Demonstration and identification of monoclonal proteins in the urine of patients with Sjögren's syndrome

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SUMMARY Fresh sera and concentrated urine from 17 patients with primary Sjögren's syndrome (SS) were fractionated by high-resolution agarose electrophoresis to investigate the presence of monoclonal immunoglobulins or their components. Homogeneous protein bands were found in the gamma-globulin region in 47% of serum samples and 76% of urine specimens of all patients tested. These monoclonal proteins were detected more often in patients with extraglandular SS (77% in serum, 100% in the urine) than in patients with glandular SS (14% in serum, 43% in the urine). Immunofixation electrophoresis showed that the majority of these monoclonal proteins were free kappa or lambda light chains. Fractionation of unconcentrated parotid salivas from five SS patients failed to reveal the presence of monoclonal light chains or immunoglobulins. The present findings further substantiate our previous observation that a monoclonal process coexists with the polyclonal activation in SS patients.

Key words: monoclonal gammopathy, free light chains, lymphoid malignancy.

Sjögren's syndrome (SS) is a chronic autoimmune disease involving the exocrine glands which results in diminished secretions and mucosal dryness. In one-third of these patients the kidneys, lungs, or muscles can be affected, and this is referred to as extraglandular SS.¹ Furthermore, this syndrome has the unique feature that it can evolve from a polyclonal B lymphocyte activation state, to B lymphocyte malignancy.^{2,3}

The immunological hallmark of this syndrome is a polyclonal B cell hyperactivity, which is expressed by hypergammaglobulinaemia, a plethora of organ or non-organ specific autoantibodies, and immune complexes.¹ Using high-resolution electrophoresis combined with immunofixation electrophoresis we have recently shown that SS patients have a high incidence of circulating monoclonal free light chains in the serum. This finding suggests that the monoclonal process starts early in a subset of B lympho-

cytes, long before the expression of the B lymphocyte neoplasm.⁴ In this report we extend this observation by demonstrating and identifying monoclonal proteins in the urine of SS patients.

Materials and methods

Pairs of fresh sera and urine were collected from 17 Greek patients with primary SS.⁵ Serum and urine were also collected from 10 normal age, race, and sex matched controls. All these patients satisfied the criteria for the diagnosis of primary Sjögren's syndrome: keratoconjunctivitis sicca (decreased flow by Schirmer's test and punctate corneal ulcerations on fluorescent staining) or xerostomia (diminished flow rate or abnormal parotid scintigraphy) or both.⁵ The diagnosis was established in all patients with the histopathological lesion in the labial salivary glands compatible with SS. None of these patients had clinical or serological abnormalities characteristic of other autoimmune diseases. The age, disease duration, and clinical manifestations of these patients are shown in Table 1. Parotid saliva

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Table 1 Clinical and laboratory manifestations in patients with Sjögren's syndrome

Patient	Age (years)	Disease duration (years)	Extraglandular manifestations	Serum studies		Urine studies	
				HRE*	Immunofixation	HRE*	Immunofixation
1	40	8	Splenomegaly, interstitial lung disease	3 bands	Free lambda	2 bands	Free lambda
2	51	5	Interstitial lung disease	2 bands	Free lambda	1 band	Free lambda
3	53	2	Raynaud's phenomenon	1 band	Free lambda	1 band	Free lambda
4	59	2	Raynaud's phenomenon	Negative	ND†	2 bands	Free kappa
5	65	15	Interstitial lung disease, Raynaud's phenomenon	2 bands	Free lambda	2 bands	Free lambda
6	40	10	Raynaud's phenomenon	2 bands	ND	2 bands	ND
7	72	30	Vasculitis, pseudolymphoma, Raynaud's phenomenon	3 bands	IgM lambda, free kappa	3 bands	Free kappa
8	60	15	Raynaud's phenomenon, lymphadenopathy, splenomegaly	Negative	—	1 band	Free lambda
9	40	4	Vasculitis	Negative	—	1 band	ND
10	50	10	Vasculitis, glomerulonephritis	3 bands	IgG kappa, IgA kappa	3 bands	Free kappa
11	65	2	None	Negative	—	1 band	ND
12	57	3	None	Negative	—	1 band	Free kappa
13	66	1	None	Negative	—	Negative	—
14	37	4	None	Negative	—	Negative	—
15	53	1	None	Negative	—	Negative	—
16	67	17	None	Negative	—	Negative	—
17	62	10	None	1 band	Lambda	1 band	Free lambda

* HRE=High resolution gel electrophoresis.

† ND=not done.

was collected in Carlson-Crittenden cups from patients 3, 4, 6, 7, and 8 (Table 1). The other patients did not have any salivary flow. All urine specimens were screened for the presence of protein by Chemstrip 7 (Boehringer-Mannheim Co., Indianapolis, Indiana). The urine specimens were concentrated 75–100 times by Amicon concentrators (Danvers, Ma, USA). Fractionation of serum, saliva, and urine proteins was performed by a high-resolution agarose gel electrophoresis technique that detects weak but discrete homogeneous protein bands in the gamma-globulin region that are undetectable by the routine protein electrophoresis techniques.⁶ With this method 0.5% agarose gel in 0.05 M barbital buffer and application of 140 volts for a 12 min electrophoresis are employed. In addition the serum sample is placed in a narrow slit, which minimises diffusion of the protein fractions and results in sharp definition of protein bands.

To identify individual monoclonal bands, immunofixation was employed after the electrophoretic separation. Identification at the site of the band was accomplished by placing a cellulose acetate strip, impregnated with antibodies specific for heavy or light chains, directly on top of the band on the unstained gel. Individual antibodies specific for human α , γ , μ heavy chains and kappa or lambda

light chains were placed separately on top of multiple replicates of each sample. Goat, burro, and rabbit antibodies (Kallestad, Austin, Texas) were used for independent confirmation of specificity. The antibodies were allowed to diffuse into the gel for 15 min at 37°C. Each slide was then immersed in saline overnight, so that the rest of the proteins were washed out and the insoluble precipitate remaining was stained with amido black. Studies with a large number of control proteins have indicated that this technique is highly sensitive and specific.^{7,8}

Results

Homogeneous protein bands were detected in the gamma-globulin region of the protein electrophoresis patterns in 8/17 (47%) serum samples and 13/17 (76%) urine specimens of all SS patients tested (Tables 1 and 2). In the extraglandular SS, bands were found in 7/10 (70%) sera, and in all urine specimens tested. In the majority of these sera and urine samples more than one homogeneous protein band was found (Table 1). An example of these homogeneous protein bands is shown in the urine electrophoresis patterns in Fig. 1. In contrast bands were found in 1/7 (14%) sera and in 3/7 (43%) of the urine specimens tested from patients with

Table 2 Incidence of monoclonal protein bands in the serum and urine of SS patients

Patients	Number	Serum %	Urine %
Sjögren's syndrome	17	47	76
glandular	7	14	43
extraglandular	10	70	100
Normal control	10	0	0

Fig. 1 Protein electrophoresis patterns of urine samples: A, of normal control; B, C, and D, from patients with Sjögren's syndrome showing 1, 2, and 3 bands respectively; E, immunofixation electrophoresis of sample D showing three monoclonal kappa chains.

glandular SS (Tables 1 and 2). In the sera and urine of this patient group only one protein band was detected.

Immunofixation electrophoresis studies were performed on 10 sera and the corresponding urine samples which contained monoclonal bands, as well as on three sera without monoclonal proteins. We could identify the monoclonal proteins only in the sera which, in the high-resolution electrophoresis, showed monoclonal bands. In eight sera and urine specimens tested the homogeneous protein bands consisted of free lambda or kappa light chains

Fig. 2 Protein electrophoresis pattern of a sample from parotid saliva of a Sjögren's patient.

(Table 1, Fig. 1). One patient showed monoclonal IgM lambda in serum and free monoclonal lambda light chains in urine, while another patient showed monoclonal IgG kappa and monoclonal IgA kappa in the serum. In the urine only monoclonal kappa chains were present (Table 1). The electrophoresis pattern of the urine specimens from normal controls revealed only a small amount of albumin (Fig. 1A). Electrophoresis of saliva revealed in all specimens tested a single homogeneous band near the point of application (Fig. 2). Immunofixation electrophoresis failed to demonstrate the presence of immunoglobulins or free light chains.

Discussion

Homogeneous protein bands that extend over a very narrow region of the gamma-globulin area have been clearly shown to be monoclonal immunoglobulins; these bands have been reported to occur in approximately 1% of the normal population.⁹ Using a very sensitive, high-resolution electrophoretic technique we demonstrated in 5% of sera from healthy National Institutes of Health employees monoclonal protein bands in the gamma-globulin region.¹⁰ Subsequently similar immunoglobulin bands were found in the sera of 57% of undifferentiated lymphoma patients and 60% of Sjögren's syndrome patients.⁴⁻¹¹ In none of these patients was one band demonstrated by routine agarose gel protein electrophoresis. These observations indicate that our high-resolution electrophoresis is more sensitive than routinely used techniques, an indication borne out by direct comparison.

In the present study it was reconfirmed that SS patients with multiorgan disease are those who had the high incidence of monoclonal light chains in the serum. Since SS patients with extraglandular manifestations are those who have a higher risk of developing lymphoma,³ the detection of monoclonal

light chains in SS patients may be an early marker of malignant transformation. Prospective serial studies will be necessary to test the validity of this hypothesis.

In this group of patients, who were all Greeks, kappa or lambda monoclonal light chains were demonstrated. This finding is in contrast to our previous observation in American SS patients, where the bands all consisted of free lambda light chains. The discrepancy between our previous study and the present one may be attributed to the different ethnic groups tested. Similar differences were observed in the monoclonal immunoglobulins found in lymphoma cells or in serum of SS patients from different ethnic groups. A study of North American SS patients with lymphoma revealed that the monoclonal lymphoid cells contained in their cytoplasm IgM kappa immunoglobulins.¹² Another study from Mexico showed that the monoclonal protein in SS patients with lymphoma showed IgM lambda monoclonal immunoglobulin.¹³ Finally, a study from Japan showed that sera from SS patients revealed IgA or IgG monoclonal immunoglobulins.¹⁴

Another observation made in this study was the higher recovery of monoclonal proteins in the urine of SS patients compared with serum. In addition it was shown in SS patients that the monoclonal protein, which was identified in the serum, could be recovered from the urine. This finding further substantiates that the monoclonal B lymphocyte process exists together with the polyclonal B lymphocyte hyperactivity in SS patients.⁴ Furthermore the high incidence of monoclonal proteins in the urine of SS patients makes the urine an excellent source of antigenic material for preparing antibodies useful for both diagnosis and treatment. We were unsuccessful in demonstrating monoclonal light chains in the saliva of the patients tested. This can be attributed to the fact that the saliva was fractionated unconcentrated.

Finally, it was shown that the sera or urine from SS patients can have more than one monoclonal protein bands. These monoclonal bands were identified by immunofixation electrophoresis as being the same monoclonal light chain. This finding suggests that perhaps slightly altered monoclonal light chains

of the same type, or fragments of the same light chain, are present in SS patients. Alternatively this finding may be attributed to the binding of the same light chain to different proteins which migrate in the gamma-globulin region. All these hypotheses are under investigation.

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