Blockade of neurokinin-1 receptor attenuates CC and CXC chemokine production in experimental acute pancreatitis and associated lung injury

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Sun J, Bhatia M. Blockade of neurokinin-1 receptor attenuates CC and CXC chemokine production in experimental acute pancreatitis and associated lung injury. Am J Physiol Gastrointest Liver Physiol 292: G143–G153, 2007. First published July 27, 2006; doi:10.1152/ajpgi.00271.2006.—Accumulating evidence suggests the neuropeptide substance P (SP) and its receptor neurokinin-1 receptor (NK-1R) play a pivotal role in the pathogenesis of acute pancreatitis (AP). However, the mechanisms remain unclear. The present study investigated whether chemokines as proinflammatory molecules are involved in SP-NK-1R-related pathogenesis of this condition. We observed temporally and spatially selective chemokine responses in secretagogue caerulein-induced AP in mice. CC chemokines monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein-1α (MIP-1α) and CXC chemokine MIP-2 were elevated after AP induction. Time-dependent, tissue-specific analysis of their mRNA and protein expression suggested that they are early mediators in the condition and mediate local as well as systemic inflammatory responses. In contrast, another CC chemokine regulated on activation, T cells expressed and secreted (RANTES) was only involved in local pancreatic inflammation at a later stage of the disease. Either prophylactic or therapeutic treatment with a potent selective NK-1R antagonist CP-96,345 significantly suppressed caerulein-induced AP in mice. CC chemokines monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein-1α (MIP-1α) and CXC chemokine MIP-2 were elevated after AP induction. Time-dependent, tissue-specific analysis of their mRNA and protein expression suggested that they are early mediators in the condition and mediate local as well as systemic inflammatory responses. In contrast, another CC chemokine regulated on activation, T cells expressed and secreted (RANTES) was only involved in local pancreatic inflammation at a later stage of the disease. Either prophylactic or therapeutic treatment with a potent selective NK-1R antagonist CP-96,345 significantly suppressed caerulein-induced increase in MCP-1, MIP-1α, and MIP-2 expression but had no apparent effect on RANTES expression. The suppression effect of CP-96,345 on MCP-1, MIP-1α, and MIP-2 expression was concordantly demonstrated by immunohistochemistry, which, additionally, suggested that chemokine immunoreactivity was localized to acinar cells and the infiltrating leukocytes in the pancreas and alveolar macrophages, epithelial cells, and endothelial cells in the lungs. Our data suggest that SP, probably by acting via NK-1R on various chemokine-secreting cells in the pancreas and lungs, stimulates the release of chemokines that aggravate local AP and the development of its systemic sequelae.

ACUTE PANCREATITIS (AP) is a common and potentially fatal disease. The majority of deaths associated with the condition result from the development of multiple organ dysfunction syndrome (1). Impairment of lung function is frequently the earliest sign of multiple organ dysfunction syndrome and manifested clinically as acute respiratory distress syndrome (3, 7). Although its pathogenesis is still incompletely resolved, various inflammatory mediators including substance P (SP) and chemokines are believed to play a pivotal role in the pathogenesis of AP and associated lung injury.

SP belongs to the tachykinin family of neuropeptides, derived from the preprotachykinin (PPT-A) gene. It is a neurotransmitter and pain mediator released from both central and peripheral endings of primary afferent neurons as well as from various inflammatory cells (13, 20). The biological actions of SP are mediated primarily by neurokinin-1 receptors (NK-1R). SP has been shown to act as a proinflammatory mediator in many inflammatory diseases. In AP, SP and NK-1R regulate the severity of the disease. Earlier study has demonstrated the presence of SP in the mouse pancreas and NK-1R on mouse pancreatic acinar cells (5). Pancreatic levels of SP and the expression of NK-1R on pancreatic acinar cells increased in experimental AP in mice (5). Gene deletion of PPT-A and NK-1R reduced the severity of AP and associated lung injury (5, 6, 11). We have earlier showed that NK-1R antagonism protected mice against caerulein-induced AP and associated lung injury (17). Further study showed that the antagonist treatment suppressed the increase in pancreatic and lung PPT-A mRNA expression and SP protein levels in the condition (16).

Despite its established role in the pathophysiology of AP, the mechanism by which SP acts to mediate the inflammatory responses in the disease is still unclear. On the basis of earlier findings that SP has chemotactic property on leukocytes (21) and that NK-1R blockade in mice induced with AP is associated with reduced leukocyte activation and infiltration in the tissues, decreased neutrophil granular enzyme myeloperoxidase activity, and reduced tissue/cellular damage (17), we hypothesized that another group of important inflammatory mediators, chemokines, may be involved in SP-NK-1R-related pathogenesis of AP and associated lung injury.

Chemokines are a family of small (8–10 kDa), inducible, secreted cytokines that have chemotactic and activating effects on different subsets of leukocytes. Leukocytes, subsequent to migration along the chemokine gradient and infiltration to damaged tissues, release various deleterious factors such as cytokines, proteolytic enzymes, and oxygen free radicals, to enhance tissue destruction and to propagate the disease to remote organs. Chemokines fall into four subfamilies: CC, CXC, C, and CX3C, based on the spacing of the amino terminal cysteine residues. Four chemokines, namely monocyte chemotactic protein (MCP)-1, regulated on activation, T cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), and MIP-2, were chosen for investigation in this study. Among the four, MCP-1, RANTES, and MIP-1α are prototypic CC chemokines principally chemotactic for monocytes and macrophages, and MIP-2 is a rodent CXC chemokine and a potent chemoattractant for polymorphonuclear neutrophils (PMN). MIP-2 induces neutrophil degranulation and release of lysozyme, leading to tissue damage. These chemokines have been implicated as important mediators in the pathogenesis of AP in different experimental models (4, 9, 19, 22, 25).

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In the present study, we aimed to investigate the participation of CC and CXC chemokines in SP-associated pathogenesis of AP and associated lung injury. To this end, we induced in mice acute necrotizing pancreatitis with the cholecystokinin (CCK) secretagogue caerulein and studied the effect of NK-1R blockade, using a specific NK-1R antagonist CP-96,345, on the mRNA and protein levels of various chemokines in the pancreas and lungs. Immunohistochemistry was also used to localize chemokine expression in the pancreas and lungs.

MATERIALS AND METHODS

Animals. Male Swiss albino mice (20–25 g) were fed with standard laboratory chow and fasted overnight before induction of pancreatitis, but water ad libitum was not withheld before and during the experiments. All experimental procedures were approved by the Animal Ethics Committee of National University of Singapore and carried out in accordance with established International Guiding Principles for Animal Research.

Reagents. Caerulein was purchased from Bachem California (Torrance, CA), and NK-1R antagonist CP-96,345 was a gift from Pfizer Diagnostics. Nembutal for euthanizing the animals was purchased from Merial Australia. TRIzol reagent for total RNA isolation was purchased from Invitrogen (Carlsbad, CA). The iScript cDNA Synthesis Kit for cDNA synthesis and iQ Supermix for polymerase chain reaction was purchased from Bio-Rad (Hercules, CA). Oligonucleotide primers were synthesized by Proligo Singapore. ELISA kits for measuring tissue and plasma chemokine levels, mouse horseradish peroxidase (HRP)-diaminobenzidine (DAB) cell and tissue staining kit for immunohistochemical staining, goat anti-mouse MCP-1, RANTES, MIP-1α, and MIP-2 primary antibodies were purchased from R&D Systems.

Caerulein-induced acute pancreatitis. Male Swiss mice were randomized into control and experimental groups of eight mice each: animals were given hourly intraperitoneal injections of normal saline or saline-containing caerulein (50 μg/kg) for 3, 6, and 10 h. CP-96,345 was administered intraperitoneally at a dose of 2.5 mg/kg either half an hour before or 1 h after the first caerulein injection. This dose of CP-96,345 has been shown to block NK-1R and consequently neurogenic inflammation. One hour after the last caerulein injection, mice were killed with a lethal dose of nembutal (pentobarbital sodium 60 mg/ml ip, Merial Australia). Pancreas and lung tissue samples and plasma samples were harvested for subsequent chemokine assays.

Total RNA isolation. For RT-PCR analysis of tissue chemokine mRNA expression, total RNA was extracted from the pancreas and lungs by using TRIzol reagent (Invitrogen) following the manufacturer’s instructions with some modifications. Briefly, pancreas and lung tissues were isolated and immediately homogenized in TRIzol reagent. Chloroform was then added to the homogenates, and samples were shaken, incubated for 5 min at 4°C, and centrifuged for 15 min at 12,000 g at −10 (pancreas samples) or 4°C (lung samples). The aqueous phase was separated and RNA was precipitated by addition of isopropanol alcohol. After RNA was pelleted by centrifugation (12,000 g for 10 min at −10 or 4°C), the pellet was washed twice in 70% ethanol, air-dried, and dissolved in RNase-free water. RNA was quantitated spectrophotometrically by absorbance at 260 nm. The purity of RNA was assessed by a 260/280 ratio between 1.6 and 2.0. The integrity of RNA was verified by the presence of distinct 28S and 18S RNA bands on a denaturing agarose gel.

Semiquantitative RT-PCR. Semiquantitative RT-PCR was performed to analyze mRNA expression levels of MCP-1, RANTES, MIP-1α, and MIP-2 in the tissues. Total RNA (1 μg) was reverse transcribed by use of the iScript cDNA synthesis kit (Bio-Rad). The cDNA synthesized was used as the template for PCR amplification by iQ Supermix (Bio-Rad). The PCR protocol consisted of optimized 33 cycles of denaturation at 95°C for 30 s, annealing for 30 s (at 65, 60, 61, and 58°C, respectively, for MCP-1, RANTES, MIP-1α, and MIP-2), and extension at 72°C for 30 s performed in MyCycler (Bio-Rad). The following specific primer pairs (Proligo, Singapore) of chemokines and were used: MCP-1 sense 5′-GGAAAAATGGATC-CACACCTTG-3′ and antisense 5′-TCTCTCTCCTCCACCCCAT- GCAG-3′ resulting in a 582-bp product; RANTES sense 5′-TCT- TCTCTGGTTGGACACACAC-3′ and antisense 5′-GCTCATCATT- CATGCTCAGTC-3′ resulting in a 215-bp product; MIP-1α sense 5′-ACTGCCCCCTTGTGCTTTCTCTTCT-3′ and antisense 5′-AGGCATCCCCCCGTTCCAGTGTA-3′ resulting in a 189-bp product; MIP-2 sense 5′-TGGCTGAAGACCTGGCAAGG-3′ and antisense 5′-CTGAGCTTGGCCTTGTGTCAGG-3′ resulting in a 150-bp product. All PCR products were analyzed on 1.5% wt/vol agarose gels containing 0.05 mg/100 ml ethidium bromide and photographed by use of Gel Doc-It Imaging System (UVP). Product sizes were identified by comparison with DNA size standards included in the gels. Densitometry results from PCR products were normalized to 18S internal controls.

ELISA analysis. ELISA was performed to examine protein levels of MCP-1, RANTES, MIP-1α, and MIP-2 in the tissues and plasma. Harvested pancreas and lung samples were thawed and homogenized (4°C) in 20 mM phosphate buffer (pH 7.4), and centrifuged (2,000 g, 15 min). The supernatants were used for measuring tissue levels of chemokines. Plasma samples prepared by centrifugation of harvested blood samples were used for measuring systemic levels of chemokines. Tissue or systemic concentrations of MCP-1, RANTES, MIP-1α, and MIP-2 were measured by specific Duetset ELISA kits (R&D Systems; detection levels 3.906, 31.250, 7.813, and 15.625 pg/ml, respectively) according to the manufacturer’s instructions. Results were expressed as picograms per microgram DNA in tissue samples or picograms per milliliter of plasma.

Immunohistochemistry. Immunohistochemistry was performed to examine the protein expression levels of chemokines and to localize chemokine expression in the tissues. Briefly, pancreas and lung samples were isolated and immediately fixed in 10% phosphate-buffered formalin solution. The fixed tissues were then embedded in paraffin and kept until use. Paraffin sections (5 μm) were deparaffinized and hydrated. Antigens were retrieved in a 10 mM sodium citrate buffer (pH 6.0) preheated to 95°C for 30 min. Immunohistochemical staining was subsequently performed on tissue sections by using mouse HRP-DAB cell and tissue staining kit (R&D Systems) following the manufacturer’s instructions with some modifications. In brief, sections were blocked with peroxidase blocking buffer, followed by serum blocking buffer, avidin blocking buffer, and biotin blocking buffer. Tissue sections were then incubated with 10 μg/ml goat anti-mouse CCL2/JE/MCP-1 or RANTES or MIP-1α or MIP-2 antibodies (R&D Systems) overnight at 4°C. Subsequently, tissue sections were washed and incubated with biotinylated anti-goat secondary antibody for 1.5 h at room temperature. After being washed, the sections were incubated with HRP-conjugated high-sensitivity streptavidin for half an hour at room temperature followed by incubation with freshly prepared 3,3′-DAB. The sections were counterstained with hematoxylin and subsequently dehydrated, mounted, and covered with coverslips. As control samples, sections of the same tissue samples were incubated with PBS in place of the primary antibody or with a nonimmunized mouse IgG antibody. In these cases, no staining was detected.

Statistical analysis. Data are expressed as means ± SE. Statistical analyses were performed with SPSS program version 13.0 (Chicago, IL). A one-way ANOVA with post hoc Tukey’s test was used when comparing three or more groups. A P value of <0.05 was considered a statistically significant difference.
RESULTS

Caerulein induced early upregulation of MCP-1 in both the pancreas and lungs. CP-96,345 administration suppressed caerulein-induced increase in MCP-1 mRNA and protein expression in a time- and dose-related manner. In both the pancreas and lungs, caerulein hyperstimulation resulted in a time- and dose-related increase in MCP-1 mRNA and protein levels. MCP-1 mRNA expression in both tissues was upregulated within 3 h after AP induction by caerulein hyperstimulation (Fig. 1, A and B). The expression level of MCP-1 was higher in the pancreas than in the lungs. MCP-1 protein levels were significantly increased in the pancreas as early as 3 h (Fig. 1C) but only after 6 h of AP induction in the lungs and plasma (Fig. 1, D and E). These results suggest that MCP-1 is an early proinflammatory mediator, probably with a more important role in local than systemic inflammatory responses.

Prophylactic or therapeutic administration of NK-1R antagonist CP-96,345 attenuated, in a time- and dose-dependent manner the elevation of MCP-1 mRNA and protein expression levels in the pancreas and lungs. The effect became significant in mice receiving six and ten hourly caerulein injections (Fig. 1, A–D). CP-96,345 treatment had no significant effect on caerulein-induced elevation of plasma MCP-1 levels (Fig. 1E).

Caerulein induced increase in RANTES mRNA and protein levels only in the pancreas. CP-96,345 administration had no effect on RANTES expression. RANTES mRNA and protein levels were significantly increased in the pancreas but not lungs 6 h after AP induction (Fig. 2, A–D). Significant increase in plasma RANTES levels was only observed after 10 h of AP induction (Fig. 2E). CP-96,345 administration had no significant effect on caerulein-induced increase in pancreatic RANTES mRNA or protein expression (Fig. 2, A and C). Also, CP-96,345 treatment had no obvious effect on lung and plasma RANTES levels (Fig. 2, B, D, and E). The results indicate RANTES may participate in the local inflammatory responses in AP at a later stage. However, RANTES is not involved in SP-NK-1R-associated pathogenesis in the condition.

Caerulein induced a time- and dose-related increase in MIP-1α mRNA and protein expression in both the pancreas and lungs. CP-96,345 administration attenuated caerulein-induced MIP-1α upregulation in both tissues. MIP-1α mRNA expression was upregulated early (3 h) in the pancreas and lungs by caerulein administration (Fig. 3, A and B). However, its protein expression was found significantly elevated in both tissues only 6 h after AP induction (Fig. 3, C and D). MIP-1α levels in the plasma remained below the level of detection even in mice given ten hourly caerulein injections (data not shown). Hence MIP-1α might not be one of the earliest mediators in the condition, but, once expressed, it is likely involved in both local and distant organ inflammatory responses. Administration of CP-96,345 significantly suppressed caerulein-induced upregulation of MIP-1α mRNA expression in the pancreas and lungs. The suppression effect became significant earlier in the pancreas (3 h at the mRNA levels and 6 h at the protein levels) than in the lungs (10 h) (Fig. 3, A and B).

Caerulein induced early upregulation of MIP-2 mRNA and protein expression in both the pancreas and lungs. CP-96,345 administration suppressed caerulein-induced MIP-2 upregulation in both tissues. MIP-2 mRNA and protein levels were significantly elevated in both the pancreas and lungs as early as 3 h after AP induction and further increased in a dose- and time-related manner up to 10 h (Fig. 4, A–D). Plasma MIP-2 levels significantly increased after 6 and 10 h of AP induction (Fig. 4E). MIP-2, similar to MCP-1, is probably involved in early events of both local and systemic inflammatory responses in AP. Administration of CP-96,345 significantly attenuated caerulein-induced increases in pancreatic and lung but not plasma MIP-2 levels (Fig. 4, A–E).

Effects of caerulein hyperstimulation and CP-96,345 treatment on immunohistochemical localization of MCP-1, MIP-1α, and MIP-2 in the pancreas and lungs. Immunohistochemical staining was performed to further confirm the attenuating effect of CP-96,345 on protein expression levels of MCP-1, MIP-1α, and MIP-2 in the pancreas and lungs and to localize these chemokines to their cellular sources in the tissues. MCP-1, MIP-1α, and MIP-2 expression was found the most in the pancreas and lungs of mice administered caerulein without CP-96,345 treatment (Figs. 5–7, A and F). There was an apparent reduction of MCP-1, MIP-1α, and MIP-2 expression in tissues of mice treated with CP-96,345 (Fig. 5–7, C, D, G, and H). The pancreas and lungs from mice administered saline showed no significant staining for these chemokines (Fig. 5–7, A and E). In the pancreas, chemokine expression was found mostly in pancreatic acinar cells and occasionally in the infiltrating leukocytes (Fig. 5–7, A–D). In the lungs, alveolar macrophages epithelial and endothelial cells are the chemokine-expressing cells (Fig. 5–7, E–H).

DISCUSSION

In an in vivo model of secretagogue-induced AP, we observed a novel interaction between two groups of proinflammatory mediators: the neuropeptide substance P and chemokines. Blockade of NK-1R, the primary receptor for SP, with a selective NK-1R antagonist CP-96,345 resulted in attenuation in pancreatitis-induced upregulation of chemokine expression. Thus the present study suggests that the proinflammatory effects of SP in this disease model are mediated by the production of chemokines, which further amplifies tissue damage and propagates the inflammatory response from a local process to a systemic inflammation.

SP is shown to have proinflammatory effects in immune and epithelial cells and participate in many inflammatory diseases. Studies in different experimental settings have revealed its proinflammatory actions: promoting lymphocyte proliferation and immunoglobulin production, enhancing leukocyte migration and accumulation, and inducing the release of cytokines as well as other inflammatory mediators such as oxygen radicals, arachidonic acid derivatives, and histamine that amplify the inflammatory responses (15, 18, 20, 21). SP is a well-known proinflammatory mediator in AP and associated lung injury. The interplay between SP and other inflammatory molecules is an important determinant of the severity of the disease. It has been earlier reported that expression of tachykinins and neurokinin receptors in AP is regulated by SP (16). Our recent study reported that SP has an important role in mediating hydrogen sulfide-induced lung inflammation (8). However, whether SP has a regulatory effect on the production of chemotactic cytokines-chemokines in the AP model has not yet been addressed. To address this question, we induced AP in mice by caerulein hyperstimulation and blocked the binding of
Fig. 1. CP-96,345 treatment attenuated caerulein (Caer)-induced increase in monocyte chemotactic protein (MCP)-1 mRNA and protein levels in the pancreas and lungs. Mice were given 3, 6, and 10 hourly injections of saline (Sa) or caerulein (Ca). CP-96,345 was administered either half an hour before (CP/Ca) or 1 h after the first caerulein injections (Ca/CP). MCP-1 mRNA expression was detected by RT-PCR, and protein levels were measured by ELISA assays as described in MATERIALS AND METHODS. A and B: semiquantitative RT-PCR detection of MCP-1 mRNA expression in the pancreas (A) and lungs (B) and the densitometry analysis. Sample loading was normalized with 18S rRNA internal control. Values are expressed relative to that of 18S and represent means ± SE for 5–8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.

C, D, and E: effect of caerulein hyperstimulation and CP-96,345 treatment on MCP-1 protein levels in the pancreas (C), lungs (D), and plasma (E). Values are expressed as pg/μg DNA of tissues and as pg/ml of plasma and represent means ± SE for 8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.
Fig. 2. Caerulein induced upregulation of regulated on activation, T cells expressed and secreted (RANTES) mRNA and protein expression in the pancreas but not lungs. CP-96,345 treatment had no effect on RANTES expression. Total RNA was isolated from mice given 3, 6, and 10 hourly injections of saline or caerulein or caerulein with prophylactic or therapeutic CP-96,345 treatment. RANTES mRNA expression was detected by RT-PCR, and protein levels were determined by ELISA assays as described in MATERIALS AND METHODS. A and B: semiquantitative RT-PCR detection of RANTES mRNA expression in the pancreas and lungs and the densitometry analysis. Sample loading was normalized with 18S rRNA internal control. Values are expressed relative to that of 18S and represent means ± SE for 5–8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.
SP to its receptor NK-1R using a specific receptor antagonist and investigated the changes in various CC and CXC chemokine expression in the pancreas and lungs (and plasma for protein levels). MCP-1, MIP-1α, and MIP-2 mRNA were found upregulated early (within 3 h) in both the pancreas and lungs upon AP induction, suggesting that they are important early mediators in both local as well as distant inflammatory responses in the condition. RANTES production was induced later than the other three as the disease progressed and only in the pancreas. This suggests its role in mediating local inflammatory responses at a later stage of AP. Either prophylactic or therapeutic CP-96,345 administration resulted in attenuation of caerulein-induced increase in MCP-1, MIP-1α, and MIP-2 but not RANTES levels in the tissues. The differential regulation of the four chemokine expression, temporally and spatially, by caerulein hyperstimulation and CP-96,345 administration, im-

Fig. 3. CP-96,345 treatment suppressed caerulein-induced upregulation of macrophage inflammatory protein (MIP)-1α mRNA and protein expression in the pancreas and lungs. Total RNA was isolated from mice given 3, 6, and 10 hourly injections of saline or caerulein or caerulein with prophylactic or therapeutic CP-96,345 treatment. MIP-1α mRNA expression was detected by RT-PCR, and protein levels were measured by ELISA assays as described in MATERIALS AND METHODS. A and B: semiquantitative RT-PCR detection of MIP-1α mRNA expression in the pancreas and lungs and the densitometry analysis. Sample loading was normalized with 18S rRNA internal control. Values are expressed relative to that of 18S and represent means ± SE for 5–8 animals in each group. C and D: effect of caerulein hyperstimulation and CP-96,345 treatment on MIP-1α protein levels in the pancreas (C) and lungs (D). Values are expressed as pg/µg DNA of tissues and as pg/ml of plasma and represent means ± SE for 8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.
Fig. 4. CP-96,345 treatment attenuated caerulein-induced increase in the MIP-2 mRNA and protein levels in the pancreas and lungs. Total RNA was isolated from mice given 3, 6, and 10 hourly injections of saline or caerulein or caerulein with prophylactic or therapeutic CP-96,345 treatment. MIP-2 mRNA expression was detected by RT-PCR, and protein levels were determined by ELISA assays as described in MATERIALS AND METHODS. A and B: semiquantitative RT-PCR detection of MIP-2 mRNA expression in the pancreas and lungs and the densitometry analysis. Sample loading was normalized with 18S rRNA internal control. Values are expressed relative to that of 18S and represent means ± SE for 5–8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.

C, D, and E: effect of caerulein hyperstimulation and CP-96,345 treatment on MIP-2 protein levels in the pancreas (C), lungs (D), and plasma (E). Values are expressed as pg/μg DNA of tissues and as pg/ml of plasma and represent means ± SE for 8 animals in each group. *P < 0.05 vs. Sa, †P < 0.05 vs. Ca.
plies their differential roles in the pathogenesis of the disease. In addition, immunohistochemical staining further suggested that chemokine expression levels were lower in antagonist treatment group than in caerulein-only treatment group.

The enhancing effect of SP on both CC and CXC chemokine production could be explained by different mechanisms. SP may directly stimulate chemokine production by pancreatic acinar cells by binding to NK-1R on the cell surface (5). SP, with vasodilatation and leukocyte chemotaxis properties, enhances the delivery and accumulation of leukocytes such as macrophages and neutrophils into the injured tissues, thereby promoting the release of chemokines from these cells (13, 21, 23). It remains possible that chemokine production is upregulated at the transcriptional level by SP via the NF-kB pathway. The role of inflammatory cells (neutrophils and mononuclear leukocytes) and other inflammatory mediators including adhesion molecules, chemokine receptors, and gas mediators such as nitric oxide...
and hydrogen sulfide (8) in the interplay between SP and chemokines may also be implicated.

The importance of chemokines in the pathogenesis of AP has been demonstrated by earlier findings (1, 3, 12, 19, 22, 24). MIP-2 is a prototype murine CXC chemokine and a potent PMN chemoattractant and activator. Neutrophils early infiltrating the tissues produce CC chemokines that lead to subsequent monocyte/macrophage influx into tissues. Neutrophils particularly are important in tissue destructive events through the release of their proteolytic enzymes such as PMN elastase and the production of lethal oxygen metabolites. MCP-1 as a prototypic proinflammatory mediator is the major chemokine in AP (19). Its mRNA expression was induced in rat pancreas after caerulein hyperstimulation (12). Both MCP-1 and RANTES were secreted from rat pancreatic acinar cells treated with CCK (25). Genetic deletion of CCR1, a receptor for MIP-1α and RANTES, protected mice from lung inflammation secondary to AP (14). MIP-2 levels were increased in serum,
pancreas, and lung tissues in AP in mice. Administration of anti-MIP-2 antibodies partially protected the mice against pancreas and lung injury (22). These earlier findings, in agreement with our findings, suggest that NK-1R blockade-associated protection effect against tissue damage in AP are due to (at least partially) decreased chemokine production, neutrophil and monocyte/macrophage infiltration and hence reduced release of deleterious factors to the tissues.

Chemokine expression by pancreatic acinar cells was demonstrated in other experimental settings. Rat pancreatic acinar cells were shown to produce MCP-1 upon AP induction (2, 9). Infiltrating neutrophils in the pancreas were reported as another cellular source for MCP-1 production (9). Isolated rat acinar cells when stimulated by CCK secreted MCP-1 and RANTES chemokines (25). We showed here that pancreatic acinar cells and infiltrating leukocytes in the pancreas are sources of

chemokine expression in caerulein-induced AP in mice. Furthermore, the alveolar macrophages, epithelial cells, and endothelial cells are chemokine-producing cells in the lungs in the condition.

Taken together, our results demonstrate a newly recognized role of the CC chemokines MCP-1 and MIP-1α and the CXC chemokine MIP-2 in SP-NK-1R-related pathway in the pathogenesis of AP. The findings with these two groups of interrelated proinflammatory mediators may have potential therapeutic implications for the treatment of the condition.

GRANTS

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