Cellular and Humoral Markers of Tissue Damage

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Introduction

Patients with sepsis who are hospitalized in intensive care units frequently develop multiple organ dysfunction failure syndrome (MODFS) with a poor prognosis (1-3). It is believed that injury in MOFDS is brought about by factors which under normal conditions play a regulatory role in homeostatic mechanisms (Table 1).

Table 1. Factors involved in septic shock

Cytokines
Growth factors
Adhesins
CD antigens
Lipid mediators
Gas mediators
Transcription factors
Oncogenes
Genes

Some of these factors are humoral like cytokines, growth factors, lipid and gas mediators while others like oncogenes and their protein products, transcription factors (i.e. nuclear factor kappa B, NFκB) or DNA nuclear material are widely recognized as cellular ones. Adhesins might be viewed both as cellular (or membrane-bound) and soluble (humoral) factors. The distinction between cellular and humoral factors tends to be more and more vague.

A widely used classification for the cluster of differentiation (CD) antigens involves not only cellular markers but also some cytokines and their receptors in membrane-bound and soluble forms (4). The best example of the dual role of cellular markers is class I major histocompatibility antigens (MHC or HLA) (5). These were first recognized as membrane-bound molecules on all nucleated

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cells, and the existence of soluble HLA antigens has also been reported. HLA antigens are known markers of severe brain damage or of the onset of transplant rejection (5, 6).

Many of the mediators associated with MODFS are generated by macrophages which being maximally stimulated already escaped physiological regulatory mechanisms and are genetically recoded to their death. This state is known as programmed cell death or apoptosis (7, 8). At a cellular level apoptosis and necrosis are responsible for the release of cellular markers to the body fluids. Apoptosis regulates cell number and eliminates damaged or infected cells. Also shedding off the cell-bound molecules from macrophages, or from other cells with the fast membrane turnover might be a source of soluble forms of cellular markers.

Baue and Faist suggested the following stages of development of MODFS: infection, increased permeability of intestinal mucosa, involvement of immune system (macrophages, cytokines, antibodies, receptors); generalized inflammation associated with a damage to endothelium, oedema and impaired oxygen availability; and finally ischaemia with reduced microcirculatory flow, necrosis and eventually MODFS (2).

This systemic inflammatory response syndrome (SIRS) caused by infection is characterized by the exacerbation of the production of pro- and anti-inflammatory mediators (9). The cascade of cellular and humoral factors (Table 1) released by endotoxin (LPS) includes thromboxane A_2 (TXA_2), nitric oxide (NO), oxygen free radicals (O_2-, OH′), lipid peroxides (LOO′), leukotrienes (LTB_4-E_4), platelet-activating factor (PAF), tumour necrosis factor (TNFα), interleukins (IL-1, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13), interferons α and γ (IFNα, IFNγ), cytoadhesins (ELAM, ICAM, sPAGEM), soluble TNF (sTNF R) and IL-1 (IL-1ra) receptors, transforming growth factor β (TGFβ) and many others.

Although in many animal models of septic shock removal of the above mediators has conferred successful protection, so far very poor results have been achieved in humans. Current understanding of the setting of an anti-inflammatory response by the host seems to indicate that the host organism is responsible for putting into motion most of the necessary regulatory processes. Indeed, both pro- and anti-inflammatory mediators appear to be markers of the severity of the disease. However, after exhaustion of adaptive mechanisms, therapeutic help in SIRS is required.

Various therapeutic approaches have been proposed to interfere with the development of SIRS (9-11). These include antibodies to: endotoxin (LPS), anti-IL-1, anti-IL-8, anti-IL-12, anti-CD14, anti-73kDa LPS receptor, anti-IFNγ, monoclonal antibodies to TNFα, macrophage migration inhibitory factor (MIF) and lymphocyte migration inhibitory factor (LIF), anti-CD11b, anti-ICAM-1, anti-ELAM-1, anti-superoxide dismutase, IL-1 receptor antagonists, sTNF R, IL-1β converting enzyme inhibitors (ICE), aprotinin, hirudin, heparin, platelet

activating factor (PAF) antagonists, cyclooxygenases (COX-1 and COX-2) inhibitors (nonsteroidal anti-inflammatory drugs, NSAID), steroid hormones, lipooxygenase inhibitors and NO synthase (NOS) inhibitors, prostacyclin analogues, tyrosine kinase inhibitors, lipid X, lipoaminoacids, as well as infusions of various electrolytes or buffers containing catecholamines, vasopressin, angiotensin II or sometimes phenoxybenzamine (10, 12-22). A relapse of time between an infection and administration of a putative drug is crucial for its efficacy. Lack of success in the treatment of MODFS with curative molecules which were effective in the experimental models of septic shock might be explained, at least partially, by a late stage of SIRS at which the therapy is usually implemented (9).

Out of a broad scope of tissue damage markers which are important in MODFS we will focus on: i) LPS/ceramide CD14 system in the induction of septic shock, ii) cytokines - inhibition of their production and cytokine-binding proteins, iii) serum HLA class I antigens, iv) lipid mediators, v) NO as a marker of septic shock and vi) apoptosis.

LPS/ceramide CD14 system

Most cases of sepsis and septic shock are secondary to infections with Gram-negative bacteria (23, 24). The Gram-negative bacterial cell wall consists of inner and outer membranes, the latter portion of which contains proteins as well as LPS (14, 25). It is useful to review briefly the structure of LPS. LPSs are complex molecules composed of three major parts: a polysaccharide side chain (O-antigen), which is attached via a bridging (core) polysaccharide to a glucosamine-based phospholipid (lipid A). The most variable part of the LPS structure is the O antigen. In contrast, the lipid A and to some extend also core region are more conserved (26). This is why antibodies are produced which may cross-react.

Endotoxin is measured utilizing the haemolymph of amoebocytes from the Limus horseshoe crab (27). This substance contains a proenzyme which is directly activated by endotoxin leading to visible gelling of the mixture. Spectrophotometric modification of the Limus amoebocyte lysate (LAL) assay detects endotoxin to less than 10 pg/dl. Unfortunately, the accuracy of the LAL assay is affected by numerous circulating plasma proteins (i.e. antithrombin III, anti-endotoxic antibodies, etc.) and it can be positive in patients with Gram-positive bacterial and fungal infections (28). This may be related to the gut leak which accompanies these infections. Measurement of LPS levels in diagnostic practice shows that endotoxin is often present in sepsis; its presence might correlate with the severity of clinical manifestations and end-organ dysfunction. However, the same literature consistently finds out that endotoxaemia, as detected by LAL assay, is not invariably present in clinical sepsis as the results of LAL assay must be viewed with scepticism (14). In

clinical practice, useful markers that may indicate the presence of Gram-negative bacteria sepsis are: blood culture for Gram-negative microbe or culture or Gram stain positive for a Gram-negative microbe at a local site of infection (26).

It has been shown that nerve growth factor (NGF), TNFα, IL-1β or Fas ligand after binding to their specific CD40 transmembrane receptors stimulate cells by releasing the intracellular messenger ceramide (29-32). This stimulation is completed through activating neutral sphingomyelinases. Agonist-induced hydrolysis of sphingomyelin puts in motion the sphingomyelin cycle analogous to the signal transduction by the phosphatidyloinositol and glycerophospholipid system (33).

Recent studies on the composition of the outer leaflet of the outer membrane of some Gram-negative bacteria emphasize the presence of a glycosphingolipid but not the LPS. This bacterium has been named *Sphingomonas* (34). The substitution of sphingolipids for LPS in this bacterium confirms that the physical properties of sphingolipids may be interchangeable with those of LPS. Indeed, the examination of the structure of LPS and ceramide have revealed a strong similarity between their molecules. Computer molecular modelling and conformational dynamics yielded a solution structure for the acylated glucosamine I of LPS with a strong similarity to that of ceramide (35). Identical chirality at two optical active carbon centres and approximately similar lengths of the hydrocarbon chains make the molecules of LPS and ceramide very much alike. LPS along with TNF and IL-1 cause common events in cells such as: (i) mitogen-activated protein kinase activation; (ii) NF-κB translocation; (iii) activator protein 1 stimulation; (iv) phospholipase A_2 activation and (v) TNF gene expression (35).

For recognition of endotoxin, a binding protein/receptor system involving LPS-binding protein (LBP) and CD14 molecule has been postulated. However, effects of LPS occur also in CD14-negative cells, and not all of them depend on the presence of LPB. This is why not all pathways in recognition of endotoxin are already defined. Several cellular structures have been found to bind LPS. For instance, the scavenger receptor or CD11/CD18 are involved in the detoxification of LPS (36). Several not well characterized membrane proteins with 18, 25, 38, 55, and 65 kDa were found to bind LPS. Also, 40- and 80 kDa, as well as 70- to 80 kDa proteins have been postulated in LPS recognition by various cell types (25). The CD14 molecule, however, has been unequivocally established to be a LPS receptor (37). CD14 is found as a 53 kDa glycoprotein on the cell surface (mCD14) of all mature myeloid cells. The gene for CD14 is located on the fifth chromosome in a region known to encode several cytokines including granulocyte/macrophage colony stimulating factor (GM-CSF), CSF-1, IL-3, endothelial cell growth factor (ECGF), as well as receptors like CSF-1 receptor, platelet-derived growth factor receptor, FMS (c-fms photooncogen) and β-adrenergic receptor. The 32 identified amino acid residues have 40 kDa and almost completely match the amino acid sequence deduced from the CD14 cDNA. In the plasma of healthy adults 4-6 μg/ml of soluble CD14 (sCD14) are

found and sCD14 is highly elevated up to 200 μg/ml in the plasma of septic patients. Two slightly different soluble forms of CD14 molecules exist which can be found in normal serum, most likely due to shedding of mCD14 as it was shown for endothelial cells (38).

Endotoxins or LPS are undoubtedly the molecules responsible for most of the pathophysiological phenomena associated with Gram-negative infections. Experimental and clinical observations indicate that endotoxin may exert its deleterious effects upon the host to a large part by provoking the release of a variety of endogenous mediators, although direct toxicity may also occur. In 1968, Chedid et al. proposed and used an antibody capable of neutralizing endotoxins. In 1982, Ziegler et al. used antiserum prepared in healthy volunteers injected with J5 LPS of rough mutant of *Escherichia coli* and substantially reduced deaths from bacteraemia (10, 11, 14). Modern technology has enabled researchers to prepare monoclonal antibodies (mAb). Many mAbs have been generated and studied. The most well known are those which reached the stage of clinical studies. E5 mAb from Xoma Corporation and HA.1A mAb from Centocor were reported to be effective for patients with sepsis and Gram-negative bacteraemia (39, 40). The neutralizing therapy is extremely expensive and the results of double blind randomized placebo controlled multicentre trials are indicative but not conclusive. The current limitations of treatment of septic patients with anti-LPS antibodies, beside the not trivial financial impact, are connected with the fact that an increasing proportion of cases of sepsis are related to infection with Gram-positive bacteria; it is difficult to demonstrate cross-reactivity against rough mutants and so far neutralization of the effect of endotoxin by antibodies has not been described. The theoretical premise that LPS antibodies are cross-protective is attractive but requires further investigation (14).

Soluble CD14 can act as an inhibitor at the monocyte/macrophage level. In contrast, sCD14 is involved in the activation of endothelial cells by LPS (41). This observation limits the therapeutic use of sCD14 but leaves room for the use of anti-CD14 antibodies. It was reported that the anti-CD14 strategy reduced hypotension, lowered cytokine production and prevented pulmonary oedema.

Proinflammatory cytokine in sepsis

While the initiating event in sepsis may be the release of endotoxin, many of the clinical symptoms of sepsis result from the release of endogenous mediators such as TNF, IL-1, IL-6, IL-8, PAF etc., mononuclear phagocytes and other cells, including endothelial cells. Each of these mediators stimulates both its own release and the release of other mediators and acts in concert to produce symptoms of sepsis. The proinflammatory cytokines TNFα and IL-1β have been studied extensively. It is generally accepted that these cytokines play a central role in the pathogenesis of sepsis (13, 19, 20, 42).

Plasma levels of TNF are a marker of sepsis and depend on the severity of disease. After bolus injection of endotoxin, blood levels of TNFα typically increase at 30 min and peak at 90 min (9, 19, 20). TNF is known to stimulate not only the release of several other cytokines (e.g. IL-1, IL-6, PAF), but also the production of endothelial adhesion molecules which promote neutrophil-endothelial cell adherences. Likewise, TNF enhances neutrophil phagocytosis and injures endothelial cells as manifested by an increase of endothelial cell permeability. TNF is rapidly cleared from the systemic circulation due to its short half-life (14-18 min in humans) and returns to base levels within a few hours (43). This is why plasma TNF activity alone is not a reliable prognostic factor in septicaemia and supports the concept that TNFα is an early mediator that initiates the changes that lead to the extensive cellular injury (44). However, the strongest evidence supporting the role of the TNF in the pathogenesis of septic shock, SIRS and MODFS comes from studies that employed anti-TNF antibodies (10). The favourable effect of anti-TNF antibodies in experimental septic shock were most prominent when antibody was administered prior to the infusion of LPS. Based on these observations, it is clear that for TNF antibodies to be effective, the antibody must be given before or very soon after the onset of bacterial infection (14).

The polypeptide hormone IL-1 is another important factor in host defence and exists in two forms: IL-1α and IL-1β. Both are potent proinflammatory monokines with biological effects that include endothelial cell activation and increase of adhesion molecule receptor expression. In addition, IL-1 promotes the release of other cytokines (TNF, IL-6, PAF) and acts synergistically with TNF in the production of many of its biological and inflammatory effects such as hypotension, endothelial cell injury, increased vascular permeability and, finally, death (45). Like TNF, serum levels of IL-1 rise after endotoxin infusion. However, in contrast with TNF, serum levels of IL-1 reach their peak 3-4 h after LPS challenge (20).

IL-6 is another cytokine in the inflammatory network. Expression of IL-6 is induced in many cells, including mononuclear phagocytes and endothelial cells, after stimulation with LPS, IL-1 and TNF. IL-6 is thought to promote neutrophil activation and accumulation at sites of inflammation (46). In keeping with these properties of IL-6, increased plasma concentrations of IL-6 have been detected in patients with sepsis and are associated with increased mortality. For that reason, most authors now agree that IL-6 is a marker of the severity of the infection (45, 47, 48).

IL-8 is a recently described peptide that is secreted by a variety of cells such as alveolar macrophages, monocytes, endothelial cells in response to endotoxin, IL-1 and TNF (15, 49). IL-8 causes chemoattraction and activation of neutrophils and is believed to mediate neutrophil recruitment in host defence and disease (50-52). It was also demonstrated that IL-8 enhances binding affinity of adhesion molecules on human neutrophils (53, 54). The accumulation of activated

neutrophils in lungs and other organs is felt to play a key role in the pathogenesis of SIRS and MODFS (55, 56).

An important first step in the process of neutrophil-mediated organ injury involves the binding of neutrophils to endothelial cells (57, 58). This interaction is largely regulated by complementary adherence molecules that are present on these cells and expressed in increasing numbers in response to endotoxin, TNF, IL-1, and IL-8. Endothelial and neutrophil cell activation by these cytokines is accompanied by enhanced expression of adhesin molecules such as ELAM1, VCAM1, ICAM1 (59). Anti-CD18 antibodies, in contrast to anti-CD11 antibodies, are able to reduce LPS-induced neutrophil sequestration in tissue and organ injury (59, 60). This antibody worsened endotoxaemia, acidosis and cardiovascular function in a canine model of LPS shock and in the baboon model of sepsis. In the same model, anti-ELAM1 therapy was beneficial. The contribution of the adhesion molecule and the interaction between endothelial and circulating cells play a major role in tissue damage and organ dysfunction. However, the use of antibodies to interfere in this process remains controversial (13).

Cytokine-binding proteins

Cytokine-binding proteins (CBPs), such as cytokine receptors and antibodies, mostly monoclonal, impair interactions of cytokines with their cellular receptors and so these agents can potentially provide a means for treating pathological conditions that have a significant cytokine involvement as is the case in septic shock. Indeed, the efficacy of such treatment was demonstrated for the first time a decade ago whereby experimental shock was prevented by antibodies against TNFα (61, 62). The cloning of genes encoding cytokine receptor chains, and the characterization of their soluble forms, has opened the way to new strategies in anticytokine therapy. These molecules clearly act as antagonists of their respective ligands through competition with the membrane receptors that transduce the biological signal into the target cell. As anticipated, the injection of sIL-1R modulate the allogenic response in vitro and prevent allograft rejection (63). Cerami's group and many others have confirmed the protective activities of anti-TNF antibodies in various models. Also in humans, anti-TNF antibodies were effective when combined with antibiotics (10, 14, 61). Counteracting the effect of another proinflammatory cytokine, IL-1 has been investigated with the IL-1 receptor antagonist (IL-1-ra) (64-66). This natural IL-1-like molecule binds to the same receptors as IL-1, but fails to transmit any signal (61). A second phase III study of IL-1ra was dropped after an interim analysis had failed to show any evidence of benefit (10). Another possibility for containing IL-1 is the use of IL-1β converting enzyme (ICE) inhibitors. ICE is responsible for cleaving a biologically inactive IL-1 precursor into the mature IL-1 active form (67). The results of the INTERSEPT placebo-controlled trial of anti-TNF mAb

(Bayx1351) in 563 patients with severe sepsis shows lack of significantly altered mortality between the studied groups (68).

In contrast, the results of the Immunex study of p75 sTNFR-Fc which have now been reported indicate an increase of mortality among patients in the treated group. In patients with sepsis, plasma levels of both sTNFR p55 and p75 are markedly increased and highly correlate with simultaneously obtained APACHE II and MODFS scores. Since the degree of increased sTNFR levels correlated poorly with patient survival, elevated sTNFR levels represent a good marker for severity of sepsis and predict an outcome (69).

However, exceptions do exist: soluble IL-6 receptor and cilliary neurotrophic factor act as agonists (70). A potential advantage of soluble receptors over high affinity mAbs is that they are of human origin and, accordingly, the problem of patient immunization is obviated. There are two drawbacks of soluble receptors: (i) their usually lower affinity than that of mAb and (ii) their shorter half-life in vivo because they are molecules smaller than antibodies. To overcome these problems, for instance, immunoadhesins have been generated that comprise two soluble receptor fragments linked genetically to a human immunoglobulin constant region (i.e. sTNFR-Fc) (71-74). Additionally, this procedure decreases the therapeutic dose of soluble CBPs in vivo. However, the major drawback of using CBPs relates to the fact that they stabilize the cytokine in the form of a cytokine-CBP complex in vivo (61). The first demonstration that CBPs are capable of stabilizing cytokines was provided by treatment with anti-IL-6 mAbs. The longer in vivo half-life of IL-6-anti-IL-6 complexes (3.5 days) provides a pharmacokinetic explanation for the accumulation of cytokine (the half-life of free IL-6 is as low as 20 min) and for their potential action as agonists (62). However, when CBPs are present in excess over the cytokine-membrane receptor, they seem to act as antagonists.

Inhibition of proinflammatory cytokine production

Rather than counteracting cytokines already generated, inhibition of their production may prevent these mediators from becoming involved in the immunoinflammatory cascade. Glucocorticoids were thought to do it; however, using them in sepsis has not been successful (13). Pentoxyphylline, a phosphodiesterase inhibitor, limits the synthesis of TNF (75). Various drugs which reduced TNF and/or IL-1 production (e.g. linomide, prostacyclin analogues or chlorpromazine) also had beneficial effects in experimental models of septic shock (76-78). More recently, it has been shown that tyrosine kinase inhibitors block LPS-induced TNF and NO production (79).

Cytokines such as IL-4, IL-10, IL-13 and TGFβ possess anti-inflammatory properties because they inhibit the generation of the most of proinflammatory cytokines in monocytes/macrophages. Moreover, these particular cytokines also

induce IL-1-ra (13). Among other putative beneficial cytokines is IFNα (80). Its usefulness in septic shock is still controversial. Maybe the natural balance between pro- and anti-inflammatory cytokines is not sufficient to slow down the running inflammatory cascade.

Serum HLA class I antigens

More than 20 years ago, MHC class I antigens were reported to be present in serum. The development of anti-HLA mAbs had a significant impact on the analysis of serum HLA class I antigens. Similar to their cell-membrane associated counterpart, the serum HLA class I molecular complex comprises a polymorphic heavy α-chain noncovalently associated with β_2 microglobulin. The level of serum HLA class I antigens markedly increases in the course of viral infections caused by cytomegalovirus, hepatitis B virus, hepatitis C virus, varicella-zostervirus, and human immunodeficiency-1 virus. During HIV-1 infection, the level of serum HLA class I antigens correlates with stages of disease and represents a good prognostic marker of the disease progression. An increase in the level of total serum HLA class I antigens has also been observed in recipients of heart, kidney or liver transplants. The rapid decrease in the level of serum HLA antigens observed following immunosuppressive therapy of acute episodes of graft rejection suggests that their level may be the result of immune system activation. The elevation of donor-derived serum HLA class I allospecificities precedes the clinical evidence of a graft rejection episode. It means that measurement of donor-derived serum HLA antigens may represent a test to diagnose graft rejection episodes (5, 6, 81, 82).

Lipid mediators

Many other mediators produced by activated cells contribute to the inflammatory syndrome. Some may be directly induced by LPS while others may be induced following target cell activation by proinflammatory cytokines. As a consequence, prostaglandins (PGs), thromboxane, leukotrienes and PAF are other potential targets for therapeutic approach (13, 21). Inhibition of PG formation by COX-2 rather than COX-1 inhibitors (e.g. ibuprofen) attenuate many alterations associated with LPS injection in animal models of endotoxin shock and inhibit TNF production. However, the experiments in human volunteers show that an injection of ibuprofen immediately before administration of endotoxin caused a significant increase in the level of circulating TNF. Prostaglandin E_1 (PGE_1) has several properties that could be beneficial for the treatment of severe sepsis. It is a potent vasodilator of the pulmonary and systemic circulation. Like prostacyclin, it has also important anti-inflammatory effects by blocking macrophage activation. It can influence coagulation by inhibiting platelet

aggregation and by inducing fibrynolysis. In dogs, PGE_1 almost entirely restored tissue oxygen extraction after endotoxin challenge (83). PAF antagonists have led to successful protection in various septic shock models induced by LPS. Recent study in patients with sepsis has shown that BN52021 (a PAF inhibitor) offered an improvement (10, 13). Another PAF antagonist, TCV-309, inhibited cytokine production in experimental endotoxaemia in chimpanzees (84).

Nitric oxide as a marker of septic shock

The free radical of NO is synthesized from L-arginine by a family of enzymes - NO synthases (NOS) (85). The continuous biosynthesis of NO by the constitutive endothelial isoform of NOS (eNOS) keeps the vasculature in active vasodilatation and reduces platelets and polymorphonuclear cell adhesion to the endothelium. The inducible isoform of NOS (iNOS) is expressed in response to immunological stimuli. iNOS produces NO at nanomolar amounts and then acts as a cytostatic and cytotoxic agent.

The role of NO in septic shock will be presented in a separate contribution and this is why only a very short summary of available data will be presented here. It has been suggested that the overproduction of NO is responsible for death during endotoxic shock or sepsis (86-91). In 1989, Vallance et al. showed that inhibition of NO synthesis by N^G-monomethyl-L-arginine (L-NMMA) had elevated blood pressure in rats (92). Furthermore, it was demonstrated that local infusion of L-NAME into brachial artery of healthy volunteers caused a dose-dependent fall in resting forearm blood flow and attenuated the dilator response to acetylcholine. The effect of L-NAME was stereospecific (D-NAME was ineffective) and reversed by supplying an excess of L-arginine (93). Ochoa et al. found elevated nitrite/nitrate levels (stable breakdown products of NO) in patients with septic shock but these levels were decreased in patients with trauma (94, 95). Recently, Wang and Chaudry pointed out the complexity of the alteration in NO production with the progression of sepsis (91). They hypothesized that NO inhibition under some septic conditions might be detrimental. We also observed ambiguous effects of NOS inhibitors in rats with LPS-induced shock. When NOS inhibitors had been administered before LPS was given, the removal of NO potentiated LPS-induced shock. Administration of NO inhibitors in the second hour after injection of LPS caused a temporary improvement. The activity of eNOS is regulated by calmodulin and changes in intracellular calcium. The enhanced formation of NO in the early stage of shock is due to the activation of eNOS. We observed the induction of iNOS mRNA in the lungs, spleen and heart of LPS-treated rats as early as 1 h after endotoxin administration. This is why we postulate that in early and late stages of shock, differential effects of nonselective NOS inhibitors might account for their action on different isoforms of NOS. So far, the role of NOS inhibitors in the treatment of sepsis remains to be defined. The availability of specific inhibitors of iNOS

would help to answer the relevant questions. Such agents are in the early stages of development but they have not as yet been studied in humans (96, 97).

Programmed cell death - apoptosis

Apoptosis constitutes an efficient system in cell biology designed to eliminate superfluous, unwanted, altered, aged, or transformed cells without eliciting damage to adjacent normal cells or surrounding tissues. The mechanism of apoptosis has long been neglected in clinical research and in clinical thinking. Nevertheless, apoptosis offers understanding of a number of pathological syndromes and clinical observations which otherwise cannot be explained by well-known biological processes. Leucocytes, monocytes and macrophages are selectively eliminated from inflammatory tissues by the occurrence of programmed cell death. The therapeutic regulation of apoptosis during and after an inflammation offers a new approach for promoting rapid healing and reduction of unwanted pathological sequelae of inflammation processes (7, 8, 98, 99).

Necrosis, or accidental cell death, occurs in response to harmful insults such as physical damage, hypoxia, hyperthermia, complement attack or chemical injury. Table 2 shows the differences between apoptosis and necrosis.

Analysis of the mechanism that prevents cell death, such as activation of the bcl-2 gene, addition of growth factors and the use of protein synthesis inhibitors or calcium entry blockers, might aid in the development of new treatment strategies. Drug and therapy designs directed at the modulation of apoptotic process will offer new opportunities for the treatment and control of tissue damage in the coming years. If these goals can be accomplished, we may finally see a reduction in the morbidity and mortality associated with MODFS.

Conclusion

Comprehension of the basic mechanisms involved in the host inflammatory response is necessary for clinicians to make educated choices and decisions regarding therapies. The discovery of the sphingomyelin cycle as a target of LPS action that mimics natural mediator ceramide promoted progress in our understanding of the events during the development of septic shock. Advances in molecular biology and in the cytokine network led to the development of novel approaches to the treatment of septic shock, SIRS and MODFS. As noted in this review, therapies are directed to distinct levels: the initiating event (i.e. endotoxin), to various mediators, and the effector cells (i.e. macrophages, endothelial cells). These factors are not merely markers of sepsis and its severity,

Table 2

	Apoptosis	Necrosis
Origin	Lack of growth factors, hormonal factors, mild toxic stimuli	Anoxia, starvation, physical and chemical damage
Occult phase	Minutes to hours	None
First manifestation	Shrinking	Swelling
Nuclear changes	Pyknosis, condensation, internucleosome cleavage, DNA laddering	Karyolysis
Chromatin	Segmentation and margination	Nuclear folding
Nucleolar changes	Intact	Granulated
Membrane integrity	Persists	Failure
Surface	Smoothing	Lysis, blebbing
Cytoskeleton	Formation of apoptotic bodies	Fragmentation
Mitochondria	Unaffected	Swelling
Endoplasmic reticulum and Golgi apparatus	Unaffected	Dilated
Organelles	Intact	Swollen, leaky
Gene expression	p53 ↑, bcl-2 ↓, c-myc ↑	No change
Protein synthesis	Blocked by cycloheximide and actinomycin D	Not affected by antibiotics
Cytoplasmic changes	Endonuclease activity ↑, transglutaminase ↑	Release of lysosome content
Cells affected	Dispersed cells	Diffuse degradation
Cell elimination	Engulfment by macrophages and endothelial cells	Inflammatory response in adjacent tissues

but play an important role in the pathophysiological mechanisms. The era of cytokine response modification in patients with severe sepsis has evolved rapidly. These therapies if they prove effective in clinical trials are in progress and it should be stressed that current interventional capacities are far ahead of our comprehension of the mechanisms involved. Moreover, we should remember that these therapies are enormously expensive. Finally, taking into account that apoptosis is a natural route of macrophage elimination, some present therapies might be ineffective because they act on cells already programmed to their death, no matter how much effort is engaged to keep them alive.

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