Chemokine receptor CCR5 deficiency exacerbates cerulein-induced acute pancreatitis in mice

Christophe Moreno,1,2 Charles Nicaise,2 Thierry Gustot,2 Eric Quertinmont,2 Nathalie Nagy,3 Marc Parmentier,4 Hubert Louis,1,2 and Jacques Devière1,2

1Division of Gastroenterology and Hepatopancreatology, Erasme Hospital; 2Laboratory of Experimental Gastroenterology, Free University of Brussels; 3Division of Pathology, Erasme Hospital; and 4Institute of Interdisciplinary Research, Free University of Brussels, Brussels, Belgium

Submitted 19 December 2005; accepted in final form 1 August 2006

Abstract

Chemokine receptor CCR5 deficiency exacerbates cerulein-induced acute pancreatitis in mice. Am J Physiol Gastrointest Liver Physiol 291: G1089–G1099, 2006. First published August 3, 2006; doi:10.1152/ajpgi.00571.2005.—Acute pancreatitis (AP) is an inflammatory disease involving the production of different cytokines and chemokines and is characterized by leukocyte infiltration. Because the chemokine receptor CCR5 and its ligands [the CC chemokines CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES] regulate leukocyte chemotaxis and activation, we investigated the expression of CCR5 ligands and the role of CCR5 and its ligands in experimental AP in mice. AP was induced by hourly intraperitoneal injections of cerulein in CCR5-deficient (CCR5<sup>−/−</sup>) or wild-type (WT) mice. Induction of AP by cerulein resulted in an early increase of pancreatic CCL2, CCL3, and CCL4 mRNA expression, whereas CCL5 mRNA expression occurred later. CCR5<sup>−/−</sup> mice developed a more severe pancreatic injury than WT mice during cerulein-induced AP, as assessed by a more pronounced increase in serum amylase and lipase levels and by more severe pancreatic edema, inflammatory infiltrates (mainly neutrophils), and necrosis. CCR5<sup>−/−</sup> mice also exhibited increased production of CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β during the course of cerulein-induced AP. In vivo simultaneous neutralization of CC chemokines with monoclonal antibodies in CCR5<sup>−/−</sup> mice reduced the severity of cerulein-induced AP, indicating a role of CC chemokines in exacerbating the course of AP in the absence of CCR5. Moreover, simultaneous neutralization of CCR5 ligands in WT mice also reduced the severity of cerulein-induced AP. In conclusion, lack of the chemokine receptor CCR5 exacerbates experimental cerulein-induced AP and leads to increased levels of CC chemokines and a more pronounced pancreatic inflammatory infiltrate, suggesting that CCR5 expression can modulate severity of AP.

Acute pancreatitis (AP) is a common disease whose incidence has been increasing over recent years. It is a disease of variable severity, from mild, self-limited, to severe disease, with substantial morbidity and mortality. Regardless of the cause, activation of digestive enzymes within pancreatic acinar cells is thought to be a critical initiating event. This noninfectious damage of pancreatic parenchyma induces an inflammatory reaction at the site of injury, which can be amplified to a generalized systemic inflammatory response, leading to distant organ damage. The severity of an attack of AP appears to be determined by the magnitude of the resultant systemic inflammatory response. A better knowledge of AP pathogenesis is necessary to allow advances in the management and specific treatment of this disease. The in vivo experimental models of AP and its associated systemic complications allow us to study the pathogenesis of AP. Administration of supramaximal doses of the cholecystokinin analog cerulein to rodents results in mild to severe AP, which develops over hours and is particularly useful for the study of early inflammatory events occurring in this disease. Another well-described model of severe lethal, necrotizing pancreatitis was induced in female mice using a choline-deficient, ethionine-supplemented (CDE) diet.

Chemokines are a family of small protein inflammatory mediators with chemotactic and activating effects on leukocytes, which provide a key stimulus for directing leukocytes to areas of injury. They can be broadly subdivided into a structural basis into the CXC subfamily, in which the first two cysteine residues are separated by another amino acid (X), and the CC subfamily, in which the first two cysteine residues are adjacent. CXC and CC chemokines are believed to have different effects. CC chemokines activate primarily monocytes, while CXC chemokines tend to preferentially activate neutrophils. Chemokines bind to a family of seven-transmembrane domain, G protein-coupled receptors on the surface of leukocytes. Chemokines appear to play a major role in the inflammatory processes governing AP and its systemic complications. It has been recently shown that CC chemokine ligand (CCL)2/monocyte chemotactic protein (MCP)-1, a CC chemokine, is produced by pancreatic acinar cells and upregulated during experimental AP. Blockade of CCL2 synthesis protects mice against AP. CCR1, a chemokine receptor for CCL3/macrophage inflammatory protein (MIP)-1α and CCL5/regulated upon activation, normal T cell expressed and secreted (RANTES), has been demonstrated to play a role in inducing pulmonary damage secondary to AP in mice.

The chemokine receptor CCR5 is a G protein-coupled receptor for the CC chemokines CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL8/MCP-2. This receptor constitutes the main coreceptor for the macrophage (M)-tropic strains of human immunodeficiency virus 1 and 2, which are responsible for disease transmission. In addition to its role in leukocyte chemotaxis, CCR5 exerts a positive regulatory effect on the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: C. Moreno, Div. of Gastroenterology and Hepato-Pancreatology, Erasme Univ. Hospital; 808, route de Lennik, Brussels B 1070, Belgium (e-mail: cmoreno@ulb.ac.be).
on T cell helper type 1 (Th1) differentiation in inflammatory processes (20). In mice, CCR5 is expressed on natural killer cells, CD4+ and CD8+ T cells, macrophages, and dendritic cells (21). Macrophages infiltrating the pancreas in human chronic pancreatitis express CCR5 (11), but the extent to which CCR5 and its ligands contribute to the pathogenesis of pancreatic diseases is unknown. Therefore, the aim of the present study was to investigate the role of CCR5 and its ligands in experimental AP in mice.

MATERIALS AND METHODS

Animals and Reagents

Six- to ten-week-old male or four- to six-week-old female (B6, 129P2-CCR5tm1Kuz/J) (16) CCR5−/− mice, and B6;129PF2 [wild-type (WT)] mice were purchased from Jackson ImmunoResearch Laboratories (Bar Harbor, ME). Animals were maintained in our animal facilities on standard laboratory chow and received care in compliance with national legal requirements and National Institutes of Health guidelines. Our experimental protocol was reviewed and approved by the Animal Well-Being Ethical Committee of the Free University of Brussels (protocol 203N). Cerulein was purchased from Sigma-Aldrich (Bornem, Belgium). CDE diet was purchased from MP Biomedicals (Irvine, CA). Anti-mouse CCL2 IgG2a (clone 123616) or isotype control (clone 141945) and anti-mouse CCL3, CCL4, and CCL5 IgG2a (clones 39624, 46907, and 53405, respectively) or isotype control (clone 54447) rat monoclonal antibodies (mAb) were purchased from R&D Systems (Minneapolis, MN).

Experimental Procedures

Cerulein-induced AP. AP was induced by hourly intraperitoneal injections of cerulein in male mice (50 μg/kg diluted in 100 μl saline) for 1, 3, 6, or 10 h. One hour after the last cerulein injection, blood was obtained, and animals were killed by cervical dislocation. Then, the pancreas was removed. One part of the pancreas (the tail) was fixed in formaldehyde and then embedded in paraffin wax for histological analysis; the other part of the pancreas (the head) was directly homogenized in the lysis solution by MagNalyser (Roche Diagnostics, Brussels, Belgium) with one run of 50 s at 6,500 rpm and stored at −80°C for RT-PCR assays. Harvested blood was centrifuged, and the serum was removed and stored at −20°C.

CCL2, CCL3, CCL4, and CCL5 were neutralized in vivo by injecting mice with 0.1 mg anti-CCL2, anti-CCL3, anti-CCL4, or anti-CCL5 mouse mAb 30 min before cerulein injections were started. CDE diet-induced AP. Young female WT or CCR5−/− mice were fasted for 12 h and then fed a choline-deficient diet supplemented with 0.5% ethionine for 72 h. All animals weighed 15 ± 1.5 g. Water was allowed ad libitum at all times. After animals were killed by cervical dislocation, the procedure was the same as described for cerulein-induced acute pancreatitis.

Blood assays. Serum amylases and lipases were measured using automated chromogenic and turbidimetric assays at 37°C. Results were expressed in international units per liter (IU/L).

Concentrations of IL-6, CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CXCL1/KC, and CXCL2/MIP-2 were determined on serum samples by commercially available ELISA kits from R&D Systems with a detection threshold of 3.1, 2, 1.5, 0.5, 0.3, and 1.5 pg/ml, respectively.

Histological grading of pancreatic lesions. Hematoxylin and eosin staining was performed on 6-μm pancreatic sections. The severity of AP was blindly graded by a semiquantitative assessment of edema, inflammatory cell infiltrate, and acinar necrosis, according to a scoring system described previously (Table 1) (35).

Immunohistochemical staining of pancreatic neutrophils. Immuno- histochemistry was performed on formalin-fixed pancreatic tissue samples according to the streptavidin-peroxidase method. Paraffin-embedded sections were deparaffinized, and endogenous peroxidases were blocked with 1% H2O2 for 30 min. To block nonspecific binding, sections were incubated in normal horse serum (Vector Laboratories, Peterborough, UK; 1:20) for 10 min. Slices were then rinsed with 50 mM Tris-buffered saline (TBS; pH 7.6). Primary antibody [polyclonal rabbit antiMPO, 1:100 diluted (Novocastra, Newcassel, Newcastle, UK)] was added for 30 min. Slices were then rinsed with TBS and incubated with biotinylated anti-rabbit IgG (1:200 diluted, Vector Laboratories) for 30 min. After being rinsed with TBS, slices were incubated with ABC complex-horseradish peroxidase (Vector Laboratories) for 30 min. Peroxidase activity was visualized by using diaminobenzidine (Liquid DAB substrate, BioGenex, San Ramon, CA). Counterstaining was performed with Mayer’s hematoxylin. Positive cells were then counted on 20 consecutive high-power fields (HPFs) on each slide at a magnification of ×400. Results are expressed as numbers of positive cells per 20 HPFs.

ChemoKine mRNA Quantification by Real-Time PCR

CCL2, CCL3, CCL4, and CCL5 mRNA were measured by real-time PCR, as previously described (34). Pancreas samples were freshly homogenized in the lysis solution by MagNalyser (Roche Diagnostics) with one run of 50 s at 6,500 rpm. Then, total RNA was extracted by the High Pure RNA Tissue kit (Roche Diagnostics), according to the manufacturer’s protocol, including DNase treatment. Reverse transcription was performed as follows: 9 μl of H2O containing 1 μg of total RNA were mixed with 4 μl of oligo-dT primer (0.1 μg/μl) and incubated at 65°C for 5 min. Samples were chilled on ice, and 7 μl of RT mix were added, in which the following components were added: 4 μl of Transcriptor 5× buffer, 2 μl of dNTP mix (10 mM each), 0.5 μl of porcine RNase inhibitor (31.75 U/ml, Amer sham Biosciences, Roosendaal, The Netherlands), and 0.5 μl of transcript reverse transcriptase (20 U/μl, Roche Diagnostics). The mixture was then incubated for 1 h at 42°C and 15 min at 70°C. Quantitative PCR was performed by using real-time fluorogenic PCR. Amplification of cDNA was performed with forward- and reverse-specific primers, and fluorogenic probes were used for the detection of amplified products (Eurogentec, Seraing, Belgium). Amplification was performed on a LightCycler (Roche Diagnostics). A total of 45 cycles was performed. For each PCR run, a standard curve was achieved. This consisted of a PCR product that included the quantified amplicon and that was prepared by “classical” PCR from cDNA positive for the concerned target mRNA. These PCR products used as standards were purified from agarose gel, at the end of which cDNA concentrations were measured by optical density spectrophotometry and the corresponding copy number was calculated, as described

Table 1. Histological scoring for acute pancreatitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td>0</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>focally increased between lobules</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>diffusely increased between lobules</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>acini disrupted and separated</td>
</tr>
<tr>
<td>Inflammatory cell infiltrate</td>
<td>0</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>rare or around ductal margins</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>in the parenchyma (&lt;50% of the lobules)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>in the parenchyma (&gt;50% of the lobules)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0</td>
<td>absent</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>architectural changes, picnotic nuclei</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>focal necrosis (&lt;10% of the parenchyma)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>diffuse parenchymal necrosis (&gt;10% of the parenchyma)</td>
</tr>
</tbody>
</table>

Histological scoring was always performed on the same part of the pancreas (the tail), which was fixed for histological analysis.

AJP-Gastrointest Liver Physiol • VOL 291 • DECEMBER 2006 • www.ajpgi.org
previously (26). Serial dilutions from the resulting PCR products were used as standard curves, with each containing a known amount of copy number. To normalize for inefficiencies in cDNA synthesis and RNA input amounts, mRNA levels of each chemokine were then normalized against housekeeping gene hypoxanthine-guanine phosphoribosyl transferase (HPRT) mRNA. Results are expressed as fold changes relative to baseline (time 0 controls), which was assigned the value of 1. Primers and probes were designed using Primer3 software. The sequences of primers and probes for the real-time PCR reactions included: HPRT sense 5'-GGA-CCT-CTC-GAA-GTG-TTG-GAT-3', HPRT antisense 5'-CCA-ACA-ACA-AAC-TTG-TCT-GGA-A-3', and HPRT probe 5'-(Fam)CAG-GCC-FAM-3'.

Fig. 1. CCL2, CCL3, CCL4, and CCL5 mRNA expression during cerulein-induced acute pancreatitis. Pancreatic CCL2, CCL3, CCL4, and CCL5 mRNA expression was measured by quantitative RT-PCR 0, 1, 3, 6, or 24 h after the first cerulein injection (n = 5 mice per group and per time point). All data are expressed as fold changes relative to baseline. HPRT, hypoxanthine-guanine phosphoribosyl transferase. The bars represent means ± SE. *P < 0.05; **P < 0.01 vs. time 0 control.

Fig. 2. CCL2, CCL3, CCL4, and CCL5 mRNA expression during CDE diet-induced acute pancreatitis. Pancreatic CCL2, CCL3, CCL4, and CCL5 mRNA expression was measured by quantitative RT-PCR 0, 24, 48, or 72 h after the first cerulein injection (n = 6 mice per group and per time point). All data are expressed as fold changes relative to baseline. The bars represent means ± SE. **P < 0.01 vs. time 0 control.

Statistical Analysis

Results are expressed as means ± SE. Statistical comparisons were made by using the two-tailed Mann-Whitney U-test. The log rank test was used to compare survival rates. Analyses were performed using SPSS 11.0 software (Chicago, IL).

RESULTS

CCR5 Ligand mRNA Expression During Experimental AP

Induction of AP by cerulein hyperstimulation (5 hourly injections) resulted in a very early increase of pancreatic CCL2, CCL3, and CCL4 mRNA expression, which peaked between 3 and 6 h after the first cerulein injection. Although occurring later in the course of AP, pancreatic CCL5 mRNA expression was also significantly elevated (Fig. 1).

On the contrary, during the course of CDE diet-induced AP, no significant increase of pancreatic CC chemokine mRNA expression was observed except for CCL3, which was only upregulated 48 h after CDE diet was started (Fig. 2).

CCR5-deficient mice develop a more severe pancreatic injury during cerulein-induced AP. The role of CCR5 in mediating acute pancreatic injury was then studied using CCR5<sup>-/-</sup> mice.
mice. Evidence of pancreatic injury in AP induced by intraperitoneal administration of cerulein at a dose of 50 μg/kg hourly for 6 or 10 h was confirmed by an increase in serum amylase and lipase levels and pancreatic histopathological changes (Figs. 3 and 4). In CCR5−/− mice, serum amylase and lipase levels were significantly elevated compared with WT mice 6 and 10 h after first cerulein injection (Fig. 3A).

Histological examination of pancreas sections confirmed more severe AP in CCR5−/− mice 6 and 10 h after cerulein injection were started in terms of edema, inflammatory cell infiltrates, and necrosis (Figs. 3A and B).

Increases in IL-6 serum levels were also significantly more pronounced in CCR5−/− mice compared with WT mice 6 h after pancreatitis induction (P < 0.05; Fig. 3C).

The effect of CCR5 deficiency was also evaluated in the severe acute necrotizing pancreatitis model induced by a CDE diet. No significant difference in the severity of CDE-induced AP was observed between CCR5−/− and WT mice. Indeed, serum amylase levels, histological analysis of the pancreas, and survival rate were not different between both groups of mice (Fig. 5).

CCR5 deficiency affects chemokine production during cerulein-induced AP. Serum levels of the chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CXCL1/KC, and CXCL2/MIP-2 were compared in CCR5−/− and WT mice (Fig. 6). Six and ten hours after the first cerulein injection, the increase in serum levels of CCL2 was significantly more pronounced in CCR5−/− mice compared with WT mice (P < 0.01 and P < 0.05, 6 and 10 h after first cerulein injection, respectively). CCL3 serum levels were only detected 1 h after cerulein injections were started and were significantly increased in CCR5−/− mice compared with WT mice (P < 0.05). One, six, and ten hours after AP began, serum levels of CCL4 were strongly increased in CCR5−/− mice compared with WT mice (P < 0.01). In contrast, CCL5 serum levels were not upregulated during AP but were different between CCR5−/− and WT mice. Serum levels of the two CXC chemokines CXCL1 and CXCL2 were not different between both groups of mice.

Pancreata from CCR5-deficient mice are characterized by a more pronounced neutrophilic infiltration. Because we observed an increase in the production of CC chemokines in CCR5−/− mice compared with WT mice, and because activated leukocytes, particularly neutrophils, are important in the development of organ damage during AP, we compared pancreatic neutrophilic infiltration between both groups of mice during AP (Fig. 7). Six hours after the first cerulein injection, the numbers of cells positive for MPO staining was significantly higher in CCR5−/− mice compared with WT mice (P < 0.05).

Role of CC chemokines in exacerbating cerulein-induced AP in CCR5-deficient mice. The striking increase in CC chemokine serum levels prompted us to neutralize these chemokines in CCR5−/− mice (Fig. 8). Six hours after the first cerulein

![Fig. 4. Pancreatic histopathological changes in WT and CCR5-deficient mice during cerulein-induced acute pancreatitis. CCR5−/− or WT mice were hourly injected with cerulein for 6 or 10 h. One hour after the last cerulein injection, animals were killed. Pancreatic tail sections were stained with hematoxylin-eosin (magnification ×100 in A and B and ×200 in C). A: baseline, with no pancreatitis. B: acute pancreatitis, at 6 h after the first cerulein injection. C: acute pancreatitis, at 10 h after the first cerulein injection.](http://ajpgi.physiology.org/)

AJPGI-Gastrointest Liver Physiol • VOL 291 • DECEMBER 2006 • www.ajpgi.org
To further investigate whether this increased production of CC chemokines plays a role in triggering pancreatic injury during AP in CCR5−/− mice, we simultaneously neutralized these four CC chemokines in CCR5−/− mice. Pancreatic injury was significantly reduced in CCR5−/− mice after neutralization of all four CC chemokines, as demonstrated by a decrease in serum amylases and lipases levels (P < 0.01) and in pancreatic histopathological scores (global score: 2.95 ± 0.26 vs. 4.1 ± 0.15 in anti-CCL2, anti-CCL3, anti-CCL4, and anti-CCL5 vs. isotype control mAb pretreatment groups, P < 0.05; Fig. 8C). This suggests that increased levels of CC chemokines are involved in exacerbating pancreatic damage in CCR5−/− mice.

Effect of CCR5 ligand neutralization on the severity of cerulein-induced AP in WT mice. To better identify the role of CCR5 ligands during the course of cerulein-induced AP, we neutralized them with mAbs in WT mice. Six hours after the first cerulein injection, pancreatic injury was significantly reduced after simultaneous CCL3, CCL4, and CCL5 neutralization, as demonstrated by a decrease in serum amylase and lipase levels (P < 0.05) and in pancreatic histopathological scores (global score: 2.98 ± 0.13 vs. 3.72 ± 0.09 in anti-CCL3, anti-CCL4, and anti-CCL5 vs. isotype control mAb pretreatment groups, P < 0.01, Fig. 9A). This suggests that CCR5 ligands are involved in the development of pancreatic injury during cerulein-induced AP.

To further investigate the role of each CCR5 ligand on pancreatic injury during cerulein-induced AP, we neutralized each CCR5 ligand individually with monoclonal antibodies. Six hours after the first cerulein injection, no statistically significant reduction in terms of serum hydrolases and histopathological changes (global score: 3.19 ± 0.17 vs. 3.08 ± 0.12 vs. 2.75 ± 0.23 vs. 3.34 ± 0.35 in anti-CCL3 vs. anti-CCL4 vs. anti-CCL5 vs. isotype control mAb pretreatment groups, P = not significant) was observed after CCL3, CCL4, or CCL5 neutralization (Fig. 9B).

DISCUSSION

The present study shows that CCR5 deficiency exacerbates the severity of pancreatic injury during cerulein-induced AP, as evidenced by a significantly higher increase in serum amylase and lipase levels, pancreatic edema, necrosis, and inflammatory infiltration.

Activated leukocytes are important in the pathogenesis of AP. Depletion of circulating neutrophils or interference with neutrophil migration reduce pancreatic damage in AP (12, 14, 36). T lymphocytes, particularly CD4+, also play a pivotal role in the development of tissue injury during AP (7).

Chemokines are key inflammatory mediators by directing leukocytes to areas of injury. CC chemokines are known to activate primarily monocytes; however, it has been suggested that CC chemokines may also be involved in neutrophil trafficking in humans (5, 18). Several pieces of evidence point to a role for CC chemokines in the pathogenesis of AP (2–4, 10). On this basis, the finding that CCR5−/− mice exhibited more severe pancreatic inflammation is intriguing. Indeed, one could expect a defect in cell recruitment in the absence of a chemokine receptor, as previously described for CCR1 and CCR2 (9, 15). However, the absence of a macrophage or T cell recruitment defect has already been reported in CCR5−/− mice (38).
Moreover, recent studies in other experimental models of disease using CCR5−/− mice support an immunoregulatory role of CCR5. In a model of influenza A virus, CCR5−/− mice developed increased pulmonary inflammation and mortality (6). CCR5−/− mice also developed worse liver injury and an enhanced recruitment of inflammatory cells into the liver in an experimental model of T cell-mediated hepatitis (24). Therefore, a more prominent pancreatic inflammatory infiltrate may be responsible for exacerbating pancreatic damage during cerulein-induced AP in CCR5−/− mice and suggests an immunomodulatory and anti-inflammatory role of CCR5 in this experimental model of AP.

The present study also shows that the expression of CCR5 ligands is increased in the pancreas during cerulein-induced AP. CCR5 is a G protein-coupled receptor for several chemokines of the CC family: CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL8/MCP-2. In the present work, we identified for the first time an early intrapancreatic production of CCL3/MIP-1α and CCL4/MIP-1β during cerulein-induced AP. Compared with CCL3/MIP-1α and CCL4/MIP-1β, CCL5/RANTES mRNA expression is delayed later in the course of AP. Thus CCR5 ligands are all upregulated but with distinct kinetics during experimental cerulein-induced AP. The observation of an early increase of CCL3 and CCL4 mRNA expression may be the result of the production of these chemokines by acinar cells, as demonstrated for CCL2/MCP-1 (2). Indeed, 1 h after cerulein injections were started, no increase in leukocyte infiltration was detected in the pancreas. In humans, it has been shown in one clinical study that CCL3/MIP-1α was significantly increased in patients with AP and multiple organ dysfunction syndrome. In this clinical study, CCL4/MIP-1β levels remained unaffected by the presence of local or systemic complications, and CCL5/RANTES was not investigated (28).

However, this increased expression of CCR5 ligands and the more severe pancreatic damage in the absence of CCR5 is only valid in the cerulein-induced AP model. Indeed, in another

![Graphs showing the expression levels of chemokines over time after cerulein injections.](http://ajpgi.physiology.org/fig6.png)
well-described model of severe lethal, necrotizing pancreatitis induced by a CDE diet (19), no significant increase of pancreatic CC chemokine mRNA expression was observed except for CCL3, which was only upregulated 48 h after CDE diet was started and at lower levels than in cerulein-induced AP. Moreover, no significant difference in the severity of CDE-induced AP was observed between CCR5−/− and WT mice. These discordant results between cerulein- and CDE-induced experimental AP models may be explained by the fact that these models have a completely different etiology and make more complex a conclusion about an eventual role of CCR5 in human disease.

Another interesting finding of this study is that CCR5 deficiency is characterized by a striking increase in CC chemokine serum levels during cerulein-induced AP. Indeed, CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β serum levels were more elevated in CCR5−/− compared with WT mice during experimental AP. We demonstrate that increased serum levels of these CC chemokines in CCR5-deficient mice play a pathological role during the course of cerulein-induced AP, as their simultaneous neutralization dramatically reduced the extent of pancreatic damage in these mice. Interestingly, when each chemokine was neutralized individually by a monoclonal antibody, only CCL2/MCP-1 neutralization could protect against pancreatic injury in CCR5−/− mice, as demonstrated by a significant reduction in serum amylase and lipase levels and a trend to decrease histopathological lesions. CCL2/MCP-1 is a CC chemokine of particular interest in the pathogenesis of AP. It has been recently shown that CCL2/MCP-1 is produced by pancreatic acinar cells and upregulated during experimental AP (2) and that blocking of CCL2/MCP-1 synthesis protects mice against AP (4). In humans, CCL2/MCP-1 serum concentrations are increased in AP and are correlated with severity of the disease (28). Moreover, a single-nucleotide polymorphism in the distal regulatory region of the MCP-1 gene (G to A) at position −2518, leading to increased CCL2/MCP-1 production when the −2518 G allele is present, has been recently shown to be a risk factor for severe AP (27).

In the present study, the protection induced by CCL2/MCP-1 neutralization on pancreatic injury seems to be only partial and milder than following simultaneous CCL2, CCL3, CCL4, and CCL5 neutralization since it does not abrogate the exacerbation in pancreatic damage observed in CCR5−/− mice. This can probably be explained by the fact that chemokines form a redundant system in which the blocking of several components is more efficient than individual neutralization.

Interestingly, this increased serum levels of CC chemokines observed in CCR5−/− mice is accompanied by a more prominent pancreatic inflammatory infiltrate, in particular neutrophils, during AP. It is generally believed that CC chemokines activate primarily monocytes, while the CXC chemokines tend to preferentially activate neutrophils. In this study, MIP-2 and KC (two CXC chemokines) serum levels were not different between CCR5−/− and WT mice. However, recent works have shown that several CC chemokines also attract neutrophils (4, 18), and CC-chemokine receptors can be expressed by neutrophils (5). Consequently, it seems plausible that the increase in CC chemokine production in CCR5−/− mice would be responsible for a more pronounced pancreatic neutrophilic infiltration and involved in the increased severity of pancreatic damage.

In the present study, we also demonstrate that CCR5 ligands play a pathological role during the course of cerulein-induced AP, as their simultaneous neutralization significantly reduced the extent of pancreatic damage in WT mice. However, individual neutralization of each CCR5 ligand does not protect against cerulein-induced AP, underlining that CCR5 ligands form a redundant system, in which the blocking of all ligands is more efficient than individual neutralization.

In humans, a 32-bp deletion in the coding region of the CCR5 gene, termed CCR5−Δ32, leads to a frame shift in the open reading frame of CCR5. Patients homozygous for this mutation cannot express CCR5 on the cell surface, whereas heterozygosity results in decreased expression of the functional CCR5 protein. The CCR5−Δ32 mutation has gained clinical interest as it confers protection against infection with the human immunodeficiency virus (30). More recently, CCR5−Δ32 mutation was found to influence disease susceptibility and severity in patients with various inflammatory diseases (8, 13, 27).
Interestingly, some of these studies suggest that CCR5 expression can play a protective role during inflammatory processes. Thus several data in humans corroborate our experimental findings of an immunoregulatory function of CCR5 in inflammatory diseases.

In summary, experimental cerulein-induced AP is severely worsened in the absence of CCR5. CCR5 deficiency induces an increase in the production of CC chemokines, thereby enhancing inflammatory infiltrate and pancreatic injury. These results highlight the role of CCR5 in the immune response, serving as...
Fig. 9. Effect of neutralization of CCR5 ligands on the course of cerulein-induced acute pancreatitis in WT mice. WT mice were hourly injected with cerulein for 6 h. One hour after the last cerulein injection, animals were killed, and serum was removed. Serum amylase and lipase levels were determined, and histological analysis was performed. A: in the first experiment, simultaneous neutralization of CCL3, CCL4, and CCL5 was performed via intravenous injection of 0.1 mg anti-mouse CCL3, anti-mouse CCL4, and anti-mouse CCL5 IgG2A rat monoclonal antibodies (α-CCL3+4+5) or 0.3 mg IgG2A isotype control rat monoclonal antibodies (ctrl) 30 min before the first cerulein injection. B: in the second experiment, intravenous 0.1 mg anti-mouse CCL3 (α-CCL3), anti-mouse CCL4 (α-CCL4), anti-mouse CCL5 (α-CCL5), or isotype control (ctrl) IgG2A rat monoclonal antibody was administered 30 min before the first cerulein injection. Results are expressed as means ± SE; n = 6 mice per group. *p < 0.05, **p < 0.01.

ACKNOWLEDGMENTS

The authors thank François-Xavier Demoor for technical assistance.

GRANTS

This work was supported in part by grants from the “Fondation Erasme” and from the Belgian “Fonds National de la Recherche Scientifique.”

REFERENCES


