博士学位論文

高脂肪食はⅠ型 IFN シグナル伝達経路の活性化を通して実験的自己免疫性膵炎を悪化させる

近畿大学大学院
医学研究科医学系専攻

瀬　海　郁　衣

Doctoral Dissertation

High-fat diet aggravates experimental autoimmune pancreatitis through the activation of type I interferon signaling pathways

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High-fat diet aggravates experimental autoimmune pancreatitis through the activation of type I interferon signaling pathways

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ABSTRACT

Autoimmune pancreatitis (AIP) is an autoimmune disorder of the pancreas characterized by enhanced IgG4 antibody responses and multiple organ involvement. AIP is a pancreatic manifestation of the systemic IgG4-related disease (IgG4-RD). Although AIP and IgG4-RD predominantly occur in middle-aged and elderly men, the roles of eating habits and lifestyle in the pathogenesis of these conditions are poorly understood. In this study, we examined whether a high-fat diet (HFD), preferred by middle-aged and elderly men, increases sensitivity to experimental AIP. We modeled AIP in MRL/MpJ mice by repeated injections of polyinosinic:polycytidylic acid. HFD exacerbated AIP development and promoted pancreatic accumulation of interferon (IFN)-α-producing plasmacytoid dendritic cells (pDCs). However, HFD did not increase the severity of autoimmune sialadenitis, another disorder associated with AIP and IgG4-RD. Neutralization of type I IFN signaling pathways prevented the development of severe AIP induced by HFD. In contrast, leaky gut was less likely to be associated with the HFD-induced exacerbation of AIP, as was evidenced by the lack of significant alterations in the jejunal or ileal expression of tight junction proteins. These data suggest that HFD exacerbates experimental AIP through the activation of pDCs producing IFN-α.

1. Introduction

IgG4-related disease (IgG4-RD) is a newly established autoimmune disorder characterized by enhanced production of IgG4 antibodies (Abs) and infiltration of IgG4-positive plasmacytes into the affected organs [1,2]. Another important feature of IgG4-RD is multiple organ involvement; the pancreas, salivary glands, and bile ducts are frequently affected [1,2]. Autoimmune pancreatitis (AIP) is classified into type 1 and 2, and the former accounts for more than 95% of all AIP cases [1]. Clinicopathological analysis identified type 1 AIP (hereafter referred to as just AIP) as a pancreatic manifestation of systemic IgG4-RD [1]. From the epidemiological standpoint, both AIP and IgG4-RD predominantly occur in middle-aged and elderly men [1,2]. This biased distribution strongly suggests the effect of some particular lifestyle features preferred by senior men, on the incidence of AIP and IgG4-RD. Notably, more than 60% of patients with AIP have pre-existing and concurrent diabetes mellitus, a prototypical lifestyle disease, at the time of diagnosis [3,4]. However, to the best of our knowledge, few studies have addressed the role of the high-fat diet (HFD), which is preferred by middle-aged and elderly men, in AIP and IgG4-RD pathogenesis.

The immunopathogenesis of AIP and IgG4-RD is poorly defined despite increasing attention of physicians and immunologists on these autoimmune disorders. The development of experimental AIP and human IgG4-RD requires activation of plasmacytoid dendritic cells (pDCs) that produce IFN-α [5–7]. pDCs and type I IFN signaling pathways are important in adipose tissue inflammation [8–10]. Therefore, we hypothesized that IFN-α-producing pDCs associated with obesity increase the sensitivity to AIP and IgG4-RD. To address this question, we utilized a well-established experimental model of AIP in combination with HFD [5,6]. Herein, we provide evidence that HFD aggravates experimental AIP through the activation of pDCs that produce IFN-α.

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Abbreviations

Ab	antibody
AIP	autoimmune pancreatitis
AIS	autoimmune sialadenitis
CCR	C–C chemokine receptor
ELISA	enzyme-linked immunosorbent assay
H&E	hematoxylin and eosin
HFD	high-fat diet
IFNAR	IFN-αβ receptor
IgG4-RD	IgG4-related disease
ND	normal diet
pDC	plasmacytoid dendritic cell
PDCA-1	pDC antigen-1
poly(I:C)	polyinosinic:polycytidylic acid
TAK1	TGF-β-activated kinase 1
TJP	tight junction protein

2. Materials and methods

2.1. Mice

Four-week-old female MRL/MpJ mice (Japan SLC, Hamamatsu, Japan) were fed a normal diet (ND) or a HFD (D12079B; Research Diets, New Brunswick, NJ, USA) that contained 13 and 41 kcal% fat, respectively [11]. Mice were fed HFD or ND for 8 weeks prior to the induction of experimental AIP (Fig. 1A). AIP was induced by repeated intraperitoneal injections of polyinosinic:polycytidylic acid (poly(I:C), 100 μg, InvivoGen, San Diego, CA) twice a week for a total of 16 times [5,6,12]. HFD or ND was continued throughout the entire experimental period. In some experiments, ND or HFD-fed mice were treated with an anti-IFN-αβ receptor Ab (anti-IFNAR Ab, 0.2 mg/mouse, BioXcell, Lebanon, NH) or a control mouse IgG (Sigma-Aldrich, St. Louis, MO) after AIP induction [5]. The mice received these Abs at the time of the poly(I:C) injection. The animal experiments were approved by the Review Board of the Kindai University Faculty of Medicine.

2.2. Pathological analysis of pancreatitis and sialadenitis samples

Mice were sacrificed 3 h after the final injection of poly(I:C). Samples from the pancreas, salivary gland, and liver were obtained from mice sacrificed 3 h after the final injection of poly(I:C). These samples were fixed in 10% formalin and stained with hematoxylin and eosin (H&E). Pathological assessment was performed using scoring systems for AIP and autoimmune sialadenitis (AIS), as previously described [5,6,12,13]. Pancreatic inflammation was scored as follows: 0, pancreas without mononuclear cell infiltration; 1, mononuclear cell aggregation and/or infiltration within the interstitium without parenchymal destruction; 2, focal parenchymal destruction with mononuclear cell infiltration; 3, diffuse parenchymal destruction with some parenchymal areas remaining intact; and 4, destruction of almost all pancreatic tissue, except for pancreatic islets, or its replacement with fibrotic or adipose tissue. Salivary gland inflammation was evaluated on a scale of 0–3 as follows: 0, normal; 1, mononuclear cell infiltration localized in the periductular regions; 2, destruction of ductules with cell infiltration extending to the parenchyma; and 3, grade 2 changes plus proliferation of the residual ductules, granulomatous lesions, and/or fibrosis in the parenchyma [13]. At least two H&E-stained sections from each slide were analyzed using a microscope (Biozero, BZ-

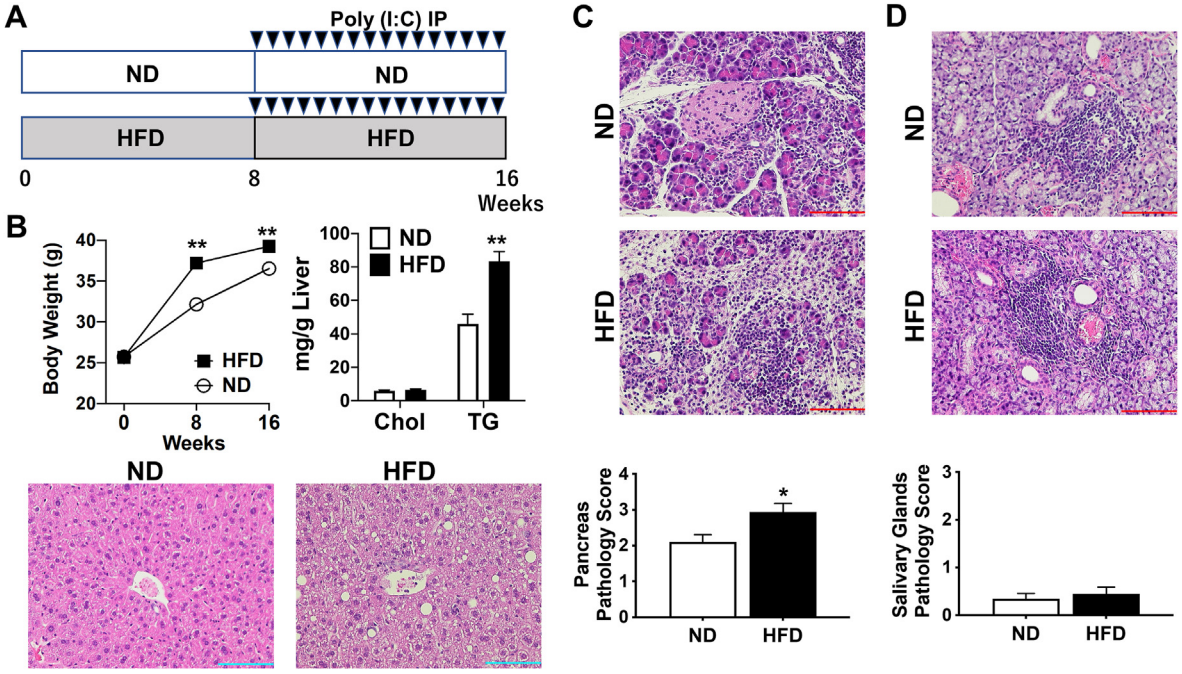

Fig. 1. High-fat diet exacerbates experimental autoimmune pancreatitis.
(A) Experimental protocols are illustrated. MRL/MpJ mice were fed normal diet (ND) or high-fat diet (HFD) during the entire experiment, starting from week 0. Mice received intraperitoneal injections of polyinosinic:polycytidylic acid (poly(I:C)) from week 8 twice a week for a total of 16 times. **(B)** Body weight and hepatic concentrations of cholesterol (Chol) and triglycerides (TG) in mice fed ND (n = 22) or HFD (n = 19) and intraperitoneally injected with poly(I:C). Bottom panel shows typical hepatic lipid droplets in the HFD-fed mice. **(C)** Pathological analyses of the pancreas of MRL/MpJ mice fed ND (n = 15) or HFD (n = 9) and treated with intraperitoneal injections of poly(I:C). Representative hematoxylin & eosin (H&E) staining and pathology scores are shown. **(D)** Pathological analyses of the salivary glands of mice fed ND (n = 10) or HFD (n = 9) and intraperitoneally injected with poly(I:C). Representative H&E staining and pathology scores are shown. Results are expressed as the mean +SEM. Scale bar; 100 μm *$P < 0.05$, **$P < 0.01$.

8100, Keyence, Osaka, Japan).

2.3. Isolation of immune cells from the pancreas, liver, and salivary glands

Mononuclear cells were prepared from the pancreas, salivary glands, and liver of mice, as previously described [5,6,12–14]. Cells were stained with FITC-conjugated anti-B220 Ab, PE-conjugated anti-pDC antigen-1 (PDCA-1) Ab, and PE-conjugated anti-CD3 Ab (all from eBioscience, San Diego, CA). Flow-cytometry analyses were performed using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) and CFlow Plus software (BD Biosciences).

2.4. Cytokine assay

Cytokine concentrations in the pancreas, salivary glands, and liver were measured using enzyme-linked immunosorbent assay (ELISA) kits [5,6,15,16]. IFN-α concentration was measured using an ELISA kit from R&D Systems (Minneapolis, MN), whereas concentrations of IFN-γ, IL-6, IL-13, IL-17, IL-33, and TNF-α were determined using ELISA kits from eBioscience [5,6,15,16].

2.5. Measurement of hepatic lipid accumulation

Liver homogenates were prepared using a kit from Cell Biolabs (Lipid Droplet Isolation Kit, San Diego, CA). Concentrations of triglycerides and cholesterol in liver homogenates were measured using Cell Biolabs kits.

2.6. Assessment of tight junction proteins in the gastrointestinal tract by quantitative PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA); cDNA was synthesized as previously described [17,18]. The mRNA levels of target genes were determined using SYBR Green-based quantitative PCR on a LightCycler 480 system (Roche, Tokyo, Japan). mRNA expression levels were normalized to that of *Gapdh* as a control gene. Primer sequences for *Cldn2, Cldn3, Cldn4, Cldn7, Cldn8, Jam1,* and *Tjp1* were as described previously [19].

2.7. Statistical analysis

The significance of differences was assessed using the Mann-Whitney *U* test. Statistical tests were performed using Prism (GraphPad Software Inc., La Jolla, CA, USA). Results were considered statistically significant if $P < 0.05$.

3. Results

3.1. HFD induces lipid accumulation in the liver

The high incidence of AIP and IgG4-RD in middle-aged and elderly men prompted us to examine the role of eating habits, especially HFD, in these disorders [1,2]. To determine the effects of HFD on the development of experimental AIP, MRL/MpJ mice were fed HFD or ND and repeatedly injected with poly(I:C) (Fig. 1A). The HFD-fed mice gained more weight than the ND-fed mice (Fig. 1B). In addition, the hepatic accumulation of triglycerides, but not cholesterol, was significantly greater in the HFD-fed mice than that in the ND-fed mice (Fig. 1B). Indeed, lipid droplet formation was augmented in the liver of the HFD-fed mice (Fig. 1B). Therefore, HFD induced steatosis in obese MRL/MpJ mice.

3.2. HFD exacerbates experimental autoimmune pancreatitis

We next examined whether the severity of AIP was altered by HFD. Repeated injections of poly(I:C) into MRL/MpJ mice led to the development of AIP, which was characterized by the destruction of pancreatic acinar architecture and infiltration of immune cells [5,6,12]. The severity of AIP was greater in the HFD-fed mice than in the ND-fed mice, as indicated by the pathological scores (Fig. 1C). AIS is one of the prototypical extrapancreatic manifestations of AIP and IgG4-RD. Diet had no significant effect on the pathological scores in AIS (Fig. 1D). The HFD-fed mice did not exhibit experimental AIP without repeated injections of poly(I:C) (data not shown). These data suggest that HFD aggravated the development of AIP, but not AIS.

3.3. HFD promotes activation of pDCs in the pancreas

The development of experimental AIP requires activation of pDCs that produce IFN-α and IL-33 [2,5,6,12]. Flow-cytometry analysis revealed that the percentage of pDCs, defined as PDCA-1^+B220^{low} cells, was higher in the pancreas of the HFD-fed mice than in the pancreas of the ND-fed mice (Fig. 2A). No significant difference was observed in the percentage of $CD3^+$ T cells in the pancreas of the HFD- and ND-fed mice, whereas the percentage of $B220^+$ B cells was significantly lower in the former group.

In agreement with the pancreatic accumulation of pDCs, the pancreatic expression levels of IFN-α and IL-33 were much higher in the HFD-fed mice than in the ND-fed mice. This accumulation of pDCs led to the robust production of pro-inflammatory and pro-fibrogenic cytokines, including IFN-γ, IL-13, and TNF-α (Fig. 2B). In contrast, the percentage of pDCs and the expression levels of IFN-α and IL-33 were comparable in the liver and salivary glands of the HFD- and ND-fed mice (Fig. 2C and D, and data not shown). HFD significantly reduced the percentage of $B220^+$ B cells in the liver. Taken together, these data suggest that the aggravation of experimental AIP by HFD was accompanied by pancreatic accumulation of pDCs producing IFN-α and IL-33.

3.4. HFD exacerbates AIP by activating type I IFN signaling pathways

Pancreatic accumulation of pDCs producing IFN-α mediates experimental AIP as shown in our previous studies [12]. The inhibition of IFN-α-mediated signaling pathways by anti-IFNAR Ab efficiently prevented the development of experimental AIP in the ND-fed mice (Fig. 3A). Administration of anti-IFNAR Ab markedly reduced pancreatic accumulation of pDCs and $CD3^+$ T cells in the ND-fed mice (Fig. 3B). In addition, pancreatic expression of IFN-α and IL-33 was markedly decreased in the ND-fed mice treated with anti-IFNAR Ab, compared to that in those treated with control Ab (Fig. 3B). These data suggest that pDCs producing IFN-α mediated the development of experimental AIP in the ND-fed mice.

Having confirmed that the percentage of pDCs producing IFN-α was higher in the pancreas of the HFD-fed mice than in the pancreas of the ND-fed mice and that the development of experimental AIP required activation of type I IFN signaling pathways in the ND-fed mice, we investigated whether HFD promotes the development of severe AIP through robust induction of type I IFN responses. For this purpose, HFD-fed mice were treated with an anti-IFNAR Ab to neutralize type I IFN responses as above [12]. Pathological examinations revealed that administration of anti-IFNAR Ab prevented the development of severe AIP in the HFD-fed mice, which was accompanied by reduced pancreatic accumulation of pDCs producing IFN-α and IL-33 (Fig. 3C and D). Thus, HFD increased the severity of experimental AIP through the

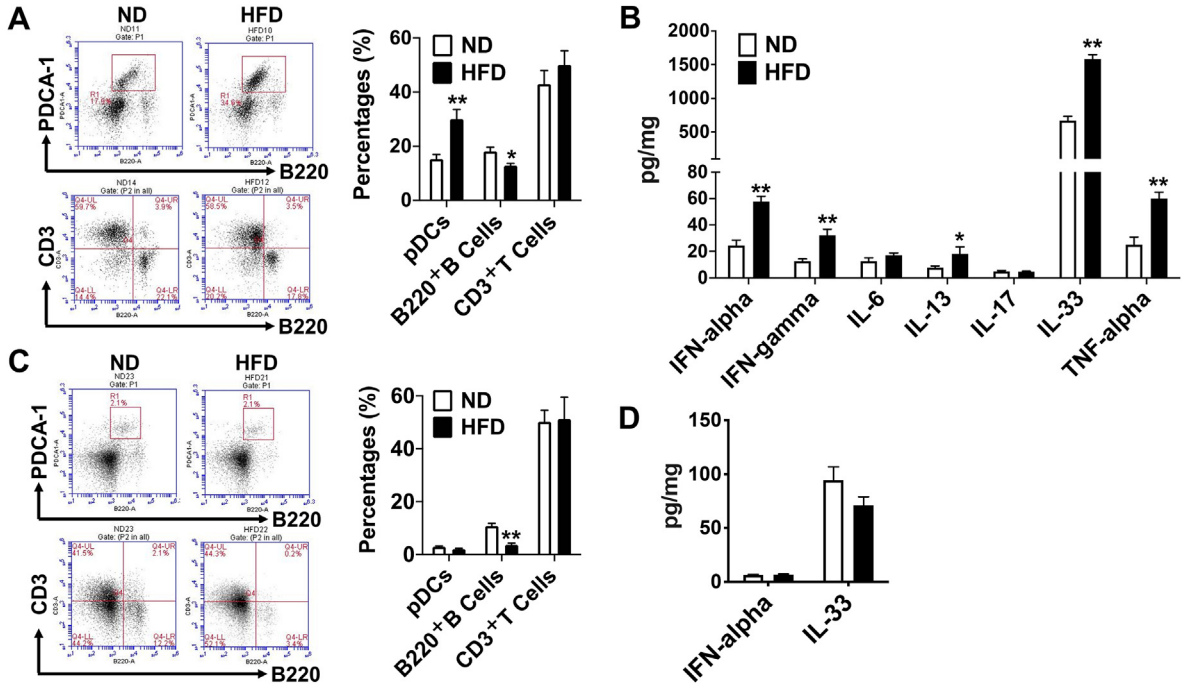

Fig. 2. High-fat diet induces pancreatic accumulation of plasmacytoid dendritic cells.
MRL/MpJ mice were fed normal diet (ND) or high-fat diet (HFD) and intraperitoneally injected with polyinosinic:polycytidylic acid, as described in Fig. 1. **(A)** The percentages of plasmacytoid dendritic cells (pDCs), $CD3^+$ T cells, and $B220^+$ B cells in the pancreas (ND; n = 10, HFD; n = 9) were determined through flow-cytometry analysis of pancreatic mononuclear cells. **(B)** Pancreatic cytokine expression levels were determined using enzyme-linked immunosorbent assays (ELISAs). **(C)** The percentages of pDCs, $CD3^+$ T cells, and $B220^+$ B cells in the liver (ND; n = 7, HFD; n = 7) were determined through flow-cytometry analysis of hepatic mononuclear cells. **(D)** Hepatic cytokine expression levels were determined using ELISAs. Results are expressed as the mean +SEM. $*P < 0.05$, $**P < 0.01$.

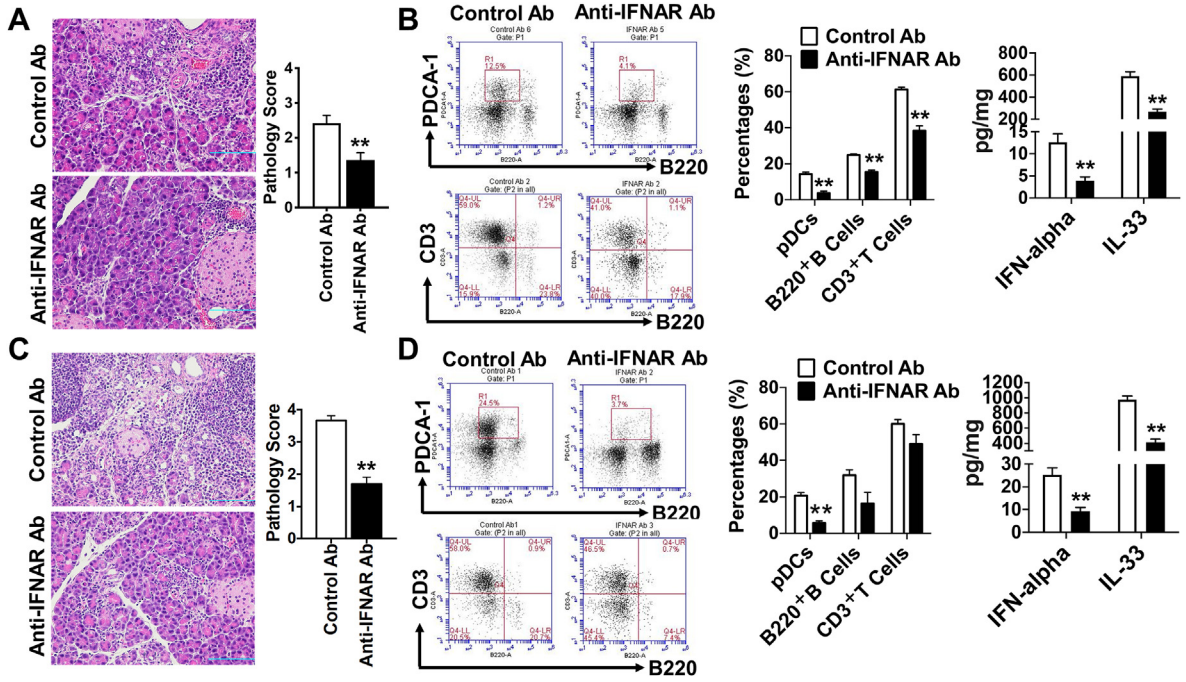

Fig. 3. Inhibition of type I IFN signaling pathways attenuates the exacerbation of autoimmune pancreatitis induced by high-fat diet.
MRL/MpJ mice were fed normal diet (ND) or high-fat diet (HFD) and intraperitoneally injected with polyinosinic:polycytidylic acid, as described in Fig. 1. **(A, B)** ND-fed mice were treated with a control Ab or an anti-IFNAR Ab after the induction of autoimmune pancreatitis (AIP). Pathological analysis of the pancreas of MRL/MpJ mice treated with a control Ab (n = 6) or an anti-IFNAR Ab (n = 5). Representative hematoxylin & eosin (H&E) staining and pathology scores are shown (A). The percentage of plasmacytoid dendritic cells (pDCs) was determined through flow-cytometry analysis of pancreatic mononuclear cells. Pancreatic cytokine expression levels were determined using enzyme-linked immunosorbent assays (ELISAs, B). **(C, D)** HFD-fed mice were treated with a control Ab or an anti-IFNAR Ab after the induction of AIP. Pathological analysis of the pancreas of MRL/MpJ mice treated with a control Ab (n = 6) or an anti-IFNAR Ab (n = 5). Representative H&E staining and pathology scores are shown (C). The percentage of pDCs was determined through flow-cytometry analysis of pancreatic mononuclear cells. Pancreatic cytokine expression levels were determined using ELISAs (D). Scale bar; 100 μm. Results are expressed as the mean +SEM. $**P < 0.01$.

activation of IFN-α-producing pDCs. Collectively, these studies utilizing anti-IFNAR Ab suggest that repeated injections of poly(I:C) led to the development of AIP in the ND or HFD-fed MRL/MpJ mice through induction of type I IFN responses. It should be noted, however, that the severity of AIP was greater in the HFD-fed mice than in the ND-fed mice due to increased accumulation of pDCs and expression of IFN-α in the pancreas of the former mice.

Although the inhibition of IFN-α-mediated signaling pathways attenuated AIP development in the HFD-fed mice, it did not alter body weight gain (data not shown) or hepatic lipid accumulation (Fig. 4A). Hepatic accumulation of triglycerides and lipid droplet formation in the HFD-fed mice were unaffected by anti-IFNAR Ab treatment. Thus, the administration of the anti-IFNAR Ab failed to protect from steatosis, which could be explained by the pro-inflammatory roles of IL-6 or TNF-α rather than that of type I IFNs in metabolic disorders [20].

3.5. HFD does not impair expression levels of tight junction proteins in the gastrointestinal tract

The HFD increases intestinal permeability through the down-regulation of tight junction protein (TJP) expression levels [21,22]. To determine the involvement of gut barrier dysfunction in the HFD-induced exacerbation of AIP, TJP expression levels were analyzed using quantitative PCR. Unexpectedly, no significant alterations were observed in the mRNA expression levels of TJPs in the jejunum, ileum, and colon of mice fed ND or HFD (Fig. 4B and data not shown). Moreover, the administration of anti-IFNAR Ab, which prevented the exacerbation of AIP induced by HFD, significantly reduced the jejunal expression of claudin 8 (Fig. 4C). These data suggest that impaired function of TJPs was not involved in the exacerbation of the experimental AIP. Collectively, the HFD-induced exacerbation of experimental AIP was likely mediated by the activation of pDCs producing IFN-α.

4. Discussion

In this study, we explored the effect of HFD on the development of AIP and AIS and found that HFD aggravated experimental AIP, but not AIS. The development of severe AIP induced by HFD was mediated by the enhanced type I IFN responses in the pancreas as shown by the higher percentage of pDCs producing IFN-α in the pancreas of the HFD-fed mice than in that of the ND-fed mice. Although the development of AIP was efficiently inhibited by the administration of anti-IFNAR Ab in the ND or HFD-fed mice, our data show that treatment with both HFD and poly(I:C) caused more severe AIP than that with poly(I:C) alone. Importantly, the inhibition of type I IFN responses protected HFD-fed mice from the development of severe AIP, but not from metabolic disorders. These data are consistent with previous findings that the development of AIP depends on signaling pathways mediated by type I IFNs, whereas that of metabolic disorders requires TNF-α and/or IL-6 production [2,20]. Although HFD is considered to increase intestinal permeability due to the downregulation of TJP function [22,23], the expression levels of TJPs in the gastrointestinal tract were comparable between the HFD- and ND-fed MRL/MpJ mice after AIP induction. Therefore, HFD promotes the development of severe AIP through the activation of type I IFN responses.

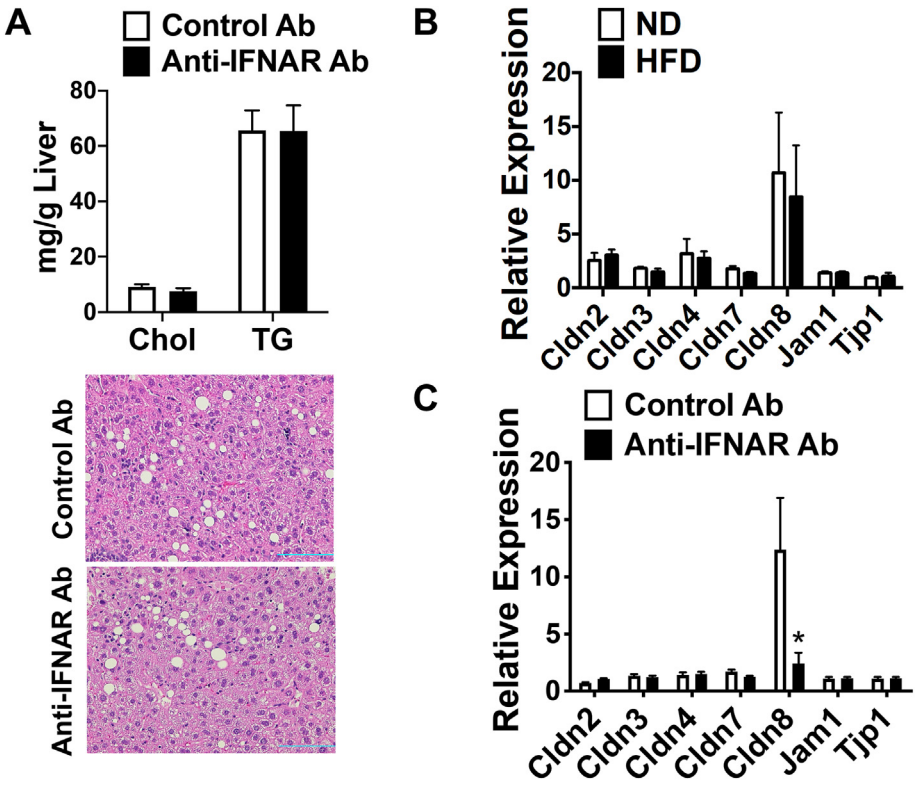

Fig. 4. High-fat diet does not impair expression levels of tight junction proteins in the gastrointestinal tract.
MRL/MpJ mice were fed normal diet (ND) or high-fat diet (HFD) and intraperitoneally injected with polyinosinic:polycytidylic acid (poly(I:C)), as described in Fig. 1. HFD-fed mice were treated with an anti-IFNAR or a control Ab after the induction of autoimmune pancreatitis. **(A)** Hepatic concentrations of cholesterol (Chol) and triglycerides (TG) in the HFD-fed mice treated with a control Ab or an anti-IFNAR Ab. Representative hepatic lipid droplets are shown in the bottom panel. **(B)** Expression of tight junction proteins (TJPs) in the jejunum of mice fed ND (n = 10) or HFD (n = 7) and intraperitoneally injected with poly(I:C). **(C)** Expression of TJPs in the jejunum of mice fed an HFD and treated with a control Ab (n = 6) or an anti-IFNAR Ab (n = 5). Results are expressed as the mean +SEM. Scale bar; 100 μm *$P < 0.05$.

AIP and IgG4-RD occur predominantly in middle-aged and elderly men [1,2]. The latest Japanese epidemiological survey of AIP revealed that the mean age of patients was 68.1 years and the male-to-female ratio was 2.94 [24]. No epidemiological data on the incidence of hyperlipidemia in patients with AIP and IgG4-RD are available, despite the fact that more than 50% of patients with these disorders have diabetes mellitus at diagnosis [3,4]. These epidemiological characteristics led us to examine the role of the HFD in experimental AIP. In this study, experimental AIP was worsened by HFD, which was accompanied by the activation of IFN-α-producing pDCs. This study provides new insights into the link between susceptibility to AIP and eating habits. Consistent with our data, Jaster et al. reported that the degree of spontaneous AIP was greater in western diet-fed MRL/MpJ mice than in those that received calorie-restricted diet [25]. In addition, they identified *Map3k7* encoding TGF-β-activated kinase 1 (TAK1) as a novel susceptibility gene for AIP driven by the western diet [25]. Given the involvement of TAK1 activation in IFN-α production by pDCs [26,27], those data together with ours support the idea that AIP is a multifactorial disease driven by dietary and genetic factors, both of which activate IFN-α-producing pDCs. This hypothesis is supported by our findings that HFD-fed MRL/MpJ mice did not develop experimental AIP without repeated injections of poly(I:C).

HFD causes gut leakiness through the downregulation of TJP expression [22,23]. However, there was no significant reduction in the expression of TJPs in the gastrointestinal tract of the HFD-fed mice. The molecular mechanisms accounting for the comparable levels of TJP expression in the HFD- or ND-fed mice remain unknown. In this regard, we speculate that strong type I IFN responses caused by the administration of poly(I:C) maintain TJP expression levels and intestinal integrity. Inhibiting type I IFN signaling pathways protected the HFD-fed mice from the aggravation of experimental AIP, despite reduced claudin 8 expression in the gastrointestinal tract. Consistent with this idea, type I IFNs upregulates TJP expression [28]. Collectively, our data suggest that HFD exacerbates the experimental AIP in a type I IFN-dependent and TJP expression-independent manner.

One question arising from the present study is how the migration of pDCs to the pancreas is accelerated upon exposure to HFD. Our preliminary data suggest that pancreatic pDCs in mice displaying AIP express CC chemokine receptor 2 (CCR2), CCR7, and CCR9 (data not shown). Based on the expression profiles of chemokine receptors, three pDC trafficking routes might be considered. First, pDCs may migrate to the pancreas from adipose tissues, where the $CCR7^{+}$ pDCs are abundant, in the HFD-fed mice [8–10]. Second, the HFD might accelerate the migration of pDCs from the gut to the pancreas due to the high expression of CCR9 in the small intestinal pDCs [29]. Finally, the liver-pancreas axis might promote the accumulation of pDCs in the pancreas, because steatosis induces CCR9 expression in myeloid cells [30]. However, this latter possibility is less likely, considering that pDC activation and production of IFN-α were comparable in the livers of the HFD- or ND-fed mice. The involvement of adipose tissue-pancreas axis and/or gut-pancreas axis in the enhanced susceptibility to AIP upon exposure to HFD requires further studies focusing on chemokine networks.

In conclusion, the development of severe AIP was accelerated by HFD. The pancreatic accumulation of IFN-α-producing pDCs contributed to the development of severe AIP. These observations provide a mechanistic link between HFD and AIP/IgG4-RD, which preferentially targets middle-aged and elderly men. Normalization of eating habits, especially limiting HFD, might protect against AIP and IgG4-RD.

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CRediT authorship contribution statement

Ikue Sekai: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. **Kosuke Minaga:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. **Akane Hara:** Investigation, Formal analysis. **Yasuo Otsuka:** Investigation, Formal analysis. **Masayuki Kurimoto:** Investigation, Formal analysis. **Naoya Omaru:** Investigation, Formal analysis. **Natsuki Okai:** Investigation, Formal analysis. **Yasuhiro Masuta:** Investigation, Formal analysis. **Ryutaro Takada:** Investigation, Formal analysis. **Tomoe Yoshikawa:** Investigation, Formal analysis. **Ken Kamata:** Investigation, Formal analysis. **Masatoshi Kudo:** Writing – review & editing. **Tomohiro Watanabe:** Conceptualization, Methodology, Investigation, Formal analysis, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no potential conflicts of interest to declare.

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