J Pharmacol Sci **101**, 189 – 198 (2006)

Journal of Pharmacological Sciences

Critical Review

Systemic Inflammatory Response Syndrome (SIRS): Molecular Pathophysiology and Gene Therapy

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Received May 14, 2006

Abstract. In recent years, extensive basic science research has led to a clear understanding of the molecular mechanisms contributing to the pathophysiology of sepsis. Sepsis is now defined as a systemic inflammatory response syndrome (SIRS) in which there is an identifiable focus of infection. SIRS can be also precipitated by non-infective events such as trauma, pancreatitis, and surgery. As a consequence of an overactive SIRS response, the function of various organ systems may be compromised, resulting in multiple organ dysfunction syndrome (MODS) and death. Production and activation of multiple proinflammatory genes are likely to play a key role in the pathogenesis of MODS development. This review article focuses on the molecular mechanisms and components involved in the pathogenesis of severe sepsis. This includes cellular targets of sepsis-inducing bacterial products and their signaling pathways with a major emphasis on transcription factors and new therapeutic approaches to severe sepsis.

Keywords: systemic inflammatory response syndrome (SIRS), sepsis, Toll-like receptor, nuclear factor-κB, activating protein-1

Introduction

Invasion of the body by infectious bacteria activates a series of mechanisms to defend against the incursion, resulting in a localized inflammatory response. When this defense response fails, and bacteria or their products, such as endotoxin, reach the vasculature, sepsis can ensue with a variety of intrinsic mediators of systemic inflammation being triggered. Traditionally, sepsis was taken to mean microbial infection even in the absence of infection proven by culture. However, the varying definitions of sepsis by different authorities have led to some confusion. This uncertainty arises partly because the underlying pathological processes represent a spectrum of responses from mild systemic toxicity to severe circulatory shock. In the American College of Chest Physicians and Society of Critical Care Medicine (ACCP/SCCM) consensus conference (1), the criteria for diagnosing sepsis were reaffirmed and a new syndrome, which was named the 'systemic inflammatory response syndrome' (SIRS), was proposed, although this was initially met with some opposition from European investigators (2). Thus, sepsis has been no longer regarded as being caused by microbial pathogenicity factors alone. In general terms, SIRS is an entirely normal response to invasion. At times, however, as a consequence of an overactive response, SIRS can compromise the function of distinct organ systems leading to multiple organ dysfunction syndrome (MODS). When SIRS results in MODS and organ failure, the mortality becomes high and can be more than 50% (3 – 5). Therefore, SIRS is the dreaded complication that far outweighs the direct toxicity of the bacterial infection itself in clinical importance.

Although the pathophysiological mechanisms responsible for SIRS are complex and are not fully understood, extensive basic science research has widely investigated inflammatory signal molecules involved in the initiation of SIRS and related conditions, and the information obtained has provided the basis for a new developmental area for novel therapeutic strategies for the treatment of severe SIRS. In this review, experimental approaches to the therapy of SIRS and sepsis that have received much

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Published online in J-STAGE: July 6, 2006
doi: 10.1254/jphs.CRJ06010X

Invited article

attention in recent years will be discussed in light of each key molecular step in the complex uncontrolled inflammatory cascade. The large body of literature has focused on the molecular pathophysiology and therapeutics of endotoxin-related sepsis and its complications. The vast majority of knowledge of such an anti-sepsis therapy on the molecular basis, however, would theoretically foster a better understanding for therapeutic approaches to all forms of severe SIRS, regardless of etiology.

Definition of SIRS

The distinction between SIRS and sepsis centers upon the presence or absence of a focus of infection. Thus, the identification of SIRS does not confirm a diagnosis of infection or sepsis since the features of SIRS can be seen in many other non-infective conditions. Non-infective causes of SIRS include acute pancreatitis, burns, trauma, or following major elective surgery (Fig. 1). On the other hand, sepsis is defined as a SIRS in which there is an identifiable focus of infection caused by bacterial pathogens, viruses, fungi, and parasites. Of the patients with SIRS associated with infection, the majority have Gram-negative sepsis (6, 7). Although differing etiologies present an identical clinical picture, the failure to identify causal pathogenic microorganisms does not necessarily mean that bacterial pathogens are absent, owing to the limitation of current diagnostic techniques.

At least two criteria are required for the identification of SIRS. Thus, SIRS is manifested by two or more of the following conditions: temperature >38°C or <36°C; heart rate >90 beats/min; respiratory rate >20 breaths /min or $PaCO_2$ <32 torr (<4.3 kPa); WBC >12,000 cells /mm^3, <4000 cells/mm^3 or >10% immature (band) forms (1). The ACCP/SCCM has also recognized a progression in the disease state from simple SIRS/sepsis to severe SIRS/sepsis in the presence of acute organ dysfunction, hypotension, or hypoperfusion (1). However, it should be noted that the SIRS criteria cannot perform much better for diagnosis or as a measure of prognosis, perhaps because they are too wide.

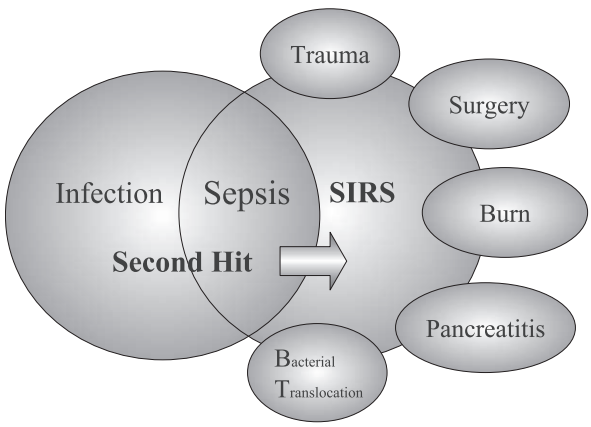

Fig. 1. The concept of SIRS as a common response to many initiating circumstances. The interrelationship between SIRS, sepsis, and infection is shown. Modified from ref. 1.

Patients with an attack of SIRS who survive the initial inflammatory insult may die following a relatively minor second event that would not normally be life-threatening. According to the two-hit hypothesis (8, 9), the initial overactive SIRS such as acute pancreatitis somehow primes the inflammatory response. Recovery is possible if no further insult occurs. Bacterial infection as a relatively minor secondary attack will, however, lead to an exaggerated secondary inflammatory response and possibly death. Thus, the septic complications of acute pancreatitis can manifest themselves as an exaggerated SIRS response with consequent multiple organ failure and death.

Cytokine storm

Cytokines are important components of the immune system that act as messages between cells, but are involved in many pathological aspects of the cascade leading to SIRS and ultimately MODS. Indeed, severe sepsis is characterized by an overwhelming production of proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β. Cytokines are a family of low-molecular weight proteins (16 – 25 kDa) that are secreted by a multitude of cells, including macrophages and monocytes. Cytokine secretion is a very closely regulated process and expression of most cytokines is modulated by transcription factors such as nuclear factor (NF)-κB. All cytokines cause their effects via highly specific cell-surface receptors. Most cytokines have pleiotropic activity and show multiple functional effects on a variety of target cells. While cytokines trigger a beneficial inflammatory response that promotes local coagulation to confine tissue damage, the excessive production of these proinflammatory cytokines can be even more dangerous than the original stimulus, overcoming the normal regulation of the immune response and producing pathological inflammatory disorders as notably seen in sepsis (10).

The term “cytokine storm” is not precisely defined, but refers to a particular kind of uncontrolled immune response. Thus, it is a fierce interplay of cytokines that can occur in a number of infectious and non-infectious diseases including SIRS (11, 12). A cytokine storm is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells. When the immune system is fighting pathogens,

cytokines signal immune cells, such as T cells and macrophages, to travel to the site of infection. In addition, cytokines activate those cells, stimulating them to produce more cytokines. This positive feedback loop reaction becomes uncontrolled, and too many immune cells are activated in a single place. Cytokine storms have potential to do significant damage to body tissues and organs. If a cytokine storm occurs in the lungs, for example, fluids and immune cells such as macrophages may accumulate and eventually block off the airways, potentially resulting in death. We thus have to consider that many cytokines contribute to the pathogenesis and progression of severe SIRS. The cytokine-antagonistic therapeutic strategies neutralizing a few cytokines have not proven to be of clinical benefit in trials (13 – 15).

Implications of Toll-like receptors (TLRs) for the pathogenesis of SIRS

The signaling induced by bacterial components occurs primarily through TLRs. TLRs have been recognized to play a key role in pathogen recognition and innate immunity (16, 17). The nomenclature arises from the toll transmembrane receptor, a homologue to TLR first described in *Drosophia* (18). To date, ten TLR family members (TLR-1 – TLR-10) have been identified in the human genome, of which the function of only six have more or less been identified. Different TLRs appear to play important roles in activation of the immune response to distinct pathogen-activated molecular patterns (Fig. 2). TLR-4 mediates responses to lipopolysaccharide (LPS), one of the toxic principles of Gram-negative bacteria (19), and a number of studies showed an additional LPS-induced response via TLR-2 (20). Since repurification of commercially available LPS led to the abrogation of TLR-2-responsiveness, indicating contamination with other compounds, the role of TLR-2 in LPS-recognition remains unclear (21). However, TLR-2 responds to peptidoglycan, a main wall component of Gram-positive bacteria (22, 23), lipopeptides, and lipoproteins. TLR-3 recognizes viral double-stranded RNA (24). TLR-5 responds to bacterial flagellin (25). The synthetic imidazoquinolines activate immune cells via TLR-7 (26), and TLR-9 recognizes specific patterns in bacterial DNA, CpG-containing DNA (27). Of the remaining TLRs identified, TLR-1 may be involved in the regulation of TLR-2 (28) and TLR-4 signaling (29). The TLR family provides the possibility for a rapid response after exposure to potential pathogens. Signaling through TLRs activates the expression of a host of cytokines, chemokines, hematopoietic factors, acute phase proteins, and antimicrobial factors.

The activation of cells by microbial components such as LPS is dependent on CD14. It has now been shown that the microbial components interact primarily with CD14 and subsequently with the TLRs. In fact, by using phosphoactivated cross-linking, it has been demonstrated that LPS becomes cross-linked to TLR-4 and MD-2 only if the latter are coexpressed with CD14 (30). The extracellular protein MD-2 is closely associated with TLR-4 and is essential for LPS binding to this receptor (31). The cytoplasmic adaptor molecule myeloid differentiation factor MyD88 is essential for TLR-2 and TLR-4 mediated signaling (32), although it seems to be the only adaptor molecule for TLR-9 (33). Defective MyD88 protein resulted in blocked or muted responses to Gram-positive and/or Gram-negative cell wall constituents (34 – 36). The MyD88-dependent pathway entails recruitment of IL-1 receptor (IL-1R)-

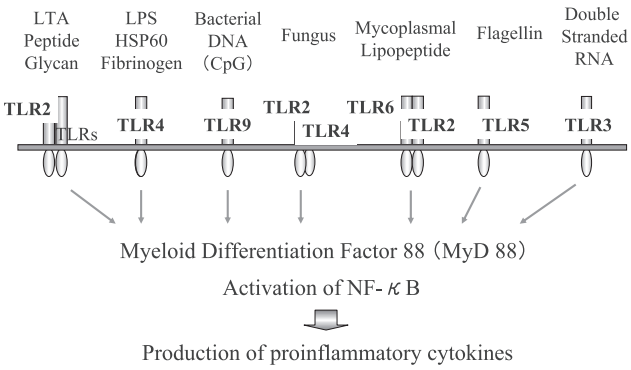

Fig. 2. The major known members of TLR family and their signaling pathways. Various distinct pathogenic motifs can signal via different TLRs. All TLRs can signal via MyD88, leading to the translocation of NF-κB and the production of proinflammatory cytokines.

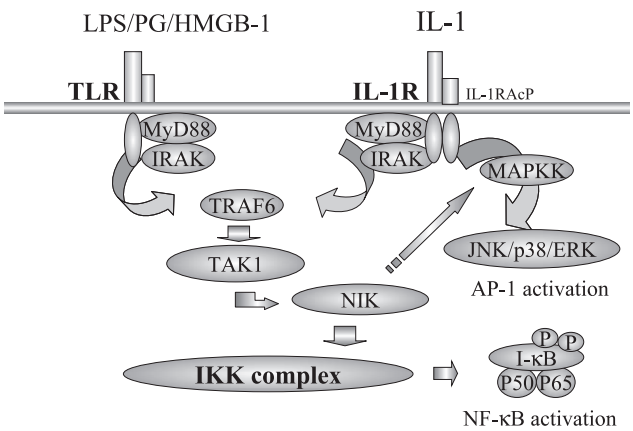

Fig. 3. TLR and IL-1R signaling pathways. The shared signaling pathway for TLR and IL-1R is depicted.

associated kinase (IRAK) isoforms (IRAK4 being particularly important (37)), tumor necrosis factor (TNF) receptor-associated factor-6 (TRAF-6) (38, 39), and transforming growth factor-β-activated kinase-1 (TAK-1) (40) and activation of the signalosome, with subsequent translocation of NF-κB to the nucleus and the transcriptional activation of numerous cytokine genes (Fig. 3). In addition, studies with knockout mice have identified MD-2 as an essentially accessory molecule in TLR-4-mediated signaling and intracellular trafficking (31, 41), whereas MD-1 is instrumental in LPS-induced B-cell proliferation and antibody production through RP105 (42, 43).

The expression patterns of TLRs vary widely, but localization of TLRs has largely been associated with immune and inflammatory cells (16, 17, 44). For example, TLR-2 and TLR-4 are represented mainly by polymorphonuclear leukocytes, monocytes, macrophages, and dendritic cells (45, 46). However, both receptors are also present on various other cell types, including epithelial and endothelial cells (38, 47, 48). The expression levels of TLR-2 and TLR-4 are modulated by LPS and other microbial components. LPS induces an increase in TLR-4 mRNA in a number of cell types, including endothelial cells (49), but surface expression of TLR-4 has been shown to be reduced on murine macrophages (50, 51) and human monocytes (52). In our preliminary study, when sepsis was induced in mice by LPS challenge or cecal ligation and puncture (CLP), TLR-4 mRNA levels were increased in the kidney and to a lesser extent in the liver, whereas the levels were decreased in the heart and lungs (unpublished observations), indicating a broader regulation by inflammation-related factors such as LPS depending on tissues. The exact mechanism(s) of expression of different subsets of TLRs in different cell types and the effects on the resulting signaling patterns remain unclear. However, TLRs may be essential innate immune receptors that alert the immune system to the presence of invading microbes. Thus, when macrophages, dendritic cells, and endothelial cells sense host invasion, they can be considered to have an excellent capacity for prompt recognition of invading pathogens via TLRs to alert other innate immune cells by producing proinflammatory cytokines.

In view of the evidence that macrophages and endothelial cells that possess TLRs respond to invading pathogens by releasing proinflammatory cytokines (16), it is most likely that some TLRs are involved in many but not all types of SIRS. Moreover, TLR-4-deficient mice were hyporesponsive to LPS (53, 54). In addition, the deficiency in TLR-4 has been reported to fully prevent endotoxemia-mediated aggravation of acute pancreatitis-associated severe outcome, acute lung injury (ALI) (55). It has also been shown that TLR-2 deficiency can attenuate *Staphylococcus aureus*-induced cardiac proinflammatory mediator production and the development of cardiac dysfunction (56). Quite recently, Johnson et al. (57) have shown that the ability of heparin sulfate and pancreatic elastase to induce SIRS is greatly diminished in TLR-4-mutant mice, suggesting that SIRS can be induced by signaling through TLR-4. These data with transgenic mice, taken together, provide further support for a contribution of some TLRs to the pathogenesis of SIRS.

Potential role of IL-1R in SIRS

The proinflammatory cytokine IL-1 can induce an increase in gene expression of many different cytokines with roles in inflammation. Its effects on innate immunity are therefore clear, and IL-1 can be considered a link between innate and adaptive immunity (58). IL-1 is strongly induced by bacterial products such as LPS and acts either directly or indirectly (via the induction of other cytokines) on inflammatory cells (32). IL-1 signals via IL-1R, resulting in activation of NF-κB, and subsequently, soon after its identification, it has been shown that TLR-4 could do so as well (59). TLRs, IL-1R, IL-18R, and a number of mammalian and non-mammalian proteins exhibit a striking similarity with respect to the Toll/IL-1R domain (TIR); hence, this family of receptors is called the TIR superfamily (32). IL-1R and IL-18R are classified into the immunoglobulin domain subgroup. MyD88 is believed to be universal (i.e., non-specific) and is required for signaling from the IL-1R and IL-18R as well as TLRs (60) (Fig. 3). It is thus likely that the presence of IL-1R and its signaling may play an important role in the pathogenesis of SIRS. To date, surprisingly, there is little information available in the literature to address this issue. Experimental evidence to provide an outlook for the potential roles of IL-1R in SIRS must await further extensive research.

NF-κB as a therapeutic target for quelling SIRS

NF-κB is a prominent nuclear transcription factor suggested to be a central regulator of genes and end-effectors of the host's inflammatory response. NF-κB belongs to members of the Relish (Rel) family that share a highly conserved Rel homology domain composed of 2 immunoglobulin-like domains (61, 62). The major form of NF-κB in cells is a heterodimeric complex composed of 50- and 65-kDa (p50/p65) protein subunits; minor complexes of p50/p50 homodimers have

also been documented (63, 64). Phosphorylation of the p65 subunit is important in optimizing transcriptional potential (65). As depicted in Fig. 4, in unstimulated cells, NF-κB is retained as a latent cytoplasmic complex bound to its inhibitor protein IκB (61, 66). When the cell is activated by various stimuli, including LPS, Gram-positive bacterial products (e.g., peptidoglycan and lipoteichoc acid), cytokines (e.g., TNF-α and IL-1), T and B cell mitogens, oxidants, and other physical and chemical stressors (67 – 69), IκB associated with NF-κB in the cytoplasm is phosphorylated, ubiquitinylated, and then degraded by the 26S proteasome (70, 71). Degradation of IκB permits NF-κB to the nucleus where it can interact with coactivator proteins, such as CBP, bind to specific sequences in the promoter regions of genes primarily involved in the inflammatory response and initiate transcription of these mediator proteins (72).

A number of genes associated with the inflammatory process, including TNF-α, inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and adhesion molecules, contain putative NF-κB binding sites within their promoters (61, 66, 73, 74), thus highlighting the importance of NF-κB as a key regulator of inflammatory gene activation and identifying it as a prime candidate for targeted inactivation. The effects of pharmacological interventions designed to inhibit activation of NF-κB have been examined in rodent models of LPS-induced sepsis. When pyrrolidine dithiocarbamate, which inhibits activation of NF-κB through an oxygen radical scavenging mechanism (75), was given to the rat by intraperitoneal injection, ALI induced by intratracheal administration of LPS was significantly attenuated (76).

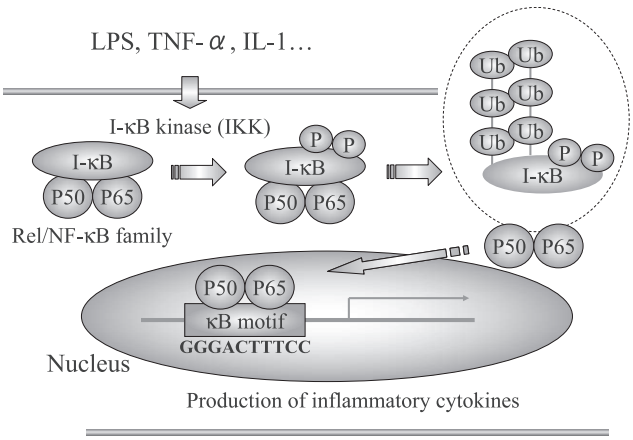

Fig. 4. NF-κB activation in SIRS. Multiple mediators associated with SIRS lead to activation of the IKK complex, which then phosphorylates IκB; this results in degradation of IκB and liberation of NF-κB dimers to move into the nucleus. In the nucleus, NF-κB binds to specific sites in promoter regions where it can initiate transcription.

Pretreatment of rats with pyrrolidine dithiocarbamate also prevented LPS-induced overexpression of TNF-α, COX-2, cytokine-inducible neutrophil chemoattractant, and intracellular adhesion molecule (ICAM)-1 mRNAs and their products as well as neutrophil sequestration in the heart, lungs, and liver (77). Furthermore, parthenolide, which inhibits NF-κB most probably by alkylating thiol groups on cysteine residues of the p65 subunit of NF-κB (78), has been shown to reduce lung sequestration of neutophils, plasma levels of NO metabolites, and gene expression of iNOS, and to improve hypotension and survival rate in rodent models of endotoxic shock (79).

To assess whether functional inactivation of NF-κB could suppress sepsis-induced ALI, we have tested the effects of decoy oligonucleotides (ODNs) directed against NF-κB on inflammatory gene overexpression and pulmonary derangements in mice with sepsis induced by LPS (80) and CLP (81). Decoy ODNs directed against NF-κB inhibit NF-κB-dependent gene transcription by competing with '*cis*'-acting elements of putative inflammatory genes that contain NF-κB binding elements. Our in vivo transfection of NF-κB decoy was confirmed to strongly reduce the increase in NF-κB activity during sepsis, as indicated by electromobility shift analysis (80, 81). Consequently, NF-κB decoy greatly diminished the expression levels of iNOS, COX-2, histidine decarboxylase, ICAM-1, platelet-activating factor receptor, and bradykinin B_1 and B_2 receptors in septic lung tissues. It is noteworthy that mice treated with NF-κB decoy ODNs displayed an improved outcome with a significant reduction in sepsis-induced ALI compared with control animals or animals treated with scrambled ODNs. We thus provide a novel therapeutic strategy with the use of NF-κB decoy ODNs to quell systemic inflammatory diseases at genetic levels. However, in vitro studies suggest that NF-κB plays a role as a survival factor, responsible in part for "turning on" genes that could block cell death by apoptosis (82). It is therefore of great importance to address a number of unresolved issues, including safety and side effects, before argument about clinical benefit in treating patients with SIRS.

Is activating protein-1 (AP-1) a new target for treating SIRS?

Like NF-κB, AP-1 is minimally activated by physiological stimuli, but is dramatically activated by many pathophysiological stimuli, including LPS, cytokines, and reactive oxygen species (83). AP-1 is a ubiquitous regulatory protein complex that interacts with AP-1 binding sites of target genes to regulate transcription

under pathophysiological conditions (83 – 86). Members of the mitogen-activated protein kinase (MAPK) family, c-Jun N-terminal kinase (JNK) and p38 MAPK, are important in the regulation of AP-1 to mediate expression of inducible genes (87). Indeed, stimulation of monocytes with LPS has been shown to enhance the transcriptional activity of AP-1 by activation of JNK and p38 MAPK (88). AP-1 is composed of protein products of members of the *jun* and *fos* proto-oncogene families, forming homodimeric (Jun/Jun) or heterodimeric (Jun /Fos) complexes (89) (Fig. 5).

The inducible transcription factor AP-1 appears to play key roles in the transcription of a number of inflammatory genes strongly involved in the pathophysiology of SIRS, including sepsis. For example, the promoter regions of ICAM-1 and COX-2 genes contain several putative AP-1 binding sites (90, 91), suggesting that initiation of the signal pathway for activation of AP-1 leads to the induction of these genes. It has been shown that in vivo challenge with LPS results in a significant increase in AP-1 DNA binding in rat lungs (92). In our preliminary work, when LPS was given to the mouse by intravenous injection, activation of the two transcription factors, NF-κB and AP-1, in lungs showed quite different time-course profiles (Fig. 6). Thus, the two inducible transcription factors may be differentially regulated under septic conditions. This differential regulation of NF-κB and AP-1 activities would imply expression of multiple proinflammatory genes and their products with different time courses, contributing to the pathogenic development of the systemic inflammatory disease.

The synthetic serine protease inhibitor gabexate mesilate was effective in endotoxin-induced pulmonary injury and coagulation abnormalities in rats (93). This effect may be partly due to inhibition of activation of AP-1, because gabexate mesilate inhibited both binding of AP-1 to target sites and the activation of MAPK pathways in human monocytes (94). However, interpretation of the results is complicated by the fact that gabexate mesilate can also inhibit the LPS-induced activation of NF-κB by inhibiting degradation of IκB (94). Thus, the experiments with the in vivo use of AP-1 decoy ODNs now in progress are aimed at delineating the role of AP-1 in the pathophysiology of SIRS and the potential usefulness of AP-1 decoy ODNs for gene therapy of this disease.

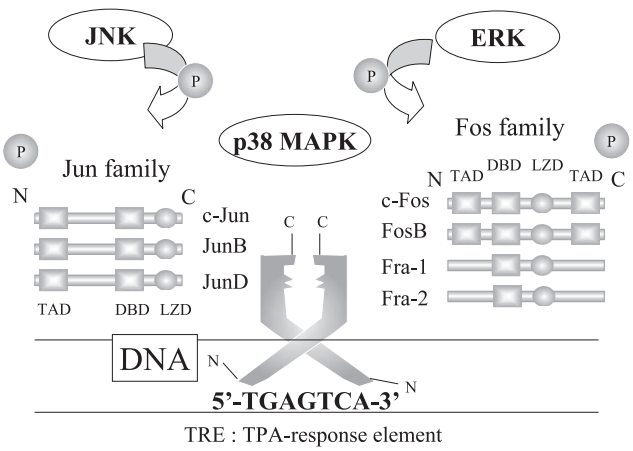

Fig. 5. AP-1 activation in SIRS. Activation of p38 MAPK that resulted from pathophysiological stimuli associated with SIRS phosphorylates JNK and ERK (extracellular signal-regulated kinase). Phosphorylated JNK activates *jun* by phosphorylating its N-terminus, whereas phosphorylated ERK activates *fos* by phosphorylating its C-terminus. Subsequently, these members of the proto-oncogene families form dimeric complexes, and interact with AP-1 binding sites to target genes to regulate transcription. TAD, transcription activating domain; DBD, DNA binding domain; LZD, leucine-zipper domain.

Role of macrophage migration inhibitory factor (MIF) in the pathophysiology of SIRS

MIF was originally identified as a cytokine derived from activated T cells that inhibits macrophage migration and promotes delayed-type hypersensitivity (95, 96). However, MIF has been newly understood to function as a proinflammatory cytokine (97, 98). In addition, MIF has been found to be a major pituitary cytokine that is released in response to physiological stress induced by LPS (98). It has been shown that recombinant mouse MIF greatly enhances lethality when co-injected with bacterial LPS, and polyclonal antibodies against the recombinant protein confer full protection to mice from LPS-induced lethal endotoxemia (98). Therefore, MIF may play a central role

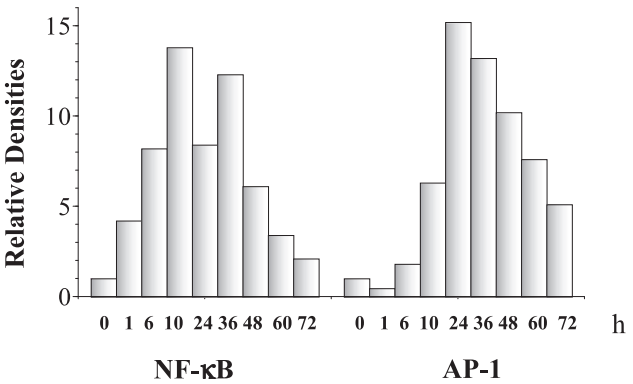

Fig. 6. Time course of LPS-induced activation of NF-κB and AP-1 in mouse lungs. Nuclear proteins extracted from lungs were tested for NF-κB and AP-1 DNA binding activity in the electrophoretic mobility shift assay. Relative band intensity as quantified using densitometry is shown.

in exacerbation of endotoxemia. Nevertheless, MIF-deficient mutant mice showed the same susceptibility to LPS for endotoxemia and the same formation level of TNF-α upon LPS stimulation as normal mice (99).

In our recent work, a mouse model of acute pancreatitis accompanied by a subsequent endotoxemia was used to gain insight into the molecular mechanisms underlying the development of multisystem organ dysfunction, including ALI, in this pathological state (100). We found that stimulation of protease-activated receptor-2 with trypsin, which could be released in large quantities following induction of acute pancreatitis, up-regulated the transcript level of MIF and the increased MIF resulted in exaggerated expression of TLR-4 in lungs. Furthermore, the study with the use of MIF knockout mice provided evidence that MIF is a strong inducer of TLR-4 and this pathway is important in the development of ALI in the setting of acute pancreatitis complicated by endotoxemia (100). We thus suggest that therapy with intervention designed to modulate MIF in patients with SIRS, such as acute pancreatitis complicated by bacterial infection, may prevent the development of multisystem organ dysfunction, thus minimizing the morbidity and mortality associated with SIRS.

Conclusions

A major hurdle in the clinical management of severe sepsis is lack of effective treatment. The significant advances in our understanding of the molecular pathophysiology of sepsis and SIRS will provide theoretical and experimental bases for the development of a novel therapeutic strategy. A central feature of the pathophysiology of severe sepsis is induction of overexpression of multiple proinflammatory genes and their products, most of which can initiate the inflammatory process and may be involved in the pathogenesis of organ dysfunction and failure. Because the transcription factors NF-κB and AP-1 play an essential role in transcriptional regulation of these proinflammatory genes, they would be appropriate targets for the treatment of sepsis and SIRS. Thus, genetic inactivation of the inducible transcription factors may be a new attractive therapeutic option in such life-threatening systemic inflammatory diseases. However, it has to be remembered that inducible transcription factors such as NF-κB are an essential component for intact immune and inflammatory responses in maintaining normal host defense mechanisms.

Acknowledgment

We wish to acknowledge a Grant-in-Aid for Scientific Research and for Exploratory Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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