Virchows Arch [Pathol Anat] (1983) 400:143–154

Virchows Archiv A

Experimental autoallergic sialadenitis in mice

Histopathological and ultrastructural studies

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Summary. Experimental autoallergic sialadenitis was induced in SL/Ni mice by one or two injections of syngeneic submandibular gland homogenate emulsified with adjuvant. Light microscopically, there were marked lymphoid cell infiltration in the submandibular glands with high incidence and proliferation of duct epithelia. Furthermore complete alteration of whole glandular lobules in some cases was observed. Ultrastructurally, small and medium sized lymphocytes and plasma cells constituted a major portion of the infiltrating cells, and lymphocytes were frequently observed inside the basal lamina of ductal and acinar regions, especially observed in the small ductal region. In the aggregates of infiltrating cells, the cell remnants of salivary gland epithelia were scattered. Furthermore some of the epithelial cell remnants in aggregates of infiltrating cells could be recognized as epithelial masses which were composed of proliferated duct epithelial cells, though no typical structure of epimyoepithelial islands seen in Sjögren's syndrome was found. Anti-salivary duct antibody was detected in only one case.

Key words: Experimental autoallergic sialadenitis – Mice – Submandibular gland – Histopathology – Ultrastructure

Introduction

Secretory gland destruction associated with lymphoid cell infiltration is responsible for impaired secretions and salivary gland swelling in patients with chronic sialadenitis, e.g. Sjögren's syndrome, chronic recurrent sialadenitis and others. Although these chronic salivary gland diseases are well studied for the investigation of pathogenesis including autoimmunity, its exact mechanism by which the secretory glands are destroyed is unknown. Experimental autoallergic diseases have been produced in various organs in order to develop animal models of immunologically mediated diseases

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in man. Recently, an experimental autoallergic sialadenitis with various grades of lesion and rapid time of onset has been reported by White et al. (1973, 1974) and Sharawy and White (1978) in rat. But atempts to obtain an experimental animal model resembling Sjögren's syndrome and other chronic sialadenitis in man have been unsuccessful. On the other hand, NZB/NZW mice, which are generally considered models for human systemic lupus erythematosus spontaneously develop generalized lymphoid cell infiltration in many organs, especially in the salivary and lacrimal glands. It has been thought that pathological changes of the salivary glands occuring in NZB/NZW mice are similar in most respects to those which characterize Sjögren's syndrome in man (Kessler 1968; Greenspan et al. 1974; Keyes et al. 1977; others). We have also attempted to obtain a new laboratory model for Sjögren's syndrome overlapping other autoimmune diseases, and have examined the salivary gland in SL/Ni mice which spontaneously develop PN-like arteritis, SLE-like glomerulonephritis and occasional malignant lymphoma. As a result of our previous histopathological examination of SL/Ni mice, slight to moderate lymphoid cell infiltration in the salivary glands spontaneously occurred (Takeda et al. 1981). The purpose of the present study is to produce more severe salivary gland lesions in SL/Ni mice.

Materials and methods

Mice. Sixty female SL/Ni mice examined used were 10 months of age. Donors were sexually mature female same mice.

Antigen. The syngeneic submandibular glands were decapsulated, minced and homogenized with an equal volume (w/v) of phosphate buffered saline in a homogenizer and used as salivary gland antigen.

Immunization procedure. The procedure was mostly according to the method of White et al. (1973, 1974). The homogenate was emulsified with an equal volume of Freund's complete adjuvant (Difco, Ltd). The emulsion was injected subcutaneously into the four foot pads of mice, followed by intravenous injection of pertussis vaccine (Takeda Chem Indust, Ltd). Controls were not treated or received adjuvant only. Thirty animals were sacrificed at 1, 2 and 4 weeks after the 1st immunization. In the other 30 animals, 2nd immunization was performed in the same manner at 2 weeks after the 1st immunization and animals were sacrificed at 1, 2 and 4 weeks after the 2nd immunization.

Histopathology. Various organs including the submandibular, sublingual and parotid salivary glands were removed and immediately fixed in 10% neutral buffered formalin. The glands were then embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for light microscopical examination.

Ultrastructure. Small pieces of submandibular gland tissues obtained from each animal were immediately fixed in 4.0% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 h at 0 to 4° C, rinsed in the same buffer, post-fixed in 1.0% osmium tetroxide, and embedded in Epon 812. Ultrathin sections were cut, stained with uranylacetate and lead citrate, and examined in a Akashi LEM 2000 ultramicroscope.

Antisalivary duct antibody (ASDA). Serum from each animal was tested for ASDA by indirect immunofluorescence as the method of Feltkamp and van Rossum (1968). The submandibular salivary gland tissue, obtained from other mouse, was frozen on a cryostat and cut into

4 μm sections. The tissue sections were thawed, overlaid with undiluted test serum for 30 min, washed in phosphate buffered saline with 3 changes, overlaid with fluoresceinated rabbit anti-mouse immunoglogulin (IgG; Dako, Ltd) for 30 min, washed with saline for 20 min with 3 changes, and coverslipped. Sera yielding definite fluorescence of duct epithelial cells was scored ASDA-positive. Sera giving equivocal fluorescence was considered ASDA-negative.

Results

1. Histopathology

Control group. In control group, slight lesions showing a few foci of lymphoid cells (more than 100 cells per focus) in the periductal areas were seen in 44.4%, and moderate lesions showing multiple confluent foci were seen in 22.2% of submandibular glands. There were no severe lesions showing extensive lymphoid cell infiltration with extensive parenchymal destruction, or complete alteration of whole lobule.

Experimental group. In experimental group, slight to severe lesions were found in 95.0% of submandibular glands (slight lesions in 33.3%; moderate lesions in 46.7%; severe lesions in 15.0%) in total (Table 1). It was clear that the incidence and grade of lesions were higher in experimental group than in control one. The severity of lymphoid cell infiltration was different in each lobule of the same gland. Figure 1a showed focal and periductal lymphoid cell infiltration assigned as a grade of "moderate", and Fig. 1b showed diffuse and widespread lymphoid cell infiltration with extensive parenchymal destruction assigned as a grade of "severe". In some lesions with severe lymphoid cell infiltration, thickened duct wall suggesting proliferation of the duct epithelia with lymphoid cell infiltration was found in the lymphoid cell aggregates (Fig. 2).

There were no important difference in incidence and grade of lymphoid cell infiltration in subgroups sacrificed at 1, 2 and 4 weeks after the 1st

Table 1. Incidence and grade of sialadenitis in submandibular glands in experimental and control mice

Groups	Mice No.	No. of mice with lesion			
		no lesion	slight	moderate	severe
1st immunization					
1 week after	10	1	4	5	0
2 weeks after	10	0	3	7	0
4 weeks after	10	0	4	6	0
2nd immunization					
1 week after	10	1	5	4	0
2 weeks after	10	0	3	3	4
4 weeks after	10	1	1	3	5
Control	9	3	4	2	0

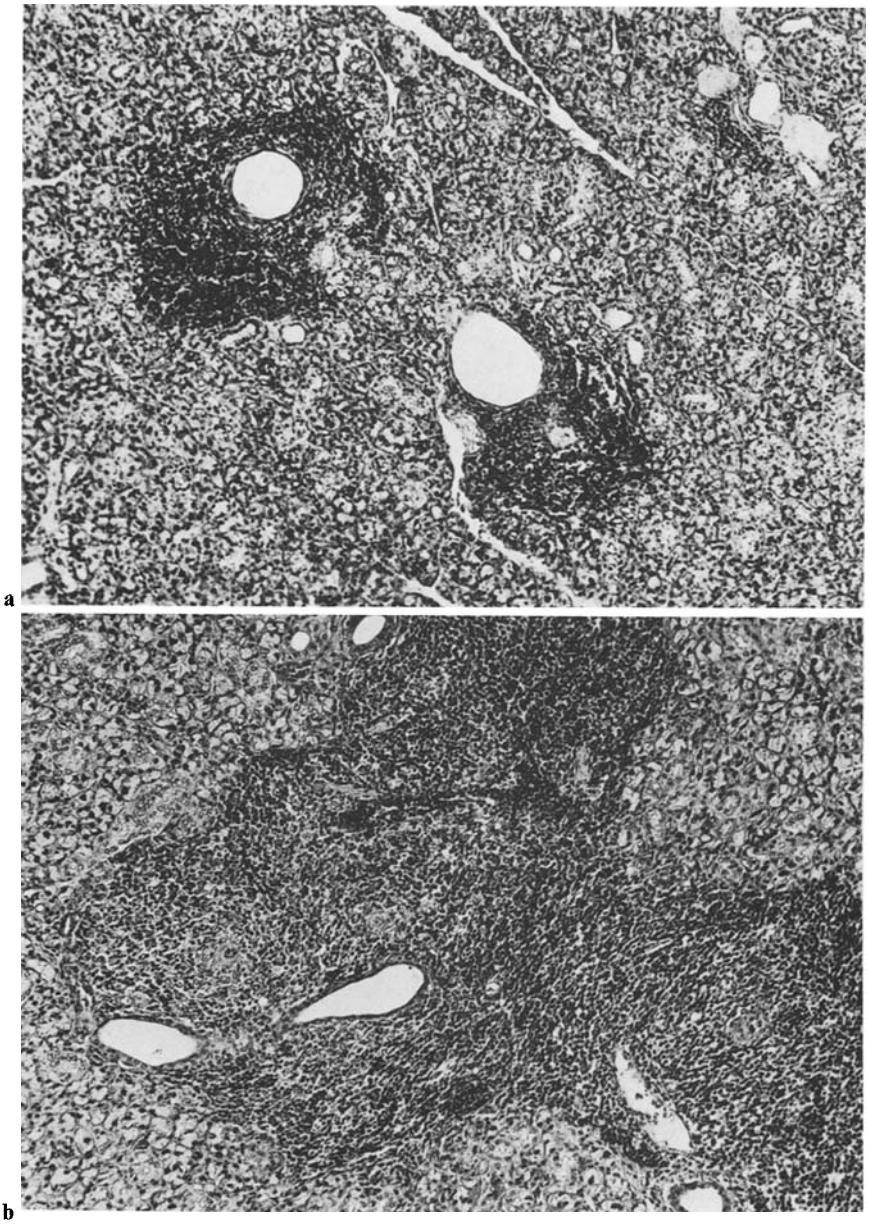


Fig. 1 a, b. Light microphotographs of submandibular salivary gland lesion in experimental mice. **a** is moderate lesion showing multifocal lymphoid cell infiltration in periductal areas. **b** is severe lesion showing extensive lymphoid cell infiltration. **a** and **b** × 70

immunization, while severe lesions were only found in subgroups sacrificed at 2 and 4 weeks after the 2nd immunization. Furthermore, complete alteration of whole glandular lobules with fibrosis, diffuse lymphoid cell infiltration and dilatation of duct lumen in various degrees was found in some cases of subgroups sacrificed at 2 and 4 weeks after the 2nd immunization

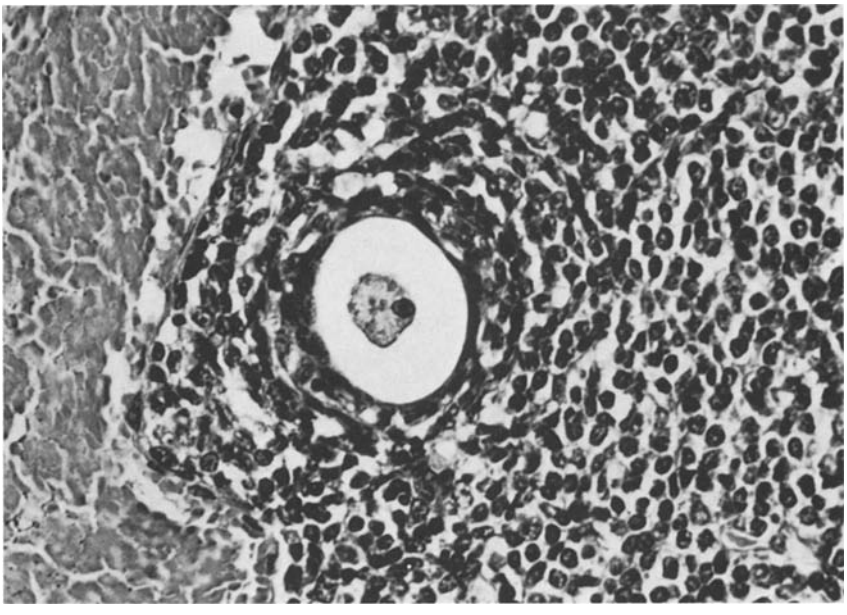


Fig. 2. Thickened duct wall suggesting proliferation of the duct epithelial cells found in the lymphoid cell aggregate. ×400

(Fig. 3a, b). Although the salivary gland lesions were the most extensive in the submandibular glands, less in the subligual glands and still less in the parotid glands.

2. Ultrastructure

At the ultrastructural level the mononuclear cell aggregates were found to be chiefly composed of lymphocytes and plasma cells (Fig. 4), and a small number of histiocytes and mast cells were also found. But leukocytes were not found in any area.

Small and medium sized lymphocytes (4 to 10 μm in diameter) were the most numerous cells, which had a sparse cytoplasm containing a little organellae. Many mature plasma cells with stacks of parallel, dilated, rough surfaced endoplasmic reticulum were also encountered. In some areas the plasma cells were more numerous than the lymphocytes.

In the areas of diffuse lymphoid cell infiltration, lymphocytes were interspersed between the epithelial cells of acini and ducts in various degrees, especially at the small ductal region. Many of the epithelial cells, in close proximity to invading lymphocytes, appeared damaged. They displayed degenerative features such as decreased cytoplasmic density, dilated or vesiculated endoplasmic reticulum, cytoplasmic vacuolization, and cellular lysis (Fig. 5).

In the area of dense aggregates of infiltrating cells, the salivary gland parenchyma was completely disappeared, and various amounts of necrotic cell debris were also found within the cell aggregates (Fig. 4). In the other areas, cell remnants of salivary gland epithelia were scattered in the aggre-

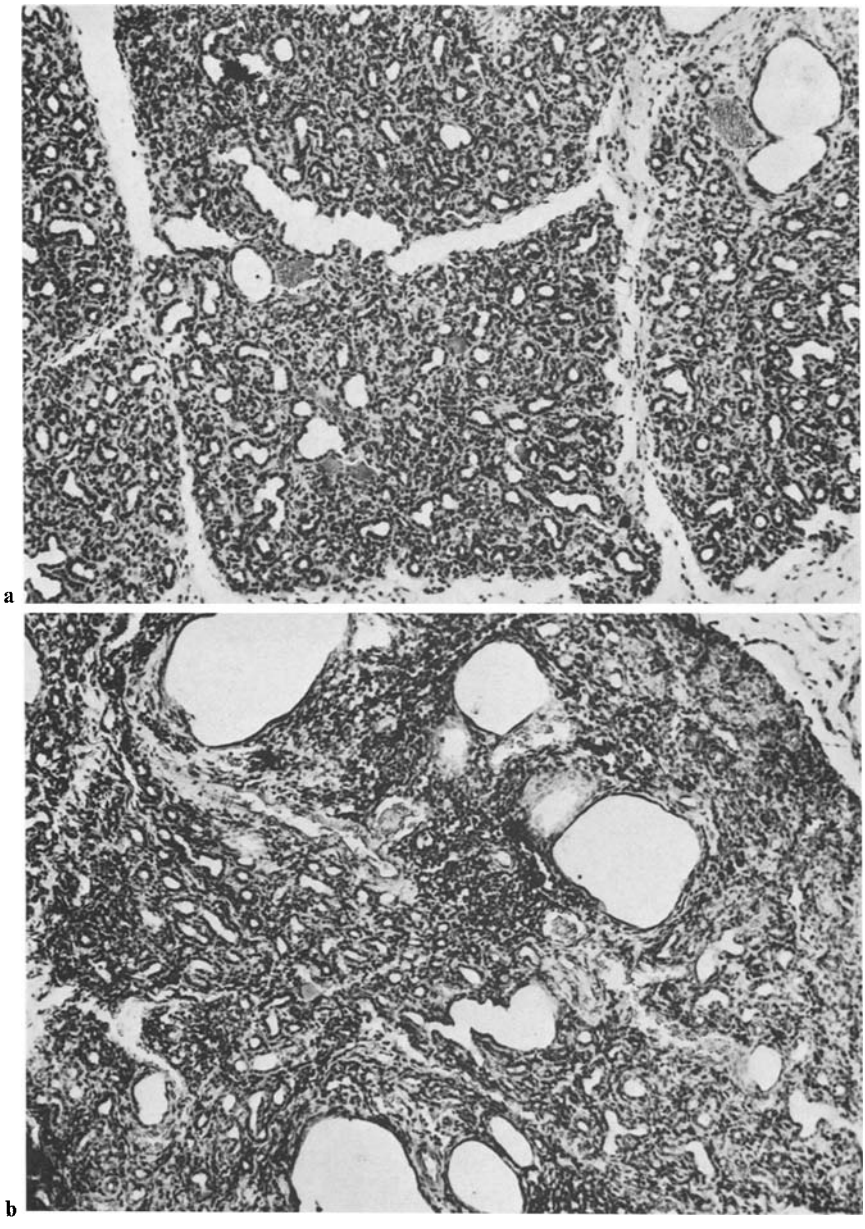
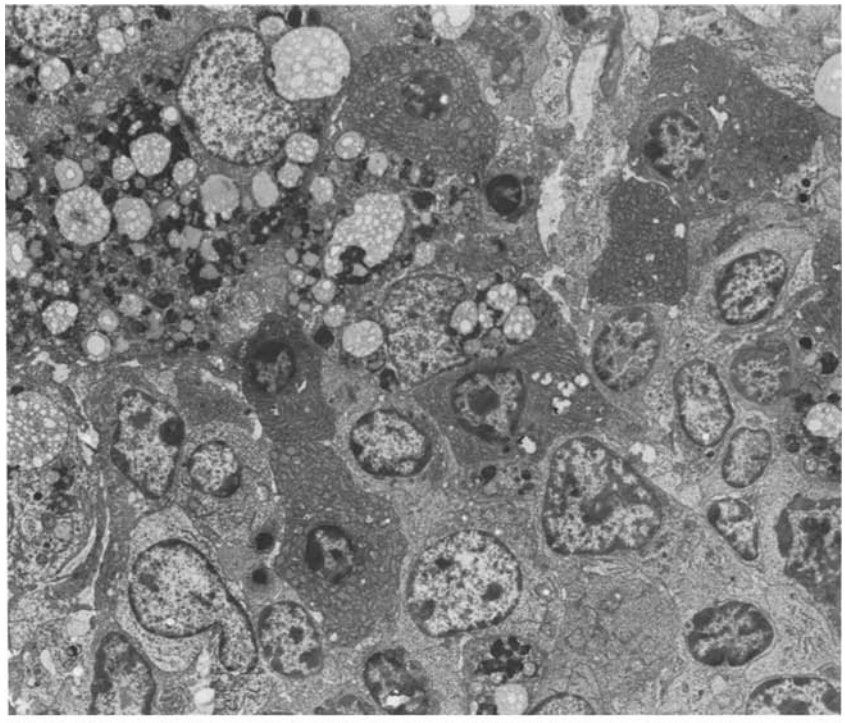


Fig. 3a, b. Complete alteration of whole gladular lobules with fibrosis, diffuse lymphoid cell infiltration, and dilatation of duct lumen in various degrees. **a** and **b** ×70

gates of infiltrating cells (Fig. 6), and lymphocytes were also interspersed between the epithelial cells (Fig. 7). Furthermore some of epithelial cell remnants recognized as epithelial masses composed of proliferated duct epithelial cells (light cells and dark cells), and a few myoepithelial cells (Fig. 8). The light cells had a round or ovoid nucleus and scanty other organellae.



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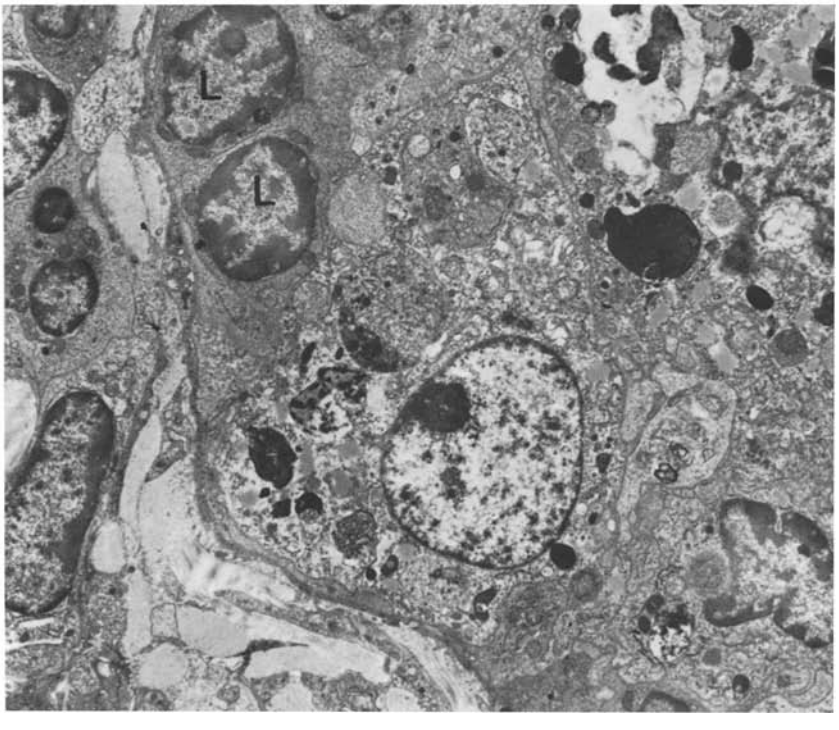

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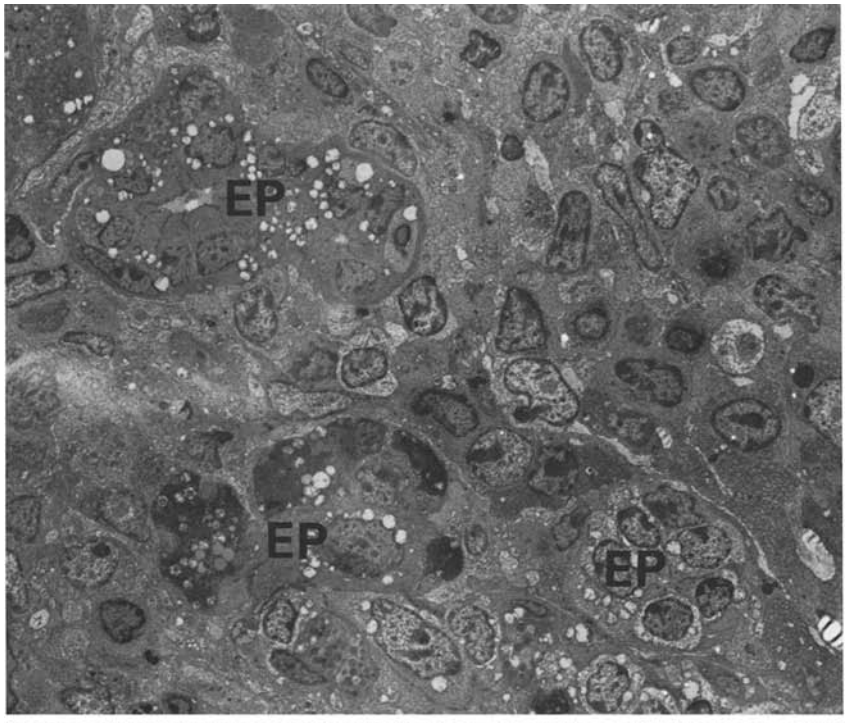
Fig. 4. Ultrastructural photograph of infiltrating cell aggregete. Infiltrating cells are chiefly composed of small and medium sized lymphocytes and plasma cells. Necrotic cell debris are seen in *upper left* of the figure. ×2,160

Fig. 5. Lymphocytes (*L*) are observed inside basal lamina of the duct, and duct epithelial cells show degenerative appearance. ×3,600



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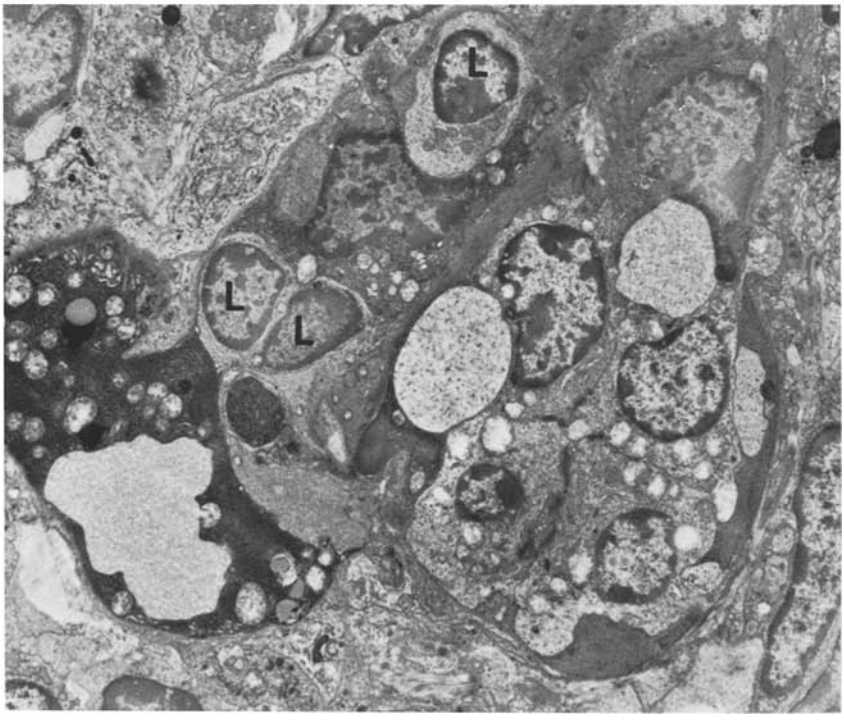

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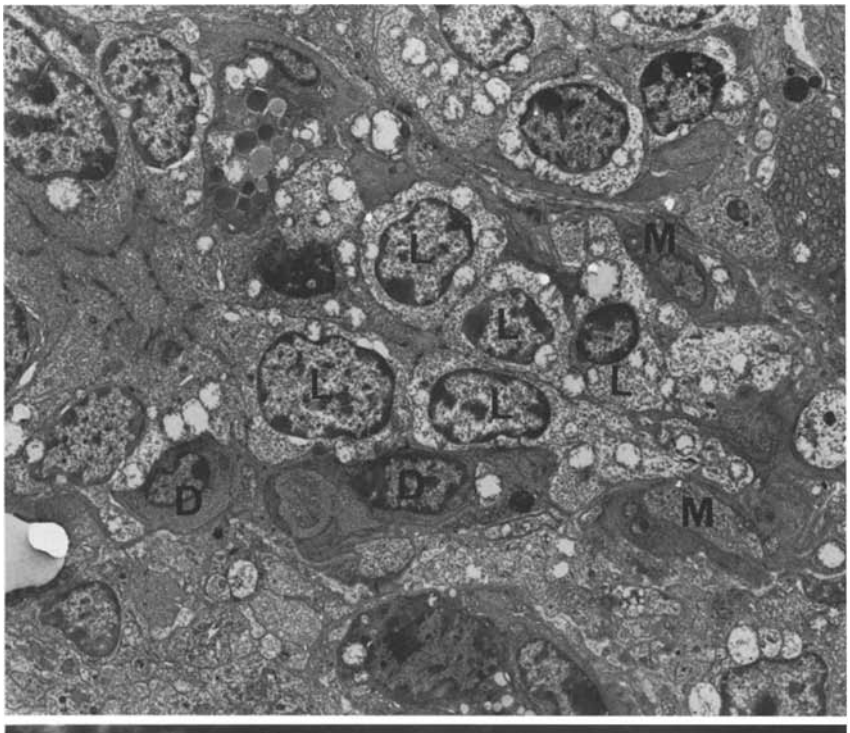
Fig. 6. Cell remnants of salivary gland epithelia (*EP*) are scattered in the aggregates of infiltrating cells. × 1,440

Fig. 7. Lymphocytes (*L*) are interspersed in the epithelial remnant (intercalated region) scattering in aggregate of infiltrating cells. × 3,600



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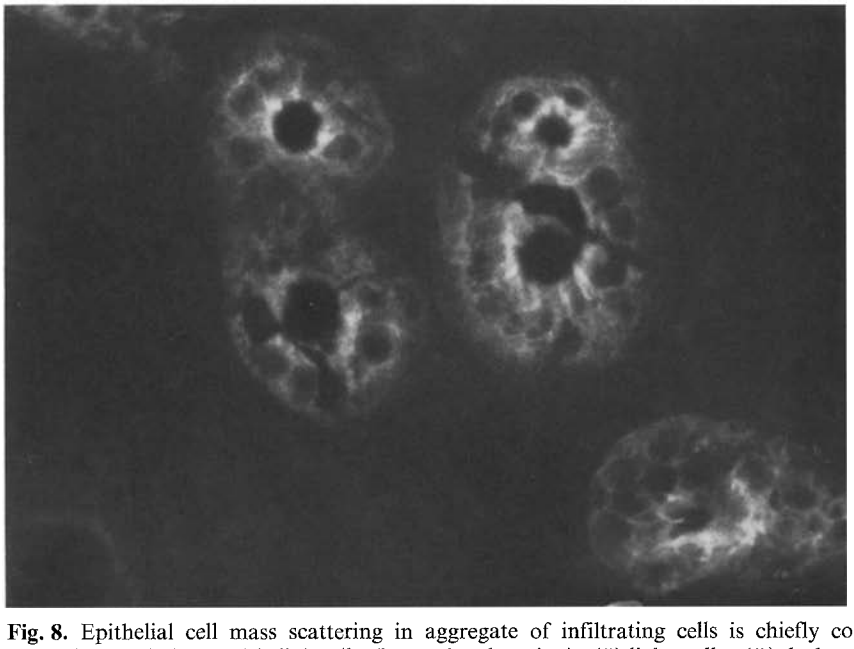

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Fig. 8. Epithelial cell mass scattering in aggregate of infiltrating cells is chiefly composed of proliferated duct epithelial cells (intercalated region). (*L*) light cells, (*D*) dark cells and (*M*) myoepithelial cells. × 2,880

Fig. 9. Antisalivary duct antibody found in 2 weeks after the 2nd immunization

The dark cells were smaller and denser than the light cells, and fine fibrils were loosely and irregularly distributed throughout the cytoplasm. A few myoepithelial cells were located in the periphery of the epithelial cell islands. Basal lamina of the epithelial cell islands were intact, while lamina densa was thickened with high electron density in part.

3. Antisalivary duct antibody (ASDA)

Test for ASDA was performed on all control and experimental mice sera by indirect immunofluorescent method, and definite fluorescence of duct epithelial cells was seen in only one case (2 weeks after the 2nd immunization, Fig. 9) of experimental mice. This ASDA-positive case had slight lymphoid cell infiltration in the salivary glands.

Discussion

There have so far been several reports on histopathological observations in experimental autoallergic sialadenitis in animals (McCabe 1961; Waterhouse 1963; Chan 1964; Whaley and MacSween 1974; Boss et al. 1977; White et al. 1973, 1974; Sharawy and White 1978). However, all failed to produce lesion in salivary glands of animals resembling Sjögren's syndrome or other chronic sialadenitis in man, since prolonged and severe lesions extending to the whole lobular areas with proliferation of salivary duct epithelia have not been produced in above experiments. On the other hand, NZB/NZW strain of mice is generally considered models for human systemic lupus erythematosus, and also develops sialadenitis which are similar to those in Sjögren's syndrome in man (Kessler 1968), though detailed histopathological and ultrastructural studies of the salivary gland lesions in NZB/NZW mice are sparse (Kessler 1968; Greenspan et al. 1974; Carlsöö and Östberg 1978), and no signs of proliferation of duct epithelial cells have observed (Carlsöö and Östberg 1978). We have attempted to obtain a new laboratory model for Sjögren's syndrome, and have examined the salivary gland changes in SL/Ni mice. SL/Ni is an inbred strain of albino mouse established in Japan. It was originally known as a strain with a high incidence of B-cell lymphoma. Since 1970s, however, after some genetic alteration, the incidence of lymphoma began to decrease and immune complex glomerulonephritis very similar to that in systemic lupus erythematosus, fibrinoid arteritis much like that of polyarteritis nodosa and various immunological disorders appeared spontaneously with considerable high incidence (Kyogoku 1980). In our previous study, it was reported that the salivary glands of female SL/Ni mice showed mild to moderate lymphoid cell infiltration arising in 2 to 4 months of age and progressing in its incidence and severity with aging (Takeda et al. 1981). The result of the present study to produce severe salivary gland lesions in SL/Ni mice showed an important difference in the incidence and the severity of the lesions between control and experimental groups, i.e. the incidence and grade of lesions were higher in experimental group than the control one. The histopathologi-

cal findings of severe lesions in the present study are similar to those observed in the salivary gland lesions from patients with chronic sialadenitis in various cases, e.g. multifocal or diffuse lymphoid cell infiltration extending to wide lobular areas, proliferation of duct epithelia in lymphoid cell aggregates, and complete alteration of whole glandular lobules. However, the salivary gland lesions in our experiment using syngeneic submandibular gland homogenate as antigen was unique to the submandibular glands, and obvious cross-reactivity with other salivary glands was not noted morphologically. The alteration of the salivary glands in cases of chronic sialadenitis in man occur predominantly in the parotid gland, therefore experimental sialadenitis in animals should be induced in parotid gland by immunization with syngeneic parotid gland component. However, it is difficult to obtain of parotid gland tissue excluding surrounding tissues as antigen, since the outline of parotid gland is irregular and diffuse, and shaped by its confines of adjacent tissues. Further studies into this problem in experimental sialadenitis are necessary.

At the ultrastructural findings of induced sialadenitis in the present study, infiltrating cells were found to consist of a rather heterogenous cell population. However, small and medium sized lymphocytes and plasma cells constituted a major portion of the infiltrating cells, histiocytes and mast cells were also frequently encountered. Infiltrating lymphocytes were also observed inside the basal lamina of the ductal and acinar regions. The epithelial cells, in close proximity to invading lymphocytes, appeared degenerated. Therefore it is possible that the duct epithelial cells represent the target cells for infiltrating lymphocytes in the disease process.

In the aggregates of infiltrating cells, the cell remnants of salivary gland epithelia were scattered. Furthermore, some of the epithelial remnants could be recognized as epithelial masses which were composed of proliferated duct epithelial cells, however, proliferation of myoepithelial cells was not found. Those epithelial masses in the aggregates of infiltrating cells showed no typical structure of epimyoepithelial island seen in Sjögren's syndrome (Boquist et al. 1970; Donath and Seifert 1972; Takeda 1980).

The antisalivary duct antibody (ASDA) has been demonstrated by indirect immunofluorescent method in patients with Sjögren's syndrome, however, the significance of the ASDA in disease pathogenesis has been questioned (MacSween et al. 1967; Chisholm and Mason 1968; others). Anderson et al. (1973) have found an inverse relationship between the presence of ASDA and the degree of lymphoid cell infiltration, while its reason has not been understood. In our study on SL/Ni mice, ASDA was detected in only one case (2 weeks after the 2nd immunization) which had slight lymphoid cell infiltration in the salivary glands. However, its significance in the present examination remains obscure.

In summary, our results of experimental autoallergic sialadenitis in SL/Ni mice show salivary gland lesions which are similar morphologically to human salivary gland lesions observed in chronic sialadenitis. However, typical structure of epimyoepithelial island seen in Sjögren's syndrome is not found, and severe lesions are found in the submandibular gland alone.

Acknowledgement. The authors are grateful to Dr. Y. Nishizuka of the Aichi Cancer Center Research Institute for his kidness in aiding this study. This study was supported in part by Grant in Aid for Developmental Scientific Research (56570627) from the Education Ministry, Japan, and Grant from Iwate Medical University-Keiryokai Research Foundation.

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Accepted March 22, 1983