Review

Critical advances in septicemia and septic shock

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Abstract

Recent advances suggest that *toll*-like receptors, various cytokines, cicosanoids, free radicals and macrophage migration inhibitory factor (MIF) play an important role in the pathobiology of septicemia and septic shock. Anti-MIF antibodies can decrease the plasma concentrations of tumor necrosis factor (TNF), lower bacterial circulating counts and enhance survival of animals with septicemia and septic shock. Monocyte expression of MHC-class II antigens, neutrophil expression of the integrin CD11b/CD18 and neutrophil activation can be related to the development of, and/or recovery from, post-operative sepsis. Thus, biological variations in the response of an individual to a given stimulus, appears to determine his/her ability or inability to develop and also recover from sepsis and septic shock. This suggests that it may be possible to predict the development of septicemia and septic shock in a given individual and take appropriate action both to prevent and treat them adequately.

Keywords: free radicals, hypotension, interleukins, macrophage migration inhibitory factor, nitric oxide, sepsis, septicemia, septic shock, superoxide anion, tumor necrosis factor

Introduction

One of the most frequent and serious problems that clinicians face is the management of serious infections that trigger a systemic inflammatory response, termed 'septicemia'. When sepsis results in hypotension and organ dysfunction, it is referred to as 'septic shock'. Septic shock is the most common cause of death in intensive care units. In the USA alone it is estimated that more than 100 000 deaths occur each year due to septicemia and septic shock [1].

The mortality rate in patients with septic shock ranges from 20 to 80%, and it can be related to both the severity of sepsis and the underlying disorder. Systemic inflammatory response can be triggered not only by infections, but also by noninfectious disorders such as trauma and pancreatitis. Sepsis associated with hypoperfusion that results in organ dysfunction syndromes, such as oliguria, lactic acidosis and altered mental function, and/or in hypotension can be referred to as 'septic shock', and has a poor prognosis [1]. As the population ages, the chances

ARDS = acute respiratory distress syndrome; IκB = inhibitor of the transcription factor nuclear-factor-κB; MIF = migration inhibitory factor; NF-κB = nuclear factor-κB; TLR = *toll*-like receptor; TNF = tumor necrosis factor.

are that physicians may have to manage more and more patients with septicemia and septic shock. In view of this, it is important that the underlying pathophysiologic mechanism(s) of this syndrome are well understood.

Innate immunity, septicemia and septic shock

The survival of humans and animals depends on their ability to recognize invading pathogenic organisms and respond to them rapidly and adequately. These defenses against microbial organisms are innate to the particular organism. The immune system is programmed to recognize the biochemical patterns displayed by these microbial organisms and to mount rapid responses to them. The innate immune system includes neutrophils, macrophages and natural killer cells, which can act directly against invading pathogens and eradicate them without involvement of the adaptive immune system. However, when necessary, these cells of the innate immune system release cytokines and express certain other stimulatory molecules that, in turn, can trigger adaptive immune responses by activating T and B cells. The adaptive immune system differs from the innate immune system in that it is highly evolved, specific in its responses and 'remembers' the antigens presented to it. Thus, the innate immune response is a nonspecific one that attempts to keep the invading foe at bay until the adaptive immune system is ready with its more specific antibodies and T cells [2•].

Septic shock is a multisystem response to infection and/or injury in which hypotension and insufficient perfusion of vital organs occurs that does not respond to fluid administration. It is believed that septic shock is due to inappropriate increase in innate immune response. Hence, to learn about septic shock, one has to understand how innate immunity functions and interacts with cell-mediated immune responses and the antibody recognition system.

Toll-like receptors, lipopolysaccharides and septicemia

The *Toll* gene was identified while screening for mutations that disrupt proper formation and development of the front and back of the fruit fly *Drosophila melanogaster*. At the time of the discovery, it was never thought that this *Toll* gene and its encoded protein(s) would have any role in the regulation of innate immunity. However, subsequent studies showed that the *Toll* gene encodes a receptor protein that can bind to pattern-recognition receptors of the infectious agents and send these signals from the cell membrane to the nucleus [3].

The innate immune system identifies the infectious agents by means of pattern-recognition receptors that are situated on the surface of macrophages. The best examples for this are the mannose receptors of macrophages, which bind to structures that contain polymannose repeats of the micro-organisms; and CD14, which has the capacity to bind to lipid-containing ligands, including lipopolysaccharides of Gram-negative organisms, and bacterial peptidoglycan and cell-wall constituents of *Mycobacterium tuberculosis* [2•]. Lipopolysaccharides induce macrophages to secrete cytokines, which in turn can act on T and B cells to upregulate the adaptive immune responses. However, this binding of lipopolysaccharides to CD14 does not transmit the signal across the cell membrane, because CD14 lacks a cytoplasmic signaling domain. Instead, a glycosyl phosphatidylinositol linkage tethers it to the cell surface, suggesting the presence of another receptor that has the capacity to signal macrophages to release cytokines. This receptor turned out to be the protein encoded by the *Toll* gene, a protein that has a crucial role in the regulation of immune response. At least six mammalian TLRs have been identified thus far. TLRs can bind to the bacterial cell-wall components and trigger a series of reactions that ultimately result in the death of the microbes and also help to switch on the adaptive immune system [4]. When this interaction between TLRs and the bacterial cell-wall components occurs in excess, septic shock may ensue [5].

Toll-like receptors and nuclear factor-κB

Inflammation, an important component of innate immunity, is dependent on the activation of nuclear factor-κB (NF-κB) genes. The activation of NF-κB involves phosphorylation and degradation of IκB, an inhibitor of NF-κB, which leads to the translocation of NF-κB heterodimer to the nucleus to bring about its action. The NF-κB/IκB system can exert transcriptional regulation on proinflammatory genes. Most genes that encode various adhesion molecules, cytokines, and other proinflammatory genes have functional NF-κB-binding elements in their promoter regions [6]. It is now known that NF-κB can be activated by several cytokines such as IL-1, TNF and IL-6, among others, that are not only proinflammatory molecules, but can also induce fever.

A protein named Dorsal, which has a role in the development of organisms, is structurally similar to NF-κB [3]. Both Dorsal and Dif, a protein that is related to Dorsal, have the ability to travel to the nucleus in response to infection, suggesting a close relationship between developmental genes and proteins and immune response. In addition, proteins that closely resemble *Toll* proteins help plants to fight infections against bacteria and fungi. The first link between the *Toll* proteins and human immunity came from the observation that TLR-4 has the capacity to activate NF-κB [4]. Furthermore, when immune cells were exposed to lipopolysaccharide, increase in the synthesis of TLR-2 was noted [6]. This increase in the formation of TLR-2 is associated with an increase in the activity of NF-κB [7,8]. Because there are at least six types of TLRs, and many more may be found in the future, it is believed that different types of TLRs are designed to respond to

different types of pathogens. Current evidence indicates that TLR-4 is essentially specific for lipopolysaccharide and perhaps lipoteichoic acid. On the other hand, TLR-2 is much less specific, and responds to a number of different antigens including peptidoglycan and Gram-positive bacteria. This suggests that it is important to look at each individual TLR separately and in combination for their possible response(s) to specific micro-organisms in order to find specific protein pathways for the different types of pathogens. This will ultimately enable us to understand the way(s) that the innate immune system responds to different stimuli, which may help us to devise methods to treat or manage different types of inflammatory diseases by selectively shutting down specific pathways.

Lipopolysaccharide, CD14, *Toll*-like receptors, nuclear factor-κB and cytokines

It is clear that various *Toll* proteins provide a link between the adaptive and innate immune systems. However, it is still not clear exactly how this relationship works, although certain generalizations are possible. For example, naïve T cells (which are constituents of the adaptive immune system) that have not been exposed to antigens need at least two signals to become active, to proliferate and to produce various cytokines. The first stimulus appears to be the binding of the unknown antigen and the second is either CD14 or a protein called B7.1 or other similar proteins such as B7.2 or CD40, which are glycoproteins expressed on antigen-presenting cells such as macrophages and human polymorphonuclear leukocytes [9]. These proteins are related to the *Toll* pathway because TLR-4 increases the production of B7.1. The importance of the TLR family lies in the fact that C3H/HeJ mice, which have defective lipopolysaccharide signaling, are homozygous for a TLR-4 mutation. Because C3H/HeJ mice are highly susceptible to Gram-negative sepsis, this suggests that TLR-4 is necessary to protect against Gram-negative infections.

Thus, the CD14 of the macrophages recruits lipopolysaccharides to TLR proteins. Because TLRs contain a cytoplasmic portion that is homologous to the IL-1 receptor, this will lead to the induction of a signaling pathway that involves the recruitment of IL-1 associated kinase 2, TNF-associated factor 6, and activation of NF-κB. This induces the synthesis and secretion of various cytokines, including TNF, by macrophages and other cells of the innate immune system. These cytokines, in turn, stimulate T and B cells of the adaptive immune system. The activated immune cells of the adaptive immune system produce several soluble factors that include various cytokines, such as TNF, and immunoglobulins in order to kill the invading organisms and protect the host. Failure of this seemingly excellent defense system in the form of over-responsiveness to the invading micro-organisms may result in septicemia and septic shock.

Macrophage, cytokines, eicosanoids, and free radicals in sepsis and septic shock

Gram-positive organisms, malarial parasite, fungi, endotoxin-containing Gram-negative organisms and other microbials can trigger septicemia. The invading micro-organism can proliferate and produce bacteremia, or may release endotoxin, exotoxin and other toxins that stimulate the monocytes, macrophages, endothelial cells, neutrophils and other cells. These stimulated cells release mediators of sepsis and septic shock, including IL-1, IL-2, IL-6, IL-8, TNF, platelet-activating factor, endorphins, various eicosanoids, nitric oxide, high mobility group 1 (HMG-1), and macrophage MIF [10–12]. These mediators have profound effects on the cardiovascular system, kidneys, lungs, liver, central nervous system, and coagulation system. As a consequence of their action(s), renal failure, myocardial dysfunction, acute respiratory distress syndrome (ARDS), hepatic failure, and disseminated intravascular coagulation can occur, which may result in death.

Administration of endotoxin results in changes in cardiovascular function that are very similar to those seen in sepsis. Also, TNF can induce depression of cardiovascular function similar to that observed in sepsis. Pretreatment with antibodies against TNF prevented death both in mice and in nonhuman primates that received endotoxin. In humans, however, monoclonal antibodies directed against TNF failed to produce substantial benefit (for review [13]), suggesting that there may be other mediators that may play a more important role in septicemia and septic shock.

In patients with septic shock, maldistribution of blood flow, aggregation of neutrophils and platelets, damage to endothelium and coagulation abnormalities are seen. Neutrophils release reactive oxygen species including superoxide anion and nitric oxide, which can damage cells. Inflammatory mediators derived from arachidonic acid such as prostaglandins and leukotrienes (referred to as eicosanoids) are also released from various cells, and these have effects on the microvasculature, resulting in microvasculature failure (for review [1]). TNF-α and IL-1β can incite the production of free radicals, nitric oxide and eicosanoids from various cells that can also produce several of the pathophysiologic changes seen during sepsis and septic shock. In view of this, it was believed that TNF-α and IL-1β may be critical mediators of septic shock [14]. Contrary to this, anti-TNF monoclonal antibody and IL-1 receptor antagonist failed to benefit patients with severe sepsis (for review [13]). This led to further studies that revealed that macrophage MIF could be a major mediator of sepsis and septic shock.

Migration inhibitory factor in septicemia and septic shock

MIF is secreted by antigen-sensitized lymphocytes, pituitary gland, cells of the brain, kidney, lung, prostate and

testis, and macrophages [15•,16•]. MIF enhanced the lethality of mice exposed to lipopolysaccharides and neutralization of MIF protected mice from the lethality of lipopolysaccharides [17–19].

The macrophage is an important source of MIF, and it is also the target of the action of MIF [20]. Low concentrations of hydrocortisone (10^{-14} mol/l) induce MIF production by macrophages, whereas at higher concentrations (10^{-7} mol/l) it completely inhibits the production of lipopolysaccharide-induced TNF-α by the same macrophages [21]. MIF is also released from the pituitary after infection and stress [18,22]. Exposure to bacterial toxins such as lipopolysaccharide, toxic shock syndrome toxin-1, and cytokines (TNF-α and IFN-γ) incites macrophages to produce MIF [22,23], which in turn promotes inflammation by stimulating the production of eicosanoids and TNF-α.

The critical role played by MIF in inflammation is evident from the fact that glucocorticoids enhance its production, whereas MIF can effectively antagonize the anti-inflammatory and immunosuppressive effects of glucocorticoids on macrophages and T cells [24,25]. Antibodies against MIF can decrease inflammation in experimental models of glomerulonephritis, arthritis and allograft rejection [26–28]. These studies suggest that MIF and glucocorticoids function as physiologic antagonists.

MIF expression is increased in experimental animals exposed to Gram-negative (ie lipopolysaccharide) and Gram-positive (ie toxic shock syndrome toxin-1 and streptococcal pyrogenic exotoxin A) bacterial toxins [29], and neutralization of MIF or deletion of MIF gene can protect mice from lethal endotoxemia or staphylococcal toxic shock [30]. High concentrations of MIF are present in the peritoneal exudate and in the systemic circulation of mice with bacterial peritonitis, and anti-MIF antibody protects TNF-α knockout mice from sepsis and septic shock [31••]. Because TNF-α knockout mice were used in that study, most of the beneficial results obtained can be attributed to the anti-MIF antibody used. However, this does not rule out an important role for TNF-α in sepsis and septic shock. A similar benefit of anti-MIF antibody even in *Escherichia coli*-induced sepsis and septic shock in mice was reported [31••]. Anti-MIF antibody protected normal mice from lethal peritonitis induced by cecal ligation and puncture and *E coli*, even when used 8 h after cecal ligation and puncture [31••]. High concentrations of MIF were detected in the plasma of patients with septicemia and septic shock, indicating that this molecule may have a role even in humans.

Furthermore, the improved survival obtained with anti-MIF antibody in sepsis and septic shock was associated with a reduction in the plasma concentrations of TNF and lower bacterial circulating counts [31••]. Even MIF gene-knockout mice showed reduced circulating levels of TNF-α [30,31••]. These results suggest that MIF can augment the synthesis and release of TNF-α, and that when this stimulus is removed the plasma levels of TNF-α should fall. MIF-knockout mice also cleared *Pseudomonas aeruginosa* from the lungs faster than did wild-type mice [30], which was not due to the effect of MIF on the growth of the bacteria, unlike IL-1β, which can enhance the growth of *E coli* [32].

Migration inhibitory factor and eicosanoids

What is the main mechanism(s) by which MIF causes sepsis/septic shock? Mitchell *et al* [25] showed that MIF stimulates the cytosolic phospholipase A_2, inducing the release of arachidonic acid, the precursor of 2 series prostaglandins and 4 series leukotrienes, which have potent proinflammatory actions. Hence, it is likely that MIF produces its systemic inflammatory response by the activation of the arachidonic acid–prostaglandin–leukotriene pathway. If this is true, it also suggests a role for n-3 fatty acids such as α-linolenic acid and eicosapentaenoic acid in the treatment of sepsis/septic shock, because n-3 fatty acids can inhibit the metabolism of arachidonic acid [33]. A diet rich in n-3 fatty acids or continuous tube feeding or intravenous infusion of n-3 fatty acids suppressed the production of proinflammatory eicosanoids and improved survival of experimental animals challenged with endotoxin [34–36]. Patients with septicemia showed low concentrations of γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid of the n-6 series and α-linolenic acid, and eicosapentaenoic acid of the n-3 series in their plasma phospholipid fraction [37]. This suggests, but does not prove, that n-3 and n-6 fatty acids may inhibit MIF secretion, similar to their suppressive effect on TNF production [38–40]. If this is true, then this suggests that n-3 and n-6 fatty acids may have some therapeutic value in the treatment of patients with sepsis and septic shock.

HLA-DR antigens, neutrophil activation and free radicals in postsurgery/trauma sepsis

It is known that, whereas some patients succumb to overwhelming sepsis after surgery, others will recover uneventfully, having received an identical course of management for the same condition. It is not yet clear why some patients never develop sepsis and why some develop sepsis but recover, whereas others succumb to sepsis with multiorgan failure. It is likely that there are some very clear biologic variations in the response of different individuals to a given stimulus that determine their ability or inability to recover from sepsis and septic shock. Some of these may include the positive and negative interactions between CD14, TLRs, MIF, TNF-α and other cytokines; TNF-α receptor density on macrophages; Fas; CD11b; DNA polymorphism for genes for the coagulation system; eicosanoids; glucocorticoid secretion; and free radical generation, including nitric oxide and antioxidant status.

Guillou [41•] showed that there could be individual variation(s) in the levels of monocyte expression of major histocompatibility complex class II (HLA-DR) antigens, neutrophil expression of the integrin CD11b/CD18 (which is necessary for adhesion of neutrophils to endothelium), and the production of hydrogen peroxide and hypochlorous acid (a marker of neutrophil activation) in patients with uncomplicated abdominal surgery. In patients with an uneventful recovery from severe trauma or surgery the level of monocyte HLA-DR expression fell within hours of trauma or surgery, but returned to normal within a week, whereas in those who developed infection but recovered it took 3 weeks for HLA-DR expression to return to normal. On the other hand, in those who developed infection and sepsis and who died as a result, HLA-DR expression fell and never returned to normal [41•,42]. Similarly, after uncomplicated elective major abdominal surgery, the expression of CD11b was unchanged throughout the postoperative period. By contrast, in patients who developed postoperative sepsis, the expression of neutrophil CD11b was significantly elevated within 24 h of surgery [41•,43]. Even the production of hydrogen peroxide by neutrophils followed a pattern similar to that of CD11b expression in these two groups of patients. Carey *et al* [44] noted that hypochlorous acid production, a marker of neutrophil activation, was reduced after uncomplicated abdominal surgery compared with those who developed sepsis 7–10 days later, in whom the hypochlorous acid production was found to be augmented to supranormal levels on postoperative day 1 [44]. These changes in HLA-DR and CD11b expression, hydrogen peroxide and hypochlorous acid production were noted even when there was no clinical or bacteriologic evidence of infection.

On the basis of these results, it was concluded that, in those patients who are destined to develop postoperative or post-trauma sepsis, neutrophil activation is an early event. The variations in neutrophil activation and HLA-DR expression between these different groups of patients was noted not preoperatively, but only after trauma or surgery. Hence, it was concluded that there is a wide biologic variation in the way that a person responds to injury. It is interesting to note that various cytokines, MIF and eicosanoids can influence neutrophil activation and HLA-DR expression. Hence, it is likely that the biologic variation observed with regard to neutrophil activation and HLA-DR expression may also exist between different types of individuals in their response to MIF, TNF-α, various interleukins, eicosanoids, free radicals and nitric oxide.

Conclusion

It is evident from the preceding discussion that there is a complex network of events that occur in sepsis and septic shock. Because there is strong evidence for the involvement of MIF in sepsis and septic shock from animal studies, it may be necessary to evaluate its role in humans. Donnelly *et al* [45] showed that MIF is detectable in the serum and the lung fluids and in alveolar macrophages of patients with ARDS. Furthermore, MIF inhibited the suppressive effects of dexamethasone on IL-8 production by lung macrophages [45]. There is a close relation between the presence of IL-8 (a neutrophil chemotactic factor [46]) in early bronchoalveolar lavage fluid samples and the development of ARDS [47]. This suggests that both IL-8 and MIF can be used as prognostic indicators for the development of ARDS, which is common in septicemia and septic shock, and reinforces the significance of macrophages in the pathobiology of both ARDS and sepsis.

It is possible that methods designed to suppress the production of TNF and interleukins may still prove to be useful in septicemia and septic shock, even though anti-TNF antibody and IL-1 receptor antagonist have failed to benefit these patients. In this context, it is interesting to note that providing adequate amounts of glucose and insulin has been shown to antagonize the harmful actions of TNF-α [48••]. It was also observed that treatment with insulin can almost completely reverse the nutritional and histopathologic toxicity of sublethal doses of TNF in rats [48••]. Furthermore, insulin may have a regulatory role in superoxide generation [49•]. In addition, the expression of MIF in adipocytes can be modulated by insulin and glucose [50]. It has also been found that MIF is secreted together with insulin from pancreatic β cells and acts as an autocrine factor to stimulate insulin release [50].

This evidence suggests that, during systemic inflammatory processes, MIF is secreted from the pituitary gland accompanied by an increase in glucocorticoid secretion (and macrophages will also produce MIF and TNF). The increase in plasma glucose concentration that occurs as a result of this glucocorticoid production is probably controlled by MIF, which has a positive effect on insulin secretion [51]. Thus, glucose homeostasis during septicemia and septic shock is maintained by glucocorticoids, insulin and TNF by inducing insulin resistance. Because there is a feedback control between MIF, glucose and insulin [50,51], it is possible that infusion of insulin and glucose can inhibit MIF production and release [51], which is similar to their action on TNF [48••]. If this hypothesis is correct, it suggests that a glucose–insulin–potassium regimen (which is used in the management of diabetic ketoacidosis) may be useful in the management of septicemia and septic shock [51], in which excess production of TNF-α and MIF seem to play an important role. However, this concept remains to be verified. It is important to measure plasma TNF and MIF concentrations both before and after the glucose–insulin–potassium regimen in these patients, and to determine whether there is any correlation between the progress and outcome of septicemia and septic shock and the concentrations of TNF and MIF.

Although animal studies indicated that anti-MIF antibody can prevent mortality due to septicemia and septic shock, many questions remain to be answered. Some of these include the following: what exactly is the relationship between MIF and TNF and other cytokines?; can the plasma levels of MIF be a guide to predict the outcome from septicemia and septic shock?; is it possible that patients who recover from septicemia and septic shock secrete substances that can neutralize the actions of MIF/TNF/interleukins?; if eicosanoids are the major mediators of the inflammatory response of MIF, why are cyclooxygenase and lipoxygenase inhibitors not useful in septicemia/septic shock?; is there a relationship between the number of TLRs expressed and MIF levels?; and, finally, can human monoclonal antibody directed against MIF prevent and/or improve prognosis in septicemia and septic shock?

References

Articles of particular interest have been highlighted as:
- • of special interest
- •• of outstanding interest

1. Parrillo JE: **Pathogenetic mechanisms of septic shock.** *N Engl J Med* 1993, **328**:1471–1477.

2. • Modlin RL, Brightbill HD, Godowski PJ: **The toll of innate immunity on microbial pathogens.** *N Engl J Med* 1999, **340**:1834–1835.

This article enumerates the importance of TLR proteins in innate immunity and suggested that agonists of TLRs in the form of vaccines may be useful as adjuvants to stimulate innate immunity and thereby enhance adaptive immunity. It is also suggested that TLRs may be useful in the treatment of septic shock.

3. Vogel G: **Fly development genes lead to immune find.** *Science* 1998, **281**:1942–1944.

4. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr: **A human homologue of the Drosophila Toll protein signals activation of adaptive immunity.** *Nature* 1997, **388**:394–397.

5. Rowe PM: **Septic shock: finding the way through the maze.** *Lancet* 1999, **354**:2058.

6. Stuhlmeier KM, Kao JJ, Bach FH: **Arachidonic acid influences proinflammatory gene induction by stabilizing the inhibitor-kBα/nuclear factor-kB (NF-kB) complex, thus supporting the nuclear translocation of NF-kB.** *J Biol Chem* 1997, **272**:24679–24683.

7. Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhang M, Goddard A, Wood WI, Gurney AL, Godowski PJ: **Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signaling.** *Nature* 1998, **395**: 284–288.

8. Kirshning CJ, Wesche H, Merrill Ayres T, Rothe M: **Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide.** *J Exp Med* 1998, **188**:2091–2097.

9. Ikemizu S, Gilbert RJ, Fennelly JA, Collins AV, Harlos K, Jones EY, Stuart DI, Davis SJ: **Structure and dimerization of a soluble form of B7-1.** *Immunity* 2000, **12**:51–60.

10. Das UN: **Free radicals: biology and relevance to disease.** *J Assoc Phys India* 1990, **38**:495–498.

11. Das UN: **Tumor necrosis factor/cachectin: biology and relevance to disease.** *J Assoc Phys India* 1991, **39**:201–204.

12. Parrillo JE: **Septic shock in humans: advances in the understanding of pathogenesis, cardiovascular dysfunction and therapy.** *Ann Intern Med* 1990, **113**:227–242.

13. Abraham E: **Why immunomodulatory therapies have not worked in sepsis?** *Intensive Care Med* 1999, **25**:556–566.

14. Glauser MP, Zanetti G, Baumgartner JD, Cohen J: **Septic shock: pathogenesis.** *Lancet* 1991, **338**:732–736.

15. • Bloom BR, Bennett B: **Mechanism of a reaction in vitro associated with delayed-type hypersensitivity.** *Science* 1966, **153**:80–82.

See [16•].

16. • David JR: **Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction.** *Proc Natl Acad Sci USA* 1966, **56**:72–77.

This paper and [15•] were the first reports that described the existence of macrophage MIF.

17. Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, Manogue KR, Cerami A, Bucala R: **MIF is a pituitary-derived cytokine that potentiates lethal endotoxemia.** *Nature* 1993, **365**:756–759.

18. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R: **MIF as a glucocorticoid-induced modulator of cytokine production.** *Nature* 1995, **377**:68–71.

19. Weiser WY, Temple PA, Witek-Giannotti JS, Remold HG, Clark SC, David JR: **Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor.** *Proc Natl Acad Sci USA* 1989, **86**:7522–7526.

20. Bucala R: **MIF, a previously unrecognized pituitary hormone and macrophage cytokine, is a pivotal mediator in endotoxic shock.** *Circ Shock* 1994, **44**:35–39.

21. Calandra T, Bucala R: **Macrophage migration inhibitory factor (MIF): a glucocorticoid counter-regulator within the immune system.** *Crit Rev Immunol* 1997, **17**:77–88.

22. Calandra T, Spiegel LA, Metz CN, Bucala R: **Macrophage migration inhibitory factor (MIF) is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria.** *Proc Natl Acad Sci USA* 1998, 95:11383–11388.

23. Calandra T, Bernhagen J, Mitchell RA, Bucala R: **The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor.** *J Exp Med* 1994, **179**:1895–1902.

24. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M, Gemsa D, Donnelly T, Bucala R: **An essential regulatory role for macrophage migration inhibitory factor in T-cell activation.** *Proc Natl Acad Sci USA* 1996, 93:7849–7854.

25. Mitchell RA, Metz CN, Peng T, Bucala R: **Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF).** *J Biol Chem* 1999, **274**:18100–18106.

26. Lan HY, Bacher M, Yang N, Mu W, Nikolic-Paterson DJ, Metz C, Meinhardt A, Bucala R, Atkins RC: **The pathogenic role of macrophage migration inhibitory factor in immunologically induced kidney disease in the rat.** *J Exp Med* 1997, 185:1455–1465.

27. Leech M, Metz C, Santos L, Peng T, Holdsworth SR, Bucala R, Morand EF: **Involvement of macrophage migration inhibitory factor in the evolution of rat adjuvant arthritis.** *Arthritis Rheum* 1998, **41**:910–917.

28. Lan HY, Yang N, Brown FG, Isbel NM, Nikolic-Paterson DJ, Mu W, Metz CN, Bacher M, Atkins RC, Bucala R: **Macrophage migration inhibitory factor expression in human renal allograft rejection.** *Transplantation* 1998, **16**:1465–1471.

29. Bacher M, Meinhardt A, Lan HY, Mu W, Metz CN, Chesney JA, Calandra T, Gemsa D, Donnelly T, Atkins RC, Bucala R: **MIF expression in experimentally-induced endotoxemia.** *Am J Pathol* 1997, **150**:235–246.

30. Bozza M, Satoskar AR, Lin G, Lu B, Humbles AA, Gerard C, David JR: **Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis.** *J Exp Med* 1999, **189**:341–346.

31. •• Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L, Heumann D, Mannel D, Bucala R, Glauser MP: **Protection from septic-shock by neutralization of macrophage migration inhibitory factor.** *Nature Med* 2000, **6**:164–170.
This important study demonstrated that monoclonal antibody against MIF can protect TNF-α-knockout experimental animals from *E coli*-induced septic shock and lethal peritonitis induced by caecal ligation and puncture, an animal model of human sepsis and septic shock. It was also demonstrated that animals treated with anti-MIF antibody show reduced levels of TNF and that the plasma of patients with septicemia and septic shock has elevated levels of MIF.

32. Porat R, Clark BD, Wolff SM, Dinarello CA: **Enhancement of growth of virulent strains of *Escherichia coli* by interleukin-1.** *Science* 1991, **254**:430–432.

33. Palombo JD, DeMichele SJ, Boyce PJ, Noursalehi M, Forse RA, Bistrian BR: **Metabolism of dietary alpha-linolenic acid vs eicosapentaenoic acid in rat immune cell phospholipids during endotoxemia.** *Lipids* 1998, **33**:1099–1105.

34. Murray MJ, Svingen BA, Holman RT, Yaksh TL: **Effects of a fish oil diet on pigs' cardiopulmonary response to bacteremia.** *JPEN J Parenter Enteral Nutr* 1991, **15**:152–158.

35. Utsunomiya T, Chavali S R, Zhong WW, Forse RA: **Effects of continuous tube feeding of dietary fat emulsions on eicosanoid production and on fatty acid composition during an acute septic shock in rats.** *Biochim Biophys Acta* 1994, 1214:333–339.

36. Mascioli E, Leader L, Flores E, Trimbo S, Bistrian B, Blackburn G: **Enhanced survival to endotoxin in guines pigs fed IV fish oil emulsion.** *Lipids* 1988, **23**:623–625.

37. Prabha PS, Das UN, Ramesh G, Kumar KV, Kalakar V: **Free radical generation, lipid peroxidation and essential fatty acids in patients with septicemia.** *Prostaglandins Leukot Essent Fatty Acids* 1991, **42**:61–65.

38. Kumar SG, Das UN, Kumar KV, Madhavi N, Das NP, Tan BKH: **Effect of n-6 and n-3 fatty acids on the proliferation and secretion of TNF and IL-2 by human lymphocytes in vitro.** *Nutrition Res* 1992, **12**:815–823.

39. Kumar SG, Das UN: **Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of tumor necrosis factor and various interleukins.** *Prostaglandins Leukot Essent Fatty Acids* 1994, **50**:331–334.

40. Madhavi N, Das UN, Prabha PS, Kumar SG, Koratkar R, Sagar PS: **Suppression of human T cell growth in vitro by cis-unsaturated fatty acids: relationship to free radicals and lipid peroxidation.** *Prostaglandins Leukot Essent Fatty Acids* 1994, **51**:33–40.

41. • Guillou PJ: **Biological variation in the development of sepsis after surgery or trauma.** *Lancet* 1993, **342**:217–220.
In this review article the author summarized the evidence available that support the notion that there are clear biologic variations in the response of different individuals to a given stimulus, especially in the levels of expression of HLA-DR antigens, neutrophil integrin CD11b/CD18 and the production of hydrogen peroxide and hypochlorous acid. The author used these variations in the individual responses to explain why some individuals develop and succumb to septicemic process, whereas others do not.

42. Hershman MJ, Cheadle WG, Wellhausen SR, Davidson PF, Polk SC: **Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient.** *Br J Surg* 1990, **77**:204–207.

43. Wakefield CH, Foulds S, Carey PD, Monson JRT, Guillou PJ: **Polymorphonuclear leukocyte activation: an early marker of the post-surgical sepsis response.** *Arch Surg* 1993, **128**:390–395.

44. Carey PD, Wakefiled CH, Thayeb A, Monson JRT, Guillou PJ: **Preservation of neutrophil antimicrobial function after laparoscopic surgery.** *Br J Surg* 1992, **79**:1223.

45. Donnelly SC, Haslett C, Reid PT, Grant IS, Wallace WA, Metz CN, Bruce LJ, Bucala R: **Regulatory role for macrophage migration inhibitory factor in the acute respiratory distress syndrome.** *Nature Med* 1997, **3**:320–323.

46. Kunkel SL, Standiford T, Kasahara K, Strieter RM: **Interleukin-8: the major neutrophil chemotactic factor in the lung.** *Exp Lung Res* 1991, **17**:17–23.

47. Donnelly SC, Strieter RM, Kunkel SL, Walz A, Robertson CR, Carter DC, Grant IS, Pollok AJ, Haslett C: **Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups.** *Lancet* 1993, **341**:643–647.

48. •• Fraker DL, Merino MJ, Norton JA: **Reversal of the toxic effects of cachectin by concurrent insulin administration.** *Am J Physiol* 1989, **256**:E725–E731.
This remarkable study showed that intraperitoneally delivered cachectin (TNF) induced a 56% decrease in food intake, a 54% decrease in nitrogen balance and a 23-g decrease in body weight gain compared with saline-treated controls. Cachectin treatment also caused a severe interstitial pneumonitis, periportal inflammation in the liver, and an increase in size of the heart, lungs, kidney and spleen. It was reported in this study that concurrent insulin administration led to near total reversal of all of the changes induced by cachectin.

49. • Boichot E, Sannomiya P, Escofier N, Germain N, Fortes ZB, Lagente V: **Endotoxin-induced acute lung injury in rats. Role of insulin.** *Pulm Pharmacol Ther* 1999, **12**:285–290.
In this important study, diabetic Wistar rats showed a 50% reduction in luminol-dependent chemiluminescence and TNF-α in the bronchoalveolar lavage fluid in response to an aerosol of lipopolysaccharide compared with controls. These parameters returned to control levels after treatment of diabetic rats with insulin, before exposure to lipopolysaccharide, suggesting that insulin might regulate superoxide anion generation and TNF-α release.

50. Waeber G, Calandra T, Bonny C, Bucala R: **A role for the endocrine and pro-inflammatory mediator MIF in the control of insulin secretion during stress.** *Diabetes Metab Res Rev* 1999, **15**:47–54.

51. Das UN: **Possible beneficial action(s) of glucose-insulin-potassium regimen in acute myocardial infarction and inflammatory conditions: a hypothesis.** *Diabetologia* 2000, in press.

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