Rheumatology 2004;43:858–862 doi:10.1093/rheumatology/keh209
Advance Access publication 4 May 2004

Spontaneous development of multiple glandular and extraglandular lesions in aged IQI/Jic mice: a model for primary Sjögren's syndrome

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***Objective*. In primary Sjögren's syndrome (SS), systemic exocrine and non-exocrine organs are frequently affected, in addition to the major target tissues of the lacrimal and salivary glands. This study aimed to examine whether the IQI/Jic mouse, an animal model of SS whose autoimmune dacryoadenitis and sialoadenitis have been documented, develops inflammatory lesions in multiple organs as in primary SS.**

***Methods*. Systemic histopathological analysis was performed on IQI/Jic mice at various ages. Phenotypes of infiltrated lymphocytes were determined using immunohistochemical techniques.**

***Results*. Inflammatory lesions were observed not only in the lacrimal and salivary glands, but also in multiple organs, including the lung, pancreas and kidney at advanced ages, and were mainly composed of $CD4^+$ T cells and B cells. The incidence and severity of the inflammatory lesions increased with age in all these organs. The histological appearance and spreading of lesions were similar to those in human primary SS.**

***Conclusions*. IQI/Jic mice spontaneously develop inflammatory cellular infiltrates in multiple exocrine and non-exocrine organs. This characteristic distinguishes IQI/Jic mice from other murine models, making them favourable for studies on the pathogenesis of systemic involvement in primary SS.**

KEY WORDS: IQI/Jic mouse, Primary Sjögren's syndrome, Autoimmune disease.

Primary Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands, resulting in the clinical complaints of oral and ocular dryness due to insufficient secretion [1]. Additional glandular tissues, including the respiratory tract, skin, external genitalia, hepatobiliary system and pancreas, are also affected. Moreover, patients with SS occasionally exhibit extraglandular manifestations, including interstitial lung disease and interstitial renal disease. According to these clinical observations, SS has been generally referred to as 'autoimmune exocrinopathy', 'dry gland syndrome' or, more widely, 'autoimmune epithelitis' [2–4]. The aetiology of this disease complex, in which multiple organs are targeted by the immune system, has not been fully elucidated.

Several animal models of SS have been proposed based on the histopathological findings of focal lymphocytic infiltrates in the lacrimal and salivary glands. They include autoimmune-prone mice that develop SS-like pathology associated with other autoimmune conditions, such as systemic lupus erythematosus, rheumatic arthritis and insulitis [5–8], and other rodent strains requiring experimental manipulations, such as antigen sensitization and neonatal thymectomy, before they develop inflammatory lesions [9–11]. However, few of these animal models bear a resemblance to primary SS, in which multiple organs, including exocrine and non-exocrine organs, are affected simultaneously.

IQI/Jic mice have been described previously as a novel model of primary SS [12–14]. They spontaneously develop autoimmune infiltration of lymphocytes into the lacrimal and salivary glands, leading to dacryoadenitis and sialoadenitis. The incidence of the disease is higher in females than in males [12]. Sialoadenitis in female mice can be detected from 2 months of age onwards [14], and significant progress of the lesions is observed after 9 months of age [12]. Inflammatory lesions occurring at young ages mainly consist of $CD4^+$ T cells with lesser abundance of $CD8^+$ T cells, B cells and macrophages, and the proportions of B cells and plasma cells are elevated in accordance with increasing magnification of the lesions. Production of antinuclear antibodies, one of the prominent pathophysiological features in patients with SS, is also observed in old IQI/Jic mice [13].

Interestingly, during our histopathological studies on IQI/Jic mice [14], we found that focal accumulations of mononuclear cells frequently occurred also in other organs at advanced ages. In the present study, we followed the development of inflammatory lesions in various organs of IQI/Jic mice histologically and immunohistochemically. We report that IQI/Jic mice develop inflammatory lesions in the lung, pancreas and kidney in addition to the lacrimal and salivary glands that are very similar to those in patients with primary SS, suggesting that the IQI/Jic strain is an animal model suitable for the investigation of the pathogenesis of SS, with progression from oral and ocular diseases to systemic disorder.

Methods

Animals

IQI/Jic mice were originally provided by Dr J. Saegusa, National Institute of Industrial Health (Kanagawa, Japan). They were bred and maintained in our own animal facility under

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Received 17 December 2003; revised version accepted 24 March 2004.

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specific-pathogen-free conditions. Female IQI/Jic mice at the ages of 2, 4, 7, 10 and 13 months ($n = 6$–10 for each age group) were used in this study. All animal experiments were carried out with the approval of the Committee of Laboratory Animal Experimentation, Graduate School of Veterinary Medicine, Hokkaido University.

Histological analysis

Various organs removed from the mice at each age were fixed in 10% formaldehyde and embedded in paraffin. Sections were stained with haematoxylin and eosin, and examined under a microscope. The severity of the inflammatory lesions in the lacrimal and salivary glands and the pancreas was classified into five grades (0–4) according to modified criteria [15] as follows: 0, no visible change; 1, mild accumulation of mononuclear cells within the interstitium; 2, focal accumulation of mononuclear cells without any parenchymal destruction; 3, focal accumulation of mononuclear cells with parenchymal destruction; 4, extensive infiltration of mononuclear cells with severe tissue damage. The lesions in the lung were similarly distinguished into grades 0–3: 0, no visible change; 1, mild accumulation of mononuclear cells surrounding blood vessels and bronchi; 2, moderate accumulation of mononuclear cells surrounding blood vessels and bronchi; 3, extensive infiltration of the interstitium with mononuclear cells. Grading of renal tissues was: 0, no visible change; 1, infiltration of the interstitium with mononuclear cells without destruction of the urinary tubules; 2, infiltration of the interstitium with mononuclear cells with the destruction of urinary tubules.

Immunohistochemistry

Immunohistochemical staining was performed on cryostat sections of various organs from 10-month-old mice using the biotin–avidin immunoperoxidase method. Briefly, the sections were fixed in acetone for 10 min, treated with 3.0% H_2O_2 in methanol for 15 min, and then blocked with 1.0% bovine serum albumin in phosphate-buffered saline (pH 7.2) for 20 min. Thereafter, they were incubated with biotinylated rat anti-mouse CD4, CD8 or B220 monoclonal antibodies (BD Pharmingen, San Diego, CA, USA) for 2 h, and then with streptavidin-conjugated peroxidase (Nichirei, Osaka, Japan). The sections were reacted with a mixture of 0.05% 3,3′-diaminobenzidine and 0.005% H_2O_2 in 50 mM Tris–HCl (pH 7.6) and 150 mM NaCl, followed by counterstaining with Mayer's haematoxylin. All control samples were incubated with normal rat serum (Nichirei) instead of the monoclonal antibodies, and showed no non-specific staining.

Results

Chronic inflammatory lesions in multiple organs

Histopathological analysis was performed on IQI/Jic mice at various ages, and demonstrated the spontaneous development of inflammatory lesions in multiple organs, including the lacrimal and salivary glands, lung, pancreas and kidney (Fig. 1). The incidence and severity of the inflammatory lesions in these organs increased gradually with age (Table 1). Generally, accumulation of mononuclear cells in the lungs, pancreas and kidneys was mild compared with the lacrimal and salivary glands at the ages examined in this study.

The initial detectable changes were minute infiltrations of mononuclear cells in the areas around ducts and veins in the lacrimal and salivary glands 2 months after birth. The histological abnormalities were more prevalent in the salivary glands at this age (Table 1). In these glands, focal infiltrations and dilatation of ducts became apparent at 7 months of age (Fig. 1A and B). Significant destructive lesions, including the displacement of acinar units, were seen in aged mice (>10 months).

The pulmonary lesions appeared much later in life, after 7 months of age, and perivascular and peribronchial cell infiltrations were obvious (Fig. 1C). Progressive accumulation of mononuclear cells was observed in the pulmonary interstitium in some aged mice (Fig. 1D).

In the pancreas, histological features were essentially similar to those found in the lacrimal and salivary glands. Foci of mononuclear cell infiltration were constitutively located in periductal and perivascular areas, resembling those in the lacrimal and salivary glands (Fig. 1E). Destruction of pancreatic acini was recognized in the magnifying processes of the foci. A few mice showed marked damage, and partial replacement with fibrils and adipose tissue (Fig. 1F). In contrast, the pancreatic islets were spared from the infiltrations in all age groups.

In the renal lesions, mononuclear cells mainly infiltrated into the interstitium, in contact with the large- and medium-sized blood vessels (Fig. 1G), and they were observed in mice older than 7 months of age (Table 1). Although mild destruction of urinary tubules was occasionally observed (Fig. 1H), the histological grade remained low throughout life (Table 1), being consistent with the absence of periodic paralysis, the typical sign of hypokalaemia due to renal tubular acidosis, in all mice examined.

Immunohistochemical analysis for infiltrating cells

The results of histological analysis were processed to examine the phenotypes of infiltrating mononuclear cells in the lacrimal glands, salivary glands, lungs, pancreas and kidneys of 10-month-old IQI/Jic mice by immunohistostaining. As shown in Fig. 2, the major populations of mononuclear cells were $CD4^+$ T cells and B cells bearing B220 antigen in all organs examined, whereas $CD8^+$ T cells were absent or scattered as extremely minor components of the infiltrates.

The proportions of $CD4^+$ T cells and B cells in infiltrates varied markedly between the lesions in exocrine organs and those in non-exocrine organs. In the salivary glands, lacrimal glands and pancreas, very small foci, comprising approximately 20 or fewer mononuclear cells, mostly consisted of $CD4^+$ T cells, whereas the major cell population was B cells in the foci with more abundant infiltrates. In large foci formed in the salivary glands (Fig. 2A–C), lacrimal glands (data not shown) and pancreas (Fig. 2D–F), areas composed predominantly of T cells and areas composed predominantly of B cells were clearly distinguishable (that is, $CD4^+$ T cells occupied the periductal areas of the foci), and they were surrounded by B220 B cells. In contrast, renal (Fig. 2G–I) and pulmonary (data not shown) lesions consisted principally of $CD4^+$ T cells, and a small number of $B220^+$ B cells were scattered throughout the foci. This configuration remained almost constant regardless of the size of the foci in the lung and kidneys.

Discussion

Our results provided evidence to show that pathological changes in IQI/Jic mice involve inflammatory infiltrations not only in the lacrimal and salivary glands, as previously reported [12–14], but also in multiple organs, including the lungs, pancreas and kidneys, and these lesions developed spontaneously with age. Human patients with primary SS show a wide spectrum of clinical symptoms, including exocrine and non-exocrine manifestations, and the symptoms usually appear relatively late in life [16]. In these patients, accumulation of lymphocytes in periductal areas and cell infiltration into the parenchyma are the most prominent observations in exocrine organs. As non-exocrine manifestations, interstitial pneumonitis and nephritis are most frequently recognized [1, 4]. Likewise, IQI/Jic mice had inflammatory lesions which

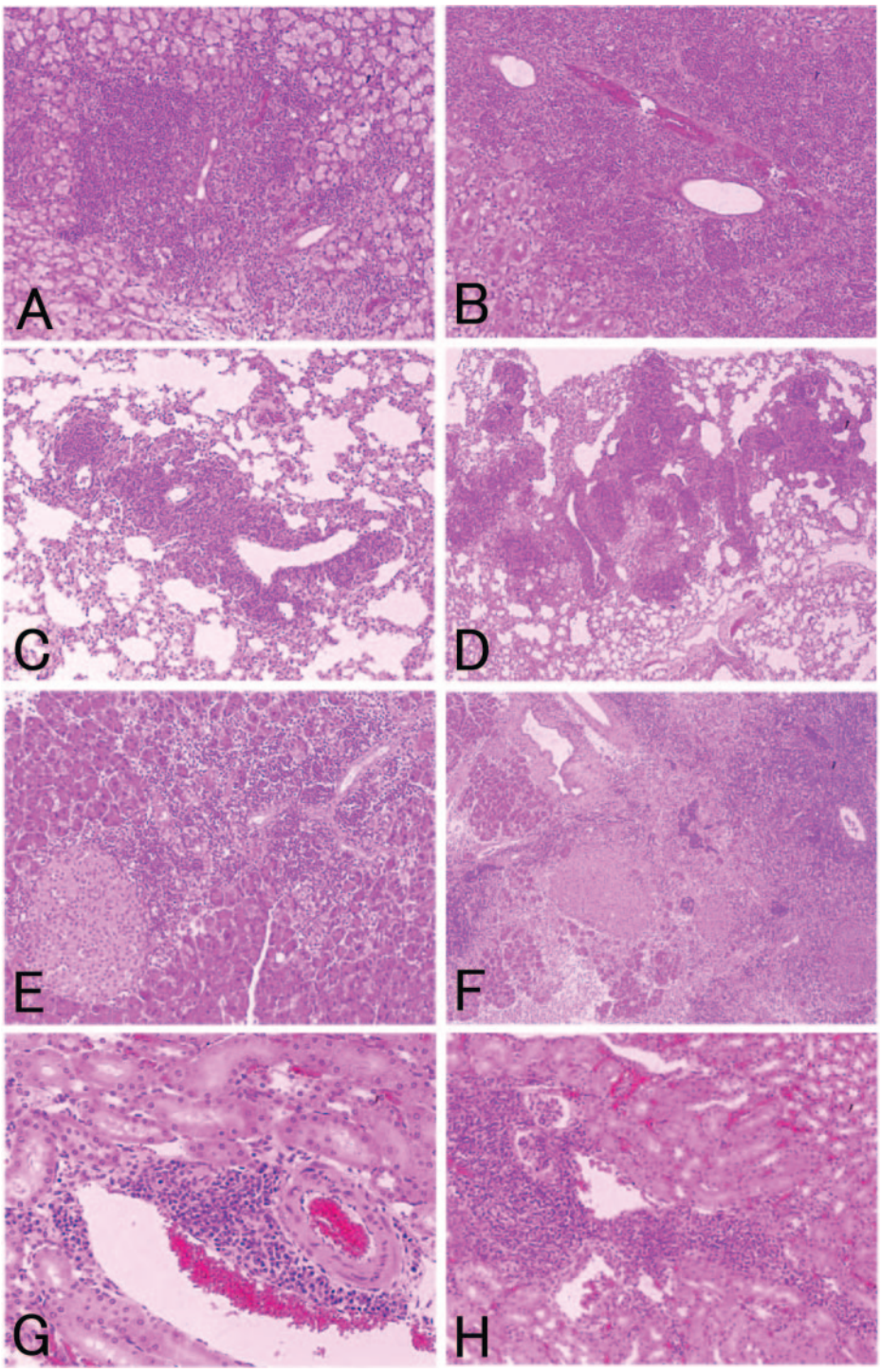

FIG. 1. Histopathology of various organs of 10-month-old IQI/Jic mice. Sections were stained with haematoxylin and eosin. (A) Periductal mononuclear cell infiltration in the lacrimal glands (grade 3). (B) Mononuclear cell infiltration in the salivary periductal regions (grade 3). (C) Focal infiltration of mononuclear cells surrounding blood vessels in the lung (grade 1). (D) Extensive infiltration of mononuclear cells in the pulmonary interstitium (grade 3). (E) Focal mononuclear cell infiltration around the duct in the pancreas. Islets were completely free of infiltration (grade 2). (F) Severe destructive lesion of the exocrine pancreas with diffuse infiltration of mononuclear cells (grade 4). (G) Accumulation of mononuclear cells in renal interstitium in contact with blood vessels (grade 1). (H) Interstitial infiltration of mononuclear cells with mild destruction of urinary tubules (grade 2). Original magnification: A, B, C, E and G, ×100; D, F and H, ×40.

surrounded ducts in the lacrimal glands, salivary glands and pancreas, or occupied the interstitium in the lungs and kidneys (Fig. 1). The similarity in histopathological findings between human patients and IQI/Jic mice suggests that this strain is suitable for studying the pathogenesis of SS, in which multiple organs are progressively affected, i.e. autoimmune epithelitis.

Many studies have demonstrated the spontaneous development of periductal foci of lymphocytic infiltration in the salivary and

TABLE 1. Incidence and mean grade of inflammatory lesions of various organs from IQI/Jic mice at different ages

	Lacrimal gland		Salivary gland		Lung		Pancreas		Kidney	
Age (months)	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]
1	0/6 (0)	0	0/6 (0)	0	0/6 (0)	0	0/6 (0)	0	0/6 (0)	0
2	2/6 (33.3)	0.2 ± 0.1	6/6 (100)	0.8 ± 0.1	0/6 (0)	0	0/6 (0)	0	0/6 (0)	0
4	4/6 (66.7)	0.3 ± 0.1	6/6 (100)	1.2 ± 0.2	0/6 (0)	0	4/6 (66.7)	0.3 ± 0.1	4/6 (66.7)	0.3 ± 0.1
7	5/6 (83.3)	1.1 ± 0.4	6/6 (100)	0.9 ± 0.1	2/6 (33.3)	0.5 ± 0.3	5/6 (83.3)	0.4 ± 0.1	6/6 (100)	0.8 ± 0.1
10	6/6 (100)	1.8 ± 0.5	6/6 (100)	2.0 ± 0.3	5/6 (83.3)	0.8 ± 0.2	4/6 (66.7)	0.5 ± 0.2	6/6 (100)	0.8 ± 0.2
13	6/6 (100)	2.0 ± 0.5	6/6 (100)	2.3 ± 0.2	9/10 (90)	1.1 ± 0.2	10/10 (100)	1.2 ± 0.3	6/6 (100)	1.0 ± 0.2

[a]Number of mice positive for lesions out of total mice examined. Numbers in parentheses indicate percentage of positive mice.
[b]Mean grade of lesion ± S.E.

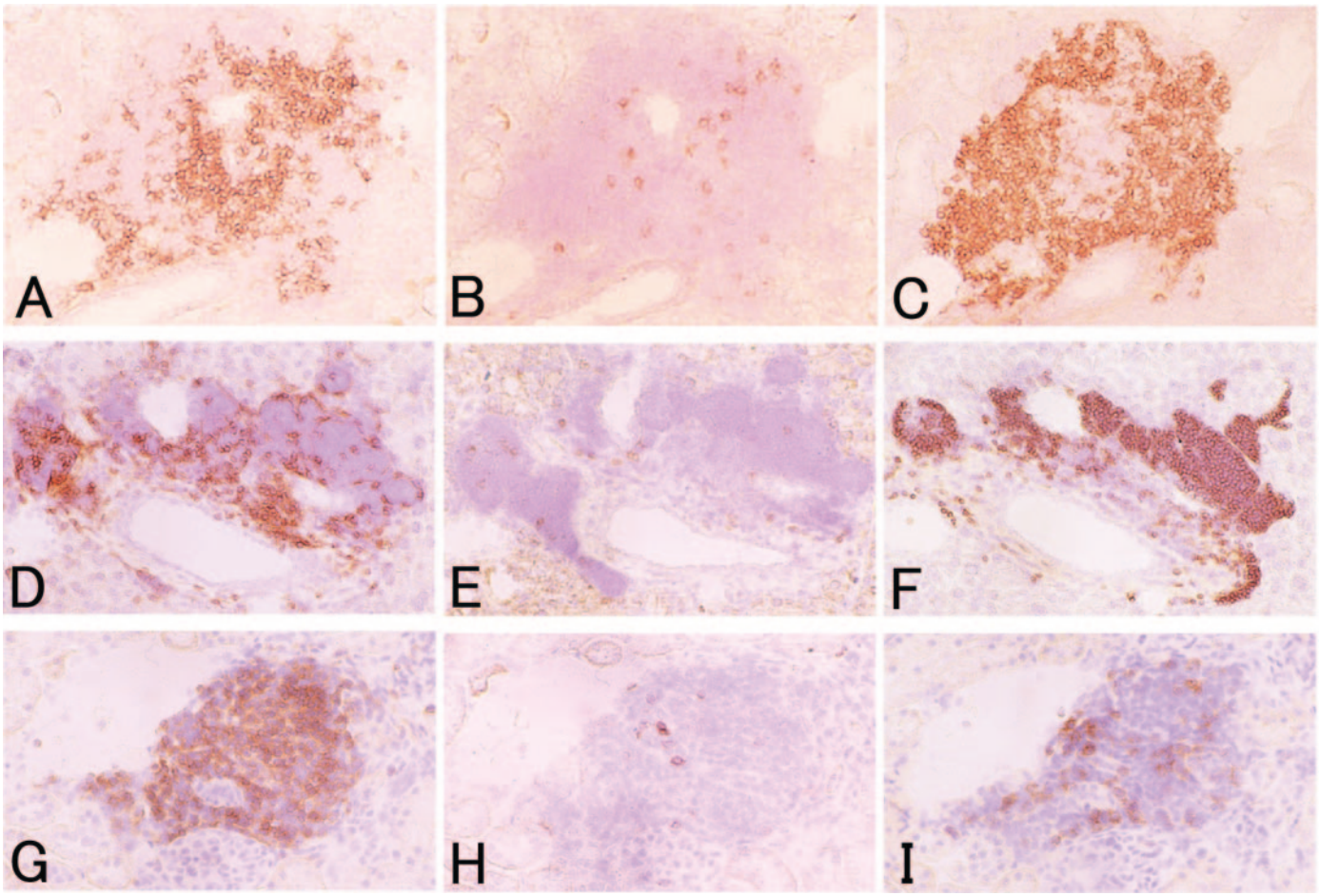

FIG. 2. Immunohistological analysis of infiltrating lymphocytes in the salivary glands (A–C), pancreas (D–F) and kidney (G–I) of 10-month-old IQI/Jic mice. Continuous sections were stained with anti-CD4 (A, D and G), anti-CD8 (B, E and H) and anti-B220 (C, F and I) antibodies. Original magnification: ×200.

lacrimal glands in several murine strains, including NOD, MRL/*lpr*, NZB/W F1 and their backcrosses [5–8, 17]. Nevertheless, there is a paucity of animal models exhibiting histological characteristics of systemic autoimmune epithelitis in SS, like those found in IQI/Jic mice. Senile C57BL/B6 mice develop inflammatory lesions in multiple organs, including the salivary glands, lungs, pancreas and kidneys [18]. However, they do not show lesions in the lacrimal glands, and pancreatic lesions in C57BL/B6 mice are insulitis in contrast to the exocrine pancreatitis found in IQI/Jic mice, where the islets are completely exempted from damage. Mice possessing homozygous mutation for alymphoplasia (*aly*/*aly*) also develop mononuclear cell infiltrations in the periductal areas of exocrine glands, such as the lacrimal and salivary glands and pancreas, as well as in the areas surrounding pulmonary veins [15]. Although the affected organs and the locations of foci in each organ are essentially similar in *aly*/*aly* and IQI/Jic mice, there are several differences. In *aly*/*aly* mice, lymphocytic foci appear in the pancreas at the relatively young age of 14 weeks, when those in the lacrimal and salivary glands develop, and progress to marked tissue damage at between 20 and 29 weeks. On the other hand, in IQI/Jic mice, pancreatic lesions appeared much later than those in the lacrimal and salivary glands, and severe destructive lesions were rarely seen in the pancreas. These characteristics, specific to strains, may imply differences in the pathogeneses of their diseases, including the origin of the autoimmune reaction and the mechanism underlying the development of the autoimmune process.

It was reported previously that the proportion of B cells in infiltrates was drastically elevated with progress of the lesions in the lacrimal and salivary glands in IQI/Jic mice [12, 14]. The present study demonstrated that a similar phenomenon also occurred in the pancreas, but not in the lungs and kidneys (Fig. 2). This is unique to IQI/Jic mice, as $CD4^+$ T cells are the predominant population infiltrating the lacrimal glands, salivary glands and pancreas throughout life in other murine models of SS, including NOD, NZB/W F1, MRL/*lpr* and *aly*/*aly* mice [13]. SS is characterized by the production of a wide range of autoantibodies, which illustrates that B cells are activated against various autoantigens in several restricted tissues or those with ubiquitous tissue distribution [19]. B-cell activation in SS often leads to monoclonal gammmopathies, pseudolymphomas and malignant lymphomas with increased frequency [20]. It has been suggested that B-cell malignancies mostly originate from infiltrates within glandular organs [21], and a sequential shift from T cells to B cells in proliferation preceding the monoclonal B-cell proliferation has been observed [22]. Therefore, investigations on IQI/Jic mice, particularly of the exact nature of infiltrating B cells, including clonality and phenotypes, could also be exploited to unravel the mechanism of B-cell lymphomagenesis in primary SS.

In conclusion, we demonstrated that IQI/Jic mice spontaneously developed lymphocyte infiltrates not only in the lacrimal and salivary glands, but also in the lungs, pancreas and kidneys, as they aged. These histopathological findings, unique to IQI/Jic mice, and their similarities with the observations reported in patients with SS suggest that this model may facilitate study of the aetiology of the progressive involvement of multiple exocrine organs and non-exocrine organs in primary SS.

Rheumatology	Key messages
	• IQI/Jic mice develop inflammatory lesions characteristic of primary Sjögren's syndrome with the progressive involvement of multiple organs, suggesting that they are suitable for the investigation of the systemic pathogenesis of Sjögren's syndrome.

Acknowledgements

We thank Dr J. Saegusa for supplying IQI/Jic mice, and K. Komabayashi, A. Nagata and K. Nishii (all from Hokkaido University) for their technical help in histopathological studies. This work was supported in part by Grants-in-Aid for Scientific Research from Japan Society for Promotion of Science (14360187 and 15658096) and a research grant from the Akiyama Foundation. KT is a recipient of a JSPS Research Fellowship for Young Scientists.

The authors have declared no conflicts of interest.

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