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ORIGINAL ARTICLE

Effects of preceding sialadenitis on the development of autoimmunity against salivary gland

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OBJECTIVE: The mechanism underlying the onset and development of autoimmune diseases such as Sjogren's syndrome is not well understood. Here, we examined the effects of preceding inflammation of the salivary gland at the onset of autoimmunity against the salivary gland.

MATERIALS AND METHODS: One side of the submandibular gland duct was ligated in mice and the effect on the contralateral gland was investigated. After histological evaluation with hematoxylin and eosin staining, the presence of autoantibodies and immune compounds was examined.

RESULTS: In all five strains of mice that were used, the salivary gland of the ligated side showed severe inflammation and atrophic change. In two mouse strains (SJL/J and PL/J), mild sialadenitis was observed on the non-ligated side 8 weeks after ligation. Autoantibodies reacting to the salivary gland were detected in three mouse strains (C3H/He, SJL/J, and PL/J). Immune complex was also detected in the duct basement membrane.

CONCLUSION: The results indicate that the autoimmune mechanism is activated by the transient inflammation in the salivary gland under a specific genetic background.

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Keywords: autoimmunity; inflammation; duct-ligation; sialadenitis

Introduction

Sjogren's syndrome is known as a salivary gland disorder related to autoimmune mechanisms. It is characterized by a focal lymphocytic infiltration of the salivary gland, resulting in symptoms of dry mouth (Fox *et al*, 1984). However, the mechanism underlying the onset and development of this autoimmune disease is not well understood.

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In general, with paired organs, when the organ on one side is damaged in some way and its function deteriorates, the contralateral organ is thought to either maintain its function unchanged or undergo a compensatory elevation of function as in the lung and kidney (Kinn and Bohman, 1983; Kelly *et al*, 1995). This phenomenon has also been reported in the salivary glands (Scott *et al*, 1999). However, in instances such as traumatic hemilateral ocular damage, there are reports of sympathetic ophthalmia resulting in blindness of the contralateral eye also due to autoimmune uveitis, although this occurs at a low frequency of 0.2–0.5% (Marak, 1976). This fact suggests the role of inflammation for the onset of autoimmunity by the presentation of sequestered antigens and/or denatured proteins. We became interested in whether preceding inflammation in the salivary gland is a trigger responsible for the activation of the autoimmune mechanism.

In this study, we used a salivary gland ductal-ligation model as a trigger of transient sialadenitis (Harrison and Garrett, 1976; Domon *et al*, 1988; Takahashi *et al*, 2000; Kurahashi, 2002) and investigated the effect on the onset of autoimmunity against the salivary gland parenchyma with reference to genetic background.

Materials and methods

Experimental animals

Eight- to 10-week-old female mice of five strains – B10A(H-2^a), BALB/c(H-2^d), C3H/He(H-2^k), SJL/J(H-2^s), and PL/J(H-2^u) – were used. The first three strains were purchased from Charles River (Yokohama, Japan) and the last two strains from Jackson Laboratory (Bar Harbor, ME, USA). All mice were kept on a 12 h:12 h light–dark cycle, given a standard pellet diet, and allowed free access to water. The following experiments were conducted in accordance with the 'Guideline for Animal Experimentation of Nagoya University.'

Salivary gland ductal ligation

Eight- to 10-week-old female mice (five mice for each strain) were anesthetized with diethyl ether. The main duct of the right submandibular gland was ligated with

silk thread, and the skin was sutured using the same thread. Sham operations were performed in five mice for each strain. Normal mice without any operation (five mice for each strain) were also analyzed and showed no autoimmune sialadenitis or anti-salivary gland antibodies up to the age of 24 weeks.

Pathological evaluation

Mice of each strain were killed at 8 weeks after ligation of the main submandibular gland duct or sham operation. Various organs (salivary gland, pancreas, liver, kidney, thyroid gland, ovaries and stomach) were extracted and fixed, embedded in paraffin, longitudinal sections were cut, and stained with hematoxylin and eosin. Histological assessments were conducted based on the method of White and Casarett (1974). Briefly, sections were examined at 150× and scored for degree of inflammatory infiltrate in the following fashion: (1) one to five foci of mononuclear cells were seen (more than 20 cells per focus), (2) more than five such foci were seen but without significant parenchymal destruction, (3) multiple confluent foci were seen with moderate degeneration of parenchymal tissue, and (4) extensive infiltration of the glands with mononuclear cells and extensive parenchymal destruction. The mean grade for each group was estimated and used for the evaluation.

Immunohistology

Detection of autoantibodies

Submandibular gland tissue of normal mice (3 months of age) was cryoembedded in OCT Compound (Sakura Finetek Japan Co. Ltd., Tokyo, Japan) and then sliced to a thickness of about 4 μm and fixed in acetone for 10 min. We used the sera of duct-ligated mice from each strain and sham-operated mice (100-fold dilution, 45-min reaction) as a primary antibody. For a secondary antibody, goat anti-mouse immunoglobulin-G (IgG) antibody fluorescein isothicyanate FITC label (300-fold dilution, 45-min reaction; Cappel Co., Durham, NC, USA) was used, and indirect immunofluorescence was conducted.

Detection of immune compounds

Eight weeks after ligation of the main submandibular gland duct in the SJL/J, PL/J and C3H/He mice, frozen sections were prepared from the non-ligated side submandibular gland. Direct immunofluorescence was conducted using goat anti-mouse IgG antibody FITC label and rat anti-mouse C3 antibody FITC label (300-fold dilution, 45-min reaction; Cappel Co.).

Results

Histological findings of sialadenitis induced by salivary gland ductal ligation, and their frequency

Duct dilatation, parenchyma damage, and inflammatory cell infiltration were observed in the salivary ducts on the ligated side in all strains of mice (Figure 1a,d,g). In the salivary glands on the non-ligated side, the tissue damage was not observed in B10A, BALB/C and C3H/He strains (representative photograph in Figure 1b). Mild tissue damage and inflammatory cell, including lymphoid cells, granulocyte and macrophage, infiltration were seen in the non-ligated side of SJL/J (H-2^s) and PL/J (H-2^u) mice at 8 weeks post-ligation (Figure 1e,h). The frequencies and severities were 100%, 2.2 in the SJL/J mice and 80%, 1.6 in the PL/J mice (Table 1). Furthermore, both fibrosis and atrophy were remarkable on the non-ligated side in the glands of the histologically affected mice. In the sham-operated group of mice, no lesions appeared in mice of any of the strains (Figure 1c,f,i). Inflammatory lesions in organs other than the salivary glands (pancreas, liver, kidney, thyroid gland, ovaries and stomach) were not detected in any of the strains of mice subjected to salivary gland ductal ligation.

Detection of anti-salivary gland (submandibular gland) antibodies

Although no salivary gland inflammation was noted in the non-ligated side in the C3H/He (H-2^k) mice, autoantibodies to salivary glands were present in the bloodstream at a frequency of 60% (3/5) at 8 weeks after ductal ligation (Table 1, Figure 2a). The autoantibodies that appeared were mainly to myoepithelial cells and excretory ducts (Figure 2a). The autoantibodies also appeared at a high frequency in the bloodstream of SJL/J and PL/J mice subjected to the same procedures, which was mainly detected at the excretory ducts (SJL/J, Figure 2b) and at the excretory ducts as well as at the basement membrane of acini (PL/J, Figure 2c). Anti-salivary gland antibodies were not found in the sham-operated group of mice (Figure 2d). In mice of all strains that underwent salivary gland ductal ligation, no antibodies to organs other than the salivary glands (pancreas, liver, kidney, thyroid gland, ovaries and stomach) were observed (data not shown).

Detection of immune complex

At 8 weeks after ductal ligation, IgG localization was observed near the basement membrane of the salivary gland ducts on the non-ligated side in C3H/He mice (Figure 3a), which showed most remarkable autoantibodies against salivary gland (Figure 2a). The background level staining for mouse IgG was faint and the difference was clearly noted (Figure 3b). Immunoreactivity against C3 was relatively intense in the salivary gland of the non-ligated side in the experimental group than that in the sham-operated control group, especially near the basement membrane (Figure 3c,d). Immunoreactivity against the immune complex was weaker in SJL/J and PL/J mice than in C3H/He mice (data not shown).

Discussion

Generally, mechanisms such as genetic background, disfunction of antigen-presenting cells, abnormality of antigen and immune tolerance disorders were reported as causes of autoimmune disease (Faustman, 1993). In the present experiment, we showed the possibility that preceding inflammation triggers off autoimmune disease, and this phenomenon might be affected by the

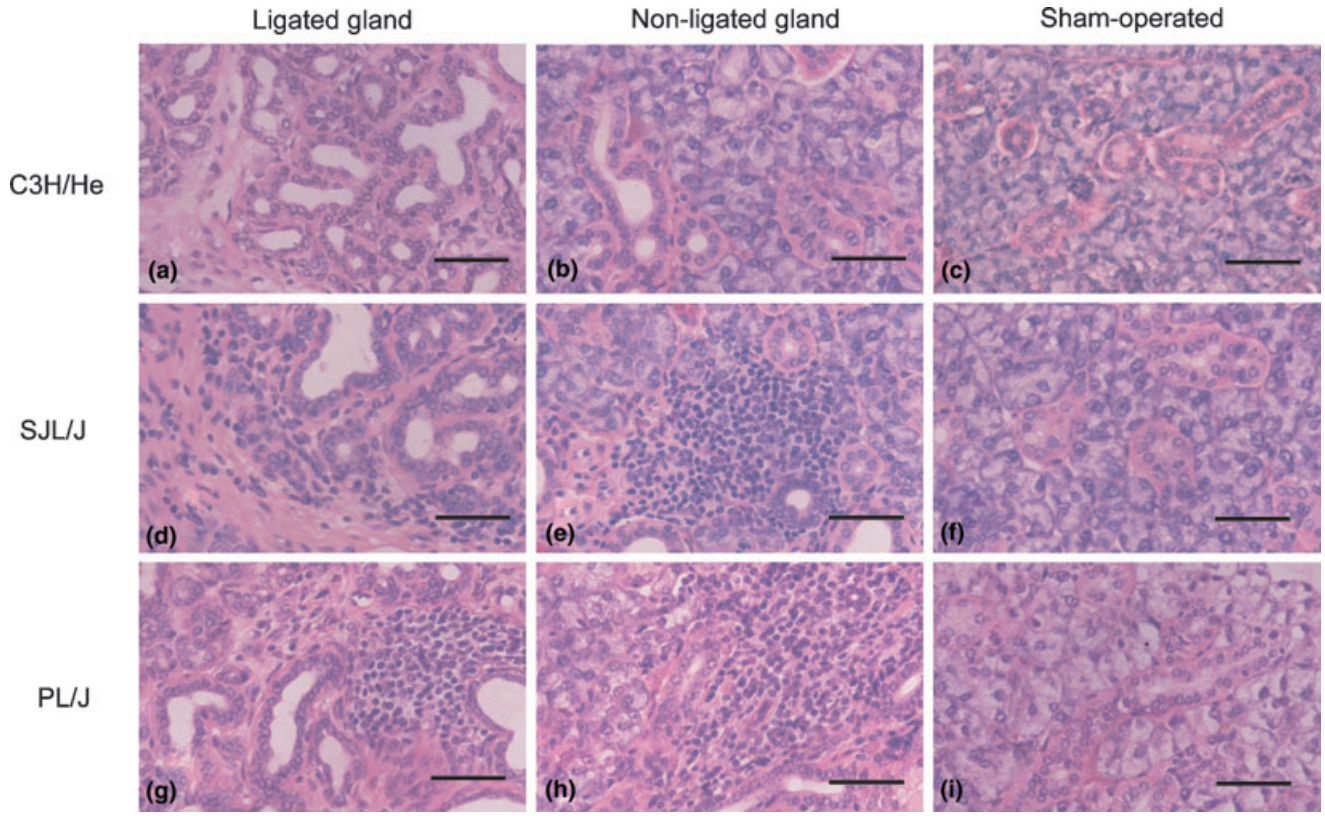

Figure 1 Histologicalimage of the salivary gland in C3H/He, SJL/J, and PL/J mice at 8 weeks post-ligation. (a) C3H/He mouse, histological image of the salivary gland on ligated side. (b) C3H/He mouse, histological image of the salivary gland on non-ligated side. (c) C3H/He mouse, histological image of the salivary gland in sham-operated mouse. (d) SJL/J mouse, histological image of the salivary gland on ligated side. (e) SJL/J mouse, histological image of the salivary gland on non-ligated side. (f) SJL/J mouse, histological image of the salivary gland in sham-operated mouse. (g) PL/J mouse, histological image of the salivary gland on ligated side. (h) PL/J mouse, histological image of the salivary gland on non-ligated side. (i) PL/J mouse, histological image of the salivary gland in sham-operated mouse. Scale bars = 20 μm

Table 1 Incidence(%) and severity of sialadenitis of the non-ligated side and anti-salivary gland antibodies in five mouse strains at 8 weeks post-ligation. Severity of sialoadenitis was assessed by the method of White and Casarett (1974)

Strain	*H-2 haplotype*	*Incidence of sialadenitis*	*Severity of sialadenitis*	*Incidence of antibodies*
B10A	a	0 (0/5)	0	0 (0/5)
BALB/c	d	0 (0/5)	0	0 (0/5)
C3H/He	k	0 (0/5)	0	60 (3/5)
PL/J	u	80 (4/5)	1.6	80 (4/5)
SJL/J	s	100 (5/5)	2.2	80 (4/5)

genetic background. Although there are reports of sympathetic ophthalmia (Marak, 1976), there is some doubt as to whether the sequestrated antigen hypothesis could be directly applied to the present experiment. However, it does seem possible that autoimmune phenomena are triggered by usual or unusual means of antibody presentation such as tissue damage due to inflammation, or micro-environmental changes such as inflammatory cytokines.

There are numerous reports that CD4-positive cells are induced in organ-specific autoimmune diseases (Nishigaki-Maki *et al*, 1999; Ohno *et al*, 1999). Although the population of CD4-positive cells was not investigated in this study, the infiltration of inflammatory cells strongly suggests the involvement of cellular immunity. In addition, IgG and C3, or immune complex depositions, were detected by direct immunofluorescence in the salivary gland in some strains of mice. Not only cellular, but also humoral immunity might be involved in the histological change of the salivary gland on the non-ligated side. However, the contribution of cellular and humoral immunity to the onset of sialadenitis is still not clear from this study and requires further investigation.

Although IgG antibodies to salivary gland were detected in the non-ligated side of the salivary gland in C3H/He mice, sialoadenitis was not observed. The appearance of autoantibodies is not completely parallel to the appearance of infiltration of inflammatory cells in some autoimmune diseases. For example, transient insulin autoantibody expression was reported independent of the development of diabetes in NOD and NOR strains (Abiru *et al*, 2001). As also shown in this study, the development of autoantibodies has some correlation with autoimmune disorders, but may be insufficient for the onset.

Among the five strains of mice (B10A, BALB/c, C3H/He, SJL/J, and PL/J), inflammatory cell infiltrations

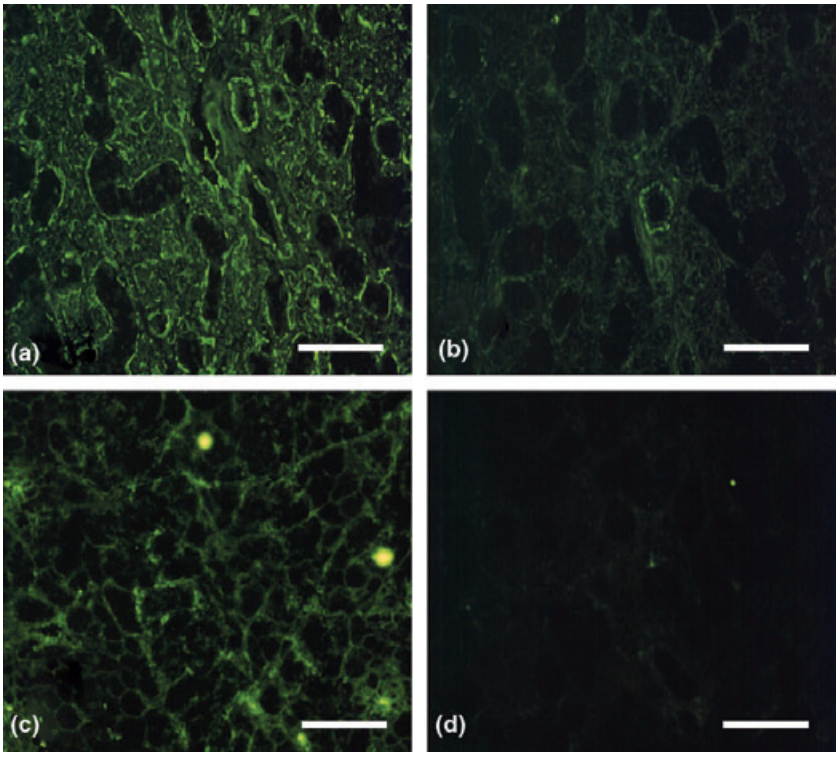

Figure 2 Detectionof anti-salivary gland antibodies in the bloodstream of C3H/He, SJL/J, and PL/J mice at 8 weeks post-ligation. (a) C3H/He mouse. (b) SJL/J mouse. (c) PL/J mouse. (d) Sham-operated mouse (C3H/He mouse). Scale bars = 100 μm

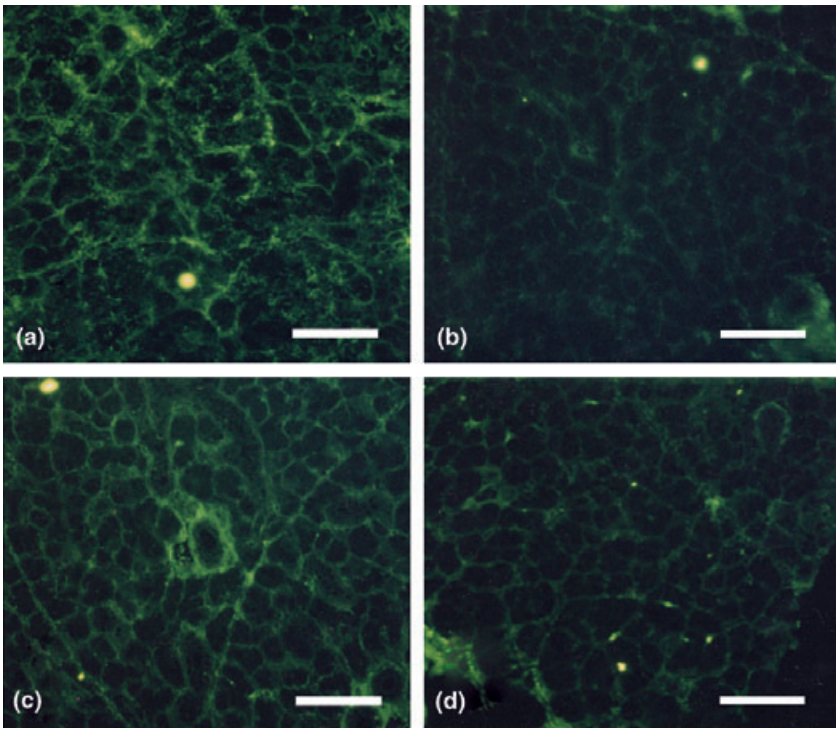

Figure 3 Detectionof immune complex (IgG and C3) in the contralateral salivary gland of C3H/He mouse at 8 weeks post-ligation. (a) IgG, post-ligated C3H/He mouse. (b) IgG, sham-operated mouse. (c) C3, post-ligated C3H/He mouse. (d) C3, sham-operated mouse. Scale bars = 100 μm

were obtained at a high frequency in only SJL/J and PL/J strains. These two strains are used in experimental allergic encephalomyelitis as an animal model of human multiple sclerosis (Fritz *et al*, 1983). Moreover, using the same mice, Nishimori *et al* (1995) succeeded in creating a model of sialadenitis by local immunization with carbonic anhydrase II that is expressed in the salivary glands and pancreas ducts together with Freund's complete adjuvant.

Autoimmune disease correlates with specific major histocompatibility complex (MHC) class II (Gebe *et al*, 2002). As discussed above, autoimmune sialadenitis due

to ductal ligation only appeared in specific mouse strains. These mice used in this study were not congenic and it might be difficult to discuss the direct relationship between the haplotype of MHC class II (H-2) and the onset of autoimmunity. However, H-2 is known to mediate antigen presentation to T cells, which is a major player of cellular immunity. It would be beneficial to investigate the relationship between H-2 haplotype and the susceptibility of autoimmunity. Furthermore, research into salivary gland antigens of duct-ligated mice, antigen complexes, and T-cell receptors will contribute to understanding this phenomenon.

In humans, the contribution of transient inflammation to the onset of autoimmune diseases is not clear. The results from this study show that the autoimmune mechanism is activated by the transient inflammation in the salivary gland under a specific genetic background. Further studies of the detailed mechanisms of this phenomenon will elucidate the potential of inflammation-induced autoimmunity in patients with Sjogren's syndrome or other autoimmune disorders.

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