Title

Comparison of neutrophil infiltration between type 1 and type 2 autoimmune pancreatitis.

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Short title

Neutrophil infiltration in AIP.

Abstract

Background

Characteristics of type 2 autoimmune pancreatitis (AIP) is granulocyte epithelial lesions, called idiopathic duct-centric pancreatitis (IDCP). To clarify pathogenesis of IDCP, we investigated mechanism of neutrophil infiltration in type 1 AIP, called lymphoplasmacytic sclerosing pancreatitis (LPSP) and IDCP.

Method

This study was performed on resected pancreata from patients with alcoholic chronic pancreatitis (ACP, n=10), LPSP (n=10) and IDCP (n=12). The number of neutrophils around the pancreatic ducts was counted. The expression of neutrophils chemoattractants granulocyte chemotactic protein-2 (GCP-2) and interleukin-8 (IL-8) in the pancreatic duct epithelia was examined using immunohistochemistry. The cell staining intensity is scored as negative (0), weak (1), moderate (2) or strong (3).

Results

The median number of neutrophils around the interlobular pancreatic ducts was significantly higher in IDCP (15.16; interquartile range [IQR]: 9.74-18.41) than in ACP (2.66; IQR: 1.33-4.33) ($P<0.05$) and LPSP (3.16; IQR: 2.74-4.57) ($P<0.01$). There was no significant difference in the median number of neutrophils around the intralobular pancreatic ducts among ACP (1.16; IQR: 0.33-3.41), LPSP (3.16; IQR: 0.74-5.5) and IDCP (3.00; IQR: 1.08-7.91). The median score of GCP-2 in the interlobular pancreatic duct epithelia was significantly higher in IDCP (1.5; IQR: 0.25-2) than in ACP (0; IQR:

0-0.75) ($P<0.05$) and LPSP (0; IQR: 0-0.75) ($P<0.05$). There was no significant difference in the median score of IL-8 in the interlobular pancreatic duct epithelia among ACP (0; IQR: 0-0.75), LPSP (1; IQR: 0-1.75) and IDCP (0.5; IQR: 0-1).

Conclusions

Significantly increased neutrophil infiltration around the interlobular pancreatic duct in IDCP may depend on GCP-2.

Key words

type 1 AIP, type 2 AIP, neutrophils, GCP-2, IL-8

Introduction

In 1961, Sarles et al. [1] observed a case of particular pancreatitis with hypergammaglobulinemia. In 1995, Yoshida et al. [2] proposed a novel concept of autoimmune pancreatitis (AIP), nowadays recognized as type 1 AIP. Type 1 AIP is the pancreatic manifestation of IgG4-related disease (IgG4-RD) [3,4], and has been recognized as a clinical entity following the significant evidence of increased serum levels of IgG4 [5]. The histologic description is called lymphoplasmacytic sclerosing pancreatitis (LPSP) [6]. However, in western countries, histological analyses using resected pancreatic samples in patients with non-alcoholic chronic pancreatitis frequently demonstrated a different histological pattern of pancreatitis from LPSP, which is called idiopathic duct-centric pancreatitis (IDCP) or AIP with granulocyte epithelial lesions (GELs) [7-9]. In 2011, the International Consensus Diagnostic Criteria (ICDC) for AIP first enabled us to diagnose and compare two distinctive subtypes [10]. Type 2 AIP has swelling of the pancreas with none or very few IgG4-positive plasma cells. Clinical features show a distinctly different profile associated with no serum IgG4 elevation or other organ involvement except for inflammatory bowel disease. The most characteristic feature of type 2 AIP is GELs, often seen with destruction and obliteration of the pancreatic duct.

Neutrophil granulocytes (also known as neutrophils) have emerged as important regulators of innate and adaptive immune responses. Recently, it has been reported that neutrophils display abnormalities in phenotype and function in various autoimmune diseases and allergic diseases, such as chronic obstructive

pulmonary disease (COPD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and asthma, and may play a central role in initiation and perpetuation of aberrant immune responses and organ damage in these conditions [11-14].

Chemokines contribute to inflammatory response by selective attraction of various leukocytic cell types.

Chemokines are classified into four subfamilies based on the position of conserved cysteines: CXC, CC,

C and CX₃C chemokines [15]. CXC chemokines can be subdivided further into two subgroups depending

on the presence or absence of the Glu-Leu-Arg sequence (ELR). ELR⁺CXC chemokines [GRO α /CXCL1,

GRO β /CXCL2, GRO γ /CXCL3, epithelial cell-derived neutrophil attractant (ENA-78)/CXCL5,

granulocyte chemotactic protein-2 (GCP2)/CXCL6, neutrophil activating protein (NAP2)/CXCL7 and

interleukin-8 (IL-8)/CXCL8] are potent activators and chemoattractants for neutrophils. Chemokines

exert their activities by binding to chemokine receptors on the target cells. For ELR⁺CXC chemokines,

neutrophils possess two receptors, CXCR1 and CXCR2. Only two chemokine members, GCP-2 and IL-8,

bind both receptors. The remainder chemokine members, GRO α , GRO β , GRO γ , ENA-78, NAP2, bind

only CXCR2 [16,17]. Due to these effects CXC chemokines can potentially attract and activate

neutrophils in inflammatory processes. Recent evidence indicates that chemokines and their receptors are

likely play a key role in the pathogenesis of various autoimmune diseases and allergic diseases [18-21].

The pathogenesis of type 1 AIP is suggested to be associated with genetic background [22-24] and

abnormal mechanisms of humoral immunity [25-29], acquired immunity [30-34], and innate immunity

[35-37]. However, there have been very few reports on the pathogenesis of type 2 AIP [38]. This study aimed to analyze the difference in level of neutrophil infiltration between type 1 AIP and type 2 AIP in pancreata. We also examined a mechanism of neutrophil infiltration in AIP.

Patients and methods

Subjects

We examined 10 patients with alcoholic chronic pancreatitis (ACP, 10 men; mean age 54 years, range 39-75 years), 10 patients with Type 1 AIP (LPSP, 6 women and 4 men; mean age 65 years, range 56-75 years), and 12 patients with Type 2 AIP (IDCP, 3 women and 9 men; mean age 40 years, range 22-65 years). These samples were obtained from surgical resection. All the type 1 AIP and type 2 AIP cases were suspected as pancreatic ductal adenocarcinoma prior to the operation, and were diagnosed histopathologically as type 1 AIP (LPSP) and type 2 AIP (IDCP) post-surgery. There were no cases of PDA with an ACP, type 1 AIP, or type 2 AIP background, either clinically or histopathologically. ACP and LPSP tissues were obtained from the Kansai Medical University. IDCP tissues were obtained from the Kansai Medical University (1 case), Kurashiki Central Hospital (2 cases), Aichi Cancer Center Hospital (1 case), and University of Verona (8 cases). The diagnosis of type 1 or type 2 AIP was made pathologically according to the ICDC. All resected specimens were obtained from patients not receiving steroids for diagnosis or treatment. Pathological diagnosis of IDCP was conducted by Japanese and Italian pathologists (two of the authors Kenji Notohara and Giuseppe Zamboni). For the control, three histologically normal sections from non-tumorous lesion of neuroendocrine tumor (NET) were used (3 men; mean age 63 years, range 48-70 years). This study was approved by Kansai Medical University

Ethics Committee.

Histopathology and immunohistochemistry

Formalin-fixed and paraffin-embedded specimens were prepared and used for histopathological and immunohistochemical studies. Serial 4- μ m thick sections were cut for hematoxylin and eosin (H&E), and immunohistochemical staining. Formalin-fixed paraffin-embedded pancreatic sections were deparaffinized and rehydrated using xylene and a graded descending series of alcohol. Immunohistochemistry was performed using the streptavidin-biotin complex indirect immunoperoxidase method. The slides containing the samples were incubated with 0.3 percent hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. After washing in distilled water, antigen retrieval was performed by microwaving for 20 minutes in 0.01M citrate buffer (pH 6.0) at 500W. The slides were incubated for 10 minutes in protein blocking reagent without serum (ProTaq Biocyc GmbH & Co., Berlin, Germany). The slides were then incubated with primary antibody overnight at 4°C. Primary antibodies used were a goat polyclonal antibody against human GCP-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:50 dilution; a goat polyclonal antibody against human IL-8 (R&D System Europe Ltd, Abingdon, UK) at a 1:40 dilution. After washing in distilled water, the slides for GCP-2 and IL-8 were incubated for 30 minutes with secondary antibodies at a 1:100 dilution using biotinylated rabbit anti-goat IgG (Vector Laboratories, Burlingame, CA). After washing in distilled water, the slides were

incubated for 30 minutes with avidin–biotin–peroxidase complex (Vectastain ABC Kit, Vector Laboratories). Finally, antibody binding was detected using 3,3'-diaminobenzidine (DAB) (Dojindo, Kumamoto, Japan). Sections were counterstained with hematoxylin. Negative controls were evaluated by replacing the primary antibody with similarly diluted non-immunized IgG. In the interlobular pancreatic ducts, 10 of the 12 IDCP cases had GELs, whereas none of the patients with LPSP had any GELs. In the intralobular pancreatic ducts, 9 of the 12 IDCP cases and one of the 10 LPSP cases had GELs.

We counted neutrophils and infiltrated cells around the inter- and intralobular pancreatic ducts in the three different inflammatory areas where neutrophils most infiltrated per high power field (hpf) ($\times 400$). The numbers of neutrophils and the ratio of neutrophils to infiltrated cells in the three different fields were averaged and compared with ACP, LPSP and IDCP respectively. These methods were performed according to our previous reports [37,39-41].

Thus, the evaluation was conducted using anti-GCP-2 and anti-IL-8 antibody. The expression of GCP-2 and IL-8 in the inter- and intralobular pancreatic duct epithelia was examined in three different active inflammatory areas. The cell staining intensity is scored as negative (0), weak (1), moderate (2) and strong (3) (Fig. 1). These methods were performed according to the previous reports [42,43].

Statistical analysis

For all studies, data are expressed as median and interquartile range. Differences were analyzed using the

Mann-Whitney U-test if the Kruskal-Wallis test led to significant results. In their tests, p-values of <0.05 were considered statistically significant.

Result

Comparison of the number of neutrophils and the ratio of neutrophils to infiltrated cells around the interlobular pancreatic ducts among ACP, LPSP and IDCP

The median number of neutrophils around the interlobular pancreatic ducts was significantly higher in IDCP (15.16; interquartile range [IQR]: 9.74-18.41; Fig. 2f) than in ACP (2.66; IQR: 1.33-4.33; Fig. 2b) ($P<0.05$; Fig. 2g) and LPSP (3.16; IQR: 2.74-4.57; Fig. 2d) ($P<0.01$; Fig. 2g). The median ratio of neutrophils to infiltrated cells around the interlobular pancreatic ducts was significantly higher in IDCP (8.29; IQR: 6.59-9.85; Fig. 2f) than in LPSP (2.28; IQR: 1.81-3.89; Fig. 2d) ($P<0.05$; Fig. 2h).

Comparison of the number of neutrophils and the ratio of neutrophils to infiltrated cells around the intralobular pancreatic ducts among ACP, LPSP and IDCP

There was no significant difference in the median number of neutrophils around the intralobular pancreatic ducts among ACP (1.16; IQR: 0.33-3.41; Fig. 3b), LPSP (3.16; IQR: 0.74-5.50; Fig. 3d) and IDCP (3.00; IQR: 1.08-7.91; Fig. 3f, g). There was no significant difference in the median ratio of neutrophils to infiltrated cells around the intralobular pancreatic ducts among ACP (2.55; IQR: 1.02-6.14; Fig. 3b), LPSP (2.54; IQR: 0.78-4.05; Fig. 3d) and IDCP (2.76; IQR: 1.18-6.22; Fig. 3f, h).

CXCR1 was expressed on the neutrophils in both LPSP and IDCP while there was no CXCR2 expression in either LPSP or IDCP

There was no significant difference in the median ratio of CXCR1-positive neutrophils to neutrophils around the interlobular pancreatic ducts among ACP (3.73; IQR: 0-8), LPSP (5.13; IQR: 0.81-9.06) and IDCP (3.66; IQR: 0.62-5.96).

There was no significant difference in the median ratio of CXCR1-positive neutrophils to neutrophils around the intralobular pancreatic ducts among ACP (0; IQR: 0-3.57), LPSP (0; IQR: 0-0.67) and IDCP (1.65; IQR: 0.25-4.62).

CXCR2 was not expressed on the neutrophils around the inter- and intralobular pancreatic ducts in ACP, LPSP and IDCP.

Comparison of the score of GCP-2 and IL-8 in the interlobular pancreatic ducts epithelia among ACP, LPSP and IDCP

The interlobular pancreatic duct epithelia were stained in reaction to anti-GCP-2 and anti-IL-8 antibody.

The median score of GCP-2 in the interlobular pancreatic duct epithelia was significantly higher in IDCP (1.5; IQR: 0.25-2; Fig. 4h) than in ACP (0; IQR: 0-0.75; Fig. 4b) ($P<0.05$; Fig. 4j) and LPSP (0; IQR: 0-0.75; Fig. 4e) ($P<0.05$; Fig. 4j). There was no significant difference in the median score of IL-8 in the interlobular pancreatic duct epithelia among ACP (0; IQR: 0-0.75; Fig. 5b), LPSP (1; IQR: 0-1.75; Fig.

5e) and IDCP (0.5; IQR: 0-1; Fig. 5h, j).

Comparison of the score of GCP-2 and IL-8 in the intralobular pancreatic ducts epithelia among ACP, LPSP and IDCP

The intralobular pancreatic duct epithelia were stained in reaction to anti-GCP-2 and anti-IL-8 antibody.

There was no significant difference in the median score of GCP-2 in the intralobular pancreatic duct epithelia among ACP (0; IQR: 0-0.75; Fig. 6b), LPSP (0; IQR: 0-1; Fig. 6e) and IDCP (0; IQR: 0-1; Fig. 6h, j). The median score of IL-8 in the intralobular pancreatic duct epithelia was significantly lower in ACP (0; IQR: 0-0.75; Fig. 7b) than in LPSP (1; IQR: 0.25-1.75; Fig. 7e) ($P<0.05$; Fig. 7j) and IDCP (1.5; IQR: 1-2.75; Fig. 7h) ($P<0.01$; Fig. 7j).

Discussion

In this study, which is focused on differences in neutrophil infiltration around the pancreatic ducts in LPSP and IDCP, the degree of neutrophil infiltration around the interlobular pancreatic duct was significantly higher in IDCP than in LPSP (Fig. 2g). Conversely, no significant differences were observed in the neutrophil infiltration around the intralobular pancreatic ducts (Fig. 3g). Based on these findings, we assumed that neutrophils infiltration around the interlobular pancreatic ducts is more important than around the intralobular pancreatic ducts for the differentiation between LPSP and IDCP, as previously reported [44]. Meanwhile, no significant difference in neutrophil infiltration around either inter- or intralobular pancreatic ducts was observed between the Italian and Japanese patients with IDCP, and there were no apparent differences in the histopathological features of IDCP between the Italian and Japanese patients (data not shown).

In the present immunohistochemical study, CXCR1 was expressed on the neutrophils in both LPSP and IDCP while there was no CXCR2 expression in either LPSP or IDCP. In view of the lack of CXCR2 expression on the neutrophils, this study focused on GCP-2 and IL-8. These two chemokines are the only ELR⁺CXC chemokines that chemoattract and activate neutrophils by binding to both CXCR1 and CXCR2, whereas the other ELR⁺CXC chemokines signal only through CXCR2 [16,17].

We noted significant differences in the number of neutrophils around the interlobular pancreatic duct and the score of GCP-2 in the interlobular pancreatic duct epithelia between LPSP and IDCP (Fig. 2g, 4j).

GCP-2 and IL-8 were expressed in the pancreatic duct epithelia in both IDCP and LPSP. In general, GCP-2 and IL-8 are potent activators and chemoattractant for neutrophils. Van Damme J, et al. [45] reported that GCP-2 complements the activity of IL-8 as neutrophil chemoattractant and activator. Meanwhile, there were no significant differences in the score of IL-8 in the interlobular pancreatic duct epithelia between LPSP and IDCP (Fig. 5j). Therefore, our findings suggest that a significantly increased infiltration of neutrophils around the interlobular pancreatic duct in IDCP may depend on secretion of GCP-2 from the pancreatic duct epithelia. Furthermore, GCP-2 may play a key role in neutrophil infiltration around the interlobular pancreatic duct in IDCP. Additionally, there were no significant differences in the score of GCP-2 in the intralobular pancreatic duct epithelia between LPSP and IDCP (Fig. 6j), so there was no significant difference in the number of neutrophils around the intralobular pancreatic duct (Fig. 3g). Therefore, our data may also suggest different characteristics between in inter- and intralobular pancreatic duct.

Chemokines are secreted by various tissue cells such as fibroblasts, inflammatory cells, epithelial cells, and vascular endothelial cells, and are involved in the pathogenesis of a variety of acute and chronic inflammatory diseases via neutrophil, monocyte and other cell migration [46]. In general, it has been recognized that GCP-2 is produced by osteosarcoma cells [47]. On the other hand, IL-8 is secreted by inflammatory cells such as monocytes, neutrophils and T-cells [48]. Additionally, there are some reports that GCP-2 and IL-8 are secreted from epithelia [45,49-51]. Our findings suggest that GCP-2 and IL-8 are

secreted from the pancreatic duct epithelia and these chemokines are involved in the migration of neutrophils around the pancreatic ducts in both LPSP and IDCP.

Moreover, there were no significant differences in the ratio of CXCR1-positive neutrophils to neutrophils between LPSP and IDCP (data not shown). Thus, our data suggest the difference in neutrophil infiltration around the pancreatic ducts between LPSP and IDCP may be due to abnormal interlobular ductal characteristics of IDCP, but not be due to leukocytes.

Alternate techniques may be required for these studies. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has widely been adopted and is an important modality for the diagnosis of pancreatic diseases. However, it is difficult to evaluate the interlobular pancreatic ducts with the small samples that can be obtained by EUS-FNA. The present study confirmed that there is no significant difference in neutrophil infiltration around the intralobular pancreatic ducts and that GELs may even be presented in the intralobular pancreatic ducts in LPSP. Furthermore there is also a report that IgG4-positive plasma cell infiltration may be found in IDCP [52]. Thus, clinicians should be careful making a differential diagnosis of LPSP and IDCP based on the number of neutrophils and IgG-4 positive plasma cells as well as the presence or absence of GELs with a small biopsied sample obtained by EUS-FNA.

In conclusion, this study identified GCP-2 and IL-8 expression in the pancreatic duct epithelia. Significantly increased neutrophil infiltration around the interlobular pancreatic duct in IDCP may depend on secretion of GCP-2.

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Legends to Figures

Fig.1 Quantitative assessment of staining intensity in the pancreatic duct epithelia.

(a). Pancreatic duct epithelia that were not stained (showed negative) were scored 0, (b). Pancreatic duct epithelia stained weakly were scored 1, (c). Pancreatic duct epithelia stained moderately were scored 2 (d). Pancreatic duct epithelia stained strongly were scored 3.

Fig.2 Histological findings of the interlobular pancreatic ducts (H&E) and comparison of neutrophils infiltration around the interlobular pancreatic ducts.

Infiltrated cells were seen around the interlobular pancreatic ducts in ACP (a, $\times 100$), LPSP (c, $\times 100$) and IDCP (e, $\times 100$). The number of neutrophils around the interlobular pancreatic ducts was significantly higher in IDCP (f, $\times 400$) than in ACP (b, $\times 400$) and LPSP (d, $\times 400$). Granulocyte epithelial lesion (GELs) were seen in IDCP (f, $\times 400$). (g). The median number of neutrophils around the interlobular pancreatic ducts was significantly higher in IDCP (15.16; IQR: 9.74-18.41) than in ACP (2.66; IQR: 1.33-4.33) ($P<0.05$) and LPSP (3.16; IQR: 2.74-4.57) ($P<0.01$). (h). The median ratio of neutrophils to infiltrated cells around the interlobular pancreatic ducts was significantly higher in IDCP (8.29; IQR: 6.59-9.85) than in LPSP (2.28; IQR: 1.81-3.89) ($P<0.05$). * $p<0.05$, ** $p<0.01$

Fig.3 Histological findings of the intralobular pancreatic ducts (H&E) and comparison of the neutrophils infiltration around the intralobular pancreatic ducts.

Infiltrated cells were seen around the intralobular pancreatic ducts in ACP (a, $\times 100$), LPSP (c, $\times 100$) and IDCP (e, $\times 100$). There was no significant difference in the number of neutrophils around the intralobular pancreatic ducts among ACP (b, $\times 400$), LPSP (d, $\times 400$) and IDCP (f, $\times 400$). Abundant infiltration of granulocytes around and into pancreatic duct with destruction of epithelia was seen in IDCP (f, $\times 400$). (g). There was no significant difference in the median number of neutrophils around the intralobular pancreatic ducts among ACP (1.16; IQR: 0.33-3.41), LPSP (3.16; IQR: 0.74-5.50), and IDCP (3.00; IQR: 1.08-7.91). (h). There was no significant difference in the median ratio of neutrophils to infiltrated cells around the intralobular pancreatic ducts among ACP (2.55; IQR: 1.02-6.14), LPSP (2.54; IQR: 0.78-4.05) and IDCP (2.76; IQR: 1.18-6.22).

Fig.4 Immunohistochemical findings of the interlobular pancreatic duct epithelia stained in reaction to anti-GCP-2 antibody and comparison of the score of staining intensity.

(a). The interlobular pancreatic duct epithelia in ACP (H&E, $\times 100$). (b). The interlobular pancreatic duct epithelia were not stained in ACP (anti-GCP-2 antibody staining, $\times 400$, score=0). (c). Negative control in ACP ($\times 400$). (d). The interlobular pancreatic duct epithelia in LPSP (H&E, $\times 100$). (e). The interlobular pancreatic duct epithelia were weakly stained in LPSP (anti-GCP-2 antibody staining, $\times 400$, score=1). (f).

Negative control in LPSP ($\times 400$). (g). The interlobular pancreatic duct epithelia in IDCP (H&E, $\times 100$). (h). The interlobular pancreatic duct epithelia were strongly stained in IDCP (anti-GCP-2 antibody staining, $\times 400$, score=3). (i). Negative control in IDCP ($\times 400$). (j). The median score of GCP-2 in the interlobular pancreatic duct epithelia was significantly higher in IDCP (1.5; IQR: 0.25-2) than in ACP (0; IQR: 0-0.75) ($P < 0.05$) and LPSP (0; IQR: 0-0.75) ($P < 0.05$). * $p < 0.05$

Fig.5 Immunohistochemical findings of the interlobular pancreatic duct epithelia stained in reaction to anti-IL-8 antibody and comparison of the score of staining intensity.

(a). The interlobular pancreatic duct epithelia in ACP (H&E, $\times 100$). (b). The interlobular pancreatic duct epithelia were not stained in ACP (anti-IL-8 antibody staining, $\times 400$, score=0). (c). Negative control in ACP ($\times 400$). (d). The interlobular pancreatic duct epithelia in LPSP (H&E, $\times 100$). (e). The interlobular pancreatic duct epithelia were weakly stained in LPSP (anti-IL-8 antibody staining, $\times 400$, score=1). (f). Negative control in LPSP ($\times 400$). (g). The interlobular pancreatic duct epithelia in IDCP (H&E, $\times 100$). (h). The interlobular pancreatic duct epithelia were weakly stained in IDCP (anti-IL-8 antibody staining, $\times 400$, score=1). (i). Negative control in IDCP ($\times 400$). (j). There were no significant difference in the median score of IL-8 in the interlobular pancreatic duct epithelia among ACP (0; IQR: 0-0.75), LPSP (1; IQR: 0-1.75), and IDCP (0.5; IQR: 0-1).

Fig.6 Immunohistochemical findings of the intralobular pancreatic duct epithelia stained in reaction to anti-GCP-2 antibody and comparison of the score of staining intensity.

(a). The intralobular pancreatic duct epithelia in ACP (H&E, $\times 100$). (b). The intralobular pancreatic duct epithelia were not stained in ACP (anti-GCP-2 antibody staining, $\times 400$, score=0). (c). Negative control in ACP ($\times 400$). (d). The intralobular pancreatic duct epithelia in LPSP (H&E, $\times 100$). (e). The intralobular pancreatic duct epithelia were not stained in LPSP (anti-GCP-2 antibody staining, $\times 400$, score=0). (f). Negative control in LPSP ($\times 400$). (g). The intralobular pancreatic duct epithelia in IDCP (H&E, $\times 100$). (h). The intralobular pancreatic duct epithelia were not stained in IDCP (anti-GCP-2 antibody staining, $\times 400$, score=0). (i). Negative control in IDCP ($\times 400$). (j). There was no significant difference in the median score of GCP-2 in the intralobular pancreatic duct epithelia among ACP (0; IQR: 0-0.75), LPSP (0; IQR: 0-1) and IDCP (0; IQR: 0-1).

Fig.7 Immunohistochemical findings of the intralobular pancreatic duct epithelia stained in reaction to anti-IL-8 antibody and comparison of the score of staining intensity.

(a). The intralobular pancreatic duct epithelia in ACP (H&E, $\times 100$). (b). The intralobular pancreatic duct epithelia were not stained in ACP (anti-IL-8 antibody staining, $\times 400$, score=0). (c). Negative control in ACP ($\times 400$). (d). The intralobular pancreatic duct epithelia in LPSP (H&E, $\times 100$). (e). The intralobular pancreatic duct epithelia were moderately stained in LPSP (anti-IL-8 antibody staining, $\times 400$, score=2).

(f). Negative control in LPSP ($\times 400$). (g). The intralobular pancreatic duct epithelia in IDCP (H&E, $\times 100$). (h). The intralobular pancreatic duct epithelia were moderately stained in IDCP (anti-IL-8 antibody staining, $\times 400$, score=2). (i). Negative control in IDCP ($\times 400$). (j). The median score of IL-8 in the intralobular pancreatic duct epithelia was significantly lower in ACP (0; IQR: 0-0.75) than in LPSP (1; IQR: 0.25-1.75) ($P<0.05$) and IDCP (1.5; IQR: 1-2.75) ($P<0.01$). * $p<0.05$, ** $p<0.01$

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7