Vasculitis and Expression of Vascular Cell Adhesion Molecule-1, Intercellular Adhesion Molecule-1, and E-Selectin in Salivary Glands of Patients with Sjögren's Syndrome

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ABSTRACT. ***Objective.*** We investigated the relationship between clinical symptoms and the grade of histopathological damage and expression of adhesion molecules in salivary glands of patients with Sjögren's syndrome (SS).

Methods. We studied untreated and recently diagnosed patients with primary (n = 20) and secondary SS [10 with SS and rheumatoid arthritis (RA); 10 with SS and systemic lupus erythematosus (SLE)] and 3 healthy controls. Salivary gland biopsies were performed in patients and controls and clinical data were obtained. Salivary gland biopsies were assessed for lymphocyte focus score and expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. In serum, antinuclear antibodies, rheumatoid factor, anti-Ro and anti-La antibodies, anti-α-fodrin IgA and IgG antibodies, and gamma-globulin concentrations were measured.

Results. In salivary gland samples, ICAM-1 was expressed on vascular endothelial cells and lymphocyte foci, while VCAM-1 was expressed on vascular endothelial cells and follicular dendritic reticulin cells. There was a positive correlation between lymphocyte focus score and ICAM-1 expression ($p < 0.05$). We detected correlation between expression of ICAM-1 and VCAM-1, and the expression of VCAM-1 was significantly related to vasculitis ($p < 0.05$). The areas of E-selectin expression and the dispersion and severity of staining were not correlated with the focus score or with patients' clinical features ($p > 0.05$). There was no correlation between the staining and autoantibody positivity and gamma-globulin levels.

Conclusion. ICAM-1 may be important for lymphocyte recruitment and glandular damage and VCAM-1 may be important for the development of vasculitis in patients with SS. (J Rheumatol 2005;32:1063–70)

Key Indexing Terms:
SJÖGREN'S SYNDROME ADHESION MOLECULES E-SELECTIN VASCULITIS
VASCULAR CELL ADHESION MOLECULE-1 INTERCELLULAR ADHESION MOLECULE-1

Sjögren's syndrome (SS) is a multisystem immune-mediated disorder characterized by chronic inflammation of the exocrine glands and clinical symptoms of dry eyes and dry mouth. The spectrum of SS extends from a largely organ-specific autoimmune disorder to a systemic process that may involve additional organ systems, and SS may be primary or secondary to various other autoimmune diseases[1,2].

The causes of the glandular damage in SS have not been confirmed. As adhesion molecules control the tissue recruitment of lymphocytes and their activation, the generation of inflammatory foci and perpetuation of the inflammatory response depend on the distribution and functions of the adhesion molecules. Thus, aberrations in the expression and function of certain adhesion molecules have been implicated in the pathogenesis of autoimmune connective tissue diseases, mainly by 2 means. First, they influence the interactions of circulating immune cells with vascular endothelium during extravasation, and lead to local retention by promoting adhesion to the extracellular matrix. Second, they affect the interactions between T cells and antigen-presenting cells, and deliver the necessary activating signals for effective T-helper, T-cytotoxic, and B cell functions[3,4]. The principal adhesion molecules with roles in inflammation of the glands are vascular cell adhesion molecule-1 (VCAM-1 or CD106), intercellular adhesion molecule-1 (ICAM-1 or CD54), and endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin or CD62E)[5-11]. These adhesion mole-

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cules' expressions and their effects have been subjects of research for several years[12-16].

We investigated the aberrant expression of ICAM-1, VCAM-1, and E-selectin in minor salivary glands from patients with primary and secondary SS. We wished to determine the relation between the expression of these molecules and vasculitic symptoms in these patient groups.

MATERIALS AND METHODS

Patients. All patients were examined at the Department of Clinical Immunology and Rheumatology outpatient clinic, School of Medicine, Ankara University. Each patient had a positive Schirmer test (without anesthesia, ≤ 5 mm in 5 min) and saliva test (unstimulated whole salivary flow ≤ 1.5 ml in 15 min). They exhibited keratoconjunctivitis sicca, and minor salivary gland biopsy was performed from the lower lip in all cases. Patients with other causes of dry mouth and eyes, including viral infections (hepatitis C, hepatitis B, human immunodeficiency virus), sarcoidosis, drug use, smoking, lymphoma, graft versus host disease, and diabetes and patients who had had radiotherapy were excluded.

All patients were evaluated by extensive clinical and laboratory studies. Organ system involvement was detected by chest radiograph and high resolution computed tomography (HRCT), magnetic resonance imaging (MRI), electroneuromyography, and echocardiography. Demographic and clinical data and blood samples were obtained from all patients. Clinical evidence of vasculitis was based on neurological signs and cutaneous findings including digital ulcers, non-healing leg ulcers, hemorrhagic macules, subcutaneous nodules, splinter hemorrhages, macular rash, palpable purpura, and vasculitic urticarial lesions. Forty-three cases (patients with SS and 3 healthy controls) were included in this study. All cases and controls were female. Patients with primary SS (n = 20, mean age 48.0 yrs) were recently diagnosed and untreated. Twenty patients had secondary SS [RA + SS: n = 10, mean age 53.3 yrs; systemic lupus erythematosus (SLE) + SS: n = 10, mean age 44.7 yrs]. Minor salivary gland biopsies and blood samples were taken from 3 healthy volunteers (mean age 52 yrs). Labial minor salivary gland biopsies were performed by the same surgeon under local anesthesia. Patients with primary SS and controls were taking no steroids or other immunosuppressive drugs. Patients with secondary SS were taking low dose steroids (prednisolone 5–10 mg/day) and disease modifying antirheumatic drugs (DMARD: methotrexate 10–20 mg/week, sulfasalazine 1.5–2 g/day, hydroxychloroquine 200–400 mg/day) for the primary disease (SLE or RA).

All patients fulfilled the revised European classification criteria for SS[17]. All patients with RA and SLE fulfilled American College of Rheumatology classification criteria for RA[18] and SLE[19]. The study, including labial salivary gland biopsies performed with informed consent, was approved by the Ethical Committee, Faculty of Medicine, Ankara University.

Laboratory measures. Measurement of Westergren erythrocyte sedimentation rate (ESR) and thyroid tests including thyroxin, thyroid stimulating hormone, anti-thyroglobulin, and anti-myeloperoxidase antibodies were performed in the laboratories of Ibni Sina Hospital, School of Medicine, Ankara University. Serum samples were evaluated for gamma-globulin concentrations (normal range 8–19 mg/dl), presence of antinuclear antibodies (ANA) by indirect immunofluorescence on HEp-2 cells using a screening dilution of 1:100 (Euroimmun, Laboratorium für Experimentelle Immunologie GmbH, Gross Grönau, Germany). Rheumatoid factor (RF; Immage, Beckman Coulter Inc., Fullerton, CA, USA) was measured by nephelometric test. Anti-Ro and anti-La antibodies were both measured by ELISA (Imtec Immunodiagnostika GmbH, Berlin, Germany). α-fodrin IgA and IgG antibodies were detected by an ELISA method employing recombinant human α-fodrin as antigen (Anti-α-fodrin IgG+IgA; Orgentec Diagnostica GmbH, Mainz, Germany) according to the manufacturer's instructions. All serum samples were studied in 1:100 diluted serum samples.

Histological and immunohistological study. After fixation in 10% buffered formalin, all salivary gland biopsies were routinely processed and embedded in paraffin. For each patient, one or 2 tissue fragments were available. Hematoxylin and eosin (H&E) stained sections were evaluated histologically using the criteria described by Chisholm and Mason and subsequently modified by Greenspan, *et al* to provide a quantitative scoring method. Grades ranged from 0 to 4 (0 = no infiltrate, 1 = slight infiltrate, 2 = moderate infiltrate, 3 = one focus of at least 50 lymphocytes/4 mm^2 of gland, 4 = more than one focus of at least 50 lymphocytes/4 mm^2 of gland). The focus score was the actual number of foci/4 mm^2 (F = 0, 1, 2, 3, 4, 6, 8, 12)[20,21]. All patients had a focus score ≥ 1 per 4 mm^2 of gland.

For immunohistochemical study, sections 4–6 μm thick were cut from the paraffin blocks. After deparaffinization, a high temperature antigen-unmasking technique was performed in a pressure cooker in 0.01 M citrate for CD54 (pH 7.3) and 1 mM EDTA (pH 8.0) for CD62E and CD106. Staining was performed with an automatic stainer (Ventana Nexes) using Ventana iView diaminobenzidine detection system for CD62E (E-selectin) (NCL-CD 62E-382; Novocastra, Newcastle, UK), CD 106 (VCAM-1) (NCL-CD106; Novocastra), and CD54 (ICAM-1) (NCL-CD54-307; Novocastra) expression. Primary antibody dilutions were 1/50, 1/25, and 1/50 for CD62E, CD106, and CD54, respectively. In all staining runs, inflamed tonsil and inflamed appendix vermiformis sections were used as positive controls. As negative controls, primary antibodies were omitted and phosphate buffered saline was used.

For all 3 antibodies membranous staining was evaluated as positive. Cytoplasmic staining alone was regarded as nonspecific. Stainings of the endothelium and epithelium were evaluated independently. The quantity and intensity of the stainings were estimated separately and subjectively evaluated on a scale of 0, 1, 2, or 3 (0: absent, 1+: weak, 2+: definite, 3+: strong staining). For each antibody a total staining score of 0, 2, 3, 4, 5 or 6 was obtained and used for statistical evaluation. In addition, stainings observed in the other cells were subjectively evaluated. Histological and immunohistochemical analyses were performed blindly for clinical data by the same histopathologist for all cases.

Statistical analysis. Correlation between focus scores and staining scores of adhesion molecules was analyzed by the Spearman correlation test. Relations between presence of vasculitis and focus/staining scores of adhesion molecules were assessed by multiple comparison test[22].

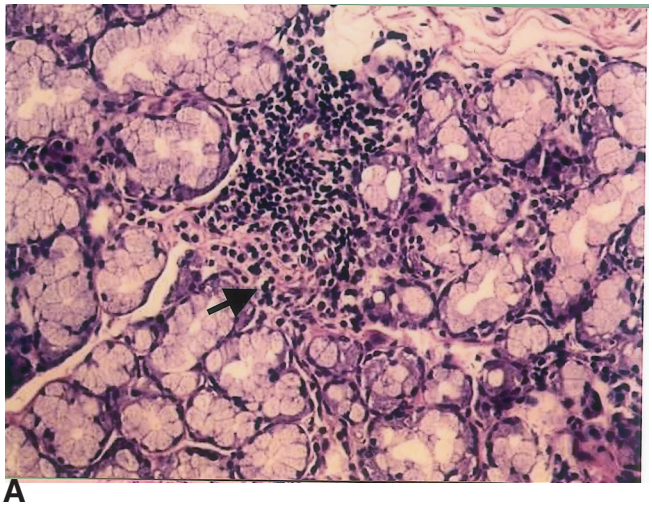
RESULTS

The demographic data and clinical and serological features of patients with primary and secondary SS and healthy controls are summarized in Table 1. The following prevalences of extraglandular involvement were found in patients with primary SS: musculoskeletal 90% (18/20), neurologic 10% (2/20), cutaneous vasculitic signs 10% (2/20), and pulmonary 5% (1/20). Autoimmune thyroiditis (Hashimoto's thyroiditis) was observed in the 20% (4/20) of patients with primary SS. No patient with primary SS was taking steroids or DMARD.

All patients with secondary SS had the symptoms of their primary diseases, and they were diagnosed with secondary SS when they were being evaluated for the primary diseases. In the patients with RA + SS, pulmonary (interstitial lung disease) and neurologic involvement was found to be 20% (2/10) and cutaneous vasculitic signs were found to be 10% (1/10). Autoimmune thyroiditis was not observed in this group. In patients with SLE + SS, cutaneous vasculitic signs and autoimmune thyroiditis were found to be 33% (3/10) and 10% (1/10), respectively. Neurologic involvement was

Table 1. Demographic and clinical data and autoantibody profiles in patients with primary (PSS) and secondary Sjögren's syndrome.

Patients	Diagnose	Age	Anti-Ro	Anti-La	ANA titer	RF	Anti-α-fodrin IgA	Anti-α-fodrin IgG	Vasculitic cutaneous signs	Neurological signs	Pulmonary signs	Hashimoto thyroiditis
1	PSS	34	Pos.	Pos.	1/1000	84.9	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.
2	PSS	67	Neg.	Neg.	Neg.	453	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
3	PSS	42	Pos.	Pos.	1/1000	276	Neg.	Neg.	Macular rash	Neg.	Nodule	Neg.
4	PSS	63	Pos.	Pos.	Neg.	81.4	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
5	PSS	48	Pos.	Pos.	1/1000	121	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
6	PSS	51	Pos.	Neg.	1/100	36.8	Neg.	Neg.	Raynaud, livedo	Mononeuritis multiplex	Interstitial pneumonitis	Neg.
7	PSS	39	Pos.	Neg.	1/1000	351	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
8	PSS	56	Neg.	Neg.	1/1000	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
9	PSS	47	Pos.	Neg.	1/100	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Pos.
10	PSS	53	Pos.	Neg.	1/100	109	Neg.	Pos.	Neg.	Neg.	Neg.	Pos.
11	PSS	36	Pos.	Neg.	1/10000	194	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
12	PSS	56	Neg.	Neg.	Neg.	37.4	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
13	PSS	39	Neg.	Neg.	Neg.	39.6	Neg.	Neg.	Raynaud, livedo	Mononeuritis multiplex	Nodule	Neg.
14	PSS	46	Pos.	Neg.	1/1000	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
15	PSS	58	Neg.	Neg.	1/1000	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Pos.
16	PSS	26	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.	Neg.	Neg	Neg.
17	PSS	67	Neg.	Neg.	1/1000	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
18	PSS	47	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
19	PSS	31	Pos.	Neg.	1/100	Neg.	Neg.	Neg.	Macular rash	Neg.	Neg.	Neg.
20	PSS	54	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Macular rash	Neg.	Neg.	Neg.
21	RA+SS	51	Neg.	Neg.	Neg.	50.9	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
22	RA+SS	43	Neg.	Neg.	1/100	70.2	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
23	RA+SS	54	Neg.	Neg.	Neg.	113	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
24	RA+ SS	64	Pos.	Neg.	1/10.000	Neg.	Neg.	Neg.	Palpable purpura	Peripheral neuropathy	Interstitial pneumonitis	Neg.
25	RA+SS	54	Neg.	Neg.	Neg.	65.4	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
26	RA+SS	43	Neg.	Neg.	1/100	64.9	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
27	RA+SS	65	Neg.	Neg.	Neg.	26.8	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
28	RA+SS	51	Pos.	Pos.	1/10.000	Neg.	Neg.	Neg.	Raynaud, livedo	Mononeuritis multiplex	Interstitial pneumonitis	Neg.
29	RA+SS	47	Pos.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
30	RA+SS	61	Pos.	Neg.	Neg.	295	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
31	SLE+SS	46	Pos.	Neg.	1/100	Neg.	Pos.	Neg.	Macular rash	Neg.	Neg	Neg.
32	SLE+SS	57	Pos.	Neg.	1/100	Neg.	Neg.	Pos.	Macular rash	Neg.	Neg.	Neg.
33	SLE+SS	37	Pos.	Neg.	1/100	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
34	SLE+SS	49	Pos.	Neg.	1/1000	Neg.	Pos.	Neg.	Splinter haemorrhage,livedo	Neg.	Neg.	Neg.
35	SLE+SS	48	Pos.	Neg.	1/100	Neg.	Neg.	Neg.	Livedo	CNS involvement	Neg.	Neg.
36	SLE+SS	36	Pos.	Neg.	1/1000	Neg.	Neg.	Neg.	Livedo	CNS involvement	Neg.	Neg.
37	SLE+SS	58	Pos.	Pos.	1/1000	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
38	SLE+SS	38	Pos.	Pos.	1/1000	54.4	Neg.	Neg.	Livedo	Peripheral neuropathy	Neg.	Neg.
39	SLE+SS	38	Neg.	Neg.	1/1000	Neg.	Neg.	Neg.	Livedo, Raynaud	Peripheral neuropathy	Neg.	Neg.
40	SLE+SS	40	Pos.	Neg.	1/1000	Neg.	Neg.	Neg.	Livedo, Raynaud	CNS involvement	Neg.	Pos.
41	Control	41	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
42	Control	42	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
43	Control	43	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.



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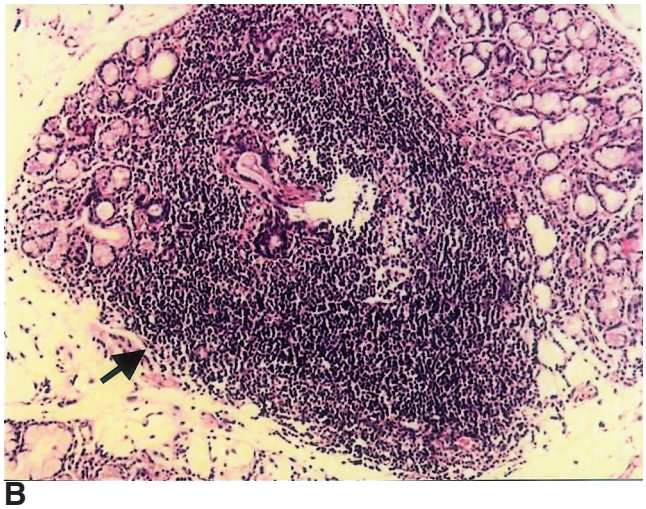

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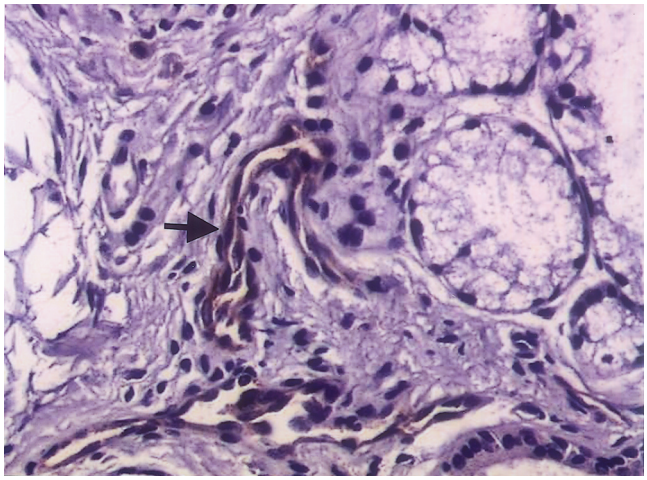

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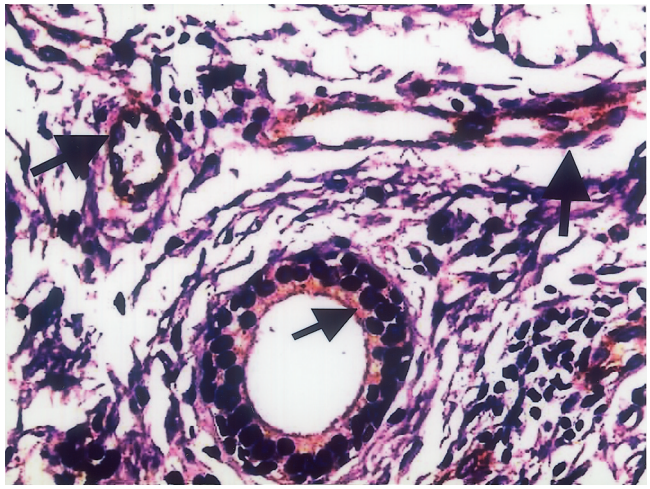

D

found to be 50% (5/10), but pulmonary involvement was not found in this group.

Immunohistochemical staining results. Focus scores of the patients with SS were 1 or higher (Figure 1A). In some patients, the biopsy specimens showed germinal center formation within the inflammatory foci (Figure 1B). Characteristically, immunohistochemical staining revealed substantial differences between patients and controls in the expression of ICAM-1, VCAM-1, and E-selectin. In the controls, ICAM-1 or VCAM-1 staining was not detected, although E-selectin was slightly positive in 2 controls. In all patients, ICAM-1 was expressed on vascular endothelial cells and mononuclear inflammatory cells. ICAM-1 staining was prominent near or within the lymphocytic foci and/or the germinal centers and on the vascular endothelial cells (Figure 1E and 1F). The intensity of VCAM-1 staining was generally low, and it was observed on ductal epithelial cells, acini, and vascular endothelial cells (Figure 1G and 1H). E-selectin staining was limited to endothelial cells, and it did not show any accentuation within or near the lymphocytic foci (Figure 1C and 1D). Histopathological grade focus scores and immunohistochemical staining scores for the adhesion molecules in biopsy specimens of controls and patients with primary and secondary SS are shown in Table 2. Total staining scores of the primary and secondary SS groups did not show statistical differences for any of the 3 antibodies ($p > 0.05$).

In the patients with primary and secondary SS, there was a significant correlation between total lymphocyte focus score and staining scores of ICAM-1 ($p < 0.05$), whereas no such correlation was observed for VCAM-1 or E-selectin ($p > 0.05$). Staining scores of VCAM-1 and ICAM-1 showed a positive correlation ($p < 0.05$). A positive correlation was found between the presence of vasculitis and VCAM-1 staining ($p < 0.001$). However, there was no correlation between the presence of vasculitis and total lymphocyte focus score and ICAM-1 and E-selectin stainings ($p > 0.05$). The relationship between the presence or absence of vasculitis and total lymphocyte focus score and total staining score for adhesion molecules is shown in Table 3.

There was no relation between the total staining score of the adhesion molecules and the positivity of autoantibodies

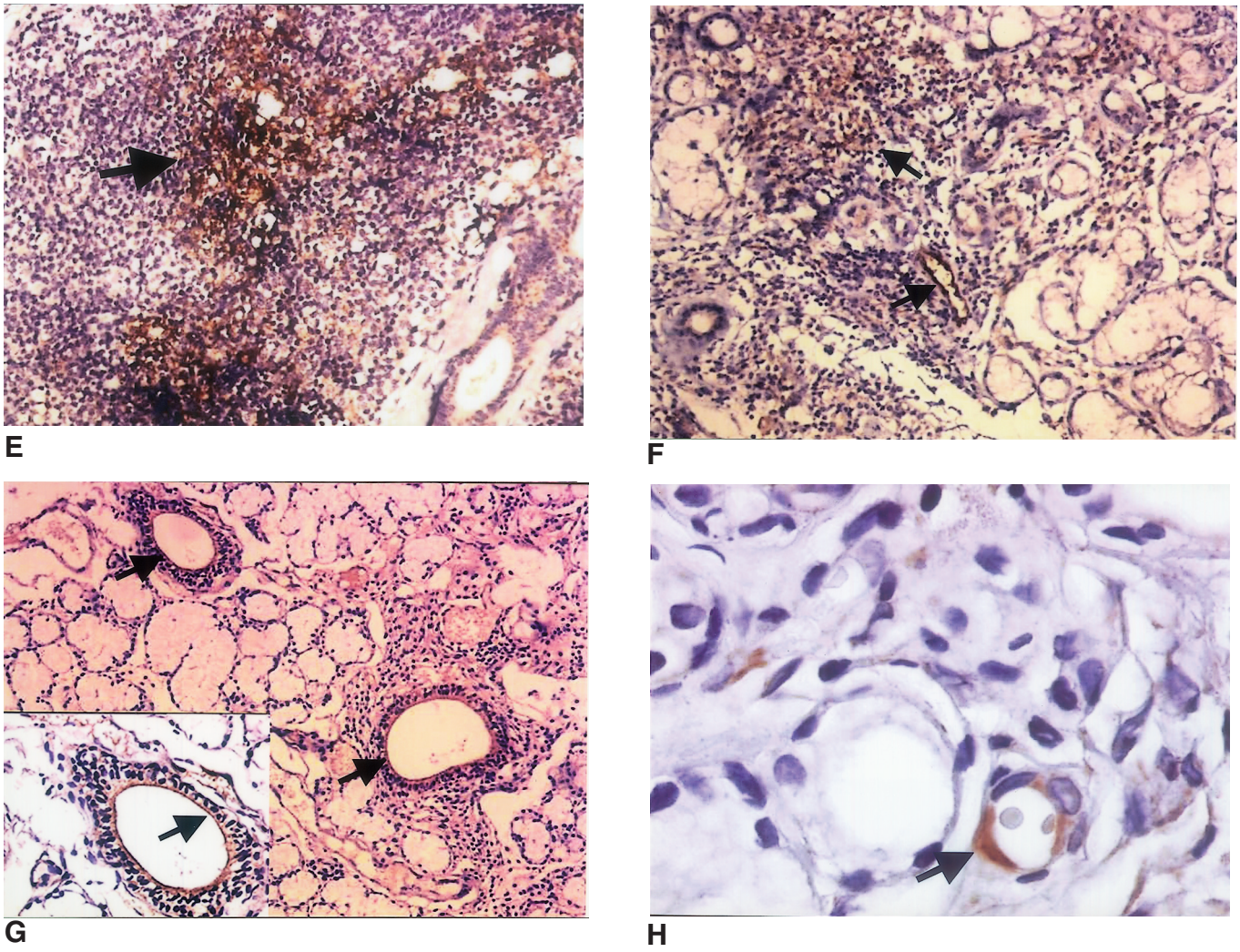

Figure 1. Histopathological and immunohistochemical findings in the patients with SS. A. A focus that shows a collection of at least 50 lymphocytes (H&E, original magnification ×100). B. A large focus destroying the lobule and showing germinal center formation (H&E, original magnification ×50). C. E-selectin (CD62), staining of vascular endothelial cells (original magnification ×200). D. E-selectin (CD62), staining of vascular endothelial cells and ductal epithelium (original magnification ×200). E. ICAM-1 (CD54), staining of mononuclear inflammatory cells within the germinal center in a focus (original magnification ×100). F. ICAM-1 (CD54), staining of vascular endothelial cells and inflammatory cells (original magnification ×100). G. VCAM-1 (CD106), apical staining of ductal epithelium (original magnification ×50, inset ×200). H. VCAM-1 (CD106), staining of vascular endothelial cells (original magnification ×500).

including anti-α-fodrin IgA and IgG, anti-SSA, anti-SSB, ANA titer, RF levels, ESR values, gamma-globulin levels, and total lymphocyte focus score ($p > 0.05$).

DISCUSSION

Adhesion of inflammatory cells to vascular endothelium is the initial step in cell migration into sites of injury or infection. Adhesion molecules and activated lymphocyte function-associated antigen type 1 (LFA-1) promote lymphocyte homing and occasionally characteristic cell clustering similar to follicular structures of the lymph nodes[4].

First, E-selectin is expressed primarily on activated endothelial cells. E-selectin mediates the adhesion of neutrophils, eosinophils, basophils, monocytes, and a subset of memory T lymphocytes to vascular endothelium by binding its ligands to the cell surface[23]. Aberrant endothelial expression of E-selectin has also been observed in the salivary glands of patients with primary and secondary SS, but E-selectin seems to be involved to a lesser extent than other adhesion molecules in the process of glandular lymphocytic infiltration[5,7,9].

In our study, E-selectin was more weakly expressed on submucosal vascular endothelium than on the glandular site far from the lymphocyte focus in the salivary glands of patients with primary and secondary SS. The sites of the expression and intensity of E-selectin were correlated with neither focus scores nor vasculitis ($p > 0.05$). Based on our

Table 2. Immunohistologic analyses of the patients with primary (PSS) and secondary Sjögren's syndrome.

Patient	Diagnoses	Grades	LFS	ICAM-1	VCAM-1	E-selectin	Vasculitis	ESR	Gamma globulin
1	PSS	4	4.57	6	3	4	Neg.	23	29.70
2	PSS	4	2.00	4	0	0	Neg.	24	15.30
3	PSS	4	3.00	4	6	2	Pos.	80	31.0
4	PSS	3	1.00	3	4	0	Neg.	54	32.6
5	PSS	3	1.00	3	4	0	Neg.	30	27.3
6	PSS	4	2.29	6	6	6	Pos.	11	16.5
7	PSS	4	7.80	6	2	3	Neg.	62	24.8
8	PSS	3	1.00	4	2	4	Neg.	30	16.4
9	PSS	4	8.00	6	0	5	Neg.	16	17.8
10	PSS	4	6.00	6	0	5	Neg.	65	19.7
11	PSS	4	9.33	6	2	0	Neg.	44	29.7
12	PSS	4	1.33	3	0	6	Neg.	39	21.6
13	PSS	3	1.00	3	5	3	Pos.	4	15.6
14	PSS	4	4.57	5	2	3	Neg.	26	24.4
15	PSS	3	1.00	2	0	3	Neg.	10	17.8
16	PSS	4	2.00	2	2	3	Neg.	13	16.8
17	PSS	3	1.00	0	0	2	Neg.	118	24.0
18	PSS	4	1.40	2	0	0	Neg.	53	16.7
19	PSS	4	2.55	5	6	2	Pos.	12	18.1
20	PSS	3	3.00	0	0	2	Neg.	3	19.7
21	RA+SS	3	1.00	2	0	0	Neg.	18	14.5
22	RA+SS	3	1.00	2	0	2	Neg.	40	20.7
23	RA+SS	4	5.33	6	0	2	Neg.	20	15.4
24	RA+ SS	4	6.67	6	6	2	Pos.	30	19.2
25	RA+SS	3	1.00	2	2	4	Neg.	26	12.8
26	RA+SS	4	5.00	5	2	0	Neg.	60	18.4
27	RA+SS	4	2.00	4	2	3	Neg.	50	22.4
28	RA+SS	4	6.30	5	6	2	Pos.	93	26.8
29	RA+SS	4	1.40	2	2	3	Neg.	30	21.6
30	RA+SS	3	1.00	2	0	2	Neg.	71	22.1
31	SLE+SS	4	2.00	2	4	2	Pos.	10	12.8
32	SLE+SS	3	1.00	2	5	2	Pos.	20	19.1
33	SLE+SS	4	1.33	3	0	2	Neg.	36	24.2
34	SLE+SS	4	4.00	5	5	0	Pos.	46	17.8
35	SLE+SS	4	1.40	5	4	3	Pos.	33	22.2
36	SLE+SS	4	1.50	4	6	2	Pos.	16	19.5
37	SLE+SS	4	1.20	4	2	4	Neg.	28	22.4
38	SLE+SS	4	1.71	3	4	2	Pos.	28	25.8
39	SLE+SS	3	1.00	3	5	2	Pos.	26	13.4
40	SLE+SS	4	2.00	4	4	0	Pos.	51	22.4
1	Control	0	0.00	0	0	0	Neg.	12	13.4
2	Control	0	0.00	0	0	2	Neg.	10	15.3
3	Control	0	0.00	0	0	0	Neg.	14	16.2

Table 3. Correlation between the presence of vasculitis and total staining scores of adhesion molecules and total lymphocyte focus scores (TLFS) in patients with primary and secondary Sjögren's syndrome.

	Vasculitis (+)		Vasculitis (–)		
	Mean ± SD	Median (range)	Mean ± SD	Median (range)	p
TLFS	2.60 ± 2.58	1.33 (0–9.33)	2.60 ± 1.85	2 (1–6.7)	0.294
ICAM-1	3.10 ± 2.06	3 (0–6)	4.07 ± 1.33	4 (2–6)	0.133
VCAM-1	1.07 ± 1.31	0 (0–4)	5.14 ± 0.86	5 (4–6)	< 0.001
E-selection	2.21 ± 1.80	2 (0–6)	2.14 ± 1.41	2 (0–6)	0.69

results, we believe that E-selectin is of minor importance in the infiltration of glandular lymphocytes for SS.

ICAM-1 and VCAM-1 are 2 molecules that play an important role in mediating T cells. Many proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1), not only upregulate ICAM-1 expression but also induce de novo VCAM-1 expression[4]. Some reports describe that elevated levels of ICAM-1 expression

were observed on ductal epithelial cells and infiltrating mononuclear cells in salivary and/or lacrimal glands of patients with SS. It has been shown that interferon-γ activated the expression of ICAM-1 and HLA-DR, while TNF-α activated the expression of ICAM-1 and VCAM-1[6,9,15,16]. Further, Flipo, *et al* described that there were increased levels of ICAM-1 mRNA and protein in the salivary glands of SS patients and patients with severe rheumatoid vasculitis, but that VCAM-1 expression was low[10]. Their data should be interpreted cautiously because the expression of these molecules might have been inhibited by immunosuppressive or steroid treatments.

We observed that ICAM-1 was strongly expressed on vascular endothelial cells and in the lymphocyte foci, and VCAM-1 was strongly expressed on vascular endothelial cells, apical duct epithelial cells, and acini in the salivary glands of patients with SS. There was a correlation between lymphocyte focus score and ICAM-1 expression ($p < 0.05$). Based on these results, the ICAM-1 molecule may be responsible for the occurrence and direction of the inflammation and for the formation of lymphocyte foci in SS. We found a correlation between expressions of VCAM-1 and of ICAM-1. In both primary and secondary SS, we found an increased VCAM-1 expression in the presence of vasculitis ($p < 0.05$). It has been suggested that VCAM-1 is an important molecule in the pathogenesis of vasculitis; however, no correlation was observed between ICAM-1 expression and vasculitis. That VCAM-1 and HLA-DR are aberrantly expressed on the same sites in SS is interesting. Hence, it is possible that VCAM-1 is an important adhesion molecule (a costimulator molecule) for antigen-presenting cells and CD4+ T cells in SS. There was no relation between the staining scores of the adhesion molecules and the positivity of autoantibodies including anti-α-fodrin IgA and IgG, anti-SSA, and anti-SSB, and ANA titers, RF levels, ESR values, gamma-globulin levels, and total lymphocyte focus scores in our study ($p > 0.05$).

In earlier studies, one or more expressions of adhesion molecules were found in either the salivary glands or in soluble forms in patients' sera. Nevertheless, we tested the expression of all 3 adhesion molecules on the minor salivary glands, and we also studied the relation between these molecules and focus scores and vasculitis in untreated patients with primary SS and secondary SS. Since we studied untreated patients with primary SS, the results from this study are important. Anticytokine therapy for autoimmune disease is currently available despite inconclusive results. New therapeutic strategies for inflammatory disease against adhesion molecules are promising.

Finally, it has been suggested that in patients with SS, ICAM-1 may be responsible for the glandular damage and VCAM-1 may be related to the vasculitis. The correlated clinical studies on our patients are insufficient and must be supported by *in vitro* experiments. Advances in this field provide new insights into the pathogenetic mechanisms leading to tissue damage in patients with autoimmune connective tissue diseases and should provide the basis for new therapeutic strategies in the near future.

REFERENCES

1. Pillemer SR, Matteson EL, Jacobson LT, et al. Incidence of physician-diagnosed primary Sjögren's syndrome in residents of Olmsted County, Minnesota. Mayo Clin Proc 2001;76:593-8.
2. Pillemer SR. Sjögren's syndrome. In: Klippel JH, editor. Primer on the rheumatic diseases. 12th ed. Atlanta: Arthritis Foundation; 2001:377-84.
3. Lenschow DJ, Walunas TI, Bluestone JA. CD28/B7 system of T cell costimulation. Ann Rev Immunol 1996;14:233-58.
4. Mojcik JF, Shevach EM. Adhesion molecules: a rheumatologic perspective. Arthritis Rheum 1997;40:991-1004.
5. Sfikakis PP, Mavrikakis M. Adhesion and lymphocyte costimulatory molecules in systemic rheumatic disease. Clin Rheumatol 1999;18:317-27.
6. St. Clair EW, Angelilo JC, Singer KH. Expression of cell-adhesion molecules in the salivary gland microenvironment of Sjögren's syndrome. Arthritis Rheum 1992;35:62-6.
7. Aziz KE, McCluskey PJ, Wakefield D. Expression of selectins (CD62 E, L, P) and cellular adhesion molecules in primary Sjögren's syndrome: questions to immunoregulation. Clin Immunol Immunopathol 1996;80:55-66.
8. Edwards JC, Wilkinson LS, Speight P, Isenberg DA. Vascular cell adhesion molecule 1 and alpha 4 and beta 1 integrins in lymphocyte aggregates in Sjögren's syndrome and rheumatoid arthritis. Ann Rheum Dis 1993;52:806-11.
9. Saito I, Terauchi K, Shimuta M, et al. Expression of cell adhesion molecules in the salivary and lacrimal glands of Sjögren's syndrome. J Clin Lab Anal 1993;7:180-7.
10. Flipo RM, Cardon T, Chopin MC, Vandecandelaere M, Duquesnoy B, Janin A. ICAM-1, E-selectin, and TNF-α expression in labial salivary glands of patients with rheumatoid vasculitis. Ann Rheum Dis 1997;56:41-4.
11. Cuida M, Halse AK, Johannessen AC, Tynning T, Jonsson R. Indicators of salivary gland inflammation in primary Sjögren's syndrome. Eur J Oral Sci 1997;105:228-33.
12. Dimitriou ID, Kapsogeorgou EK, Moutsopoulos HM, Manoussakis MN. CD40 on salivary gland epithelial cells: high constitutive expression by cultured cells from Sjögren's syndrome patients indicating their intrinsic activation. Clin Exp Immunol 2002;127:386-92.
13. Tanaka Y, Masashi N, Fujii K, et al. Intracellular adhesion molecule 1 underlies the functional heterogeneity of synovial cells in patients with rheumatoid arthritis. Arthritis Rheum 2000;43:2513-22.
14. Mikulowska-Mennis A, Baohui Xu, Berberian JM, Michie SA. Lymphocyte migration to inflamed lacrimal glands is mediated by vascular cell adhesion molecule-1/$\alpha_4\beta_1$ integrin, peripheral node addressin/L-selectin, and lymphocyte function-associated antigen-1 adhesion pathways. Am J Pathol 2001;159:671-81.
15. Kapsogeorgou EK, Dimitriou ID, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Activation of epithelial and myoepithelial cells in the salivary glands of patients with Sjögren's syndrome: high expression of intercellular adhesion molecule-1 (ICAM-1) in biopsy specimens and cultured cells. Clin Exp Immunol 2001;124:126-33.
16. Tsunawaki S, Nakamura S, Ohyama Y, et al. Possible function of salivary gland epithelial cells as nonprofessional antigen-presenting cells in the development of Sjögren's syndrome. J Rheumatol 2002;29:1884-96.
17. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria

proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-8.
18. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
19. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
20. Chislom DM, Mason DK. Labial salivary gland biopsy in Sjögren's disease. J Clin Pathol 1968;21:656-60.
21. Greenspan JS, Daniels TE, Talal N, Sylvester RA. The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg 1974;37:217-30.
22. Conover WJ. Multiple comparison test. In: Conover WJ. Practical nonparametric statistics. 2nd ed. Philadelphia: John Wiley & Sons; 1980:229-39.
23. Ley K, Tedder TF. Leukocyte interactions with vascular endothelium. New insights into selectin-mediated attachment and rolling. J Immunol 1995;155:525-8.