Detection of the soluble form of Fas ligand (sFasL) and sFas in the saliva from patients with Sjögren's syndrome

Sirs,

We examined the possible involvement of the soluble form of Fas ligand (sFasL) and sFas in the saliva from Sjögren's syndrome (SS) patients in the process of salivary gland destruction.

Sera were obtained from 40 primary SS at our hospital. All the patients fulfilled the revised European criteria for the diagnosis of SS (1). Anti-SS-A and anti-SS-B seropositivity were 54.6% and 19.4%, respectively. Saliva was obtained from 25 primary SS patients by catheter, which was inserted 1-2 cm into the Stensen's duct as previously described (2). 20 subjects (for sera examination) and 12 subjects (for saliva examination), who were age-matched normal controls, also enrolled. Informed consent was obtained, and the study was conducted in accordance with the human experimental guidelines of our institution.

Protein concentrations of sFasL and sFas were measured by a sandwich enzyme-linked immunosorbent assay kit using anti-human FasL monoclonal antibodies (mAbs) or anti-Fas mAbs (MBL, Nagoya, Japan). Sialography of the parotid gland was carried out to assess the radiographic grading of glandular destruction in patients with SS. Radiographic grading of glandular destruction was determined according to Rubin & Holt (from stage 0; normal to stage IV; destructive) (3). Statistical analysis was performed using Student *t*-test.

sFasL was detected in all the sera; however, their concentrations were not different between the SS patients and control subjects (mean ± SD; SS patients: 0.16 ± 0.07 ng/ml, control subjects: 0.16 ± 0.08 ng/ml, p = 0.83) (Fig.1A). sFas in sera was also detected in all the SS patients (mean ± SD; 3.94± 1.20 ng/ml), which was higher than the control subjects (mean ± SD; 2.12 ± 0.59 ng/ml, p< 0.01) (Fig.1B). sFasL protein concentration in saliva was significantly higher in the SS patients than the control subjects (Fig. 1C). Interestingly, sFasL in saliva in SS was higher than that of sera (compare Fig. 1C to Fig. 1A). sFas in saliva from SS patients was also higher than the control subjects. However, sFas in saliva was significantly less than in sera concentration (compare Fig. 1D to Fig. 1B).

Parotid gland sialography was examined in 22 SS patients, all of whom were also evaluated for protein concentration of sFasL and sFas in saliva. We classified 22 SS patients into 2 groups according to Rubin & Holt radiographic grading, as 11 early-stage patients (stage 0 and stage I) and 11 advanced-stage patients (stage II to IV). A notable data shown in Fig. 2 was that sFasL protein concentration was markedly high in advanced-stage SS patients as compared with early stage SS patients. In contrast to sFasL, sFas protein concentration was not different between early-stage patients and advanced-stage patients.

The above data may indicate that sFasL in saliva appears to be involved in the salivary gland destruction of SS. Since sFasL concentration in sera from the SS patients was not different from the normal subjects, the increased amount of sFasL in saliva from SS patients appears to be associated with the microenvironment of the salivary glands from SS patients. Furthermore, it is interesting to note that sFasL concentration of saliva was clearly increased in the radiographic advanced-stage of SS patients. sFasL is cleaved from membrane-bound (mFasL) by MMP-like enzyme, and also can induce Fas-mediated apoptosis (4, 5). Although the lymphocyte infiltrates may also influence apoptotic cell death of salivary epithelial cells (6), we speculate that, salivary "cytokine-rich" microenvironment of SS patients facilitates the release of sFasL in saliva from acinar cells, and are closely involved in the apoptotic cell death of ductal epithelial cells.

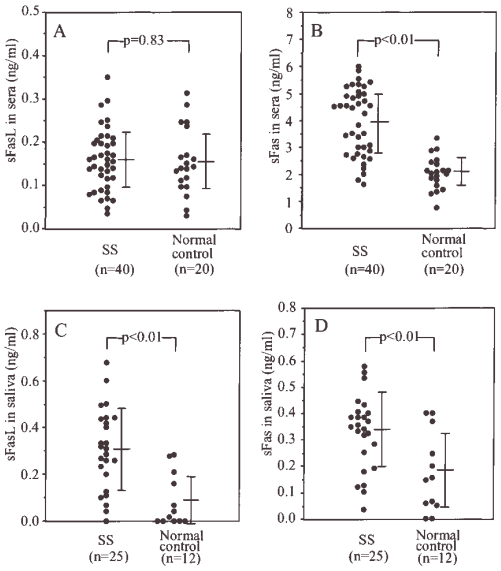

Fig. 1. Levels of sFasL and sFas in the sera and saliva from SS patients determined by ELISA.
A: Levels of sFasL in sera of SS patients (n = 40) determined by ELISA compared with normal subjects (n = 20). No significant difference was observed between SS and normal subjects (p = 0.83). **B**: Levels of sFas in sera of SS patients (n = 40) determined by ELISA, which were higher than those of normal subjects (n = 20) (p < 0.01). **C**: Levels of sFasL in saliva of SS patients (n = 25) were significantly elevated compared to normal subjects (n = 12) (p < 0.01). In SS patients, sFasL in saliva was higher than that in sera (compare Fig. 1C to Fig. 1A). **D**: Levels of sFas of saliva in SS patients (n = 25) were also higher than normal subjects (n = 12) (p < 0.01). However, sFas in saliva was significantly less than in sera concentration (compare Fig. 1D to Fig. 1B). Error bar indicated mean ± SD.

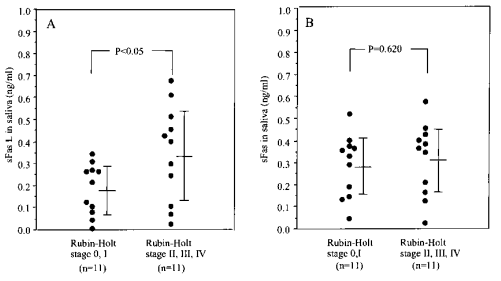

Fig. 2. Relationship between the protein concentrations of sFasL/sFas in saliva of SS patients and radiographic grading of glandular destruction. Although no significant difference was determined in sFas concentration of saliva between radiographic early-stage SS patients and advanced-stage SS patients (**B**, p = 0.62), sFasL concentration in radiographic advanced-stage SS patients was significantly higher than in early-stage patients (**A**, p < 0.05). Error bar indicates mean ± SD.

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Abbreviations: ELISA: enzyme-linked immunosorbent assay; MMP: matrix metalloprotease; SS: Sjögren's syndrome; sFasL: soluble form of Fas ligand; mFasL: membrane-bound Fas ligand; mAbs: monoclonal antibodies.

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