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Spontaneous Development of Autoimmune Sialadenitis in Aging BDF1 Mice

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This study reports that spontaneous autoimmune sialadenitis developed in aging female, rather than male, BDF1 mice. The lesions first appeared in 6-month-old female BDF1 mice and were aggravated with advancing age, especially in 24-month-old and 30-month-old senescent mice. In contrast, significant inflammatory changes did not develop in aging male BDF1 mice. The presence of antisalivary duct antibody was found in sera from mice with sialadenitis. The infiltrating cells in the lesions of submandibular salivary glands were mainly composed of T cells, especially Lyt 1^+ and L3T4$^+$ cells. Moreover, mild inflammatory lesions were observed in parotid, sublingual salivary glands, pancreas, or kidneys in some mice that developed spontaneously occurring sialadenitis. (Am J Pathol 1988, 132:173–179)

IN PREVIOUS COMMUNICATIONS, we presented experimental evidence that allergic sialadenitis developed in female CRJ:CD-1 mice thymectomized 3 days after birth and later immunized with a homogenate of the submandibular salivary gland emulsified with complete Freund's adjuvant,[1] or immunized with murine submandibular salivary gland cells that were infected with mumps virus *in vitro*.[2] These findings suggest that a deficit of T-cell-dependent immune response is a possible cause of destructive and long-lasting lesions of the salivary glands. In addition to the experimental evidence, the incidence of focal lymphocytic infiltration in the human submandibular salivary gland, probably due to an autoimmune process, showed a trend to increase with advancing age, as reported previously.[3] Previous immunologic investigations in aging mice have shown that T-cell-dependent immune responses decrease with advancing age and the decline in immunologic activities appears to be responsible for the increase in the incidence of diseases such as cancer, infection, or autoimmune disorder.[4,5] Thus, we were interested in whether any pathologic states of autoimmune nature including sialadenitis can develop in aging BDF1 mice that show an apparent T-cell-dependent immunologic disorder with advancing age.

The present study reports spontaneously occurring autoimmune sialadenitis in aging female BDF1 mice and discusses the possible immune mechanism based on autoimmunity associated with the aging process.

Materials and Methods

Animals

Female and male (C57BL/6NCrj × DBA/2NCrj)F1 (BDF1) mice at 4 weeks of age were purchased from Charles River Japan, Inc. (Atsugi, Japan) and used in the present study. Their mean life span was 28.2 months in females and 31.8 months in males. The mice were reared in our specific pathogen-free mouse colony. At 3, 6, 12, 18, 24, and 30 month of age, BDF1 mice of both sexes (a total of 133 mice) were killed and provided for histologic and immunohistochemical examination.

Histology

All organs were removed from the mice, fixed with 4% phosphate-buffered formaldehyde (pH 7.2), and

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Table 1—Degree of Inflammatory Infiltrate in Autoimmune Sialadenitis in BDF1 Mice

Months	No. of mice	No. of mice with lesion (grade 0–4)* 0	1	2	3	4	Mean grade of lesion ± SD†	Percent incidence‡
3 M								
Female	10	10	–	–	–	–	–	–
Male	10	10	–	–	–	–	–	–
6 M								
Female	10	6	2	2	–	–	0.60 ± 0.80	20.0%
Male	10	10	–	–	–	–	–	–
12 M								
Female	10	7	1	1	1	–	0.60 ± 1.02	20.0%
Male	10	8	2	–	–	–	0.20 ± 0.40	–
18 M								
Female	14	–	3	8	3	–	2.00 ± 0.65[a]	78.6%
Male	10	7	2	1	–	–	0.40 ± 0.66[b]	10.0%
24 M								
Female	15	–	2	7	3	3	2.47 ± 0.96[c]	86.7%
Male	10	3	5	2	–	–	0.90 ± 0.70[d]	20.0%
30 M								
Female	14	–	2	8	3	1	2.21 ± 0.77[e]	85.7%
Male	10	4	4	2	–	–	0.80 ± 0.75[f]	20.0%

* Inflammatory lesions were divided into four grades according to the method of White and Casarett.[6]

† Standard deviation.

‡ Percent incidence indicates the proportion of numbers of mice with inflammatory lesions with Grade 2 or more versus the total number of mice examined.

Difference between a and b, c and d, or e and f was statistically significant ($P < 0.02$, Mann–Whitney U test).

prepared for histologic examination. The sections were stained with the hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) method. Grading of the inflammatory lesions of submandibular salivary glands was classified according to the method proposed by White and Casarett.[6] Longitudinal sections of all glands were examined under ×150 magnification and scored for the degree of infiltration of mononuclear cells, including lymphocyte, plasma cells, and macrophages, as follows: Grade 1 indicates that 1–5 foci composed of more than 20 mononuclear cells per focus were seen; Grade 2 indicates that more than 5 such foci were seen but without significant parenchymal destruction; Grade 3 indicates that multiple confluent foci were seen in moderate degeneration of parenchymal tissue; and Grade 4 indicates extensive infiltration of the glands with mononuclear cells and extensive parenchymal destruction.

Immunohistochemical Staining

Immunohistochemical staining with monoclonal antibodies was performed on freshly frozen sections using the biotin-avidin immunoperoxidase method. Briefly, frozen sections approximately 4 μ thick were fixed in acetone and rinsed in cold phosphate buffered saline (PBS, pH 7.2). To inactivate endogeneous peroxidases, sections were treated in 0.3% H_2O_2 in methanol for 30 minutes. After washing with PBS, they were incubated for 1 hour with each of biotinylated rat monoclonal antibodies to Thy 1.2, Lyt 1, L3T4, and Lyt2 (Becton-Dickinson, Inc., Sunnyvale, CA. L3T4 was biotinylated in our laboratory). They were then washed with cold PBS for 30 minutes and incubated with avidin and biotinylated horseradish peroxidase complex (ABC reagent, Vector, Burmingham, CA) for 30 minutes.

After being washed 3 times with PBS, the sections were reacted with a fresh mixture of 0.05% 3,3-diaminobenzidine and 0.005% H_2O_2 in Tris-HCl buffer (0.05M, pH 7.6) for 5 minutes, washed with distilled water, and were lightly counterstained with hematoxylin. To detect the B-cell lineage, the sections were incubated with rabbit serum to each of mouse IgG, IgM, or IgA (Miles Laboratories, Erkhart, IN) at a dilution of 1:60 for 30 minutes at room temperature. Thereafter, anti-rabbit IgG labeled with horseradish peroxidase (Miles Laboratories) was applied for 30 minutes at room temperature. The peroxidase was localized in the manner described above.

Detection of Anti-Salivary Duct Antibody

The presence of antisalivary duct antibody in sera was examined by the indirect immunofluorescent antibody staining technique for the presence of antisalivary duct antibody as described previously.[1,2] Briefly, frozen sections of submandibular salivary gland from 3-month-old female BDF1 mice were prepared and reacted with a testing serum and fluorescein isothiocyanate (FITC)-labeled rabbit antisera to mouse IgG (Miles Laboratories). The titer of antisalivary duct antibody was expressed as the highest dilution of testing serum giving positive staining on the frozen sections. To confirm specificity of the reaction, absorption tests were carried out as follows. The submandibular salivary glands, liver, kidney, and adrenals obtained from 3-month-old mice were chopped with scissors. They were homogenized gently in an equal volume (weight/volume) of cold PBS and centrifuged at 5000*g* for 30 minutes. The resulting pellets were suspended in an appropriate volume (volume/volume) of twofold dilution of a testing serum and stood for 1 hour at 37 C. This mixture was centrifuged at 8000*g* for 20 minutes, and the supernatant was used as a testing sample. In addition, tissue sections from the pancreas, thyroid, brain, and kidney of the aging mice were examined for immunoreactivity of antisalivary duct antibody.

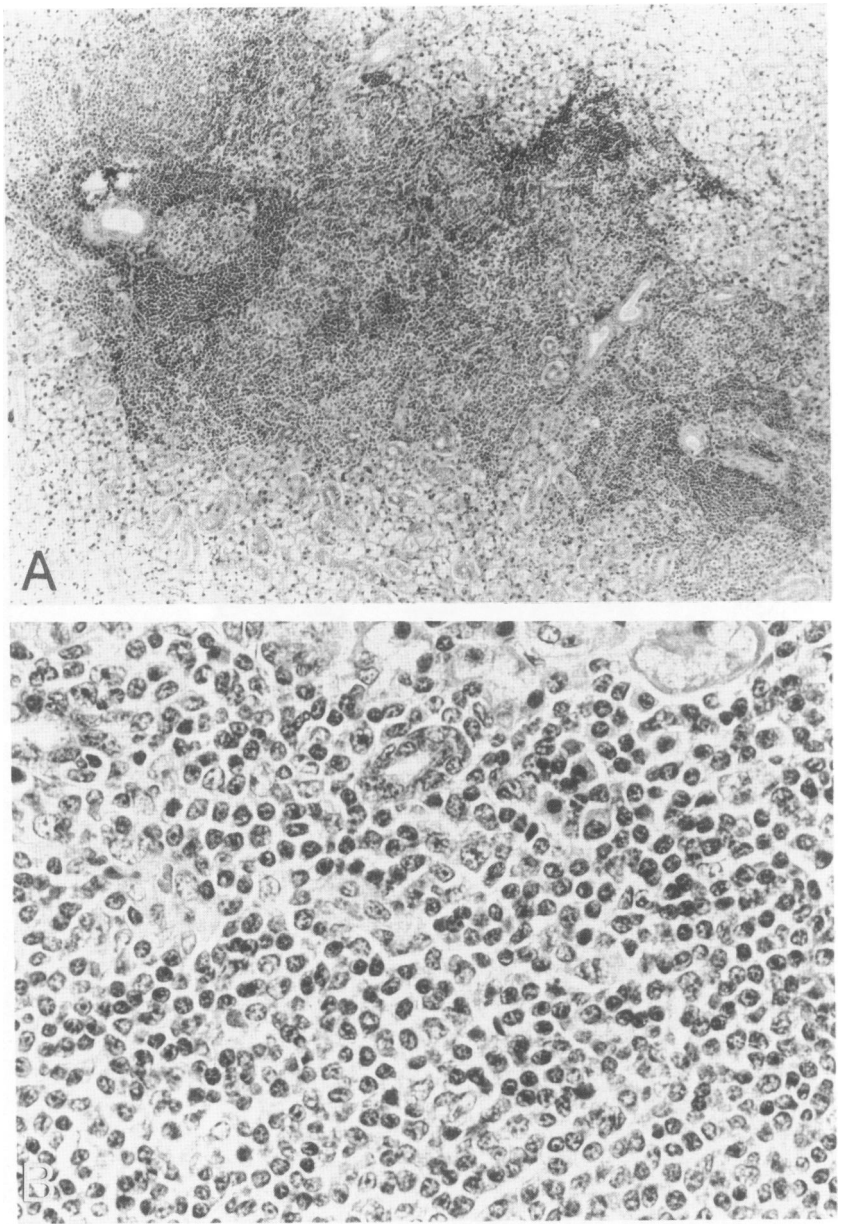

Figure 1—Histologic appearance of spontaneously occurring sialadenitis in 24-month-old female BDF1 mouse. **A and B**—Photomicrographs demonstrating a severe destructive lesion (grade 4) of the submandibular salivary gland with extensive infiltration of mononuclear cells. Infiltrative mononuclear cells consists of small and medium-sized lymphocytes. (A, H&E, ×70; B, H&E, ×360)

Results and Discussion

It has been well known that sialadenitis appears spontaneously in NZB/NZW and SL/Ni mice, with lesions resembling human diseases such as systemic lupus erythematosus and polyarteritis nodosa.[7–12] Moreover, we have found that allergic sialadenitis is induced in the submandibular salivary gland of female CRJ:CD-1 mice by thymectomy 3 days after birth and later is immunized with a homogenate of the submandibular salivary gland of female CRJ:CD-1 mice, emulsified with Freund's complete adjuvant,[1] or immunized with murine submandibular salivary gland cells that were infected with mumps virus *in vitro* without the use of any adjuvant materials.[2] Sialadenitis observed in these mice was similar to that reported in the salivary gland of the patients with Sjögren's syndrome.[13]

In addition, it has been reported that neonatal thymectomy in certain strain of mice results in the spontaneous development of inflammatory lesions similar to human autoimmune diseases in the thyroid, ovary, kidney, testis, and stomach.[14–18] These data imply that a deficit of cellular immune function may be responsible for the development of organ-localized autoimmune diseases.

The decline in T-cell-dependent immune response with advancing age has been well documented in humans, rats, guinea pigs, and mice.[19–21] In the BDF1 mice used for this study we found that T-cell-depen-

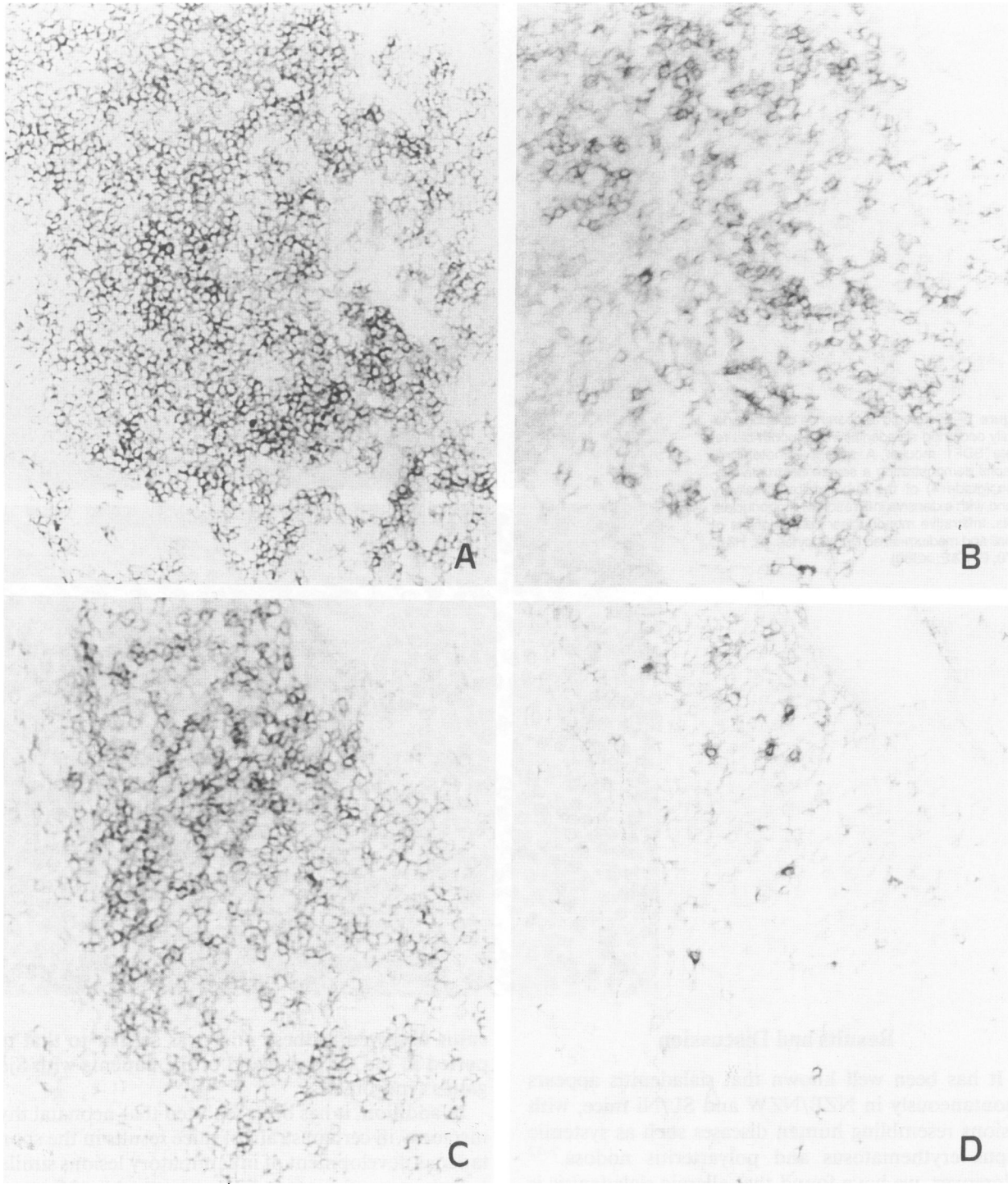

Figure 2—Immunohistochemical detection of infiltrative lymphocytes in spontaneously occurring sialadenitis. Positive staining of Thy1.2 (**A**), Lyt 1 (**B**), L3T4 (**C**), and Lyt 2 (**D**) in 4 serial sections. The majority of lymphocytes recognized as T cells (**A**), which were composed chiefly of Lyt 1^+, L3T4$^+$ cells (**B,C**) and a lesser number of Lyt 2^+ cells (**D**). (A–D, Immunoperoxidase staining, ×300)

dent immunologic activities assessed by phytohemagglutinin (PHA), concanavalin A (Con A), and mixed leukocyte reaction (MLR) showed a decline with advancing age, as reported previously.[22,23] However, histopathologic and immunohistochemical studies in aging BDF1 mice have not been reported. Table 1 summarized the degree of mononuclear cell infiltration and parenchymal destruction of the subman-

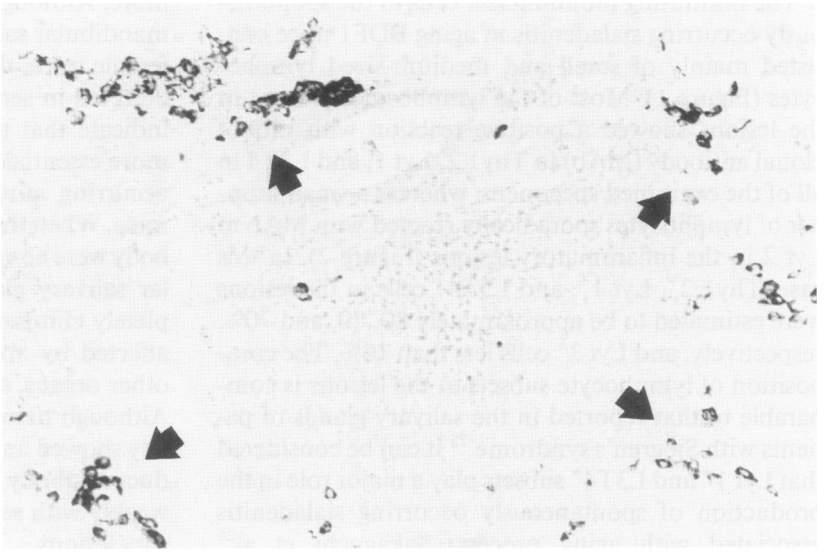

Figure 3—Detection of Ig-bearing cells in infiltrative mononuclear cells in spontaneously occurring sialadenitis. IgG-bearing cells (arrows) appear in the periphery of the inflammatory lesions in autoimmune sialadenitis. (Immunoperoxidase staining, ×240)

dibular salivary glands in individual mice of each age group.

The incidence and severity of spontaneously occurring autoimmune sialadenitis in BDF1 mice increased gradually with advancing age, and were observed clearly in more often females than males. Significant sex differences in occurrence of sialadenitis were observed at 18, 24, and 30 months of age ($P < 0.02$). The sex difference in the incidence of sialadenitis has been acknowledged as a distinct feature of the human autoimmune diseases. A significantly higher incidence in females has been reported in spontaneous thyroiditis in the rat.[24] The Grade 4 inflammatory lesion, indicating extensive infiltration of mononuclear cells and destruction of parenchymal tissues, was seen in 3 of 15 and 1 of 14 female mice examined at 24 and 30 months of age. None of the 3- and 6-month-old mice of both sexes and none of the aging male mice had an inflammatory lesion of more than Grade 3 in the submandibular salivary gland. Mild inflammatory lesions could be observed in some organs, such as the parotid and sublingual salivary gland, pancreas, and kidney. Inflammatory lesions could not be observed in any of the mice of any different age in organs such as the thyroid, ovary, testis, adrenal, and stomach.

Table 2—Search for Antisalivary Duct Antibody in Sera* from Mice of Different Month of Age

Months	Sex	No. of testing sera	No. of mice giving FA-positive reaction (Antibody titer†) 1:5	1:20	1:80	1:160
3 M	Female	4	–	–	–	–
	Male	4	–	–	–	–
6 M	Female	5	–	–	–	–
	Male	5	–	–	–	–
12 M	Female	5	1‡‖	–	–	–
	Male	5	–	–	–	–
18 M	Female	5	1‖	1‖	1¶	–
	Male	5	1§	–	–	–
24 M	Female	5	–	2§‖	2¶**	1**
	Male	5	1§	–	–	–
30 M	Female	5	1§	2§‖	1¶	1¶
	Male	5	1§	–	–	–

* Each testing serum was harvested from individual mice.

† Titer of antisalivary duct antibody is expressed as the highest serum dilution giving FA-positive reaction.

‡ Inflammatory lesions devided into four grades according to the method of White and Casarett (6).

§ Grade 0 or Grade 1.

‖ Grade 2.

¶ Grade 3.

** Grade 4. See the text for details.

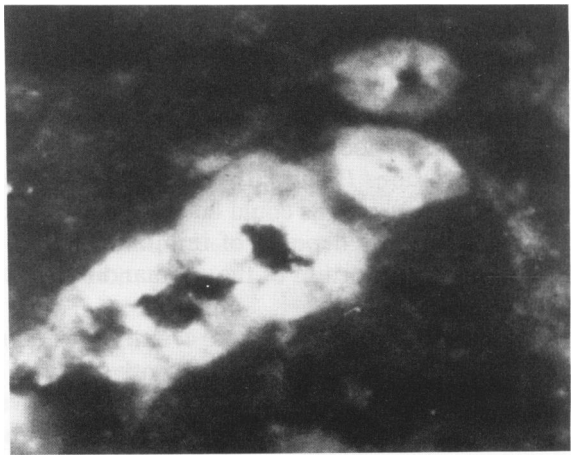

Figure 4—Antisalivary duct antibody detected in the serum from a 24-month-old female BDF1 mouse. The dilution of testing serum used was 80-fold.

The infiltrating mononuclear cells in the spontaneously occurring sialadenitis in aging BDF1 mice consisted mainly of small and medium-sized lymphocytes (Figure 1). Most of the lymphocytes present in the lesions showed a positive reaction with monoclonal antibody (MAb) to Thy1.2, Lyt 1, and L3T4 in all of the examined specimens, whereas a small number of lymphocytes sporadically reacted with MAb to Lyt 2 in the inflammatory lesions (Figure 2). In this case, Thy1.2$^+$, Lyt 1$^+$, and L3T4$^+$ cells in the lesions were estimated to be approximately 80, 70, and 70%, respectively, and Lyt 2$^+$ cells less than 10%. The composition of lymphocyte subsets in the lesions is comparable to that reported in the salivary glands of patients with Sjögren's syndrome.[25] It can be considered that Lyt 1$^+$ and L3T4$^+$ subsets play a major role in the production of spontaneously occurring sialadenitis associated with aging process. Sakaguchi et al[26] showed that autoimmune diseases such as oophoritis and thyroiditis could be adoptively transferred to syngeneic mice or nude mice by Lyt 1$^+$ cells only. In addition, Sakaguchi et al[27] described that the cells responsible for disease induction are believed to be Thy 1$^+$, Lyt 1$^-$, and Lyt 2$^-$. They concluded that T cells are required as effector cells, and that these may develop from Lyt 1$^-$, Lyt 2$^-$ cells, although the lymphocytes that infiltrated the inflammatory lesions were not examined. Jabs et al reported that the majority of infiltrating lymphocytes in both the lacrimal gland inflammatory lesions and renal vasculitis of MRL/lpr mice expressed L3T4.[28] However, it appears likely that a Lyt 2$^+$ subset directly destroying parenchymal tissues was observed in allergic sialadenitis induced by various immunizations, as reported previously.[1,2]

This suggests that the pathogenic mechanisms of the allergic lesions induced in the submandibular salivary glands may be different from spontaneously occurring sialadenitis associated with aging process.

Moreover, immunoglobulin (Ig)-bearing cells were demonstrated in the specimens with inflammatory lesions in the submandibular salivary glands. The IgG-bearing cells were located in the periphery of the inflammatory lesions (Figure 3), although only a few IgM- or IgA-bearing cells could be observed. In addition, there were no deposits of immunoglobulin in any parenchymal portion of the submandibular salivary glands affected.

As shown in Table 2 and Figure 4, antisalivary duct antibody of IgG type was more frequently detected in aging female rather than male BDF1 mice. Moreover, a good correlation was noticed between the presence of antisalivary duct antibodies and the severity of the lesions in individual mice. Eleven of 17 (65%) mice with positive sera had sialadenitis with Grade 2 or more. Although the inflammatory lesions in the submandibular salivary glands appeared in 6-month-old female mice, this antisalivary duct antibody was first detected in sera from 12-month-old mice. This may indicate that the cellular immune responsiveness is more essential for the development of spontaneously occurring autoimmune sialadenitis in aging BDF1 mice. When the sera containing antisalivary duct antibody were absorbed by a homogenate of submandibular salivary gland, this antibody activity was completely eliminated, whereas the sera activity was not affected by absorption with the homogenate of the other organs, such as the liver, kidney, and adrenals. Although tissues such as the thyroid, brain, and kidney showed an FA-negative reaction with antisalivary duct antibody, the parotid gland or pancreas stained weakly with sera from mice with the mild inflammatory lesions.

The inflammatory lesions that developed in the submandibular salivary gland of aging BDF1 mice can be considered to be autoimmune sialadenitis but were not caused by nonspecific infection in the salivary gland. This is derived from the presence of antisalivary duct antibody in aging BDF1 mice with sialadenitis. Keyes et al[11] described an age-related decline in responsiveness to various mitogens such as PHA, Con A, and lipopolysaccharide in NZB/NZW mice that was associated with an increase in the onset of the lesion resembling Sjögren's syndrome in humans and the progression of lymphoid cell infiltration into the salivary glands. Also, it is well known that there is an age-related increase in the incidence of serum autoantibodies in humans and rodents associated with age-related decline in various normal immune functions.[29,30] In addition, it was shown that the percentage of Lyt 2$^+$ cells in the spleen decreased with advancing age, whereas that of L3T4$^+$ cells was relatively constant throughout the lifespan in mice.[31] These findings indicate that some sort of autoimmune mechanism associated with the aging process functions for the formation of the inflammatory lesions in the submandibular salivary glands.

To our best of knowledge, spontaneously occurring sialadenitis in nonautoimmune prone mice has not yet been reported except in this strain of aging mice. This may become an useful animal model in searching for the pathogenesis and/or genetics of human susceptibility to Sjögren's syndrome.

References

1. Hayashi Y, Sato M, Hirokawa K: Induction of experimental allergic sialadenitis in mice. Am J Pathol 1985, 118:476–483

2. Hayashi Y, Yura Y, Yoshida H, Yanagawa T, Sato M: Development of allergic sialadenitis in mice immunized with mumps virus-infected submandibular salivary gland. Am J Pathol 1986, 123:271–279
3. Kurashima C, Hirokawa K: Age-related increase of focal lymphocytic infiltration in the human submandibular glands. J Oral Pathol 1986, 15:172–178
4. Walford RL: The immunologic Theory of Aging. Copenhagen, Munkusgaard, 1969
5. Makinodan T, Kay MMB: Age influence on the immune system. Adv Immunol 1980, 29:287–330
6. White SC, Casarett GW: Induction of experimental autoallergic sialadenitis. J Immunol 1974, 112:178–185
7. Kessler HS: A laboratory model for Sjögren's syndrome. Am J Pathol 1968, 52:671–685
8. Kyogoku M: Pathogenesis of vasculitis in the SL/Ni mouse, systemic lupus erythematosus. Edited by Japan Medical Research Foundation. Tokyo, Tokyo Press, 1980, pp 281–294
9. Greenspan JS, Gutman GA, Weissman IL, Talal N: Thymus-antigen and immunoglobulin-positive lymphocytes in tissue infiltrates of NZB/NZW mice. Clin Immunol Immunopathol 1974, 3:16–31
10. Carlosöö B, Östberg Y: Ultrastructural observation on the parotitis autoimmunica in the NZB/NZW hybrid mice. Acta Otolaryngol 1978, 85:298–305
11. Keyes GG, Vicker RA, Kersey JH: Immunopathology of Sjögren-like disease in NZB/NZW mice. J Oral Pathol 1977, 6:288–295
12. Takeda Y, Ishikawa G: Experimental alloallergic sialadenitis in mice: Histopathological and ultrastructural studies. Virchows Arch[Pathol Anat] 1983, 400:143–154
13. Greenspan JS, Daniels TE, Talal N, Silvester RA: The histopathology of Sjögren's syndrome in labial salivary gland biopsies. Oral Surg 1974, 37:217–221
14. Kojima A, Tanaka-Kojima A, Sakakura T, Nishizuka Y: Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. Lab Invest 1976, 34:550–557
15. Welch P, Rose NR, Kite JH: Neonatal thymectomy increase spontaneous thyroiditis. J Immunol 1973, 110: 575–577
16. Nishizuka Y, Sakakura T: Ovarian dysgenesis induced by neonatal thymectomy in the mouse. Endocrinol 1971, 89:866–893
17. Taguchi O, Nishizuka Y, Sakakura T, Kojima A: Autoimmune oophoritis in thymectomized mice; detection of circulating antibodies against oocytes. Clin Exp Immunol 1980, 40:540–553
18. Taguchi O, Nishizuka Y: Experimental autoimmune orchitis after neonatal thymectomy in the mice. Clin Exp Immunol 1981, 46:425–434
19. Walford RL: Immunologic theory of aging: Current status. Fed Proc 1974, 33:2017–2019
20. Makinodan T, Good RA, Kay MMB: Cellular basis of immunosenescence, Immunology and Aging. New York, Plenum Medical Book Co., 1977
21. Segre D, Segre M: Age-related changes in B and T lymphocytes and decline in humoral immune responsiveness in aged mice. Mech Ageing Dev 1977, 6:115–129
22. Hirokawa K, Utsuyama M, Goto H, Kuramoto K: Differential rate of age-related decline in immune functions in genetically defined mice with different tumor incidence and life span. Gerontology 1984, 30:223–233
23. Hirokawa K: Autoimmunity and Aging, Concepts in Immunopathology. Basel, Karger, 1985, pp 251–288
24. Silverman DA, Rose NR: Spontaneous and methylcholanthrene-enhanced thyroiditis in BUF rats. J Immunol 1975, 114:145–147
25. Adamson, TC, Fox RI, Frisman DM, Howell FV: Immunohistological analysis of lymphoid infiltrates in primary Sjögren's syndrome using monoclonal antibodies. J Immunol 1983, 130:203–207
26. Sakaguchi S, Takahashi T, Nishizuka Y: Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage after adoptive transfer. J Exp Med 1982, 156:1565–1576
27. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T: Organ-specific autoimmune diseases in mice by elimination of T cell subsets. J Exp Med 1985, 161:72–87
28. Jabs DA, Prendergast RA: Reactive lymphocytes in lacrimal gland and vasculitic renal lesions of autoimmune MRL/lpr mice express L3T4. J Exp Med 1987, 166: 1198–1203
29. Rowley MJ, Buchman H, Machay IR: Reciprocal change with age in antibody to extrinsic and intrinsic antigens. Lancet 1968, 2:24–26
30. Bullock JY, Booth RJ, Wilson JD: Tissue antibodies in a healthy New Zealand population. NZ Med J 1979, 89: 11–13
31. Utsuyama M, Hirokawa K: Age-related changes of splenic T cells in mice: A flow cytometric analysis. Mech Aging Dev 1987, 40:89–102