Mast cells, extracellular matrix components, TGFβ isoforms and TGFβ receptor expression in labial salivary glands in systemic sclerosis

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Abstract

***Objective*—To determine whether there was altered elaboration of non-collagenous extracellular matrix proteins or expression of TGFβ isoforms and their receptors in salivary glands of patients with systemic sclerosis (SSc) and Raynaud's phenomenon (RP). Because of the possible role of mast cells in the early stages of SSc their presence was also investigated.**

***Methods*—Sections of normal labial salivary glands (n=10) and glands from patients with SSc (n=13) and RP (n=5) were stained immunohistochemically and using acid toluidine blue.**

***Results*—SSc glands contained more mast cells than control tissues ($p<0.005$) and similar numbers to those found in RP specimens. There were no differences in the pattern or amount of non-collagenous matrix proteins detected. Tenascin and elastin were predominantly found surrounding ducts whereas fibronectin had a more general distribution. TGFβ isoforms and receptors were expressed by glandular epithelium, fibroblasts, vascular endothelium and inflammatory cells. Cell counts showed no differences in expression of TGFβ1 or TGFβ receptors between groups. However, the percentage of TGFβ2 positive fibroblasts was significantly higher in SSc glands compared with controls ($p<0.004$). RP glands showed an intermediate level of expression. By contrast, a lower percentage of RP fibroblasts expressed TGFβ3 compared with controls with SSc glands showing an intermediate level of expression.**

***Conclusions*—These results show that (a) there are no changes in glandular expression of tenascin, elastin and fibronectin in SSc and RP and (b) both conditions are associated with an increased salivary gland mast cell population and changes in expression of TGFβ2 and β3 isoforms by glandular fibroblasts.**

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Systemic sclerosis (SSc) is a generalised connective tissue disorder with an unknown cause.[1] It is characterised by cutaneous sclerosis attributable to increased synthesis of collagen, glycosaminoglycans and other connective tissue substances by dermal fibroblasts.[2] This leads to progressive fibrosis of the skin, the epidermis becomes fixed to the deeper subcutaneous tissues and vascular insufficiency occurs through abnormalities in arterioles and capillaries. Skin lesions usually occur first on the face, hands or trunk, but all the skin surfaces can become involved. Progressive fibrosis also occurs in many internal organs, which results in disruption of their normal architecture and ultimately leads to their dysfunction and failure. It has been shown that salivary glands are also subject to fibrosis in SSc patients and that the disease may be associated with Sjogren's syndrome.[3–6]

Transforming growth factor β (TGFβ) regulates cell growth and differentiation and stimulates the synthesis and secretion of protein constituents of the extracellular matrix by fibroblasts. Although TGFβ inhibits proliferation of many cells including endothelial and epithelial cells it is a potent mitogen for fibroblasts, a powerful chemoattractant for monocytes and fibroblasts and a growth stimulant for immature fibroblasts.[7–9] TGFβ is therefore important in regulation of fibroblast activity and is known to be a determining factor in fibrosis and wound healing in the presence or absence of scarring.[10 11] The finding that SSc fibroblasts produce more collagens, fibronectin and TIMPs than normal fibroblasts indicates that TGFβ, via autocrine or paracrine pathways, or both, may be the central mediator of fibrosis in systemic sclerosis.[12]

Most studies of SSc have concentrated on cutaneous lesions and investigated growth factors, fibroblast and matrix metalloproteinase activities associated with collagen turnover.[12] However, an increase in the amount of elastic fibres in the skin of SSc patients has been noted in the one published study of non-collagenous extracellular matrix proteins.[14] Fibronectin and tenascin are two extracellular matrix proteins that have not been investigated in SSc. Fibronectin binds to collagen and membrane bound adhesion molecules and is important in guided migration of a variety of cells types within connective tissue.[15 16] Tenascin is expressed during tissue restructuring, is anti-adhesive and is therefore in competition with the adhesive properties of fibronectin[17] and consequently can influence cell migration. Changed expression of these proteins, both under the control of growth factors such as TGFβ, may play an important part in disease progression.

Little is known about salivary gland involvement in SSc. Information in the literature is limited to comparisons with Sjogren's syndrome[3 4] and a study showing that salivary

Table 1 Clinical and laboratory features of the patient populations

Patient/ sex/age*	Diagnosis	Disease duration	Sicca signs/ symptoms	RF	ANA	Ro	La	Histology†
1/M/51	SSc [D]‡	1	−/−	NK§	+	−	−	+
2/M/43	SSc [D]	3	−/−	−	NK	−	−	−
3/F/66	SSc [D]	2	−/−	NK	+	−	−	+D
4/F/52	SSc [D]	2	−/+	−	+	+	+	+
5/M/53	SSc [D]	3	−/−	NK	+	−	−	+
6/F/47	SSc [D]	5	−/−	NK	+	−	−	−
7/F/34	SSc [D]	NK	−/−	NK	+	−	−	−
8/F/74	SSc [D]	7	+/+	+	+	+	+	+
9/M/56	SSc [L]	8	−/+	−	+	−	−	−
10/M/81	SSc [L]*	5	−/−	NK	−	NK	NK	−
11/M/48	SSc [L]*	18	−/+	NK	+	−	−	+D
12/F/39	SSc [L]*	13	−/+	NK	−	−	−	−
13/M/49	SSc [L]*	6	−/−	NK	+	−	−	−
14/F/80	RP	30	−/+	NK	−	NK	NK	−
15/F/60	RP	>20	+/+	NK	+	−	−	+
16/M/52	RP	3	+/−	+	+	NK	NK	+
17/F/34	RP	25	−/−	NK	+	−	−	+
18/F/74¶	RP	6	−/+	NK	+	−	−	+

*Patients 8 and 15 fulfilled EU criteria for Sjogren's syndrome. †Presence or absence of focal lymphocytic sialadenitis; D, indicates presence of diffuse lymphoid infiltrates not consistent with a diagnosis of Sjogren's syndrome. ‡D = diffuse and L = limited disease; * = CREST. §NK = not known. ¶Patient also had primary biliary cirrhosis with raised serum IgM.

gland changes (increased expression of E-selectin and TNFα and infiltration by mast cells) occur in the very early stages of SSc, before the onset of skin changes.[13] This latter finding was based on a one to seven year follow up study of patients with Raynaud's phenomenon (RP) who failed to satisfy the criteria for a diagnosis of SSc.

The purpose of this immunohistochemical study was to discover if there was any changed elaboration of non-collagenous extracellular matrix proteins or expression of TGFβ isoforms and their receptors in the salivary glands of patients with SSc. Because of the possible role of mast cells in fibrosis in early stages of SSc and progression to RP,[13 18] the presence of mast cells was also investigated as were salivary glands obtained from a small group of patients with RP.

Methods

PATIENTS AND TISSUES

Labial salivary glands were obtained, after informed consent, from 13 patients with SSc and five patients with RP (table 1). Two patients (one SSc and one RP) fulfilled the European criteria for a diagnosis of Sjogren's syndrome.[19] The diagnosis of RP was based on the patients' medical history and the observation of bilateral, episodic, cold induced pallor and/or cyanosis of the fingers. None of the patients with RP have gone on to develop SSc/CREST during the 9–14 year follow up period since labial biopsy. All tissues were fixed in neutral buffered formalin (18–24 hours) and processed under standard conditions via ethanol and xylene to paraffin wax. Paraffin blocks (n=10) of labial salivary glands showing normal histological structure that had been removed from patients complaining of dry mouth and under investigation for possible Sjogren's syndrome (seven women and three men; mean age, 44.4 years) were used as "normal" control tissue. Of the 10 control subjects only four (one woman and three men) had any underlying medical history and none were taking drugs known to affect salivary gland function. The woman was asthmatic using a salbutamol inhaler and taking the antidepressant, flupenthixol; two of the male controls had rheumatoid arthritis and the other polyarthritis. None of these patients have developed Sjogren's syndrome, SSc, CREST or RP in the 12–15 year follow up period after labial biopsy.

HISTOLOGY AND IMMUNOHISTOLOGY

Five micron sections were cut for histological examination and immunocytochemical analysis. Sections were dried for two hours at 56°C, before being dewaxed in xylene and rehydrated through a graded alcohol series to phosphate buffered saline (PBS). Conventional stains included haematoxylin and eosin, Van Gieson for the demonstration of the fibrous tissue and acid toluidine (0.5% toluidine blue in 0.5M HCl) to detect mast cells.

Affinity purified polyclonal antibodies (Santa Cruz) to TGFβ isoforms (sc82, sc90, sc146) and receptors I (R20, V22) and II (L21) were used at 0.5–2.0 μg/ml. Mouse monoclonal antibodies to tenascin (clone BC-24) and elastin (clone BA-4) were obtained from Sigma and used at 3.75 and 10 μg/ml respectively. Monoclonal antibodies to fibronectin (clone 568, 0.8 μg/ml), vimentin (clone 3B4, 0.05 μg/ml) and CD antigens (clones 2B11PD7/6, CD45, 9 μg/ml; clone PG-M1, CD68, 1.8 μg/ml) were obtained from Novocastra, Boehringer Mannheim Biochemica and Dako. Immunocytochemical staining was performed on trypsin treated sections (0.1% trypsin, Difco, 1: 250 grade; 30 minutes at room temperature). Endogenous peroxidase activity was quenched using a 3% hydrogen peroxide in water and any non-specific antibody up take by proteins was blocked using a 20% dilution of goat serum in PBS. The primary antibodies were also diluted in PBS containing 1% bovine serum albumin. Immunoperoxidase staining was carried out using a biotin-streptavidin system (StrAviGen, Multilink; Biogenex) and the site of antibody binding was visualised using diaminobenzidine reagent. The brown reaction product was darkened using 0.5% $CuSO_4$ in saline (five minutes). The sections were counterstained with Meyer's haematoxylin and mounted in XAM.

Immunostaining for each antigen was performed on all specimens at the same time to maximise comparability of staining between specimens. Negative staining controls included omission of the primary layer and substitution of the primary layer with PBS. Specificity of the TGFβ isoform and receptor antibodies was confirmed by checkerboard peptide blocking experiments. The working dilution of each antibody was incubated with 10-fold excess (by weight) of peptide (Santa Cruz) overnight at 4°C, before staining. In all cases staining was abolished by homologous peptide but unaffected by pre-incubation with peptides corresponding to other isoforms or receptor types.

EVALUATION OF STAINED SECTIONS

Cell counts and determination of the frequency of occurrence of extracellular matrix proteins around ducts were performed on coded slides.

Table 2 Histological features of salivary glands

		Number of specimens showing:			
Salivary glands	*Number*	*fibrosis*	*acinar atrophy*	*duct dilatation*	*lymphocytic infiltration**
Systemic sclerosis	13	10	4	9	6 (4)
Raynaud's phenomenon	5	5	2	4	4 (4)
Controls	10	0	0	0	0

*Number containing periductal lymphocytic foci characteristic of Sjogren's syndrome are shown in parentheses.

Thus the observer knew what the sections had been stained for but not from which patient group they had originated. Mast cell numbers were determined at a magnification of × 400 using sections stained with acid toluidine blue. Apart from diffuse pale blue staining of some mucous acinar cells this stain is specific for mast cell granules. All positive granular cells were counted throughout the entire biopsy specimen consisting of three or more glands (>12 mm^2 total area) and reported as cells/mm^2 salivary gland tissue. Gland areas were determined using TV image analysis (Seescan Prism running TPL 4v20; Seescan Imaging, Cambridge). Objectivity of mast cell counts was verified by an initial investigation using five labial gland specimens to determine the relation between mast cell density and the area of salivary gland observed. It was found that reproducible values, not affected by further counts, were achieved by observing a minimum area of 12 mm^2.

Deposition of both tenascin and elastin around striated ducts was assessed by counting 100 ducts and calculating the percentage showing deposition of these extracellular proteins in both the SSc glands and the control sections. The percentage of ducts (dilated and collecting ducts) containing vimentin positive dendritic cells, identified morphologically, was determined for each group of specimens. TGFβ isoform and receptor staining of fibroblasts was quantified by counting 100 fibroblasts per salivary gland lobule (3–5 lobules per specimen) and calculating the percentage showing positive staining per specimen. All counts were performed manually on randomly selected fields at a magnification of × 100 for assessment of the extracellular matrix proteins and at × 400 for quantification of dendritic cells and fibroblast staining for TGFβ isoforms and receptors. Counts were performed using a microscope fitted with a 10 mm × 10 mm ocular grid and × 10 oculars. Starting from the top left hand corner of each lobule from each specimen, alternate fields were evaluated until 100 ducts or 100 fibroblasts had been counted. Because the number of large ducts was small in labial gland specimens the results for dendritic cell containing ducts was based on counting all such ducts in all specimens from each patient group and reported as a group percentage.

STATISTICS

Data were analysed using Minitab (version 9) and the significance of differences determined using the Mann-Whitney U test unless stated otherwise.

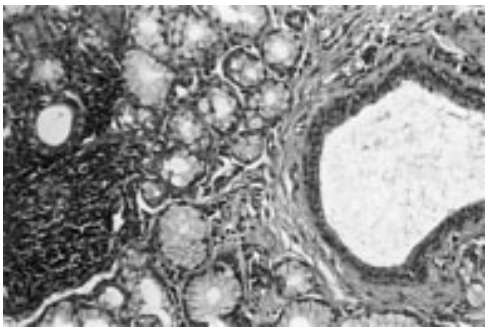

Figure 1 Labial salivary gland from a patient with SSc showing a dilated duct surrounded by fibrous tissue separated from a focal collection of lymphocytes by a few residual acini (haematoxylin and eosin × 200).

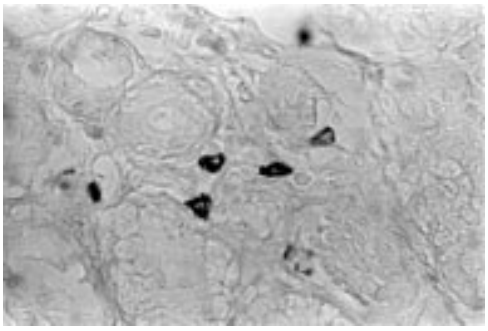

Figure 2 Toluidine blue stained mast cells within a labial salivary gland (× 320).

Results

HISTOLOGY

Table 2 shows the results from the histological review of haematoxylin and eosin stained sections. Fibrosis, duct dilatation and presence of lymphocytic foci (fig 1) were common features in the glands from the SSc and RP patients. None of these features were observed in haematoxylin and eosin stained sections from the control group of specimens.

MAST CELLS

Cell counts on toluidine blue stained sections (fig 2) showed that there were significant differences between groups (p=0.018; AOVO) and that SSc glands contained significantly more mast cells (mean (SD) 60.0 (8.0) per mm^2) than control tissues (32.2 (12.3) per mm^2, p<0.005). A high mast cell density, similar to that in SSc glands, was found in RP tissues (56.5 (31.6) per mm^2) but was not statistically different from normal controls.

IMMUNOHISTOLOGY

Extracellular matrix proteins

In all specimens, elastin staining was extracellular and observed in the connective tissue surrounding the glands and the fibrous septae, around blood vessels and encircling variable numbers of striated ducts. There was no significant difference between SSc, RP and control glands in respect of the percentage of striated ducts surrounded by fine elastin fibres (table 3; p=0.59, AOVO; fig 3A). When present, larger collecting ducts were either surrounded by a wide band of positive fibres (fig 3B) or a few single lines of staining. Elastin was not seen between secretory acini in any of the specimens.

Table 3 Percentage incidence of elastin and tenascin deposition around ductal epithelium

% ducts surrounded by:	*Systemic sclerosis*	*Raynaud's phenomenon*	*Controls*
Tenascin	58.8 (31.6)	57.6 (25.9)	66.5 (22.6)
Elastin	29.1 (18.7)	21.7 (18.5)	24.1 (6.5)

Data shown as mean (SD).

Only small amounts of tenascin, mainly localised around blood vessels, was observed in the connective tissue around the glands or within the fibrous septae of both the control and the SSc tissues. However, dark staining fibres were found surrounding many of the salivary ducts (figs 4 and 5). The percentage of striated ducts surrounded by a definite line of tenascin did not differ between SSc, RP and control glands (table 3; p=0.79, AOVO) and the amount of tenascin staining encircling the ducts did not seem to be related to the amount or position of any lymphocyte infiltration. Although staining was often confined to areas encircling ducts, in some specimens linear staining was also seen surrounding some secretory acini in areas of histologically normal gland (fig 4A).

There were no apparent differences in fibronectin expression between SSc, RP and control glands. All specimens showed varying amounts of positive staining of extracellular fibres and the cytoplasm of endothelial cells in the connective tissue both within and surrounding the gland. Unlike elastin and tenascin, there was no association between fibronectin staining and ducts.

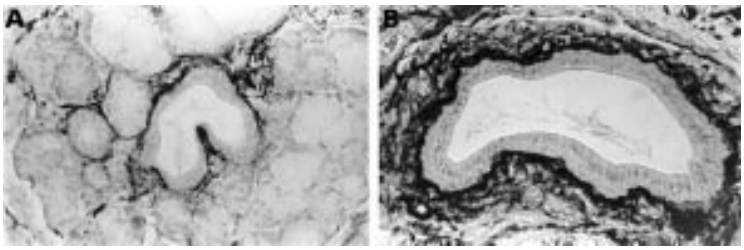

Figure 3 Elastin deposition around striated ducts (A) and a large collecting duct (B) within a labial gland from a patient with systemic sclerosis (× 760).

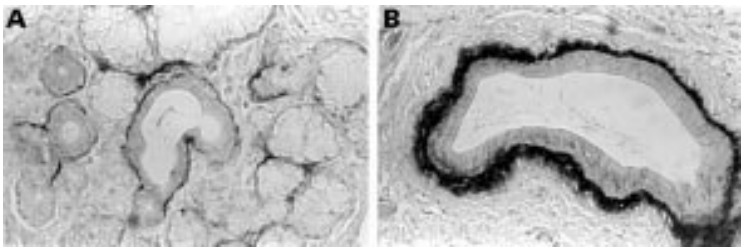

Figure 4 (A) Linear tenascin deposition characteristically found around striated ducts and acini compared with (B) broad bands of staining seen around large collecting ducts. Serial sections to figure 3 (× 760).

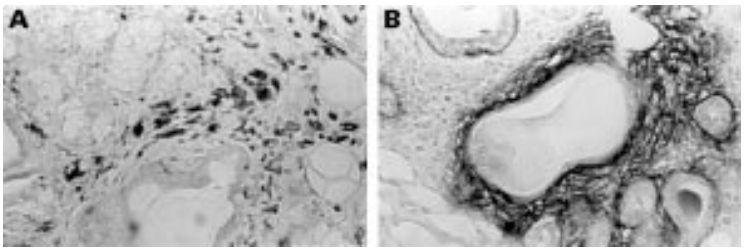

Figure 5 (A) CD68 positive macrophages containing phagocytic vacuoles adjacent to (B) a dilated duct surrounded by an wide band of tenascin (CREST;× 760).

TGFβ isoforms

In all specimens all three TGFβ isoforms were detected in the cytoplasm of fibroblasts, endothelial cells, duct and acinar cells (fig 6) and, when present, lymphocytes. The intensity of staining for the isoforms varied in a consistent manner with TGFβ3 reactivity being darker than that of the other two isoforms. Subjectively, there seemed to be no difference in the staining patterns of the three isoforms between the SSc, RP and control tissues. Expression of all three TGFβ isoforms by duct and acinar cells was variable in all glands, with both positive and negative ducts and acini within the same section. Comparison between serial sections stained for TGFβ isoforms, tenascin, elastin and CD45 indicated that there was no association between ductal expression of TGFβs and the presence or absence of extracellular matrix proteins or lymphoid infiltration. Many fibroblasts in the connective tissue around and within the gland were positive for all TGFβs in both the SSc group and the control group. Cell counts (table 4) showed that there were no significant differences between the percentage of TGFβ1 positive fibroblasts in sections from SSc, RP and control glands (p=0.86; AOVO). By contrast, TGFβ2 expression differed between groups (p<0.002, AOVO) with the percentage of fibroblasts expressing TGFβ2 being significantly higher in the SSc group (mean (SD) 65.2 (17.4)%) compared with the control sections (38.4 (13.6)%; p<0.004). The mean percentage of TGFβ2 positive fibroblasts in RP glands (52.2 (8.3)%) was intermediate between those for normal and SSc glands but comparison between medians showed that the differences did not quite reach statistical significance (p<0.058 and p<0.084 respectively). TGFβ3 expression also differed between groups (p<0.05, AOVO). However, the percentage of fibroblasts positive for TGFβ3 in SSc tissues was similar to that in normal gland whereas RP glands contained significantly fewer positive cells (p<0.02) than normal tissues.

TGFβ receptors

Antibodies to receptors 1 and II gave similar patterns of staining to those obtained using isoform specific antibodies. Receptors were detected in fibroblasts, acinar cells and ductal epithelium (fig 7). Cell counts (table 4) showed that there were no significant differences in the percentage of fibroblasts stained for either type of TGFβ receptor.

Vimentin positive ductal dendritic cells

Sections of all specimens were stained for vimentin as an adjunct to screening sections for connective tissue components. In addition to vimentin positive fibroblasts, lymphocytes, endothelial cells and dendritic cells in overlying oral epithelium all but two specimens (both from SSc patients) contained vimentin positive dendritic cells within the walls of large ducts

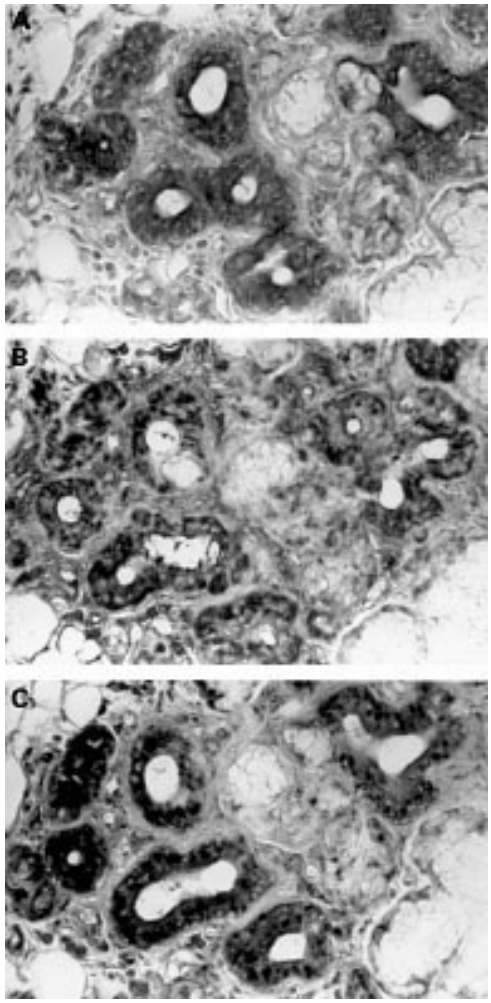

Figure 6 Glandular expression of TGFβ1 (A), TGFβ2 (B) and TGFβ3 (C) in a specimen from a patient with SSc (× 760).

Table 4 Percentage expression of TGFβ isoforms and receptors by stromal fibroblasts

% fibroblasts stained for:	*Systemic sclerosis*	*Raynaud's phenomenon*	*Controls*
TGFβ1	43.7 (16.2)	45.9 (13.2)	40.9 (19.2)
TGFβ2	65.2 (17.4)	52.2 (8.3)	38.4 (13.6)
TGFβ3	61.7 (17.8)	49.9 (16.9)	71.3 (11.2)
TGFβ receptor I	94.1 (6.4)	ND*	92.4 (8.4)
TGFβ receptor II	90.1 (8.5)	ND	89.0 (10.0)

*Not determined. Data shown as mean (SD).

(fig 8). These cells were particularly noticeable in the walls of ducts containing intraepithelial lymphocytes. The overall percentage of ducts containing these dendritic cells appeared to be higher in RP glands (42 of 65 ducts; 64.6%) than SSc (54 of 117 ducts; 46.2%) or control (33 of 108; 30.6%) glands. It was not possible to compare groups as the number of large ducts was very variable between individual specimens (range, 2–26).

Discussion

Most studies of extracellular matrix proteins and growth factors in SSc have been performed on skin lesions and dermal fibroblasts[12] and those investigating salivary glands have concentrated on characterising histological parameters for the diagnosis of Sjogren's syndrome in

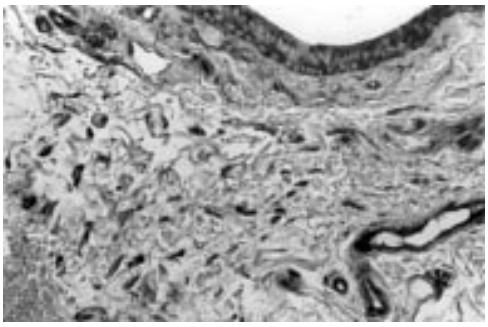

Figure 7 TGFβ receptor I (V22) expression by ductal epithelium, vascular endothelium and fibroblasts with the connective tissue stroma of a salivary gland from a patient with SSc (× 760).

Figure 8 Vimentin positive dendritic cells within the wall of a large collecting duct (RP (× 760).

SSc[4 5] or growth factor expression in Sjogren's syndrome.[20–22] Thus, this study is the first to investigate extracellular matrix proteins and TGFβ expression in salivary glands from patients with SSc. The histological features noted in salivary glands from patients with SSc and RP in this study were qualitatively and quantitatively similar to those previously noted.[4 13]

Apart from staining in lymphoid foci, there were no differences in expression of elastin, tenascin or fibronectin between SSc, RP and histologically normal control glands. Occasionally, increased tenascin or fibronectin deposition, or both, was noted within some inflammatory cell foci. This was particularly noticeable for tenascin when macrophages were present. The finding of both fibronectin and tenascin within lymphoid foci, one with adhesive and the other with anti-adhesive properties, perhaps indicates a role in localisation or migration of lymphocytes in glandular tissue. However, there was no association between elastin or tenascin staining around ducts and the presence of adjacent lymphocyte foci. A similar lack of association has been reported in the lungs in people with asthma[23] and in inflammatory and neoplastic diseases of the large bowel.[24] Overall, the results indicate that local glandular synthesis and deposition of these non-collagenous extracellular matrix proteins is unaffected in SSc and RP. This contrasts with the finding of increased numbers of elastin fibres, many showing ultrastructural abnormalities, reported in the skin of SSc patients,[14] which may suggest deregulation of extracellular matrix production.

All three TGFβ isoforms and receptors I and II were expressed by glandular epithelium,

endothelium, fibroblasts and inflammatory cells in all glands irrespective of the donor. The staining obtained was predominantly cellular and cytoplasmic with little staining of the extracellular matrix. We have found similar localisation patterns of TGFβs and TGFβ receptors using the same primary antibodies on other tissues including oral mucosa, dental pulp and developing tooth germs.[25–27] The overall patterns of staining did not differ between glands from the different patient and control groups. However, cell counts showed that the proportion of fibroblasts expressing TGFβ2 and TGFβ3 was altered in SSc and RP compared with controls, suggesting upregulation of TGFβ2 and downregulation of TGFβ3. It has been shown that TGFβ3 can restrict the deposition of fibrous tissue during wound healing.[10] Furthermore, Sfikakis and coworkers[28] detected extracellular staining for TGFβ2, but not TGFβ1 or TGFβ3, in the dermis of skin lesions in SSc that was not present in normal control tissues or non-involved skin. Thus, it is possible that the fibrous replacement detected in RP and SSc glands is related to these changes in fibroblast isoform expression, especially as receptor expression was maintained.

Our immunohistochemical findings do not allow any firm conclusions to be drawn in respect of differences in synthesis or secretion of growth factors by positively stained fibroblasts or the densities of their cell surface receptors. Clarification of the situation requires studies investigating the expression of isotype specific mRNAs and in vitro cell response studies. Surprisingly little is known about TGFβ isoform and TGFβ receptor expression by fibroblasts in SSc. Differential gene expression in fibroblasts (not TGFβ related) has been detected between lesional fibroblasts and those isolated from clinically unaffected sites.[29] More specifically, recent studies have indicated that SSc fibroblasts show increased expression (twofold) of both type I and II TGFβ receptor mRNAs compared with normal fibroblasts.[30] Furthermore, in transient transfection studies, overexpression of receptors in dermal fibroblasts significantly increased α2 (I) collagen promoter activity with TGFβ acting in an autocrine manner. Thus, although we could not find a difference in the proportion of fibroblasts expressing type I and type II receptors there is evidence that increased collagen synthesis by fibroblasts in SSc is attributable to autocrine effects of TGFβ and increased receptor densities. Similar studies on TGFβ gene expression are needed to determine the presence and potential role of differential expression of growth factor isoforms in SSc.

Published data on the immunohistochemical expression of TGFβ in both skin lesions of SSc[28 31] and within labial glands from patients with Sjogren's syndrome or non-specific sialadenitis[20–22] are inconsistent. Although the variable results obtained in SSc may be explained by the possibility that TGFβ expression in lesional skin is dependent upon the stage of disease at the site,[12] they could also be attributed to differences in primary antibodies, tissues and immunohistochemical methods resulting in variable intracellular and extracellular detection of TGFβs. The latter possibility seems to be the only one that can explain the contradictory results reported in the three published studies of TGFβ in labial glands from patients with Sjogren's syndrome. Koski *et al*,[20] in their study of TGFβ2 found no staining of glandular epithelium in any specimens but showed increased expression by fibroblasts, endothelium and mononuclear cells compared with control glands. By contrast, Cauli *et al*,[21] using an uncharacterised monoclonal antibody to "TGFβ", showed staining of all glandular epithelium in both patient and control glands with the staining being weaker in controls. Staining of inflammatory cells and endothelium was also noted. The third study that examined TGFβ1 expression[22] found no staining of stromal cells in any specimens. However, glandular expression of TGFβ1 was found in all normal control glands but in only 2 of 12 glands from Sjogren's syndrome patients. Our results, using well characterised antibodies and methods including specific peptide blocking experiments, embrace all three studies and indicate expression of the three TGFβ isoforms by both parenchymal and stroma cells in health and disease (SSc and RP).

Mast cells have been long associated with atypical fibrosis, including SSc, because many of their products (for example, heparin, histamine and TNFα) are able to stimulate fibroblast proliferation and collagen synthesis.[6 32–34] Studies of mast cell numbers in skin from SSc patients have given variable results because of their uneven distribution in tissues especially if quantified using only randomly selected high power fields.[35] However, it has been shown that mast cell numbers increase in the early years of the illness but reduce to normal values as the disease progresses.[18] Our results clearly show increased mast cell densities in salivary glands in SSc (60 (18) cells/mm^2) and RP (57 (32) cells/mm^2) although the increase in RP was not statistically significant from controls (32 (12) cells/mm^2). In broad terms our data are similar to those of Hebbar *et al*[13] who found increased mast cell densities (10–40 cells/mm^2) in glands from RP patients who went on to develop SSc/CREST compared with those (<10 cells/mm^2) in glands from patients whose disease did not progress. However, our results, obtained using similar staining methods, differ in that all mast cell densities, including those for controls, fall within the "prognostic" range determined by Hebbar *et al*.[13] The lack of data on normal, non-RP control glands and absence of information on the methods used by Hebbar and coworkers to quantify and verify the objectivity of their mast cell densities do not allow any meaningful comparisons between the studies. Further quantitative histological studies are required to clearly define mast cell densities in labial glands before such data could be of clinical use as a marker for development of SSc. Thus, our findings support the idea that mast cells are increased and play a part in salivary gland changes seen in SSc and RP but indicate

caution with respect to using glandular mast cell densities as a predictive marker for progression of RP to SSc.

Presence of vimentin positive dendritic cells within the walls of large ducts in all but two specimens was an incidental finding and, as far as we are aware, is only the second time such cells have been reported in ductal epithelium. In a previous study by our group[36] CD1 positive intraepithelial cells, some showing a dendritic morphology, were found in labial salivary glands from patients with Sjogren's syndrome. The true identity of these cells and the possibility that they may be increased in Sjogren's syndrome, SSc and RP is deserving of further study. If they represent dendritic antigen presenting cells, they could be important in local activation of lymphocytes and modulate the glandular changes associated with these diseases.

In conclusion, these results indicate that there are no changes in glandular expression of tenascin, elastin or fibronectin in SSc and RP and that both conditions are associated with an increased salivary gland mast cell population and changes in expression of TGFβ2 and TGFβ3 by glandular fibroblasts.

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