LE PRIX CLOËTTA 2001
ESTDÉCERNÉ À

MONSIEUR

# THIERRY CALANDRA

DOCTEUR EN MÉDECINE

NÉ EN 1956 À LAUSANNE
PROFESSEUR ASSOCIÉ À L'UNIVERSITÉ DE LAUSANNE
ETMÉDECIN-ADJOINTDE LADIVISION
DES MALADIES INFECTIEUSES
AU CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS, LAUSANNE,
POUR SES REMARQUABLES TRAVAUX DANS LE DOMAINE DE LA
PHYSIOPATHOLOGIE DU CHOC SEPTIQUE.

ZURICH, LE 28 NOVEMBRE 2001

AU NOM DU CONSEILDE FONDATION:

LE PRÉSIDENT

LE VICE-PRÉSIDENT

UN MEMBRE

Dr. Thierry François Calandra

# CURRICULUM VITAE

| | |
|---|---|
| Name: | Thierry François Calandra, M.D., Ph.D. |
| Date and place of birth: | June 16, 1956, Lausanne, Switzerland |
| Citizenship: | Switzerland |
| Civil status: | Married, two children |

*Education:*

| | |
|---|---|
| 1974 | Baccalauréat in mathematics and science |
| 1974 – 1980 | Medical school (Faculty of Medicine, University of Lausanne) |
| 1980 | Graduation in Medicine (MD Degree), Swiss Federal Licence |
| 1990 | Doctor of Medicine – University of Lausanne, Switzerland |
| 1990 | PhD degree – University of Utrecht, The Netherlands |
| 1995 | Swiss Board Certificate in Internal Medicine |
| 1999 | Swiss Board Certificate in Infectious Diseases |

*Fellowships, post-doctoral record and appointments*

| | |
|---|---|
| April 1981 – Sept. 1983 | Resident<br>Department of Internal Medicine,<br>Centre Hospitalier Universitaire Vaudois,<br>Lausanne, Switzerland |
| Oct. 1983 – Sept. 1986 | Research fellow<br>Laboratory of Infectious Diseases,<br>Department of Internal Medicine,<br>Centre Hospitalier Universitaire Vaudois,<br>Lausanne, Switzerland |
| Oct. 1986 – Dec. 1986 | Resident<br>Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois,<br>Lausanne, Switzerland |
| Jan. 1987 – August 1991 | Chief resident<br>Department of Internal Medicine and Division of Infectious Diseases,<br>Centre Hospitalier Universitaire Vaudois,<br>Lausanne, Switzerland |
| Sept. 1991– August 1992 | Post-doctoral fellow<br>Laboratory of Cellular Physiology and Immunology, The Rockefeller University,<br>New York, USA |
| Sept. 1992 – August 1995<br>Sept. 1995 – Sept. 1996 | Senior Scientist<br>Assistant Professor<br>The Picower Institute for Medical Research, Manhasset, New York, USA |

| | |
|---|---|
| Oct. 1996 – March 2000 | Médecin-associé |
| April 2000 – August 2001 | Assistant Professor |
| Sept. 2001 – to date | Associate Professor |
| | Division of Infectious Diseases, |
| | Department of Internal Medicine |
| | Centre Hospitalier Universitaire Vaudois, |
| | Lausanne, Switzerland |

*Research activities*

Innate Immune Responses of Macrophages to Microbial Products

Pathophysiology of Sepsis and Septic Shock: Role of Cytokines and other Pro-inflammatory Mediators

Pathogenesis and Treatment of Infections in Neutropenic Cancer Patients

Prevention and Treatment of Candida Infections in Surgical and Intensive Care Units Patients

*Grants*

Swiss National Science Foundation

«Characterisation of the Role of Macrophage Migration Inhibitory Factor (MIF) in Host Defenses»
Grants# 32-49129.96 and 3231-048916.96

«Mechanisms of defense against endotoxins and Gram-positive cell walls»
Grant# 32-055829.98 (co-investigator)

Swiss Foundation for Cardiology
(1997 – 1998)

«Expression and Pathogenic Role of Macrophage Migration Inhibitory Factor (MIF) in Heart Transplant Patients with Infections and Graft Rejection»

*Awards*

| | |
|---|---|
| 1993 | Thesis Award, Faculty of Medicine, University of Lausanne, Switzerland |
| 1996 | SCORE A Program from the Swiss National Science Foundation |
| 1999 | Hoechst-Marion Roussel Award of the Swiss Society of Infectious Diseases |
| 2000 | Bourse Bridge-relève, Fondation Leenaards, Lausanne, Switzerland |

*Memberships*

Fédération des médecins suisses

Société suisse de médecine interne

Société suisse d'infectiologie

Société vaudoise de médecine

International Immunocompromised Host Society (Member of the Council)

American Society for Microbiology

Infectious Disease Society of America

European Organization for Research and Treatment of Cancer (EORTC)

International Antimicrobial Therapy Cooperative Group
(Member of the Advisory Board)

European Society of Clinical Microbiology and Infectious Diseases

*Editorial activities*

| | |
|---|---|
| 1990 – 1994 | Intensive Care Medicine (Advisory Board) |
| 1997 – present | Sepsis (Editorial Board) |

*Reviewing*

On ad hoc basis:

Nature, Nature Medicine, Nature Immunology, Lancet, JAMA, Journal of Immunology, European Journal of Immunology, Journal of Clinical Investigation, Molecular Medicine, Infection and Immunity, Journal of Infectious Diseases, Clinical Infectious Diseases, European Journal of Clinical Microbiology and Infectious Diseases, Antimicrobial Agents and Chemotherapy, Intensive Care Medicine, Critical Care Medicine, European Journal of Cancer, American Journal of Respiratory and Critical Care Medicine, Journal of Antimicrobial Chemotherapy

# SELECTED PUBLICATIONS


01. Calandra T, Glauser MP, Schellekens J, Verhoef J and the Swiss-Dutch J5 Immunoglobulin Study Group.
*Treatment of Gram-negative septic shock with human IgG antibody to Escherichia coli J5: a prospective, double-blind, randomized trial.*
Journal of Infectious Diseases 1988;158:312–319

02. Calandra T, Baumgartner JD, Grau GE, Wu MM, Lambert PH, Schellekens J, Verhoef J, Glauser MPand the Swiss-Dutch J5 immunoglobulin study group.
*Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-α and interferon-γ in the serum of patients with septic shock.*
Journal of Infectious Diseases 1990;161:982–987

03. Calandra T, Gerain J, Heumann D, Baumgartner JD, Glauser MP and the Swiss-Dutch J5 study group
*High circulating levels of interleukin-6 in patients with septic shock : evolution during sepsis, prognostic value and interplay with other cytokines.*
American Journal of Medicine 1991;91:23–29

04. 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 endotoxaemia.*
Nature 1993;365:756–759

05. Calandra T, Bernhagen J, Mitchell RA, Bucala R.
*The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor (MIF).*
The Journal of Experimental Medicine 1994;179:1895–1902

06. 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

07. Waeber G, Calandra T, Roduit R, Haefliger J-A, Thompson N, Thorens B, Temler E, Meinhardt A, Bacher M, Metz C, Nicod P, Bucala R.
*Insulin secretion is regulated by the glucose-dependent production of islet βcell macro - phage migration inhibitory factor.*
Proceedings of the National Academy of Science USA1997;94:4782–4787

08. Calandra T, Spiegel L, 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*.
Proceedings of the National Academy of Science USA1998;95:11383–11388

09. Calandra T, Echtenacher B, Leroy D, Pugin J, Metz CN, Hültner L, Heumann D, Männel D, Bucala R, Glauser MP
*Protection from septic shock by neutralization of macrophage migration inhibitory factor.*
Nature Medicine 2000;6:16–170

10. Roger T, David J, Glauser MP, Calandra T
*MIF regulates innate immune responses to endotoxin and Gram-negative bacteria through modulation of Toll-like receptor 4.*
Nature in press

# INNATE IMMUNE RESPONSES TO BACTERIALINFECTIONS: A PARADIGM FOR EXPLORING THE PATHOGENESIS OF SEPTIC SHOCK

*Dr Thierry Calandra*

Severe sepsis and septic shock are life-threatening complications of infections and the most common cause of death in intensive care units. Yet, it has been difficult to obtain accurate estimates of the frequency of sepsis, severe sepsis and septic shock, mainly because of a lack of authoritative case definitions of these heterogenous clinical syndromes. Recent data suggest that the frequency of septic complications may be increasing. A study published in 1990 by the Center for Diseases Controls in the United Stated indicated that the incidence of septicemia had increased from 73.6 per 100,000 patients in 1979 to 175.9 per 100,000 patients in 1987. Based on several recent US and European hospital-based surveys, it is estimated that severe sepsis accounts for 2% to 11% of all hospital or ICU admissions (1). Unfortunately, there are no data on the incidence of severe sepsis and septic shock in Swiss hospitals. Population-based epidemiological studies are thus needed to obtain a more precise appreciation of the true incidence of sepsis in the general population.

Over the last twenty years, considerable progress has been made in the understanding of the pathogenesis of inflammation and sepsis (2). Bacteria, mainly Gram-negative bacilli and Gram-positive cocci, are the most frequent microbial agents isolated from patients with septic shock. However, other microorganisms such as spirochetes, rickettsia, viruses, fungi or parasites also may occasionally cause septic shock. A pathogen can be isolated from blood or the primary site of infection in more than two thirds of the patients with severe sepsis (3). Whereas Gram-negative infections were predominant in the 1960s and early 1970s, the frequency of Gram-positive infections has increased during the last two decades and are now responsible for about half of all cases of severe sepsis. Gram-positive bacteria are even more frequent than Gram-negative bacteria in certain centers. Fungal infections are also

increasing in many countries. One of the most significant achievement in the understanding of the pathogenesis of sepsis has been the recognition of the central role played by the innate immune system in the host defenses against invasive pathogens. Sadly, however, despite better supportive care, the hospital mortality of severe sepsis and septic shock has not changed significantly over the last decades and much remained to be accomplished to improve the poor outcome of these patients. In fact, death rates of sepsis still range between 20% to 80% depending upon the severity of the sepsis syndrome.

For about two decades, one of the research domains of our laboratory has been the study of the pathophysiology of septic shock. Over the last 15 years, I have had the privilege to participate, both in the laboratory and in the clinic, in the investigations of the role played by microbial products (endotoxin and Gram-positive cell walls), acute phase proteins (the LPS-binding protein, LBP), receptors (CD14 and recently the Toll-like receptors) and cytokines in the pathogenesis of sepsis. This review article will describe the central role played by the innate immune system in the host defenses against invasive pathogens. We will focus our attention on the components of the innate immune system involved in the recognition of microbial products. Once activated by the microbes, immune cells release a broad-range of effectors molecules, including cytokines. We will examine how cytokines contribute to the host defenses and pathogenesis of sepsis. Finally, we will discuss what might be future therapeutic strategies for the management of severely ill septic patients.

## *1. Innate Immunity*

Across species of the plant and the animal taxa, the innate immune system plays an essential role in the host defenses against invasive pathogens (4-6). This ancient defense system constitutes the first line of the host defenses. Evolutionary, the innate immune system has appeared before the separation of vertebrates and invertebrates, whereas the acquired immune system has emerged approximately 450 millions years ago. The innate immune system comprises both soluble fac-

tors (such as the alternative and the mannan-binding lectin pathways of the complement, acute phase proteins and cytokines) and cellular components (such as monocytes, macrophages, granulocytes, dendritic cells and NK cells), that are activated as soon as a pathogen crosses the host defense barriers (Fig. 1). The host innate immune response lasts for a few hours and usually results in the elimination of the micro-

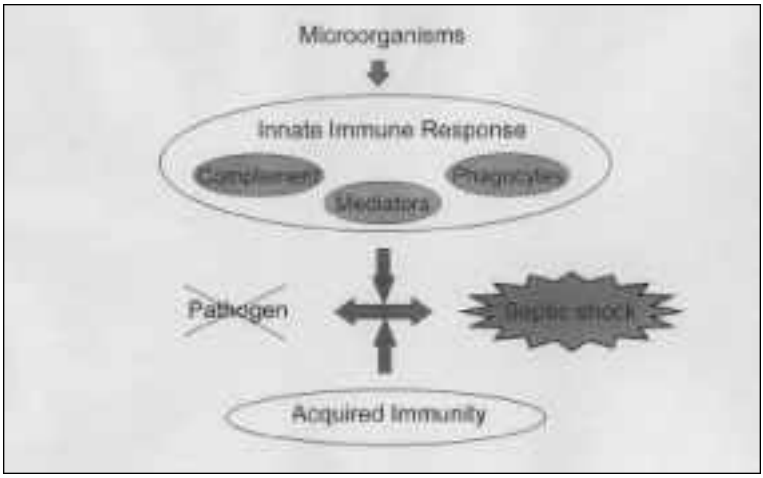


Figure 1. *Schematic representation of the host defenses against infections.* Invasive microbial pathogens crossing the epithelial or mucosal surfaces of the host instantly encounter cellular (phagocytes) and soluble (alternative and mannan-binding lectin pathways of the complement) components that will mount the host defensive innate immune response. Binding of common molecular motifs of pathogens to cell surface receptors activates phagocytes to engulf and eliminate the microorganism and to release mediators that will initiate the inflammatory response. In most instances, the host innate immune response will result in the removal of the microorganism. Occasionally, however, the pathogen resists to this initial attack and may cause severe sepsis or septic shock. Persistence of the pathogen triggers an adaptive immune response with activation of T and B lymphocytes.

organisms. A fundamental characteristic of innate immunity is its capacity to recognize and eliminate a broad range of microorganisms. Recognition of microbial pathogens is mediated by germ-line encoded pattern recognition receptors expressed on the surface of innate immune cells that recognize pathogen-associated molecular motifs. Upon

binding of the ligand, these receptors activate a cascade of intracellular events resulting in the production of a large array of effectors molecules, including pro-inflammatory mediators. Among these, the cytokines play an essential role in orchestrating the cellular and humoral responses designed to eradicate the pathogens. A precise understanding of the molecular and cellular basis of host-pathogen interactions is an essential step for the development of new treatment modalities for patients with sepsis. Let us first review how the innate immune system senses the presence of invasive pathogens, such as the Gram-negative and Gram-positive bacteria.

## 2. *Interaction between Microbial Products, Plasma Proteins and Immune Cells Receptors*

*2.1. Gram-negative sepsis.* Considerable progress has been made in recent years in understanding how bacterial products initiate sepsis. In the case of Gram-negative infections, the endoxin (lipopolysaccharide, LPS) moeity of the outer membrane is a pivotal activator of both immune and non-immune cells (7). The exploration of the interaction between LPS and host cells has led to the identification of key molecules such as the LPS binding protein (LBP) and the CD14 receptor (8;9). LBP and CD14 have been shown to work in concert to alarm the host to the presence of LPS or Gram-negative bacteria and to initiate the inflammatory response. LBP belongs to a group of lipid-binding and transferring molecule. It binds LPS monomer from LPS aggregates and transfers LPS either to lipoproteins, which results in the inactivation of LPS, or to CD14 on the surface of myeloid cells, which results in cellular activation and cytokine production (Figure 2). LPS may also bind to a soluble form of CD14 that serves to activate CD14-negative cells, such as epithelial or endothelial cells. Thus, LBP functions as an opsonin and amplifies the host response to LPS. Neutralization of LBP or CD14 prevents cellular activation, cytokine production and protects animals from lethal experimental shock (10). CD14 is an important component of the LPS receptor complex. However, CD14 is a glycosylphosphatidylinositol-anchored ligand-binding membrane protein and therefore is not in direct communicati-

on with the signal transduction host cellular system. Accordingly, LPS must interact, directly or indirectly, with a trans-membrane molecule to initiate signal transduction. Hence the hypothesis made years ago that CD14 was part of a multiprotein receptor complex. Many research efforts have been devoted to the identification of the signal-transducing molecule to which LPS binds.

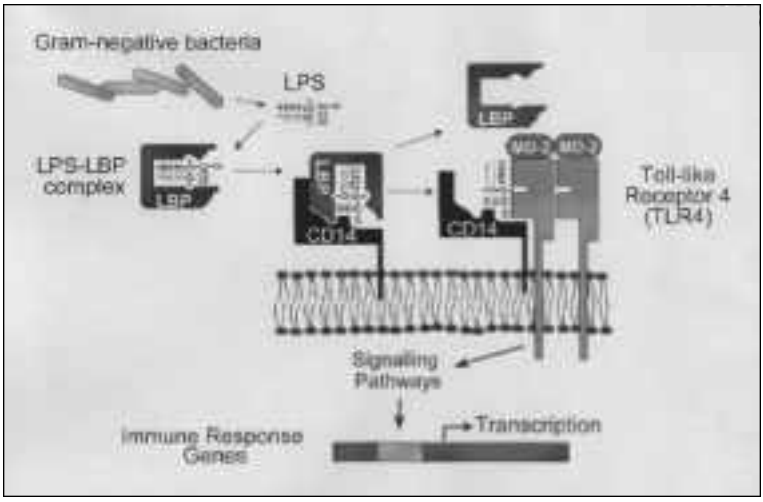


Figure 2. *Host response to endotoxin and Gram-negative bacteria.* This schematic representation describes the events involved in the recognition of and the response to endotoxin (lipopolysaccharide, LPS) and Gram-negative bacteria by immune cells. LBP: lipopolysaccharide-binding protein; CD14: receptor expressed on myeloid cells that binds the LPS-LBP complex; TLR4: Toll-like receptor 4 : signal transducing molecule of the LPS receptor complex. MD-2: a secreted protein that associates with the extracellular domain of TLR4 and augments the cellular response to LPS.

Important clues regarding the identity of this molecule came from studies of the innate immunity of *Drosophila melanogaster* (5). In a series of fascinating studies investigators uncovered that a family of proteins related to the *Drosophila* Toll receptor were critically involved in innate immune responses. Originally, the Toll receptor had been discovered for their central role in dorso-ventral polarity during embryogenesis in *Drosophila.* Subsequently, Toll was found to be an essential element

of the innate host defenses of flies. In 1996, Lemaitre *et al.* reported that the Toll receptor and downstream elements of the Toll signaling pathway were implicated in the control of the expression of antifungal peptides in *Drosophila* (11). The signaling pathways activated by the Toll receptor showed striking analogy with a mammalian signaling cascade activating the transcription factor NF-κB, that was known to play an important role in the activation of pro-inflammatory and immunoregulatory genes. A human homologue of the *Drosophila* Toll (hToll), subsequently referred to as Toll-like receptor 4 (TLR4), was first reported by Medzhitov *et al.* in 1997 (12). Expression of hToll mRNA was detected in many tissues and human cell-lines and was shown to participate in the activation of the transcription factor NF-κB and release of pro-inflammatory cytokines. Today, 10 *Tlr* genes have been identified in mammals. Human TLRs are type I transmembrane protein with an extracellular leucine-rich repeat domain and an intracellular domain homologous to the interleukin 1 (IL-1) receptor. TLR4 has been shown to be the LPS signal transducing molecule of the LPS receptor complex (Fig. 2). Indeed, Beutler and colleagues provided powerful genetic evidence linking resistance of C3H/HeJ or C57Bl/10ScCr mice to LPS to mutations in the *Tlr4* gene (13). This critical observation and a series of studies in mice and humans have unequivocally demonstrated that TLR4 is an essential component of the LPS receptor complex (14-17). MD-2, a secreted protein that associates with the extracellular domain of TLR4 has been shown to augment responses to LPS (18;19). Thus, strong evidence supports a critical role for LBP, CD14, TLR4 and MD-2 in cellular response to LPS and Gram-negative bacteria (Fig. 2).

*2.2. Gram-positive Sepsis.* Staphylococcal and streptococcal toxic-shock syndromes (TSS) are examples of fulminant and often fatal complications of Gram-positive sepsis. In comparison with Gram-negative sepsis, less is known about how Gram-positive bacteria stimulate immune cells. Gram-positive bacteria can provoke severe sepsis and septic shock by at least two distinct mechanisms. Firstly, staphylococci or streptococci have been shown to release exotoxins that act as superantigens (20;21). Secondly, cell wall components of Gram-positive bacteria were found to activate monocytes and macrophages to release pro-inflammatory mediators (22;23).

In 1984, Ikejima *et al.* showed that filtrate from TSS-associated *Staphylococcus aureus* strains was capable of inducing IL-1 production by monocytes (24). A staphylococcal exotoxin referred to as toxic-shock syndrome toxin 1 (TSST-1) was then found to stimulate tumor necrosis factor alpha (TNF-$\alpha$) and IL-1 secretion from human mononuclear cells and to cause a shock-like syndrome when injected in rabbits. Later, exotoxins of Gram-positive bacteria (the staphylococcal enterotoxins, exfoliating toxins or TSST-1, and the streptococcal pyrogenic exotoxins) have been identified to act as superantigens. They stimulate a large pool of T cell by cross-linking class II MHC proteins of antigen-presenting cells to particular V$\beta$ chains of the T cell receptor (20). Activation of macrophages and these T cells lead to an abundant production of pro-inflammatory cytokines (TNF-$\alpha$, IL-1, lymphotoxin, IL-2, and IFN$\gamma$), producing the fulminant clinical manifestations of the toxic shock syndromes with severe and often prolonged shock and multiorgan failure. However, as not all Gram-positive bacteria isolated from patients with septic shock produce exotoxins, other pathogenic mechanisms are likely to also play an important role in the pathogenesis of Gram-positive sepsis.

By analogy with Gram-negative bacteria, it was postulated that cell walls of Gram-positive bacteria might contain moieties capable of interacting with pattern recognition receptors on myeloid cells to induce signal transducing events resulting in the release of pro-inflammatory mediators. Recent studies conducted by various research groups have shown that peptidoglycan (PGN), a large mesh-like polymer structure composed of glycan chains, consisting of repeating units of N-acetylglucosamine and N-acetylmuramic acid, cross-linked by tetrapeptides, and lipoteichoic acids (LTA), a similar type of polymer linked to plasma membrane via a diacyl-glycerol molecule, display pro-inflammatory properties (22;23). Work by Moreillon and colleagues has shown that PGN preparations of *Streptococcus pneumoniae* induced the release of TNF-$\alpha$ and IL-6 by human peripheral blood mononuclear cells (25). Cell activation was enhanced by the presence of plasma and was mediated by the pattern recognition receptor CD14. Importantly, on a weight basis, 1000-times more Gram-positive cell walls than Gram-negative LPS were required to stimulate the produc-

tion of equal amounts of the pro-inflammatory cytokine TNF-$\alpha$. As these initial studies were performed with crude and rather insoluble PGN preparations, it was hypothesized that sub-components of PGN might be more active. In fact, tripeptides and more complex peptides were found to be 100-fold more active than native PGN. These results therefore suggested that activation of immune cells by Gram-positive peptidoglycan stem peptides might participate in the pathogenesis of Gram-positive sepsis (25).

Studies using cell-lines transfected with TLR2, a member of the TLR family of receptors, and NF-$\kappa$B-luciferase reporter constructs, or macrophages from TLR2 knockout mice have shown that TLR2 is

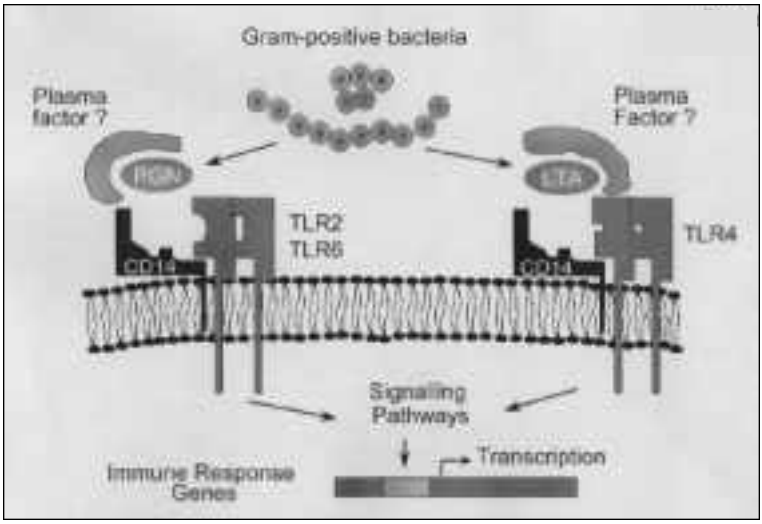


Figure 3. *Host response to Gram-positive bacteria and cell-wall fragments.* The molecular mechanisms underlying the recognition of Gram-positive bacteria or cell walls (peptidoglycan, PGN or lipoteichoic acids, LTA) by immune cells are not well defined. An as yet unidentified plasma factor and CD14 augment the responses of myeloid cells to Gram-positive cell wall components. Toll-like receptor 2 (TLR2) and 6 (TLR6) are involved in the recognition of PGN and Gram-positive bacteria. Data obtained with cells isolated from TLR2 and TLR4 knockout mice suggest that TLR4 is involved in the recognition of LTA.

implicated in the host response to whole Gram-positive bacteria (staphylococci, streptococci, *Listeria monocytogenes*, micrococci), soluble PGN and perhaps also LTA, though the latter is controversial (16;26-30) (Fig. 3). Similarly to what has been observed with LPS, CD14 was found to augment cellular responses to Gram-positive cell walls. Interestingly, cooperation between TLRs, namely TLR2 and TLR6, was shown to be necessary for recognition of PGN by mouse macrophages. Both TLR2 and TLR6 were required for induction of NF-κB-luciferease activity and TNF-$\alpha$ production in response to Gram-positive bacteria, PGN or the yeast particle zymosan (31). Of note, mice with deletion of the *Tlr6* gene were resistant to stimulation with PGN, indicating that TLR6 is critical for host responses to PGN. In constrast, recognition of bacterial lipopeptides requires TLR2 alone or in combination with a TLR other than TLR6 (32). In addition to its involvement in cellular responses to Gram-positive bacteria, TLR2 has been shown to mediate host responses to a broad-spectrum of microorganisms, including mycobacteria, mycoplasma, spirochetes *(Leptospira)* and yeast cell wall particles (i.e. zymosan). Likewise TLR2 is implicated in innate immune responses to microbial lipoproteins and lipopeptides and to mycobacterial cell wall components (lipoarabinomannan, mycolylarabinogalactan-peptidoglycan complex and *Mycobacterium tuberculosis* total lipids) (33-38).

*2.3.Role of Other Toll-like Receptors in Sepsis*

Computer analyses of the mouse and human public databases have revealed the presence of more than ten putative members of the TLR family (39). However, ten TLRs have been cloned so far. Beside TLR2 and TLR4, three other TLRs (TLR5, TLR6 and TLR9) have been shown to serve as receptors for microbial-derived ligands (Fig. 4). Aderem and colleagues have recently reported that TLR5 is a receptor for flagellin, a 55-kD flagellar protein of Gram-positive and Gram-negative bacteria (40). As mentioned above, TLR6 appears to be required for host responses to Gram-positive bacteria, peptidoglycan and zymosan (31). Using TLR9 knockout mice ($TLR9^{-/-}$), Akira and colleagues convincingly demonstrated that TLR9 was essential for recognition of bacterial DNA by cells of the innate immune system (41).

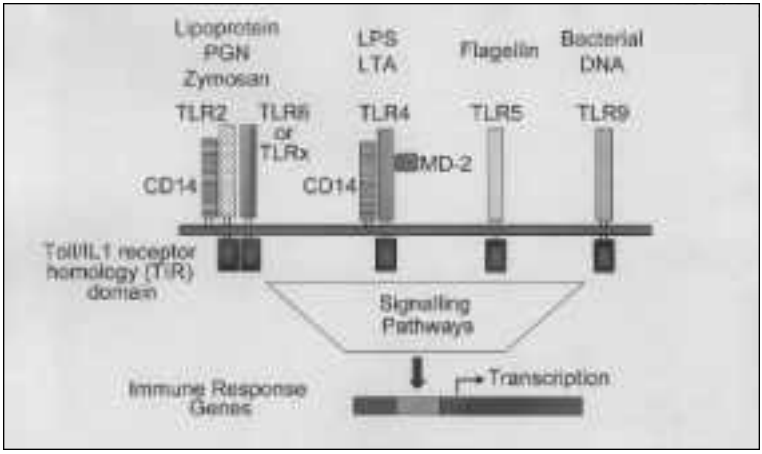


Figure 4. *Ligands of Toll-like receptors (TLR).* Among the ten TLRs cloned to date, five have been shown to bind microbial-derived ligands. TLR2 binds lipopeptides, Gram-positive peptidoglycan (PGN) , the yeast particle zymosan and a variety of other microbial products (for a review see reference #39). TLR4 binds LPS and possibly also lipoteichoic acids (LTA). TLR5 serves as a receptor for flagellin. TLR6 has been shown to contribute to signal transduction induced by Gram-positive bacteria, peptidoglycan and yeast. TLR9 plays an essential role in cellular response to bacterial DNA(i.e unmethylated CpG).

Macrophages, B cells and dendritic isolated from TLR9$^{-/-}$ mice were found to be completely unresponsive to unmethylated CpG DNA. Likewise, D-galactosamine-sensitized TLR9$^{-/-}$ mice were protected from shock induced by CpG DNA.

### *3. Cytokines*

Binding of microbial products to pattern recognition receptors, such as the TLRs, on innate immune cells activates several intracellular signaling pathways. This cascade of intracellular events results in the activation of transcription factors (NF-κB, AP-1) implicated in the expression of immune response genes and in the release of a broad range of effectors molecules. Pro-inflammatory mediators play a critical role in the eradication of the invading microorganisms. They serve to initiate

the inflammatory reaction and orchestrate the cellular and humoral responses. Cytokines are an important class of mediators released by diverse cell types, including lymphocytes, monocytes, macrophages and non-immune cells. Tumor necrosis factor, interleukins, chemokines, interferons and colony stimulating factors are main members of the cytokine family of messenger molecules. Cytokines are small proteins (usually of less than 30 kDa), whose expression is, with few exceptions, induced rather than constitutive. Pleiotropism (i.e. the capacity for a given cytokine to stimulate several cell types) and redundancy (i.e. the ability of different cytokines to exert similar effects) are typical features shared by many cytokines. Moreover, cytokines have the capacity to induce each other's expression, giving rise to a broad network of interacting molecules. They display autocrine, paracrine, and endocrine activities mediated by the interaction with specific receptors expressed on target cells. Cytokines exert chemotactic effects for immune cells, enhance the expression of the MHC class I and II molecules, and participate in the activation and proliferation of B and T lymphocytes. These pleiotropic immuno-modulating effects play an essential role in coordinating the innate and acquired immune responses necessary to eliminate invading pathogens. It is beyond the scope of this review article to discuss the implication of pro-inflammatory and anti-inflammatory cytokines in the pathogenesis of sepsis [for a review see (42)]. We will, however, briefly examine the salient features of one of the first cytokines identified, macrophage migration inhibitory factor, which might be a target for future therapeutic interventions in patients with septic shock.

*Macrophage migration inhibitory factor (MIF).*

The name MIF was coined after the observations by Bloom and David in 1966 that a soluble material released by sensitized lymphocytes was able to inhibit the random migration of peritoneal exsudate cells (43;44). Subsequently, MIF was found to stimulate several macrophage functions, such as cell adhesion, phagocytosis and killing of intracellular parasites. However, the biological activities of MIF remained undefined until the cloning of a human MIF cDNA in 1989 (45). Recently, MIF has emerged recently as an essential effector molecule of the innate immune system. It is constitutively expressed in large

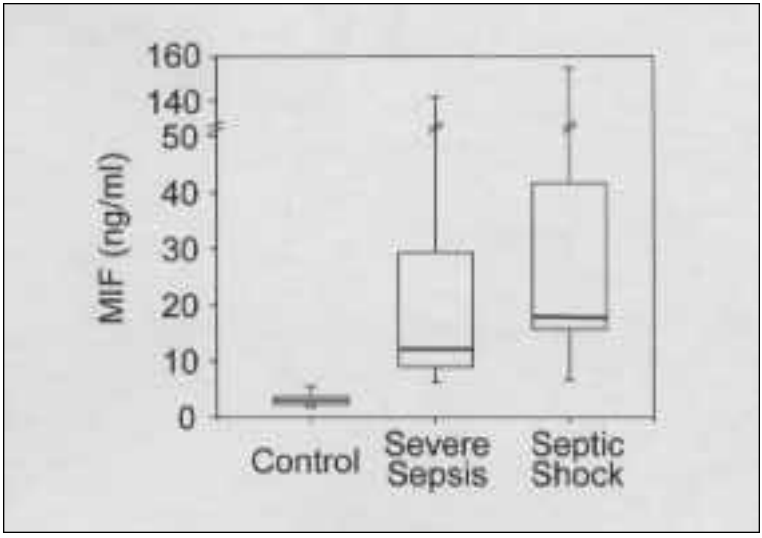


Figure 5. *Plasma concentrations of MIF in healthy controls and in patients with severe sepsis or septic shock.* The bottom, median and top lines of the box mark the 25th, 50th and 75th percentiles, respectively. The vertical line shows the range of values comprised between the 5th and 95th percentiles. From reference #51.

amounts by many cells, including immune cells (monocytes, macrophages, T and B lymphocytes), endocrine cells (pituitary and adrenal glands) and epithelial cells (46–48). During the course of inflammatory and infectious diseases, exposure of these cells to microbial products and pro-inflammatory cytokines induces the release of preformed MIF as well as *de novo* protein synthesis (47;49). When secreted into tissues or into the systemic circulation, MIF plays an important role in promoting inflammatory reactions and innate and adaptive immune responses. Moreover, it acts as a physiological antagonist of the anti-inflammatory and immunosuppressive effects of glucocorticoids (50). High blood levels of MIF are found in patients with inflammatory and infectious diseases, including severe sepsis and septic shock (51;52) (Fig. 5). Immunoneutralization of MIF or deletion of the Mif gene protects mice against lethal endotoxemia, Gram-positive toxic shock syn-

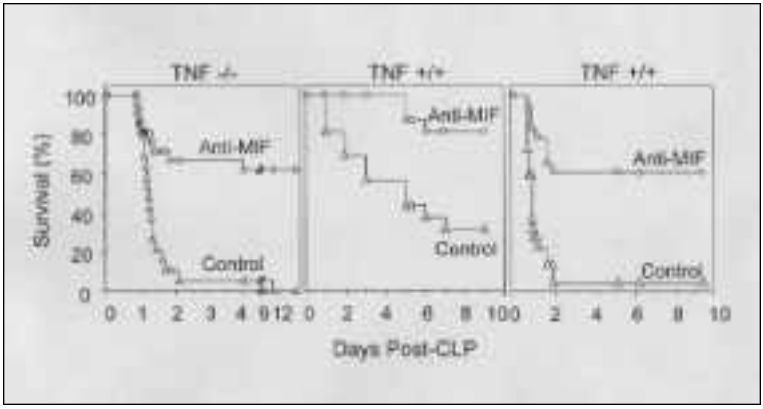


Figure 6. *Anti-MIF monoclonal antibodies (mAb) protect mice from septic shock induced by cecal ligation and puncture.* $TNF^{-/-}$: TNF knockout mice, $TNF^{+/+}$: wild-type mice. Left panel: Anti-MIF mAb was given at the time of surgery (t=0h), survival: Anti-MIF mAb: 62%, control antibody: 0%, P=0.00002. Middle panel: Anti-MIF mAb was given at the time of surgery (t=0h), survival : Anti-MIF mAb: 81%, control antibody: 31%, P=0.01. Right panel: Anti-MIF mAb was given 4.5 h after surgery, survival: Anti-MIF mAb 61%, control antibody: 5%, P=0.00008. From reference #51.

dromes and experimental bacterial peritonitis (Fig. 6). Conversely, administration of recombinant MIF increases the lethality of endotoxemia and bacterial sepsis (46;49;51;53). It seems, therefore, that MIF has the potential to endanger life when expressed in excess during sepsis. Most recently, MIF was observed to be a critical regulator of macrophage responses to endotoxin. MIF-deficient macrophages were hypo-responsive to stimulation with LPS and Gram-negative bacteria. The impaired response was due to a down-regulation of TLR4, providing a molecular basis for the resistance of MIF-deficient mice to endotoxic shock. Altogether, these results suggest that development of strategies that either block MIF production or inhibit its function may have a role in the treatment of severe sepsis and inflammatory diseases.

## 4. *Strategies for Prevention and Treatment of Severe Sepsis and Septic Shock*

The innate immune response must be tightly regulated. Failure to mount an appropriate defense response might have dramatic consequences for the host. In fact, severe sepsis and septic shock can be viewed as clinical manifestations of a failing innate immune response and may result either from a defective or a hyper-responsive host response to infection. Any inherited or acquired quantitative or qualitative defects of the innate immune system would facilitate microbial invasion and growth. Indeed, severe underlying diseases or loss-of-function mutations of any component of the innate immune system, such as the TLRs, may affect the capacity of the host to combat infection and may result in overwhelming infections. Alternatively, septic shock may be caused by an over-reactive host response, triggered by a highly virulent pathogen or possibly also due to gain-of-function mutations of one or more of the molecules of the innate immune system. The fact that severe sepsis and septic shock do not occur in epidemics suggests that the host rather than the micro-organism *per se* is often the major determinant of the sepsis equation.

Whatever the underlying pathogenic basis of the disease, septic shock is characterized at the acute phase of the disease by a powerful inflammatory response. Numerous studies have shown that high levels of pro-inflammatory mediators are associated with poor outcome in patients with septic shock (54;55). These observations have been the basis of the concept of blocking pro-inflammatory mediators as a mean of improving the survival of patients with septic shock. Despite encouraging results in pre-clinical investigations, all recent experimental anti-cytokine therapies have had a modest impact on the outcome of patients with severe sepsis and septic shock. Several factors may account for the limited efficacy of anti-TNF and anti-IL-1 therapies in sepsis [reviewed in (56;57)]. Firstly, it is perhaps not so surprising, because of the complexity of the cytokine network cascade induced by sepsis, that immunomodulatory therapies targeting one single mediator did not markedly improve patients' survival. Although TNF-$\alpha$ and IL-1 are important cytokines of the host defence system, many other effector

molecules are likely to contribute to the pathogenesis of the sepsis syndrome. Secondly, beyond factors associated with species disparity, patients enrolled in clinical trials also differ quite significantly from experimental animals with sepsis. Pre-clinical studies have always been carried out in healthy animals with identical genetic backgrounds. By contrast, patients randomized in clinical trials differ with respect to their genetic make-up, age, underlying diseases, presence of co-morbidities, and other key characteristics including the microbial species causing the infection as well as the site, type and severity of infection. Moreover, in animal models, anti-cytokine therapy was most often administered before or together with the microbial extracts or bacterial inoculum. In many models, the protective effect was lost when treatment was started after the injurious stimuli. The timing of therapeutic intervention was very different in clinical trials, as the immunomodulating agent was almost always infused at least hours, and sometimes days, after the onset of infection. Therefore, most likely experimental therapy was administered after the peak of cytokine release. Cytokine measurements were performed in a limited number of trials. When available, these retrospective analyses have revealed that a minority of randomized patients had detectable levels of cytokines in the blood when anti-TNF$\alpha$ or anti-IL-1 therapy was administered to the patients. Time-course studies of cytokine release in septic patients have indicated that sepsis also induces a powerful anti-inflammatory response. Thus, anti-cytokine therapies administered during this compensatory, anti-inflammatory phase could impair the host defenses. Indeed, blockade of pro-inflammatory cytokines was efficacious in models of hyperacute inflammation or fulminant sepsis coupled with high death rate. However, when given to animals with focal rather than systemic infections, such as peritonitis or pneumonia for example, the same agents often proved to be detrimental and increased lethality. Phase III clinical trials have included patients with both focal and systemic infections associated with different death rates. In theory, it is possible that the sickest patients benefited from anti-cytokine therapy, whereas those with less severe infections might have been harmed by it. The net overall results might have been an absence of protection due to opposite effects occurring in these highly heterogeneous groups of patients. The currently used definitions of sepsis, severe sepsis and septic shock may

thus not be very useful to identify patients likely to respond to novel therapeutic interventions. Classification of patients based primarily on biological factors of interest rather than on the sole clinical parameters may be an approach worth pursuing to detect those individuals most likely to respond to defined therapy. However, despite the lack of encouraging results obtained with anti-TNF-$\alpha$ and anti-IL-1 therapies in sepsis the concept of blocking cytokine has yieldid impressive results for the treatment of patients with rheumatoid arthritis and Crohn's disease. It remains critical to identify new therapeutic targets for the treatment of septic shock. Recent studies have revealed the critical role played by two pro-inflammatory macrophage mediators, namely macrophage migration inhibitory factor (MIF) and high mobility group-1 (HMG-1) in innate immune responses to microbial toxins. Neutralization of the biological activities of these two cytokines, especially MIF, was shown to improve outcome in experimental models of endotoxemia and septic shock, also when given after the administration of toxins or the onset of infection (46;51;58;59).

Designing new therapeutic approaches aimed at blocking the activation of immune cells by microbial products remains an attractive concept, even though previous studies with anti-endotoxin antibodies have not yielded the anticipated results (60). It is possible that a relatively limited number of pathogen-associated molecular motifs serve as ligands for pattern recognition receptors. Treatment strategies design to block these ligand-receptor interactions would have the advantage to act at the most proximal point of the cascade of events induced by the microbes. LBP, CD14 and TLR4 for Gram-negative sepsis, and CD14, TLR2 and TLR6 for Gram-positive sepsis are potential targets for drug development. Phase I studies of anti-CD14 monoclonal antibodies (IC14) are ongoing. Component of the cellular signalling pathways, such as the mitogen-activated protein (MAP) kinase might be other targets for drug development. However, any intervention with the aim to inhibit the innate immune response carries with it the inherent risk of harming the patient. Indeed, C3H/HeJ and C57BL/10ScCr mice with spontaneous mutations or deletions of *Tlr4* gene survive when exposed to lethal doses of LPS, yet they exhibit an increased sensitivity to bacterial infections and succumb quickly when exposed to otherwise non-

lethal bacterial inocula (61;62). Thus, appropriate selection of patients with severe infections associated with a high probability of death are of the utmost importance.

The pathogenesis of sepsis is far more complex that was initially anticipated. However, combined research efforts of basic scientists and clinical investigators continue to provide critical information for the identification of novel therapeutic targets. Microbial products also have the capacity to directly activate the complement and coagulation cascades. The exciting results obtained recently with novel treatment strategies, especially the activated protein C (63), designed to correct coagulation abnormalities induced by sepsis have reminded us of the possibility of developing therapeutic interventions acting on various systems activated by the pathogens. Yet, identification of new therapeutic targets for the management of septic shock remains imperative. The search must go on.

*Thanks and acknowledgements*

Let me first express my sincere gratitude towards the members of the Council of the Max Cloëtta Foundation for the allocation of this prestigious prize. During my clinical and research training in Lausanne and in New York, I have had the immense privilege to be guided by outstanding individuals who have had a major impact on my career. I would like to take this opportunity to express my profound gratefulness to Michel P. Glauser, a truly exceptional mentor and friend, whose relentless enthusiasm, continuous support and guidance have been essential driving forces over the last twenty years, to Patrick Francioli for his teaching, precious advice, long-time support and friendship, and who in fact was responsible for (he recruited me) and contributed to much of what happened over the years, and to Philippe Moreillon, a fantastic colleague and dear friend, with whom I have been very fortunate to share much more than just my clinical and research training.

I would like to warmly thank Richard Bucala, Anthony Cerami, Samuel D. Wright and the late Zanvil A. Cohn for their leadership and help

during the years I spent with them at the Picower Institute and the Rockefeller University in New York. I express my thankfulness to Ernst Rietschel for many thoughtful discussions, judicious recommendations and full support over many years. I would like to acknowledge the contribution and help of all the members of the Division of Infectious Diseases of the Centre Hospitalier Universitaire Vaudois (CHUV) in Lausanne and of many colleagues around the world, especially in Switzerland, in the Netherlands, and in the United States, for their invaluable help, discussions, suggestions and critiques. I also wish to acknowledge the support of the Fondation de recherche dans le domaine des maladies infectieuses, the Fonds de perfectionnement du CHUV, the Swiss National Science Foundation, the Bristol-Myers Squibb Foundation and the Leenards Foundation whose contributions have been essential for the accomplishment of my work.

Finally, I thank my wife, Ellen, and my children Noémie and Matthieu, who deserve much of the credit that I am getting today for their endless support and for so much understanding and patience over the years.

# REFERENCES


01. Angus DC, Wax RS. Epidemiology of sepsis: an update. Crit Care Med. 2001;29:S109-S116.

02. Glauser MP, Zanetti G, Baumgartner JD, Cohen J. Septic shock: pathogenesis. Lancet. 1991;338:732-36.

03. Bochud PY, Glauser M, Calandra T. Antibiotics in sepsis. Intensive Care Med. 2001;27:S33-S48.

04. Medzhitov R, Janeway CA, Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell. 1997;91:295-98.

05. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. Phylogenetic perspectives in innate immunity. Science. 1999;284:1313-18.

06. Kimbrell DA, Beutler B. The evolution and genetics of innate immunity. Nat Rev Genet. 2001;2:256-67.

07. Alexander, C. and Rietschel, E. T. Bacterial lipopolysaccharides and innate immunity. J Endotoxin Res. 7, 165. 2001.

08. Ulevitch RJ, Tobias PS. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr Opin Immunol. 1999;11:19-22.

09. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 1990;249:1431-33.

10. Gallay P, Heumann D, Le Roy D, Barras C, Glauser MP. Lipopolysaccharide-binding protein as a major plasma protein responsible of endotoxemic shock. PNAS. 1993;90:9935-38.

11. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996;86:973-83.

12. Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997;388:394-97.

13. Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. Science. 1998;282:2085-88.

14. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem. 1999;274:10689-92.

15. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y et al. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. J Immunol. 1999;162:3749-52.

16. Heine H, Kirschning CJ, Lien E, Monks BG, Rothe M, Golenbock DT. Cutting edge: cells that carry A null allele for toll-like receptor 2 are capable of responding to endotoxin. J Immunol. 1999;162:6971-75.

17. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet. 2000;25:187-91.

18. Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll- like receptor 4. J Exp Med. 1999;189:1777-82.

19. Schromm AB, Lien E, Henneke P, Chow JC, Yoshimura A, Heine H et al. Molecular genetic analysis of an endotoxin nonresponder mutant cell line: a point mutation in a conserved region of MD-2 abolishes endotoxin-induced signaling. J Exp Med. 2001;194:79-88.

20. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. Science. 1990;248:705-11.

21. Kappler J, Kotzin B, Herron L, Gelfand EW, Bigler RD, Boylston A et al. V$\beta$-specific stimulation of human T cells by staphylococcal toxins. Science. 1989;244:811-13.

22. Mattsson E, Verhage L, Rollof J, Fleer A, Verhoef J, Van Dijk H. Peptidoglycan and teichoic acid from Staphylococcus epidermidis stimulate human monocytes to release tumour necrosis factor-alpha, interleukin-1 beta and interleukin-6. FEMS Immunol Med Microbiol. 1993;7:281-87.

23. Heumann D, Barras C, Severin A, Glauser MP, Tomasz A. Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. Infect Immun. 1994;62:2715-21.

24. Ikejima T, Dinarello CA, Gill M, Wolff SM. Induction of interleukin-1 by a product of Staphylococcus aureus associated with toxic shock syndrome. J Clin Invest. 1984;73:1312-20.

25. Majcherczyk PA, Langen H, Heumann D, Fountoulakis M, Glauser MP, Moreillon P. Digestion of Streptococcus pneumoniae cell walls with its major peptidoglycan hydrolase releases branched stem peptides carrying proinflammatory activity. J Biol Chem. 1999;274:12537-43.

26. Flo TH, Halaas O, Lien E, Ryan L, Teti G, Golenbock DT et al. Human Toll-like receptor 2 mediates monocyte activation by Listeria monocytogenes, but not by group B streptococci or lipopolysaccharide. J Immunol. 2000;164:2064-69.

27. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999;11:443-51.

28. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature. 1999;401:811-15.

29. Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D. Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. J Immunol. 1999;163:1-5.

30. Wang Q, Dziarski R, Kirschning CJ, Muzio M, Gupta D. Micrococci and peptidoglycan activate TLR2—>MyD88—>IRAK—>TRAF—>NIK—>IKK—>NF-kappaB signal transduction pathway that induces transcription of interleukin-8. Infect Immun. 2001;69:2270-2276.

31. Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB et al. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci U S A. 2000;97:13766-71.

32. Takeuchi O, Kawai T, Muhlradt PF, Morr M, Radolf JD, Zychlinsky A et al. Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol. 2001;13:933-40.

33. Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ. Human toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. J Immunol. 1999;163:3920-3927.

34. Means TK, Lien E, Yoshimura A, Wang S, Golenbock DT, Fenton MJ. The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. J Immunol. 1999;163:6748-55.

35. Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc Natl Acad Sci USA. 1999;96:14459-63.

36. Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD et al. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. Science. 1999;285:736-39.

37. Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR et al. Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. Science. 1999;285:732-36.

38. Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint G, I et al. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat Immunol. 2001;2:346-52.

39. Akira S, Takeda K, Kaisho T.Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2:675-80.

40. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001;410:1099-103.

41. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H et al. A Toll-like receptor recognizes bacterial DNA. Nature. 2000;408:740-745.

42. Calandra T. Cytokines in septic shock. In: Remington JS, Swartz M, eds. Current Clinical Topics in Infectious Diseases. In press.

43. David J. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. PNAS. 1966;56:72-77.

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

45. Weiser WY, Temple PA, Witek-Gianotti JS, Remold HG, Clark SC, David JR. Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. PNAS. 1989;86:7522-26.

46. Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature. 1993; 365:756-59.

47. 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;179:1895-902.

48. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M et al. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. PNAS. 1996;93:7849-54.

49. 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. PNAS. 1998;95:11383-88.

50. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature. 1995;377:68-71.

51. Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hultner L et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med. 2000;6:164-70.

52. Beishuizen A, Thijs LG, Haanen C, Vermes I. Macrophage migration inhibitory factor and hypothalamo-pituitary- adrenal function during critical illness. J Clin Endocrinol Metab. 2001;86:2811-16.

53. Bozza M, Satoskar AR, Lin G, Lu B, Humbles AA, Gerard C et al. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. J Exp Med. 1999;189:341-46.

54. Girardin E, Grau G, Dayer J, Roux-Lombard P, J5 Study group, Lambert PH. Tumor necrosis factor and interleukin-1 in serum of children with severe infectious purpura. N Engl J Med. 1988;319:397-400.

55. Calandra T, Baumgartner JD, Grau GE, Wu MM, Lambert PH, Schellekens J et al. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, alpha-interferon and gamma-interferon in the serum of patients with septic shock. J Infect Dis. 1990;161:982-87.

56. Abraham E. Why immunomodulatory therapies have not worked in sepsis? Intensive Care Med. 1999;25:556-66.

57. Glauser MP. Pathophysiologic basis of sepsis: considerations for future strategies of intervention. Crit Care Med. 2000;28:S4-S8.

58. Calandra T, Spiegel LA, Metz CN, Bucala R. Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria. Proc Natl Acad Sci U S A . 1998;95:11383-88.

59. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248-51.

60. Calandra T, Baumgartner JD, Glauser MP. Anti-lipopolysaccharide and anti-tumor necrosis factor/cachectin antibodies for the treatment of gram-negative bacteremia and septic shock. In: Sturk A, van Deventer SJH, Ten Cate JW, Büller HR, Levin J, eds. Bacterial endotoxins. Cytokine mediators and new therapies for sepsis. New York: Wiley-Riss; 1991: 141-59.

61. Cross AS, Sadoff JC, Kelly N, Bernton E, Gemski P. Pretreatment with recombinant murine tumor necosis factoralpha /cachectin and murine interleukin 1 alpha protects mice from lethal bacterial infection. J Exp Med. 1989;169:2021-27.

62. O'Brien AD, Rosenstreich DL, Scher I, Campbell GH, MacDermott RP, Formal SB. Genetic control of susceptibility to Salmonella typhimurium in mice: role of the LPS gene. J Immunol. 1980;124:20-24.

63. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001;344:699-709.