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## Molecular mechanisms in the early phase of hemorrhagic shock

Received: 19 April 2001  
Accepted: 21 April 2001  
Published online: 20 June 2001  
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**Abstract** Hemorrhagic shock (HS) results in the initiation of an inflammatory cascade that is critical for survival following successful resuscitation. We identified a complex sequence of molecular events including shock-dependent and reperfusion-dependent responses that offer a new comprehensive approach for consequences of HS. Shock-dependent initializing mechanisms include the induction of inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and CD14 and play a catalyzing role for subsequent phenotypic changes following resuscitation. The early immediate response genes iNOS and COX-2 promote the inflammatory response by the rapid and excessive production of nitric oxide (NO) and prostaglandins. The transcription factor hypoxia-inducible factor-1 (HIF-1) may regulate the induction of iNOS during the

ischemic phase of shock. NO is an important signaling molecule which is involved in redox-sensitive mechanisms including the downstream activation of nuclear factor (NF)- $\kappa$ B. NO-dependent NF- $\kappa$ B activation promotes the induction of inflammatory cytokine expression during the reperfusion phase. Peroxynitrite-mediated direct toxicity and NO-mediated inflammatory toxicity contribute to organ injury. Patients suffering consequences of severe HS are susceptible to systemic inflammation, organ injury, and mortality if physiologic and therapeutic mechanisms are ineffective in limiting the activation of the inflammatory cascade.

**Keywords** Hemorrhagic shock · Inflammatory cascade · Shock-dependent and reperfusion-dependent mechanisms · Inflammatory signaling

### Introduction

Hemorrhagic shock (HS) initiates an inflammatory response that contributes to organ injury and early multi organ failure. The focus of this review was to delineate molecular mechanisms in the early phase of HS that promote the inflammatory response. HS can be characterized by two early phases: the shock phase followed by the post-resuscitation phase. We examined initiating mechanisms with the goal of identifying shock- and reperfusion-dependent responses [1]. The inflammatory response is defined by the upregulation of inflammatory cytokines [2], leukocyte adhesion molecules, and infil-

tration of neutrophils into tissues [3]. These changes occur in organs such as the lungs, the liver, and the gut and are likely to contribute to end organ damage and resultant dysfunction following resuscitation from shock. Our understanding of the mechanisms by which hemorrhage triggers this inflammatory response are evolving gradually. Heightened adrenergic activity [4], tissue hypoxia, and systemic release of pro-inflammatory agents from the gut [5, 6] have been hypothesized to contribute to the activation of inflammatory pathways and to acute organ injury following hemorrhage. In addition, reactive radicals are produced and have been implicated in a number of signal transduction pathways [7].

### **Shock-dependent events: iNOS, COX-2, CD14, HIF-1**

Inflammatory signaling pathways are activated very early following the induction of shock. In a recent study, we have shown that Jun kinase (JNK) is activated in the liver within 30 min of the initiation of shock [8]. This is followed by early upregulation of inflammatory response genes during the shock or ischemic phase and these genes participate in subsequent phenotypic changes that follow resuscitation. We have demonstrated the upregulation of three genes iNOS, cyclooxygenase (COX)-2, and CD14 during shock. We have recently identified a clear role for inducible nitric oxide synthase (iNOS) in the upregulation of the inflammatory response following resuscitation [1]. As early as 30 min, iNOS mRNA can be seen in the liver and protein is evident in hepatocytes shortly thereafter. Studies using isolated liver cells have shown that iNOS expression is regulated at the level of transcription as well as mRNA stability [9].

Induction of iNOS in HS may involve the activation of the hypoxia-inducible factor-1 (HIF-1). The transcriptional factor HIF-1 was discovered in hepatocytes subjected to hypoxia while studying the oxygen-dependent regulation of erythropoietin [10]. HIF-1 consists of an  $\alpha/\beta$  heterodimer and is widely expressed in hypoxic tissues [11]. HIF-1 binding sites were found in a number of genes including erythropoietin (EPO), vascular endothelial growth factor (VEGF), heme oxygenase (HO-1) and iNOS, as well as glycolytic enzymes [12]. HIF-1 controls the cellular adaptation to hypoxia including erythropoiesis, angiogenesis, vasodilatation and anaerobic metabolism.

The promoter region of iNOS contains a HIF-1 binding element [12]. Increased binding to the HIF-1 binding element may promote iNOS gene expression. HIF-1 activation occurred in an *in vivo* model of HS demonstrating HIF-1 activation in the lungs of rats subjected to ischemia [13]. We therefore propose a novel mechanism of HIF-1-dependent iNOS induction during ischemia in our rat model of HS [14]. Although HIF-1 is a key regulator of hypoxic gene expression, additional transcriptional factors including activator protein (AP)-1, nuclear factor (NF)- $\kappa$ B and p53 modulate the systemic response to hypoxia [12].

The release of prostaglandins is part of the inflammatory response and may mediate tissue inflammation and damage. COX-2 is the inducible enzyme that is capable of producing large amounts of prostaglandins. In previous reports, COX-2 upregulation occurred in conditions of redox stress, such as focal cerebral ischemia and reperfusion [15], and hypoxia. The expression of COX-2 increased in the lungs of animals subjected to compensated (1 h), decompensated (2.5 h), and irreversible (3.5 h) ischemic shock relative to sham control animals. Upregulation of COX-2 mRNA expression was dependent on the severity of shock but independent of the reperfusion phase [13].

The mechanisms leading to increased COX-2 expression in HS have not been identified. The promoter region of COX-2 contains NF- $\kappa$ B binding elements. The activation of NF- $\kappa$ B occurs during HS [1] and has been shown to play a regulatory role in the induction of cytokines. Reduced NF- $\kappa$ B activation was accompanied by reduced cytokine expression of interleukin (IL)-6 and granulocyte colony-stimulating factor (G-CSF). Thus, increased NF- $\kappa$ B binding activity during HS may promote the induction of COX-2 expression.

Another potential stimulus for the inflammatory response in HS is the release and recognition of gut-derived products. Several studies have implicated endotoxin or gut-derived products in cytokine upregulation. Clinical studies have failed to identify endotoxin or bacterial translocation in human trauma victims [6]. However, recent evidence points to the lymphatics as an important route for gut-derived products [16]. Endotoxin recognition occurs primarily through interaction of the lipopolysaccharide (LPS) LPS/LPS binding protein (LBP) complex with endotoxin-sensitive receptors. The expression of CD14 and Tlr4 surface receptors are two key components for LPS recognition and mononuclear cell activation. We found that CD14 was upregulated during the ischemic phase of shock and that CD14 expression correlated with the severity of shock. In endotoxin-resistant (HeJ) and endotoxin-sensitive (HeN) mice as well as CD14-deficient (CD14 KO) or CD14-competent (CD14 WT) mice, the circulatory regulation and survival as well as upregulation of CD14 mRNA expression following HS was independent of the capacity to respond to LPS. Increased LBP mRNA expression was independent of Tlr4-mediated LPS recognition. IL-6, tumor necrosis factor (TNF) $\alpha$ , and intercellular adhesion molecule (ICAM-1) as markers of inflammation were increased in CD14-deficient and CD14-competent mice in HS. These results suggest that early upregulation of cytokines may be independent of the LPS recognition system CD14/ Tlr4 receptor expression (T.R. Billiar, unpublished observations).

The activation of inflammatory cascades following HS is a complex systemic response. However, the accumulated evidence strongly suggests that important changes begin during shock alone and do not require resuscitation.

### **Reperfusion-dependent events: IL-6, G-CSF, PMN infiltration**

Resuscitation results in reperfusion of ischemic tissues. Reperfusion occurs in the setting of changes in gene expression during shock. Furthermore, with resuscitation, a number of reperfusion-dependent changes take place. We examined the importance of the reperfusion phase for the upregulation of proinflammatory cytokines and tran-

scription factors [17, 18]. We focused on cytokines such as IL-6 and G-CSF that are chemotactic for polymorphonuclear neutrophilic granulocytes (PMN) and contribute to PMN infiltration into tissues, a hallmark of the inflammatory response [3]. Activation of PMN leads to enhanced adhesiveness of PMN to endothelium associated with release of reactive oxygen intermediates (ROIs), and degranulation with protease release [19]. In a rodent model of unresuscitated and resuscitated HS, IL-6 and G-CSF were locally produced in the lungs. Shock and resuscitation were required to increase cytokine levels above those of sham control animals. Cytokine expression was dependent on the duration of the ischemic phase of resuscitated shock and was accompanied by signs of acute lung injury characterized by PMN infiltration, pulmonary capillary leakage, and hypoxia. These results suggest that increased IL-6 and G-CSF expression may contribute to increased lung damage and hypoxia observed in resuscitated HS.

Local production of G-CSF may affect tissue accumulation of PMN through its demonstrated ability to enhance PMN adhesion to endothelium via a mechanism involving integrin affinity conversion, as well as through its ability to enhance PMN chemotaxis and chemokinesis [20]. IL-6 is part of the acute inflammatory response, stimulates neutrophilia, and induces the synthesis of acute phase proteins [21]. IL-6 contributes to tissue recruitment of PMN by induction of chemokines [22]. The promoter region of IP-10, a member of the C-X-C subgroup of chemokines contains an interferon (IFN)-stimulated response element (ISRE) capable of binding STAT proteins [23]. IL-6 activated Stat3 may contribute to local chemokine production through transcriptional activation of chemokine genes.

The pathogenesis of acute respiratory distress syndrome (ARDS) includes diffuse alveolar-capillary injury. Activated neutrophils have been implicated in the pathogenesis of microvascular injury [24]. Intratracheal instillation of IL-6 or G-CSF protein into the lungs of normal rats caused PMN infiltration, pulmonary edema, and lung damage. These results indicate that the presence of IL-6 or G-CSF alone can induce lung injury. A recent study using IL-6 KO mice demonstrated that IL-6 was essential for the early recruitment of PMN into the lungs and liver [25]. The reduction of PMN accumulation was associated with less pulmonary edema following resuscitation from shock. Thus, increased IL-6 and G-CSF production in the lungs of patients suffering from hemorrhagic shock may be an additional mechanism that contributes to PMN-induced lung injury and possibly ARDS.

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### Activation of STAT proteins

Signal transducers and activators of transcription (STAT) proteins are intracellular transcription factors that are

activated following receptor binding of cytokines. Stat3 was the isoform predominantly activated in resuscitated HS. The activation of Stat3 is linked to cytokine activation pathways and IL-6 and G-CSF are among the factors capable of stimulating Stat3 [26]. The activation of Stat3 exhibited the same pattern as G-CSF and IL-6 expression in HS [27]. Stat3 activation occurred in resuscitated HS and was dependent on the severity of shock suggesting that the activation of this intracellular signaling cascade is dependent on the production of cytokines and requires both shock and resuscitation.

The activation of Stat3 in HS may be used as a new sensitive parameter for tissue inflammation. Stat3 may activate genes important for regulating differentiation of PMN [28] and the expression of the Fc $\gamma$ RI receptors on PMN. STAT proteins also mediate INF $\gamma$  and TNF $\alpha$ -induced expression of gene products such as interferon regulatory factor (IRF)-1, ICAM-1, Mig and RANTES during inflammation [29].

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### Gut response to HS

Shock-induced gut injury and loss of gut barrier function contribute to the systemic inflammatory response. The gut is considered to become a cytokine-generating organ in HS. The release of gut-derived cytokines contributes to systemic increases in circulating cytokines. HS results in the activation of a dense network of resident macrophages in the muscularis externa and mucosa [30]. These resident cells may be the source of early local cytokine production. The activation of NF- $\kappa$ B and Stat3 has been demonstrated in muscularis and mucosa layer of the gut in resuscitated HS. Since Stat3 activation is known to activate ICAM-1 gene expression [31], this upregulation of Stat3 activity could contribute to increased ICAM-1 expression and consequently to PMN recruitment in HS.

Recovery from hemorrhagic shock is associated with decreased gut motility leading to postinjury atony and functional ileus. HS has been shown to result in reduced spontaneous contractility of the circular muscle layer [30]. In a recent study, animals subjected to shock and resuscitation demonstrated a decrease in slow-wave amplitudes [30]. In addition to alteration in spontaneous activity, bethanechol-stimulated contractile activity was significantly depressed and correlated with the rapid onset of cytokine induction and PMN infiltration into the intestinal muscularis. This inflammatory response including PMN extravasation contributed to altered muscularis function. A leukocyte-mediated suppression of contractile function has also been demonstrated in a rat model of small-bowel transplantation, in surgical manipulation, and sepsis-induced ileus [32]. Possible mechanisms of PMN-mediated smooth muscle dysfunction include inhibition of Na/K ATPase, Ca-stimulated ATPase, and myofibril ATPase [32]. The early upregulation of

iNOS and IL-6 also appear to contribute to muscular dysfunction.

Recent studies have shown that mesenteric lymph generated following HS is toxic to endothelial cells and results in cell injury, increased endothelial cell permeability, and activation of neutrophils [33]. In addition, gut-derived factors carried in the lymph following shock contribute to lung injury. These studies demonstrated that interruption of lymphatic flow prevented the onset of hemorrhagic shock-induced lung injury [16].

HS reduces intestinal perfusion and the resulting tissue hypoxia causes permeability changes in the mucosal lining leading to intestinal barrier dysfunction [34]. The loss of gut barrier function contributes to development of systemic inflammation and distant organ failure. Recent studies indicated that elevated NO levels are critically involved in both shock and endotoxin-induced gut injury and demonstrated an important role for iNOS in the regulation of bacterial translocation and intestinal barrier function [35]. iNOS KO mice were protected against both endotoxin- and intestinal ischemia-reperfusion-induced bacterial translocation and mucosal injury [36]. Increase in endotoxin- and shock-induced mucosal permeability is dependent on elevated NOS activity. The mechanisms of iNOS dependent increase in gut permeability are not completely understood but include a peroxynitrite-mediated direct cytotoxic effect on epithelial cells that is NO dependent.

In summary, hemorrhagic shock initiates the rapid local production and action of inflammatory cytokines within resident cells of the muscularis externa and mucosa. The inflammatory response within the muscularis was also characterized by a cellular component that includes PMN infiltration. The resulting motility changes may promote delayed infection. More importantly, the gut seems to be an important source of inflammatory products that enter the systemic circulation via the lymphatics. We demonstrated that the complex sequence of events initiated by HS resulted in a significant inhibition in circular smooth muscle contractile activity.

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### More on the roles of NO and iNOS in HS

The production and generation of reactive radicals play an important role in promoting the inflammatory response. Among the radicals produced during hemorrhagic shock is the bio-regulatory molecule NO. NO is generated catalytically in tissues by one of three enzymes collectively termed NOSs. The inflammatory or inducible NOS (iNOS or NOS2) is upregulated in organ systems such as the lungs, liver, and gut during shock and remains elevated following resuscitation [37, 38]. This isoform is capable of catalyzing the sustained production of NO. NO can have both direct effects on cell signaling as well as indirect effects mediated by reaction products with oxygen or superoxide [39].

Studies using the selective iNOS inhibitor L-N<sup>6</sup>-(iminoethyl)-L-lysine (L-NIL) or iNOS KO mice demonstrated that iNOS contributes to the expression of cytokines in the lung and liver following resuscitation from hemorrhagic shock. The iNOS-dependent increase in cytokine mRNA levels was associated with iNOS-dependent increase in NF- $\kappa$ B and Stat3 activation in these tissues. An association between the upregulation of these proinflammatory events by NO and organ injury was shown by experiments demonstrating reduced PMN accumulation and edema formation in the lungs, and reduced plasma levels of liver enzymes with iNOS suppression. These data suggest that induction of NO synthesis is a key event in the subsequent activation of inflammatory cascades following resuscitation.

The current data support the idea that NO can increase cytokine expression through the activation of NF- $\kappa$ B, and that the activation of Stat3 may be the result of local cytokine expression [1]. NO-mediated signaling events that are initiated in early phases of resuscitation result in rapid activation of inflammatory cascades.

NO is an important signaling molecule that signals through a cGMP-dependent pathway [40] or through direct protein modification, such as S-nitrosylation of proteins containing cysteine residues [41]. NO-mediated signaling in HS is characterized by the associated redox stress. Reduction in antioxidant defense, increased NO production, and formation of peroxynitrite (ONOO<sup>-</sup>) can activate redox-sensitive signaling pathways. Under conditions of redox stress, such as HS, NO has been shown to activate p21<sup>ras</sup> through S-nitrosylation resulting in downstream activation of NF- $\kappa$ B. Thus, NO-mediated activation of NF- $\kappa$ B may be a potential mechanism for redox-dependent signaling in HS [7, 42].

NO may mediate vascular decompensation associated with prolonged hemorrhagic shock. Inhibiting NO may improve vascular tone and increase blood flow to vital tissues [38]. Alternatively, the iNOS expression pattern is consistent with the possibility that iNOS may contribute to the progressive vascular dysfunction seen with sustained shock [37]. Evidence presented by Szabo and coworkers suggest that ONOO<sup>-</sup> is formed. ONOO<sup>-</sup> in turn may activate poly (ADP-ribose) synthase (PARS), leading to energy failure in vascular tissues.

HS results in increased production of free radicals and impairs antioxidant defense mechanisms. Excessive amounts of oxygen radicals such as superoxide are produced during the resuscitation phase by xanthine oxidase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The enzymatic and non-enzymatic antioxidant capacity is suppressed as indicated by reduced levels of superoxide dismutase, catalase, and glutathione peroxidase [39] as well as reduced levels of vitamin E, ascorbic acid, and  $\alpha$ -tocopherol. Reduced antioxidant defense renders cells and tissues more susceptible to oxidant-mediated injury [9].



## Therapeutic approaches

Nonspecific NOS inhibition increased organ injury in hemorrhagic shock [43] while selective iNOS inhibition suppressed inflammation and decreased organ injury. At present, the results indicate that basal levels of NO produced by endothelial constitutive (ec)NOS are organ protective but that increased quantities of NO generated by iNOS cause injury. Several studies have used the strategy of scavenging free radicals by administration of antioxidants during resuscitation. These studies have shown reduced organ injury and improved survival. Addition of scavengers to standard resuscitation fluids may be beneficial in removing increased amounts of free oxygen radicals during the resuscitation phase [9].

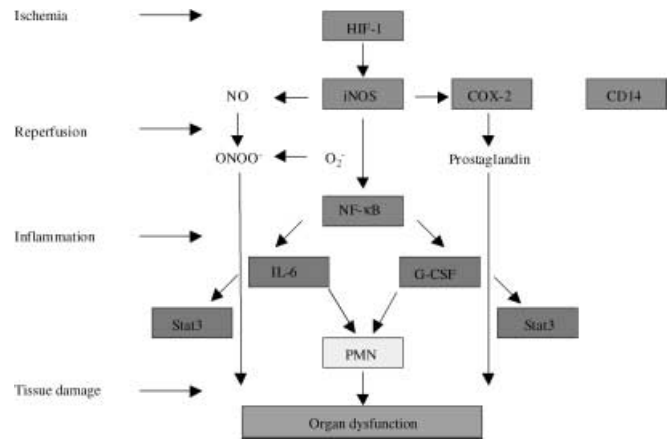
Approaches to remove NO in hemorrhagic shock could have therapeutic benefit. Patients suffering severe or sustained hemorrhagic shock following trauma or due to other causes of bleeding (e.g. ruptured aortic aneurysm) can develop organ injury and dysfunction. Inhibitors of the NO synthase such as L-NIL are likely to be beneficial if they are highly selective for iNOS and preserve ecNOS activity. It is unclear at this time whether selective iNOS inhibition with L-NIL could also lead to unwanted consequences.

As an alternative approach, NO scavengers may preserve adequate levels of NO required to maintain perfusion while removing excess NO. We have shown that the NO scavenger, NOX-100, prevents inflammatory signaling in resuscitated HS and limits early organ injury [44]. NOX is a dithiocarbamate that is water soluble and that readily binds NO [45]. In addition, NOX has a short half-life of 15 min. Due to the short half-life of NOX, removal of excessive amounts of NO can be controlled and limited to the initial period of NO activity that is critical for the initiation of the inflammatory response.

In conclusion, we have demonstrated that induced NO contributes to the inflammatory response and organ injury following resuscitation from hemorrhagic shock and that the NO scavenger NOX may offer a new therapeutic approach to block self-amplifying circles of inflammation and to suppress NO-mediated toxicity in the early phases of shock.

## Late mechanisms in HS

Recovery from HS is complicated by increased incidence of infection and multi-organ dysfunction syndrome (MODS). The immune response in HS includes the state of acute inflammation and, possibly, the development of immune hypo-responsiveness and dysfunction. Immune dysfunction is characterized by impaired major histocompatibility class II (MHC class II) antigen presentation by macrophages including reduced MHC class-II molecule expression, and antigenic processing, and may be followed by the release of anti-inflammatory



**Fig. 1** Sequence of molecular events in the early phase of hemorrhagic shock. Shock-dependent initializing mechanisms include the induction of iNOS, COX-2, and CD14. The early immediate response genes iNOS and COX-2 promote the inflammatory response by the rapid and excessive production of NO and prostaglandins. The transcription factor HIF-1 may regulate the induction of iNOS during the shock phase. NO-dependent NF-κB activation modulates inflammatory cytokine expression during the reperfusion phase. Activated PMN are important cellular mediators of inflammation. Peroxynitrite-mediated direct toxicity and NO-mediated inflammatory toxicity contribute to organ injury. Following HS patients are susceptible to MODS if physiologic and therapeutic mechanisms are ineffective in limiting the activation of the inflammatory cascade

mediators such as IL-10, IL-4, and transforming growth factor beta (TGFβ). These mechanisms occur in later phases of HS. The reader is referred to a specialized review [46].

## Summary

We have identified a complex sequence of molecular events that offers a paradigm for understanding the untoward events associated with HS. We propose that the rapid activation of signaling pathways during shock leads to early phenotypic changes. These changes include the rapid upregulation of key genes that in turn upregulate inflammatory mechanisms at the time of resuscitation. iNOS appears to be an important gene in this response and represents a viable therapeutic target (Fig. 1).

Undoubtedly, many other events yet to be fully defined take place in both shock and following resuscitation. Accepting the paradigm of the two phases of early inflammatory signaling in HS permits a rational approach to study the molecular events leading to organ damage and dysfunction.

## References

1. Hierholzer C, Harbrecht B, Menezes JM, et al (1998) Essential role of induced nitric oxide in the initiation of the inflammatory response following hemorrhagic shock. *J Exp Med* 187:917–928
2. Chaudry IH, Ertel W, Ayala A (1993) Alterations in inflammatory cytokine production following hemorrhage and resuscitation. In: Schlag G, Redl H, Traber DL, (eds) Shock, sepsis, and organ failure, Third Wiggers Bernard Conference. Springer, Berlin Heidelberg New York, pp 73–127
3. Cotran RS, Kumar V, Robbins SL (1989) Robbins pathologic basis of disease. W.B. Saunders Company, Philadelphia, pp 39–61
4. Le Tulzo Y, Shenkar R, Kaneko D, et al (1997) Hemorrhage increases cytokine expression in lung mononuclear cells in mice: involvement of catecholamines in nuclear factor-kappa B regulation and cytokine expression. *J Clin Invest* 99:1516–1524
5. Deitch EA, Morrison J, Berg R, Specian RD (1990) Effect of hemorrhagic shock on bacterial translocation, intestinal morphology, and intestinal permeability in conventional and antibiotic-decontaminated rats. *Crit Care Med* 18:529–536
6. Peitzman AB, Udekwu AO, Ochoa J, Smith S (1991) Bacterial translocation in trauma patients. *J Trauma* 31:1083–1086
7. Lander HM (1997) An essential role for free radicals and derived species in signal transduction. *FASEB J* 11:118–124
8. McCloskey CA, Billiar TR (2000) Activation of C-Jun N-terminal kinase in mouse liver during hemorrhagic shock. *Surg Forum* 51:131–133
9. Szabo C, Billiar TR (1999) Novel roles of nitric oxide in hemorrhagic shock. *Shock* 12:1–9
10. Semenza GL, Roth PH, Fang HM, Wang GL (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J Biol Chem* 269:23757–23763
11. Wang GL, Jiang BH, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O<sub>2</sub> tension. *Proc Natl Acad Sci USA* 92:5510–5514
12. Guillemin K, Krasnow MA (1997) The hypoxic response: huffing and HIFing. *Cell* 89:9–12
13. Hierholzer C, Harbrecht B, Billiar TR, Tweardy DJ (2001) Induction of reperfusion-independent early events in inflammatory cascade of hemorrhagic shock. *Arch Orthop Trauma Surg* 121:219–222
14. Palmer LA, Semenza GL, Stoler MH, Johns RA (1998) Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. *Am J Physiol* 274:L212–L219
15. Nogawa S, Forster C, Zhang F, Nagayama M, Ross ME, Iadecola C (1998) Interaction between inducible nitric oxide synthase and cyclooxygenase-2 after cerebral ischemia. *Proc Natl Acad Sci USA* 95:10966–10971
16. Sambol JT, Xu DZ, Adams CA, Magnotti LJ, Deitch EA (2000) Mesenteric lymph duct ligation provides long term protection against hemorrhagic shock-induced lung injury. *Shock* 14:416–419
17. Hierholzer C, Kelly E, Billiar TR, Tweardy DJ (1997) Granulocyte colony-stimulating factor (G-CSF) production in hemorrhagic shock requires both the ischemic and resuscitation phase. *Arch Orthop Trauma Surg* 116:173–176
18. Hierholzer C, Kalff JC, Omert L, et al (1998) Interleukin-6 production in hemorrhagic shock is accompanied by neutrophil recruitment and lung injury. *Am J Physiol* 275:L611–L621
19. Klebanoff SJ, Vadas MA, Harlan JM, et al (1986) Stimulation of neutrophils by tumour necrosis factor. *J Immunol* 136:4200–4205
20. Smith WB, Gamble JR, Vadas MA (1994) The role of granulocyte-macrophage and granulocyte colony-stimulating factors in neutrophil transendothelial migration: comparison with interleukin-8. *Exp Hematol* 22:329–334
21. Kishimoto T (1992) Interleukin-6 and its receptor in autoimmunity, (review). *J Autoimmun* 5:123–132
22. Romano M, Sironi M, Toniatti C, et al (1997) Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* 6:315–325
23. Nazar AS, Cheng G, Shin HS, et al (1997) Induction of IP-10 chemokine promoter by measles virus: comparison with interferon-gamma shows the use of the same response element but with differential DNA-protein binding profiles. *J Neuroimmunol* 77:116–127
24. Tate RM, Repine JE (1983) Neutrophils and the adult respiratory distress syndrome. *Am Rev Respir Dis* 128:552–559
25. Meng ZH, Dyer K, Billiar TR, Tweardy DJ (2001) Essential role for IL-6 in postresuscitation inflammation in hemorrhagic shock. *Am J Physiol Cell Physiol* 280:C343–C351
26. Tweardy DJ, Wright TM, Ziegler SF, et al (1995) Granulocyte colony-stimulating factor rapidly activates a distinct STAT-like protein in normal myeloid cells. *Blood* 86:4409–4416
27. Hierholzer C, Kalff JC, Bednarski B, et al (1999) Rapid and simultaneous activation of Stat3 and production of interleukin 6 in resuscitated hemorrhagic shock. *Arch Orthop Trauma Surg* 119:332–336
28. Leaman DW, Leung S, Li X, Stark GR (1996) Regulation of STAT-dependent pathways by growth factors and cytokines. *FASEB J* 10:1578–1588
29. Bovolenta C, Gasperini S, Cassatella MA (1996) Granulocyte colony-stimulating factor induces the binding of stat1 and stat3 to the ifn-gamma response region within the promoter of the Fc-gamma-Ri/Cd64 gene in human neutrophils. *FEBS Letters* 386:239–242
30. Hierholzer C, Kalff JC, Menezes JM, et al (1999) The nitric oxide scavenger, NOX, reduces intestinal inflammation and organ damage following hemorrhagic shock. *Surg Forum* 50:212–214
31. Caldenhoven E, van Dijk T, Raaijmakers JA, Lammers JW, Koenderman L, De Groot RP (1995) Activation of the STAT3/acute phase response factor transcription factor by interleukin-5. *J Biol Chem* 270:25778–25784
32. Eskandari MK, Kalff JC, Billiar TR, Lee KKW, Bauer AJ (1997) Lipopolysaccharide activates the muscularis macrophage network and suppresses circular smooth muscle activity. *Am J Physiol* 36:G727–G734
33. Upperman JS, Deitch EA, Guo W, Lu Q, Xu D (1998) Post-hemorrhagic shock mesenteric lymph is cytotoxic to endothelial cells and activates neutrophils. *Shock* 10:407–414
34. Wattanasirichaigoon S, Menconi MJ, Delude RL, Fink MP (1999) Effect of mesenteric ischemia and reperfusion or hemorrhagic shock on intestinal mucosal permeability and ATP content in rats. *Shock* 12:127–133
35. Suzuki Y, Deitch EA, Mishima S, Duran WN, Xu DZ (2000) Endotoxin-induced mesenteric microvascular changes involve iNOS-derived nitric oxide: results from a study using iNOS knock out mice. *Shock* 13:397–403
36. Suzuki Y, Deitch EA, Mishima S, Lu Q, Xu D (2000) Inducible nitric oxide synthase gene knockout mice have increased resistance to gut injury and bacterial translocation after an intestinal ischemia-reperfusion injury. *Crit Care Med* 28:3692–3696
37. Kelly E, Shah NS, Morgan NN, Watkins SC, Peitzman AB, Billiar TR (1997) Physiologic and molecular characterization of the role of nitric oxide in hemorrhagic shock: evidence that type II nitric oxide synthase does not regulate vascular decompensation. *Shock* 7:157–163

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38. Thiemermann C, Szabo C, Mitchell JA, Vane JR (1993) Vascular hyporeactivity to vasoconstrictor agents and hemodynamic decompensation in hemorrhagic shock is mediated by nitric oxide. *Proc Natl Acad Sci USA* 90: 267–271
  39. Szabo C (1996) The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. *Shock* 6:79–88
  40. Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J* 6:3051–3064
  41. Lander HM, Hajjar DP, Hempstead BL, et al (1997) A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. *J Biol Chem* 272:4323–4326
  42. Lander HM, Milbank AJ, Tauras JM, et al (1996) Redox regulation of cell signalling (letter). *Nature* 381:380–381
  43. Harbrecht BG, Wu B, Watkins SC, Billiar TR, Peitzman AB (1997) Inhibition of nitric oxide synthesis during severe shock but not after resuscitation increases hepatic injury and neutrophil accumulation in hemorrhaged rats. *Shock* 8:415–421
  44. Menezes J, Hierholzer C, Watkins SC, et al (1999) A novel nitric oxide scavenger decreases liver injury and improves survival after hemorrhagic shock. *Am J Physiol* 277:G144–G151
  45. Paschenko SV, Khramtsov VV, Skatchkov MP, Plyusnin VF, Bassenge E (1996) EPR and laser flash photolysis studies of the reaction of nitric oxide with water soluble NO trap Fe(II)-proline-dithiocarbamate complex. *Biochem Biophys Res Commun* 225: 577–584
  46. Ayala A, Ertel W, Chaudry IH (1996) Trauma-induced suppression of antigen presentation and expression of major histocompatibility class II antigen complex in leukocytes. *Shock* 5:79–90