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Modulation in the expression of SHP-1, SHP-2 and PTP1B due to the inhibition of MAPKs, cAMP and neutrophils early on in the development of cerulein-induced acute pancreatitis in rats

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ABSTRACT

The protein tyrosine phosphatases (PTPs) SHP-1, SHP-2 and PTP1B are overexpressed early on during the development of cerulein -induced acute pancreatitis (AP) in rats, and their levels can be modulated by some species of mitogen-activated protein kinases (MAPKs), the intracellular levels of cAMP and by general leukocyte infiltration, the latter at least for SHP-2 and PTP1B. In this study we show that cerulein treatment activates extracellular signal-regulated kinase (ERK) and c-Jun NH_2-terminal kinase (JNK) but not p38 MAPK during the early phase of cerulein-induced AP (2 h after the first injection of cerulein). Therefore, by using the MAPK inhibitors SP600125 (a specific JNK inhibitor) and PD98059 (a specific ERK inhibitor), we have unmasked the particular MAPK that underlies the modulation of the expression levels of these PTPs. JNK would act by preventing SHP-1 protein expression from increasing beyond a certain level. ERK 1/2 was the main MAPK involved in the increase in SHP-2 protein expression due to cerulein. JNK negatively modulated the SH2-domain containing PTPs. Both MAPKs played a role in the increase in PTP1B protein expression due to cerulein. Finally, by using the white blood cell inhibitors vinblastine sulfate, gadolinium chloride and FK506 (tacrolimus), we show that the macrophage activity or T-lymphocytes does not modulate the expression of any of the PTPs, although neutrophil infiltration was found to be a regulator of SHP-2 and PTP1B protein expression due to cerulein.

1. Introduction

During the course of acute pancreatitis (AP), different pathophysiological responses occur in intrapancreatic regions, including the activation of pancreatic enzymes and inflammatory mediators, the formation of free radicals and changes in blood flow. Some of these factors may also affect peripancreatic tissue or even distant organs. Nevertheless, the molecular mechanisms involved in the development of AP remain incompletely understood, especially the early acinar events, although these signaling mechanisms would determine the development of pancreatitis [1].

One of the animal models of the edematous form of AP is that induced by cerulein (Cer). Under the conditions used here the manifestations of pancreatitis include hyperamylasemia, increased pancreatic weight and pancreatic cell size, edema acinar cell damage, and inflammatory cell infiltration [2–5]. The intracellular mechanisms by which Cer regulate pancreatic acinar function are very complex. Cer modulates tyrosine phosphorylation [6,7], a central regulatory mechanism of many cell functions, including the maintenance of cell adhesion complexes [8], that are known to be dissociated in pancreatitis [9,10]. Cer-induced AP also activates the mitogen-activated protein kinase (MAPK) cascade, which requires the phosphorylation of both tyrosine and threonine residues [11,12]. The adenosine A1-receptor pathway [13], which decreases intracellular cAMP levels, also becomes activated in AP, and increases in intracellular cAMP are known to enhance secretagogue-sensitive zymogen activation. Recently, we have reported that the expression of three different protein tyrosine phosphatases (PTP)—the SH2-domain containing PTPs SHP-1 and SHP-2, as well as PTP1B, is increased at both the gene and protein levels early on in the development of Cer-induced AP [14,15]. It is known that in AP PTPs play roles in the regulation of exocytotic processes in exocrine pancreatic acinar cells [16] and in different inflammatory diseases. Additionally, they may become inactivated by reactive oxygen species or secondary products of oxidation [17] that may form during the development of AP [18], thus affecting protein tyrosine phosphorylation levels (although such a redox-mediated activity modulation has never been confirmed in AP). SHP-1 has been implicated in the Jak–Stat and MAPK pathways [19]. SHP-2 is involved in at least the Ras–Raf–MAPK, Jak–Stat, PI3 kinase and NF-κB pathways [20]. PTP1B is a key controller

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of several cytokine signaling pathways through its influence on members of the Janus kinases/signal transducers and activators of transcription (JAK/STAT) pathway [21]. All the above PTP signaling pathways have been implicated in the development of AP. We have also previously reported that the increases observed in the protein levels of the three PTPs during the early phase of Cer-induced AP may somehow be modulated by inhibition of MAPK pathways [14,15], although the specific MAPK implicated in such modulation is unknown. Moreover, leukocyte infiltration seems to affect at least SHP-2 and PTP1B expression in some way [14,15]. It has been recently confirmed that the infiltration of inflammatory cells into the pancreas is an early, central and trigger event in AP [22]. This infiltration causes local and systemic injury in the disease. Not only neutrophils, which are key players in acute inflammatory diseases, but also inflammatory monocytes and macrophages, and even lymphocytes, are involved in the development of acute experimental pancreatitis [23]. In this regard, the attenuation of the proinflammatory immune response by specifically targeting neutrophils, monocytes or lymphocytes improves the early course of several models of experimental pancreatitis [24–26].

In light of the above, here we unmask the particular MAPK that underlies modulation of the expression levels of SHP-1, SHP-2 and PTP1B in the early phase of Cer-induced AP development, by using the MAPK inhibitors SP600125 and PD98059. For comparison, the effect of the increase of cAMP (by inhibiting type IV phosphodiesterase) in the expression levels of the PTPs was also analyzed. Finally, we also studied the influence of different inflammatory cells in the changes in PTP expression by using vinblastine sulfate, which elicits a neutropenic state, gadolinium chloride ($GdCl_3$), which inactivates macrophage activity, and FK506 (tacrolimus) an immunomodulatory drug that preferentially decreases T-lymphocyte proliferation.

2. Materials and methods

2.1. Reagents

Bovine serum albumin (BSA), cerulein (Cer), dithiothreitol (DTT), FK506, gadolinium chloride ($GdCl_3$), phenylmethylsulfonyl fluoride (PMSF), PD98059, Protein Inhibitor Cocktail, rolipram, soybean trypsin inhibitor, SP600125, and vinblastine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) membranes were obtained from Amersham Biosciences, Spain. Monoclonal antibodies anti-PTP-1 (D-11), anti-p-ERK (E-4) and p38α/β (A-12), and polyclonal antibodies anti-ERK 1 (K-23) and JNK1 (FL) were obtained from Santa Cruz Biotechnology, Inc., CA, USA. Monoclonal antibody anti-PTP-2 was obtained from BD Biosciences Pharmingen, San Diego, CA, USA. Monoclonal antibody anti-PTP1B (ab52650) was obtained from Abcam, Cambridge, UK. Polyclonal antibodies anti-p-SAPK/JNK (Thr183/Tyr185) and anti-p-p38 (Thr180/Tyr182) were acquired from Cell Signalling Technology (Danvers, MA, USA). Monoclonal antibody anti-GAPDH was acquired from Ambion, Inc., Applied Biosystems, Madrid, Spain. Biotinylated anti-rabbit immunogammaglobulin and avidin–biotin–peroxidase complex were purchased from Vector (Burlingame, CA, USA). The Myeloperoxidase (MPO) Peroxidation Assay Kit was purchased from Cayman Chemical Company, MI, USA. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymatic assay kits were obtained from RAL, Barcelona, Spain.

2.2. Animals

Young male Wistar rats weighing 250–280 g were used. The animals were housed in rooms maintained at 22 ± 1 °C using a 12-h light/dark cycle and they were fasted for 24 h before experiments, with free access to water. Care was provided in accordance with the procedures outlined in European Community guidelines on ethical animal research (86/609/EEC), and protocols were approved by the Animal Care Committee of the University of Salamanca.

2.3. Induction of AP and preparation of samples

The early and intermediate phases of AP were induced as described previously [14,15]. Briefly, rats received 2 (early phase) or 4 (intermediate phase) s.c. injections of 20 μg Cer/kg body weight or its vehicle (0.9% NaCl) at hourly intervals. At 2 or 4 h after the first injection, the animals were anesthetized with sodium pentobarbital (100 mg/kg), blood samples were taken from the tail and by cardiac puncture and animals were then killed. The pancreata, which were used immediately for experiments, were rapidly dissected out from the surrounding fat tissue and lymph nodes, and their wet weights rapidly measured. A small portion of the tissue was used for histological studies [14]. The remaining parts of the pancreata were homogenized individually with a Potter Elvehjem device in 4 ml of homogenization buffer (3 mM imidazole buffer, pH 7.4 containing 0.25 M sucrose, 1 mM EDTA, 1 mM PMSF, 100 μg/ml trypsin inhibitor, 100 μM TPCK and TLCK, 2 μg/ml Protease Inhibitor Cocktail, 1 mM NaF and 1 mM Na_3VO_4). Homogenization buffer was added to reach a ratio of 10 volumes (w/v) and a postnuclear homogenate of each pancreas was obtained as described before [27]. Serum samples were stored at −80 °C until amylase determination with a Gernon Star Modular Analyzer, using a RAL assay kit, as reported previously [14]. Protein concentrations were assayed by the method of Bradford [28] using BSA as standard. Special care was taken with this assay in order to minimize deviations in the amount of proteins loaded in each lane of the SDS-PAGE gels. Quality control of the assays was ensured by repeating them at least three times with five different volumes of three-to-five different sample dilutions.

2.4. Light microscopy and immunohistochemistry

Paraffin-embedded tissue sections (8- to 10-μm thick) were stained with hematoxylin and eosin (H&E staining), as indicated previously [15], or subjected to immunostaining using the streptavidin peroxidase technique. For the latter, the sections were incubated for 30 min at room temperature in phosphate-buffered saline (0.15 M, pH 7.2) containing 1% normal horse serum and 0.3% Triton X-100 (buffer A) before overnight incubation at 4 °C with anti-PTP1B polyclonal antibody diluted 1:50. Bound antibody was detected with a biotinylated anti-rabbit immunoglobulin (Ig) G (1 h, 1:200 dilution in buffer A at room temperature) and avidin–biotin–peroxidase complex (1 h, 1:100 dilution in buffer A at room temperature). The tissue-bound peroxidase was developed with H_2O_2, using 3,3-diaminobenzidine as chromogen. To ensure the specificity of the primary antibody, we incubated sections in either the absence of the primary antibody or with a non-immunized rabbit IgG antibody. In these cases, no immunostaining was detected.

2.5. Inhibition of MAPKs by SP600125 and PD98059 or of type IV phosphodiesterase by rolipram in Cer-induced AP

Rats received intraperitoneal (i.p.) injections of SP600125 (15 mg/kg), PD98059 (5 mg/kg), rolipram (5 mg/kg) or their vehicles [1 ml/kg of a 10% DMSO/NaCl solution (SP600125 and rolipram) or 1% Pluronic F-68 in DMSO (PD98059)] both 2 h before and 30 min after (SP600125) [12], 1 h before (PD98059) [29], or 30 min before and 30 min after (rolipram) [30] the first Cer injection. At 2 h, 1 h or 30 min after the first injection of SP600125, PD98059 or rolipram, respectively, the rats were injected subcutaneously with Cer (20 μg Cer/kg) or its vehicle (0.9% NaCl) at hourly intervals. The animals were sacrificed 2 h after the first Cer injection (early phase of Cer-induced AP).

2.6. White blood cell depletion

Vinblastine sulfate was dissolved in sterile 10 mM sodium phosphate buffer, 147 mM NaCl, and 2.7 mM KCl (pH 7.4) and administered to rats intravenously (i.v.) at a dose of 0.75 mg/kg on day 1, as previously described [31]. At this dose, the animals become neutropenic between days 4 and 6 [31]. On day 5 after vinblastine sulfate or saline administration, the animals were treated with 4 doses of Cer (20 μg Cer/kg, administered at hourly intervals) to induce AP. FK506 and $GdCl_3$ were dissolved in 1 ml of the same buffer as the vinblastine sulfate. FK506 was administered to rats subcutaneously (0.3 mg/kg body weight) 48 h before the induction of the pancreatitis in 24 h intervals [26]. $GdCl_3$ (1 mg/100 g of body weight) was injected into the rat tail vein 12 h and 1 h before the start of Cer or saline administration [32].

2.7. Whole and differential white blood cell (WBC) count

Whole WBC and neutrophils were counted from cardiac puncture blood samples by flow cytometry using a Sysmex XT-2000i automated hematology analyzer. Differential WBC counts were done using peripheral blood smears made from drops of blood from the tail vein. The slides were stained using the May–Grünwald–Giemsa method. One hundred cells/blood sample were counted to obtain the leukocyte formula.

2.8. Myeloperoxidase (MPO) assay

MPO activity was detected in the pancreatic postnuclear homogenates immediately after their collection using the Myeloperoxidase Peroxidation Assay Kit and following the manufacturer's instructions.

2.9. Serum AST and ALT determination

Serum levels of AST and ALT were determined with enzymatic assay kits, using a Gernon Star Modular Bio-Chemical analyzer and following the manufacturer's instructions.

2.10. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

PTPs were analyzed by SDS-PAGE using 10% gels [33]. The proteins present in the gels were transferred to PVDF membranes. After blocking non-specific binding with 5% non-fat dry milk (dissolved in buffer 10 mM Tris–HCl, 100 mM NaCl, 0.1% Tween 20, pH 7.5), Western blots were probed with anti-SHP-1, anti-SHP-2 and anti-PTP1B monoclonal antibodies, or anti-GAPDH polyclonal antibodies, diluted 1:150, 1:2500, 1:1000 or 1:40,000, respectively, in the blocking solution. Blots were visualized by chemiluminescence. Analyses of the spots on all the blots were performed as described previously [34]. The densities corresponding to the different PTP bands in the different treatments were normalized to the GAPDH band densities (used as a loading control). The images shown in the figures are from blots whose films were exposed for optimal reproduction rather than for the linearity of band densities.

2.11. ERK, JNK and p38 phosphorylation

Activation of MAP kinases was assessed as previously described [35]. ERK, JNK and p38 kinase activation was evaluated with use of antibodies that recognize dually phosphorylated (Thr/Tyr) MAP kinase, diluted 1:200, 1:1000 and 1:1000, respectively. The blots were stripped and then normalized to total ERK, JNK or p38 kinases with the use of antibodies that recognize the MAP kinases regardless of its phosphorylation state.

2.12. Statistical analysis

Data are expressed as means ± SD. They were analyzed (version 19.0 of the SPSS program for MS Windows, SPSS, Chicago, Ill) using the non-parametric Mann–Whitney *U* test. Statistical significance was considered for a p value <0.05.

3. Results

We have previously divided the development of our animal model of AP into the early, intermediate and later (2, 4 and 9 h after the first injection of Cer) phases of AP [14,15]. In this work, serum amylase determination and histological assessment were used for monitoring the establishment of initial and intermediate phases of clinically relevant relatively mild edematous pancreatitis. Similarly to our previously reported data [15,36], as early as 2 h after the first injection of Cer pancreatic acinar cell disruption and some lymphocyte infiltration were observed. A higher degree of leukocyte infiltration, higher numbers of unstructured acini and some neutrophils were detected during the intermediate phase of Cer-induced AP (Supporting Information 1).

3.1. Effect of MAPK inhibitors and rolipram on PTP expression and ERK 1/2 and JNK activation

Figs. 1 and 2 show the expression levels of the very similar SH2-domain containing PTPs SHP-1 and SHP-2, and PTP1B, respectively. They also illustrate the effect on such levels of two different MAPK inhibitors—SP600125 and PD98059 (Fig. 1)—and rolipram, an inhibitor of type IV phosphodiesterase (Fig. 2), during the early phase of Cer-induced AP. In all groups of rats, PTP expression levels (Western blots in whole postnuclear pancreatic homogenates) were quantified (graphics) in two different ways: (1) for an equal amount of proteins (open bars), (2) with respect to the total amount of proteins in the whole pancreas (shaded bars). It is well known that pancreatitis is associated with the neutrophil infiltration and cell death that differentiate the cell composition of the pancreata of the groups of rats. Accordingly, we considered that a better interpretation of the results would require the expression of the data with respect to the total amount of proteins in the pancreas. We have previously reported an increase in the protein levels of the three PTPs during the early phase of Cer-induced AP that may in some way be modulated by MAPK inhibition and the intracellular cAMP pathway [14,15], although the specific MAPK implicated in such modulation is unknown. It is known that such signaling pathways play essential roles in the pathogenesis of AP, in part due to an amelioration of the severity of Cer-induced AP after inhibition of JNK and ERK 1/2 kinases or after increasing cAMP levels in neutrophils [12,30]. In Fig. 1 it can be observed that rather than reducing the increase in the expression of SH2-domain containing PTPs, SP600125 pretreatment significantly increased the expression of both SHP-1 and SHP-2 after Cer administration. With respect to PTP1B, SP600125 pretreatment mostly reduced the increase in its expression after Cer treatment. In Fig. 1 it is also shown that, by contrast, PD98059 pretreatment did not affect the increase in expression of SHP-1 and was able to reduce significantly the increase in SHP-2 expression observed after Cer administration. As in the case of SP600125, PD98059 pretreatment mostly reduced the increase in PTP1B expression after the Cer treatment. In Fig. 2 it can be observed that in comparison with the non-pretreated animals SHP-1 protein expression due to Cer was not modified and SHP-2 protein expression due to Cer was significantly suppressed in the pancreata obtained from Cer-injected rats pretreated with rolipram. With respect to PTP1B, rolipram pretreatment mostly reduced the increase in the PTP protein expression due to Cer.

Once it had been confirmed that the increases in the expression levels of the PTPs due to Cer during the early phase of AP were affected by the signaling pathways studied, we analyzed the activation of ERK 1/2 and JNK in the different groups of rats. Fig. 3 shows that

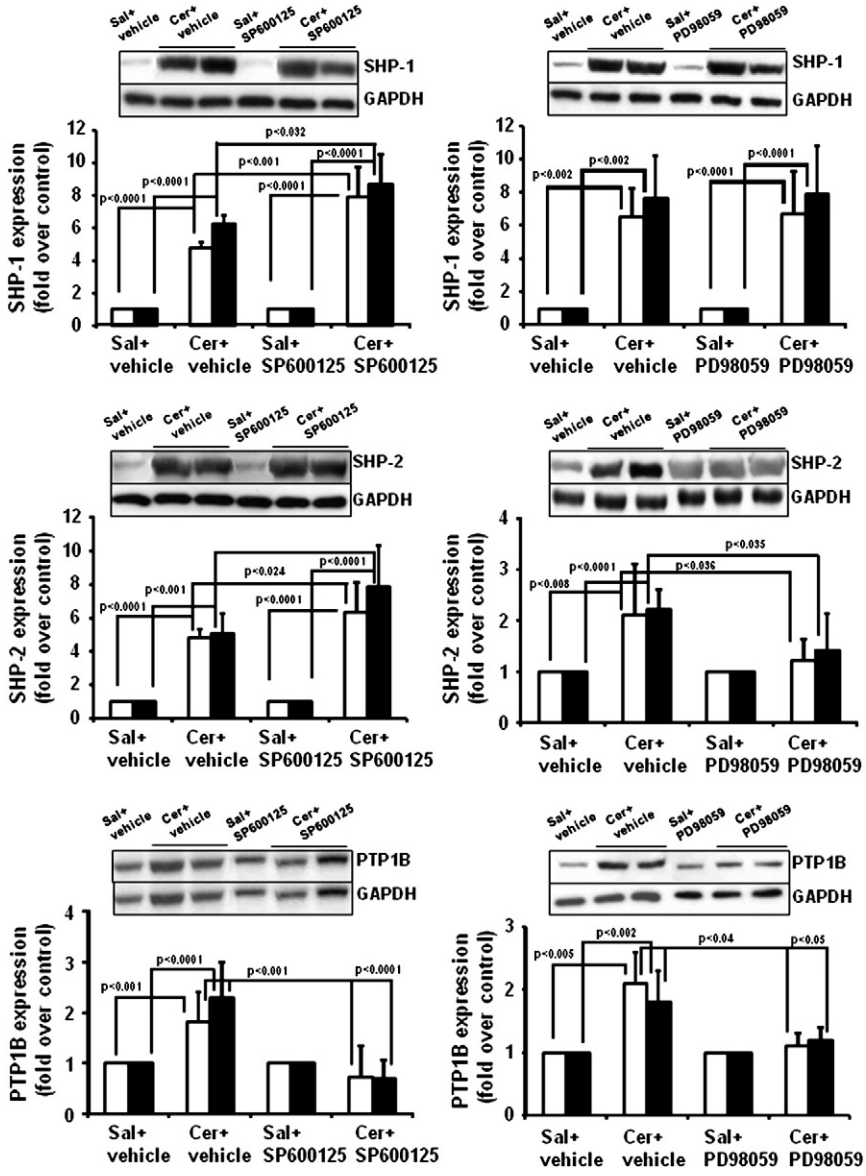

Fig. 1. Effect of MAPK inhibitor pretreatments on SHP-1, SHP-2 and PTP1B protein expression in the early phase of Cer-induced AP. Rats were pretreated with SP600125, PD98059 or its vehicle as indicated in the "Materials and methods" section. Then, the animals were injected subcutaneously with either Cer or its vehicle [20 mg/kg or saline (0.9% NaCl, Sal) in two injections at hourly intervals, respectively]. Quantification (graphics, values given as SHP1/GAPDH, SHP-2/GAPDH and PTP1B/GAPDH ratios, and considering both controls: Sal + vehicle and Sal + inhibitor as 1) of expression (Western blots in whole postnuclear pancreatic homogenates — a representative is shown — where the 2 animals pretreated with the inhibitors are representative of the higher and lower levels of the standard deviation of the experiment) was carried out both per mg of proteins (□) and considering the whole pancreas (■). Each lane in the blots contained 25 μg of proteins. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

Cer administration led to the increase in the activation of both ERK 1/2 and JNK. Although SP600125 and PD98059 are respectively specific JNK and ERK 1/2 inhibitors, their use in animals may lead to mixed inhibitions of both MAPKs [12]. Accordingly, it was necessary to confirm whether under the conditions used in this study they led to a selective inhibition of one of the MAPKs. As shown in Fig. 3, in vivo both inhibitors maintained their selective inhibition. Thus, SP600125 and PD98059 pretreatments led to specific reductions of JNK and ERK1/2 activation, respectively. Although to our knowledge it has never been reported that SP600125 or PD98059 might inhibit p38 MAPK, we also analyzed the activation of this latter kinase in the different groups of rats. As shown in Supporting Information 2, during the early phase of Cer-induced AP the phosphorylation of p38 was not significantly affected, and neither was it affected after SP600125 or PD98059 pretreatments. The lack of response of p38 to Cer is consistent with previously reported data in a similar rat model of AP [29]. Since the phosphorylation of ERK, JNK and p38 determines the activity of MAPK signaling, these data support the notion that, as expected, p38 does not play a role in the modulation of PTP expression after ERK or JNK inhibition. Therefore, such modulation must be carried by one or both of the two latter MAPKs. With respect to rolipram pretreatment, the activation of the MAPKs did not change with respect to the increase detected after Cer treatment.

3.2. Effect of white blood cell inhibitors on blood cell counts

The development of inflammation and cell death in pancreatitis is somehow influenced by neutrophil infiltration. Additionally, the pancreas probably contains an unknown proportion of resident macrophages, whose activity, as well as that of liver Kupffer cells should be blocked by $GdCl_3$ [23]. Nevertheless, the effects of $GdCl_3$ on resident macrophages in the pancreas remain unknown. It is also known that lymphocytes are involved in AP since the calcineurin antagonist FK506 reduces the severity of the disease [23]. In some cases, rats were rendered neutropenic with vinblastine sulfate before treatment with

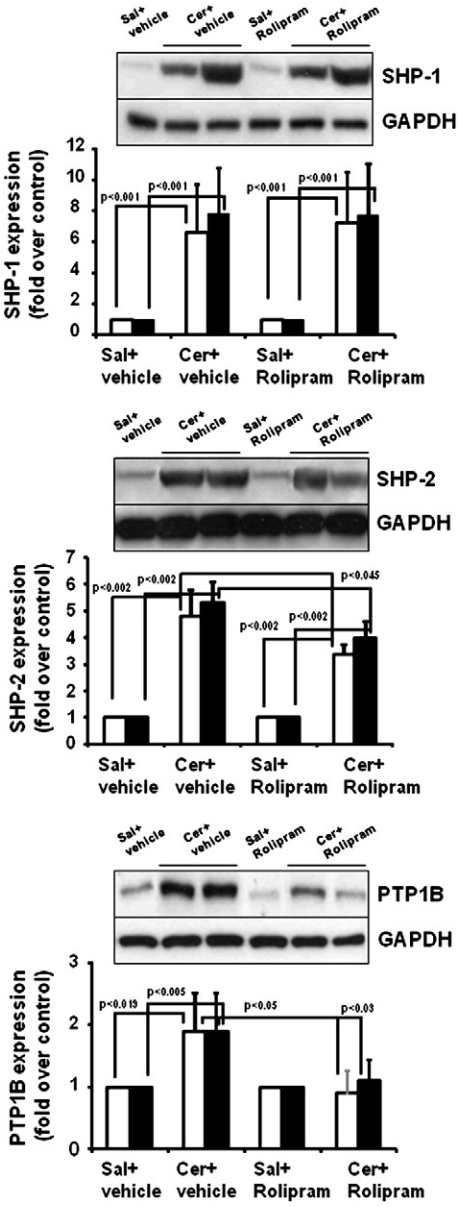

Fig. 2. Effect of PDE4 inhibitor pretreatment on SHP-1, SHP-2 and PTP1B protein expression in the early phase of Cer-induced AP. Rats were pretreated with rolipram or its vehicle as indicated in the "Materials and methods" section. Then, the animals were injected subcutaneously with either Cer or its vehicle [20 mg/kg or saline (0.9% NaCl, Sal) in two injections at hourly intervals, respectively]. Quantification of expression was carried out as indicated in the legend of Fig. 1. Each lane in the blots contained 25 μg of proteins. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

$GdCl_3$ or FK506. For this purpose, we chose conditions previously described by us and others [14,15,31]. A count of the cells in the blood of the rats used in our experiments is shown in Supporting Information 3. As expected, a differential leukocyte count in the blood revealed a significant decrease in the percentage of neutrophils only in the groups pretreated with vinblastine sulfate (Supporting Information 4).

3.3. Effect of MAPK inhibitors, rolipram and white blood cell inhibitors on serum amylase, edema and pancreatic MPO levels

Figs. 4 and 5 illustrate the levels of serum amylase in all groups. PD98059 was not able to reduce, SP600125 showed a tendency (although not significant) to reduce, and rolipram significantly reduced the serum amylase activity observed in the animals given Cer alone (2 h after the first injection of Cer). Regarding white blood cell inhibitors, we decided to study the intermediate phase of AP (4 h) because it has been reported that inflammatory cell infiltration has already taken place at this time [37]. In this case, only the group of rats pretreated with vinblastine sulfate and then treated with $GdCl_3$ showed a significant reduction in serum amylase activity with respect to the animals given Cer alone. Therefore, the lack of effect of $GdCl_3$ itself on serum amylase activity differs from previously reported data [32].

Supporting Information 5 shows that Cer treatments also resulted in significant organ edema, as evidenced by the increased pancreatic wet weight. Regarding the MAPK inhibitors, pretreatments with SP600125 and PD98059 were associated with an 80 and 57% reduction in the Cer-induced edema, respectively, which supports the notion of an early protective effect of MAPK inhibition on intrapancreatic damage. By contrast, rolipram pretreatment did not reduce the extent of the edema during the early phase of Cer-induced AP, although such a protective effect has been reported in fully developed Cer-induced AP [30]. This is probably due to the fact that neutrophil infiltration is still low during this early phase (2 h after the first Cer injection).

Leukocyte accumulation in the pancreas was investigated by measuring MPO activity (4 h after the first injection of Cer). In Fig. 5 it may be seen that while Cer treatment caused leukocyte accumulation in the pancreas, the groups of rats that were pretreated with vinblastine sulfate had blunted pancreatic MPO levels. Unlike previously reported data [32], $GdCl_3$ also significantly decreased pancreatic MPO activity, although the decrease was much more pronounced in the group of rats pretreated with vinblastine sulfate.

3.4. Effect of white blood cell inhibitors on hepatic functions

No significant changes were found on the hepatic functions of $GdCl_3$- or FK506-treated animals measured as serum values of ALT and AST (Supporting Information 6).

3.5. Effect of white blood cell inhibitors on PTP expression

We have previously reported that the early increase in the expression of both SHP-1 and PTP1B (2 h after the first injection of Cer) persists during the intermediate phase of AP (4 h after the first injection of Cer), although to a lower extent. By contrast, SHP-2 expression falls to levels similar to those of the controls from 4 h onwards [14,15]. Fig. 6 shows that $GdCl_3$ alone did not significantly affect the changes in protein expression of any of the PTPs after Cer treatment. Nevertheless, the depletion of neutrophils in the rats (rats pretreated with vinblastine sulfate) specifically increased SHP-2 protein expression during the intermediate phase of AP to a significant extent. Also, FK506 alone did not significantly affect the changes in protein expression of any of the PTPs after Cer treatment. In this case, pretreatment with vinblastine sulfate was specifically associated with the decrease in PTP1B expression to levels similar to those of the controls. Since this might indicate that the increase in PTP1B expression due to Cer could occur mainly in leukocytes associated with the inflammatory infiltrate of the pancreas rather than with the acinar cells, immunohistochemical analyses were performed. Supporting Information 7 shows the increase in PTP1B immunoreactivity during the early and intermediate phases of Cer-induced AP; this can be mainly ascribed to the acinar cells and not to the infiltration. The data support a kind of crosstalk between infiltration and acinar cells that needs to be addressed in the future.

4. Discussion

It seems clear that one of the important factors in the development of AP must be the differential change in gene and/or protein expressions in the pancreas during the initial phase of the disease [1]. Such changes might transfer the early activated signaling pathways in pancreatic acinar cells towards final responses even in extrapancreatic tissues. The

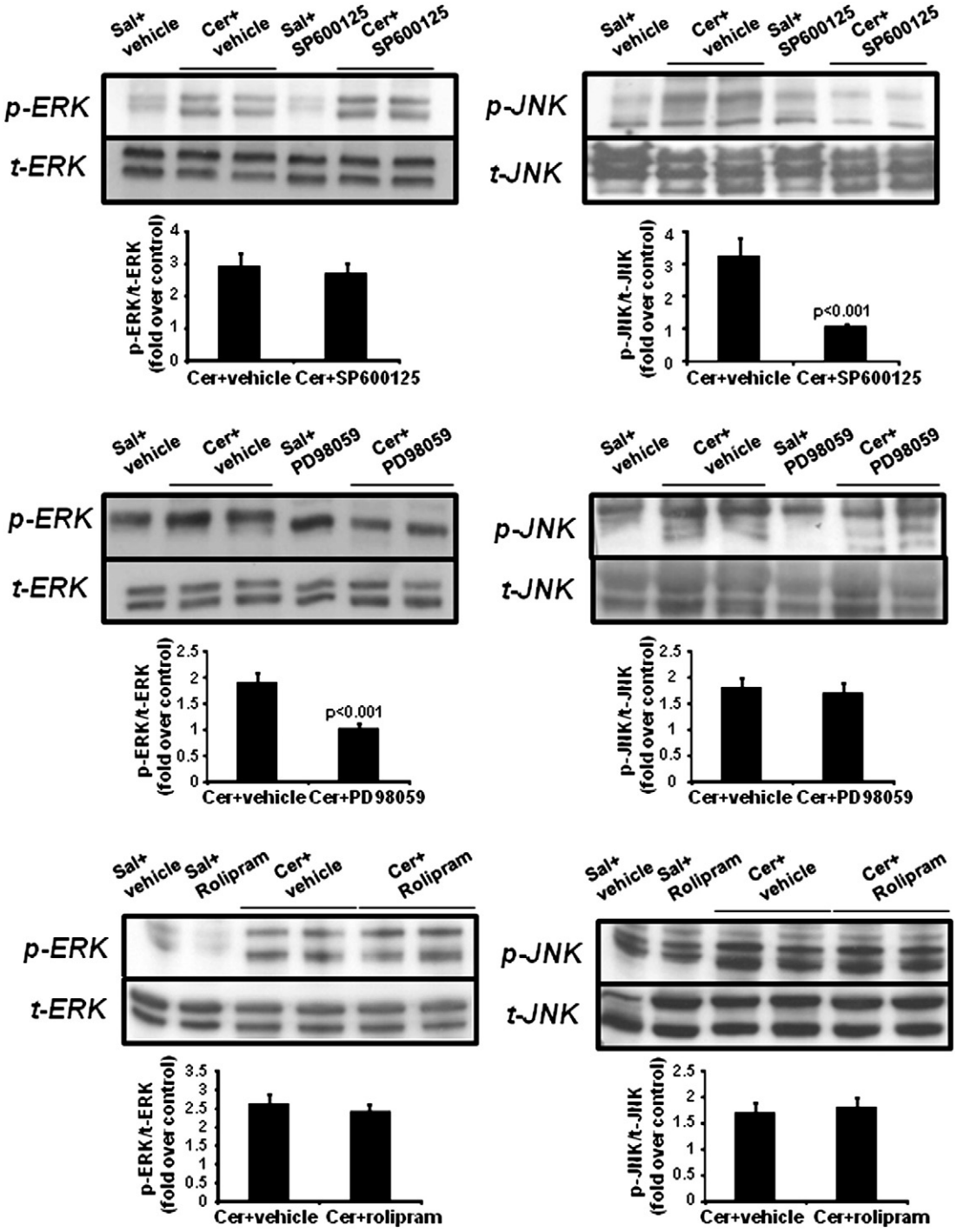

Fig. 3. MAPK activation state in control (Sal) or pancreatitic (Cer, early phase of Cer-induced AP) rats pretreated or non-pretreated with MAPKs or PDE4 inhibitors. Activation of signaling cascades was analyzed by immunoblotting with antibodies that recognized the phosphorylated/active forms of ERK and JNK. The same membranes were stripped and reprobed to check the total levels of these proteins. The levels of the phosphorylated/active forms were quantified and normalized with respect to the total levels of these proteins (graphics). Each lane in the blots contained 20 μg of proteins. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

activation of the stress kinases JNK and ERK1/2 plays an important mediating role during the early phase (first 1–1.5 h) of AP [12,31,38], and might regulate transcription factors (TFs) induced early on during the onset of AP [1], as well as protein translation [39]. Regarding this, we have previously reported an increase in SHP-1, SHP-2 and PTP1B in the pancreas at the level of both protein and mRNA as an early event during the development of Cer-induced AP [14,15]. The fact that out of this three PTPs SHP-2 also increased its protein expression in in vivo models of AP brought about by sodium-taurocholate duct infusion and in bile-pancreatic duct obstruction highlights the probable general importance of this PTP in AP [14,15]. We have also reported that the ERK 1/2 and/or JNK kinases, cAMP levels, and general infiltration of the pancreas modulate the protein expression of all or some of the PTPs in the early phase of Cer-induced AP [14,15]. Nevertheless, the specific MAPK involved in each case, as well as the influence of different types of leukocytes is unknown.

The study of the MAPK involved in PTP protein modulation was accomplished using different inhibitors: SP600125, a JNK inhibitor, and PD98059, an ERK 1/2 inhibitor. It should be noted that although these molecules are specific inhibitors of one type of MAPK, their use in

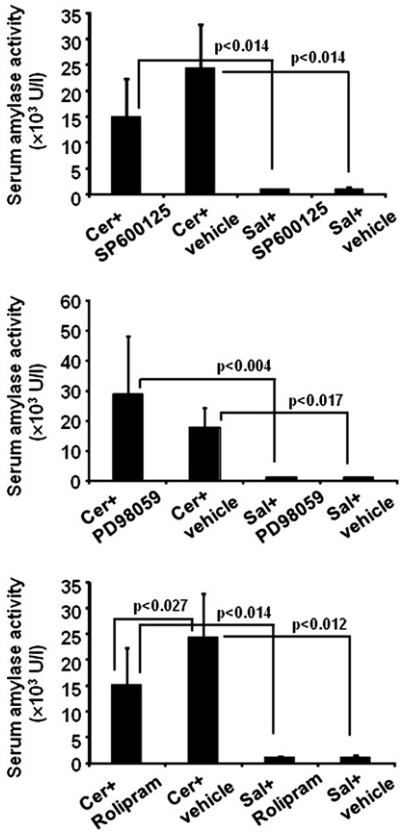

Fig. 4. Effect of MAPKs and PDE4 inhibitor pretreatments on serum amylase activity in the early phase of Cer-induced AP. Rats were pretreated with SP600125, PD98059 or rolipram as indicated in the "Materials and methods" section. Then, the animals were injected subcutaneously with either Cer or its vehicle [20 mg/kg or saline (0.9% NaCl, Sal) in two injections at hourly intervals, respectively]. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

animals might elicit mixed inhibitions of both MAPKs [12]. Accordingly, analysis of the activation state of the kinases after treatment with the inhibitors was necessary to clearly ascribe the modulation of a particular PTP to a specific MAPK. Such an analysis was also performed for rolipram, a specific strong inhibitor of type IV phosphodiesterase. Under the conditions used in this work, SP600125 and PD98059 respectively almost completely inhibited the activation of Cer-induced pancreatic JNK and ERK 1/2, the only two MAPKs that became activated after Cer treatment. Rolipram pretreatment did not affect the MAPK activation state. These results led us to conclude that in the early phase of Cer-induced AP: (i) JNK would be acting by preventing SHP-1 protein expression from increasing beyond a certain level, because the further increase in SHP-1 expression with respect to that observed after Cer treatment occurred only after SP600125, but not after PD98059 pretreatment. Moreover, ERK 1/2 does not seem to be involved in the increase in SHP-1 protein expression due to Cer. (ii) ERK 1/2 was the main MAPK involved in the increase in SHP-2 protein expression due to Cer, because such an effect was blunted only after PD98059 pretreatment. For this PTP, JNK seems to play a role similar to the one describe for SHP-1. In light of the above, JNK seems to negatively modulate the SH2-domain containing PTPs. (iii) Both MAPKs seem to play a coordinated role in the increase in PTP1B protein expression due to Cer, because such an effect was equally blunted after SP600125 or PD98059 pretreatments. These roles of JNK are interesting also in relation to our recent observation that STRAP, an inhibitor of transforming growth factor β (TGF-β), that acts as a negative regulator of the apoptosis signal-regulating kinase 1 (ASK-1)-mediated JNK signaling cascade, is overexpressed during the early phase of Cer-induced AP [35]. (iv) The experiments with rolipram were done for comparison and as an internal quality control of our results. The results concerning SHP-1 and PTP1B coincided with those previously reported by us [14,15]. The SHP-2 results were partly different because, here, the suppression of the increase in SHP-2 protein expression due to Cer in rolipram-pretreated rats, although significant, was much lower than in our previous experiments [14]. It is known that MAPKs [12] and the increases in intracellular

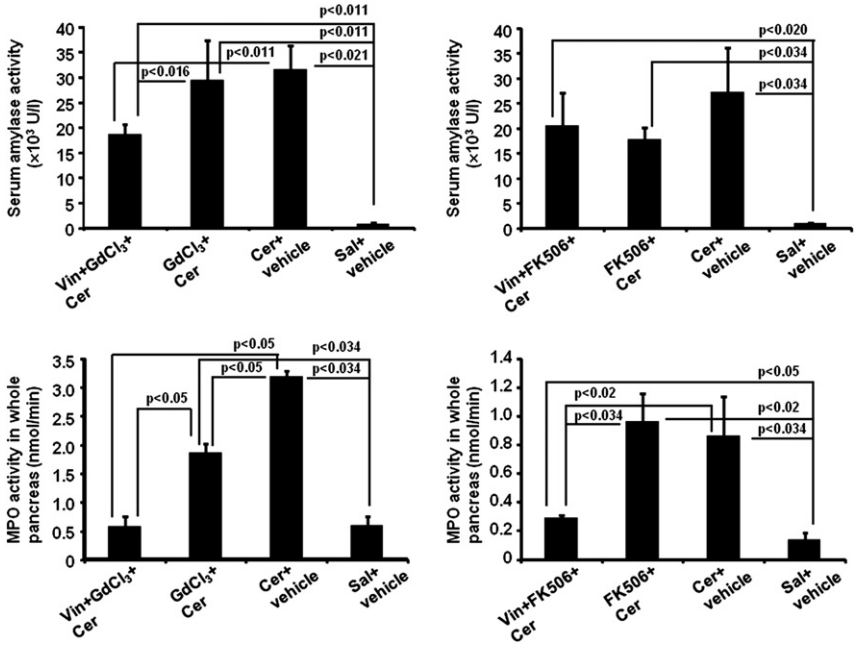

Fig. 5. Effect of white blood cell inhibitor pretreatments on serum amylase and pancreatic MPO levels in the intermediate phase of Cer-induced AP. Rats were pretreated with $GdCl_3$, FK506 or their vehicles as indicated in the "Materials and methods" section. Then, the animals were injected subcutaneously with either Cer or its vehicle [20 mg/kg or saline (0.9% NaCl, Sal) in four injections at hourly intervals, respectively]. One group of rats (Vin) was given 0.75 mg/kg i.v. vinblastine sulfate to induce neutropenia before pretreatments with $GdCl_3$ or FK506 as indicated in the "Materials and methods" section. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

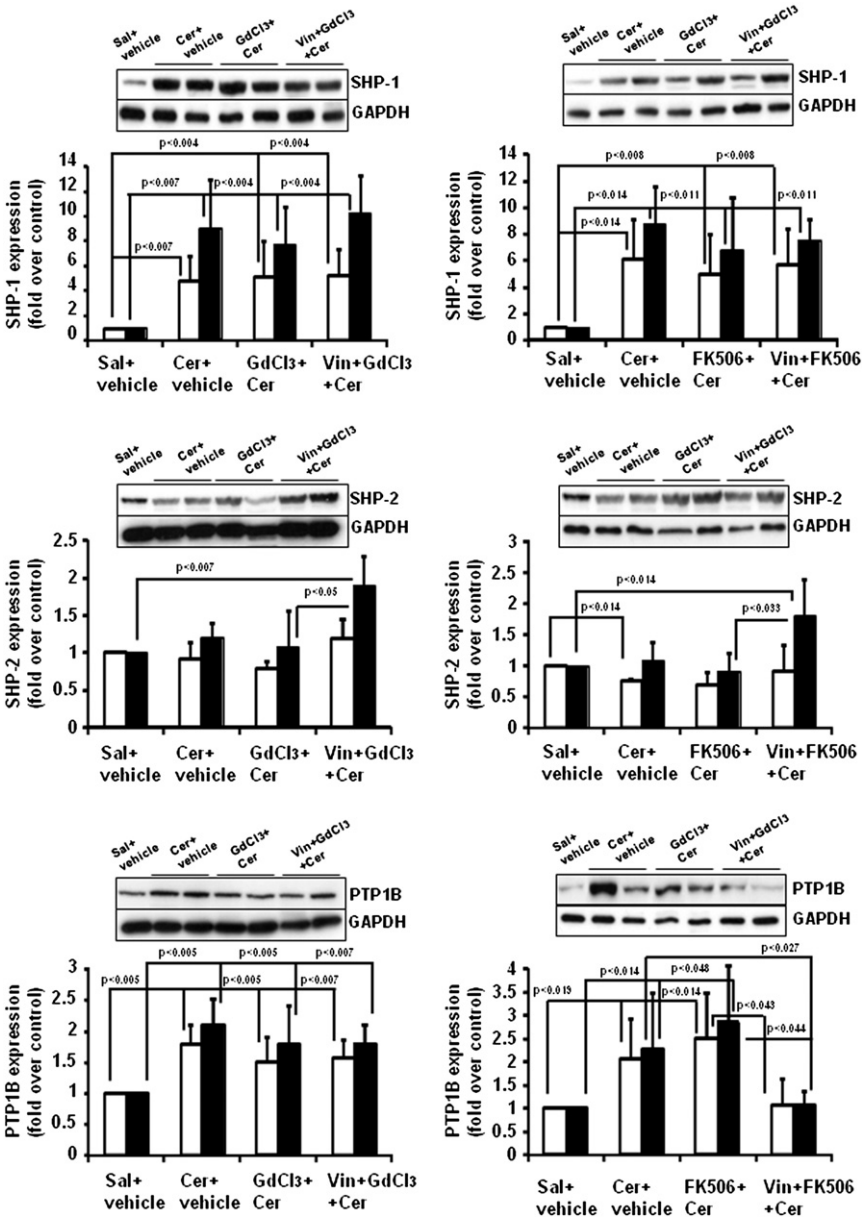

Fig. 6. Effect of white blood cell inhibitor pretreatments on SHP-1, SHP-2 and PTP1B protein expression in the intermediate phase of Cer-induced AP. Rats were pretreated with vinblastine sulfate, $GdCl_3$, FK506 or their vehicles as indicated in the legend of Fig. 5. Quantification (graphics, values given as SHP1/GAPDH, SHP-2/GAPDH and PTP1B/GAPDH ratios) of expression (Western blots in whole postnuclear pancreatic homogenates — a representative is shown) was carried out both per mg of proteins (□) and considering the whole pancreas (■). Each lane in the blots contained 25 μg of proteins. Data are means ± S.D. of 3 experiments with 3 rats per group in each experiment.

cAMP levels [40] either up-regulate or reduce the expression of inflammatory cytokines, such as TNF-α, that prime cell infiltration in the pancreas, respectively. Also, the identification of a pool of a soluble adenylyl cyclase associated with isolated nuclei in unstimulated pancreatic acinar cells, raises the possibility of the implication of this cyclase, and of a compartmentalized cAMP signal, in transcriptional responses [41].

It is known that neutrophil depletion due to an antineutrophil serum or vinblastine sulfate administration reduces the severity of AP because neutrophil infiltration into tissues plays an important role in its development. However, the protection remains partial, perhaps reflecting the presence of local pancreatic macrophages [32]. $GdCl_3$, a compound that is not toxic at the dose use in this work [23,42], has been employed to inactivate Kupffer cells [32], the resident macrophages in the liver, although it is also possible that gadolinium might have effects on resident macrophages in the pancreas [23]. Kupffer cells are regarded as the predominant source of inflammatory cytokines producing systemic injury in AP [43]. Also, activated lymphocytes, especially T lymphocytes, probably play a role in the development of AP [23], although a less important one than that of neutrophils. Thus, in Cer-induced AP in mice it has been reported that the $CD4^+$ T helper subset is a mediator of local tissue injury [44]. FK506, a drug without significant adverse effects on the exocrine pancreas, exerts its action on T-helper lymphocytes after binding to intracellular immunophilins, thus inhibiting calcineurin phosphatase [45]. This phosphatase is involved in the activation of the transcription nuclear factor of activated T cells that modulates the expression of cytokine genes. Nevertheless, the influence of FK506 in AP is not well understood [46,47] because both attenuation and no changes in intrapancreatic damage have been reported, depending on the animal model used for the induction of pancreatitis [47,48].

Our results indicate that $GdCl_3$ by itself did not modulate the expression of any of the PTP due to Cer. The positive modulation of SHP-2 protein expression observed in the whole pancreas of rats

pretreated with both vinblastine sulfate and $GdCl_3$ should be ascribed to the neutrophil depletion due to vinblastine administration, because we have previously demonstrated that such an effect is already produced in rats pretreated only with vinblastine [14]. Accordingly, no effect of cytokines produced by the resident macrophages of the liver, and probably also by the resident macrophages of the pancreas, seems to be involved in the increase in PTP protein expression due to Cer. With respect to FK506, similar conclusions to those observed for $GdCl_3$ can be drawn for the SH2-domain containing phosphatases: (i) FK506 alone did not modulate their expression due to Cer, and (ii) the changes observed in SHP-2 protein expression in the Vin + FK506 + Cer group of rats should be ascribed to the neutrophil depletion due to vinblastine [14]. Regarding PTP1B, the increase in the expression of PTP1B protein due to Cer was blunted in the Vin + FK506 + Cer group of rats. This effect should also be ascribed to the neutropenic state, because it was not observed in the FK506 + Cer group (this work) whereas it was in our previous work using rats pretreated only with vinblastine [15]. These data indicate a role of neutrophil infiltration as a regulator of PTP1B protein expression and are in good agreement with those for rolipram, because type 4 phosphodiesterase is abundantly expressed in neutrophils. Accordingly, rolipram will target at least neutrophils, probably exerting anti-inflammatory effects through the inhibition of diverse leukocyte functions such as the production/secretion of proinflammatory cytokines, as indicated above. The data are also in accordance with previous work reporting that rolipram suppressed neutrophil infiltration in the pancreas due to Cer [13].

Concerning serum amylase activity, the lack of an effect of MAPK inhibitors and rolipram on its increase due to Cer is coincident with previously reported data [14,15]. By contrast, in fully developed Cer-induced AP it has been reported that SP600125 significantly reduces the increase in serum amylase activity due to Cer [12]. Taken together, the data support the notion that the reduction in amylase activity after MAPK inhibition would not occur during the early phase of AP but later in its development, as indicated elsewhere [14,15]. $GdCl_3$ and FK506 alone did not decrease serum amylase activity. The lack of effect of FK506 regarding the decrease in serum amylase activity due to Cer has been also reported in a rat model of fully developed Cer-induced AP [26]. By contrast, in a mice model of fully developed Cer-induced AP the prevention of Kupffer cell activation by $GdCl_3$ significantly reduced the increase in serum amylase activity due to Cer [32]. Accordingly, a reduction in such activity would probably also occur later in the development of AP. Neutrophil infiltration (assessed by MPO activity) was already observed during the intermediate phase of Cer-induced AP, as previously reported [14,15]. Since $GdCl_3$ is specifically taken up by Kupffer cells [32], the fact that $GdCl_3$ alone reduced MPO activity probably reflects the presence of local pancreatic macrophages. MPO activity was completely reduced to the control level when $GdCl_3$ treatment was associated with a neutropenic state. The fact that FK506 treatment also associated to a neutropenic state reduced MPO activity almost completely, although not totally, further supports the notion that the residual MPO activity might reflect the existence of macrophages in the pancreas.

In sum, here we unmask the particular MAPK that underlies the modulation of the expression levels of SHP-1, SHP-2 and PTP1B in the early phase of Cer-induced AP development. JNK did not allow SHP-1 protein expression to increase beyond a certain level. ERK 1/2 was the main MAPK involved in the increase in SHP-2 protein expression due to Cer, but was not involved in the increase in SHP-1 protein expression. JNK negatively modulated the SH2-domain containing PTPs. Both MAPKs played a coordinated role in the increase in PTP1B protein expression due to Cer. Finally, neither macrophage activity nor T-lymphocytes modulated the expression of any of the PTPs, although neutrophil infiltration was a regulator of SHP-2 and PTP1B protein expression due to Cer.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2013.11.003.

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References

[1] B. Ji, X. Chen, D.E. Misek, R. Kuick, S. Hanash, S. Ernst, R. Najarian, C.D. Logsdon, Pancreatic gene expression during the initiation of acute pancreatitis: identification of EGR-1 as a key regulator, Physiol. Genomics 14 (2003) 59–72.
[2] R. Alonso, A. Montero, M. Arévalo, L.J. García, C. Sánchez-Vicente, F. Rodríguez-Nodal, J.M. López-Novoa, J.J. Calvo, Platelet-activating factor mediates pancreatic function derangement in caerulein-induced pancreatitis in rats, Clin. Sci. 87 (1994) 85–90.
[3] R. Pescador, M.A. Manso, A.J. Revollo, I. De Dios, Effect of chronic administration of hydrocortisone on the induction and evolution of acute pancreatitis induced by cerulein, Pancreas 11 (1995) 165–172.
[4] N. Yönetçi, N. Oruç, A.O. Ozütemiz, H.A. Celik, G. Yüce, Effects of mast-cell stabilization in caerulein-induced acute pancreatitis in rats, Int. J. Pancreatol. 29 (2001) 163–171.
[5] M. Zhao, D.B. Xue, B. Zheng, W.H. Zhang, S.H. Pan, B. Sun, Induction of apoptosis by artemisin relieving the severity of inflammation in caerulein-induced acute pancreatitis, World J. Gastroenterol. 14 (2007) 5612–5617.
[6] N. Rivard, G. Rydzewska, J.-S. Lods, J. Martinez, J. Morisset, Pancreas growth, tyrosine kinase, PtdIns 3-kinase, and PLD involve high-affinity CCK-receptor occupation, Am. J. Physiol. 266 (1994) G62–G70.
[7] N. Rivard, G. Rydzewska, J.S. Lods, J. Morisset, Novel model of integration of signalling pathways in rat pancreatic acinar cells, Am. J. Physiol. 269 (1995) G352–G362.
[8] T. Muller, A. Choidas, E. Reichmann, A. Ullrich, Phosphorylation and free pool of beta-catenin are regulated by tyrosine kinases and tyrosine phosphatases during epithelial cell migration, J. Biol. Chem. 274 (1999) 10173–10183.
[9] J. Schnekenburger, J. Mayerle, B. Krüger, I. Buchwalow, F.U. Weiss, E. Albrecht, V.E. Samoilova, W. Domschke, M.M. Lerch, Protein tyrosine phosphatase κ and SHP-1 are involved in the regulation of cell–cell contacts at adherent junctions in the exocrine pancreas, Gut 54 (2005) 1445–1455.
[10] M.M. Lerch, M.P. Lutz, H. Weidenbach, F. Muller-Pillasch, T.M. Gress, J. Leser, G. Adler, Dissociation and reassembly of adherent junctions during experimental acute pancreatitis, Gastroenterology 113 (1997) 1355–1366.
[11] C. Widmann, S. Gibson, M.B. Jarpe, G.L. Johnson, Mitogen activated protein kinases: conservation of a three kinase module from yeast to human, Physiol. Rev. 79 (1999) 143–180.
[12] L. Minutoli, D. Altavilla, H. Marini, M. Passaniti, A. Bitto, P. Seminara, F.S. Venuti, C. Famulari, A. Macri, A. Versaci, F. Squadrito, Protective effects of SP600125 a new inhibitor of c-jun N-terminal kinase (JNK) and extracellular-regulated kinase (ERK1/2) in an experimental model of cerulein-induced pancreatitis, Life Sci. 75 (2004) 2853–2866.
[13] A. Satoh, T. Shimosegawa, K. Satoh, H. Ito, Y. Kohno, A. Masamune, M. Fujita, T. Toyota, Activation of A1-receptor pathway induces edema formation in the pancreas of rats, Gastroenterology 119 (2000) 829–836.
[14] N. Sarmiento, M.C. Sánchez-Bernal, M. Ayra, N. Pérez, A. Hernández-Hernández, J.J. Calvo, J. Sánchez-Yagüe, Changes in the expression and dynamics of SHP-1 and SHP-2 during caerulein-induced acute pancreatitis in rats, Biochim. Biophys. Acta 1782 (2008) 271–279.
[15] N. Sarmiento, M.C. Sánchez-Bernal, M.N. Pérez-González, J.L. Sardina, A. Mangas, J.J. Calvo, J. Sánchez-Yagüe, Rolipram and SP600125 suppress the early increase in PTP1B expression during cerulein induced pancreatitis in rats, Pancreas 39 (2010) 639–645.
[16] P. Feick, S. Gilhaus, I.R. Blum, F. Hofmann, I. Just, I. Schulz, Inhibition of amylase secretion from differentiated AR4-2J pancreatic acinar cells by an actin cytoskeleton controlled protein tyrosine phosphatase activity, FEBS Lett. 451 (1999) 269–274.
[17] A. Hernández-Hernández, M.N. Garabatos, M.C. Rodríguez, M.L. Vidal, A. López-Revuelta, J.I. Sánchez-Gallego, M. Llanillo, J. Sánchez-Yagüe, Structural characteristics of a lipid peroxidation product, trans-2-nonenal, that favour inhibition of membrane associated phosphotyrosine phosphatase activity, Biochim. Biophys. Acta 1726 (2005) 317–325.
[18] C. Sánchez-Bernal, O.H. García-Morales, C. Domínguez, P. Martín-Gallán, J.J. Calvo, L. Ferreira, N. Pérez-González, Nitric oxide protects against pancreatic subcellular damage in acute pancreatitis, Pancreas 28 (2004) e9–e15.
[19] M. You, Z. Zhao, Positive effects of SH2 domain-containing tyrosine phosphatase SHP-1 on epidermal growth factor- and interferon-γ-stimulated activation of STAT transcription factors in HeLa cells, J. Biol. Chem. 272 (1997) 23376–23381.
[20] C.-K. Qu, Role of the SHP-2 tyrosine phosphatase in cytokine-induced signalling cellular response, Biochim. Biophys. Acta 1592 (2002) 297–301.

[21] A. Bourdeau, N. Dubé, M.L. Tremblay, Cytoplasmic protein tyrosine phosphatases, regulation and function: the roles of PTP1B and TC-PTP, Curr. Opin. Cell Biol. 17 (2005) 203–209.
[22] M. Sendler, A. Dummer, F.U. Weiss, B. Krüger, T. Wartmann, K. Scharffetter-Kochanek, N. van Rooijen, S.R. Malla, A. Aghdassi, W. Halangk, M.M. Lerch, J. Mayerle, Tumour necrosis factor α secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice, Gut 62 (2013) 430–439.
[23] A. Vonlaufen, M.V. Apte, B.A. Imhof, J.L. Frossard, The role of inflammatory and parenchymal cells in acute pancreatitis, J. Pathol. 213 (2007) 239–248.
[24] J.L. Frossard, A. Saluja, L. Bhagat, H.S. Lee, M. Bhatia, B. Hofbauer, M.L. Steer, The role of intercellular adhesion molecule I and neutrophils in acute pancreatitis and pancreatitis-associated lung injury, Gastroenterology 116 (1999) 694–701.
[25] Y. Mikami, K. Takeda, K. Shibuya, H. Qiu-Feng, H. Shimamura, J. Yamauchi, S. Egawa, M. Sunamura, H. Yagi, Y. Endo, S. Matsuno, Do peritoneal macrophages play an essential role in the progression of acute pancreatitis in rats? Pancreas 27 (2003) 253–260.
[26] B.M. Rau, M.C. Krüger, C. Hasel, V. Oliveira, C. Rubie, H.G. Beger, M.K. Schilling, Effects of immunosuppressive and immunostimulative treatment on pancreatic injury and mortality in severe acute experimental pancreatitis, Pancreas 33 (2006) 174–182.
[27] N. Sarmiento, J. Sánchez-Yagüe, P.P. Juanes, N. Pérez, L. Ferreira, V. García-Hernández, A. Mangas, J.J. Calvo, C. Sánchez-Bernal, Changes in the morphology and lability of lysosomal subpopulations in caerulein-induced acute pancreatitis, Dig. Liver Dis. 43 (2011) 132–138.
[28] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[29] W. Namkung, J.S. Yoon, K.H. Kim, M.G. Lee, PAR2 exerts local protection against acute pancreatitis via modulation of MAP kinase and MAP kinase phosphatase signalling, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G886–G894.
[30] T. Sato, M. Otaka, M. Odashima, S. Kato, M. Jin, N. Konishi, T. Matsuhashi, S. Watanabe, Specific type IV phosphodiesterase inhibitor ameliorates caerulein-induced pancreatitis in rats, Biochem. Biophys. Res. Commun. 346 (2006) 339–344.
[31] A.P. Clemons, D.M. Holstein, A. Galli, C. Saunders, Cerulein-induced acute pancreatitis in the rat is significantly ameliorated by treatment with MEK1/2 inhibitors U0126 and PD98059, Pancreas 25 (2002) 251–259.
[32] C.M. Pastor, A. Vonlaufen, F. Georgi, A. Hadengue, P. Morel, J.-L. Frossard, Neutrophil depletion—but not prevention of Kupffer cell activation—decreases the severity of cerulein-induced acute pancreatitis, World J. Gastroenterol. 28 (2006) 1219–1224.
[33] A. Hernández-Hernández, M. Llanillo, M.C. Rodríguez, F. Gómez, J. Sánchez-Yagüe, Amphiphilic and hydrophilic nature of sheep and human platelet phosphotyrosine phosphatase forms, Biochim. Biophys. Acta 1419 (1999) 195–206.
[34] A. Hernández-Hernández, M.C. Rodríguez, A. López-Revuelta, J.I. Sánchez-Gallego, V. Shnyrov, M. Llanillo, J. Sánchez-Yagüe, Alterations in erythrocyte membrane protein composition in advanced non-small cell lung cancer, Blood Cells Mol. Dis. 36 (2006) 355–363.
[35] J.L. Sardina, G. López-Ruano, L.I. Sánchez-Abarca, J.A. Pérez-Simón, A. Gaztelumendi, C. Trigueros, M. Llanillo, J. Sánchez-Yagüe, A. Hernández-Hernández, $p22^{phox}$-dependent NADPH oxidase activity is required for megakaryocytic differentiation, Cell Death Differ. 17 (2010) 1842–1854.
[36] V. García-Hernández, C. Sánchez-Bernal, N. Sarmiento, Raul A. Viana, L. Ferreira, N. Pérez, J.J. Calvo, J. Sánchez-Yagüe, Proteomic analysis of the soluble and the lysosomal + mitochondrial fractions from rat pancreas: implications for cerulein-induced acute pancreatitis, Biochim. Biophys. Acta 1824 (2012) 1058–1067.
[37] J. Mayerle, J. Schnekenburger, B. Kruger, J. Kellermann, M. Ruthenburger, F.U. Weiss, A. Nalli, W. Domschke, M.M. Lerch, Extracellular cleavage of E-cadherin by leukocyte elastase during acute experimental pancreatitis in rats, Gastroenterology 129 (2005) 1251–1267.
[38] T. Hofken, N. Keller, F. Fleischer, B. Goke, A.C. Wagner, Map Kinase Phosphatases (MKP's) are early responsive genes during induction of cerulein hyperstimulation pancreatitis, Biochem. Biophys. Res. Commun. 276 (2000) 680–685.
[39] J.A. Williams, Intracellular signalling mechanisms activated by cholecystokinin-regulating synthesis and secretion of digestive enzymes in pancreatic acinar cells, Annu. Rev. Physiol. 63 (2001) 77–97.
[40] J. Semmler, H. Wachtel, S. Endres, The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-α production by human mononuclear cells, Int. J. Immunopharmacol. 15 (1993) 409–413.
[41] T.R. Kolodecik, C.A. Shugrue, E.C. Thrower, L.R. Levin, J. Buck, F.S. Gorelick, Activation of soluble adenylyl cyclase protects against secretagogue stimulated zymogen activation in rat pancreatic acinar cells, PLoS One 7 (7) (2012) e41320.
[42] B. Gloor, K.E. Todd, J.S. Lane, M.P. Lewis, H.A. Reber, Hepatic Kupffer cell blockade reduces mortality of acute hemorrhagic pancreatitis in mice, J. Gastrointest. Surg. 2 (1998) 430–435.
[43] B. Gloor, T.A. Blinman, D.A. Rigberg, K.E. Todd, J.S. Lane, O.J. Hines, H.A. Reber, Kupffer cell blockade reduces hepatic and systemic cytokine levels and lung injury in hemorrhagic pancreatitis in rats, Pancreas 21 (2000) 414–420.
[44] A. Demols, O. La Moine, F. Desalle, E. Quertinmont, J.L. Van Laethem, J. Devière, DC4(+) T cells plat an important role in acute experimental pancreatitis in mice, Gastroenterology 118 (2000) 582–590.
[45] F.J. Dumont, FK506, an immunosuppressant targeting calcineurin function, Curr. Med. Chem. 7 (2000) 731–748.
[46] J.M. Mayer, V.J. Laine, A. Gezgin, S. Kolodziej, T.J. Nevaleinen, M. Storck, H.G. Beger, Single doses of FK506 and OKT3 reduce severity in early experimental acute pancreatitis, Eur. J. Surg. 9 (2000) 734–741.
[47] C. Liu, K. Dou, C. Dou, J. Liu, Q. Zhao, Anti-inflammatory effects of tacrolimus in a rat model of acute pancreatitis, Med. Chem. 6 (2010) 37–43.
[48] Y. Echigo, K. Inoue, M. Kogire, R. Doi, S. Higashide, S. Sumi, H. Kaji, M. Imamura, Effects of cyclosporine and tacrolimus (FK 506) on acute pancreatitis in mice, Arch. Surg. 130 (1995) 64–68.