Published in final edited form as:

Gastroenterology. 2013 January ; 144(1): 26–29. doi:10.1053/j.gastro.2012.11.016.

Nuclear Factor- κ B in Pancreatitis: Jack-of-All-Trades, But Which One Is More Important?

ILYA GUKOVSKYANNA GUKOVSKAYA

VA Greater Los Angeles Healthcare System and University of California at Los Angeles Los Angeles, California

Abstract

See “Deletion of I κ B α activates RelA to reduce acute pancreatitis in mice through up-regulation of Spi2A,” by Neuhöfer P, Liang S, Einwächter H, et al, on page 192; and “Activation of nuclear factor- κ B in acinar cells increases the severity of pancreatitis in mice,” by Huang H, Liu Y, Daniluk J, et al, on page 202.

The nuclear factor (NF)- κ B inducible transcription factors are critical regulators of cell behavior both in health and disease.^{1–7} NF- κ B mediates the expression of hundreds of genes that play key roles in inflammation, immunity, cell survival and proliferation, and is induced by a great variety of stresses. There are 5 NF- κ B family members in mammals: RelA/p65, RelB, c-Rel, p50 (derived from its precursor p105/NF- κ B1), and p52. NF- κ B proteins bind to their cognate DNA sites as homo- or heterodimers, such as the prototypical p50-p65, and activate (in most cases) or negatively regulate target gene transcription. In most “resting” cells, NF- κ B is kept inactive in the cytoplasm through sequestration in complexes with the inhibitor of NF- κ B (I κ B) proteins, such as I κ B α and I κ B β . The sequestration prevents NF- κ B migration into the nucleus, its’ binding to DNA, and transcriptional activation. Typically, in response to an inducing stimulus the I κ Bs are phosphorylated on specific Ser residues by I κ B kinases (IKKs), which results in I κ B ubiquitylation and proteasome-mediated degradation, allowing NF- κ B nuclear translocation. The IKK complex is composed of 2 related kinases, IKK1 (or IKK α) and IKK2 (or IKK β), and a scaffolding protein NEMO/IKK γ , which is critical for IKK activation. IKK activation involves recruitment of adaptor proteins and multiple ubiquitination events. The main (“canonical”) pathway, which is triggered by proinflammatory cytokines, for example, tumor necrosis factor- α , is primarily mediated by IKK2, and results in activation of p50-p65 and induction of genes regulating inflammation and cell survival. Transcriptional activity of NF- κ B dimers is also regulated by their posttranslational modification, for example, C-terminal phosphorylation of p65. Thus, NF- κ B activation is mediated and regulated at 3 levels: IKK, I κ B, and the NF- κ B dimers.

Inflammation and parenchymal cell death are key pathologic responses of pancreatitis and determinants of disease severity.⁸ Although the mechanisms initiating pancreatitis are not

Address requests for reprints to: Ilya Gukovsky, Pancreatic Research Group, West Los Angeles VA Healthcare Center, 11301 Wilshire Blvd, Bldg 258, Rm 340, Los Angeles, California 90073. igukovsk@ucla.edu.

Conflicts of interest

The authors disclose no conflicts.

clear, the disease is believed to originate in injured acinar cells. In most patients, the ensuing inflammatory response and pancreatic damage ultimately resolve, but in severe cases unrelenting systemic inflammatory response syndrome leads to multiple organ (especially lung) failure, a major cause of mortality in acute pancreatitis.⁸ Uncontrolled inflammation also contributes to parenchymal necrosis, greater amounts of which bode a worse prognosis.

Dramatic increases in cytokines and chemokines were found in 1990s in both human and experimental pancreatitis.⁹ It was further shown that injured acinar cells themselves produce and release a variety of inflammatory mediators,^{10,11} followed by recruitment of inflammatory cells into the pancreas and systemic inflammation.¹² Prompted by these results, an early pancreatic NF- κ B activation was found in the “classical” cerulein model of acute pancreatitis and the ex vivo model of CCK-hyperstimulated acinar cells.^{13,14} Since then, NF- κ B activation in pancreatitis has been studied in >250 publications¹⁵; it occurs in all experimental models, involves p50-p65 activation (i.e., the canonical pathway) mediated by degradation of both I κ B α and I κ B β , and is associated with induction of cytokines and other inflammatory mediators. Mechanisms mediating NF- κ B activation in acinar cells involve cytosolic Ca²⁺ increase and novel PKC isoforms.¹⁵ The p50-p65 and p50-p50 dimers are the only activated NF- κ B complexes shown in pancreatitis.

In most studies, pharmacologic NF- κ B inhibition ameliorated the inflammatory response, necrosis, and other parameters of pancreatitis severity.¹⁵ However, the pharmacologic agents were largely nonspecific, such as antioxidants and proteasomal inhibitors. The first knockout study¹⁶ found amelioration of cerulein pancreatitis in mice deficient in p50/p105, with decreased tumor necrosis factor- α level and leukocyte accumulation. In accord with these data, adenoviral transfer of RelA/p65 to elevate NF- κ B in pancreas resulted in neutrophil infiltration and pancreatic and lung injury.¹⁷ Thus, a consensus was building that NF- κ B activation in pancreatitis is detrimental. The next significant development came in 2007 in 2 studies,^{18,19} which both used the Cre/loxP technology, but came to mutually opposing conclusions. In one of them,¹⁸ acinar-cell-specific, inducible expression of constitutively active (CA) IKK2 was sufficient to trigger severe pancreatitis in the transgenic mice, and also aggravated cerulein pancreatitis. Furthermore, acinar-cell-specific expression of dominant negative IKK2 significantly improved cerulein pancreatitis. These results confirmed and expanded the previous findings.¹⁵ In stark contrast, the study¹⁹ from Schmid’s group found aggravation of cerulein pancreatitis in mice with pancreas-specific ablation of *rela* gene (termed *rela*^{panc}), with more parenchymal necrosis and more inflammation in pancreas and lung. The authors concluded that p65 protects acinar cells from death associated with inflammation. Their findings revealed a previously unappreciated complexity of NF- κ B roles in pancreatitis. However, the results of these 2 studies^{18,19} created a paradox in that both activation and inhibition of NF- κ B in acinar cells aggravated disease.

The 2 new studies^{20,21} in this issue of GASTROENTEROLOGY continue along these “parallel tracks”— one²⁰ using mostly inhibition, and the other²¹ activation of the IKK/NF- κ B pathway. Neuhöfer et al²⁰ (the Schmid-Algül group) generated mice with pancreas specific ablation of I κ B α (termed *I κ B α* ^{panc}), with the rationale that this would lead to constitutive activation of p65. The *I κ B α* ^{panc} mice, indeed, showed some basal NF- κ B

activation and small increases in pancreatic cytokines and chemokines, which did not cause any overt phenotype. But cerulein- and L-arginine-induced pancreatitis was ameliorated in *I κ B α ^{panc}* mice, with less pancreatic and lung injury and improvements in serum amylase, pancreatic necrosis, and trypsin activity.²⁰ To further prove that the protective effects of *I κ B α* ablation were through p65, the authors crossed *I κ B α ^{panc}* with *rela^{panc}* mice and showed that amelioration of cerulein pancreatitis was lost in the double-knockout *I κ B α ^{panc} × rela^{panc}* mice. The results reinforce this group's previous conclusion¹⁹ that acinar cell NF- κ B activation is protective in acute pancreatitis. Microarray analysis implicated serine protease inhibitor 2A (Spi2A) in the protective effect of NF- κ B, and lentiviral-mediated expression of Spi2A improved cerulein pancreatitis.²⁰

By contrast, Huang et al²¹ (the Logsdon-Ji group) pursued the strategy of directly activating acinar cell NF- κ B by developing several strains of transgenic mice. One strain was generated by crossing mice expressing “loxP-stop-loxP-p65” (LSL-p65) construct and mice with inducible Cre recombinase under a strong acinar-cell-specific promoter. LSL excision in the resultant LSL-p65/Cre mice, indeed, caused an increase in p65, but this was compensated by greatly upregulated *I κ B α* (the *I κ B α* gene expression is induced by NF- κ B) and did not lead to any obvious phenotype. However, upon induction of cerulein pancreatitis the LSL-p65/Cre mice displayed greater pancreatic NF- κ B activation, early and dramatic induction of proinflammatory cytokines, and more severe disease, compared with controls, that did not express transgenic p65 (eg, harboring Cre alone). Thus, in contrast with Neuhöfer et al,²⁰ the high level of acinar cell p65 did not protect, but aggravated pancreatitis. Furthermore, repeated administration of modest amounts of cerulein, which did not cause pancreatic damage in control mice, resulted in chronic pancreatitis injury in LSL-p65/Cre mice, with persistent inflammation and fibrosis.²¹ The second strain of transgenic mice, LSL-IKK2/Cre, similarly allowed inducible, acinar-cell-specific expression of IKK2-CA. In these mice, there was no compensatory up-regulation of *I κ B α* , and the induction of IKK2-CA resulted in spontaneous pancreatitis within 7 days. The pancreas damage and inflammation increased with longer times of IKK2-CA expression. Furthermore, the injury was worse in triple transgenic LSL-IKK2/LSL-p65/Cre mice that combined elevated levels of p65 and IKK2-CA expression. Within several days, these mice spontaneously developed severe inflammation as well as pancreatic fibrosis (in particular, increased α -smooth muscle actin). The results²¹ are in accord with the previous findings from Logsdon's group¹⁷ and the results from Baumann et al¹⁸ on IKK2-CA and dominant negative IKK2 transgenic mice.

The 2 studies^{20,21} perpetuate the paradox that activation of IKK2 or p65 leads to both aggravation (even induction) and amelioration of pancreatitis. However, this paradox can be resolved, at least in part, by realizing that acinar cell NF- κ B activation, indeed, triggers both pro- and anti-inflammatory pathways (Figure 1). A major pro-inflammatory mechanism, emphasized in Ref. 21, is the initial release of cytokines and other mediators induced by NF- κ B. The opposite effect of NF- κ B^{19,20} is protection against acinar cell necroptosis that can limit pancreatic inflammation through as-yet unclear mechanisms. These mechanisms may involve limiting inflammasome activation by factors released from damaged or necrotic cells.²² Of note, the role of inflammasome in pancreatitis is just beginning to be explored.²³ The protective effect of NF- κ B could also be through limiting interleukin (IL)-1 β processing

and secretion.²⁴ In this regard, it would be instructive to compare changes in the pancreatic and circulating levels of IL-1 β between the transgenic NF- κ B strains at different stages of cerulein pancreatitis.

The balance between these—as well as other yet undefined—mechanisms determines the extent, pattern, and kinetics of the inflammatory response, thus “integrating” the various effects of NF- κ B activation. The higher level of NF- κ B activity in transgenic mice generated in Huang et al²¹ clearly favors the proinflammatory “arm”; and increased local inflammation promotes acinar cell necrosis,^{25,26} which may overpower the protective, prosurvival effect of NF- κ B. It is not so clear whether pancreatitis-induced inflammation is less or more in the *I κ B α ^{panc}* mice, as the authors themselves state.²⁰ In addition, the different approaches targeting IKK2, I κ B α and p65 in^{20,21} may have nonoverlapping effects (see below).

The complexities of the IKK/I κ B/NF- κ B system, in particular both pro- and anti-inflammatory effects, are discussed in numerous reviews that appeared in 2012 to commemorate 25 years since the discovery of NF- κ B (eg, a special issue of *Immunological Reviews*^{4–7}). The following are some of the aspects relevant to^{20,21} (1) both the extent and the mode of NF- κ B manipulation can have pro-foundly different consequences, as shown in the liver and intestine, and in particular on mice with NEMO ablation (essentially complete blockade of NF- κ B) versus IKK2 ablation (less NF- κ B inhibition) versus manipulating I κ B (variable extent of NF- κ B inhibition).^{1,2,6} In this regard, it will be interesting to assess the effects of NEMO ablation on experimental pancreatitis. (2) Modulating the NF- κ B pathway has very different effects on the acute versus chronic inflammation and tissue damage, as shown by IKK2 ablation in intestinal epithelial cells.^{27,28} That is why it would be important to carefully examine the time-course of the effects of manipulating NF- κ B in pancreatitis, in particular at the early, acute stage versus the repair/resolution stage. Equally important is elucidation of the role of NF- κ B activation in inflammatory cells, which is often opposite to that in epithelial (ie, acinar) cells. Myeloid-cell specific NF- κ B inhibition has been utilized for this purpose in other organs (eg, Eckmann et al²⁸) and was shown in a chronic pancreatitis model to decrease overall inflammation and ameliorate disease.²⁹ (3) Manipulating the IKK/I κ B/NF- κ B system at 1 level can perturb pathways that would not be affected by modulation at other levels. For example, the IKKs have targets beyond the NF- κ B pathway, and some of their effects are independent of IKK kinase activity.^{1–3} Such pathways would not be affected by modulating “lower” levels, namely, p65. On the other hand, p65 ablation affects pathways involving p65 posttranslational modifications and interactions with other transcription factors^{1–5} (such as p53, shown to play a role in pancreatitis³⁰). The absence of p65 can be compensated by other NF- κ B dimers,⁵ which obviously does not occur when manipulating the IKKs.

One objective of Neuhöfer et al²⁰ was to explore the interrelations between intra-acinar NF- κ B and trypsinogen activation, both early events in pancreatitis. Findings from different studies^{17,31–33} show that intra-acinar trypsin does not affect NF- κ B; however, whether NF- κ B activation in acinar cells can or cannot directly modulate trypsin activity is not quite clear. One way of testing this would be to measure agonist (eg, CCK)-induced trypsin activity in acinar cells isolated from NF- κ B transgenic mice,^{20,21} to bypass the effects of infiltrating inflammatory cells on trypsinogen activation in vivo.^{26,34} In our studies

(unpublished observations), the blockade of NF- κ B activation in p50^{-/-} p65^{+/-} acinar cells had no effect on CCK-induced trypsin activity, indicating there is no direct connection between the 2 events.

A common theme in the reviews on IKK/I κ B/NF- κ B¹⁻⁷ is that this system is strikingly “context-dependent” and its’ fine-tuning will require exhaustive characterization of the underlying mechanisms. The studies in this issue of GASTROENTEROLOGY^{20,21} expand our knowledge of NF- κ B in pancreatitis, but also underscore both its complexity and the gaps in our understanding of this pathway. Building on these results, elucidation of the many and diverse “trades” of NF- κ B will clarify its role in the pathogenesis of pancreatitis and, hopefully, harness the therapeutic potential of this system for treatment of pancreatitis.

References

- Hayden MS, Ghosh S. NF- κ B, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev* 2012;26:203–234. [PubMed: 22302935]
- Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol* 2007;8:49–62. [PubMed: 17183360]
- Chariot A. The NF-kappaB-independent functions of IKK subunits in immunity and cancer. *Trends Cell Biol* 2009;19:404–413. [PubMed: 19648011]
- Hinz M, Arslan SÇ, Scheidereit C. It takes two to tango: I κ Bs, the multifunctional partners of NF- κ B. *Immunol Rev* 2012;246: 59–76. [PubMed: 22435547]
- Smale ST. Dimer-specific regulatory mechanisms within the NF- κ B family of transcription factors. *Immunol Rev* 2012;246:193–204. [PubMed: 22435556]
- Pasparakis M. Role of NF- κ B in epithelial biology. *Immunol Rev* 2012;246:346–358. [PubMed: 22435565]
- DiDonato JA, Mercurio F, Karin M. NF- κ B and the link between inflammation and cancer. *Immunol Rev* 2012;246:379–400. [PubMed: 22435567]
- Pandol SJ, Saluja AK, Imrie CW, et al. Acute pancreatitis: bench to the bedside. *Gastroenterology* 2007;132:1127–1151. Erratum: *ibid*, 133:1056. [PubMed: 17383433]
- Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. *Am J Surg* 1998;175:76–83. [PubMed: 9445247]
- Gukovskaya AS, Gukovsky I, Zaninovic V, et al. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor- α . Role in regulating cell death and pancreatitis. *J Clin Invest* 1997;100:1853–1862. [PubMed: 9312187]
- Grady T, Liang P, Ernst SA, et al. Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. *Gastroenterology* 1997;113:1966–1975. [PubMed: 9394737]
- Bhatia M, Brady M, Shokuhi S, et al. Inflammatory mediators in acute pancreatitis. *J Pathol* 2000;190:117–125. [PubMed: 10657008]
- Gukovsky I, Gukovskaya AS, Blinman TA, et al. Early NF-kappaB activation is associated with hormone-induced pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 1998;275:G1402–G1414.
- Steinle AU, Weidenbach H, Wagner M, et al. NF-kappaB/Rel activation in cerulein pancreatitis. *Gastroenterology* 1999;116:420–430. [PubMed: 9922324]
- Rakonczay Z Jr., Hegyi P, Takacs T, et al. The role of NF-kappaB activation in the pathogenesis of acute pancreatitis. *Gut* 2008; 57:259–267. [PubMed: 17675325]
- Altavilla D, Famulari C, Passaniti M, et al. Attenuated cerulein-induced pancreatitis in nuclear factor-kappaB-deficient mice. *Lab Invest* 2003;83:1723–1732. [PubMed: 14691290]
- Chen X, Ji B, Han B, et al. NF-kappaB activation in pancreas induces pancreatic and systemic inflammatory response. *Gastroenterology* 2002;122:448–457. [PubMed: 11832459]

18. Baumann B, Wagner M, Aleksic T, et al. Constitutive IKK2 activation in acinar cells is sufficient to induce pancreatitis in vivo. *J Clin Invest* 2007;117:1502–1513. [PubMed: 17525799]
19. Algul H, Treiber M, Lesina M, et al. Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. *J Clin Invest* 2007;117:1490–1501. [PubMed: 17525802]
20. Neuhöfer P, Liang S, Einwächter H, et al. Deletion of I κ B α activates RelA to reduce acute pancreatitis in mice through up-regulation of Spi2A. *Gastroenterology* 2013;144:192–201. [PubMed: 23041330]
21. Huang H, Liu Y, Daniluk J, et al. Activation of nuclear factor- κ B in acinar cells increases the severity of pancreatitis in mice. *Gastroenterology* 2013;144:202–210. [PubMed: 23041324]
22. Strowig T, Henao-Mejia J, Elinav E, et al. Inflammasomes in health and disease. *Nature* 2012;481:278–286. [PubMed: 22258606]
23. Hoque R, Sohail M, Malik A, et al. TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. *Gastroenterology* 2011;141:358–369. [PubMed: 21439959]
24. Greten FR, Arkan MC, Bollrath J, et al. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. *Cell* 2007;130:918–931. [PubMed: 17803913]
25. Sandoval D, Gukovskaya A, Reavey P, et al. The role of neutrophils and platelet-activating factor in mediating experimental pancreatitis. *Gastroenterology* 1996;111:1081–1091. [PubMed: 8831604]
26. Sandler M, Dummer A, Weiss FU, et al. Tumour necrosis factor alpha secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. *Gut* 2012 4 5 [Epub ahead of print].
27. Chen LW, Egan L, Li ZW, et al. The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. *Nat Med* 2003;9:575–581. [PubMed: 12692538]
28. Eckmann L, Nebelsiek T, Fingerle AA, et al. Opposing functions of IKKbeta during acute and chronic intestinal inflammation. *Proc Natl Acad Sci U S A* 2008;105:15058–15063. [PubMed: 18815378]
29. Treiber M, Neuhöfer P, Anetsberger E, et al. Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 2011;141:1473–1485. [PubMed: 21763242]
30. Nakamura Y, Do JH, Yuan J, et al. Inflammatory cells regulate p53 and caspases in acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2010;298:G92–100. [PubMed: 19850968]
31. Tando Y, Algül H, Schneider G, et al. Induction of IkappaB-kinase by cholecystokinin is mediated by trypsinogen activation in rat pancreatic lobules. *Digestion* 2002;66:237–245. [PubMed: 12592100]
32. Ji B, Gaiser S, Chen X, et al. Intracellular trypsin induces pancreatic acinar cell death but not NF-kappaB activation. *J Biol Chem* 2009;284:17488–17498. [PubMed: 19383608]
33. Dawra R, Sah RP, Dudeja V, et al. Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. *Gastroenterology* 2011;141:2210–2217. [PubMed: 21875495]
34. Gukovskaya AS, Vaquero E, Zaninovic V, et al. Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis. *Gastroenterology* 2002; 122:974–984. [PubMed: 11910350]
35. Sung KF, Odinokova IV, Mareninova OA, et al. Prosurvival Bcl-2 proteins stabilize pancreatic mitochondria and protect against necrosis in experimental pancreatitis. *Exp Cell Res* 2009; 315:1975–1989. [PubMed: 19331832]

Figure 1.

Pathways through which acinar cell NF- κ B activation regulates inflammation in pancreatitis. A major proinflammatory mechanism is the induction of cytokines and other mediators, as emphasized by Huang et al.²¹ In contrast, Neuhofer et al.²⁰ emphasize the protective effect of NF- κ B against acinar cell necroptosis, limiting pancreatic inflammation. The mechanisms underlying this prosurvival effect of NF- κ B are not clear but may involve PAP1,¹⁹ Spi2A,²⁰ Bcl-xL,³⁵ and other proteins. One mechanism whereby acinar cell necrosis may promote pancreatic inflammation is through activation of the inflammasome.^{22,23} The protective effect of NF- κ B activation could also be through limiting IL-1 β processing and secretion.²⁴