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Early Detection of Lymphomas in Sjögren's Syndrome by *in situ* Hybridisation for κ and λ Light Chain mRNA in Labial Salivary Glands

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Sjögren's syndrome (SS) is an autoimmune disease characterised by a generalised lymphoproliferation. Patients have an increased risk of developing lymphomas which are usually of the type associated with mucosa-associated lymphoid tissue (MALT). Histological examination of the minor salivary glands of the lower lip is a common and useful diagnostic test for SS but has not been able to provide information with regard to potential malignant change. In this study, a sensitive *in situ* hybridisation technique for the detection of κ and λ immunoglobulin light-chain mRNA was applied to labial salivary glands of 14 patients with SS. 7 cases showed light chain restriction, in 5 cases this was κ(κ:λ ratio >8.0) and in 2 it was λ(κ:λ ratio <0.6). Of these 7, 5 developed lymphomas—4 were low grade lymphomas of MALT type and the fifth patient died of disseminated lymphoma. The finding of light chain restriction in lip minor salivary glands is strong evidence of a monoclonal population of B-cells at this site. It is concluded that in patients with SS who develop lymphomas, dissemination of malignant cells may result in detectable disease in the minor salivary glands. Determination of κ:λ ratios in labial minor salivary glands may thus provide important prognostic information.

Keywords: immunoglobulin light chains, mRNA, lymphoma of mucosa-associated lymphoid tissues, MALT, labial gland biopsy, *in situ* hybridisation
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INTRODUCTION

SJÖGREN'S SYNDROME is an autoimmune disease characterised by dry eyes and dry mouth and in about 50% of cases is associated with another autoimmune disorder, usually rheumatoid arthritis. Although the characteristic pathological lesion of Sjögren's syndrome (SS) is a lymphoepithelial lesion of the parotid glands there is a generalised lymphoproliferation and many sites may be affected [1]. Patients have a risk of developing lymphoma that is about 44 times greater than the general population [2]. Lymphomas may arise within lymphoepithelial lesions of the parotid gland or at extraglandular sites, but they are usually low grade lymphomas of the type associated with mucosa-associated lymphoid tissue (MALT) [3–5].

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Gland involvement is usually widespread and histopathological examination of the minor salivary glands of the lower lip is a common and useful diagnostic test for SS [1, 6, 7] but has provided very little prognostic information with regard to potential malignant change. Recently we demonstrated an increased proportion of IgM-positive plasma cells in lip glands of SS patients [6] and in preliminary immunocytochemical studies found evidence of immunoglobulin light chain restriction in the same glands [8]. This suggests that a neoplastic monoclonal process can involve the minor glands but the findings need to be correlated with disease outcome for individual patients. In attempting to further this work we have found immunocytochemistry to be unsatisfactory since non-specific staining and high background often make interpretation of sections or quantification of cell numbers difficult or impossible.

In this study we have applied a sensitive *in situ* hybridisation technique for the detection of kappa (κ) and lambda (λ) immunoglobulin light chain mRNA to paraffin sections of labial salivary glands from patients with SS. The purpose was to establish a correlation between light chain restriction in the minor glands and lymphoma development.

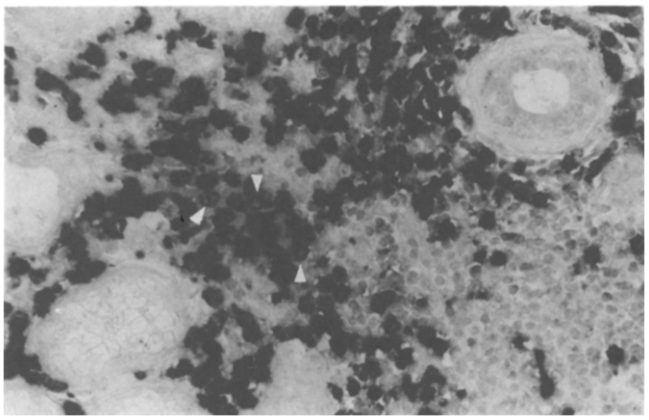

Fig. 1. *In situ* hybridisation for κ mRNA. There is a dark black cytoplasmic reaction product in both plasma cells and smaller immunoglobulin-producing lymphocytes (arrows) (× 100).

MATERIALS AND METHODS

Labial minor salivary glands were obtained from 14 patients with SS. Six had primary SS diagnosed on the basis of symptomatic dry eyes and mouth, a positive Schirmer's test and focal lymphocytic infiltrates in the minor glands. The remaining 8 had secondary SS associated with either rheumatoid arthritis or systemic lupus erythematosus. All had dry mouth and focal lymphocytic infiltrates in the minor glands. At the outset 3 cases were selected because it was known that they had developed a lymphoma. The remaining 11 cases of Sjögren's syndrome were selected at random. 2 cases developed a lymphoma during the course of the study. Four inflamed lip glands from patients without SS were also included to determine the light chain distribution in non-specific sialadenitis. These were obtained from the depth of lip biopsies taken for other, unrelated lesions.

Specimens were routinely formalin-fixed and paraffin-embedded, 5-μm sections were mounted on coated slides and coded prior to staining and quantification. The *in situ* hybridisation technique has been described elsewhere [9]. Briefly, after pretreatment with DEPC-treated water and incubation in 5 μg/ml proteinase K the sections were again washed and incubated in prehybridisation buffer. Sections were then incubated for 3 h or overnight in a cocktail of either κ or λ specific digoxigenin-labelled oligonucleotide probes. Bound probe was detected using an anti-digoxigenin alkaline phosphatase conjugate visualised with bromo-chloro-indolyl-phosphate (BCIP) enzyme substrate and nitroblue tetrazolium (NBT) salt to give a dark blue/black reaction product (Fig. 1). Controls included RNase pretreatment and omission of the probe.

Positive cells were quantified using a computerised image analysis system (Seescan Plc, Cambridge, U.K.). An enhanced image was captured on the computer screen and individual gland lobules were identified and defined. By direct reference to the original sections the grey levels were adjusted to define the positive cells and delete background. The computer then scanned the image and calculated the proportion of κ and λ positive cells in adjacent serial sections.

RESULTS

An intense black cytoplasmic reaction product was seen in plasma cells and in immunoglobulin secreting B-cells (Fig. 1).

Table 1. Results of cell quantification and clinical outcome in the Sjögren's syndrome patients

Patient no.	κ:λ	%κ+ cells	Outcome	Time*
1	9.8	91	Gastric lymphoma of MALT	15
2	8.3	89	Disseminated lymphoma. ? of MALT	4
3	10.0	91	Oral lymphoma of MALT	6
4	0.6	37	MALT lymphoma in lymph node	1
5	0.4	28	NEL	
6	26.5	96	MALT lymphoma in lymph node	23
7	9.9	91	Lost to follow-up	
8	1.8	64	NEL	
9	1.5	60	NEL	
10	2.6	72	NEL	
11	1.0	50	NEL	
12	2.2	68	NEL	
13	1.5	60	NEL	
14	1.4	60	NEL	

Patients 1–7 showed evidence of light-chain restriction. NEL = no evidence of lymphoma. *Time = time interval, in months, between detection of light chain restriction in the labial salivary glands and subsequent diagnosis of lymphoma.

In the inflamed glands the proportion of κ positive cells was within the normal range [10] and varied from 50 to 69% (mean ± S.D. 59.8 ± 7.8). In the SS cases the range was 28–96% (Table 1). 7 cases showed light chain restriction. In 5, the restricted light chain was κ with a κ:λ ratio of 8:1 or greater (Table 1; Fig. 2). 2 cases showed λ light chain restriction with a κ:λ ratio of less than 0.6. The mean proportion of κ positive cells in the SS cases without light chain restriction was 62 ± 7.0%.

Of the 7 patients whose glands showed light chain restriction, 1 has emigrated and is lost to follow-up, 1 is alive and apparently well but the remaining 5 have subsequently developed a clinically detectable lymphoma. In 4 cases these were low grade B-cell lymphomas of MALT type, 1 in the stomach, 1 in the palate and 2 in cervical lymph nodes. The fifth patient died of disseminated lymphoma involving bone marrow. Where tissue was available the lymphomas and corresponding lip glands showed the same light chain restriction. In all cases the diagnosis of lymphoma was made after the lip biopsies were taken, the time interval ranging from 1 to 23 months (Table 1). Patients without light chain restriction have been followed for between 2 and 5 years but none have developed lymphoma.

DISCUSSION

The finding of light chain restriction in lip minor salivary glands is strong evidence for the presence of a monoclonal population of cells at this site. Monoclonal populations of B-cells have previously been detected in major glands by anti-idiotype antibodies [11], heavy chain gene rearrangements [12–14] and immunocytochemistry [15] and appear to be a good prognostic indicator of a malignant monoclonal lymphoproliferation. However, the origin of the clonally expanded cells remains unknown. The cells may selectively home to the tissues or there may be local clonal expansion of B-cells, possibly driven by antigen. Such clones may remain under control of the immune system but subsequently may escape

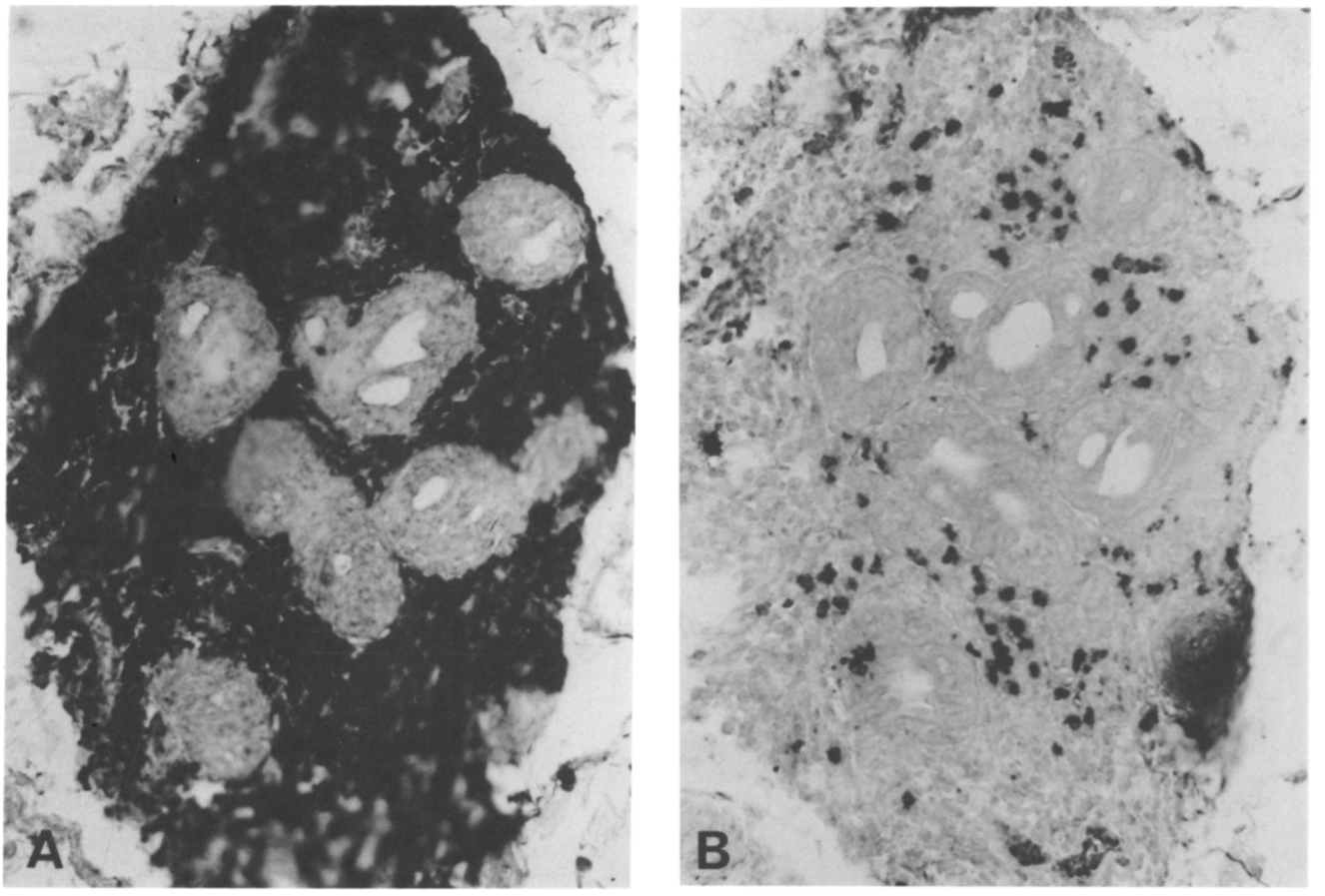

Fig. 2. Serial sections of a lobule of minor labial salivary gland from patient 2 stained by *in situ* hybridisation for (A) κ and (B) λ mRNA. There is κ light chain restriction with a κ:λ ratio of 8.3 (×40).

and lead to lymphoma development [12]. Others regard the presence of a monoclonal population to be direct evidence for the presence of a lymphoma [13–15].

Recently, Bodeutsch *et al.* [16], using immunohistochemistry, have also detected light chain restriction in labial salivary glands of patients with Sjögren's syndrome. 3 out of 10 patients with light chain restriction developed a systemic monoclonal lymphoproliferation. One of these developed a lymphoma but the immunoglobulin isotype was not the same as that seen in the lip glands. They concluded that the monotypic cells in the labial glands were not neoplastic but that primitive B-cells home to the exocrine glands and then undergo clonal expansion after prolonged antigenic stimulation by parenchymal cells. This is then associated with an increased risk of developing a systemic lymphoproliferative disorder.

Our results, however, suggest that the restricted cell population is of neoplastic origin. The finding that 5 out of 7 patients with a monoclonal population of cells in their lip glands harboured lymphomas elsewhere is good evidence that the cells in the lip are neoplastic and have arisen as a result of lymphoma dissemination. In 4 of the cases this is probably due to the tendency of MALT lymphomas to migrate, via an unknown homing mechanism, to other mucosal sites [17]. One of our patients (case 1) developed gastric symptoms 15 months after lip biopsy and a low grade B-cell lymphoma was confirmed in a gastric biopsy and bone marrow trephine. Review of a previous gastric biopsy, diagnosed as 'peptic ulcer' 11 years previously showed similar histology. DNA was extracted from tissue from all four biopsy sites, all showed an identical heavy chain gene rearrangement and sequencing of the PCR product confirmed that the cells were derived from the same clone [18].

We conclude from this study that in patients with SS who develop lymphomas dissemination of lymphoma cells may result in detectable disease in the minor salivary glands of the lower lip before clinical symptoms become apparent. Since SS patients have a significantly increased risk of developing lymphomas [2] and lip biopsy is often performed as a diagnostic procedure [1], quantification of κ:λ ratios may provide a valuable method for the early detection of a malignant lymphoproliferative disorder.

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