	2	3	3	3	1	2	2	6
SS	9	—	4	—	5	2	3	1	3	2	2	4	1
RA	6	1	3	2	—	5	1	—	—	—	3	—	3
SLE	4	—	3	1	—	3	1	—	—	1	2	1	—
Other	4	—	2	1	1	3	1	—	—	—	2	1	1
Total	34	1	15	6	12	15	9	4	6	4	11	8	11

all patients, only those with RA (with or without SS) and only those with SS (with or without RA). For all patients and for those with RA, synthesis of IgG and IgM correlated with lymphoid infiltration ($P < 0.05$ for IgG, $P < 0.001$ for IgM). This relationship was not found in the patients with SS. However, in this latter group, IgA synthesis was negatively correlated with infiltration ($P < 0.05$). Synthesis of IgG and IgM tended to vary together in all comparisons ($P < 0.001$ for all patients, $P < 0.05$ for RA, $P < 0.02$ for SS). There was no correlation between serum immunoglobulin concentration and synthesis of that immunoglobulin in the cultures ($P > 0.10$).

Paraprotein (IgM) synthesis by lip biopsy tissue. Certain patients with SS have coexisting primary (Waldenström-type) macroglobulinemia (7). One such patient (V. B.), was included in this study. At the time of lip biopsy, her disease was well controlled on prednisone and chlorambucil. The serum IgM concentration had decreased from 30 to 6 mg/ml. Radioimmunoelectrophoretic analysis of cultures of bone marrow, lip, and peripheral blood leukocytes, as analyzed with a polyvalent antiserum or one specific for IgM, are shown in Fig. 4. A large amount of IgM was produced by cells in the lip as well as in the bone marrow. This newly synthesized IgM showed restricted electrophoretic mobility, identical with that of the serum paraprotein. It was almost entirely of light chain type K, the same as the serum paraprotein. No IgM synthesis was detected in leukocyte cultures.

Diagnostic value of lip biopsy. One of the patients with RA (P. H.) was not considered to have SS until the results of lip biopsy were known. The degree of lymphoid infiltration and immunoglobulin synthesis was highly suggestive of SS. Even though she was asymptomatic, keratoconjunctivitis sicca was present. Salivary flow was normal at this time and will be followed in the future.

DISCUSSION

This study demonstrates the immunologic competence of the lymphoid cells infiltrating the salivary glands in SS. Since SS is characterized by hypergammaglobulinemia and autoantibody formation, the augmented synthesis of immunoglobulins by salivary lymphoid cells is not surprising. However, peripheral blood lymphocytes in SS may show a decreased response to mitogenic stimulation and impaired delayed hypersensitivity reactions (8). In some patients, SS may evolve into reticulum cell sarcoma and hypogammaglobulinemia (9). Thus, the salivary lymphocytes might have shown a diminished capacity to synthesize immunoglobulins.

In general, immunoglobulin synthesis by salivary gland lymphoid cells did not correlate with the serum immunoglobulin concentration. Apparently, the local production of immunoglobulin by the infiltrating cells is not a direct reflection of the state of systemic immunoglobulin formation.

The marked synthesis of IgM in the salivary gland is remarkable because certain patients with SS develop macroglobulinemia, sometimes associated with pulmonary lymphoid infiltrates and the immunologic and histologic findings of Waldenström's disease. One such patient included in this report was producing the paraprotein both in the bone marrow and salivary gland. We have previously observed rheumatoid factor formation in the parotid gland of a patient with SS (9).

Local synthesis of IgG and IgM were related and independent of IgA formation. Indeed, in the patients with

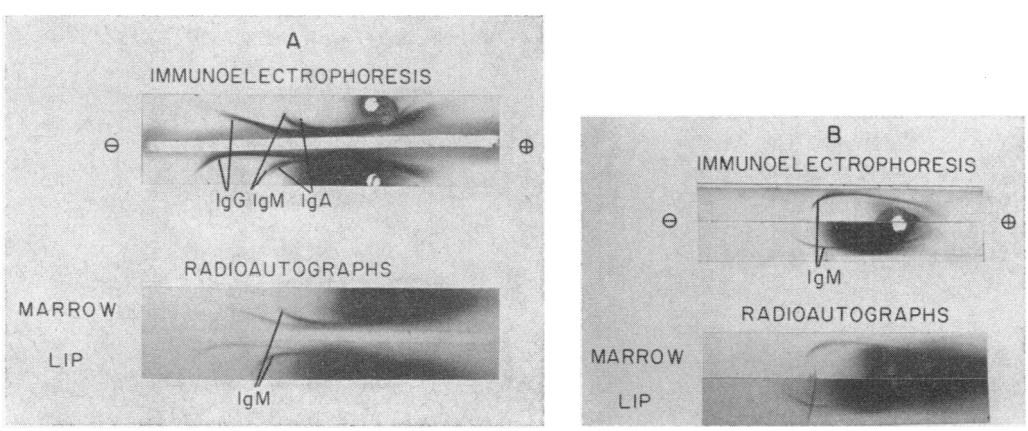

FIGURE 4 Immunoelectrophoretic and radioautographic analysis of culture fluid from patient V. B. who had Sjögren's syndrome and Waldenström's macroglobulinemia. Precipitin lines are developed with polyvalent (A) or IgM-specific (B) antisera. Culture fluids from bone marrow contain a nondialyzable material which appears to inhibit electroendosmosis. Note the anodal shift of all proteins, including those in the carrier.

SS alone, there was a negative correlation between IgA production and lymphoid infiltration. Normally, IgA is the major immunoglobulin produced by salivary gland tissue (10). A relative decrease in the proportion of cells synthesizing IgA, or a destruction of acinar tissue which is producing the "T-chain" component of secretory IgA (10) or both, could account for this negative correlation. These results suggest that the lymphoid cells normally synthesizing IgA are not the origin of the infiltrating cells. The latter probably arise from the systemic lymphoid pool.

While our investigation was underway, Chisholm and Mason published a histologic study of lip biopsies in 10 patients with SS, 30 additional control patients, and 60 postmortem subjects (11). As in our series, the most extensive lymphoid infiltrates were seen in SS.

The lip biopsy promises to be of diagnostic value in SS and an easily obtained source of salivary and lymphoid tissue for further investigations in this illness.

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