Mice lacking the transcription factor Mist1 exhibit an altered stress response and increased sensitivity to caerulein-induced pancreatitis

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PANCREATITIS is a disease that targets exocrine pancreas cells, leading to inflammation, fibrosis, and general tissue disruption. Pancreatitis is often the result of environmental agents such as excessive alcoholic consumption or the appearance of gall stones and can also be a complication in cystic fibrosis (51) and acute pancreatitis (41, 51). However, little is known about the molecular factors that promote increased sensitivity to the environmental agents that cause pancreatitis.

Several animal models have been developed to study pancreatitis, and these models have been instrumental in identifying specific biochemical and cellular changes that occur during the initiation and progression of pancreatitis (16, 30, 38, 47). One such model involves the supramaximal stimulation of the CCK signaling pathway with caerulein, a CCK analog, to produce a mild edematous form of pancreatitis (17, 46, 48). Following the initiation of caerulein-induced pancreatitis (CIP), various signaling pathways are activated including JNK/SAPK pathways (8). Active JNK signaling can cause alterations in gene expression and promote apoptosis (7). JNK activation has also been reported to enhance the phosphorylation of both protein kinase R (PKR)-like endoplasmic reticulum (ER) kinase (PERK) and inositol-required enzyme 1 (IRE1), which are mediators of the ER stress pathway (29, 33). ER stress is activated by a number of conditions, including alterations in Ca2+ homeostasis, and leads to a block in general translation through the phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α), a member of the translational elongation complex (54). In confirmation that activation of the ER stress response pathway may play an important role in pancreatitis, a recent study (28) has shown that this pathway becomes activated following the administration of l-arginine, which promotes severe necrotizing pancreatitis. In addition, stress response genes such as heat shock protein 70 (Hsp70) (3) and early growth response 1 (EGR1) (20) are upregulated following CIP.

While the importance of these pancreatitis models cannot be overestimated in the study of disease, to date few genes have been identified that specifically increase the sensitivity to pancreatitis. Targeted deletion of the genes that encode connexin32 (Cx32) and α2-macroglobulin have both resulted in an increased severity of pancreatitis induced by CIP or a choline- and methionine-deficient, ethionine-supplemented diet, respectively, but neither of these proteins are important for acinar cell differentiation (11, 50). Given the reactivation of the developmental program during regeneration from pancreatitis (19), it seems likely that disruption of a developmentally important gene would affect the severity of pancreatic damage.

Mist1 is a basic helix-loop-helix transcription factor that is specifically expressed in serous secreting exocrine cells (42). In the pancreas, Mist1 is restricted to acinar cells throughout development, and it is specifically expressed in the exocrine pancreas during pancreatic ductal cell differentiation (42). Mist1 is initially expressed in the ductal epithelium and is later expressed in the acinar cells, indicating that Mist1 may have a role in acinar cell differentiation (42).

Mist1 is an exocrine specific transcription factor important for the complete differentiation and function of pancreatic acinar cells. Mice lacking the Mist1 gene [Mist1 knockout (KO) mice] exhibit cellular disorganization and functional defects in the exocrine pancreas but no gross morphological defects. Following the induction of pancreatitis with caerulein, a CCK analog, we observed elevated serum amylase levels, necrosis, and tissue damage in Mist1 KO mice, indicating increased pancreatic damage. There was also a delay in the regeneration of acinar tissue in Mist1 KO animals. Molecular profiling revealed an altered activation of stress response genes in Mist1 KO pancreatic tissue compared with wild-type (WT) tissue following the induction of pancreatitis. In particular, Western blot analysis for activating transcription factor 3 and phosphorylated eukaryotic initiation factor 2α (eIF2α), mediators of endoplasmic reticulum (ER) stress, indicated limited activation of this pathway in Mist1 KO animals compared with WT controls. Conversely, Mist1 KO pancreatic tissue exhibits increased expression of growth arrest and DNA damage inducible 34 protein, an inhibitor of eIF2α phosphorylation, before and after the induction of pancreatitis. This finding suggests that activation of the ER stress pathway is a protective event in the progression of pancreatitis and highlights the Mist1 KO mouse line as an important new model for studying the molecular events that contribute to the sensitivity to pancreatic injury.

activating transcription factor 3
development and in adult tissue (10, 43). Targeted ablation of the Mist1 gene [Mist1 knockout (KO)] through homologous recombination results in incomplete acinar cell maturation (43), disruption of cellular function (34), and altered expression of genes involved in cell communication (44), granule secretion (23), and Ca^{2+} handling (34). In addition, Mist1 KO pancreatic tissue exhibits premature enzyme activation and stellate cell activation and increased expression of pancreatitis-associated proteins (43), which have been identified as initiating events for acute pancreatitis (45, 56). Surprisingly, the fibrosis and inflammation typically associated with pancreatitis is absent in Mist1 KO mice, suggesting that additional events are required for the progression of pancreatitis.

Given the appearance of pancreatitis-related factors in Mist1 KO pancreatic tissue, we hypothesized that these mice would have increased susceptibility for exocrine pancreatic damage. Therefore, we characterized the sensitivity of Mist1 KO mice to supramaximal CCK stimulation compared with wild-type (WT) mice. Our results clearly showed that Mist1 KO mice exhibited an increased severity of pancreatitis and a longer recovery period compared with WT mice and that the severity could be linked to a block in the activation of the stress response pathway. These findings further our understanding of key mediators involved in pancreatitis and offer a new model by which to study the severity and susceptibility of this disease.

**MATERIALS AND METHODS**

**Materials.** All chemicals were purchased from Sigma (St. Louis, MO) unless otherwise stated.

**Mice.** Protocols were approved by the Animal Care Committee at The University of Western Ontario, and mice were handled according to regulations stipulated by the Canadian Council on Animal Care. Mice deficient for Mist1 (Mist1 KO) mice were previously generated by targeted homologous recombination (43) and back crossed for at least seven generations into a C57BL/6 background. All mice were fed a standard diet and water ad libitum. Two- to four-month-old WT and Mist1 KO litters were used for all experiments.

**Induction of pancreatitis and histological analysis of pancreatic tissue.** To induce mild, edematous, acute pancreatitis, male Mist1 KO and WT litters were given one to seven hourly intraperitoneal injections of caerulein (Sigma, 50 μg/kg body wt). As a control, WT and Mist1 KO animals were given saline injections (n = 8). Mice were killed at 1 h (n = 4), 4 h (n = 7–11), 8 h (n = 4), 24 h (n = 7), 48 h (n = 4), 72 h (n = 6–8), and 7 days (n = 4) after the initiation of pancreatitis. For susceptibility studies, 3.1–50 μg caerulein/kg body wt was administered for 4 h (n = 3–4 for WT and 5 for Mist1 KO animals). To assay serum amylase levels, blood was obtained from WT and Mist1 KO mice, placed on ice for 15 min, and centrifuged at 3,000 rpm and 4°C for 10 min. The supernatant was retained, and amylase levels were determined using the Phadebas amylase test (Pharmacia Diagnostics) as per the manufacturer’s instructions.

For histological analysis, pancreatic tissue was fixed in cold 10% formalin for several hours followed by paraffin embedding. Tissues were sectioned to 6 μm and stained with hematoxylin or eosin (H&E) or Gomori’s trichrome stain. Sections were assayed for the expression of apoptotic bodies per millimeter squared of exocrine pancreatic tissue, and the apoptotic index was calculated as the proportion of apoptosis was determined by counting the numbers of apoptotic bodies per millimeter squared of exocrine tissue, and the apoptotic index was calculated as the proportion of apoptotic nuclei within 10 random microscopic fields of exocrine tissue stained with H&E was assayed, and the amount of necrotic area based on cellular swelling and the presence of inflammatory cells was calculated as a percentage of the entire exocrine tissue. In both cases, sections from 4, 8, 24, and 72 h after the initiation of CIP were assayed for WT and Mist1 KO mice.

**Acinar cell isolation.** Acini were purified as described by Yule and Williams (53) from female mouse pancreatic tissue with the following modifications. To digest the pancreas, 200 U/10 ml of chromatographically purified collagenase (code CLSPA from Clostridium histolyticum, Worthington Biochemicals, Lakewood, NJ) and 2.5 mg/ml of BSA were diluted in DMEM-Krebs-Ringer solution. DMEM was used for all washes, and the final wash contained 1% BSA. Cells were plated in DMEM containing 1% penicillin-streptomycin. To determine cell viability, acinar cells from WT and Mist1 KO cultures were stained with trypan blue, and the percentage of live cells was determined. Only cultures exhibiting >95% viability in intact acini were used for further experiments. Aliquots of isolated acini were stimulated with PBS and 30 μM physiological levels) or 10 mM CCK for 30 min. Alternatively, acinar cells were incubated in 0.2 μM thapsigargin for 30 min or 2 μg/ml tunicamycin for 1 h before the isolation of RNA.

**TUNEL assay.** Following stimulation, isolated acinar cells were placed on fibronectin-coated coverslips. Cells were washed three times for 5 min with PBS and fixed for 1 h with 4% formaldehyde. Tissues were obtained from caerulein-injected mice at 4 h into caerulein treatment, embedded in cryomatrix, and sectioned to 6 μm. Sections were fixed as described, and all samples were washed three times for 5 min with PBS and permeabilized with 0.2% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Samples were again washed three times for 5 min with PBS, and the TUNEL reaction mixture (Roche Diagnostics, Laval, PQ, Canada) was applied for 1 h at 37°C in the dark. Samples were counterstained with a 5-min wash containing 4’,6-diamidino-2-phenylindole, mounted on slides in Vectashield, and coverslipped. Cells and tissue samples were viewed using a Leica DMlM upright microscope, and images were captured using the Open Lab 4 imaging program (Improvision).

**Protein isolation and Western blot analysis.** Protein was isolated from WT and Mist1 KO acinar cells or pancreatic tissue 4 h after the initiation of CIP using an isolation buffer containing 5% (wt/vol) Nonidet P-40, 5 mM EDTA, 30 mM Na fluoride, 2 mM Na orthovanadate, 10 μM leupeptin, 5 μM pepstatin, 1 mM PMSF, 20 mM MOPS, and one Complete Mini-Tablet (Roche Diagnostics) per 7 ml of isolation buffer. Cells were homogenized, sonicated, and centrifuged at 5,000 rpm for 5 min at 4°C. The supernatant was collected, and protein was quantified using the Bradford protein quantification assay according to the manufacturer’s instructions.

Protein (10–50 μg) was resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) at 200 mA for 90 min. Membranes were washed in Tris-buffered saline (TBS) and blocked with 5% nonfat dry milk in TBS with 0.1% Tween 20 (TBST) for 1 h at room temperature. Primary rabbit antibodies specific for phosphorylated c-Jun, growth arrest and DNA damage inducible 34 (Gadd34), activating transcription factor 3 (ATF3), phosphorylated and total eIF2α (Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated JNK, or amylase (Calbiochem) were diluted to 1:10,000 in blocking solution and incubated at 4°C overnight. Membranes were washed three times for 5 min with TBST and then incubated in horseradish peroxidase-conjugated donkey α-rabbit IgG (1:2,000, Sigma) for 1 h at room temperature. Blots were washed five times for 5 min with TBST, incubated in chemiluminescent substrate (Perkin-Elmer, Woodbridge, ON, Canada), and exposed to Kodak X-ray film.

**RNA isolation, Northern blot analysis, and microarray analysis.** RNA was isolated from the splenic portion of the pancreas as previously described (43) or from acinar cell cultures using TRIzol (Invitrogen). Northern blot analysis was performed as previously described. Blots were hybridized overnight at 42°C with [α-32P]dCTP-labeled probes (1 × 10^6 counts · min⁻¹ · ml⁻¹).
with cDNA obtained from MRC Gene Service (UK) for c-Fos (no. 2582234), EGR1 (no. 6488118), gadd34 (no. 3710924), and ler2 (no. 3590296) or a probe specific for 18S rRNA (as a normalization control).

For microarray analysis, total pancreatic RNA was used to generate cRNA probes as per the manufacturers’ instructions (Affymetrix, Santa Clara, CA). Labeled cRNA was isolated using an RNeasy Mini Kit column (Qiagen, Mississauga, ON, Canada). Purified cRNA probes were hybridized to MOE430 oligonucleotide arrays (Affymetrix). Expression values for individual genes were calculated using Affymetrix GeneChip software MAS 5.0. Hybridization experiments were performed on pancreatic tissue from WT and Mist1 KO mice treated with saline or caerulein (n = 3 mice/sample group). Analysis of the microarray was performed using GeneSpring 6.2 software (Silicon Genetics, Foster City, CA). Gene differences exhibiting a P value of <0.05 (by Benjamini and Hochberg multiple correction) were considered significant.

Statistical analysis. Quantitative results are depicted as means ± SE, and significance was identified using two-way ANOVA followed by a Bonferroni post hoc analysis. A P value of <0.05 was identified as significant. A Tukey’s post hoc test was used for susceptibility analysis.

RESULTS

Mist1 KO mice exhibit increased sensitivity to CIP. To determine the response of Mist1 KO mice to supramaximal CCK signaling, Mist1 KO and WT mice were given 7 hourly injections of 50 μg/kg caerulein or saline, and serum amylase levels were assessed at various time points after the initial injection (Fig. 1A). WT mice injected with caerulein began to show significantly elevated levels of serum amylase at 4 h after the initiation pancreatitis that peaked at 8 h. In comparison, Mist1 KO mice had significantly increased levels of serum amylase within 1 h of the initial caerulein injections. At both 4 and 8 h, Mist1 KO mice had elevated levels of serum amylase compared with both WT-treated and saline-injected Mist1 KO mice. At 24 h postinjection, serum amylase levels were higher for both caerulein-treated WT and Mist1 KO mice compared with their untreated counterparts, but, by 72 h postinjection, only Mist1 KO mice still exhibited a small but significant elevation in serum amylase levels compared with untreated mice (data not shown). This increase in serum amylase was not simply reflective of increased tissue amylase in Mist1 KO mice because previous analysis (43) has indicated that amylase expression is slightly reduced in these animals compared with WT mice.

To assess the susceptibility of Mist1 KO mice to caerulein-induced pancreatic injury, various doses of caerulein were injected for 4 h (Fig. 1B). Significantly higher levels of serum amylase were observed in Mist1 KO mice injected with levels of caerulein ≥6.3 μg/kg compared with saline-treated animals. In contrast, only WT animals injected with the maximal level of caerulein (50 μg/kg) showed significantly higher levels of serum amylase compared with saline-treated control animals. Together, these results suggest that Mist1 KO mice exhibit increased susceptibility and display more severe pancreatitis initiated by supramaximal stimulation of the CCK pathway.

To further investigate the severity of pancreatitis, we examined the morphology of pancreatic tissue sections at various time points following CIP (Figs. 2 and 3). A low-magnification examination of saline-injected pancreatic tissue revealed typical acinar cell morphology for WT tissue (Fig. 2A), where Mist1 KO tissue was disorganized (Fig. 2B), as described previously (23). Four hours after the initiation of pancreatitis, there was increased tissue and cellular disorganization in Mist1 KO tissue (Fig. 2D) compared with WT tissue (Fig. 2C). Mild edema was observed in both WT and Mist1 KO mice; however, there was also some distension of acinar lumens in Mist1 KO tissue (Fig. 2D). A similar analysis at 72 h revealed widespread damage in Mist1 KO tissue (Fig. 2F), whereas WT tissue was completely recovered back to the normal pancreatic phenotype (Fig. 2E). Histological analysis of tissue 72 h after the initiation of CIP with Gomori’s trichrome stain identified large areas of fibrosis in Mist1 KO tissue (Fig. 2H) compared with WT tissue (Fig. 2G), consistent with the increased pancreatic damage in these animals. By 7 days after caerulein treatment, Mist1 KO tissue had reverted back to its original morphology but with increased adipose cell accumulations (data not shown).

Closer examination of H&E-stained sections revealed several morphological defects associated with increased pancre-
KO tissue based on all criteria examined, the severity of pancreatitis was greater in Mist1 KO mice compared with WT mice.

The molecular response to CIP is altered in Mist1 KO mice. The examination of physiological and morphological changes during CIP strongly supports the hypothesis that Mist1 KO mice have increased sensitivity to pancreatic damage. Since Mist1 is a transcription factor, one would expect a significant difference in the molecular response to CIP as well. To identify these molecular differences, RNA was isolated from WT and Mist1 KO mice treated with saline or caerulein (50 μg/kg) for 4 h was analyzed by molecular profiling of Affymetrix MOE430 microarray gene chips. The complete array results can be found at www.ncbi.nlm.nih.gov/geo (Accession No. GSE3644). To validate the array data that was generated, genes that have previously been identified by Northern and Western blot analysis as being differentially expressed between WT and Mist1 KO pancreatic tissue were examined (Supplemental Table 1). As previously reported, there were significant differences between WT and Mist1 KO animals in the expression values for Mist1 and CCK-A receptor (43), Cx32 (44), Rab3D (23), andinositol (1,4,5)-trisphosphate receptor 3 (43).

Using GeneSpring 6.0, we identified a number of genes that were significantly increased or decreased following 4 h of caerulein treatment in WT and Mist1 KO mice (Supplemental Appendixes 1 and 2). Details of how these lists were generated can be found in the Supplemental Information. Examination of the gene expression changes that occurred following caerulein treatment indicated a difference in the response between WT and Mist1 KO mice (Supplemental Table 2). These genes were categorized based on function determined through GNF SymAtlas (symatlas.gnf.org/SymAtlas; Supplemental Appendixes 3 and 4).

From this analysis, it became clear that the expression of numerous stress-related genes was increased following the induction of pancreatitis in WT mice. Surprisingly, many of these genes, including c-fos and c-jun (activator protein 1 transcription complex), EGR1, Ier1, Ier2, Ier5, and numerous Hsps, failed to accumulate in Mist1 KO pancreatic tissue following caerulein treatment (Table 1). As a validation of the array data, Northern blot analysis for EGR1 and Ier2 verified increased expression in WT tissue at 4 h into caerulein treatment and limited, to no response in Mist1 KO tissue (Fig. 4A).

Previous studies (29, 31) have identified an induction of JNK signaling following supramaximal stimulation with CCK, and JNK is linked to numerous stress response pathways. Therefore, we examined the activation of JNK through examination of its phosphorylation status. In addition, the expression of c-fos and activation of c-Jun, which are both downstream targets of the JNK pathway, were also examined. Northern blot

1 Supplemental information for this article is available at the American Journal of Physiology-Gastrointestinal and Liver Physiology website.
analysis confirmed an increase in c-fos expression by 4 h into caerulein treatment of WT mice with no increased expression in Mist1 KO tissue (Fig. 4A). An examination of active JNK and c-Jun protein by Western blot analysis also confirmed an increase in the activation of both proteins associated with CIP after 4 h in WT mice (Fig. 4B). Whereas Mist1 KO mice exhibited increased activity of c-Jun, there was significantly less activity compared with WT mice. No increase was observed in the phosphorylation state of JNK, indicating limited activation of this signaling pathway in Mist1 KO mice. Therefore, the increased accumulation of immediate response genes that occurs during CIP in WT mice was absent in Mist1 KO mice, suggesting a deficit in the ability of these animals to activate stress response pathways.

### Table 1. Genes involved in the stress response that were identified as differentially expressed in WT but not Mist1 KO pancreatic tissue following caerulein-induced pancreatitis

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Relative Increase in WT Tissue</th>
<th>Relative Increase in Mist1 KO Tissue</th>
<th>Fold Difference in Expression Between WT and Mist1 KO Tissue</th>
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<tbody>
<tr>
<td>Sesn2/Hif95</td>
<td>8.4</td>
<td>1.1</td>
<td>13.8</td>
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<tr>
<td>Cebpβ/Nf-il6</td>
<td>8.1</td>
<td>1.2</td>
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<td>Herpud1</td>
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<td>0.3</td>
<td>9.58</td>
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<td>Nfat3/E4BP4</td>
<td>108.3</td>
<td>14.4</td>
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Relative increases in wild-type (WT) tissue gene expression levels were calculated from raw array data in WT animals treated with caerulein/those treated with saline. Relative increases in Mist1 knockout (KO) tissue gene expression levels were calculated from raw array data in Mist1 KO animals treated with caerulein/those treated with saline. Fold differences in expression levels between WT and Mist1 KO tissue were calculated from raw array data from WT animals treated with caerulein/Mist 1 KO animals treated with caerulein.

Fig. 3. Mist1 KO pancreatic tissue exhibits cellular and tissue damage during CIP. A–F: hematoxylin and eosin (H&E)-stained sections from WT (A and C) and Mist1 KO mice (B and D–F) at 8 h (A and B) and 24 h (C–F) after the initiation of CIP revealed significant cellular damage in Mist1 KO tissue, including vascular leakage (black arrowheads), intracellular vacuolation (black arrows), and distended lumen of acini (*). WT tissue exhibited some acinar cell disorganization and apoptotic bodies (white arrows) but limited tissue damage. Magnification bar = 50 μm. G and H: evaluation of pancreatic tissue architecture in WT (G) and Mist1 KO (H) pancreatic tissue revealed necrosis (white arrows) in Mist1 KO tissue at 24 h after the initial caerulein treatment. Necrosis was significantly reduced in WT and Mist1 KO tissue at 4, 8, 24, and 72 h (*P < 0.05) after the initiation of pancreatitis (n = 3 for all time points). All mice received 7 hourly injections except those killed at 4 h. Values represent means ± SE.
The ER stress response is altered in pancreatic acini from Mist1 KO mice. We observed that several genes that are part of the ER stress response pathway were upregulated to a much higher degree in WT mice following CIP, including ATF3, ATF4, Hsp68, immunoglobulin heavy chain binding protein (BiP)/glucose-regulated protein 78 (GRP78), and gadd34 (see Table 1). To confirm that the ER stress pathway was differentially activated in the Mist1 KO pancreas during CIP, the expression of ATF3, which is activated following phosphorylation of eIF2α/H9251, was determined by Western blot analysis. In WT tissue, a dramatic increase in the level of ATF3 protein was observed within 1 h after the initiation of CIP, suggesting an activation of the stress response pathway (Fig. 5A). Alternatively, there was only a mild and transient increase in ATF3 accumulation in Mist1 KO tissue, supporting the idea that the stress response pathway is altered in the absence of Mist1.

Similar analysis of Gadd34 expression revealed a strikingly different pattern of expression. During the ER stress response, gadd34 is activated by ATF3 and acts as a negative feedback mechanism by combining with protein phosphatase 1 (PP1) and causing the dephosphorylation of eIF2α (40). In WT tissue, there was only a slight increase in protein accumulation at 4 h following CIP (Fig. 5A). Conversely, Gadd34 protein accumulated to higher levels in Mist1 KO pancreatic tissue at all time points examined. This was somewhat surprising given our array data, which indicated that Gadd34 mRNA does not accumulate in Mist1 KO tissue. Therefore, we examined the expression of Gadd34 mRNA by Northern blot analysis. As indicated by the array data, there was significantly more Gadd34 mRNA accumulating at 4 h of CIP in WT mice compared with Mist1 KO tissue (Fig. 5B). However, at 1 h after the induction of CIP, there was a robust accumulation of Gadd34 mRNA in both WT and Mist1 KO samples. These results further indicate that there are alterations in the ER stress response pathway to CCK stimulation in Mist1 KO mice likely leading to a muted response.

If the increased expression of Gadd34 is causing a block in the ER stress pathway, then one would expect a block in the phosphorylation of eIF2α. As would be expected following the activation of ER stress, Western blot analysis of WT protein extracts confirmed increased levels of phosphorylated eIF2α 1 and 4 h after the induction of pancreatitis. Conversely, examination of protein extracts from the Mist1 KO pancreas revealed no increase in eIF2α phosphorylation (Fig. 5A). To determine if the lack of stress response was due to an alteration
in the CCK signaling pathway or to a more general defect in Mist1 KO acinar cells, primary acinar cells were exposed to thapsigargin and tunicamycin (Fig. 5C), which stimulate ER stress. In both cases, WT acinar cells exhibited an increase in ATF3 accumulation following the introduction of the stress. However, neither thapsigargin nor tunicamycin stimulated increased ATF3 accumulation in isolated Mist1 KO acinar cells. Therefore, in the absence of Mist1, pancreatic acinar cells are unable to appreciably activate the ER stress pathway.

Acinar cells from Mist1 KO mice are more resistant to CCK-stimulated apoptosis. One of the primary outcomes of stimulating ER stress is activation of the apoptotic program (21) (see Fig. 8A). If the ER stress response is affected in Mist1 KO pancreatic tissue, then a decrease in apoptosis should be observed in Mist1 KO acinar cells. Acinar cells were purified from pancreatic tissue and stimulated with physiological (30 pM) or pathological (10 nM) concentrations of CCK (Fig. 6). Approximately 3% of all WT acinar cells were apoptotic following the incubation in PBS alone, whereas only 1% of Mist1 KO acinar cells were apoptotic under similar conditions ($P < 0.05$). Stimulation with physiological levels of CCK did not affect apoptosis levels, whereas stimulation with 10 nM CCK significantly increased apoptosis in WT cells (4.5%), confirming that supramaximal stimulation with CCK can induce apoptosis. Conversely, Mist1 KO acinar cells showed no increase in apoptosis compared with PBS-stimulated acinar cells. Therefore, as predicted, Mist1 KO acinar cells have an inherent defect in their ability to undergo apoptosis following increased CCK signaling.

Similar differences in the amount of apoptosis were also observed between WT and Mist1 KO acinar cells in vivo following the initiation of pancreatitis. Histological analysis revealed the presence of apoptotic bodies in both WT and Mist1 KO pancreatic tissue (Fig. 7, A and B). However, numbers of apoptotic bodies were lower in Mist1 KO pancreatic sections (Fig. 7C). At both 4 and 8 h, there were significantly more apoptotic bodies in WT tissue compared with Mist1 KO tissue. By 24 h, the numbers of apoptotic bodies decreased to negligible amounts in both lines. Fewer apoptotic cells were also identified by TUNEL analysis 4 h after the initial caerulein injection (data not shown). The amount of acinar cell apoptosis following the induction of pancreatitis is inversely correlated to the severity of pancreatitis (4), and these results support the model that the ER stress response is altered in Mist1 KO mice leading to increased sensitivity to pancreatic injury.

**DISCUSSION**

The Mist1 KO mouse model is the first example described where a mouse harboring a simple deletion of a gene necessary for proper acinar cell development has been assessed for increased sensitivity to pancreatic insult. Through the analysis of serum amylase levels and tissue morphology, we have clearly shown that Mist1 KO mice are more sensitive to...
pancreatic insult, develop more severe disease symptoms, and require a longer period of time in which to recover compared with WT mice. The increased sensitivity to pancreatitis may be due to the inability to activate the ER stress response in Mist1 KO acinar cells, which would typically lead to increased protein degradation and apoptosis that would be protective against widespread tissue damage. Importantly, this study highlights the importance of proper acinar cell maturation and function in protecting the exocrine pancreas from significant damage during pancreatic insult.

**ER stress as a mediator of pancreatitis severity.** Based on our data and previously published reports, we suggest a mechanism that accounts for the deficient stress response in Mist1 KO mice (Fig. 8A). Under conditions that promote acinar cell stress, the ER stress response pathway is activated with BiP/GRP78 dissociating from IRE1 and PERK (2). The activation of IRE1 results in the increased expression of apoptosis signal-regulating kinase 1 (39), promoting JNK activation and increased apoptosis (35, 49), whereas the activation of PERK leads to the phosphorylation of eIF2α (14, 15). The phosphorylation of eIF2α blocks its ability to associate with the translational complex, thereby causing a general repression of translation (21). At the same time, there is increased expression of the transcription factors ATF3 and ATF4 (6, 22), which activate gadd34 expression (22, 40). Gadd34 acts as a negative feedback mechanism by recruiting PP1 to eIF2α (40), promoting dephosphorylation and restoration of translation. Under continued exposure to the stress, Gadd34 is translated at low levels to prevent it from providing negative feedback at the level of eIF2α phosphorylation.

In support of such a model, our array data have identified increased expression of most of these factors during CIP in WT pancreatic tissue (Table 1), and we have confirmed the increased accumulation of phospho-eIF2α, ATF3, and Gadd34. A recent study (28) has revealed that the ER stress response pathway is activated during L-arginine-induced pancreatitis, although the importance of this pathway to promoting or limiting disease severity was not determined. However, during CIP, Hsp70.1, a chaperone molecule activated by ER stress, is expressed to higher levels and is believed to be protective against the progression of pancreatic damage (3). In addition, several studies (4, 5, 25) have identified an inverse correlation between the amount of acinar cell apoptosis and severity of pancreatitis, likely due to a concomitant increase in necrosis within the pancreas.

In Mist1 KO acinar cells, these events do not occur (Fig. 8B). There is a general suppression of ER stress-mediated transcription, decreased activation of JNK and its targets, and decreased acinar cell apoptosis. We propose that these deficits are linked to a block in the phosphorylation of eIF2α, which would not allow for the activation of the acinar cell stress response mechanism. Mist1 KO pancreatic tissue contains elevated amounts of Gadd34 protein, which would prevent the inactivation of eIF2α and the increased expression of ATF3 and other genes in this pathway (i.e., ATF4, Hsp70, etc.). At the same time, JNK is not activated, therefore not allowing for the induction of immediate-early response genes (Ier2, EGR1, and c-fos), increased activation of c-Jun, or the activation of the apoptotic pathway. JNK can also be activated directly by CCK receptor signaling in pancreatic acinar cells (8), so the decreased activation of c-Jun may also be attributed to alterations in this signaling pathway.

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**Fig. 8.** Model for differential activation of the ER stress pathway in WT and Mist1 KO acinar cells. A: in WT acinar cells, pancreatitis activates protein kinase R (PKR) like ER kinase (PERK), causing the phosphorylation of eIF2α, leading to a general decrease in the translation and activation of ATF3 gene expression. ATF3 expression causes an upregulation of gadd34, which, when translated, acts as a negative feedback mechanism on the phosphorylation eIF2α. At the same time, IRE1 is activated, leading to increased expression of apoptosis signal-regulating kinase 1 (ASK1), which promotes JNK activation. JNK signaling activates the expression of immediate-early response genes such as EGR1, Ier2, and c-fos and, combined with ER stress, leads to acinar cell apoptosis. B: in Mist1 KO acinar cells, JNK activation is limited, there is no increase in eIF2α phosphorylation and ATF3 and ASK1 expression, and there are already elevated levels of Gadd34 protein. These events result in a block in the expression of immediate-early response genes, muting of the ER stress pathway, and an inability to activate the apoptotic pathway, thereby leading to increased severity of pancreatitis. The solid arrows represent alterations to this pathway identified in this study, whereas the open arrows represent inferred changes based on the array data. The dashed arrows represent a reduction in these pathways.
Previous results from our laboratory and others (34, 43) have shown this pathway to be affected in the absence of Mist1.

So how would activation of the ER stress pathway protect against pancreatic injury? During pancreatitis, Ca\(^{2+}\) homeostasis is greatly altered, with higher than normal levels maintained in the cytosol (26, 27, 37). Increased cytosolic Ca\(^{2+}\) levels are maintained at the expense of the ER and Golgi complex, where protein processing occurs. Proper processing and folding of proteins is critical for sorting into appropriate cell compartments and vesicles, includingzymogens targeted for secretion, and this is heavily dependent on intraorganelle Ca\(^{2+}\) concentrations. Since the primary outcome of activating the ER stress response is a general cessation of protein translation, activation of this pathway prevents the accumulation of improperly folded proteins and limits premature enzyme activation and cellular damage. Decreased activation of ER stress is observed in aged cells (31), and deficits in this pathway have been linked to degenerative disorders other than pancreatic, such as Parkinson’s disease and Alzheimer’s disease (18, 25) and diabetes (36).

**Link of Mist1 to the disease process.** It is unclear if the CIP effects observed in Mist1 KO mice are due to the specific loss of Mist1 transcriptional activity or due to the development of a more susceptible environment in its absence. Deletion of Mist1 results in abnormal acinar cell organization, gene expression, and function. However, Mist1 KO mice are viable, fertile, and indistinguishable from WT littermates at a gross morphological level (43). To date, it has been difficult to establish a molecular hierarchy by which the loss of Mist1 leads to exocrine cell defects. This is probably due to fact that Mist1 regulates a number of exocrine-specific genes that affect numerous cellular functions (23, 43, 44). Our array analysis has revealed a large number of genes that are differentially expressed between WT and Mist1 KO pancreatic tissue, but likely these changes represent a combination of direct targets of Mist1 as well as the consequences of an altered pancreatic environment.

Regardless of how the loss of Mist1 leads to the pancreatic phenotypes observed in Mist1 KO mice, these mice still respond to pancreatitis in a dramatically different way at the molecular level compared with WT mice (Supplemental Appendixes 3 and 4). It is possible that these changes are directly due to an absence in Mist1 transcriptional activity in Mist1 KO mice. However, it is likely that the altered acinar and pancreatic environments that exist in Mist1 KO mice also contribute to disease severity. For example, Mist1 KO pancreatic tissue has increased numbers of stellate cells that are activated during the early stages of pancreatitis and promote the fibrosis that is associated with the disease (43). In addition, even though Mist1 is expressed exclusively in the exocrine pancreas, work from our laboratory (10) has indicated that older Mist1 KO mice develop a reduced tolerance to elevated glucose levels, likely due to specific effects on the endocrine tissue.

While it can be argued that the presence of stellate cells within the Mist1 KO pancreas prior to stimulation may be responsible for the increased pancreatitis severity, there is compelling evidence that deficits within the acinar cells themselves contribute to the pathogenesis of the disease. We have documented a reduced ability for Mist1 KO acinar cells to initiate apoptosis, which is crucial for the protection of pancreatic tissue from more widespread disease (4). Importantly, the inability to initiate apoptosis is a cell autonomous defect since purified acinar cells from Mist1 KO pancreatic tissue exhibit a decreased ability to undergo apoptosis either before or after stimulation with high levels of CCK. We have also shown that the inability to activate stress response genes is also an acinar-specific defect for Mist1 KO acinar cells. Tunicamycin and thapsigargin, both of which stimulate ER stress (32, 52), cause activation of ATF3 only in WT acinar cell cultures. This would suggest that the increased disease severity we observed in Mist1 KO mice is not limited to the caerulein model of the induction of pancreatitis.

In conclusion, this study identifies the Mist1 KO mouse as an important new model for studying the environmental and molecular factors affecting the initiation, progression, and severity of acute pancreatitis. Continued analysis of Mist1 KO mice will enable us to better understand the pathways involved in pancreatic disease and how these pathways can be manipulated to ameliorate symptoms of the disease.

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