T lymphocyte activation state in the minor salivary glands of patients with Sjögren's syndrome

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SUMMARY Local lymphoplasmacytoid infiltration of the diseased exocrine glands is a cardinal sign of Sjögren’s syndrome (SS). The state of T lymphocyte activation present in these local infiltrations was studied by three different techniques: determination of interleukin 2 (IL2) receptor (Tac) on cell surface membrane; autoradiography combined with immunoperoxidase staining of T cell epitopes; and electron microscopic analysis of the lymphoblast subclasses. Although 64 (SEM 4)% of the local inflammatory cells expressed Ia antigen, only 4 (SEM 1)% of them displayed the T cell activation antigen Tac. Autoradiography-immunoperoxidase double labelling showed that less than 1% of all T cells in situ were [3H]thymidine incorporating blasts. This finding suggests that although T lymphocyte is the dominant cell in situ, only a few of these cells have passed the G0/G1 interphase, and even fewer have been pushed to the S phase of the cell cycle by IL2. Transmission electron microscopy showed that few T blasts were present, even though there were many plasma cells. This result further confirms the impression that only a minor T cell subpopulation in situ is blast transformed despite the fact that many of the local T lymphocytes in the diseased salivary glands in SS are Ia positive.

Sjögren’s syndrome (SS) is an autoimmune exocrinopathy clinically characterised by xerostomia and keratoconjunctivitis sicca.1 2 SS is characterised by lymphoidcytoid cell infiltration of the lachrymal and salivary glands3-5; therefore the composition of this infiltration has been analysed in many cyto-adherence, histochemical, and immunohistological studies.6-13 The presence of B lymphocytes and plasma cells in situ has been well recorded.7-10 T lymphocytes, however, especially those belonging to the T4+ inducer/helper subset, seem to be the predominant cells in situ.11-13 In this work we studied the activation state of T lymphocytes in situ in the diseased glands in SS.

The cardinal feature of SS is a chronic monocellular cell infiltration of the exocrine glands. Many of the local lymphoid cells are of B cell origin and there is much evidence indicating active B cell involvement in the pathogenesis of SS.7-10 This includes hypergammaglobulinaemia, autoantibodies, demonstration of plasma cells in situ, an active immunoglobulin and rheumatoid factor synthesis in diseased glandular tissue, and the development of B cell derived malignancies in SS.14 In most patients with SS, however, T lymphocyte is the predominant immune inflammatory cell in the diseased salivary glands.11-13 There is little information on the state of activation of this lymphocyte subset in situ. We therefore studied the state of T cell activation in the labial salivary glands of patients with SS by three different techniques to assess Tac expression, lymphocyte ultramorphology, and DNA synthesis.

Patients and methods

PATIENTS AND BIOPSIES

Clinical characteristics of the patients studied are given in Table 1. In addition, labial salivary glands were also obtained from four patients without SS (patients 9-12; Table 1) and without any inflammatory systemic or local inflammatory disease.
Six labial salivary glands were removed from each patient through a 2 cm long horizontal incision on the inside of the lower lip 1 cm from the vermillion border to the right of the midline. The local anaesthesia was 1·8 ml of Citanest-Octapressin.

Immediately after removal two glands processed for transmission electron microscopy were fixed with cold (4°C) 1·5% phosphate buffered glutaraldehyde for one hour, washed, then postfixed in cold 1·5% osmium acid for one hour. After alcohol dehydration the specimens were treated twice with propylene oxide and embedded in Epon 812. Two glands were immediately embedded in OCT compound (Lab–Tek Products, Division Miles Laboratories, Elkhart, IN), snap frozen, and stored at −20°C for immunohistochemical lymphocyte activation marker profile analysis. Immediately after removal two glands processed for autoradiography-avidin-biotin-peroxidase complex (ABC) double labelling were put into RPMI 1640 solution containing 111 kBq/ml or [3H]thymidine (185 MBq/mmol; Radiochemical Centre, Amersham, UK) and 0·5 mg/ml of thymidine phosphorylase inhibitor 5-nitouracil, incubated at 37°C for two hours, and snap frozen in OCT.

**HISTOCHEMISTRY**

Cryostat sections 6 μm thick were prepared and fixed in cold (4°C) acetone for 5 minutes in preparation for immunostaining. The sections were washed in phosphate buffered saline (0·1 M, pH 7·3) and stained with monoclonal antibodies using the ABC method introduced by Hsu et al. Technical details and staining and slide controls have been described in detail elsewhere. The preparations were studied with several monoclonal antibodies given in Table 2. As a further control we used inappropriate mouse IgG myeloma protein NS1 from P3×63NS1 line (Cappel Laboratories, Cochraneville, PA) instead of the specific primary antibodies. Exogenous, peroxidase positive, specifically stained cells were brown and thus readily

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Therapy</th>
<th>Schirmer’s test</th>
<th>Focus score</th>
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<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>4/5</td>
<td>3·5</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>3/6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>3/0</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>F</td>
<td>2°SS/RA</td>
<td>NSAID</td>
<td>5/2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>F</td>
<td>2°SS/PMR</td>
<td>Prednisone</td>
<td>0/0</td>
<td>2</td>
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<tr>
<td>6</td>
<td>67</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>5/3</td>
<td>1·7</td>
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<tr>
<td>7</td>
<td>30</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>0/0</td>
<td>4·2</td>
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<tr>
<td>8</td>
<td>56</td>
<td>F</td>
<td>1°SS</td>
<td>None</td>
<td>1/0</td>
<td>4·4</td>
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<tr>
<td>9</td>
<td>32</td>
<td>M</td>
<td>Fibrositis</td>
<td>None</td>
<td>19/22</td>
<td>0</td>
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<tr>
<td>10</td>
<td>39</td>
<td>F</td>
<td>Iatrogenic</td>
<td>None</td>
<td>0/0</td>
<td>0</td>
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<tr>
<td>11</td>
<td>74</td>
<td>F</td>
<td>Iatrogenic</td>
<td>None</td>
<td>ND</td>
<td>0·3</td>
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<tr>
<td>12</td>
<td>46</td>
<td>F</td>
<td>Psychogenic</td>
<td>None</td>
<td>20/24</td>
<td>0</td>
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1°SS=primary Sjögren’s syndrome; 2°SS=secondary Sjögren’s syndrome; RA=rheumatoid arthritis; PMR=polymyalgia rheumatica; NSAID=non-steroidal anti-inflammatory drug.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Specificity</th>
<th>Reference</th>
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<tbody>
<tr>
<td>OKT11</td>
<td>Total T cells</td>
<td>17</td>
</tr>
<tr>
<td>OKT4</td>
<td>Helper/inducer T cells</td>
<td>18</td>
</tr>
<tr>
<td>OKT8</td>
<td>Suppressor/cytotoxic T cells</td>
<td>19</td>
</tr>
<tr>
<td>OKM1</td>
<td>Monocytes, null cells, granulocytes</td>
<td>20</td>
</tr>
<tr>
<td>pan-B</td>
<td>B cells</td>
<td>21</td>
</tr>
<tr>
<td>OKLa</td>
<td>Activated T cells, B cells, monocytes</td>
<td>22</td>
</tr>
<tr>
<td>Tac</td>
<td>Activated lymphocytes (T cell growth factor receptor)</td>
<td>23, 24</td>
</tr>
<tr>
<td>OKT9</td>
<td>Activated lymphocytes (transferrin receptor early haematopoietic stem cells)</td>
<td>25</td>
</tr>
<tr>
<td>4F2</td>
<td>Activated lymphocytes, monocytes</td>
<td>26</td>
</tr>
</tbody>
</table>
were further staining test. Mann-Whitney dispersion, and square counting counterstained tissue counted from were mean between error Standard immersion objective. After DOUBLE AUTORADIOGRAPHY-ABC microscope. The cells distinguishable from unstained cells under a light microscope. The cells analysed by light microscopy were counted from 6 μm thick haematoxylin counterstained tissue sections with an ocular counting square (20 squares × 20 squares) and an oil immersion objective (×1000 magnification). Standard error of the mean was used to expression dispersion, and the significance of the differences between mean values was determined with the Mann–Whitney test.

AUTORADIOGRAPHY-ABC DOUBLE LABELLING
After incorporation of [3H]thymidine and ABC staining some OKT4, OKT8, and Tac stained slides were further processed for autoradiography. They were dipped in NTB2 nuclear track emulsion (Eastman Kodak Co, Rochester, NY), exposed in darkness for 12 days, developed (Developer D–76; Eastman Kodak) for four minutes, and fixed (Rapid Fix; Eastman Kodak) before mounting.27 Cells were regarded as [3H]thymidine labelled when they contained numerous grains over the cell nucleus (Fig. 1). The results of autoradiography-ABC double labelling are given as a percentage of all [3H]thymidine incorporating blasts.

TRANSMISSION ELECTRON MICROSCOPY
Ultrathin sections prepared from specimens embedded in Epon were heated to 60°C, after which the sections were stained with uranyl acetate and lead citrate for the electron microscopic study (Jeal CX–100 transmission electron microscope). Ultramorphologically, cells were regarded as lymphoblasts if they were greater than 10 μm in diameter and if the nucleus: cytoplasm ratio was less than 0.5; cells were classified as T blasts and plasma cells according to the criteria of Ishikawa and Ziff.28

Results
STAINING CONTROLS
The staining and slide controls indicated the speci-

Table 3  The intensity, but not the composition, of the cellular infiltrates differs in minor salivary glands of patients with Sjögren’s syndrome compared with those of healthy controls

<table>
<thead>
<tr>
<th>Source of biopsy specimen</th>
<th>Focus score</th>
<th>All inflammatory cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T11</td>
<td>T4</td>
</tr>
<tr>
<td>Patients with SS</td>
<td>3-5 (0-9)†</td>
<td>67 (4)</td>
</tr>
<tr>
<td>Controls</td>
<td>0-1 (0-1)</td>
<td>53 (3)</td>
</tr>
<tr>
<td>p (Mann–Whitney’s U test)</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p<0.01; NS = not significant.
† Values are mean (SEM).
ficity of the staining and showed that the inhibition of endogenous peroxidase was successful. Staining for endogenous peroxidase alone showed that the proportion of macrophages and monocytes was less than 10% of all inflammatory cells in situ in labial salivary glands. T4+ inducer/helper T lymphocytes were the predominant cells (Table 3). Although there was a more intense cellular inflammation in the patients with SS than in those without focal sialadenitis, the composition of the sparse diffuse infiltrates was similar in both (Table 3).

**ACTIVATION MARKER PROFILE ASSAY**

The proportions of Ia+, Tac+, T9+, and 4F2+ cells were higher in the diseased salivary glands than in normal salivary glands (Fig. 2). The proportion of Ia+ and 4F2+ cells was higher than that of Tac+ and T9+ cells both in patients with SS and controls (Fig. 2).

**AUTORADIOGRAPHY-ABC DOUBLE LABELLING**

The labelling index of the inflammatory mononuclear cells in situ in the salivary glands of patients with SS was less than 1%. Most of the [3H]thymidine incorporating blast cells belonged to the T4+ subset (69 (SEM 3)%), whereas only 26 (2) % of the [3H]thymidine incorporating blasts in situ were from the T8+ subset. The T4/T8 ratio, calculated from the proportion of the respective T cell subsets in all the inflammatory mononuclear cells, was 2.3 (SEM 0.2). The ratio was surprisingly similar to the activated T4/T8 ratio calculated from the [3H]thymidine incorporating blast cell fraction (2.3 (0.2) v 2.7 (0.3)) (Fig. 3). Normal glands contained hardly any [3H]thymidine incorporating blasts.

**TRANSMISSION ELECTRON MICROASSAY**

Transmission electron microscopy showed only occasional T blasts (less than 1% of all inflammatory mononuclear cells in situ) in the specimens from patients with SS. Plasma cells were found peripherally to the lymphocyte foci and between the glandular acini, and they formed about 10–15% of all inflammatory mononuclear cells in situ. In the control slides normal salivary glands contained hardly any T blasts or plasma cells.

**Discussion**

The active involvement of T lymphocytes in the local pathogenetic mechanisms in SS has been suggested by higher percentages of Ia+ lymphocytes in salivary glands than in peripheral blood lymphocytes of the same patients.11 We also detected many Ia+ cells in situ, but it should be remembered that many other cells—such as B lymphocytes, plasma cells, monocytes, macrophages, dendritic cells, and even some resident cells—may display Ia. Surprisingly, immunohistochemical findings showed low Tac expression, confirming the earlier findings of Fox et al.29 Furthermore, because interleukin 2 receptor has recently been reported on B cells and macrophages,24 it is impossible, in the absence of double marker studies, to state that all interleukin 2 receptor bearing cells are T cells. The low percentage of IL2 receptor carrying cells in situ, however, might have been due to the blocking of immunoreactive epitopes by receptor bound IL2. This was refuted by experiments in which we used [3H]thymidine to assess the proportion of T cells that synthesised DNA in the diseased salivary glands of patients with SS;27; less than 1% of all local lymphocytes carrying T cell markers were [3H]thymidine incorporating blasts.

Resting lymphocytes change morphologically upon activation. Furthermore, plasma blasts and plasma cells show ultramorphological characteristics distinct from those of T cell derived activated blasts. Our electron microscopic findings on the rarity of lymphoblasts with T cell characteristics, in contrast with those with plasma blast/plasma cell characteristics, strengthen the conclusion based on the immunohistochemical findings for Tac expression and on the autoradiographical findings for DNA synthesis.
Active B cell involvement in the aetiopathogenesis of SS is amply recorded, but recent observations using monoclonal antibodies to detect lymphocytes with T cell phenotype have shown this to be the predominant immune inflammatory cell in situ. According to our findings, based on observations made with three different assays, only a minor fraction of these T cells, though 1a positive, are blast transformed. This may be related to the chronicity of the disease and does not necessarily imply that most of the local T lymphocytes are innocent bystanders. In any case, T lymphocyte activation and blast transformation seem to be more extensive in glandular tissue in SS than in normal control glands.

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References
25 Trombridge I S, Omary M B. Human cell surface glycophorin related to cell proliferation is the receptor for transferrin. Proc Natl Acad Sci USA 1981; 78: 3034–43.
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