Regulation of HSP60 and the role of MK2 in a new model of severe experimental pancreatitis

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In recent years the important role of inflammatory mediators and cytokines has been demonstrated in the pathogenesis of acute pancreatitis, especially, systemic complications have been studied during pancreatitis, such as lung injury and multiple-organ dysfunction syndrome (MODS) (3). It is obviously of high interest to understand in more detail the physiologic of underlying processes in such fatal complications. MK2, also known as MAKAP kinase-2, Map2k2, MEK2, and Prkml2, is a member of the mitogen-activated protein kinase (MAPK)-activated protein kinases. It plays an important role in the regulation of the expression of many cytokine genes, including TNF-α and IL-6 (11, 31). Recently, it has been revealed that the p38/MK2 pathway participates in the regulation of synthesis and secretion of TNF-α and IL-6, and the cellular migration of mouse macrophages (11, 19, 23). This has an important impact on inflammatory responses. Therefore, MK2 deletion putatively interferes with a variety of inflammatory reactions, possibly reducing inflammation in the pancreas (36), neuroinflammation (35), and arthritis (10).

The regulation of protective proteins such as HSPs is of great importance in this context. Heat shock proteins (HSPs) belong to a large family of specialized proteins present in various cellular compartments. They are divided into four main families: the HSP90 family (molecular mass 83–100 kDa), HSP70 family (molecular mass 66–78 kDa), HSP60 family (molecular mass 60 kDa), and small HSP family (molecular mass 12–43 kDa) (27). The expression of HSPs increases when the body is challenged by a variety of stress such as heat, acid, osmotic shock, and others. Their upregulation returns to normal when the disease-promoting agents ameliorate; hence they are considered an adaptive cytoprotection of the body (20, 26, 27), putatively connected to signal systems, such as inflammatory mediators and cytokines.

A wide variety of HSPs is persistently present in the pancreas and can be induced by multiple types of stress. We have previously shown that these stimuli cause not only the synthesis of HSP27, but also its phosphorylation in pancreatic exocrine cells (12). MK2 is the primary upstream kinase that phosphorylates HSP27 in pancreatic acinar cells as it does in other cells. The phosphorylation of HSP27 and its overexpression provided protection against acute pancreatitis induced by cerulein (12). In addition, MK2 and its substrate HSP27 synergize to participate in the regulation of NF-κB activation (9), the known hub for controlling inflammatory responses (1, 6). A wealth of findings confirms the linkage between MK2 and HSP27 and the important role they jointly play in inflammatory processes.

HSP60, also called chaperonin 60, is involved in protein folding, assembly, disassembly, and degradation under normal conditions. An increased transcription and production of HSP60 with protective actions has been suggested in pancreatitis (20, 22). However, details are lacking explaining the significance of HSP60 during acute pancreatitis. Thus far, the relationship of the p38/MK2 signal cascade and HSP60 has not been addressed properly yet.
This study induced acute pancreatitis in mice, producing different degrees of severity by repeated injections of cerulein with or without lipopolysaccharide (LPS). This approach adds another level of complexity to the already existing model of experimental pancreatitis (7). Addition of LPS resembles another independent and severe model, allowing to better focus on systemic complication during the onset of acute pancreatitis. The role of MK2 was studied by using mice with a homozygous MK2 gene deletion and wild-type C57BL mice as control group. Local alterations of the pancreas were assessed by established parameters, and systemic inflammation was determined through assaying the serum IL-6 levels and lung myeloperoxidase (MPO) activity. The effects of MK2 on HSP60 expression and their possible regulation in acute pancreatitis were studied in detail. Our findings may provide clues to fresh targets for novel anti-inflammatory treatments in acute pancreatitis.

MATERIALS AND METHODS

Animals. C57BL wild-type mice and MK2−/− mice weighing 25–30 g were used in this study. The MK2−/− animals were generated on a C57BL/6J genetic background as described previously (11). During the experiments the animals were housed and maintained under controlled environmental conditions. All animal studies were in accordance with the national and international guidelines as outlined in the Guide for the Care and Use of Laboratory Animals, and the study protocols were approved by Institutional Clinical Care and Local Veterinarian Offices.

C57BL wild-type mice and MK2−/− mice were fed with a standard diet before being fasted for 16 h prior to the experiment. Each type of mice was divided at random into four groups, i.e., the control group (NS), the cerulein group (Cer), the LPS group, and the cerulein + LPS group (Cer+LPS), with at least 10 mice in each group.

Induction of acute pancreatitis. The induction of acute pancreatitis was performed according to Ding et al. (7). A small modification was introduced in the amount of liquid injected. The mice (wild-type and MK2−/−) received hourly ipertinonuclear injections, with a total of six, of either normal saline (20 ml/kg) for the NS group or 50 μg/kg of cerulein (Sigma Aldrich, Taufkirchen, Germany) in normal saline for the Cer group. Mice in the LPS group were injected intraperitoneally with normal saline the same way as those in the NS group except that 10 mg/kg of LPS (Sigma Aldrich) was added into the last saline injection. Mice in the Cer+LPS group were injected intraperitoneally with cerulein in the same way as those in the Cer group except that LPS was added (10 mg/kg) into the last cerulein injection.

Three hours after the final injection, all animals were euthanized by decapitation under isoflurane anesthesia, and blood was collected. Pancreas and lung tissue were carefully dissected and removed. Both organs were rinsed with normal saline, put on a Whatman paper, weighed, and divided into several portions. One portion of the pancreas was fixed in 10% formalin immediately, whereas others were shock frozen in liquid nitrogen and stored at −80°C for later analysis.

Histological studies and evaluation of pancreatic pathological changes. The pancreas fixed in 10% formalin as described above was freeze sliced, paraffin embedded, and hematoxylin and eosin stained. The slides were studied under a light microscope. Multiple randomly chosen microscopic fields from at least three mice in each group were examined and scored for semiquantitative assessment on a scale of 0–3 (0 being normal and 3 being severe) blindly by a pathologist. The scoring was based on the number of acinar cell necroses, presence of vacuolization, interstitial edema, interstitial inflammation as represented by infiltrating inflammatory cells, congestion of blood vessels, and extent to which these characteristics affected the organ as has been previously described (11).

Quantification of serum amylase activity. Blood was processed as described previously (12). A Phadebas test was used for serum amylase assay according to the manufacturer’s instructions (Magle, Lund, Sweden). Results were measured as units per liter and presented as % of control.

Quantification of pancreatic trypsinogen activation. Trypsinogen activation was measured as earlier reported (36). In brief, one portion of the pancreatic tissue was homogenized in ice-cold 3-(N-morpholino)-propanesulfonic acid (MOPS) buffer (pH 6.0, 250 mM sucrose, 5 mM MOPS, 1 mM magnesium sulfate) by use of a Teflon glass homogenizer. The resulting homogenate was centrifuged, and the supernatant was used for the assay. Trypsin activity was measured fluorometrically using Boc-Glu-Ala-Arg-AMC-HCl (Bachem, Heidelberg, Germany) as substrate, and the activity was calculated from the slope by using a standard curve generated with purified trypsin. Protein content was measured with the Bio-Rad protein assay. The trypsin activity was expressed as femtomoles per milligram protein and calculated as % of control.

Quantification of lung MPO activity. MPO activity in the lung tissue was measured as described previously (36). Tissue samples were homogenized with an Elmer Potter homogenizer at 2,400 rpm. An aliquot of this homogenate was taken for protein determination with the Bio-Rad protein assay; the rest was centrifuged at 10,000 g and 4°C for 10 min. Each pellet was resuspended in 0.5 ml of extraction buffer and snap frozen and thawed four times. Samples were subsequently sonicated twice for 10 s and centrifuged for 5 min at 10,000 g and 4°C. The supernatant was used for MPO measurement. The reaction mixture consisted of 1 ml KH2PO4 buffer (pH 6.0), 10 μl o-dianisidine, 10 μl H2O2, and 100 μl of the supernatant. The supernatant was added to the mixture after 1 min of monitoring the absorbance at 460 nm, and absorbance was monitored for an additional 4 min. The activity was calculated from the slope and expressed as milliunits per milligram of wet weight of lung tissue and calculated as % of control.

Determination of serum IL-6 levels. IL-6 levels were measured by using a commercially available ELISA kit for mouse IL-6 (Quantikine; R&D Systems, Minneapolis, MN) following the manufacturer’s protocol. Each sample was measured in triplicate with a microplate reader and expressed as means ± SE (pg/ml serum).

Western blotting for measuring HSP expression. Western blotting experiments as well as the initial preparation of lysates were performed as described previously (12, 17). Primary antibodies used in this study included polyclonal HSP25, monoclonal HSP60, and HSP90, as well as HSP32 and HSP70 (Stressgen, Victoria, BC, Canada) in a 1:1,000 dilution individually. After a washing of the nitrocellulose membranes (Whatman, Dassel, Germany), the appropriate secondary antibody conjugated to horseradish peroxidase was applied in a 1:2,000 dilution and incubated for 1.5 h at room temperature. For internal reference, a monoclonal mouse anti-mouse β-actin antibody (1:2,000 dilution) (Sigma-Aldrich) was used. Finally, antibody binding was detected by enhanced chemiluminescent detection system and recorded on film. ImageJ analysis system (U. S. National Institutes of Health) was applied for analysis of the optical density of the protein bands. The relative expression quantity of HSP25, HSP60, or HSP90 protein was illustrated as the percentage of the optical density (OD) of HSP25, HSP60, or HSP90, adjusted with the corresponding β-actin OD, vs. that of the normal control, respectively.

Statistical analysis. Results are means ± SE. Values were obtained from multiple determinations in five or more separate experiments with two or three animals in every experiment with the variously treated groups. All data were analyzed by ANOVA (SPSS 12.0 software), unless stated otherwise. Differences were considered as
significant at P values of P < 0.05 (significant) or higher (P < 0.01).

RESULTS

Effects and histological analysis of MK2 gene deletion in experimental pancreatitis. Cerulein-treated mice displayed histological signs of acute pancreatitis characterized by interstitial edema, vacuolization, and infiltration of neutrophil and mononuclear cells with little parenchyma necrosis and hemorrhage (Fig. 1C). The treatment of cerulein in combination with LPS caused more severe pathological changes in the pancreatic tissue, with an obvious edema, inflammation, vacuolization, and many local necroses of acinar cells (indicated by the asterisks in Fig. 1D). In contrast, treatment of MK2−/− mice by injections of

![Image of histological sections](http://ajpgi.physiology.org/)

Fig. 1. Histological evaluation of pancreatic morphology in wild-type and MK2−/− mice (hematoxylin and eosin, ×400). The pancreatic tissues were harvested and examined by light microscopy as described in MATERIALS AND METHODS. A–D: representative sections from the pancreatic tissues of wild-type mice. E–H: sections from MK2−/− mice. NS, normal saline; Cer, 6 hourly injections of cerulein 50 µg/kg; Cer+LPS, 6 hourly injections of cerulein 50 µg/kg with LPS (10 mg/kg) being added into the last injection; LPS, 6 hourly injections of normal saline with LPS (10 mg/kg) being added into the last injection. Increased inflammatory cell infiltration is indicated by the arrows; asterisks indicate pancreatic acini cells with increased necrosis and vacuolization.
HSP60 AND MK2−/− PROTECTS AGAINST SEVERE PANCREATITIS
cerulein, or cerulein + LPS, led to markedly attenuated inflammatory changes with reduced edema, fewer necroses, and modest signs of inflammation and vacuolization in the pancreatic tissues (Fig. 1, G and H). In controls, wild-type, or MK2−/− mice, after saline injections no effect appeared on pancreatic morphology and only small morphological changes were seen after LPS with few inflammatory cells detectable in the interstitial tissue and with normal acinar morphology (Fig. 1, A, B, E, and F). To assess these changes in a semiquantitative manner, histological slides of the pancreas were scored blindly as described in MATERIALS AND METHODS. The results showed significant difference in the appearance of morphology between the wild-type and MK2−/− mice as likewise in the Cer group as well as in the Cer+LPS group with decreased inflammation in the MK2−/− animals (Table 1). These results confirm our previously reported results on protective effects of MK2 deficiency on cerulein-induced pancreatitis (36). It demonstrates that MK2 deficiency offers protection against the morphological deleterious effects in a more severe and systemic model of acute pancreatitis.

Expression of HSP25 and HSP60 in the pancreas. The amount of HSP protein expression in both wild-type and MK2−/− mice is presented as the percentage of expression in the NS group, with the individual ratio of the OD of the HSP band vs. the OD of the corresponding β-actin band. The findings showed that, regardless whether MK2−/− or wild-type mice were studied, the expression of HSP25 or HSP60 in pancreatic tissue was robustly elevated by cerulein stimulation alone or in combination of cerulein + LPS (Fig. 2, A and B). In wild-type mice the expression of HSP60 showed a significant increase in the Cer+LPS group. Also in MK2−/− mice, an enhancement was found nearly five times higher as controls in the Cer+LPS group (P < 0.05). Although the increased levels of HSP60 expression in the MK2−/− mice treated with Cer+LPS was somewhat higher compared with the wild-type mice, the difference was not statistically significant (P > 0.05; Fig. 2E). In both the wild-type and MK2−/− mice groups, LPS caused a small but significant increase of HSP60 expression compared with the saline group. No significance was detectable between the wild-type and MK2−/− (Fig. 2C). Similarly, there were no different expression levels detectable of HSP60 after treatment with cerulein (Fig. 2D). These results revealed that the increased expression of HSP60 and HSP25 was induced during induction of an inflammatory pancreatitis, and these changes were not affected by the homozygous deletion of the MK2 gene. Furthermore, the expression of HSP32, HSP70, and HSP90 was analyzed. Here, no change of protein expression of these HSPs was observed after stimulation with cerulein, LPS, or cerulein + LPS (data not shown).

Effects of MK2 gene deletion on amylase activity and trypsinogen activation. Treatment of wild-type mice by cerulein or with or without LPS led to enhanced serum amylase levels compared with control mice (P < 0.05), with a slightly more pronounced increase after cerulein + LPS injection (Fig. 3, B and C). Likewise, effects were seen on the activation of pancreatic trypsinogen (P < 0.05; Fig. 3, E and F). In contrast, the homozygous deletion of MK2 showed significantly reduced levels of serum amylase compared with wild-type mice (P < 0.01; Fig. 3, B and C) and reduced pancreatic trypsinogen activation (P < 0.01 and P < 0.05; Fig. 3, E and F) after cerulein or Cer+LPS treatment. Sole LPS treatment did not change pancreatic enzymes significant in either type of mice (Fig. 3, A and D).

Effects of MK2 homozygous deletion on serum IL-6 levels. To assess the protein levels of IL-6 in the serum, ELISA assays were performed in both MK2−/− and wild-type mice. The IL-6 serum levels revealed a pronounced increase after LPS or cerulein + LPS treatment compared with control animals (P < 0.01; Fig. 4, A and C). Cerulein alone caused moderately elevated IL-6 levels in wild-type animals. This increase was significantly reduced in the MK2-deficient mice (Fig. 4, A–C). MK2−/− mice expressed nearly 60% less IL-6 in the Cer group (P < 0.01) and ~30% less IL-6 in the LPS group (P < 0.01; Fig. 4, B and A). The protective effects in MK2−/− mice after cerulein + LPS treatment were less pronounced but still statistically significant (P < 0.05) compared with wild-type mice (Fig. 4C). Injection of normal saline did not affect basal IL-6 levels (Fig. 4, A–C). These data demonstrate that the increased serum IL-6 levels induced by cerulein, LPS, or cerulein + LPS are significantly reduced by MK2 deficiency.

MPO activity in lung tissues. To further evaluate systemic complications of acute pancreatitis, we assessed MPO activation, a marker for neutrophil sequestration, in lung tissues of wild-type and MK2−/− mice after treatment with LPS and after induction of acute experimental pancreatitis. The characteristics of MPO activation mirrored those of the serum IL-6 levels. In wild-type mice, the administration of LPS, cerulein, or cerulein + LPS induced a significant increase in MPO activity compared with control animals (P < 0.05, and P < 0.01 respectively; Fig. 5, A–C) whereas in MK2−/− mice the MPO activity was reduced by more than 70% in LPS-treated mice (P < 0.01) and ~65% after cerulein + LPS injection (P < 0.01; Fig. 5, A and C). Treatment with cerulein alone induced a small but significant increase in MPO activity in lung tissue compared with controls. Only a trend of reduction was detectable in the MK2−/− mice (Fig. 5B).

DISCUSSION

The development of acute pancreatitis is a complex multistep process, accompanied by an activation of intracellular trypsinogen early on. Subsequently, other downstream enzymes are activated in an enzyme cascade such as phospholipase A, chymotrypsino-

**Table 1. Morphological analysis and scoring by a blinded pathologist of the effects of MK2 homozygous gene deletion on pancreatic inflammatory changes in acute pancreatitis (means ± SE)**

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<thead>
<tr>
<th>Treatment/Group</th>
<th>WT Mice</th>
<th>MK2−/− Mice</th>
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<tr>
<td>NS</td>
<td>0.3±0.1</td>
<td>0.3±0.05</td>
</tr>
<tr>
<td>LPS</td>
<td>0.7±0.1</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Cer</td>
<td>5.6±1.8*</td>
<td>3.1±1.0**</td>
</tr>
<tr>
<td>Cer+LPS</td>
<td>7.1±2.1*</td>
<td>4.9±1.2†</td>
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Pancreatic tissues were harvested 3 h after hourly injections of either normal saline (NS), LPS, cerulein (Cer), or cerulein plus LPS, from wild-type (WT) or MK2−/−, mice, fixed, paraffin embedded, sliced, and stained by hematoxylin and eosin for microscopic analysis. The histological changes were scored semiquantitatively by an independent and blinded pathologist from 0 (normal) to 3 (severe) on the basis of the number of acinar cell necrosis and the presence of vacuolization, interstitial edema, and interstitial inflammation (see MATERIALS AND METHODS). The severity scoring presented here is the integrated data of the scores for edema, inflammation, vacuolization, and necrosis. Results are expressed as means ± SE for 6 slides at least from 3 mice in each experimental group with *P < 0.05 compared with the NS control group; †P < 0.05 compared with the same treatment group of wild-type mice.
gen, and others, triggering further early events in acinar cells to solicit digestive injuries in pancreatic tissues (15, 26). As the disease proceeds, the local inflammatory reactions continue into a systemic inflammatory disease. At this stage, cytokines, including TNF-α and IL-6, play a major role (4, 8, 26).

In this study, we induced acute pancreatitis in two different mouse models characterized by varying degrees of severity. Our results demonstrate that acute pancreatitis induced with cerulein alone shows localized lesions of acute inflammation with vacuolization and necrosis, elevated levels of serum amylase, IL-6, pancreatic trypsinogen activation, and moderate increased leukocyte infiltration in lung tissues. In contrast, acute pancreatitis induced by a combination of cerulein plus LPS injection showed a more severe course with deteriorated

Fig. 2. Analysis of heat shock proteins (HSPs) HSP25 and HSP60 expression in pancreatic tissue in wild-type and MK2−/− mice by Western blotting. Representative Western blots from at least 4 independent experiments with profiling of HSP25 (A) and HSP60 (B) expression in mouse pancreatic tissue from normal mouse without any experimental treatment (Nor) and NS, Cer, LPS, Cer+LPS mice. Densitometric quantification of HSP60 expression and comparison between wild-type and MK2−/− for LPS (C), Cer (D), and Cer+LPS (E) treatment. In each experimental group, at least 6 animals were included. n.s., Not significant.
pancreatic inflammation, evident local acinar necrosis, as well as drastic systemic inflammatory responses. Our data confirm that the combination of cerulein plus LPS induces a much more severe acute pancreatitis as cerulein alone. In addition, LPS is an aggravating agent and impairs the local and systemic inflammatory response during acute experimental pancreatitis. Therefore, the model of cerulein plus LPS-induced pancreatitis seemed useful to study the role of the MK2 signaling pathway in inflammatory responses.

Although presently we have only limited knowledge about the exact pathophysiological mechanism of acute pancreatitis, it has at least been proven that inflammation is critically influenced by the impact of cytokines, very likely facilitating complications such as lung injury and MODS (24, 28, 30). It is accepted that the signal transduction pathways of MAP kinases among others are involved in the regulation of response to environmental stress, inflammatory reactions, as well as regulation of the synthesis and secretion of TNL-α and IL-6 (4, 11, 15, 19, 23, 35, 36). Wagner et al. (37) reported that inhibitors of JNK and p38 could ameliorate the severity of rat acute pancreatitis induced by cerulein. Furthermore, we have shown that overexpression of HSP27 as well as gene deletion of MK2 protects against cerulein-induced pancreatitis (12, 36).

Fig. 3. Serum amylase activity and pancreatic trypsinogen activation in wild-type and MK2−/− mice. The effects of MK2 homozygous gene deletion on cerulein-induced pancreatitis were evaluated by serum amylase activity (A–C) and pancreatic trypsinogen activation (D–F). Comparison and analysis showed that acute pancreatitis was induced in wild-type and MK2−/− mice after 6 hourly injections of 50 μg/kg cerulein, or Cer + LPS (solid bars) to a various extent. Control animals received normal saline (open bars). Results are expressed as % of control in each group with means ± SE. In each experimental group at least 8 animals were included. *P < 0.05 and **P < 0.01 when wild-type mice were compared with the MK2−/− mice after treatment (solid bars).
To prove in a more severe model of pancreatitis whether a MK2 knockout can protect against local or systemic complications of acute pancreatitis, we used a combination of cerulein injections plus LPS as described by Ding et al. (7). Interestingly, LPS injections alone induced some systemic reactions with elevated IL-6 levels but no effects on the pancreas morphology or enzyme activation. The MK2−/− mice were more resistant against acute pancreatitis, showing preserved morphology, less pancreatic enzyme activation, and less systemic inflammatory reactions compared with wild-type mice. This suggests that the p38/MK2 signal transduction pathway is mandatory for the regulation of other critical downstream signals influencing systemic inflammatory reactions in acute pancreatitis.

Acute pancreatitis induces inflammatory stress, which is frequently accompanied by induction of several HSPs. In addition, a more pronounced induction of selective HSPs can protect against acute pancreatitis, depending on the model of acute pancreatitis used (32–34). In this context, HSP27 and HSP70 have been extensively studied. We and others have

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**Fig. 4.** Serum IL-6 levels in wild-type and MK2−/− mice. Serum IL-6 was analyzed with ELISA technique after 6 hourly injections of normal saline, LPS, cerulein, or cerulein + LPS (A–C), respectively. Results are expressed as means ± SE with at least 8 animals in each group with *P < 0.05 and **P < 0.01 when wild-type mice were compared with the MK2−/− mice after treatment (solid bars). The normal saline control group is shown in open bars.

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**Fig. 5.** Myeloperoxidase (MPO) activity in lung tissue of wild-type and MK2−/− mice. MPO activity in lung tissue was measured after 6 hourly injections of either normal saline (control, open bars, A–C), LPS (A), cerulein (B), or cerulein + LPS (C) into wild-type or MK2−/− mice. Data are expressed as % of control in each group with means ± SE from 8 mice in each experimental group, *P < 0.01 compared with wild-type mice and MK2−/− mice after treatment with LPS (A) or Cer+LPS (C); there were no significant differences between wild-type and MK2−/− mice after treatment with Cer (B).
Moreover, the expression of HSP60 decreased at 1 h but lipase levels remained at a relatively high level (18). Significantly decreased in severe acute pancreatitis, whereas trypsin treatments suggested that expression of pancreatic HSP60 significantly decreased after induction of acute pancreatitis in mice. This intimate chaperoning may have important biological significance, ensuring the natural synthesis, folding, and secretion of the enzymes and preventing them from premature activation in acinar cells. Lee et al. (14) reported that the treatment of rats with cold-water immersion could specifically induce the synthesis of HSP60 and thereby ameliorate cerulein-induced pancreatitis. A recently published study showed that bee venom treatment increases HSP60 and HSP70 expression and protects against CCK-induced pancreatitis in rats (29). Here, HSP60 in turn might act as a lysosomal protein-hydrolase cathepsin B from displacing to the zymogen granule-rich areas and stop the colocalization of cathepsin B and digestive enzymes, thereby inhibiting the activation of trypsinogen in pancreatic acinar cells. Later, Rakonczay et al. (22) confirmed that cold-water immersion could specifically induce the synthesis of HSP60 and partly enable rats to resist hemorrhage-necrotizing pancreatitis. A recently published study showed that bee venom and LPS separately. The similar phenomenon was observed in all groups of mice with no significant difference of HSP60 protein expression in pancreatic tissue of the wild-type or MK2−/− mice. Expression of HSP90 was evident in all groups of mice with no significant difference among the different mouse groups (data not shown). These results demonstrate that LPS fails to cause localized pathological changes in the pancreas, or to alter the expression of pancreatic HSP25 or HSP60, although it does trigger some systemic inflammatory responses in the experimental animals. However, when the pancreas is in a pathological state of acute pancreatitis, LPS deteriorates the acute pancreatitis induced by cerulein and pushes the HSP25 or HSP60 expression levels even higher. To our knowledge, to date there is no report exploring the relationship between the roles of MK2 in regulating HSP60 expression in mouse pancreatic acini during acute pancreatitis. Some investigators propose that HSP60 can activate via the Toll/IL-1 receptor kinase signal transduction pathway, in which the adaptor molecules MyD88 and TRAF6 are being involved, the downstream kinase pathways of p38, JNK, ERK, and NF-κB. In this context, it is of interest that TLR4 plays a significant proinflammatory role independently of LPS in the progression of acute pancreatitis shown by Sharif et al. (30).

It seems that the above-described changes of HSPs are independent of the MK2 gene status of the mice, suggesting that MK2 does not affect the cerulein-induced expression of HSP60. Therefore, the protective effects of MK2 are most likely due to a regulation of the cytokine expression in pancreatic acinar cells, independently of the regulation of HSP60 and HSP27. Although some functions of HSP27 need to be regulated by activated p38/MK2 transduction pathways (12, 21, 25, 36, 38), our data indicate that MK2 is not involved in the expression and regulation of HSP60 in a new severe model of acute pancreatitis.

In summary, a MK2 homozygous gene deletion ameliorates systemic inflammatory responses in a severe model of acute pancreatitis induced by cerulein + LPS, indicating that this MAP kinase signal-transduction pathway can regulate inflammatory cytokines. The expression of stress proteins, including HSP25 and HSP60, thus impacts the localized and systemic inflammatory responses induced by cerulein and LPS. Nevertheless, the increased expression of HSP25 and HSP60 by itself is not sufficient to protect in a severe model of acute pancreatitis. More research efforts are required to pinpoint the precise link and elucidate the exact mechanisms.

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