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ORIGINAL ARTICLE

Expression of Interleukin-8 in Human Obstructive Pancreatitis

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ABSTRACT

Context Obstructive pancreatitis is a specific form of pancreatitis, which is caused by the obstruction of the main pancreatic duct due to tumors or some other causes. Interleukin-8 is induced in acute pancreatitis, but its expression in obstructive pancreatitis has not been clarified.

Objective We attempted to provide some insight into the significance of interleukin -8 in the pathogenesis of pancreatic fibrosis.

Patients Fifteen cases of pancreatic cancer, 7 cases of mucinous cystadenoma, 3 cases of Vater's papilla cancer and 9 normal pancreases were included in this study.

Main outcome measures The obstructive pancreatitis portions of the above pathologies were evaluated for interleukin-8 expression by means of immunohistochemistry and *in situ* hybridization.

Results Interleukin-8 was positive in 72% of cases of obstructive pancreatitis. The positive rate was not significantly related to the etiology of the obstruction (P=0.972). Interleukin-8 was expressed in infiltrating cells, proliferating ductular cells and acinar cells. In contrast, normal pancreases and tumor cells lacked interleukin-8 expression (P<0.001 vs. obstructive pancreatitis). Both immunohistochemistry and *in situ* hybridization demonstrated that interleukin-8 was expressed mostly in acinar cells in mild pancreatic fibrosis, whereas it was expressed in stromal and ductular cells in moderate and severe pancreatic fibrosis.

Conclusions These results suggest that interleukin-8 expression is related to the fibrotic process in obstructive pancreatitis.

INTRODUCTION

Interleukin (IL)-8 is a member of the C-X-C chemokine family which mediates the recruitment of polymorphonuclear neutrophils, basophils, eosinophils and lymphocytes to inflammatory sites [1]. IL-8 acts as both a neutrophil activator and a chemoattractant [2]. Growth-regulated gene product/cytokine-induced neutrophil chemoattractant (GRO/CINC)-1 in rats corresponds to IL-8 in humans [3]. GRO/CINC-1 consists of 72 amino acids which are homologous to human peptides with melanoma growth stimulatory activities, indicating that GRO/CINC-1 has a structural and functional homology to human IL-8 [3]. GRO/CINC-1 or IL-8 is expressed in experimental models such as gastric ulcer [4], ischemia-reperfused liver [5] and acute pancreatitis [6].

In humans, serum levels of IL-8 are reported to be an early predictor of disease severity and complications in acute pancreatitis [7]. An anti-IL-8 neutralizing antibody inhibited cytokine response and acute lung injury in

experimental acute pancreatitis [8]. Although IL-8 mRNA was recently reported to be expressed in human chronic pancreatitis [9, 10], the role of IL-8 in the pathogenesis of chronic pancreatitis is still unclear. It is often difficult to obtain chronic pancreatitis tissue, especially surgical specimens, in clinical settings. Obstructive pancreatitis is a specific form of pancreatitis, which is caused by the obstruction of the main pancreatic duct due to tumors or some other causes. Obstructive pancreatitis is different from chronic pancreatitis caused by alcohol abuse or cholelithiasis. However, obstructive pancreatitis tissue is often available at pancreatic surgery, and analysis of IL-8 expression in obstructive pancreatitis might provide some insight into the significance of IL-8 in the pathogenesis of chronic pancreatitis or pancreatic fibrosis.
In the present study, we attempted to assess the involvement of IL-8 in human obstructive pancreatitis.

MATERIALS AND METHODS

Patients

Fifteen cases of pancreatic cancer (14 cases of ductal adenocarcinoma and 1 case of islet cell carcinoma), 7 cases of mucinous cystadenoma, and 3 cases of Vater's papilla cancer were studied. All cases were diagnosed histopathologically. The mean age of the 25 analyzed patients was 63.0±6.6 years (range: 51-74 years); 15 were males and 10 were females. Nine patients with normal pancreases were also included in this study; the gender (5 males, 4 females) and ages (mean 61.0±5.1 years; range: 54-69 years) of these patients were not significantly different from those of pancreatic cancer patients (P=1.000 and P=0.401, respectively).

Pancreatic Tissue

All pancreatic tissue was obtained at surgery; 25 specimens of obstructive pancreatitis portions from the patients with the above diseases and 9 specimens of normal pancreatic tissue were analyzed. Normal pancreatic tissue was obtained from patients with illnesses other than pancreatic disease and was confirmed to be histologically normal.
Immediately after surgical removal, all pancreatic tissue samples were fixed in 10% formalin, and sectioned at a thickness of 4 μm. The sections were stained with hematoxylin and eosin (H&E), and the severity of obstructive pancreatitis was graded from the viewpoint of pancreatic fibrosis as follows: grade 1 (mild), the percentage of fibrotic area in the total specimen was less than 25%; grade 2 (moderate), the fibrotic involvement was between 25 and 50%; grade 3 (severe), the fibrotic involvement was greater than 50%.

Immunohistochemistry

We used a DAKO LSAB kit (DAKO, Carpinteria, CA, USA) for *in situ* hybridization (ISH). The sections were incubated with a monoclonal anti-human IL-8 antibody (1:25 dilution, Genzyme, Minneapolis, MN, USA) overnight at 4°C. Normal mouse IgG was used as a negative control. The immunohistochemical reactivity of IL-8 was evaluated in each cell type as the frequency (in percentage) of pancreatic lobules displaying more than 20 cells which were positive for IL-8 expression [9].

In Situ Hybridization

An ISH kit (Maxim Biotech, Inc., San Francisco, CA, USA) was used. The hybridization was done at 42°C overnight, with 250 ng/mL of an oligonucleotide (30 mer) antisense human IL-8 probe (Biognostik GmbH, Göttingen, Germany). As negative control experiments, serial sections were hybridized with a sense probe. These probes were labeled with biotin using a DNA labeling kit (Biotin-High Prime, Boehringer Mannheim GmbH, Mannheim, Germany).

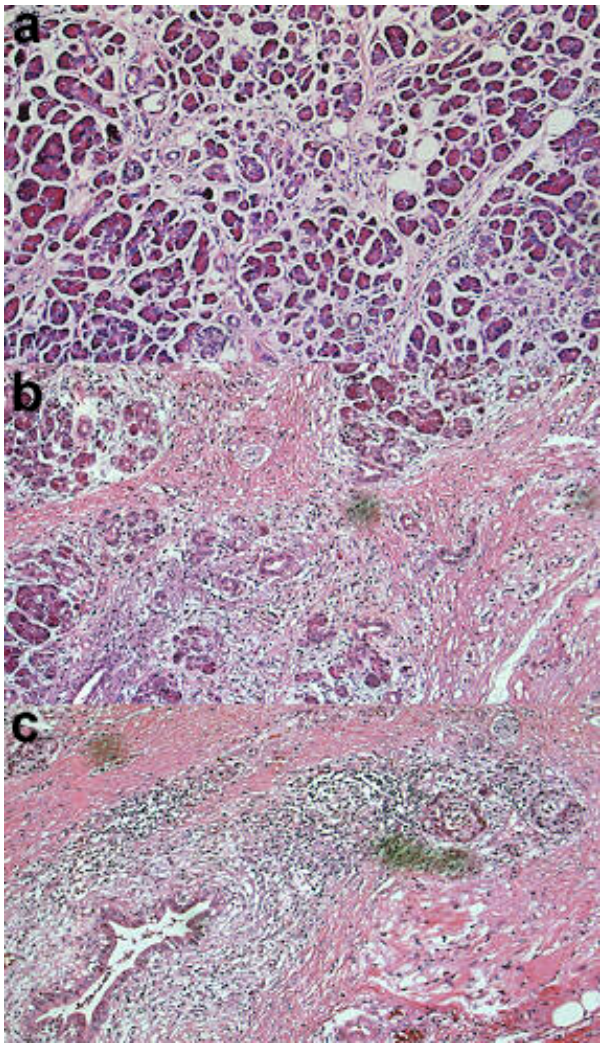

Figure 1. Different grades of pancreatic fibrosis in human chronic obstructive pancreatitis as defined in the section "Materials and Methods: Pancreatic Tissue" by hematoxylin and eosin staining. Mild, grade 1 (a); moderate, grade 2 (b); severe, grade 3 (c). (Original magnification: ×25).

ETHICS

Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as revised in 1983, as reflected in a priori approval by the Kanazawa University School of Medicine Review Committee.

STATISTICS

All data were expressed as mean, standard deviations, range, and frequencies. The Mann-Whitney, the Pearson chi-square, the Fisher's exact, and the McNemar tests were used to analyze differences in human IL-8 expression. Two-tailed P values less than 0.05 were considered statistically significant. The SPSS version 8.0 software (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis.

RESULTS

Histological Evaluation of Human Obstructive Pancreatitis

Pathological analysis revealed the histological characteristics of obstructive pancreatitis with heterogeneously extended fibrosis in the vicinity of damaged parenchyma. Mononuclear cell infiltrates and ductal proliferation were observed. As for histological severity, a representative figure for each grade is shown in Figure 1: grade 1 (mild, Figure 1a), 10 cases; graded 2

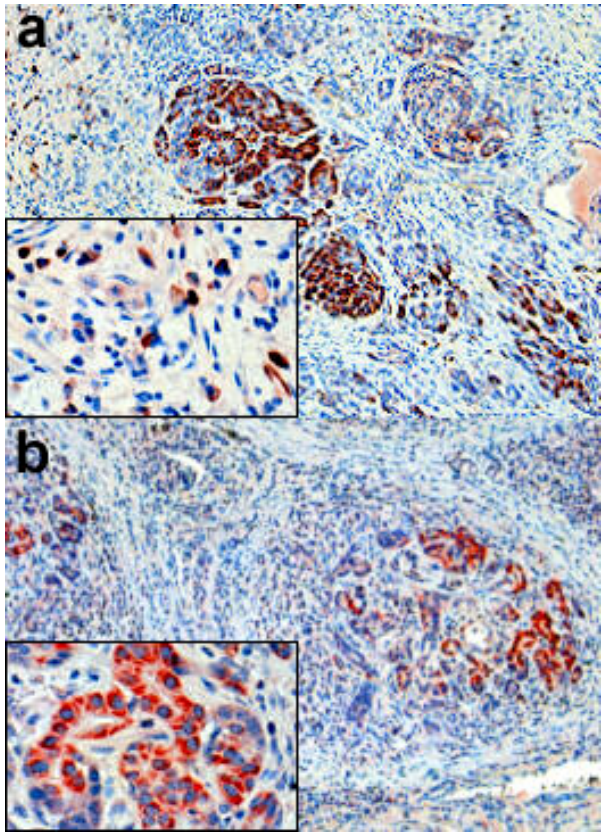

Figure 2. IL-8 expression in pancreatic tissue sections from patients with CP. IL-8 protein is localized in the cytoplasm of acinar and stromal infiltrating cells (a). Proliferating ductular cells were also positive for IL-8 (b). Immunohistochemistry (magnification: ×25, inset ×100).

(moderate, Figure 1b), 6 cases; grade 3 (severe, Figure 1c), 9 cases. The 9 control specimens showed normal pancreatic histology (grade 0).

IL-8 Expression in Human Obstructive Pancreatitis

IL-8 was expressed in 72.0% (18/25) of obstructive pancreatitis specimens although the frequency of positive cells varied considerably among the samples. IL-8 was localized in the cytoplasm of acinar, ductal and inflammatory cells (Figure 2). Most IL-8-positive-infiltrates were seen in fibrotic areas. The relationship between the expression of IL-8 and the histological stage of chronic obstructive pancreatitis is shown in Figure 3. In grade 1 (mild), IL-8 was expressed mostly in acinar cells, whereas in grade 2 (moderate) and 3 (severe), IL-8 expression was found to be increased in the inflammatory infiltrates and ductal cells.

IL-8 immunoreactivity data are shown in Table 1. The positivity of IL-8 was not significantly related to the etiology of obstruction (P=0.972). In the normal human pancreases, IL-8 immunoreactivity was absent (P<0.001 vs. obstructive pancreatitis) and it was not expressed in any adenocarcinoma tissue (P<0.001 vs. obstructive pancreatitis). In 3 (21.4%) of the 14 adenocarcinoma lesions examined, IL-8 immunoreactivity was present in infiltrates around cancer cells (P=0.003 vs. obstructive pancreatitis; P=0.253 vs. normal pancreas; P=0.016 vs. cancer portion of adenocarcinoma). These 3 cases also had positive IL-8 immunoreactivity in the obstructive pancreatitis portions.

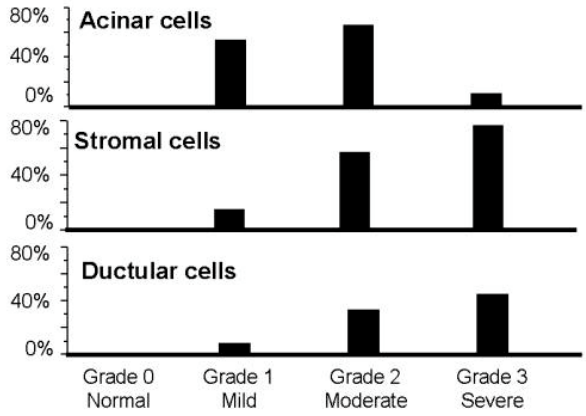

Figure 3. IL-8 protein expression in pancreatic acinar, stromal and ductular cells in different grades of pancreatic fibrosis. IL-8 expression was semi-quantified as defined in the section "Materials and Methods: Immunohistochemistry".

IL-8 mRNA Expression in Human Obstructive Pancreatitis

The localization of the mRNA was similar to that of the protein. IL-8 mRNA was localized in acinar, inflammatory and ductal cells in serial sections of obstructive pancreatitis (Figure 4), whereas it was not expressed in the normal pancreas and cancer cells.

Table 1. Expression of IL-8 in human pancreatic tissues.

Disease	IL-8 positivity
Obstructive pancreatitis	18/25 (72.0%)
Etiology (P=0.972; chi-square test):	
- Pancreatic cancer	11/15 (73.3%)
- Mucinous cystadenoma	5/7 (71.4%)
- Vater's papilla cancer	2/3 (66.7%)
Adenocarcinoma (cancer portion*)	0/14 (0%)[a]
Adenocarcinoma (infiltrate around cancer)	3/14 (21.4%)[bcd]
Normal pancreas	0/9 (0%)[a]

* Positivity in cancer cells
[a] P<0.001 vs. obstructive pancreatitis (Fisher's exact test)
[b] P=0.003 vs. obstructive pancreatitis (Fisher's exact test)
[c] P=0.253 vs. normal pancreas (Fisher's exact test)
[d] P=0.016 vs. cancer portion (McNemar test)

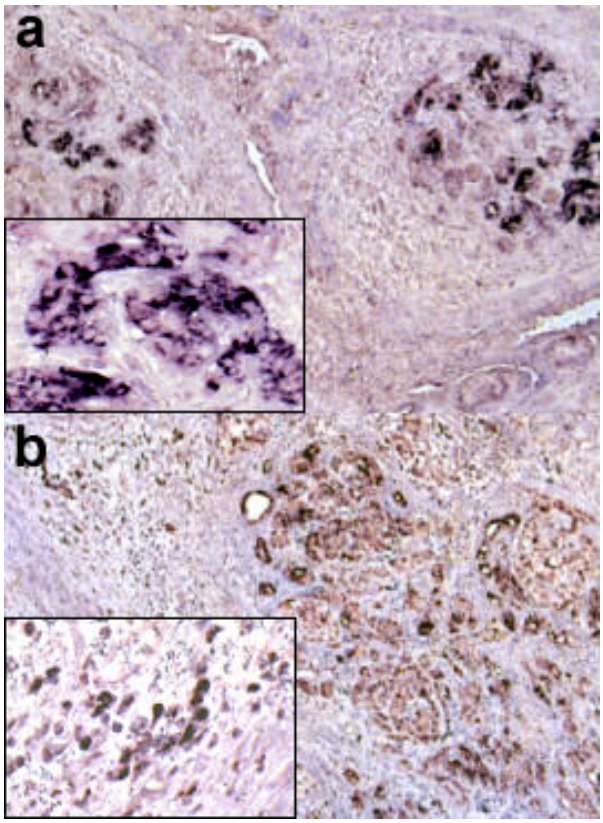

Figure 4. IL-8 mRNA is expressed in acinar cells (a) and infiltrating inflammatory cells (b). *In situ* hybridization (magnification: ×25; inset: ×100).

DISCUSSION

Major etiologies of chronic pancreatitis are alcohol abuse and gallstones, but obstructive pancreatitis [11] develops due to the stricture or obstruction of the main pancreatic duct by post-inflammatory fibrosis, stones or tumors [12]. We attempted to assess whether IL-8 expression is involved in the pathophysiology of obstructive pancreatitis.

In previous reports, the main source of IL-8 was inflammatory cells, including neutrophils [13]. Saurer *et al*. [9] demonstrated that IL-8 mRNA is expressed in acinar, ductal and infiltrating cells in human chronic pancreatitis using *in situ* hybridization, which is consistent with our results. However, they did not study the expression of IL-8 protein. Different results for IL-8 protein and mRNA have been reported in alcoholic liver disease [14]. In the present study, we have clearly shown that the expression of IL-8 protein is up-regulated in pancreatic lobules with histopathologic alterations of obstructive pancreatitis. The distribution of IL-8 protein was consistent with that of IL-8 mRNA. Therefore, IL-8 was obviously expressed in obstructive pancreatitis, whereas it was not found in the normal pancreases and in pancreatic cancer. In other human chronic diseases, including idiopathic pulmonary fibrosis [15], and cystic fibrosis [16], an enhanced expression of IL-8 has been associated with fibrosis. Although no direct link between IL-8 and pancreatic fibrosis has been proven, Andoh *et al*. [17] reported that TNF-alpha and IL-1beta regulate IL-8 production in pancreatic periacinar myofibroblasts and that most IL-8-positive infiltrates were located in the vicinity of fibrotic areas.

IL-8 expression is related to the histological activity of inflammation in inflammatory bowel diseases [18]. In the pancreas, it is reported that IL-8 gene expression in the pancreatic parenchyma is frequently observed in advanced grades of the disease. Our results do not fully support this report. In the present study, IL-8 was expressed mostly in acinar cells in mild obstructive pancreatitis, whereas its expression was increased in interstitial infiltrating cells in moderate and severe obstructive pancreatitis, suggesting that the type of cells expressing IL-8 differs depending on the histological grade of obstructive pancreatitis. The differences might be explained by the etiologies of pancreatitis. Saurer's study [9] was carried out, for the most part, on alcoholic chronic pancreatitis, whereas our study was on obstructive pancreatitis.

The important early mechanism of progressive fibrosis in chronic obstructive pancreatitis is considered to be increased ductular pressure and acinar damage releasing local proinflammatory cytokines which stimulate peri-acinar stellate cells to accelerate pancreatic fibrosis. Animal experiments and some clinical studies show that obstructive pancreatitis can be reversible if the obstruction is released. This

reversibility could partly explain the changing source of IL-8 during the development of fibrosis.

The overexpression of IL-8 in pancreatic cancer is predominant in regions exposed to hypoxia after orthotopic implantation in the pancreases of nude mice [19]. During the development and progression of pancreatic cancer, the expression of iNOS and protein tyrosine nitration is increased, indicating the potential involvement of oxidative stress [20]. The overexpression of iNOS has been reported in human chronic pancreatitis [21]. A significant association between NO and IL-8 is also reported in human chronic obstructive pulmonary disease [22]. We speculate that IL-8 induction is associated with oxidative stress in obstructive pancreatitis.

The pathophysiology of pain in chronic pancreatitis is not yet fully understood, but it was recently reported that IL-8 is expressed in macrophages surrounding enlarged pancreatic nerves [10]. Substance P is released from sensory pancreatic nerves and directly stimulates the release of IL-8 from macrophages [23]. Such interaction between inflammatory cells and nerves could be called "neuroimmune cross talk" [23], and may be involved in the generation of pain in chronic pancreatitis. Our study showing the changing source of IL-8 in different cell types in the course of obstructive pancreatitis would suggest a role of IL-8 in the intrinsic maintenance of the inflammatory response, thus sustaining the progression of obstructive pancreatitis.

In conclusion, the present study shows that IL-8 is expressed in the pancreas of patients with obstructive pancreatitis with different expression sites depending on the grade of pancreatic fibrosis.

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Keywords Fibrosis; Gene Expression Profiling; Immunohistochemistry; *In Situ* Hybridization; Interleukin-8; Pancreatic Diseases; Pancreatitis

Abbreviations GRO/CINC: growth-regulated gene product/cytokine-induced neutrophil chemoattractant; ISH: *in situ* hybridization

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