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Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism

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SUMMARY

MRL/Mp mice are known to have autoimmune disease-prone genetic background, which contributes to the development of a lethal autoimmune disease at an early age in association with the lymphoproliferative gene, *lpr*. In this study, we found that MRL/Mp mice, not bearing *lpr* (MRL/Mp-+/+), spontaneously developed pancreatitis at a late stage of life, which was histopathologically characterized by destruction of pancreatic acinar cells with mononuclear cell infiltration. In female 34–38-weeks-old mice the incidence of pancreatitis reached 74%, whereas the male mice developed the disease with a reduced incidence, at a later stage of life and with a reduced severity. Cell infiltrates in the affected lesions were composed predominantly of $CD4^+$ cells and to lesser extent Mac-2^+ macrophages. Adoptive transfer of the spleen cells obtained from pancreatitis-bearing female mice generated pancreatitis in female normal mice, but not in the male mice. Transfer of the serum of pancreatitis-bearing mice failed to induce any pancreatic lesions. These findings indicate that pancreatitis in MRL/Mp-+/+ mice may be mediated by cellular autoimmune mechanism. This may present a useful concept for analysis of the developmental mechanisms of human chronic pancreatitis in an aspect of autoimmunity.

Keywords autoimmune pancreatitis adoptive transfer lupus mice

INTRODUCTION

MRL/Mp mice bearing a lymphoproliferative gene, *lpr* (MRL/Mp-*lpr*/*lpr*) spontaneously develop severe autoimmune diseases such as glomerulonephritis, arteritis and arthritis at an early stage of life, associated with autoantibody production and T cell dysfunction [1–3]. These mice also generate inflammatory destructive lesions in lachrymal and salivary glands, resembling Sjögren's syndrome [4]. However, MRL/Mp mice not bearing the *lpr* gene (MRL/Mp-+/+) also develop these diseases, but at a much later stage of life, and with reduced incidence and severity [2,3]. Genetical analyses of these mice [5–7] suggest that the MRL/Mp strain of mice have autoimmune disease-prone genetic background and the *lpr* gene acts as an accelerator gene for the development of these diseases.

Recently, we found severe inflammatory lesions in the pancreas of aged MRL/Mp-+/+ mice. Considering the genetic background of these mice, this may be mediated by autoimmunity. Here we examined this possibility in immunohistochemical studies and by adoptive transfer of spleen cells or serum. Our results indicate that pancreatitis in MRL/Mp-+/+ mice may be generated in autoimmune mechanism.

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MATERIALS AND METHODS

Mice

MRL/Mp-+/+ mice used in this study were originally derived from The Jackson Laboratories, Bar Harbor, ME in 1985, and have been bred in a closed colony and housed in clean rooms in the Animal Research Institute of Tohoku University School of Medicine.

Histopathologic studies

Mice were anaesthetized with ether. After blood sampling, mice were killed with cervical dislocation, and pancreas, heart, lungs, liver, spleen, submandibular glands and kidneys of each mouse were removed for histopathologic examinations. Tissues were fixed in 10% formalin in 0·01 M phosphate buffer (pH 7·2) and embedded in paraffin. They were stained with haematoxylin and eosin or with Elastica-Masson.

Histopathologic evaluation of pancreatic lesions was performed under a light microscopy. Severity of the lesions in each mouse was scored on a 0–4+ grade based on the histopathologic changes as follows: 0, pancreas without mononuclear cell

infiltration, indicating almost normal; 1+, mononuclear cell aggregation and/or infiltration within the interstitium without any parenchymal destruction; 2+, focal parenchymal destruction with mononuclear cell infiltration; 3+, diffuse parenchymal destruction but retained some intact parenchymal residue; and 4+, almost whole pancreatic tissue, except pancreatic islets, destroyed or replaced with adipose tissue. To estimate the incidence of pancreatitis in MRL/Mp-+/+ mice, mice with pancreatic lesions that scored >2+ was defined as positive for pancreatitis.

To examine the health status of each mouse, other tissue specimens, including lungs and liver, were studied under a light microscopy. We did not find any lesions suggesting bronchitis, pneumonia, hepatitis or other infectious diseases.

Immunohistochemical studies

At autopsy, pancreas samples were fixed in periodate–lysine–paraformaldehyde fixative [8], and then embedded in OCT compound (Miles, Elkhart, IN) and frozen. Immunohistochemical examinations were performed as described elsewhere [9]. To detect activated macrophages or $CD4^+$ T cells, tissue sections were reacted with rat anti-Mac-2 (clone M3/38; Hybritech, San Diego, CA) [10] or rat anti-L3T4 (culture supernatant of clone GK1.5; a generous gift of Dr K. Okumura) IgG MoAb, respectively, followed by biotinylated rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA) and horseradish peroxidase (HRP) conjugated streptoavidin (Bio Genex Laboratories, Dublin, CA). $CD8^+$ T cells were directly stained with biotinylated anti-Lyt.2 antibody (Becton Dickinson, Mountain View, CA), followed by HRP–streptoavidin (Vector Labs).

Adoptive transfer of spleen cells

Spleen cells (1×10^7 cells/ml) from donor MRL/Mp-+/+ mice were cultured in the RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (Flow Laboratories, Irvine, UK), 50 μg/ml of gentamycin in the presence or absence of 25 μg/ml of phytohaemagglutinin (PHA; ICN Immuno Biologicals, Lisle, IL) [11]. After 3 days, these cells were provided for adoptive transfer. Recipient MRL/Mp-+/+ mice had been injected intraperitoneally with cyclophosphamide (Endoxan; Shionogi Pharmaceutical, Osaka, Japan) as a single dose of 200 mg/kg body weight 7 days before adoptive transfer [11]. Cell transfer was performed by i.v. injection of the cultured spleen cells (1×10^7 cells) suspended in 0·5 ml of HBSS. After 6 weeks these mice were killed and pancreatic tissue was provided for histological and immunohistochemical studies.

Serum transfer

Pooled sera from MRL/Mp-+/+ mice (32-week-old female or 5–8 week-old male) were used. After sterilization using 0·22-μm pore filters (Milipore, Bedford, MA), 0·5 ml of the serum was injected intravenously to unmanipulated recipient MRL/Mp-+/+ mice repeatedly on days 0, 2 and 4. These mice were killed on day 6 or 16 after the last injections and pancreatic tissue was examined histopathologically.

Statistical analysis

Statistical significance was determined by the Student's *t*-test. $P < 0.05$ was taken as significant.

RESULTS

MRL/Mp-+/+ mice develop chronic pancreatitis spontaneously

We found severe inflammatory lesions in pancreas of female MRL/Mp-+/+ mice aged more than 22 weeks. These pancreatic lesions were characterized histopathologically in order of appearance by mononuclear cell infiltration, destruction of acini and replacement of parenchyma with adipose tissue, but they were not associated with necrotic or haemorrhagic lesions (Fig. 1). Pancreatic islets were unaffected and remained intact throughout the progression (Fig. 1b). Sometimes, granulomatous inflammatory lesions were observed in advanced stage (data not shown).

The incidence of pancreatitis increased up to 74% in the female mice at 34–38 weeks (Table 1). The male mice also

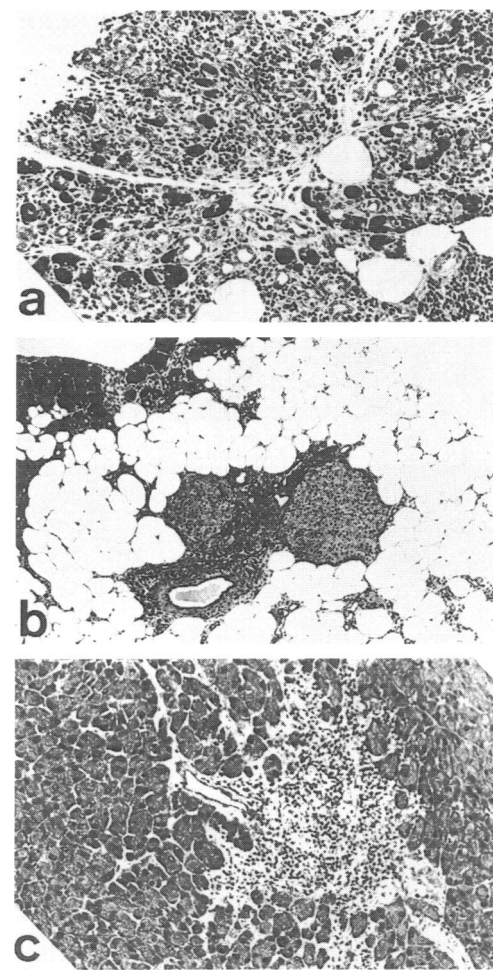

Fig. 1. Histopathologic manifestations of pancreatitis spontaneously developed in MRL/Mp-+/+ mice. (a) Pancreatitis in a female 36-week-old mouse shows diffuse infiltration of mononuclear cells and resultant severe destruction of pancreatic parenchyma. In part, the replacement of parenchyma with adipose tissue is observed. This lesion corresponds to grade 3+. Haematoxylin and eosin stain; magnification × 90; (b) an advanced lesion of pancreatitis in a 48-week-old female mouse, corresponding to grade 4+. This lesion is characterized by the replacement of almost the entire part of pancreatic parenchyma except the islets with adipose tissue. Haematoxylin and eosin stain; magnification × 45; (c) an early lesion of pancreatitis in a 36-week-old female mouse, characteristic of focal parenchymal destruction following inflammatory cell infiltration in the interstitium (grade 2+). Haematoxylin and eosin stain; magnification × 90.

Table 1. Incidence and severity of pancreatitis in MRL/Mp-+/+ mice

Age (weeks)	Female				Male				*P* (female *versus* male)
			Incidence				Incidence		
	n	Severity*	*n*	%	*n*	Severity*	*n*	%	
18–22	16	1·13	3	19	13	0·23	0	0	
22–26	16	1·56	9	56	15	0·47	1	7	
26–30	29	1·88	19	66	22	0·32	0	0	
34–38	19	2·16	14	74	14	1·07	5	36	<0·005
42–46	11	2·55	8	73		ND			
46–50	64	2·31	41	64	38	1·55	14	37	<0·01

*Average grade of pancreatic lesions in each mouse.

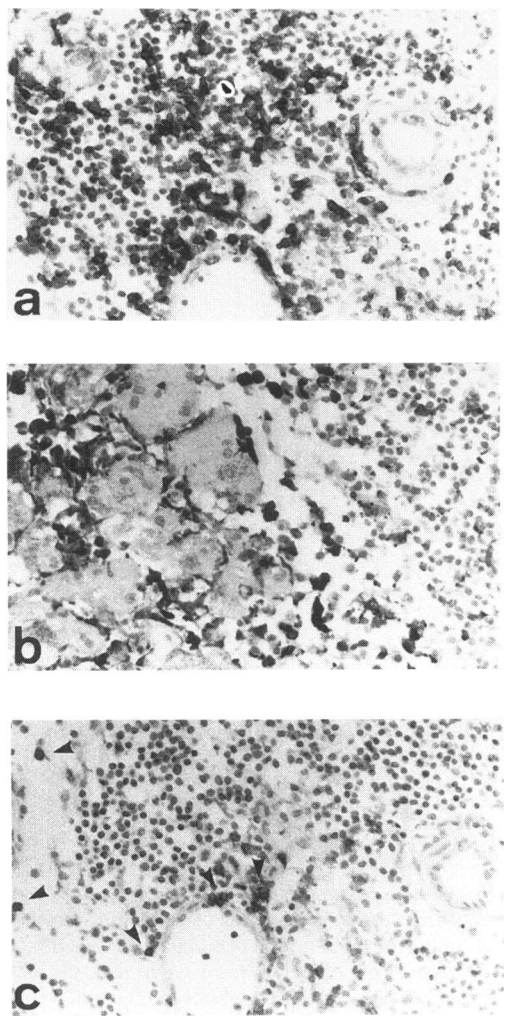

Fig. 2. Immunohistochemical findings of pancreatitis spontaneously developed in a 36-week-old female MRL/Mp-+/+ mouse, whose lesions were graded 3+ in histopathological examinations. (a) $CD4^+$ cells in the interstitium partly infiltrating to the parenchyma (upper left). Anti-L3T4, magnification × 240; (b) Mac-2^+ cells diffusely infiltrate to the parenchyma, some attached to acinar cells (left). Anti-Mac-2; magnification × 240; (c) a few $CD8^+$ cells are observed in the interstitium (arrow head). Anti-Lyt.2, magnification × 240.

developed pancreatitis with similar histopathological manifestations, but at a much later stage in life and with a reduced incidence, namely less than 40% in 46–50-week-old mice. Moreover, the average grade of pancreatic lesions through the life was remarkably lower (Table 1).

$CD4^+$ cells are dominant in infiltrating cells

Inflammatory lesions in the pancreas of the older female mice contained mononuclear cells positive for L3T4, Lyt.2 or Mac-2 antigens. A major population of these cells was the L3T4 positive cells, which were localized in the interstitium and diffusely infiltrating to the parenchyma (Fig. 2a). Mac-2^+ cells were dispersed in the parenchyma, and some of them seemed to be attached to acinar cells (Fig. 2b). A few Lyt.2^+ cells were observed in the interstitium sporadically (Fig. 2c).

Adoptive transfer of spleen cells generates pancreatitis

To examine adoptive transfer for pancreatitis, donor spleen cells from pancreatitis-bearing mice were treated with PHA and injected to the recipient mice. As shown in Table 2, PHA

Table 2. Phytohaemagglutinin (PHA) treatment of donor spleen cells on adoptive transfer

Donor spleen cells	Treated with	Recipients*				
				Incidence		
		n	Severity†	*n*	%	*P*‡
28-week-old female MRL/Mp-+/+	PHA	7	0·86	3	43	>0·1
	—	9	0·22	1	11	
8-week-old male MRL/Mp-+/+	PHA	10	0·10	0	0	

*Female 7–8-week-old MRL/Mp-+/+ mice were killed for histopathologic examinations 6 weeks after the transfer.

†Average grade of pancreatic lesions in each mouse.

‡With PHA *versus* without PHA.

Table 3. Adoptive transfer of spleen cells to female or male MRL/Mp-+/+ mice

Donor*	Recipient (*n*)†	Severity‡	Incidence		*P* (female *versus* male)
			n	%	
Female	Female (7)	1·57	5	71	<0·05
Female	Male (6)	0·17	0	0	

*Spleen cells obtained from female 36-week-old mice were cultured with phytohaemagglutinin for 3 days before transfer.

†Female 9-week-old or male 7-week-old mice were killed for histopathologic examinations 6 weeks after the transfer.

‡Average grade of pancreatic lesions in each mouse.

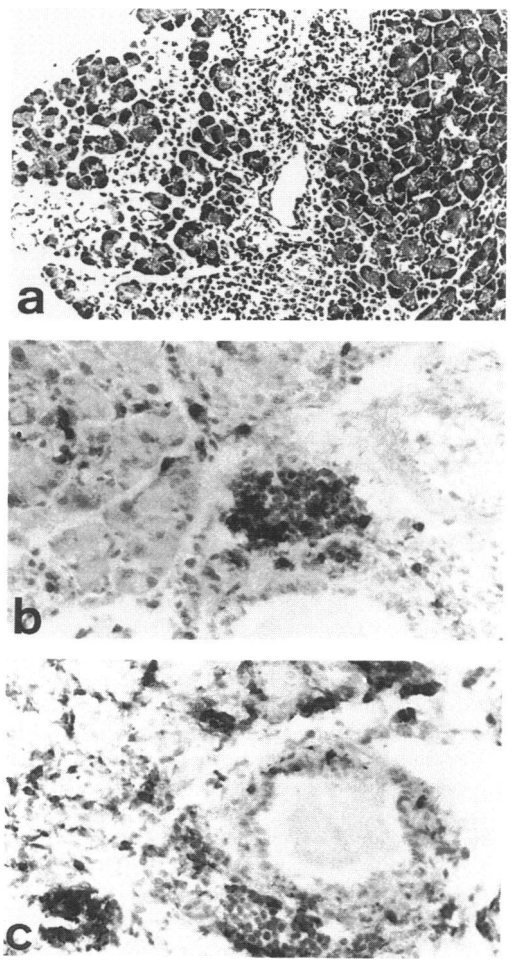

Fig. 3. Pancreatitis in 9-week-old female MRL/Mp-+/+ mice induced by adoptive transfer of spleen cells which were obtained from 36-week-old female MRL/Mp-+/+ mice. (a) Relatively focal parenchymal destruction associated with mononuclear cell infiltration is seen. Such a lesion is similar to that observed in unmanipulated aged MRL/Mp-+/+ mice, corresponding to grade 2+. Haematoxylin and eosin stain; magnification × 150; (b) $CD4^+$ cells are present in an aggregated form in the interstitium and in a diffuse manner in the parenchyma. Anti-L3T4, magnification × 240; (c) $Mac\text{-}2^+$ cells located in the parenchyma similar to those in Fig. 2b. Sometimes these cells localize in an aggregated form (bottom left). Anti-Mac-2, magnification × 240.

treatment increased the severity of pancreatitis in the recipient mice. This result was not due to the PHA treatment by itself, since PHA treatment of spleen cells from young male mice without pancreatitis could not generate the disease in the recipient mice (Table 2). Then, all donor spleen cells in further experiments were pretreated with 25 μg/ml of PHA for 3 days before adoptive transfer.

Spleen cells from the female 36-week-old mice with pancreatitis generated inflammatory lesions in the pancreas of female 9-week-old mice (Table 3), which were histopathologically characterized by diffuse mononuclear cell infiltration and acinar cell destruction, corresponding to grade 2+ (Fig. 3a). The infiltrating cells in these lesions were mainly $CD4^+$ cells and to a lesser extent $Mac\text{-}2^+$ cells (Fig. 3b,c), similarly to the unmanipulated older female mice. In this system, the incidence of pancreatitis in the recipient mice reached 71%, almost the same as that in the female 34–46-week-old mice (see Table 1), although the score of the severity was lower. However, when the recipient mice were male, these donor spleen cells completely failed to generate pancreatitis (Table 3).

Some of the recipient female mice generating pancreatitis developed inflammatory lesions in submandibular glands, which were histopathologically similar to those observed in most of older female MRL/Mp-+/+ mice with pancreatitis (Fig. 4).

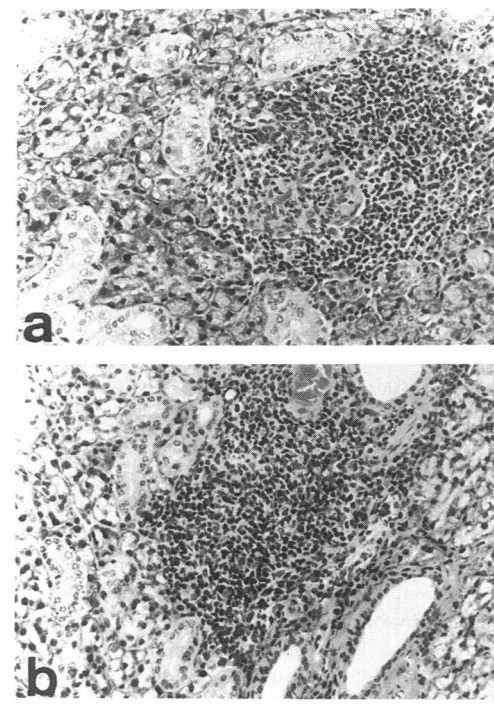

Fig. 4. (a) Inflammatory lesions in submandibular gland of a 9-week-old female MRL/Mp-+/+ mouse generated by adoptive transfer of spleen cells which were obtained from 36-week-old female MRL/Mp-+/+ mice. In this recipient pancreatitis also was generated, corresponding to grade 2+. Haematoxylin and eosin stain; magnification × 160; (b) representative histopathological manifestations of sialoadenitis of submandibular glands observed in an unmanipulated 36-week-old female MRL/Mp-+/+ mouse with pancreatitis. Haematoxylin and eosin stain; magnification × 160. Both lesions are similar and characterized by the destruction of serous glands associated with severe mononuclear cell infiltration.

Serum transfer fails to generate pancreatitis

Autoantibodies for pancreatic acinar cells are found in some patients with chronic pancreatitis [12–15]. In our preliminary study, IgG fractions obtained from older female MRL/Mp-+/+ mice, but not from the young male mice, were found under immunohistochemical examination to react with pancreatic acinar cells of the older female mice. Thus, to examine a possible role of such autoantibodies in the development of pancreatitis in MRL/Mp-+/+ mice, we performed experiments of serum transfer. The sera from the 32-week-old female mice with pancreatitis did not generate pancreatitis in all five 4-week-old female mice when they were examined histopathologically on day 10 or on day 20 after the transfer.

DISCUSSION

This is the first report on spontaneously developed pancreatitis in mice. We found severe and progressive chronic pancreatitis in MRL/Mp-+/+ mice. Moreover, our results indicate that pancreatic inflammatory lesions in these mice are mediated essentially by cellular autoimmune mechanism, as the inflammatory lesions were transferable with spleen cells obtained from the mice with pancreatitis, but not with the sera from these mice.

It became clear from immunohistochemical analysis that $CD4^+$ cells were predominant in the pancreatic inflammatory sites of the unmanipulated mice as well as the recipient mice with adoptive transfer of spleen cells. We also found a large amount of Mac-2^+ macrophages in the destructive sites in parenchyma. These findings suggest that $CD4^+$ cells play a major role *in situ* for the activation of macrophages. These activated macrophages may induce destruction of pancreatic parenchyma directly or via antibody-dependent cellular cytotoxicity (ADCC) associated with autoantibodies reacting with acinar cells. Macrophages of MRL/Mp-*lpr*/*lpr* mice, but not of C3H/HeJ-*lpr*/*lpr* mice, show increased tumour cytolytic activity, ADCC activity, superoxide production and phagocytic activity [16,17]. Moreover, both MRL/Mp-+/+ and MRL/Mp-*lpr*/*lpr* mice display impaired Fc receptor-mediated clearance [18]. Therefore, macrophages in the MRL/Mp strain of mice may play important roles in the progression of pancreatic inflammatory lesions under a particular genetic background.

Another important point presented here is the fact that female MRL/Mp-+/+ mice develop pancreatitis at an earlier age, with a higher incidence and with more severity, compared with male mice. Moreover, we observed a remarkable difference in the incidence of pancreatitis between the female and male mice treated with adoptive transfer of spleen cells (Table 3). Brick *et al.* [19] reported that sex hormones influence the titres of autoantibodies in MRL/Mp-+/+ mice. Moreover, administration of androgens retards autoimmune disease in female MRL/Mp-*lpr*/*lpr* mice [20]. Therefore, sex-related factors in these mice seem to influence the development of pancreatitis.

There have been several reports suggesting the contribution of immunological mechanisms to the development of chronic pancreatitis in humans. Forbes *et al.* [15] pointed out the significance of immunological factors, especially HLA-A25 and -Cw1, in the development of idiopathic chronic pancreatitis. The antigen HLA-A1 is also associated with non-alcoholic chronic pancreatitis [21]. In immunohistochemical studies, Bedossa *et al.* [22] detected a large amount of $CD8^+$ cells in the inflammatory lesions of human chronic pancreatitis. As for autoantibodies in human chronic pancreatitis, several groups found anti-acinar cell autoantibodies in some patients of chronic pancreatitis [13–15]. However, it is still unclear whether an autoimmune mechanism is involved in the aetiopathogenesis of human chronic pancreatitis. Our results indicate that MRL/Mp-+/+ mice are a useful animal model for autoimmune pancreatitis, and may advance the analysis of autoimmune mechanisms in human chronic pancreatitis.

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