

## Expression of metalloproteinase-2 (gelatinase A) in labial salivary glands of patients with Sjögren's syndrome with HTLV-I infection

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### Key words:

Sjögren's syndrome, matrix metalloproteinases, HTLV-I, HAM.

### ABSTRACT.

#### Objective

To determine whether gelatinase A (MMP-2) plays a significant role in the pathogenesis of Sjögren's syndrome (SS) with or without HTLV-I infection.

#### Methods

We examined 24 patients with SS (14 HTLV-I-seropositive and 8 HTLV-I-seronegative). Labial salivary gland tissue samples were analysed immuno-histochemically using anti-MMP-2 monoclonal antibodies.

#### Results

In normal salivary glands, MMP-2 expression was not detected. All biopsy samples of 8 SS patients with HTLV-I-associated myelopathy (HAM) and 3 of 6 HTLV-I-seropositive SS patients without manifestation of HAM stained positively for MMP-2. However, the other samples were negative for MMP-2.

#### Conclusion

Our study showed the MMP-2 expression in labial salivary glands of HTLV-I seropositive SS patients, especially in all SS patients with HAM. The presence of MMP-2 in the salivary glands of these patients suggests that it may play a role in cellular infiltration and destruction in salivary glands of SS.

#### Introduction

Sjögren's syndrome (SS) is an autoimmune disease characterised by lymphocytic infiltration and destruction of the salivary and lacrimal glands (1). Although several aetiological mechanisms have been proposed, the pathogenesis of SS remains unknown. Viruses, including retroviruses, have been considered as potential aetiological agents inducing SS (2, 3). We reported previously a high seroprevalence of human T cell lymphoma virus type -I (HTLV-I) in female SS patients, compared with female blood donors residing in Nagasaki, which is heavily endemic for HTLV-I infection (4). These findings suggest a close relationship between HTLV-I infection and SS.

Matrix metalloproteinases (MMPs) are a family of enzymes that degrade most

of the macromolecules making up the extracellular matrix (ECM) (5). MMPs are implicated in the pathogenesis of autoimmune diseases and are responsible for tissue destruction. Gelatinase (MMP-2) is capable of degrading type IV and type V collagens, which are major components of the basal lamina and are thought to play an important role in cell migration and tumor cell metastasis (6). In the present study, we examined the expression of gelatinase in the labial salivary glands of SS patients with or without HTLV-I infection.

#### Materials and methods

##### Patients

We examined 24 female patients diagnosed as having primary Sjögren's syndrome using the European Community criteria (7). The mean age of the patients was 60.4 years. Eight had HTLV-I-associated myelopathy (HAM) (mean age, 63.1 yrs.) diagnosed according to the criteria proposed by Osame *et al.* (8). Six patients who were seropositive for anti-HTLV-I antibody had no clinical manifestations of HAM (mean age 56.5 yrs.). Eight patients were seronegative for anti-HTLV-I antibody (mean age 58.1 yrs.). Three patients with sicca syndrome but who did not fulfill the European Community diagnostic criteria for SS (including no foci on histology) were also examined in this study. Informed consent was obtained from all participating patients and the study was conducted in accordance with the guidelines on human experimentation of our institution.

##### Monoclonal antibodies

Anti-hMMP-2 monoclonal Ab [Clone No. 42-5D11 (9)] was obtained from Fuji Chemical Co. (Takaoka, Japan). Anti-CD3 mAb (Leu-4) was purchased from Becton Dickinson Monoclonal Center (Mountain View, CA). Anti-CD68 mAb was purchased from DAKO (Glostrup, Denmark).

##### Immunohistochemical method

Labial salivary glands were obtained under local anaesthesia, and fixed in 4% paraformaldehyde (PFA) in PBS (pH 7.4). Cryosections (5-7 µm) were cut and mounted on glass slides. The binding of mouse monoclonal antibody (MMP-2)

**Table I.** Immunohistopathological findings in the minor salivary glands of Sjögren's syndrome patients with or without HTLV-I infection.

	SS patients (No.)	Age/Sex	Histological features* (Grade)	Antinuclear- antibody**	Anti-SSA/ SS B***	MMP-2**** lymphoid cell	MMP-2**** macrophage- like cell
HAM	No. 1	69/F	3	-	+ / -	+	+
	No. 2	73/F	3	x 160	- / -	+	++
	No. 3	61/F	3	-	- / -	+	+
	No. 4	68/F	4	-	- / -	++	+
	No. 5	66/F	4	-	- / -	+	+
	No. 6	72/F	3	-	- / -	++	++
	No. 7	62 /F	3	x 80	- / -	+	+
	No. 8	40/F	4	-	+ / -	++	++
Anti-HTLV-I Ab(+)	No. 9	72/F	3	x 1280	+ / +	++	+
	No. 10	40/F	4	x 640	+ / -	-	-
	No. 11	48/F	4	x 640	+ / -	+	+
	No. 12	43/F	4	x 320	+ / +	-	-
	No. 13	68/F	3	x 320	+ / -	+	+
	No. 14	68/F	3	x 40	+ / -	-	-
Anti-HTLV-I Ab(-)	No. 15	63/F	4	x 160	+ / +	-	-
	No. 16	48/F	4	x 1280	+ / -	-	-
	No. 17	58/F	4	-	+ / -	-	-
	No. 18	75/F	4	x 40	+ / -	-	-
	No. 19	86/F	4	x 640	- / -	-	-
	No. 20	64/F	3	x 5120	- / -	-	-
	No. 21	30/F	3	x 160	+ / -	-	-
	No. 22	61/F	4	x 320	+ / -	-	-
	No. 23	56/F	3	x 640	- / -	-	-
	No. 24	40/F	4	x 2560	- / -	-	-

\*Criteria defined by Chisholm and Mason: The presence of at least one focus of mononuclear cells in 4 mm<sup>2</sup> sections, defined as 3.

\*\*The titre of antinuclear antibodies. \*\*\*Antibodies to SS-A(Ro) and SS-B(La) determined by ELISA.

\*\*\*\*Evaluation of cells which stained positive on immunohistochemistry. The relative frequency of positively stained cells in the salivary gland tissues was estimated as follows: (-) 0%; (+) 0 - 20%; (++) > 20%.

was detected by the labelled-streptavidin-biotin method (HISTOFINE staining kit, Nichirei Co., Tokyo), as described previously (10). Color was developed using 3,3-diamino-benzidine and H<sub>2</sub>O<sub>2</sub>. The slides were counterstained with haematoxylin. Negative control sections were treated with normal mouse IgG. All specimens were evaluated with a microscope using x200 magnification power. All infiltrated cells were counted and the number of cells positive for MMP-2 was graded as follows: (-) 0%; (+) 0 - 20%; (++) > 20% (Table I).

## Results

*Expression of matrix metalloproteinases*  
Immunohistochemical staining by anti-MMP-2 on the normal salivary glands was carried out. As shown in Figure 1B, MMP-2 was not localized in normal salivary gland tissues. Also no positive staining was noted in control sections stained with murine monoclonal IgG in the place

of anti-MMP-2 mAb (Fig. 1A). Salivary gland tissues from 24 SS patients with or without HTLV-I infection were examined immunohistochemically using specific antibody against MMP-2. Labial salivary gland from SS patients with HAM were positively stained with anti-MMP-2 monoclonal antibodies. MMP-2 was mainly stained in the mononuclear cells infiltrating in the interstitium of the salivary gland (Fig. 1D). In contrast, staining with anti-MMP-2 mAb was not observed in salivary gland specimens from HTLV-I- seronegative SS patients (Fig. 1C).

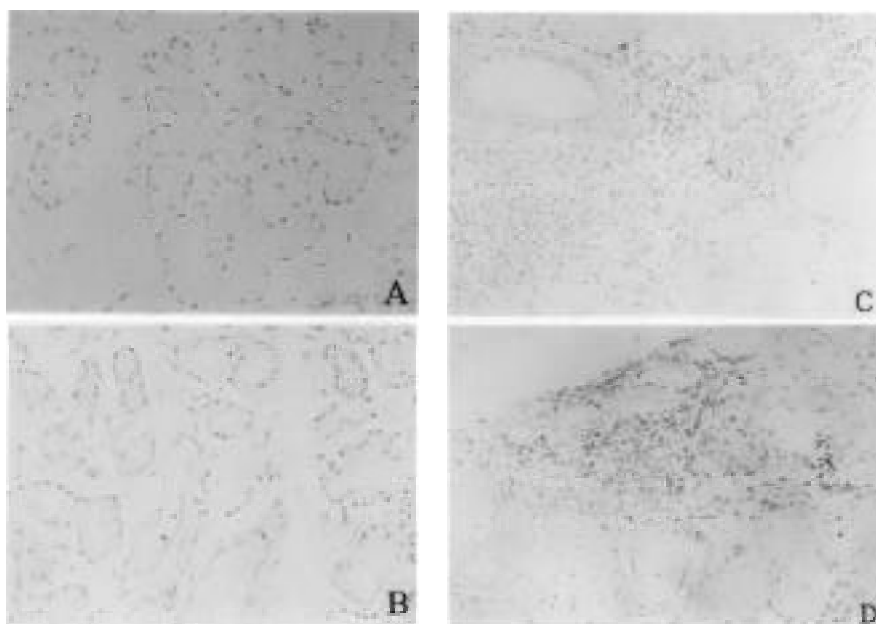
Consecutive sections of the same specimens from HTLV-I- seropositive SS patients were stained with anti-MMP-2, anti-CD-3 and anti-CD-68 monoclonal antibodies. MMP-2 expressing cells (Fig. 2A) were mostly positively stained with anti-CD-3 mAb (Fig. 2B) and anti-CD-68 mAb (Fig. 2C). These results indicate that MMP-2 positive cells were

predominantly T lymphocytes and macrophage-like cells. By contrast, staining with anti-MMP-9 mAb was not detected in salivary gland specimens from SS patients with or without HTLV-I infection.

Table I shows the MMP-2 expression of salivary gland specimens in SS patients with or without HTLV-I infection. MMP-2 staining of salivary glands was detected in all 8 SS patients with HAM and 3 of 6 HTLV-I-seropositive SS patients. No MMP-2 staining of salivary glands was observed in all 10 HTLV-I- seronegative SS patients.

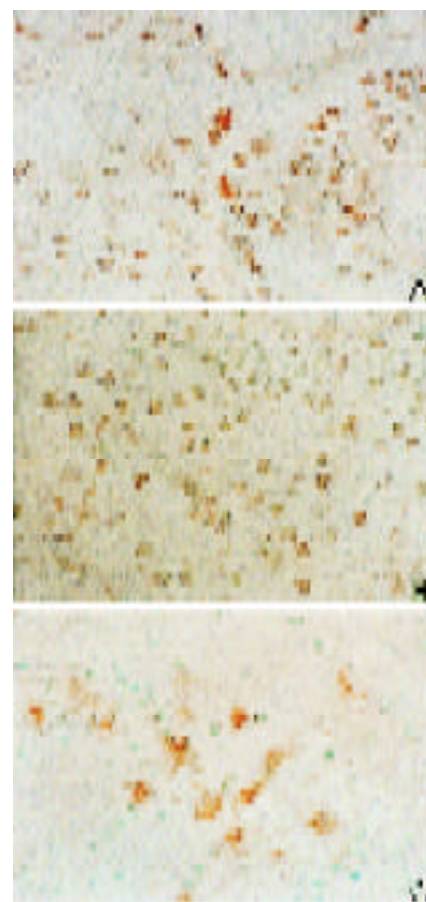
## Discussion

We have demonstrated in this study the presence of MMP-2 in the labial salivary glands of SS patients with HAM as well as in those of HTLV-I-seropositive SS patients by immunohistochemical analysis. In contrast, MMP-2 was not present in the salivary glands of HTLV-I- seron-



**Fig. 1.** Immunohistochemical staining of normal labial salivary glands using anti-MMP-2 monoclonal antibodies. (A) No cells were stained with anti-MMP-2 monoclonal antibodies in normal salivary glands; (B) no positive staining was noted in the same sections stained with murine monoclonal IgG in the place of anti-MMP-2 mAb (original magnification x 200)

Immunohistochemical staining of labial salivary glands from SS patients using anti-MMP-2 monoclonal antibodies. (C) No cells were stained with anti-MMP-2 monoclonal antibodies in minor salivary glands from HTLV-I-seronegative SS patients; (D) MMP-2 staining was detected in the infiltrating mononuclear cells in minor salivary glands from HTLV-I-seropositive SS patients (original magnification x 200).



**Fig. 2.** MMP-2 expression in labial salivary glands from HTLV-I-seropositive SS patients. The consecutive sections of the same salivary gland specimens from HTLV-I-seropositive SS patients were stained with anti-MMP-2 (A), anti-CD3 (B), and anti-CD68 (C) monoclonal antibodies. The majority of MMP-2 positive cells (A) are CD3 positive T lymphocytes (B) and CD68 positive macrophage-like cells (C) (original magnification; x 500)

egative SS patients using the same analysis.

In SS, migration of lymphocytes to salivary glands could be triggered by antigen challenge (11). MMP-2 degrades type IV collagen, a major component of the basal lamina, and may enhance the penetration and transmigration of antigen-stimulated lymphocytes through the subendothelial basal lamina (12). Thus, it is likely that the high expression of MMP-2 promotes infiltration and accumulation of lymphocytes and macrophages in the extra-lymphoid tissues.

In HTLV-I Tax transgenic mice, lymphocytic infiltration is observed in salivary glands, and the histological findings resemble those in SS patients (13). HTLV-I tax gene upregulates inflammatory cytokine genes by activating NF- $\kappa$ B, a transcriptional factor (14). Production of these cytokines may result in lymphocyte activation, and migration to the salivary glands (15). HTLV-I-infected lymphocytes and macrophages are known to secrete large amounts of cytokines including IL-2, IFN- $\gamma$ , TGF- $\beta$  (16) which are known to induce MMP-2 production from lymphoid and fibroblastic cells

(17). Therefore, it is possible that HTLV-I infection may contribute to increased MMP-2 expression in lymphocytes and macrophages via these cytokines, and cause the infiltration and destruction of salivary glands. In this regard, Azuma *et al.* (18) recently reported the presence of a positive relationship between activation of MMP-2 and NF- $\kappa$ B activity in salivary glands of SS patients.

In summary, our data demonstrated that MMP-2 was predominantly expressed in salivary glands of HTLV-I-seropositive patients with SS. These data suggest that HTLV-I-infection could contribute to MMP-2 expression by salivary infiltrating cells and suggest a potential role for MMP-2 in lymphocyte trafficking process as well as destruction of the connective tissue of salivary glands in HTLV-I seropositive patients with SS.

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