



**Review**

## Immunotherapy in the Management of Sepsis

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**Abstract.** This work presents the role of Gram-negative bacteria endotoxins, pro- and anti-inflammatory cytokines and reactive oxygen species (ROS) in the complex and not fully explained pathogenesis of sepsis. The so-called “respiratory burst” of neutrophils and the antioxidant mechanisms of the host are also discussed. The work focuses on possible approaches to the management of sepsis connected with immunotherapy. Neutralization of endotoxin lipopolysaccharide (LPS), anti-tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) therapy with monoclonal antibodies or pentoxifylline (PTXF), as well as soluble recombinant cytokine agonists and antagonists used in clinical trials are taken into consideration. In addition, cytokine manipulation therapy, anti-adhesion techniques, glucocorticoides and antioxidant barrier interference are also described. So far there has been no immunotherapy of sepsis in children of proven clinical efficacy, which prompts an aggressive examination of the immune system aimed at affecting its function.

**Key words:** sepsis; cytokines; reactive oxygen species; monoclonal antibodies; cytokine agonists and antagonists; immunotherapy.

### Introduction

Sepsis, the systemic inflammatory response to infection, is a major cause of death of hospitalized patients, despite the availability of potent antibiotics, supportive care and advanced medical therapies. The pathogenesis of sepsis is complex and its mechanisms are still being elucidated. The discovery of the complex cytokine cascade resulting from the interaction of microbial pathogens with cells of the immune system has been a major advance. Knowledge of the cytokine cascade has facilitated the understanding of the basic mechanisms of the immunological and metabolic consequences of infection and has opened new areas of potential therapeutic manipulation.

The endotoxin of Gram-negative bacteria elicits humoral and cellular defense mechanisms; it activates coagulation and fibrinolysis, macrophages, neutrophils and the release of endogenous inflammatory mediators. The key role in the pathogenesis of sepsis recently has been attributed to pro-inflammatory cytokines released by numerous cells, including lipopolysaccharide (LPS)-activated macrophages. Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukins (IL-1, IL-6, IL-8) are the most important factors. LPS released by Gram-negative bacteria activate metabolic processes in monocytes triggering the translation of mRNA for TNF- $\alpha$  and other cytokines in their cytoplasm. Matrix RNA for all cytokines is constantly present in the cytoplasm and, thus, these appear as soon as few minutes after the activation

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of monocytes. *In vivo* high concentrations of TNF- $\alpha$  and IL-1 have been observed after 1–2 h. Activated monocytes, macrophages and other cells release IL-6 within several hours. IL-6, IL-1 and TNF- $\alpha$  are responsible for the release of acute-phase proteins (e.g. C-reactive protein) during systemic infection. The increased serum levels of these cytokines were found to correlate with severity and mortality in the course of sepsis<sup>18, 27</sup>.

In the first stage of inflammation the activity of pro-inflammatory cytokines is biologically favorable, as they stimulate the non-specific defensive mechanisms. The clinical value of these cytokines is of particular importance in conditions where they lose their original defensive properties and become active against the host's tissues. A significant role in the pathogenesis of sepsis is played by TNF- $\alpha$ , which appears as the first of pro-inflammatory cytokines in reaction to the infectious factor triggering the cytokine cascade. TNF- $\alpha$ , IL-1, IL-6, and IL-8 released by activated macrophages may accelerate septic conditions and bring about a fatal outcome caused by uncontrolled immune response to infectious factors. TNF- $\alpha$  can induce oxidative burst in neutrophils and activate the production of reactive oxygen species (ROS) in patients with systemic infection<sup>45</sup>. Due to the oxidase system of NADPH + H<sup>+</sup>, which catalyses one-electron oxygen reduction to the superoxide anion, neutrophils release ROS. An excessive production of pro-inflammatory cytokines by activated macrophages may stimulate neutrophils to an enhanced generation of ROS, which further leads to tissue damage and multiorgan failure, i.e. multiple organ dysfunction syndrome (MODS), in the course of sepsis<sup>23, 61</sup>.

The anti-inflammatory mechanisms associated with the control of the immune response, elicited to maintain system homeostasis, involve the release of anti-inflammatory cytokines (IL-4, IL-10, IL-13) and soluble cytokine receptors or receptor antagonists<sup>25, 28, 44, 53, 65</sup>. Particular attention is focused on IL-10 and IL-13, which inhibit the release of pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1, IL-6, IL-8, by activated macrophages<sup>28, 34, 41</sup>. DOUGHTY et al.<sup>10</sup> reported increased IL-10 levels in the blood sera of children with multiorgan failure in the course of sepsis, which correlated with IL-6 levels. Other authors have demonstrated increased levels of soluble p55 TNF- $\alpha$  receptor (sTNFR1), soluble p75 TNF- $\alpha$  receptor (sTNFR2) and IL-1 receptor antagonist (IL-1ra), which were found in experimentally elicited infection<sup>44</sup>. There have been literature reports on the identification of the inhibitors of TNF- $\alpha$ , IL-1 and IL-6 along with anti-inflammatory cytokines in patients with systemic infection<sup>2, 19, 28, 34, 41, 44, 53</sup>. The release of anti-inflammatory cytokines

seems to be the initial stage for the further control and limitation of the immune response in these patients<sup>19, 34</sup>.

### Respiratory Burst

The evaluation of neutrophil oxidative burst during sepsis was a subject of controversy. LLOYD et al.<sup>33</sup> were the first to demonstrate an increased hydroxyl radical generation *in vivo* in patients with sepsis. However, the studies performed by VESPASIANO et al.<sup>59</sup> implied that a decrease in neutrophil oxidative burst and decreased ROS generation is observed in particularly serious courses of sepsis, often complicated by endotoxemic shock. DROSSOU et al.<sup>12</sup> also observed decreased oxidative metabolism in neutrophils in children with serious infection. Our own results concerning the decreased oxidative burst of neutrophils in children prior to sepsis therapy, which correlated with an increased serum concentration of sTNFR1 before the treatment and with an increased expression of the p75 TNF- $\alpha$  receptor, should be explained by the prolonged stimulation with bacterial antigens and the functional exhaustion of neutrophils in the course of serious systemic infection. Moreover, our studies may confirm the thesis of IL-1 as a potent regulator of immune response *in vivo* in children with a serious course of sepsis with decreased oxidative metabolism in neutrophils<sup>52</sup>.

### Anti-Oxidative Mechanisms

The damaging activity of pro-inflammatory cytokines is counterbalanced (i.e. anti-inflammatory cytokines are produced and soluble receptors for these cytokines and inhibitors of cytokines are released into the blood stream), and, at the same time, antioxidative mechanisms are activated. These are, among others, the inactivation of ROS by increasing the activity of enzymes – the so-called first-order antioxidants, for example superoxide dismutase, catalase, and glutathione peroxidase. However, in generalized sepsis these mechanisms are not sufficient and, as a result, tissues are damaged by ROS and the development of MODS<sup>22, 65</sup>. In the course of sepsis, decreased concentrations of second-rate antioxidants (tocopherol, retinol,  $\beta$ -carotenes) have also been observed which prevents the amplification of free radicals in the sera of these patients<sup>21</sup>. Thus, increasing the enzymatic activity of the first and second-rate antioxidants may be therapeutically important in limiting damage to tissues and multiorgan failure in the course of sepsis.

## Treatment

The standard treatment of sepsis and septic shock consists of antibiotics, surgical treatment of the inflammatory focus, monitoring of the patients in intensive care units, normalization of metabolic disturbances, and proper nutrition. It should be noted that although antibiotics are key to the specific control of infection, their administration may result in signal enhancement in transduction pathways and an initial burst of cytotoxic mediators, damaging to the host. The possibility of modifying the immunological reaction has become an attractive challenge for many scientists in their studies on sepsis. It appeared that modification of the activity of one cytokine did not lead to the expected results in the majority of cases, probably due to the complex character of the cytokine cascade. The standard treatment of sepsis fails when cells become damaged by bacterial toxins and when endogenous inflammatory mediators, such as pro-inflammatory cytokines, reactive oxygen species or nitric oxide are released.

Thus, new strategies in the treatment of sepsis and septic shock have been directed towards neutralization of the bacterial toxins and endogenous inflammation mediators. Numerous attempts at an immunomodulation of the cytokine cascade have been undertaken by using endogenous antagonists of cytokines and anti-inflammatory cytokines<sup>7, 15, 17, 18, 32, 35, 37</sup>. Also, numerous experimental studies have used monoclonal antibodies against endotoxin or lipid A and against pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, IL-6, IL-8), which block the receptors and attempts at inhibition of the synthesis of some mediators of inflammation (e.g. NO) have also been undertaken<sup>5, 16, 39, 46, 48, 54, 56, 58</sup>. Due to the significant role played by ROS in the pathogenesis of sepsis, studies on the application of free oxygen radical scavengers (for example of supraoxide dismutase) to limit the damage to tissues and organs have been attempted<sup>22</sup>. These therapeutic methods are still in the first stage of clinical study, and the results of experiments on animals are not encouraging. There have also been attempts at changing the response of neutrophils in the course of sepsis and septic shock by application of growth factors for granulocytes (G-CSF)<sup>11, 40</sup>.

## Anti-Endotoxin Therapy

Release of a specific endotoxin from Gram-negative bacteria is thought to be the first event in the initiation of the sepsis cascade. This endotoxin initiates the release of a wide variety of inflammatory mediators.

Thus, a reasonable therapeutic approach would involve enhancing the elimination of such bacterial products. An experimental study by McCABE et al.<sup>38</sup> suggested that antibodies directed towards epitopes in the inner core-sugar region of the LPS are highly protective and could be used to treat or prevent infections. Other clinical trials with either polyclonal antisera or plasma were performed later and demonstrated a reduction of mortality in patients with Gram-negative infection or a significant prevention of shock and death due to these pathogens<sup>3</sup>. However, these anti-serums have not been applied on a wider scale, for various reasons (e.g. lack of standardization, the risk of transmission of infection, and the high number of necessary donors).

In other studies, purified immunoglobulin (Ig) preparations were used. In one study with anti-core intravenous immunoglobulins (IVIG), patients at high risk of post-surgical infection were given IVIG, an IgG preparation enriched with anti-Re LPS antibodies (Re-IVIG), or a placebo. The mortality rate did not significantly differ among the three groups<sup>9</sup>. It seemed that polyclonal anti-endotoxin antiserum exerted some effect via an unknown mechanism, although studies with purified IgG failed to confirm any specific effect.

Subsequent clinical investigations examining monoclonal antibodies (MoAbs) to endotoxin were formulated. Two MoAbs, the human IgM HA-1A (Centoxin) and the murine IgM E5 (Xomen), have been studied in patients. HA-1A was studied in two placebo-controlled clinical trials. Patients with Gram-negative sepsis showed significantly reduced mortality following HA-1A treatment (30%) versus placebo (49%) and results within the subgroup of patients who developed septic shock were even more favorable<sup>64</sup>. However, in the second trial no difference was noted between the treatment and placebo groups<sup>39</sup>. Numerous points of controversy were subsequently raised, including the fact that the epitope of core endotoxin was not characterized precisely at the time of these clinical investigations, that the mode of action of anticore endotoxin monoclonal antibodies was unclear, and, finally, that this approach was extremely expensive.

Other studies have examined the role of naturally occurring endotoxin antibodies (EndoCAbs) and their correlation with outcome in septic patients. GOLDIE et al.<sup>20</sup> revealed that IgM EndoCAb levels correlated negatively with 28-day mortality in septic patients and that IgG EndoCAb depletion was predictive of mortality.

## Anti-TNF Therapy

TNF- $\alpha$  is a potent pleiotropic cytokine active in host-defense inflammation and cell death. TNF- $\alpha$  plays

the main pathogenic role in sepsis and septic shock. The biological activity of TNF- $\alpha$  is mediated by the cross-linking of membrane-bound receptors which exist in two isoforms: TNF receptor I (TNFRI, p55) and TNF receptor II (TNFRII, p75)<sup>4</sup>. The extracellular domains of both receptors can be shed such that the soluble forms retain their ability to bind to TNF- $\alpha$  and neutralize its activity, thus limiting acute TNF- $\alpha$ -induced effects<sup>14</sup>.

Therapy with antibodies against endotoxin may prove successful in sepsis caused by Gram-negative bacteria. In cases of other etiology elimination of the damaging effect of TNF- $\alpha$  has been attempted. However, anti-TNF- $\alpha$  strategies have so far failed to be of clear benefit in studies on human sepsis despite favorable predictions based on animal models<sup>24</sup>. The reasons why these outcomes have been difficult to predict are due to the complexity of the molecular structure and function of TNF- $\alpha$  and the extraordinary diversity of its biological activities. Also, the time span from the exposition to pathogens to the administration of antibodies is important. Thus, good therapeutic effect should be expected when anti-TNF- $\alpha$  antibodies are administered shortly before, or directly after, the initiation of the cytokine cascade triggered by the development of sepsis. It is known that serum TNF- $\alpha$  levels increase when animals or humans are given endotoxin. Similarly, elevated levels of TNF- $\alpha$  are often observed in patients with sepsis<sup>18, 25, 27, 52</sup>. It is thought that this cytokine may be the mediator of the hypotension, disseminated intravascular coagulation and organ failure observed during sepsis. The hypothesis that neutralization of TNF- $\alpha$  in such patients might prevent or reverse some of the detrimental findings has been tested using MoAbs which block TNF- $\alpha$ .

In the past decade a number of clinical trials were conducted using anti-TNF- $\alpha$  monoclonal antibodies – cA2 (Centocor) or TNF receptor – IgG (TNFR p55 IgG Fc) fusion protein constructs or “immunoadhesins” to treat patients with a diagnosis of presumed sepsis<sup>62</sup>. In a series of phase I and II trials, cA2 was administered to patients with a diagnosis of presumed sepsis in two large studies<sup>1, 16</sup>. There were no major differences in adverse events, and the difference in mortality between those receiving a placebo (62%) versus those receiving cA2 (37%) was not statistically significant. Thus, the role of TNF- $\alpha$  as a major mediator of organ damage and tissue injury in sepsis has not been formally proven by these clinical studies, and it is possible that the simultaneous blockade of other mediators may be necessary to reveal the clinical benefit of blocking TNF- $\alpha$ .

Two basic mechanisms of action have been pro-

posed for cA2. In the first, complexing of soluble TNF- $\alpha$  with cA2 leads to the loss of TNF- $\alpha$  bioactivity and downregulation of the downstream inflammatory events. In the second, binding of cA2 to transmembrane TNF- $\alpha$  results in the destruction of TNF- $\alpha$ -producing cells by Fc-mediated mechanisms, leading to a reduction in inflammatory and immune cell numbers.

### **Pentoxifylline**

Recently, the immunomodulatory activity of pentoxifylline (PTXF) has been the focus of more and more attention. LAUTERBACH and ZEMBALA<sup>30</sup> demonstrated that this drug decreased the concentration of TNF- $\alpha$  in the blood serum of premature newborns in the course of sepsis, increasing their survival. Other researchers reported an inhibitory effect of PTXF on the production of TNF- $\alpha$  in experimental studies on animals<sup>31</sup>. ZENI et al.<sup>63</sup> showed that PTXF decreased the concentration of TNF- $\alpha$  present in the blood stream (without affecting the concentration of IL-6 and IL-8) in patients with septic shock. It seems that PTXF may be a promising new agent in the sepsis syndrome therapy. However, a multicenter clinical study is needed to evaluate the efficacy of this drug in terms of survival in patients with severe sepsis.

OISMULLER et al.<sup>43</sup> demonstrated that PTXF administered intravenously was able to reduce granulocyte respiratory burst activity in patients fulfilling the established criteria of sepsis. Other authors observed that in the presence of PTXF, TNF- $\alpha$  and recombinant human granulocyte-colony stimulating factor (rhG-CSF) were unable to stimulate respiratory burst activity of the neutrophil<sup>13</sup>. These results suggest that by blocking the inflammatory action of TNF- $\alpha$  on neutrophils, PTXF may diminish endothelial damage caused by inhibited neutrophil chemotaxis.

### **Cytokine Agonists and Antagonists**

Another direction of research is the application of recombinant soluble receptor for TNF- $\alpha$ , which would bind the excess of this cytokine in body fluids. However, the promising results of experimental studies have not been confirmed in clinical studies. A phase II blind, randomized trial with recombinant human dimeric p75 TNF receptor was conducted in patients with sepsis syndrome; enhanced mortality was observed in the group treated with high doses (53%) versus the placebo group (30%)<sup>50</sup>. This suggests that anti-cytokine therapy

needs to be approached with care, because of possible harmful effects.

Recently, the effect of treatment with rhG-CSF on the neutrophil count and function of pre-term neonates with documented sepsis has been investigated<sup>11</sup>. The authors revealed that the administration of rhG-CSF to septic neonates significantly increased the absolute granulocyte count and enhanced the neutrophil respiratory burst and  $\beta 2$  integrin expression.

The IL-1ra is a naturally occurring protein that binds to human IL-1 receptor but has no agonist activity. In the presence of IL-1ra, IL-1 cannot bind to its receptor and cannot exert its pro-inflammatory activity. Elevated IL-1ra levels were also found in pediatric sepsis syndrome<sup>51</sup>. The IL-1ra level was more than 1000 times higher than the IL-1 $\beta$  level: a concentration known to block IL-1 receptors. Thus, an additional benefit from exogenous IL-1ra therapy would be questionable. However, the authors think that further studies are indicated to determine whether there is a population of patients with sepsis who could benefit from the administration of exogenous IL-1ra.

### Cytokine Manipulation Therapy

A potentially beneficial role of IL-10 in the pathogenesis of various infectious diseases has been suggested by a number of studies using animal models. In healthy volunteers subjected to experimental endotoxemia, IL-10 reduced the LPS-stimulated *ex vivo* production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-8<sup>7, 17</sup>.

MANCUSO et al.<sup>35</sup> revealed that either prophylactically or therapeutically administered recombinant IL-12 (rIL-12) significantly improved survival time in experimental neonatal sepsis caused by group B streptococci (GBS). They postulated that rIL-12 was a possible candidate for the treatment of GBS sepsis.

MATSUKAWA et al.<sup>37</sup> elucidated the role of endogenous IL-13 in a murine model of septic peritonitis. They observed that IL-13 blockade with anti-IL-13 antibodies significantly decreased the survival rate of mice from 53 to 14% on day 7, compared with the control. Thus, endogenous IL-13 protected mice from lethality in this experimental study by modulating inflammatory responses via the suppression of an overzealous production of inflammatory cytokines in tissues. Although the *in vitro* anti-inflammatory effects of IL-13 and IL-10 are fascinating, we must await more *in vivo* studies of these cytokines before their manipulation becomes possible or practical in septic patients.

NO has been implicated as the major cause of the profound vasodilation and hypotension associated with sepsis and trauma<sup>23, 65</sup>. An inducible form of NO synthetase (NOS) is expressed in response to various stimuli, including bacterial endotoxin. N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) and N<sup>G</sup>-monomethyl-L-arginine (L-NMMA) are competitive inhibitors of NOS and have been studied in experimental sepsis and humans with septic shock. Since NO plays an important role in the bactericidal activity of neutrophils, modulation of its synthesis could be detrimental to the infected host. Thus, other investigators have attempted to modulate the effects of NO by inhibiting soluble guanylate cyclase through the use of methylene blue. This represents another potential therapeutic approach to counteract excessive NO production during sepsis<sup>47</sup>.

### Anti-Adhesion Techniques

The inhibition of neutrophils by agents, which inhibit adhesion of these cells to tissues, may become another possibility in the management of sepsis. Monoclonal antibody 1B4 directed against CD18 integrins prevented tissue damage in a rabbit model<sup>57</sup>. Recently, it was shown that peptides derived from the FHA region binding to CD11b/CD18 inhibited adherence and transendothelial migration of neutrophils *in vitro* and prevented recruitment of leukocytes into the cerebrospinal fluid in an experimental model of meningitis in rabbits<sup>49</sup>.

### Glucocorticoides and Antioxidant Barrier Manipulation

Mechanisms regulating the activity of antioxidative enzymes have been extensively studied. It seems that among the mechanisms of protein and enzyme synthesis, the regulation of the transcription rate of genes plays a fundamental role<sup>60</sup>. The mechanism of activity of corticosteroides is probably connected with the activation of promoters for the transcription of many antioxidative enzymes. Corticosteroides have been implied in the inhibition of mRNA degradation or activation of translation<sup>42</sup>. Also, the place of binding of the complex steroid-receptor to DNA has been the subject of study. It has been demonstrated that within the gene for superoxide dismutase there are places similar to those activated by glucocorticoides<sup>26</sup>. It is thus possible that glucocorticoides activate the transcription of the gene for superoxide dismutase at these regions. Glucocorti-

coides also inhibit the production of cytokines, for instance by decreasing the transcription rates of the genes for IL-6 and IL-1 and the stability of the corresponding mRNA<sup>8</sup>.

Despite the completion of multiple animal and human trials, the use of dexamethasone adjuvant therapy in patients with sepsis (meningitis) remains one of the most debated topics in modern pediatrics and this kind of treatment is still controversial.

Due to the important role played by reactive oxygen species in the pathogenesis of sepsis, studies on the application of first and second rate antioxidants in the treatment of generalized infections are still being conducted. Antioxidant defenses may be compromised in sepsis, so enhancement of the antioxidant barrier activity may turn out to be the best approach. Literature reports suggest that these methods of treatment are still in the first stage of clinical study, and that the results of *ex vivo* experiments and the trials on animals are not numerous and cannot be applied to humans<sup>36, 55</sup>.

## Conclusions

A large variety of new approaches are being formulated for the treatment of sepsis. A number of these strategies are being tested in clinical trials. Due to the fact that cytokines play both a pathogenic and a protective role in sepsis, immunotherapy directed at their neutralization will probably not be the key to success. Unfortunately, our knowledge of the pathogenesis of sepsis remains incomplete, and further studies directed at the elucidation of it will lead to the development of new therapeutic methods. Besides this, the true nature of the immune system *in vivo* must be better understood before any precise clinical manipulation is possible.

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