Exacerbated experimental pancreatitis in interleukin-19 knockout mice

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Special Issue: Academic seeds for drugs

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Mice

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to generate IL-19 KO mice [14]. C57BL/6-IL-19 heterozygous mice were intercrossed to generate mutant and control mice. Age-matched mice (9–12 weeks old) were used in all experiments. All procedures used in this study complied with institutional policies of the Osaka Prefecture University Animal Care and Use Committee.

**Cerulein-induced pancreatitis**

Mice were fasted for 16 h before the experiment and received eight intra-peritoneal injections of cerulein at 50 µg/kg or 75 µg/kg at hourly intervals over 7 h [21]. Control mice were administered saline.

**Serum amylase level**

Blood samples collected from tail vein were centrifuged at 10,000×g for 10 min and then stored at -20°C. The serum amylase assay was determined using the Amylase Assay (MaxDiscovery™) (Bioo Scientific Corporation, Austin, TX).

**Histology and immunohistochemistry**

The pancreas was fixed with 10% neutral buffered formalin and embedded in paraffin. Sections were routinely stained with hematoxylin and eosin (HE). MPO activity and TUNEL staining were routinely detected according to the manufacturer’s instructions [22]. Immunohistochemistry was performed as previously described [22]. Primary antibodies against p62, cleaved caspase-3, and Ki-67 were used.

**Statistical analysis**

Results are expressed as the mean ± standard error of the mean (SEM). Differences in parametric data were evaluated using Student’s t tests. Differences with P values of less than 0.05 were considered significant.

**Results**

**Mild condition of pancreatitis induced by cerulein at low dose**

In the first experiments, mice were injected with cerulein at 50 µg/kg. We examined the serum amylase activity in mice with cerulein-induced pancreatitis. Cerulein induced the serum amylase activities in WT and IL-19 KO mice compared to saline-injected mice. Serum amylase level was slightly higher but not significant (p=0.114) in IL-19 KO mice with cerulein-induced pancreatitis than in WT mice (Figure 1A). In addition, mice given cerulein (50 µg/kg) showed no significant differences in body weight change between WT and IL-19 KO mice (Figure 1B).

We next examined histological changes in the respective mice. IL-19 KO mice after saline administration showed no vacuolization, no degeneration, and no omission (loss) in acinar cells compared to WT mice. Mice after cerulein (50 µg/kg) administration showed the detachment, degeneration, and atrophy of acinar cells with interstitial edema and inflammatory cells infiltration (Figure 2). However, there was no notable difference in the severity of histological characterization when the pancreas from WT and IL-19 KO mice were compared. As shown in Figure 3, cerulein (50 µg/kg) injection increased pancreatic MPO activity. However, the level of MPO activity was similar in WT and IL-19 KO mice. In addition, cerulein (50 µg/kg) injection resulted in a small number of apoptosis in acinar cells. However, there was no notable difference in the number of apoptosis evaluated by TUNEL staining (Figure 3 right panels).
Severe condition of pancreatitis induced by cerulein at high dose

Next, mice were injected with cerulein at 75 µg/kg. Serum amylase level was significantly higher in IL-19 KO mice with high dose cerulein-induced pancreatitis than in WT mice (Figure 4A). In accordance with the observed significance in amylase activities, IL-19 KO mice given a high dose of cerulein showed a severe weight loss compared to WT mice given the same dose of cerulein (Figure 4B).

Further histological analysis of the pancreas from IL-19 KO mice with cerulein-induced pancreatitis showed severe interstitial edema and severe degeneration in acinar cells (Figure 5 left panels). In contrast, there was no notable difference in infiltrating cells by histological evaluation. Cerulein (75 µg/kg) injection more increased pancreatic MPO activity compared to cerulein (50 µg/kg) (Figure 5 right panels and Figure 3). However, the level of MPO activity was similar in WT and IL-19 KO mice.

We performed immunohistochemical detections of p62 (autophagy-associated factor), cleaved caspase-3 (apoptosis-associated factor), and Ki-67 (cellular proliferation-associated protein). As shown in Figure 6, the pancreas of IL-19 KO mice contained a high level of p62...
following cerulein (75 g/kg) administration. In addition, the detection of cleaved caspase-3 was increased in IL-19 KO mice with cerulein-induced pancreatitis (Figure 6 upper right panels). Strikingly, the activity of proliferation in the pancreas of IL-19 KO mice with cerulein-induced pancreatitis (evaluated using anti-Ki-67 antibody) was similar to that of WT mice (Figure 6 lower panels).

Discussion

In this study, our experiments highlighted several novel aspects of the anti-inflammatory effects of IL-19 in cerulein-induced pancreatitis in vivo. We show that cerulein-induced pancreatitis was exacerbated in IL-19 KO mice. The severe phenotype following genetic ablation of IL-19 was evident only when it was administered at 75 µg/kg, as mice given cerulein at 50 µg/kg showed no marked change in amylase activity, body weight, or histological damage. The more severe pancreatic inflammation following genetic ablation of IL-19 was accompanied by an increased level of autophagy, which has been implicated in the pathogenesis of pancreatitis. Cerulin is the CCK analogue and, when it is given to the animals, the excess stimulation leads to abnormally high secretion of digestive enzymes, resulting in acute pancreatitis. The pancreas of this model is histologically quite similar to the early phase of acute pancreatitis in humans [23]. The autophagy, as well as stress and inflammation, are constant features in the pathology of human pancreatitis [24]. It is well known that p62 plays a role in a cellular autophagic process [25]. Furthermore, p62 has been found to play an important role in the autophagy of pancreatitis [26,27]. The increased level of p62 suggests facilitated autophagy in cerulin-induced pancreatitis. The autophagy has been suggested to be beneficial to minimize tissue damages by preventing inflammation accompanied with necrosis [28]. However, to date, there have been no reports examining the role of IL-19 in cellular autophagy. We press that this is first reported to show the relation of IL-19 with autophagy. These reports examining the role of IL-19 in cellular autophagy. We press that accompanied with necrosis [28]. However, to date, there have been no reports examining the role of IL-19 in cellular autophagy. We press that this is first reported to show the relation of IL-19 with autophagy. These reports suggest that the acinar cell damage in the pancreas of IL-19 KO mice results in enhanced autophagy.

Apoptosis, a programmed cell death, is thought to be a final protective mechanism to minimize tissue damages by preventing the release of digestive enzymes and proteases into the extracellular spaces [29]. Our analysis of IL-19 KO mice showed that there was an increase in the expression of cleaved caspase-3, an apoptosis marker. In the previous study, treatment of monocytes with mouse IL-19 induced mouse monocyte apoptosis by the production of IL-6, TNF-α, and reactive oxygen species [30]. This previous report did not support our data. These results suggest that IL-19 plays differential roles in different types of cell. Inflammatory responses also are related to onset and development of animals with pancreatitis [31,32]. We examined whether the deficiency of IL-19 affects neutrophils infiltration. Strikingly, the infiltration of neutrophils in the pancreas of IL-19 KO mice (evaluated by MPO activity) was similar to that of WT mice. These results further show that increased infiltration of neutrophils may not be absolutely required for the acinar cell damage of IL-19 KO mice. Therefore, our results suggest that IL-19 may protect pancreatic inflammation through the regulation of autophagy and apoptosis in an acinar cell. It is possible that IL-19 may not regulate the infiltration of inflammatory cells in the pancreas.

Our strength of this study is that there is an interaction of IL-19 with pancreatitis. Up to the present, little has been reported on possible relation of IL-19 in pancreatic diseases including pancreatitis. Our results indicate that although IL-19 may play an important role in controlling pancreatic inflammation as a new function, it may constitute one of several potential cellular and immunological mechanisms that can contribute to pancreatic pathology.

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