Glandular and extraglandular expression of costimulatory molecules in patients with Sjögren's syndrome

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Abstract

Objectives—To investigate the expression and regulation of CD80, CD86, and CD28 costimulatory molecules in sialoadenitis and interstitial nephritis in patients with Sjögren's syndrome (SS).

Methods—Expression of CD80, CD86, and CD28 molecules was studied by immunohistochemical staining of lip biopsy specimens obtained from patients who had sialoadenitis associated with SS, and renal biopsy specimens obtained from patients who had interstitial nephritis associated with SS. To elucidate the mechanism of de novo expression of CD80 and CD86 antigens, their induction by cytokines in human salivary duct cell line (HSG) and renal cortical epithelial cells (HRCE) by cell enzyme linked immunosorbent assay (ELISA) was quantitatively investigated.

Results—In patients with severe sialoadenitis, CD80 and CD86 were strongly expressed on ductal epithelial cells. In contrast, these antigens were not found in the minor salivary glands of normal subjects or of patients with mild sialoadenitis. Some infiltrating cells expressed CD28. In patients who had interstitial nephritis associated with SS, some tubular epithelial cells expressed CD86 but not the CD80 antigen. Unstimulated HSG cells did not express CD80 or CD86. Interferon γ (IFN γ) consistently up regulated levels of CD80 and CD86. In contrast, tumour necrosis factor α (TNF α), interleukin 1 β (IL1 β), IL2, and IL4 had no effect on either CD80 or CD86 levels. Unstimulated HRCE did not express CD80 or CD86. IFN γ consistently up regulated CD86 expression. No CD80 expression was found on tubular cells. TNF α , IL1 β , IL2, and IL4 had no discernible effects.

Conclusions—Salivary ductal cells in patients with SS can express CD80 and CD86 costimulatory molecules in response to IFN γ . Tubular epithelial cells in patients who have interstitial nephritis associated with SS express only CD86 molecules. In patients with SS, salivary ductal cells and tubular epithelial cells may activate infiltrating CD28 positive T lymphocytes by presenting antigens to T cells, potentially leading to tissue destruction.

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Sjögren's syndrome (SS) is a chronic autoimmune disorder characterised by dysfunction and destruction of the salivary, lachrymal, and other exocrine glands. Histologically, marked infiltration of lymphoid cells is commonly found in the lachrymal and salivary glands. Most infiltrating lymphocytes are activated CD4+ T cells¹ and produce cytokines such as interleukin 2 (IL2), interferon γ (IFN γ), tumour necrosis factor α (TNF α), and IL1 β .^{2-3 41} Recently, several studies have shown that glandular epithelial cells, especially ductal cells in SS lesions, are activated and may play a part in induction and progression of the inflammatory process.⁴ Class II type major histocompatibility antigen (MHC) HLA-DR protein and intercellular adhesion molecules (ICAM-1) are also expressed on ductal epithelial cells in SS.⁵ We previously reported that ductal epithelial cells show aberrant Fas expression and apoptosis induced by Fas ligand of infiltrated cells.^{6 7}

In some patients with SS, renal disease, especially interstitial nephritis, develops, possibly as a consequence of immunological renal injury.⁸ Histologically interstitial nephritis is characterised by interstitial mononuclear cell infiltration and interstitial oedema with fibrosis. These histological changes are similar to those in the salivary glands.⁹

In interstitial nephritis associated with SS, most infiltrating lymphocytes are activated CD4+ T cells.¹⁰ Recently, several studies have reported that tubular epithelial cells in interstitial nephritis, both associated¹¹ and not associated¹² with SS, express HLA-DR and ICAM-1 and may have a role in induction and progression of the inflammatory process.

The differentiation of activated T cells requires not only an antigen-specific signal mediated by the T cell receptor but also a costimulatory signal.¹³⁻¹⁵ A minor T cell costimulatory pathway involves surface interactions between the ICAM-1 on antigen-presenting cells (APC) and lymphocyte function associated antigen-1 molecules on T cells. CD28 on T cells is thought to play an important part in the transmission of costimulatory signals. CD80 (B7.1) and CD86 (B7.2) molecules have been identified as CD28 ligands. Blockade of the B7-CD28 pathway in vitro results in T cell clonal anergy or in immunosuppression.¹⁶

CD80 and CD86 molecules have been reported to be expressed by specialised APC, including monocytes, macrophages, dendritic cells, and activated B cells. Recently, however,

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some non-specialised APC, such as keratinocytes,¹⁷ myocytes,¹⁸ vascular endothelial cells,¹⁹ synovial cells,^{20, 21} and peripheral T cells²² were also reported to express CD80 or CD86 molecules or both. Such expression of costimulatory molecules may be sufficient to induce primary T cell responses.

In SS, Manoussakis *et al* studied costimulatory molecules in lip biopsy specimens and in long term cultures of salivary glands obtained from patients with SS.³⁰ CD80 was strongly expressed on the surface of salivary gland epithelial cells in most patients. This aberrant expression of costimulatory molecules on salivary gland epithelial cells in SS may signify a central role of these cells in the immunopathogenesis of this disease. However, extraglandular expression of costimulatory molecules in SS remains unknown. The correlation between the histological grade of sialoadenitis and the grade of epithelial expression of costimulatory molecules was unclear in the study by Manoussakis *et al*.

Although aberrant costimulatory molecule expression has been reported, the mechanism inducing such expression remains unknown. Using immunoperoxidase cell enzyme linked immunosorbent assay (ELISA),^{23, 24} we quantitatively examined the expression of Fas antigen in salivary ductal cells stimulated by cytokines. A human salivary gland duct cell line (HSG) was established from an irradiated human submandibular salivary gland.²⁵ It has characteristics of intercalated duct cells. HSG cells can mimic several features of SS disease in vivo.²⁶ Cytokines such as IFN γ and TNF α induce increased expression of ICAM-1 and HLA-DR in the HSG cell line. In addition, renal cortical epithelial cells (HRCE) were derived from normal human kidney and showed enzymatic properties of distal renal tubules.²⁷ We used immunoperoxidase cell ELISA to investigate the CD80 and CD86 expression on HSG and HRCE stimulated by cytokines.

We sought to determine the cellular expression and distribution of CD80 and CD86 molecules in biopsy specimens of the salivary glands and kidney and investigated induction mechanisms for the ductal/tubular expression of these molecules.

Patients and methods

PATIENTS

The minor salivary glands of 20 patients (18 women, two men, aged 25–75 years, mean 49.4) with SS were studied. All patients had sicca symptoms with at least one of the following abnormalities: (a) keratoconjunctivitis sicca (positive results of Schirmer's test and rose bengal test), (b) lymphocytic infiltration of a specimen of the salivary gland obtained by lip biopsy, and (c) characteristic findings indicative of duct-acinar destruction on sialography. Secondary SS was diagnosed in two patients fulfilling the 1984 revised criteria for classification of systemic lupus erythematosus, two fulfilling the American Rheumatism Association criteria for rheumatoid arthritis, one fulfilling preliminary criteria for systemic sclerosis, and one fulfilling preliminary criteria for mixed

connective tissue disease.²⁸ The 14 other patients were given a diagnosis of primary SS. As control, normal minor salivary glands were obtained for diagnostic examination from four patients with fever of unknown origin; there were no abnormal changes in the lip biopsy specimens.

Renal biopsy specimens obtained from six patients (four women, two men, aged 44–66 years, mean 55.5) who had interstitial nephritis associated with SS were also studied. All patients had sicca symptoms, with at least one of the abnormalities described above. One patient fulfilling the criteria for systemic lupus erythematosus and one with rheumatoid arthritis were considered to have secondary SS. The other four patients were diagnosed as primary SS. At the time of the biopsies, no patients were undergoing any immunomodulative treatment with anti-inflammatory drugs and corticosteroids, except one patient who received 5 mg prednisolone daily. As control, normal specimens of renal tissue obtained from the non-affected portions of surgically removed kidneys in four patients with renal cell carcinoma were examined. The control specimens showed minimal changes on light microscopic and immunofluorescence studies—namely, mild shrinkage of the glomerular tuft probably owing to the ligation of the renal artery.

IMMUNOPEROXIDASE STAINING

Samples of minor salivary glands were obtained by lip biopsy. Tissue sections were prepared for immunoperoxidase staining as follows: 6 μ m thick cryostat sections were dried, fixed in acetone for 15 minutes at 4°C, and rinsed with phosphate buffered saline (PBS). After treatment with normal goat serum, the sections were successively incubated in the following solutions washed with PBS: monoclonal antibody to CD80 (Immunotech, Marseille, France), CD86 (Ancell, MN, USA), or CD28 (Dako Japan, Kyoto, Japan); Envision solution (peroxidase binding dextran polymer labelled with goat antimouse immunoglobulins; Dako Japan, Kyoto, Japan); 0.02% 3,3'-diaminobenzidine containing 0.03% H₂O₂ and 10 mM sodium azide. The sections were subsequently stained with methyl green.

A surgically removed lymph node showing non-specific chronic lymphadenitis was used as a positive control. The following negative controls were used: (a) omission of primary antibody or (b) appropriately diluted mouse IgG as a first layer. In all negative controls, only a few granulocytes showed endogenous peroxidase activity.

HISTOLOGICAL GRADING

To assess inflammation in the salivary gland biopsy specimens, the number of focal aggregates including at least 50 mononuclear cells per gland was counted. We graded the ductal CD80 or CD86 expression according to the following criteria: no CD80 or CD86 expression, grade 0; CD80 or CD86 expression by a few ductal cells, grade 1; and CD80 or CD86

Figure 1 Immunohistochemical staining of CD80 and CD86 in the salivary gland. (A) CD80 was not seen in normal controls ($\times 180$); (B) CD86 was not seen in normal controls ($\times 180$); (C) CD80 on ductal epithelial cells (arrow) and some infiltrating mononuclear cells in severe sialoadenitis ($\times 180$); (D) CD86 was expressed on ductal epithelial cells (arrow) and some infiltrating mononuclear cells in severe sialoadenitis ($\times 360$); (E) CD28 was seen on many infiltrating mononuclear cells in severe sialoadenitis around the duct expressing CD80; (F) serial section of (E) ($\times 180$).

expression by all cells of one duct or by many ductal cells, grade 2.

TISSUE CULTURE

HSG cells (a gift from Dr Mitsunobu Sato, Tokushima University, Japan) were cultured in HAM F-12 Medium (Life Technologies, NY, USA) supplemented with 10% fetal calf serum (Biowhittaker MD, USA) at 37°C with 5% CO_2 .

HRCE cells purchased from Clonetics (San Diego, Ca, USA) were cultured in a renal epithelial cell growth medium bullet kit (Clonetics) at 37°C with 5% CO_2 .

TREATMENT OF CELLS WITH CYTOKINES

Recombinant human $\text{IFN}\gamma$ (R&D Systems, MN, USA), recombinant human $\text{TNF}\alpha$ (Pharmingen, CA, USA), recombinant human $\text{IL1}\beta$ (R&D Systems), human IL2 (Roche Diagnostics GmbH Mannheim, Germany), and recombinant human IL4 (Genzyme, MA, USA) were used. Cells were cultured in the

presence of $\text{IFN}\gamma$, 1–100 U/ml; $\text{TNF}\alpha$, 1–100 U/ml; $\text{IL1}\beta$, 0.001–0.1 U/ml; IL2 , 0.02–2 U/ml; or IL4 , 3–30 U/ml for 24 hours.

To examine the time course of CD80 or CD86 expression on HSG or HRCE, the cells were cultured in the presence of $\text{IFN}\gamma$ (100 U/ml) for 4–72 hours.

CELL ELISA

A total of 8×10^3 cells/well were seeded onto gelatin coated, 96 well, flat bottomed microtitre plates (Falcon 3072, Becton Dickinson, NJ, USA) in 0.2 ml of medium. After reaching confluence (~ 48 hours), the culture medium was replaced with 0.2 ml of fresh medium containing various concentrations of cytokines and incubated for various periods of time at 37°C in 5% CO_2 . The cultures were fixed with 3% paraformaldehyde in PBS containing 8% sucrose for 30 minutes at room temperature. Subsequent washing steps were performed with five changes of PBS with 0.05% Tween 20 (Wako Osaka, Japan). To avoid non-specific

binding of antibodies, the assays were performed after pretreatment with PBS and 50% Blockace (Yukizirushi, Sapporo, Japan). After washing, the fixed monolayer was incubated with 0.1 ml/well of 5% normal goat serum PBS for one hour. The blocking solution was added with 0.4 mg of mouse monoclonal antibody to CD80 (Immunotech) or with 2 mg of mouse monoclonal antibody to CD86 (Ancell) for 120 minutes at 37°C. After washing, the plates were treated with 0.1 ml/well of Envision solution (peroxidase binding dextran polymer labelled with goat antimouse immunoglobulins, Dako Japan) for 75 minutes at room temperature. The plates were washed again and incubated with 0.1 ml of 3,3',5,5'-tetramethyl benzidine (Dako Japan) for 10–30 minutes at room temperature. The plates were read on a microtitre plate reader (SLT Labinstruments, Salzburg, Austria) at 650 nm. For the dose dependent, time course and blocking experiments, the data are reported as the optical density (OD) achieved in a given experiment (mean (SD) of triplicate wells) after subtracting the background OD of wells for the negative control. As positive control for the cell ELISA system, epithelial membrane antibody (Dako Japan) was used. The following negative controls were used: (a) omission of primary

antibody or (b) appropriately diluted mouse IgG as a first layer.

STATISTICAL ANALYSIS

Cell ELISA data were analysed with Student's *t* test.

IMMUNOPEROXIDASE STAINING OF CULTURED HSG AND HRCE ON SLIDE GLASS

A total of 1.6×10^4 cells/well were seeded onto Lab-Tek II chamber slides (Nunc, IL, USA) in 1 ml of medium. After reaching confluence (~48 hours), the culture medium was replaced with 1 ml of fresh medium with and without IFN γ and incubated for 48 hours (HSG cells) or six hours (HRCE cells) at 37°C in 5% CO $_2$. To prepare for immunoperoxidase staining, the tissue sections were fixed with 3% paraformaldehyde in PBS containing 8% sucrose for 30 minutes at room temperature, and rinsed with PBS. After treatment with normal goat serum, the sections were successively incubated with the following solutions, and washed with PBS: 0.8 mg of monoclonal antibody to CD80 (Immunotech) or 2 mg of monoclonal antibody to CD86 (Ancell), Envision solution (peroxidase binding dextran polymer labelled with goat antimouse immunoglobulins, Dako Japan), 0.02% 3,3'-diaminobenzidine containing 0.03% H $_2$ O $_2$ and 10 mM sodium azide. The sections were subsequently stained with methyl green.

Results

CD80 AND CD86 EXPRESSION ON SALIVARY GLANDS IN PATIENTS WITH SS

In normal salivary glands, CD80 and CD86 were not expressed on either acinar cells or ductal cells (figs 1A and B). No cells expressing CD28 were found in normal salivary glands. In patients who had SS with mild mononuclear cell infiltration and moderate interstitial fibrosis, CD80 was not expressed on minor salivary glands. In contrast, in patients with massive mononuclear cell infiltration, CD80 was expressed on ductal epithelial cells and a few acinar epithelial cells in the lip biopsy specimens (fig 1C). CD80 was seen on some infiltrating mononuclear cells of the minor salivary glands in all patients with SS. Ductal CD86 expression was also observed in patients who had massive mononuclear cell infiltration (fig 1D). In patients with mild mononuclear cell infiltration, CD86 was not expressed on minor salivary glands. CD86 positive cells were seen on some mononuclear cells infiltrating the minor salivary glands. Several CD28 positive cells (probably T cells) infiltrated (fig 1E) around the CD80 (fig 1F) or CD86 positive ducts of the minor salivary glands. Ductal CD80 or CD86 expression was positively correlated with the grade of inflammation of the salivary glands (figs 2A and B). In severe lymphocytic infiltration, CD80 and CD86 expression was seen in many ductal cells. There was no difference in ductal CD80 and CD86 expression between primary and secondary SS.

Figure 2 Ductal CD80 (A) and CD86 (B) expression and salivary gland lymphocytic infiltration. Ductal CD80 and CD86 expression was shown by the grading scale. CD80 and CD86 expression of ductal cells correlated with interstitial infiltration. $p < 0.01$ in Spearman rank correlation.

Figure 3 Immunostaining of CD80 and CD86 in the kidney. (A) CD80 was not seen on tubular epithelial cells in control tissue ($\times 180$); (B) CD86 was not seen on tubular epithelial cells in control tissue ($\times 180$); (C) CD86 expression on tubular epithelial cells (basal side, arrows) in interstitial nephritis associated with Sjögren's syndrome (SS) ($\times 360$); (D) the several tubular cells showed CD86 antigen in SS kidney (arrow) ($\times 180$); (E) CD80 was not seen on tubular epithelial cells in interstitial nephritis associated with SS ($\times 180$); (F) CD28 expressed on some infiltrating cells (the same portion of the serial section of (C) and (E) ($\times 180$)).

CD80 AND CD86 EXPRESSION IN INTERSTITIAL NEPHRITIS ASSOCIATED WITH SS

In the normal kidney, CD80 and CD86 were not expressed on tubular epithelial cells in normal control (figs 3A and B). No cells expressing CD28 were seen in normal renal interstitium. In five patients who had interstitial nephritis associated with SS, CD86 was expressed on tubular epithelial cells near the foci of mononuclear cell infiltration in renal biopsy specimens (figs 3C and D). Otherwise, few CD80 positive tubular cells were seen (fig 3E). CD80 or CD86 was seen on some infiltrating mononuclear cells in the renal interstitium of all patients with SS. Several CD28 positive cells were infiltrating (fig 3F) around the tubules in all cases. Figures 3C, E, and F show serial sections of the same case of interstitial nephritis. There was no distinct correlation between tubular CD86 expression and interstitial inflammation, probably because of the small number of cases examined.

EXPRESSION OF CD80 AND CD86 MOLECULES IN HSG CELL LINE

On immunohistochemical staining, CD80 and CD86 were not expressed on unstimulated HSG cells but were expressed on many HSG cells stimulated by 100 U/ml of IFN γ (figs 4A, B, C, and D).

Using an immunoperoxidase cell ELISA, we found that unstimulated HSG cells did not express CD80 or CD86 molecules. IFN γ (1–100 U/ml) consistently up regulated constitutively expressed levels of CD80 and CD86 in a dose dependent fashion (figs 5A and B). Figures 5C and D show the time courses of CD80 and CD86 expression induced by IFN γ . A significant increase in CD80 or CD86 expression was seen 48 hours after the addition of IFN γ (100 U/ml).

To examine the specificity of IFN γ , we tested the ability of antibody to IFN γ to block the expression of CD80 and CD86. Pretreatment of IFN γ with anti-IFN γ for 30 minutes before

Figure 4 Immunohistochemical staining of anti-CD80 or CD86 antibody on cultured human salivary gland duct (HSG) cell line ($\times 180$). Without cytokine stimulation, CD80 (A) or CD86 (B) expression was not seen on HSG. Many HSG cells stimulated by 100 U/ml of interferon γ for 24 hours showed CD80 (C) or CD86 (D) expression.

stimulation resulted in a dose dependent elimination of the up regulation of CD80 and CD86 expression (figs 5E and F).

Six to 48 hours' incubation with IL1 β , IL2, or IL4 did not influence the expression of CD80 and CD86 on HSG cells (data not shown).

EXPRESSION OF CD80 AND CD86 MOLECULES ON HRCE CELLS

On immunohistochemical staining, CD80 and CD86 were not expressed on unstimulated HRCE cells. CD86 expression was seen on many HRCE cells stimulated by 100 U/ml of IFN γ (figs 6A, B, C, and D).

Using an immunoperoxidase cell ELISA, we found that unstimulated renal cortical epithelial cells did not express CD80 or CD86 molecules. IFN γ (1–100 U/ml) consistently up regulated constitutively expressed levels of CD86 in a dose dependent fashion (fig 7A). However, the levels of CD80 did not change in response to IFN γ (1–100 U/ml) stimulation (fig 7B). Figure 7C shows the time course of CD86 expression induced by IFN γ . A significant increase in CD86 expression was seen 6–12 hours after the addition of IFN γ .

To examine the specificity of IFN γ , we tested the ability of antibody to IFN γ to block the expression of CD86. Pretreatment of IFN γ with antibody to IFN γ for 30 minutes before stimulation with IFN γ resulted in elimination of the up regulation of CD86 expression (fig 7D).

Six to 48 hours' incubation with IL1 β , IL2, or IL4 did not influence the expression of CD80 or CD86 on HRCE cells (data not shown).

Discussion

The role of costimulatory molecules in autoimmune diseases has received considerable attention. The expression of CD80 or CD86 molecules by APC infiltrating the synovia of patients with rheumatoid arthritis²¹ and skin lesions of patients with systemic lupus erythematosus¹⁷ has been recently described. The aberrant expression of CD80 or CD86 molecules by epithelial cells has been implicated in the pathogenesis of autoimmune disorders. Thyroid epithelial cells from patients with Graves's thyroiditis have been found to express CD80.²⁹

For the first time, Manoussakis *et al* reported that CD80 was strongly expressed on the surface of salivary gland epithelial cells in most patients with SS and on long term cultures of salivary glands obtained from patients with SS.^{30–31} However, extraglandular expression of costimulatory molecules in SS remains unknown. The correlation between the histological grade of sialoadenitis and the grade of epithelial expression of costimulatory molecules was unclear in this study. We therefore examined the expression of CD80 and CD86 molecules in sialoadenitis and interstitial nephritis, conditions affecting glandular and extraglandular organs respectively. We also studied the correlation of ductal CD80 and CD86 expression with the grade of sialoadenitis.

The induction and regulation of CD80 and CD86 expression on salivary ductal cells and tubular epithelial cells are thought to be the keys to in situ T cell activation associated with organ involvement in SS. Quantitative measurements of CD80 and CD86 expression on

Figure 5 Effect of interferon γ (IFN γ) on human salivary gland duct (HSG) CD80 or CD86 expression as measured by immunoperoxidase cell ELISA. Cells were cultured and stimulated with IFN γ . (A) CD80 expression after 24 hours' exposure to increasing concentrations of IFN γ ; (B) CD86 expression after 24 hours' exposure to increasing concentrations of IFN γ ; (C) time course of induction of CD80 by IFN γ (100 U/ml); (D) time course of induction of CD86 by IFN γ (100 U/ml); (E, F) effect of blockade of CD80 (E) and CD86 (F) expression by anti-IFN γ . HSG cells were cultured and stimulated with 100 U/ml of IFN γ and with 0.01, 0.1, and 1 μ g/ml of monoclonal anti-IFN γ antibody (R&D). Addition of 1 μ g/ml of anti-IFN γ inhibited the up regulation of IFN γ . All values are expressed as means (SD) of triplicate wells. * p <0.05, ** p <0.01 indicate significant difference from the control (untreated) OD. Absence of an error bar indicates that the error was less than the symbol size.

these epithelial cells are therefore necessary. A variety of methods, including fluorescence activated cell sorter (FACS) and radioimmunoassay, have been used to measure cell surface antigen expression. Cell ELISA was compared with immunofluorescence staining by Grunow *et al*,²³ and both assays were found to be similarly sensitive. CD4 positive and CD4 negative cell lines were mixed at different cell ratios. Anti-CD4 curves in the cell ELISA and mean immunofluorescence intensities both

reached cut off values at a cell ratio of 12.5%. Those results underline the advantage of cell ELISA for antigen detection. In contrast to FACS and radioimmunoassay, our approach avoids mechanical disruption of cell monolayer, trypsinisation, and the use of radioactivity.

In severe sialoadenitis in patients with SS, ductal epithelial cells expressed aberrant CD80 and CD86 molecules. Ductal expression was correlated with interstitial cellular infiltration. Human salivary ductal cells stimulated by

Figure 6 Immunohistochemical staining of anti-CD80 or CD86 antibody on cultured renal cortical epithelial cells (HRCE) cells ($\times 180$). Without cytokine stimulation, CD80 (A) expression was not seen on HRCE and a few CD86 positive cells were seen (C). CD80 expression was not induced by interferon γ ($\text{IFN}\gamma$) (B). Many HRCE cells stimulated by 100 U/ml $\text{IFN}\gamma$ for 24 hours showed only CD86 (D) expression.

$\text{IFN}\gamma$ showed CD80 and CD86. Therefore, $\text{IFN}\gamma$ secreted by infiltrating lymphocytes in salivary tissue can induce CD80 and CD86 expression by ductal cells. This aberrant expression of CD80 and CD86 may activate T cells around the ducts. Activated T cells reacting with antigen on ductal cells might destroy the salivary tissues by Fas-Fas ligand system or perforin mechanism.⁷ Some activated T cells may produce cytokines such as $\text{IFN}\gamma$. Fox *et al* reported that $\text{TNF}\alpha$, $\text{IL1}\beta$, IL2 , IL4 , IL6 , and IL10 were secreted in salivary glands in addition to $\text{IFN}\gamma$.² In contrast, $\text{IL1}\beta$, IL2 , IL4 , and granulocyte macrophage colony stimulating factor did not influence the levels of CD80 and CD86 in salivary ductal cells.

In interstitial nephritis associated with SS, tubular epithelial cells expressed only CD86. Human tubular epithelial cells stimulated by $\text{IFN}\gamma$ showed only CD86. The expression of costimulatory molecules on tubular epithelial cells has been controversial. Hagerty reported that tubular cells of MRL-lpr mouse did not express CD80 or CD86 antigens on fluorescence cytometry.³² CD80 and CD86 were not found on human tubular cells by Schulz *et al*.³³ On the other hand, Banu and Meyers reported that $\text{IFN}\gamma$ and lipopolysaccharide differentially modulate CD80 expression on murine renal tubular epithelial cells.³⁴ These disparate results may arise from differences in mouse strains and in monoclonal antibodies to costimulatory molecules. There were also some methodological differences. Hagerty and Schulz *et al* used trypsin to prepare single cell suspensions of tubular cells, whereas Banu and Meyers used cold EDTA. We therefore used cell ELISA for assay of CD80 and CD86

expression to avoid mechanical disruption of cell monolayer and trypsinisation.

Aberrant HLA-DR and ICAM-1 molecules are expressed on tubular epithelial cells in interstitial nephritis associated with SS.¹¹ In patients with SS who had interstitial nephritis, tubular epithelial cells expressed aberrant CD86 molecules. Human tubular cells stimulated by $\text{IFN}\gamma$ showed CD86. Therefore, $\text{IFN}\gamma$ secreted by lymphocytes infiltrating renal tissue can induce CD86 expression by tubular cells. This aberrant expression of CD86 may activate T cells around the tubules.

Available evidence thus indicates that the expression of CD80 or CD86 molecules by SS epithelial cells involves intracellular activation processes. Specific expression of CD80 or CD86 by the salivary and tubular epithelial cells of patients with SS provides evidence that these cells function as APC in the development of the lymphocytic infiltration that characterises the disease.

Functional differences between CD80 and CD86 have received attention. Kuchroo *et al* reported that costimulation of CD80 differentially activates the Th1 development pathway.³⁵ Freeman *et al* suggested that CD86 but not CD80 preferentially costimulates the initial production of IL4 .³⁶ However, CD80 and CD86 were reported to stimulate similar patterns of cytokine secretion by Levine *et al*.³⁷ Azuma *et al* found that CD86 costimulation was more important than CD80 costimulation and suggested that monocytes that constitutively express CD86 but not CD80 act as the predominant APC.³⁸ On costimulation with

Figure 7 Effect of interferon γ (IFN γ) in CD80 or CD86 expression on renal cortical epithelial (HRCE) cells as measured by immunoperoxidase cell ELISA. Cells were cultured and stimulated with IFN γ . (A) CD86 expression after 12 hours' exposure to increasing concentrations of IFN γ ; (B) CD80 expression was not induced by 12 hours' exposure to IFN γ ; (C) Time course of induction of CD86 by IFN γ (100 U/ml); (D) effect of blockade of CD86 expression by anti-IFN γ (a-IFN). HRCE cells were cultured and stimulated with 100 U/ml of IFN γ and 1 μ g/ml polyclonal anti-IFN γ (Genzyme). Addition of 1 μ g/ml of anti-IFN γ inhibited the up regulation of IFN γ . All values are expressed as means (SD) of triplicate wells. * $p < 0.05$, ** $p < 0.01$ indicate a significant difference from control (untreated) OD. No error bar indicates that the error was less than the symbol size.

non-specialised APC, vascular endothelial cells principally expressed CD86 in the culture medium, and anti-CD86 monoclonal antibody induced no response in T cells stimulated by endothelial cells.¹⁹ However, the functional differences between CD80 and CD86 in SS remain unknown.

Murata *et al* reported that the repertoire of the T cell receptor V β gene on T cells infiltrating the kidneys of patients with SS who have interstitial nephritis is more restricted than that of peripheral blood lymphocytes.³⁹ The same V β 2 clones as those found in the kidney were not detected in the labial salivary glands of the same patients with SS. They concluded that T cells infiltrating the kidneys of patients who have SS with interstitial nephritis might recognise different autoantigens⁴⁰ from those infiltrating the labial salivary glands. In our study, tubular epithelial cells showed only CD86 protein, whereas salivary ductal cells expressed both CD80 and CD86. Interstitial nephritis is a major extraglandular disease that does not develop in most patients with SS. In patients with renal tubules that express CD86 molecules in response to activation by IFN γ , tubular epithelium might activate T cells through

interactions between MHC + tubular antigen (?) and T cell receptor, associated with CD86 and CD28 binding.

In conclusion, salivary ductal cells and tubular epithelial cells in patients with SS can express CD80, CD86, or both. Such epithelial cells may be suitably equipped for the presentation of antigens to CD28-expressing T lymphocytes as well as MHC molecule expression after activation. Whether CD80 and CD86 molecules expressed by salivary ductal and tubular epithelium can provide optimal costimulation remains to be established. Further functional studies of costimulatory molecules in SS may lead to the identification of mechanisms responsible for organ involvement and to the development of new treatment for SS.

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