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Just the right amount of JNK – how NFkB and downstream mediators prevent burn-induced intestinal injury

Jessica A. Clark, PhD and Craig M. Coopersmith, MD, FCCM

Department of Surgery, Washington University School of Medicine, 660 S. Euclid Ave. St. Louis, MO 63110

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NFkB is a highly regulated transcription factor that controls the expression of an enormous number of genes involved in the immune response, inflammation, and cell survival (1). Mounting evidence suggests that NFkB plays a crucial role in the pathophysiology of critical illness by modulating expression of genes (cytokines, chemokines, receptors, etc.) that collectively determine the host response (2).

Under basal conditions, NFkB resides in the cytosol in an inactive form via interactions with inhibitor of kB (IkB) proteins. The classical NFkB signaling cascade is activated in response to diverse stimuli including injury, infection, and cellular stress signals. Upon activation, IkB kinase (IKK) initiates proteolytic degradation of the IkB complex, allowing NFkB to translocate into the nucleus and initiate gene transcription (3). IKK is a large complex of proteins that consist of two catalytic subunits (IKK α and IKK β) and a regulatory subunit. The classical NFkB pathway is dependent on IKK β (4).

Under pathophysiological conditions, cell fate depends on the integration of input from several pathways, including NFkB and the mitogen activated protein kinase (MAPK) signaling cascade (5). In general, NFkB prevents apoptosis by initiating transcription of caspase inhibitors and other anti-apoptotic molecules. In contrast, cell death is induced, in part, by activation of MAPKs such as the c-Jun-N-terminal kinase (JNK), which is activated following exposure to proinflammatory cytokines such as TNF- α (6). Suppression of JNK activation has been demonstrated to protect against TNF- α -induced cell death (7;8).

In this issue of *Critical Care Medicine*, Dr. Chen and colleagues report that knocking out NFkB in the intestinal epithelium by tissue-specific ablation of IKK β induces a higher degree of intestinal injury following burn than would ordinarily be seen following thermal injury (9). Following a 30% body surface area burn, mice lacking intestinal epithelial NFkB signaling (*Vil-Cre/Ikk^{F/A}* mice) have increased intestinal permeability, edema, villous tip sloughing, caspase 3, JNK and pp38 activity with decreased expression of the anti-apoptotic molecules Bcl-xL and cFLIP compared to burned wild type (WT) animals. Importantly, there is a differential effect of JNK inhibition in the intestine of burned *Vil-Cre/Ikk^{F/A}* and WT mice. Treatment of *Vil-Cre/Ikk^{F/A}* mice with SP600125 (a specific JNK inhibitor) following burn decreases intestinal permeability and increases pp38 and TRAF2. However, treatment with the same JNK inhibitor following burn in WT mice results in increased intestinal permeability.

Address correspondence to: Craig M. Coopersmith, MD Washington University School of Medicine 660 South Euclid Avenue Campus Box 8109 St. Louis, Missouri 63110 Phone: (314) 362–9342 Fax: (314) 362–1602 E-mail: coopersmithc@wustl.edu.

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These studies demonstrate that both NFkB and JNK signaling are critical in determining the gut's response to thermal-induced intestinal injury. In the setting of intact NFkB signaling, suppression of JNK phosphorylation worsens burn-induced barrier failure. However, in the absence of intestinal NFkB signaling, thermal injury increases JNK and worsens barrier failure which can be partially abrogated by JNK inhibition. This suggests that either too much or too little JNK signaling can be harmful in the intestine following burn injury.

Despite the many strengths of this study, a number of questions remain unanswered. On an organ scale, it is not clear what the connection is between worsened intestinal permeability, villous sloughing and caspase 3 expression in burned *Vil-Cre/Ikk^{F/A}* mice. The authors imply that increased caspase 3 is reflective of increased epithelial apoptosis and injury which, in turn, leads to worsening barrier function. However, they never actually demonstrate increased epithelial apoptosis. Elevated caspase 3 levels by Western blot taken from mucosal tissue can be reflective of epithelial or immune cell death. While NFkB ablation is epithelial specific, there is substantial evidence of epithelial-immune crosstalk within the intestine, and it is unclear from the data presented whether elevations in caspase 3 activation are of epithelial or immune (or both) origin. If gut epithelial apoptosis is increased in *Vil-Cre/Ikk^{F/A}* mice, this allows for further examination of the complex interplay between the NFkB and JNK pathways. Recent studies indicate that TNF- α -induced apoptosis relies upon JNK-dependent phosphorylation of the E3 ligase ITCH, which promotes cell death by degrading the NFkB-induced anti-apoptotic protein cFLIP (10). This link is poorly studied in critical illness and the model system used by Chen et al. could potentially be exploited to determine whether similar pathways exist in an injured host.

Further, although the authors convincingly show that permeability and gross histologic injury are worse following thermal injury in *Vil-Cre/Ikk^{F/A}* than WT mice, it is unclear if this is related to gut epithelial apoptosis. It has previously been demonstrated that barrier function is worsened and cell death is increased following burn (11). However, while there is associative evidence linking increased apoptosis with increased permeability following injury *in vivo*, a causal link between them has only been demonstrated *in vitro* (12;13).

More importantly, the functional significance of worsened burn-induced gut injury in *Vil-Cre/Ikk^{F/A}* mice is unclear. The intestine has been described as the “motor” of SIRS, and worsening intestinal permeability, injury and apoptosis are likely maladaptive in isolation since gut epithelial cell death is associated with increased mortality in sepsis (14). However, NFkB modulates a number of crucial genes, independent of gut homeostasis. Previous experiments on *Vil-Cre/Ikk^{F/A}* mice demonstrate that not only do they have a marked upregulation in gut epithelial apoptosis following ischemia/reperfusion, they fail to develop organ dysfunction and lung edema that are seen in WT mice subjected to the same insult (15). It is unknown if gut-specific ablation of NFkB has the same systemic effects in thermal injury as it does in ischemia/reperfusion. Even if it does, it is unclear whether preventing systemic inflammation at the cost of a marked increase in intestinal injury represents a beneficial or detrimental trade-off to the host in terms of mortality.

Because of the clinically relevant genes it induces, the NFkB signaling cascade is an appealing therapeutic target in critical illness. However, its double-edged nature -- upregulating inflammation while inhibiting cell death -- makes translating mechanistic insights into therapeutics a daunting task. One logical approach is to focus on targeting specific downstream mediators such as JNK and pp38 in an effort to design rationale targets at a molecular level that have beneficial effects at the whole body level. While more questions than answers remain, Dr. Chen and colleagues have taken us one step closer towards reaching this goal.

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Reference List

1. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol* 2002;3:221–227. [PubMed: 11875461]
2. Abraham E. NF-kappaB activation. *Crit Care Med* 2000;28:N100–N104. [PubMed: 10807322]
3. Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* 2006;357:13.
4. Li ZW, Chu W, Hu Y, et al. The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med* 1999;189:1839–1845. [PubMed: 10359587]
5. Tang G, Minemoto Y, Dibling B, et al. Inhibition of JNK activation through NF-kappaB target genes. *Nature* 2001;414:313–317. [PubMed: 11713531]
6. Papa S, Bubici C, Zazzeroni F, et al. The NF-kappaB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. *Cell Death Differ* 2006;13:712–729. [PubMed: 16456579]
7. Deng Y, Ren X, Yang L, et al. A JNK-dependent pathway is required for TNFalpha-induced apoptosis. *Cell* 2003;115:61–70. [PubMed: 14532003]
8. Ventura JJ, Cogswell P, Flavell RA, et al. JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. *Genes Dev* 2004;18:2905–2915. [PubMed: 15545623]
9. Chen L, Chen P, Chang W, et al. Ikb-kinase/NF-kB signaling prevents thermal injury-induced gut damage by inhibiting JNK activation. *Crit Care Med*. 2007THIS ISSUE DETAILS TO BE FILLED OUT BY CCM EDITORIAL STAFF
10. Chang L, Kamata H, Solinas G, et al. The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. *Cell* 2006;124:601–613. [PubMed: 16469705]
11. Spies M, Chappell VL, Dasu MR, et al. Role of TNF-alpha in gut mucosal changes after severe burn. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G703–G708. [PubMed: 12181186]
12. Yasuda T, Takeyama Y, Ueda T, et al. Breakdown of intestinal mucosa via accelerated apoptosis increases intestinal permeability in experimental severe acute pancreatitis. *J Surg Res* 2006;135:18–26. [PubMed: 16603187]
13. Abreu MT, Palladino AA, Arnold ET, et al. Modulation of barrier function during Fas-mediated apoptosis in human intestinal epithelial cells. *Gastroenterology* 2000;119:1524–1536. [PubMed: 11113074]
14. Coopersmith CM, Chang KC, Swanson PE, et al. Overexpression of Bcl-2 in the intestinal epithelium improves survival in septic mice. *Crit Care Med* 2002;30:195–201. [PubMed: 11902262]
15. 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]