

# Enhanced Expression of Programmed Death-1 (PD-1)/PD-L1 in Salivary Glands of Patients with Sjögren's Syndrome

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**ABSTRACT. Objective.** Programmed death-1 (PD-1) mediates a negative signal and introduces tolerance for lymphocytes. Dysfunction of the PD-1 pathway is thought to result in autoimmune diseases such as rheumatoid arthritis (RA). To investigate the role of the PD-1/PD-L system in the pathology of Sjögren's syndrome (SS), we examined the expression of PD-1 and its ligand PD-L1 in salivary lymphocytes and salivary glands from patients with SS.

**Methods.** Flow cytometry analysis was used to determine expression of PD-1 in SS salivary lymphocytes. Intracellular staining of interleukin 10 (IL-10) was performed after stimulation with PMA and ionomycin. Indirect immunohistochemistry was used to investigate the expression of PD-1 and PD-L1.

**Results.** The mean fluorescence intensity of PD-1 expression in SS salivary lymphocytes was significantly higher than that from healthy controls and patients with RA or systemic lupus erythematosus. PD-1-positive SS salivary lymphocytes expressed IL-10 intracellularly upon PMA/ionomycin stimulation. Immunohistochemical analysis showed that PD-1 was expressed on infiltrating lymphocytes in salivary gland from 52% of SS patients, and PD-L1 was expressed on ductal and acinar epithelial cells from 68% of SS patients. *In vitro* analysis using HSG cells revealed that PD-L1 was induced by interferon- $\gamma$  but not by tumor necrosis factor- $\alpha$  and IL-1 $\beta$ .

**Conclusion.** PD-1 is expressed on T lymphocytes and PD-L1 on epithelial cells from inflamed salivary glands of patients with SS, which suggests that dysfunction of the PD-1/PD-L1 pathway may be related to tolerance for lymphocytes, which causes SS. (J Rheumatol 2005;32:2156-63)

## Key Index Terms:

SJÖGREN'S SYNDROME  
PROGRAMMED DEATH-1 LIGAND

PROGRAMMED DEATH-1  
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Sjögren's syndrome (SS) is an autoimmune disease in which the immune system attacks the body's own moisture-producing glands. The hallmark symptoms are dry eyes and dry mouth. SS is also a systemic disease, causing dryness of other organs and infiltration of lymphocytes into kidney, blood vessels, lung, liver, pancreas, and central nervous system. Histologically, salivary glands in SS show characteristic lymphocyte infiltration, which causes the destruction of glandular cells. Immunologically, the common characteristics of SS include polyclonal hypergammaglobulinemia<sup>1</sup>, as in systemic lupus erythematosus (SLE), and a sizable production of autoantibodies to the intracellular ribonucleoprotein particles (Ro/SSA and La/SSB).

Although the key trigger of SS remains unidentified, recent studies have proposed several candidates for specific autoantigens in SS. One of these is  $\alpha$ -fodrin, which provides membrane intracellular scaffolding. Sera from patients with SS react positively with purified  $\alpha$ -fodrin antigen and recombinant human  $\alpha$ -fodrin protein, whereas sera from patients with SLE and rheumatoid arthritis (RA) do not<sup>2</sup>. Golgi complexes<sup>3</sup> and muscarinic acetylcholine receptors<sup>4</sup>

are other candidates for autoantigens in SS. Identification of the processing mechanisms can be expected to become an important future issue in treatment of SS.

Programmed death-1 (PD-1) is a 55 kDa transmembrane protein with 24% amino acid homology to CTLA-4, a member of the CD28 family<sup>5</sup>. PD-1 is expressed on activated T cells, B cells, and monocytes<sup>6</sup>, and delivers a negative signal by means of recruitment of src homology 2-domain-containing tyrosine phosphatase 2 (SHP-2) to the phosphorylated tyrosine residue in the cytoplasmic region<sup>5,7</sup>. Interestingly, PD-1<sup>-/-</sup> mice develop autoimmune diseases. Those with a C57BL/6 background develop lupus-like glomerulonephritis and arthritis<sup>8</sup>, and those with a BALB/c background autoimmune dilated cardiomyopathy caused by the autoantibody<sup>9</sup>. These findings emphasize the importance of PD-1 in downregulating immune responses and in maintaining peripheral tolerance.

Recent studies have shown that single nucleotide polymorphisms (SNP) of the PD-1 gene are linked to susceptibility to SLE and RA. One SNP on intron 4 of the PD-1 gene was found to be associated with development of SLE in Europeans and Mexicans<sup>10</sup>. This SNP, located in an intronic enhancer, alters its binding capacity for runt-related transcription factor 1. Another study showed that one silent SNP on exon 5 of the PD-1 gene was linked to susceptibility to RA, but not SLE, in a Chinese population<sup>11</sup>. Although SS has not been examined for links to SNP of the PD-1 or PD-L1 genes, the defective or enhanced expression of PD-1 or its ligand PD-L1 may be genetically controlled and define the susceptibility to salivary tissue destruction by T cells as well as to lymphoproliferative diseases.

The ligands for PD-1, PD-L1 and PD-L2, are members of the B7 family<sup>12,13</sup>. PD-L1 has 20% amino acid homology with the extracellular domains of B7-1 (CD80) and 15% with B7-2 (CD86). PD-L1 is not expressed on resting cells, but its cell surface expression is induced by stimulation with interferon- $\gamma$  (IFN- $\gamma$ ) in nonhematopoietic cells as well as hematopoietic cells, while the expression of PD-L2 is induced only on monocytes, macrophages, and dendritic cells<sup>12,14</sup>. Some reports have suggested that the function of PD-L1 is to inhibit T cell proliferation and to inactivate T cell proliferation via PD-1 by upregulating the production of interleukin 10 (IL-10) and downregulating the production of IL-2 and IFN- $\gamma$ <sup>15,16</sup>. PD-1 and its ligands are therefore thought to play a critical role in downregulating immune responses by inhibiting T cell proliferation and activation, which would indicate the possible involvement of PD-1/PD-L interaction in autoimmune diseases. We used flow cytometry and immunohistochemistry analysis to examine the expression for PD-1 and PD-L1 on lymphocytes and glandular epithelia in patients with SS, in order to determine the influence of PD-1/PD-L1 expression on pathogenesis of SS.

## MATERIALS AND METHODS

**Antibodies.** Phycoerythrin (PE) conjugated anti-human mouse CD4 (RPA-T4) and CD8 (RPA-T8) were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Anti-human PD-1 mouse Mab (J116) was generated by Honjo Chemical Co. (Tokyo, Japan), and anti-human PD-L1 (18B7) mouse mAb was by Minato Medical Science (Osaka, Japan). FITC conjugated anti-human PD-1 was purchased from eBioscience (San Diego, CA, USA). Goat anti-mouse IgG1-FITC was obtained from Southern Biotechnology (Birmingham, AL, USA), while PE conjugated anti-human mouse CD95L and FITC conjugated anti-human mouse CD80 were purchased from Caltag Laboratories (Burlingame, CA, USA). FITC conjugated anti-human mouse CD86 was obtained from Serotec Ltd. (Oxford, UK) and anti-human mouse APO-1/FAS, CD95-FITC conjugated, from Dako Japan (Kyoto, Japan).

**Salivary gland samples.** Diagnosis of SS was established from objective evidence of dryness of the eyes and mouth, systemic autoimmune abnormalities, salivary gland focus scores, and serological data<sup>17</sup>. With the approval of the Committee for Human Research at Kobe University, salivary gland samples were obtained from patients who had given informed consent for the samples to be used in this study.

**Flow cytometry analysis of PD-1 expression.** Lymphocytes in saliva were obtained from healthy controls and patients with SS, RA, and SLE. Saliva was collected with the gum test for 10 min, and filtered with a cell strainer (Falcon 352340, Becton Dickinson) to remove large epithelial cells and debris. Flow cytometry analysis was performed as described<sup>16</sup>. After 2 washes with phosphate buffered saline (PBS) containing 2% fetal bovine serum (FBS), the purified cells ( $1 \times 10^5$  cells/sample) were used for flow cytometry analysis. Briefly, cells were incubated with PBS containing 20% human AB serum for 5 min on ice, and incubated with biotinylated anti-PD-1 Mab (J116) or biotinylated anti-mIgG1 (for controls) for 30 min at 4°C, and then with cychrome conjugated streptavidin under the same conditions. Flow cytometric analysis used a FacsCalibur<sup>®</sup> machine with CellQuest<sup>®</sup> software (Becton Dickinson).

**Intracellular staining of IL-10.** To detect intracellular cytokine production, cells ( $1 \times 10^6$ ) were activated with 50 ng/ml phorbol myristate acetate (PMA; Sigma, St. Louis, MO, USA), 0.5  $\mu$ g/ml ionomycin (Sigma) and monensin (GolgiStop; PharMingen, San Diego, CA, USA) in plastic tissue culture dishes at 37°C for 4 h. After activation, cells were washed with PBS containing 2% FBS and incubated with PBS containing 20% human AB serum for 5 min on ice, and then incubated with FITC conjugated anti-PD-1 Mab or biotinylated anti-mIgG1 (for controls) for 30 min at 4°C. After 2 washes with PBS containing 2% FBS, cells were resuspended thoroughly with Cytofix/Cytoperm solution (Becton Dickinson) for 20 min at 4°C. After washing 2 times in Perm/Wash solution (Becton Dickinson), cells were resuspended in Perm/Wash solution containing PE conjugated anti-IL-10 Mab (Caltag). After 2 washes with Perm/Wash solution, purified cells ( $1 \times 10^5$  cells/sample) were used for flow cytometry.

**Immunohistochemical analysis.** Lip biopsy specimens from patients with SS were fixed with 3% paraformaldehyde, and then frozen in OCT compound and stored at -100°C. Cryostat sections (3  $\mu$ m) were incubated with FITC conjugated anti-PD-1 Mab and serum containing anti-PD-L1 or anti-mouse IgG1 Mab overnight at 4°C, followed by incubation with biotin conjugated rabbit anti-mouse Ig for 30 min at room temperature (RT) and use of the avidin-biotin technique. Sections were then incubated with anti-mouse IgG1-FITC for 30 min at RT, and analyzed for PD-1 and PD-L1 expression under confocal microscopy (LSM5 Pascal V3.0; Zeiss, Jena, Germany). A lip biopsy specimen from a patient who eventually was found to have no autoimmune disease was used as the normal reference salivary gland.

**Histopathology of salivary glands.** Lip biopsy specimens were assessed histologically for lymphocytic infiltration according to the criteria described by Chisholm and Mason<sup>18</sup> and modified by Greenspan, *et al*<sup>19</sup>. In brief, grades ranged from 0 to 4, 0 representing absence of infiltration of the gland, 1 slight infiltration, 2 moderate infiltration, 3 one focus of at least 50 lymphocytes/4 mm<sup>3</sup>, and grade 4 more than one focus of at least 50 lymphocytes/4 mm<sup>3</sup>.

*Expression of costimulatory molecules on HSG cells.* The HSG cell line established from salivary epithelial cancer was kindly donated by Dr. M. Sato of Tokushima University, Tokushima, Japan. HSG cells ( $5 \times 10^4$ /well) were cultured in the presence or absence of recombinant human IFN- $\gamma$ , TNF- $\alpha$ , or IL-1 $\beta$  for 24 h with the aid of the Lab-Tek<sup>®</sup> chamber slide system (Apogent Technologies, Portsmouth, NH, USA). After cultivation, expression of PD-L1, CD80, and CD86 on HSG cells was determined by confocal microscopy.

*Statistical analysis.* Statistical analysis was performed with StatView software. Data are given as the mean  $\pm$  SD of the number of samples studied. Comparative statistical analysis of 2 independent groups was performed with the Mann-Whitney U test and that of multiple, independent diagnostic groups with the Kruskal-Wallis test. Results were considered statistically significant at  $p < 0.05$ .

## RESULTS

*PD-1 is expressed on infiltrating lymphocytes of salivary glands from patients with SS.* The total volume of saliva from patients with SS was less than that from control samples, and the concentration of lymphocytes in saliva was significantly higher in SS patients (mean  $\pm$  SD,  $5.65 \pm 4.63 \times 10^5$ /ml in SS patients vs  $0.53 \pm 0.27 \times 10^5$ /ml in healthy controls;  $p < 0.01$ ). In contrast, concentrations of lymphocytes in saliva from patients with RA and SLE were not increased (data not shown).

Flow cytometry analysis using salivary lymphocytes from controls and patients with SS, SLE, or RA indicated that PD-1 expression was clearly upregulated in SS patients (Figure 1A), with high expression levels detected in 12 of 27 SS patients (44%). Statistical analysis showed that the mean fluorescence intensity for PD-1 expression of SS patients was significantly higher than that for controls and SLE and RA patients (Figure 1B). We also examined PD-1 expression with the lip biopsy sections from 21 SS patients. PD-1 expression was found in 52% of SS patients, and especially on infiltrating lymphocytes (Figure 1C). We were able to examine PD-1 expression of both salivary lymphocytes and salivary glands in 9 patients. Five patients had PD-1 expression on both materials, one in salivary gland only, one in infiltrating lymphocytes only, and 2 with no PD-1 expression on either sample. This indicated PD-1 expression on salivary lymphocytes tended to be correlated with the PD-1 expression on infiltrating lymphocytes from SS patients. When we compared PD-1 expression on lymphocytes in lip biopsies with the degree of lymphocyte infiltration graded by Greenspan's classification, PD-1 positivity was not statistically correlated with the focus scores (data not shown).

Two-color flow cytometry analysis of salivary lymphocytes, detected by anti-PD-1 and anti-CD4 or anti-CD8 antibodies, showed that both CD4+ and CD8+ T cells expressed PD-1 (Figures 2A, 2B). There was no statistical difference in the percentage of PD-1+ cells between CD4+ T and CD8+ T cells. Then we performed intracellular cytokine analysis of PD-1+ salivary lymphocytes. Salivary lymphocytes from SS patients cultured with PMA/ionomycin for 4 h produced IL-10 from PD-1+ lymphocytes, while lympho-

cytes from normal saliva did not (Figure 2C). The positive percentages of intracellular IL-10 on PD-1+ salivary lymphocytes from SS patients and controls were  $18.0\% \pm 9.4\%$  (mean  $\pm$  SD,  $n = 9$ ) and  $5.8 \pm 2.2\%$  ( $n = 6$ ), respectively ( $p < 0.01$ ).

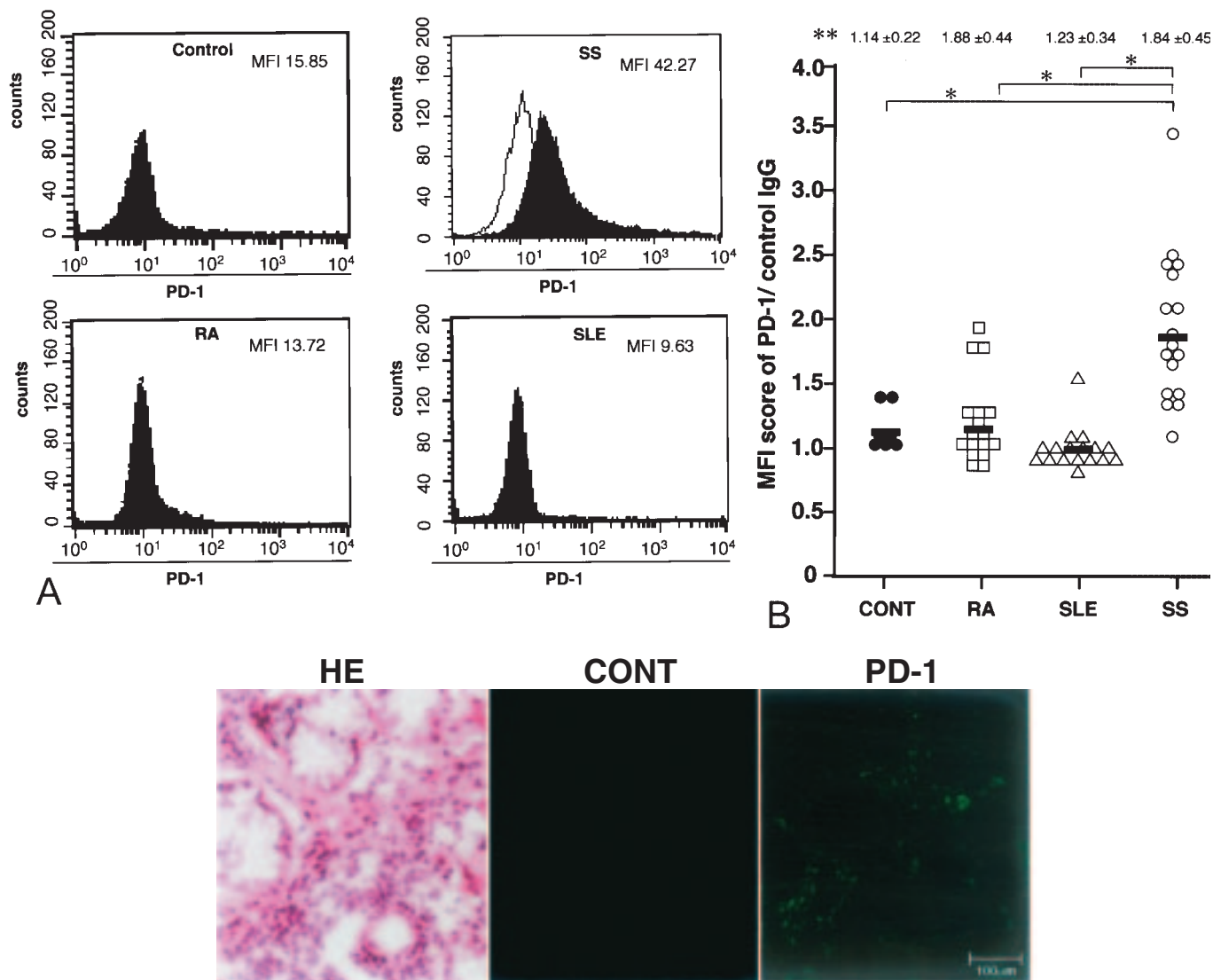
We also analyzed the correlations between PD-1 expression and various immunological markers, namely, antinuclear antibody, IgG, IgA, IgM, C-reactive protein, white blood cell count, platelet count, red blood cell count, hemoglobin level, erythrocyte sedimentation rate, and amylase concentration. However, no clear relationship between the expression of PD-1 and any of these markers could be established (data not shown).

*PD-L1 is expressed on salivary gland epithelium.* To investigate the expression of PD-L1 in human salivary gland, we immunohistochemically stained the lip biopsy sections from 34 SS patients with anti-PD-L1 Mab. PD-L1 was strongly expressed in the salivary gland ductal and acinar epithelial cells from SS patients (Figures 3A, 3B), but not in normal salivary gland. PD-L1 expression was found in 68% of SS patients, and this expression paralleled the degree of lymphocyte infiltration detected with the method of Greenspan, *et al*<sup>19</sup>. Statistical analysis showed that the percentage of PD-L1 expression on the salivary glands with lymphocytic infiltration was higher than on those without lymphocyte infiltration (Figure 4). PD-L1 expression was also detected on epithelia that were distant from the foci of lymphocyte infiltration, suggesting that cytokines or chemokines such as IFN- $\gamma$  produced by infiltrating T cells upregulated PD-L1 expression on surrounding tissues.

*PD-L1 expression induced by IFN- $\gamma$  on HSG cells.* It has been reported that PD-L1 expression was not detected on resting macrophages, but was induced after stimulation with IFN- $\gamma$ <sup>20</sup>. We analyzed the expression of PD-L1, CD80, and CD86 on HSG cells after stimulation with IFN- $\gamma$ , TNF- $\alpha$ , or IL-1 $\beta$ . HSG cells strongly expressed PD-L1 after IFN- $\gamma$  stimulation (Figure 5). Surface expression of CD80 and CD86 was upregulated slightly by IFN- $\gamma$ . However, expression of PD-L1 on HSG cells did not change after stimulation with TNF- $\alpha$  or IL-1 $\beta$ , while the surface expression of CD80 and CD86 was strongly upregulated after the same stimulation. To our knowledge, this is the first description that PD-L1 expression cannot be induced by IL-1 $\beta$ , in contrast to that of CD80/86. These results suggest the possibility that PD-L1 may be expressed under conditions or circumstances different from those of the other B7 molecules, CD80 and CD86.

## DISCUSSION

Most autoimmune diseases generally result from the destruction of sophisticated immune mechanisms. Recent studies using murine models have shown that the PD-1 pathway is critical for prevention of the autoimmune process, and also plays an important role in antiviral immunity by



**Figure 1.** PD-1 expression on salivary and infiltrated lymphocytes from SS patients. (A) Salivary lymphocytes from healthy controls and patients with SS, RA, and SLE were stained with anti-hPD-1 (solid lines) or control IgG (broken lines) and analyzed with flow cytometry. Upregulation of PD-1 expression was detected only in SS samples (12 of 27 cases). (B) MFI score of PD-1 expression/control IgG was higher for SS samples than for others (\* $p < 0.01$ ; \*\*data are means  $\pm$  SD). (C) Fluorescence staining also shows PD-1 expression on infiltrating lymphocytes. MFI: mean fluorescence intensity, HE: hematoxylin and eosin, CONT: control.

inducing T cell tolerance at the effector phase in the liver<sup>21</sup>. PD-1 is therefore thought to be a key molecule in the negative regulation of immune response and in the maintenance of peripheral tolerance. Another interesting finding is that PD-L1/2, ligands for PD-1, contribute to tumor immunity by means of CD8+ T cell mediated rejection of tumor cells<sup>22</sup>. For this reason, PD-1/PD-L interaction is regarded as an important event in many immune responses. We demonstrated the expression of PD-1 on salivary and infiltrating lymphocytes and the expression of PD-L1 on salivary epithelial cells, and the positive correlation between PD-L1 expression on epithelial cells and lymphocytic infiltration into salivary gland. Interestingly, PD-L1 expression on epithelial cells, but not on lymphocytes, was correlated with

the degree of infiltrating lymphocytes in SS salivary glands. In contrast, in a murine model of SS, NOD mice were found to have exacerbated sialoadenitis when they were backcrossed with PD-1<sup>-/-</sup> mice in our recent study (data not shown), supporting the idea that PD-1+ T cells control the development of SS in a murine model. Thus, the presence of PD-1+ T cells in human SS tissue, with no correlation to the degree of focus score, suggests that the dysfunction or insufficiency of PD-1+ T cells in maintaining tolerance may occur in the course of SS development. Further studies, such as analyzing SNP of the PD-1 gene locus for susceptibility to SS, are needed to verify the involvement of PD-1 in the pathogenesis of human SS.

It has been reported that the expression of CD28/B7 fam-

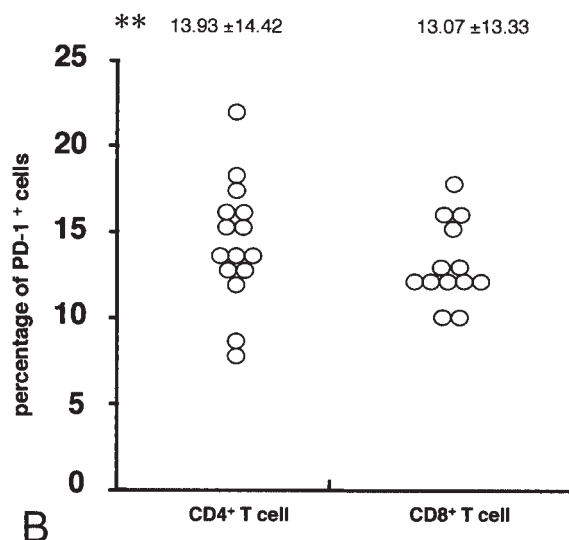
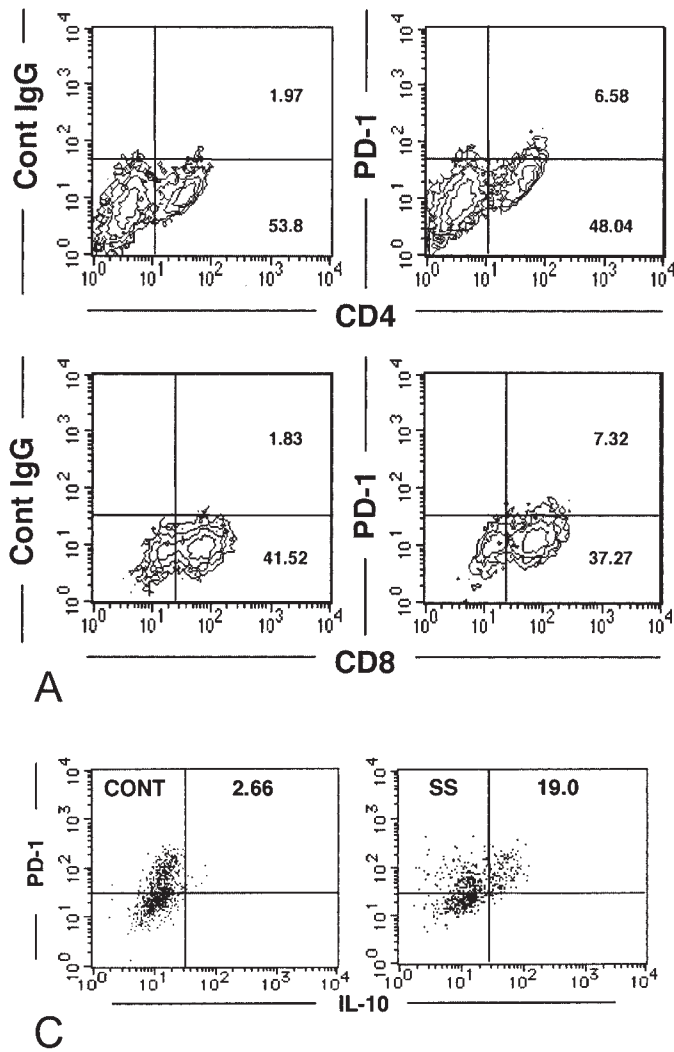
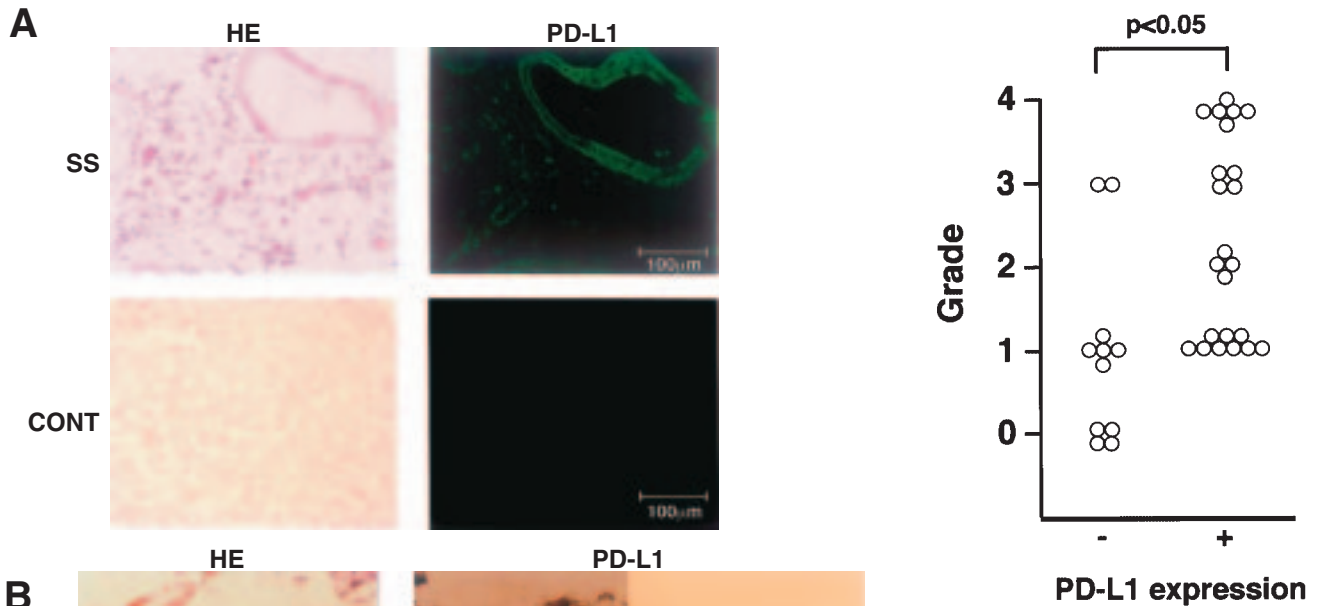


Figure 2. PD-1 and IL-10 expression by lymphocytes from SS patients. Two-color staining was performed with flow cytometry using anti-PD-1 and anti-IL-10, anti-CD4 or anti-CD8 Mab. (A) PD-1 expression (vertical axis) was detected on both CD4+ and CD8+ T cells. (B) No statistically significant difference was detected between CD4+ and CD8+ T cells in terms of PD-1 expression (\* $p > 0.10$ ; \*\*data are means  $\pm$  SD). (C) Intracellular IL-10 production was detected on PD-1+ salivary lymphocytes, and not on PD-1- lymphocytes. CONT: control.

ily molecules on nonhematological cells, also known as nonprofessional antigen-presenting cells (nonprofessional APC), is important in autoimmune diseases. Expression of CD80 (B7.1) and CD86 (B7.2), the ligands for CD28, as well as of Class II MHC molecules has been detected on acinar and ductal cells of salivary glands from SS patients<sup>23-25</sup>. These salivary epithelial cells have therefore been postulated to function as nonprofessional APC. On the other hand, Bolstad, *et al*<sup>26</sup> reported that immunoregulatory receptors such as PD-1 and CTLA-4 are expressed in salivary tissue<sup>26</sup>. We have shown that PD-L1 was also expressed on salivary epithelial cells from SS patients, which suggests that PD-L1 expression may regulate the T cell or B cell activation evoked by cell-cell contact with epithelial cells. In addition, we also show that the PD-L1 expression was higher in patients with a higher score. Therefore, PD-L1 expression by salivary epithelial cells, nonprofessional APC, may be a key immunoregulatory system at the site of sialoadenitis.

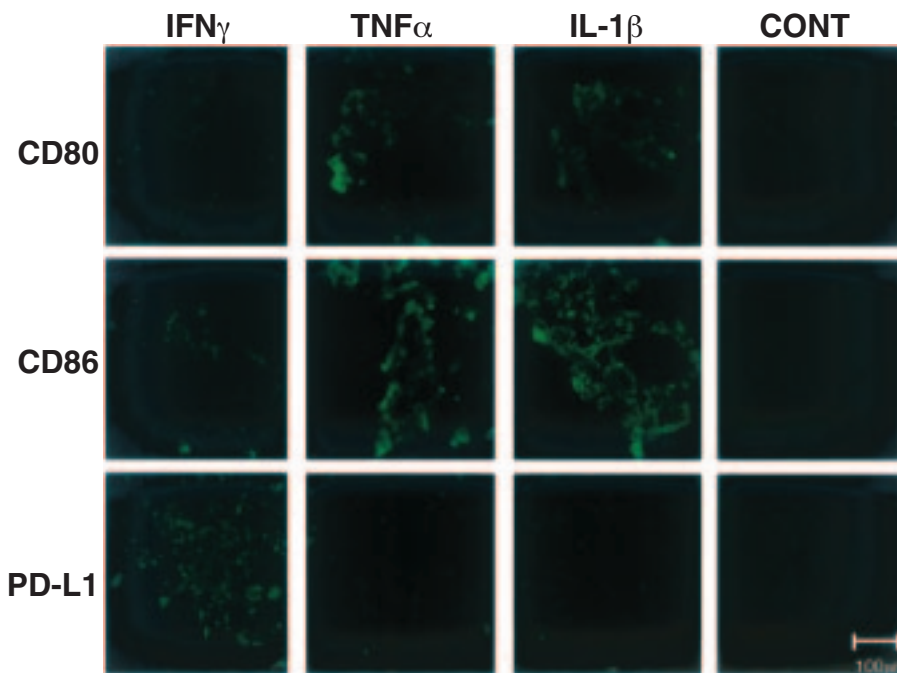
We demonstrated that both CD4+ and CD8+ T lymphocytes from saliva expressed PD-1. It has been reported that

the majority of infiltrating cells in SS salivary glands are CD4+ CD45RO+ memory T cells<sup>27</sup>. These CD4+ T cells, which may be activated by self-antigens presented with class II MHC molecules on salivary epithelial cells, thus generate cytokines and cell-cell interactions that activate cytotoxic CD8+ T cells and B cells. We previously reported that PD-1+ T cells of synovial fluid from patients with RA produced IL-10, but PD-1- T cells did not<sup>16</sup>. In this study, we showed that PD-1+ T cells in saliva from SS patients were capable of producing IL-10, while PD-1- T cells were not. Thus, we have redefined the IL-10-producing lymphocytes in SS salivary glands, which had been described as CD4+ T cells<sup>28</sup>, as PD-1+ T cells. These observations suggest that PD-1+ T cells should be recognized as immunoregulatory T cells, rather than simply activated ones. It may be paradoxical that sialoadenitis persists in the presence of immunoregulatory cells like IL-10-producing PD-1+ T cells and PD-L1+ epithelial cells. One possible explanation may be that a strong immune stimulation such as viral reactivation may override the effect of negative regulators including



**Figure 3.** PD-L1 expression on salivary gland tissue from a patient with SS. (A) Indirect immunofluorescence staining shows PD-L1 expression on most acinar epithelial cells, but not on infiltrating lymphocytes from SS patients. Normal salivary gland did not express PD-L1. (B) Cryostat sections were incubated with serum containing anti-PD-L1 Mab and analyzed with the avidin-biotin technique. A representative sample shows that PD-L1 was highly expressed on ductal epithelial cells (arrow). HE: hematoxylin and eosin, CONT: control.

**Figure 4.** Relationship between PD-L1 expression and lymphocyte infiltration. PD-L1 expression was found in 68% (23 of 34) of SS patients and strongly correlated with their focus scores. Statistical analysis showed that PD-L1 expression was higher in patients with a higher score (\* $p < 0.01$ ). The score for lymphocyte infiltration (focus score) of salivary glands was established according to criteria as described<sup>18</sup> and modified<sup>19</sup>.



**Figure 5.** PD-L1, CD80, and CD86 expression on HSG cells after stimulation with IFN- $\gamma$ , TNF- $\alpha$ , or IL-1 $\beta$ . HSG cells were incubated with IFN- $\gamma$ , TNF- $\alpha$ , or IL-1 $\beta$ , and then cultured with serum containing anti-PD-L1, anti-CD80, or anti-CD86 Mab and analyzed by means of confocal microscopy. HSG cells expressed CD80 and CD86 after TNF- $\alpha$  or IL-1 $\beta$  stimulation, while they expressed PD-L1 after IFN- $\gamma$  stimulation. CONT: control.

the PD-1/PD-L system or IL-10, which then results in perpetuation of the inflammatory process. In accord with this idea, there are reports that IL-10 concentrations were high in saliva and sera from SS patients, and concentrations in saliva and sera were positively correlated with the degree of sicca symptom and lymphocyte infiltration, respectively<sup>29,30</sup>. These reports indicate that high IL-10 production may not be sufficient in maintaining tolerance in patients with SS.

PD-L1 is constitutively expressed by dendritic cells and is also strongly upregulated by IFN- $\gamma$  stimulation<sup>31</sup>. Our results showed that PD-L1 was expressed in salivary glands from patients with SS, and that HSG expressed PD-L1 upon stimulation with IFN- $\gamma$  but not with TNF- $\alpha$  or IL-1 $\beta$ . In HSG cells, the induction pattern of PD-L1 was different from those of CD80/CD86, which are ligands for CTLA-4 and are more effectively upregulated by TNF- $\alpha$  or IL-1 $\beta$ . The class of CD28 family molecules such as PD-1, CTLA-4, and B/T cell lymphocyte attenuator (BTLA) are regarded as major immunoinhibitory receptors<sup>32</sup>. IFN- $\gamma$  is reported to be abundantly expressed in CD4+ T cells from SS salivary glands<sup>33</sup>. The specific induction of PD-L1 by IFN- $\gamma$  implies that PD-L1 expression may be directly induced by infiltrating T cells. We have shown that PD-1+ CD4+ T cells from synovial fluid from patients with RA coexpressed CTLA-4<sup>16</sup>. Immunological tolerance is induced when T cell receptor signaling is switched on in the absence of CD28/B7 mediated costimulatory signals, while another mechanism for inducing peripheral tolerance regardless of CD80/CD86 expression includes the PD-1/PD-L system<sup>34-37</sup>. Thus, further investigations to clarify the different functions between PD-1/PD-L and CTLA-4/B7 may elucidate the failure of peripheral tolerance in Sjögren's syndrome.

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