THE IMMUNOLOGY OF SJOGREN'S SYNDROME: EMPHASIS ON THE ROLE OF SALIVARY GLAND AUTOANTIGEN

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SUMMARY
• Primary Sjogren’s syndrome (SS) in humans is an autoimmune disease characterized by diffuse lymphoid cell infiltrates in the salivary and lacrimal glands, resulting in symptoms of dry mouth and dry eye due to insufficient secretion. The spectrum of presentation of the disease is broad, ranging from the organ-localized dysfunction of exocrine gland to systemic complications such as liver, kidney and lung involvement. A significant proportion of the SS patients may develop malignant lymphoproliferative disorders such as B cell lymphoma and macroglobulinemia. Although it has been assumed that a combination of immunologic, genetic, and environmental factors may play a key role on the development of autoimmune lesion in the salivary and lacrimal gland, little is known about the pathogenesis of primary SS. The hypofunction of the salivary glands is associated with lymphocytic infiltration, in which autoreactive T cells recognize unknown self-antigen and play a central role in the pathobiology of SS. This disease is also characterized by systemic production of auto antibodies to ribonucleoprotein particles SS-A/Ro and SS-B/La, however, the specificity for this immunogenicity remains to be defined. The 120 kD α-fodrin is an important salivary gland autoantigen implicated in the development of SS in both animal model and SS patients. This article will review recent observations of the immunological aspects of autoimmune sialadenitis as it occurs in SS patients, and in murine model for SS, and particularly emphasize on the role of salivary gland autoantigen during development of autoimmune lesions in SS. (Biomed Rev 1998; 9:131-141)

INTRODUCTION
• Sjogren’s syndrome (SS) is a chronic autoimmune disorder affecting the salivary and lacrimal glands and leading to clinical symptoms of dryness of the mouth and eyes (sicca syndrome) (1-3). The syndrome is clinically subdivided into primary SS and secondary SS. In primary SS patients, a wide spectrum of extraglandular manifestations may occur, including vasculitis, thyroiditis, nephritis, pneumonitis, neuropathy, and lymphoproliferation, besides sicca syndrome (4-6). In secondary SS, the sicca symptoms are associated with an additional well-defined systemic autoimmune disorder, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS), or primary biliary cirrhosis (PBC). The histopathological changes in the minor salivary gland biopsy are characterized by focal and/or diffuse lymphoid cell infiltrates and parenchymal destruction. The majority of lymphoid cells in the salivary biopsy are CD4+ T cells with a small proportion of CD8+ T cells (7). These T cells express the antibody antigen receptor and cell surface antigens associated with mature memory T cells. Recent studies suggest a preferential use of specific variable region segments of the antigen receptor (3 chain by salivary gland T cells (8). It can be suggested that an unknown organ-specific autoantigen targeted by
Autoimmune T cells may be present in the salivary glands. It was previously established and characterized an animal model for primary SS in NFS/sld mutant mice thymectomized 3 day after birth (3d-Tx), bearing autosomal recessive gene with sublingual gland differentiation arrest (9,10). When the repertoire of T cell receptor (TCR) Vb genes transcribed and expressed within the inflammatory infiltrates was analysed in this animal model, a preferential utilization of TCR Vb gene was detected in these lesions from the onset of disease. Moreover, the antisali-vari duct autoantibodies were detected in sera from mice with autoimmune lesions. Recently, we succeeded in purifying a 120 kD organ-specific autoantigen from the salivary gland tissues of this animal model. The sequence of the first 20 N-terminal residues was found to be identical to that of the human cyto-
skeletal protein α-fodrin(11). Furthermore, sera from patients with SS reacted positively with purified 120 kD antigen, and proliferative response of peripheral blood mononuclear cells from SS patients to the purified autoantigen was detected, but not from SLE or RA patients, and healthy controls. These results indicate that the anti-120 kD oc-fodrin immune response plays a critical role in the development of primary SS.

**IMMUNOREGULATION IN THE PATIENTS WITH SJOGREN'S SYNDROME**

- Although primary SS is characterized by autoimmune destruction of the salivary and lacrimal glands, a wide variety of extraglandular manifestation may occur. The spectrum of clinical manifestation as autoimmune disorder found in SLE patients may also occur in SS patients (12). The tendency toward lymphoproliferation appears greater in SS than SLE, and the incidence of interstitial nephritis is higher in SS than SLE. Dryness of the skin has been attributed to a decrease in the secretory capacity of the sebaceous glands, and the dryness of the skin and upper airways leads to particular clinical symptoms in the S S patients. Difficulty in swallowing is a frequent occurrence in primary SS patients due to decreased saliva production. Ab normal esophageal motility may also contribute to dysphagia in some primary SS patients. Clinical evidence of liver dysfunction is found to be 5% to 10% of primary SS patients (12). In a small proportion of primary SS patients, the presence of antimitochondrial antibodies indicates coexistence with PBC. PBC is uncommon for primary SS patients, the presence of anti-B antigen, and thus the PBC-associated sicca syndrome probably represents a form of secondary SS. Primary SS patients may exhibit central and peripheral nervous system manifestations. It was reported that central nervous system abnormalities may occur in approximately 20% of primary SS patients. Peripheral neuropathies among primary SS include symmetric peripheral neuropathies and mononeuritis multiplex. The symmetric neuropathies frequently present as sensory and motor components occur less frequently. The relative risk for primary SS patients of developing lymphoma has been estimated much higher than that of age-matched control subjects (13). The lymphomas are predominantly non-Hodgkin B cell (IgM-k) neoplasms that arise in the salivary gland and/or cervical lymph nodes. The distinction between malignant lymphomas and "pseudolymphoma" in SS patients is often quite difficult, even when recombinant DNA methods are clinically utilized (14,15).

The most common autoantibodies in primary SS patients are antinuclear antibodies (ANA) and rheumatoid factor (12). The positive ANA is due to the presence of antibodies against SS-A/ Ro and SS-B/La antigens. Antibodies against SS-A/Ro react with a ribonucleoprotein (RNP) complex that contains a novel class of small RNA designated hY1-hY5 (h for human; Y for cytoplastic) RNA, and it is now evident that such RNAs are also intranuclear components. The hY RNAs share many features including sequence, and size (83-112 bp). However, the specificity for the immunogenicity of SS-A/Ro and SS-B/La remains unclear. It has been proposed that viral transcripts at tached to S S-B may lead to immune responses against the RNP complex in genetically predisposed individuals. A proposed, molecular mimicry hypothesis is that an immune response against any virus may cross-react with SS-B/La proteins. Although functional studies have shown that salivary gland lymphocytes can produce autoantibodies in vitro (16,17), organ-specific autoantibodies have not yet been detected in primary SS patients.

**MURINE MODEL FOR SJOGREH'S SYNDROME**

- Several animal models for studying this autoimmune phenomenon in primary SS include autoimmune-prone mice which develop as an associated lesion (18-21), and spontaneously occurring age-related lesion in certain strain of mice (22, 23). We have provided evidence that autoimmune salivade-nitis could be induced in non-autoimmune strain of 3d-Tx mice fol lowed by later immunization with salivary gland cells or tissue homogenate (24-26). A significantly higher incidence of autoimmune lesions in females was found, and the antisalivary duct autoantibodies were frequently detected in sera from mice with autoimmune lesions. The salivary gland autoimmune lesions first appeared in 4-week-old mice, and were aggravated with age until 20-week-old. Periductal inflammatory lesions quite resembled human primary SS in the salivary and lacrimal gland developed exclusively in 3d-Tx NFS/sld mice. Extensive inflammatory lesions with parenchymal destruction were frequently observed in these mice.

The inflammatory infiltration into the salivary and lacrimal glands consisted mainly of CD3-CD4- T cells with lesserproportion of CD8-T cells and B220-B cells. Immunohistochemically, a large number of TCR Vb6 cells was evident in all samples during the course of disease, and a small number of TCR Vb6~ T cells was also identified in the salivary and lacrimal gland.
Immunoreactive cells with mAbs to other TCRs examined were not clearly detected within these lesions. In control mice, significant numbers of detectable T cells could not be observed in both the salivary and lacrimal glands. FACS analysis of surface phenotype in the spleen cells showed a significant decrease of CD8- T cells in 3d-TXNFs/sld mice, compared with nontreated mice. Tissue infiltrating lymphocytes obtained from the inflamed subintradibular glands were analysed by flow cytometry. A predominance of CD4~Vb8~cells could be identified at each age, and a minor population of CD4~Vb6~ T cells was also identified. When the repertoire of TCR Vb genes transcribed within the inflammatory infiltrates was analysed by reverse-transcribed polymerase-chain reaction (RT-PCR), a preferential utilization of TCR Vb gene (Vb8 and Vb6 predominant) was detected in these lesions from the onset of disease. In experimental autoimmune encephalomyelitis, it has been shown that the repertoire of the autoreactive T cells is restricted. The Vb8.2 and Vb4 seg-ments were dominantly utilized by myelin basic protein-specific T cells in H-2umice, while Vb 17a was frequently observed in H-2S mice (27-29). Recent studies of human autoimmune diseases have provided evidence for oligoclonality by the restriction fragment length polymorphism method. It was demonstrated that T cells of synovial fluid in patients with rheumatoid arthritis expressed TCR Vb7 transcripts (30) and brain biopsy specimens from multiple sclerosis preferentially expressed TCR Vb 10 gene (31).

Immunohistochemical analysis was carried out to identify the particular cells responsible for production of various kinds of cytokines (TNF-α, IL-2, IFN-γ, IL-4, IL-6, and IL-10), and cell adhesion molecules (CAM) (ICAM-1, LFA-1, CD44, and Mel-14) in the salivary glands of 3d-TX NF/SLD mice. A variable proportion of cytokine-positive cells (TNF-α, IL-2, IFN-γ) was detected in the salivary glands from the onset of inflammatory lesions at 4 weeks of age. A smaller number of an anti-IL-10-positive cells were found within the inflammatory infiltrates after 4 weeks of age. Anti-IL-6-positive mononuclear cells were detected in the periductal inflammatory infiltrates in the salivary glands at 12 weeks of age or more. In contrast, ICAM-1-positive cells were observed mainly in vascular endothelial cells at age of 3 weeks before the onset of inflammatory infiltrates in the salivary glands. At 4 weeks of age or more, a considerable number of ICAM-1-positive cells were seen in the inflammatory lesions in addition to the endothelial cells. LFA-1-, CD44-, and Mel-14-positive cells were constantly detected within the inflammatory lesions during the course of disease.

We found the upregulation of cytokine genes (Fig. 1) and CAM genes (ICAM-1, LFA-1, CD44, Mel-14) in the salivary glands by RT-PCR, which were supported by immunohistochemistry. The expression of IL-4 mRNA was not detected throughout the course of autoimmune disease in this animal model. FACS analysis demonstrated that a significant proportion of splenic CD4~T cells express activation markers (CD44, LFA-1, Mel-14low, CD45RBlow) at a high level, and an increase in expression of B220- B cells bearing I-Aq class-II molecules. Therefore, it is possible that the organ-specific autoantigen is targeted by clonally expanded autoreactive CD4~T cells with Th 1 cytokine profile. In this animal model, autoimmune lesions develop exclusively in the salivary and lacrimal glands, indicating that the organ-specific autoreactive CD4T cell clone generated in the periphery before 3 days after birth play an essential role for the development of salivary and lacrimal gland autoimmunity. It is now considered that the autoreactive CD4T cells are controlled by CD4~T cells with suppressor or regulatory activity that can only be generated after the first week of life (32). To un-

**Figure 1.** Expressions of cytokine, TCR Cb, and f-actin mRNA in the tissue-infiltrating mononuclear cells into the salivary glands of 3dTx NF/SLD mice of various ages demonstrated by Southern blot analysis of RT-PCR product.
Understand the pathogenic mechanism leading to spontaneous autoimmune lesions in the salivary glands, we attempted to clarify whether CD4+ T cells are activated after the 3 d-TX treatment in the periphery. It is important to know whether autoreactive T cells generated in the periphery are spontaneously activated or not. We found that significant proportions of splenic CD4+ T cells from this mouse model expressed activation markers CD44, LFA-1, Mel-141low, CD45RBlow at a high level. Many previous studies demonstrated that mature T cell populations include cells that recognize and respond to self-antigens, but these cells are normally harmless because they are not activated (3, 35). It has been recently reported that only very small fractions of the large numbers of potential disease-causing T cells are spontaneously activated in TCR transgenic model (34, 35). We suggest that local upregulation of Th1 cytokines and CAM may be involved in the cascade of events that initiate and accelerate organ-specific autoimmunity in the salivary glands. Autoreactive T cells in this animal model may be triggered by an in situ activation through unknown autoantigen stimuli in the salivary glands.

Moreover, we confirmed these experimental evidences using another SS model (36). The non-obese diabetic (NOD) mouse develops spontaneous autoimmune sialadenitis besides a well-characterized T cell-mediated autoimmune insulitis (37-40). Immunohistochemical analysis showed that the vast majority of inflammatory infiltrates in the salivary glands were CD4+Vb8+ and CD4+Vb6+ T cells, whereas CD8+ T cells and B220+B cells were fewer in number. We used RT-PCR to analyse the repertoire of TCR Vb chain genes expressed in the isolated infiltrating cells from affected salivary glands. Predominant expression of the Vb8 and Vb6 gene segment was detected in the infiltrating cells in the salivary glands very early, and age-related diversity of TCR Vb gene usage was observed. Single-strand conformation polymorphism (SSCP) analysis demonstrated a strikingly symmetrical distribution of expanded clones in the PCR products of the Vb8 and Vb6 gene in the cells infiltrating the salivary glands. Nucleotide sequencing of amplified TCR Vb cDNA revealed that T-cell clonotypes had a high incidence of identical clones, indicating that the immune response in NODsialadenitis is driven by common stimuli. From these findings, we concluded that in autoimmune sialadenitis of murine models, there might be a restricted usage of TCR Vb elements in the early stage of the autoimmune response to recognize unknown self-antigen. Our recent study has demonstrated that a cleavage product of 120 kD a-fodrin may be an important salivary gland autoantigen in the development of SS, and thus anti-a-fodrin antibodies have been frequently detected in sera from patients with primary SS.

IDENTIFICATION OF SALIVARY GLAND AUTOANTIGEN AND ITS CLINICAL APPLICATION

- The TCR Vb gene is preferentially used in the salivary gland autoimmune lesions from the onset of disease, and high concentrations of anti-salivary duct autoantibodies of IgG type were detected in sera from these mice (Fig. 2A). To identify an organ-specific antigen, the salivary gland autoantigen reactive with affinity purified IgG from sera of 3d-TxNFS/sld mice was detected by Western blotting; no reactivity was detected in tissue homogenates from the lung, liver, heart, kidney, pancreas, spleen, and brain. Moreover, no reactivity was expressed in tissue homogenates from the salivary glands in other mouse strains such as BALB/c and C3H/He. To purify the salivary gland autoantigen, the tissue homogenates of these mice were subjected to fast protein liquid chromatography (FPLC) on Superose 12 HR. The fraction containing autoantigen activity was further purified by ion exchange high performance liquid chromatography (HPLC) on DEAE Cosmogel. Autoantigen activity was recovered in fractions 27 and 28. Western blotting of these fractions gave a major band of 120 kD (Fig. 2B). To identify the 120 kD salivary gland protein, we electrophoresed the band onto an Immobilon membrane and directly sequenced it with an Applied Biosystems 477A Protein Sequencer. The bands were shown to possess a single N-terminal sequence. This sequence was identical to the N-terminal sequence of human a-fodrin:RQKLEDYSRITOPQRDSTAEEL(41-43).

Proliferative T cell responses of spleen cells from 3d-TxNFS/sld mice to the identified 120 kD antigen developed spontaneously at 4 weeks of age, consistent with the onset of the autoimmune lesions in the salivary gland. This response increased at 8 and 12 weeks of age, then declined by week 16. In contrast, no response was detected with control antigens (fraction 40, lypozy-me, and a-amylase). The spontaneous development of a pro-liferative T cell response to the 120 kD antigen is consistent with endogenous priming. We tested the 120 kD antigen-reactive T cells for additional properties to confirm the specific T cell responses. Splenic T cells from 8-week-old 3d-TxNFS/sldmice challenged with the 120 kD antigen produced IL-2 and IFN-y. Thus, a potentially pathogenic Th1-type T cell population may be spontaneously primed to the 120 kD salivary gland autoantigen early in the development of autoimmune lesions in the salivary glands of this mouse model. To examine the organ-specificity of the 120 kD a-fodrin, we investigated various tissue homogenates in 3d-Tx NFS/sld mice immunoblotted with a commercially available monoclonal antibody to a-fodrin. More of a-fodrin was detected in the salivary gland homogenates than in homogenates from other organs. These data suggest that the salivary gland expression of a-fodrin may be an initial response in the development of autoimmune lesions in the salivary glands. Polyclonal rabbit antibodies to a synthesized a-fodrin N-terminal peptide reacted with the 120 kD salivary gland antigen upon Western blotting. Epithelial duct cells were intensely stained for antibodies against synthetic a-fodrin peptide in the salivary glands with autoimmune lesions, but not in non-thymectomized and normal control mice.
Salivary glands, autoantigen and Sjögren’s syndrome

Figure 2. Detection of anti-salivary gland autoantibodies in the salivary glands by the indirect immunofluorescence antibody technique (A). HPLC fractionation on DEAE Cosmogel (B). The purified 120 kD antigen was detected by Western blotting in fraction 27 and 28 (insert). From Ref 11.

120kDa-fodrin and the autoimmunity of Sjögren’s syndrome

To test the specificity of the α-fodrin as the salivary gland autoantigen, we constructed a recombinant α-fodrin fusion protein using the glutathione S-transferase (GST) fusion system in Escherichia coli. JS-1 cDNA, encoding the N-terminal portion, was constructed by inserting cDNA (1-1784bp) into the EcoRI site of pGEX-2T. Purified recombinant JS-1 protein was determined by examining its reactivity with sera from 3d-TxNFS/sld mice. The reactivity with sera containing antibodies to 120 kDa-fodrin was confirmed by Western blotting. Spleen cells from 3d-Tx mice proliferated to the recombinant JS-1 fusion protein. It was also investigated whether the intra-venous injection of the recombinant α-fodrin protein protects animals against the development of autoimmune lesions. The treatment with intravenous injection of recombinant JS-1 protein (25 mg) inhibited the development of autoimmune conditions including lymphocytic infiltration and autoantibody production to the whole 120 kD molecules. To confirm the disease specificity of α-fodrin and the recombinant JS-1 protein as an autoantigen in primary SS, we investigated sera from other autoimmune diseases, including secondary SS, such as SLE, RA, and from normal healthy individuals, in addition to patients with primary SS. As a result, 41 sera among 43 patients with primary SS were positive for both the purified 120 kD antigen and the recombinant JS-1 protein on Western blots (95.35%), whereas sera from all patients with SLE and RA and from normal healthy individuals were negative (Fig. 3). Moreover, α-fodrin was detected in tissue homogenates of lip biopsies from patients with primary SS, but not in control materials. These data support the hypothesis that α-fodrin may be expressed in the salivary glands of both the mouse model of SS and the patients with SS. Furthermore, we detected proliferative T cell response to the purified 120 kD antigen using peripheral blood mononuclear cells from patients with primary SS. These data indicate that α-fodrin is a key target autoantigen in the induction of primary SS in humans.

Fodrin is a major component of the cortical cytoskeleton of most eukaryotic cells, and it forms heterodimers aligned in a side to side manner composed of one a (240 kD) and one β (235 kD) subunit (41,42). α-fodrin is an actin-binding protein that is found at the periphery of chromaffin cells and may be involved in secretion (44-46). The stimulation of secretion in parotid acinar cells is associated with dramatic rearrangements of the subplasmalemmal cytoskeleton, particularly of α-fodrin (47). Fodrin possesses binding sites for various proteins including actin (46), calmodulin (48-51), and CD45 (52,53). Proteolytic cleavage of α-fodrin resulting from the activation of a neutral calcium-activated protease (calpain) could be responsible for these conformational changes (54). The fodrin a-subunit is cleaved in association with apoptosis, and the 120 kD fragment is a breakdown product of the fodrin a-subunit (55). The proteolysis of fodrin during apoptosis may be a consequence of unknown protease activation, α-fodrin is recently found to be cleaved by the calcium-activated protease calpain in apoptotic T cells, and by calpain and caspases in anti-Fas-stimulated Jurkat cells and/or neuronal apoptosis (56). We therefore speculate that an increase in the activity of apoptotic proteases is involved in the progression of a-fodrin proteolysis during development of SS. Fodrin cleavage during apoptosis may have implications for the membrane blebbing seen during this process. When human T cell leukemia CEM cells were induced to undergo apoptosis by exposure to either anti-Fas Ab, C2-ceramide, or staurosporin, the 240 kD α-fodrin was cleaved to a single detectable fragment of 120 kD, whereas in cultures.
containing fewer apoptotic cells, a larger fragment of 150 kD was observed (57). It is plausible that the 120 kD fragment is a breakdown product of the 150 kD a-fodrin cleavage (55,56). Analysis of proteolytic events associated with apoptosis to define the mechanisms leading to protease activation, and to identify key substrates whose cleavage might be linked to the profound changes in cellular architecture. There is increasing evidence that calpain is overactivated in autoimmune conditions and subsequent tissue destruction (58,59).

Altogether, an increase in the activity of cysteine proteases through unknown mechanisms is probably involved in the progression of a-fodrin proteolysis during the initial stages in the development of primary SS.

Autoantibodies are produced to the pyruvate dehydrogenase complex in PBC (60), thyroid peroxidase in Hashimoto's disease (61), glutamic acid decarboxylase in insulin-dependent diabetes (39,40), FT/IC-ATPase in pernicious anaemia (62), and RNA polymerase 1 (RNA pol I) in SLE and RA (63). In SS, autoantibodies are generated to RNP particles SS-A/Ro and SS-B/La. Among them, disease-specific autoantibodies have only rarely been described. Despite the recent acquisition of extensive information relating to the mechanisms of self-tolerance, understanding of those leading to pathogenic autoimmune immunity remains obscure. Autoantigens can stimulate primary T cell proliferative responses, which suggests that many autoreactive T cells escape deletion and remain in an unresponsive state. The identification of the salivary gland 120 kD a-fodrin is of relevance in elucidating the crucial role of this autoantigen in the development of primary SS.

**IMMUNE RESPONSE AGAINST THE SALIVARY GLAND AUTOANTIGEN**

- A correlation between the cellular manifestations and the production of autoantibodies during the course of autoimmune diseases has not yet been analysed. To elucidate the mechanisms of the anti-120 kD a-fodrin immune response *in vivo*, we analysed NOD mice as a model for SS during development of autoimmune sialadenitis. We found a specific autoantibody production against a-fodrin, and its production correlates closely with autoimmune sialadenitis. Protein immunoblotting revealed that sera from NOD mice were positive for a-fodrin from the onset of disease at 10 weeks of age.

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**Figure 3.** Western blot analysis of the 120 kD a-fodrin and recombinant fusion protein, JS-l, with human sera from autoimmune diseases. Sera from patients with primary SS were positive for the 120 kD antigen, whereas those from all patients with SLE, RA, and normal healthy donors were negative. Representative immunoblotting profiles were selected from each group. Western blots of purified 120 kD a-fodrin (A). Western blots of the recombinant fusion protein, JS-l (70 kD protein is an artifact band of *Escherichia coli* degradation) (B). From Ref 11.
antibody production con-elated closely with inflammatory infiltration in the salivary glands in NOD mice until 30 weeks of age. To examine the organ specificity of a-fodrin, we immuno-blotted salivary gland homogenates with polyclonal antibodies to synthetic a-fodrin of the purified 120 kD antigen. More a-fodrin was detected in the salivary gland homogenates from NOD and NFS/sld mice, but not from control BALB/c mice. The content of IL-2, IL-4 and IFN-y by ELISA in culture supernatants of spleen cells from 8-, 12-, and 16-week-old NOD mice 48 h after challenge with a-fodrin antigen was determined. We detected a significant level of IL-2, IFN-y, but not of IL-4 produced in culture supernatants measured by ELISA. Proliferative T cell response to a-fodrin antigen develops spontaneously in NOD mice in a defined chronological order. Antigen-stimulated blastogenesis was detected in spleen cells from 10- to 16- week-old female NOD mice, as the same level of NFS/sld mouse model. A specific T cell response of splenocytes against a-fodrin autoantigen was observed in NOD mice from the early onset of autoimmune sialadenitis, and in vitro cytokine production by splenic T cells such as IL-2 and IFN-y, but not IL-4, was detected by ELISA. A variable proportion of cytokine-positive cells (TNF-a, IL-2, IFN-y, and IL-10) was detected in the salivary glands at 10 weeks of age, and their numbers increased with age until 30 weeks. IL-6-positive mononuclear cells were found in the affected glands from 16 weeks of age. No IL-4-positive cells were detected in any of the inflammatory lesions. Figure 4 shows the representative immunohistochemical features of the infiltrating cells positive for cytokines in the salivary glands from a 16-week-old NOD mouse. We detected significant levels of cytokine mRNA expression of IL-1 (3, TNF-a, IL-10, and IL-12 (p40) in the mononuclear cells isolated from salivary glands of NOD mice at 8 weeks of age, before the development of inflammatory lesions (Fig. 5). Because Cb mRNA was clearly detected at 8 weeks of age, very early T-cell infiltrates may develop in the salivary glands of NOD mice before the onset of the histological changes. In particular, a significantly higher level of IL-12 (p40) expression was observed during early stage of disease. We found the upregulation of local cytokine genes including Th1 cell type (IL-1 (3, TNF-a, IL-2, IFN-y, IL-6, IL-10, and IL-12 (p40) in the tissue-infiltrating cells at various ages determined by RT-PCR and Southern blot analysis, but expression of IL-4 mRNA was not detected at any age. The mRNA expression

Figure 4. Immunoperoxidase staining of infiltrating mononuclear cells in the salivary gland of a 16-week-old female NOD mouse. A variable number of cells were positive for IL-2 (A), IFN-y (B), IL-6 (C), and IL-10 (D). x 240.
was associated with detectable mRNA\textsuperscript{m} and mRNA\textsuperscript{mRNA} expressions. This pattern of cytokine mRNA expression in the salivary glands of NOD mice differs from those reported in organ-specific autoimmune diseases (64). We have demonstrated that the autoreactive CD4\textsuperscript{T} cells in the salivary glands composed mainly of Th1 cells during the course of autoimmune lesions. Likewise, these cells exhibit cytokine mRNA expression including IL-2, IFN-\(\gamma\), and IL-10 (65,66). In the BB rat, it was suggested that IFN-\(\gamma\) and IL-12 may play a major role in the expression of insulitis and thyroiditis (67). Also, IL-10 and IL-12 play a critical role in the initial phase of development of autoimmune sialadenitis in MRL/lpr mice (68). Although IL-10 is known to have multiple suppressive effects on various effector phases of the immune responses, recent reports have demonstrated that IL-10 accelerates the development of autoimmune lesions in NOD insulitis and NZBWF1 mice (69,70), and that Th1 development of naive CD4\textsuperscript{~T} cells depends on the coordinate action on IL-12 and IFN-\(\gamma\) (71). Since IL-12 has a striking effect in increasing production of IFN-\(\gamma\) by T cells (72), local IL-12 production may play an important role in the development of Th1-type autoimmune sialadenitis in NOD mice through IFN-\(\gamma\) production. It is possible that in spontaneous autoimmune sialadenitis in NOD mice, there may be a specific anti-a-fodrin immune responses on the development of autoimmune lesions, and that the autoreactive CD4\textsuperscript{~T} cells possess an unregulated Th1-cytokine profile besides IL-10 and IL-12.

CONCLUSION

- The identification of the salivary gland autoantigen, 120 kD a-fodrin, is of relevance in elucidating its increasingly crucial role in the development of primary SS. Additional information about fine epitope mapping in both T and B cells, the nature of the etiologic autopeptide in immune recognition, and the function of autoreactive T cells in tissue destruction will provide new insights into the appropriate diagnosis and the therapy for autoimmune diseases in the salivary glands.

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**Figure 5.** Expression of cytokine, ICR Ch, and jJ-actin mRNA in the tissue-infiltrating mononuclear cells isolated from the salivary glands of female NOD mice at various ages, as demonstrated by Southern blot analysis of RT-PCR products.


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