

Sepsis Immunopathology: Perspectives of Monitoring and Modulation of the Immune Disturbances

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Abstract Septic syndromes are the main cause of death in the intensive care units and although the mortality rates is slowly decreasing, the occurrence of the disease has been increasing. The pathogenesis of sepsis includes countless disturbances of the host immune system starting with a harmful, infection-triggered exaggerated inflammatory cascade, followed by the development of an immunoparalysis state. The latter contributes to the failure in pathogen eradication and leads to secondary infections, which are often the cause of fatal complications. In this review, we consider different novel therapeutic strategies for restoration of immune function. The use of glucocorticoids, intravenous immunoglobulins, heparin, recombinant human activated protein C, granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor, interferon- γ , statins, macrolides and high-volume hemofiltration are discussed. Even though some clinical trials of these regimens are promising, the key to their successful application seems to be the precise monitoring of the status of immune system followed by implementation of the adequate therapy. Thus, in this paper we present disturbances in the immune system in the course of human sepsis, with special attention to the parameters that could be monitored and serve as markers for immunomodulatory therapies. We conclude by briefly presenting the current sepsis treatment strategy.

Keywords Sepsis · Monitoring · Flow cytometry · Immunomodulation

Abbreviations

APC	Activated protein C
ARDS	Acute respiratory distress syndrome
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
TF	Tissue factor
HVHF	High-volume hemofiltration
HLA	Human leukocyte antigen
ICU	Intensive care units
IFN- γ	Interferon gamma
IVIg	Intravenous immunoglobulins
LPS	Lipopolysaccharide
NETs	Neutrophil extracellular traps
PAMPs	Pathogen-associated molecular patterns
PARRs	Pattern recognition receptors
PCT	Procalcitonin
PMNs	Polymorphonuclear cells
SIRS	Systemic inflammatory response syndrome
TLR	Toll-like receptor
TNF	Tumor necrosis factor

Introduction

Sepsis, severe sepsis and septic shock still constitute a major and underestimated clinical problem. Despite the progress in basic science, both understanding of the pathomechanisms and the therapy of sepsis remains challenging. Septic syndromes are the number one cause of deaths in the intensive care units (ICU) in Europe and US.

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The mortality rates vary between 30 and 60%, due to a range of definitions applied in the reviews about sepsis and its complications (Angus and Linde-Zwirble 2001; Levy et al. 2010). Another reason for the broad range of estimates lies in the differences between studied populations. According to 131 studies on septic shock and conducted between 1958 and 1997, the overall mortality rate was 49.7%. About one-quarter of deaths can be classified as a complication of bacteremia while another 10% is directly caused by the disease itself. Growing invasiveness of medical procedures, expanding number of immunocompromised patients, massive use of broad-spectrum antibiotics and aging of societies are the main reasons for the increasing occurrence of sepsis currently estimated to be the cause of 1.5% of deaths each year. It was calculated that the diagnosis of sepsis is made in 1.8 million patients a year (Angus and Linde-Zwirble 2001; Levy et al. 2010).

In the early 1990s a strong connection between sepsis and the systemic inflammatory response syndrome (SIRS) was noted (Bone et al. 1992). Local inflammation is definitely a beneficial mechanism of the host defense response against infection. However, when it is followed by the massive release of proinflammatory mediators, such as tumor necrosis factor (TNF), interleukin 1 β (IL-1 β), IL-6, IL-12, anaphylotoxins, uncontrolled SIRS can be developed. This excessive inflammatory response was thought to be the main cause of death. Such deduction led to numerous trials to block the inflammatory cascade by high doses of glucocorticosteroids, neutralizing antibodies directed against endotoxin and early inflammatory cytokines such as TNF or IL-1. Although results of studies in animal models were encouraging, clinical trials failed to improve outcome of sepsis (Opal et al. 1997; Reinhart et al. 2001), which led to increasing doubts in the whole hypothesis (Bone 1996). Next, the attention of researchers was drawn by the observation that most deaths of sepsis patients occurs in the later phase of the disease, which is probably due to the development of secondary infection in the course of the multi-organ dysfunction syndrome. It is known from autopsy studies that most of the patients that died of sepsis had active sites of infection, in spite of intensive antimicrobial treatment (Torgersen et al. 2009). In fact, the immune response is a dynamic process disturbing the homeostatic mechanisms, rather than a simple state of hyperactivity. The primary activation of inflammatory pathways is followed by multi-organ dysfunction, hypotension and hypoperfusion, but it also activates opposite mechanisms, known as the compensatory anti-inflammatory response syndrome (Bone 1996). First, in order to restore the immune balance, this process operates as a part of homeostasis. Only later a form of immunosuppression, called “immunoparalysis” is evoked and is the probable reason for therapeutic failures. At present,

many immunomodulating therapies are considered, but because of the dynamics of the host defense system, introduction of such regimens requires individual and precise analysis of the actual state of the patient immune system (Adib-Conquy and Cavaillon 2009; Draisma et al. 2008; Monneret et al. 2008). The possibilities of monitoring the immune system disturbances are discussed below and summarized in Table 1.

Immune System and Its Activation in Sepsis

Various microorganisms may be recognized by the cells of innate immune system because of their expression of the so called pathogen-associated molecular patterns (PAMPs), i.e. murein and lipopolysaccharide (LPS) in bacteria, mannans in fungi and double-strand RNA in some viruses. These PAMPs are recognized by pattern recognition receptors (PARRs), including Toll-like receptors (TLR). Activation of PARRs is the first step of activating immune response (Bone 1996; Draisma et al. 2008; Torgersen et al. 2009).

Mechanisms of the initiation of the inflammatory cascade are well studied. Numerous exogenous (endo- and exotoxin) and endogenous mediators, such as activated complement factor (C5a), active Hagemann factor (XIIa) or damaged cells, are capable of activating monocytes and macrophages, which synthesize cytokines responsible for the development of SIRS, like TNF, IL-1 β , IL-6 and interferon γ (IFN- γ). The intracellular inflammatory pathway is commonly started by a non-specific oxidative stress in the following mechanism: exogenous stimuli (i.e. LPS) activate serine phosphokinase via ligation to PRR, which evokes oxidative reactions that in turn activate nuclear factor κ B (NF- κ B) by splitting off its inhibitor. The inhibitor of κ B active dimer migrates to the nuclei, where it regulates transcription of genes for proinflammatory cytokines (TNF, IL-1 β) and proinflammatory enzymes: inducible nitric oxide synthase (iNOS) and cyclooxygenase 2. The iNOS synthase is a key player in septic circulatory dysfunctions through both strong cardiodepressive effect and vasodilatory effect in microcirculation (Adib-Conquy and Cavaillon 2009; Bone 1996; Draisma et al. 2008; Monneret et al. 2008).

Targets for Monitoring Immune Dysfunctions

Circulating Mediators

Monitoring the patient's plasma concentration of proinflammatory cytokines is a relatively cheap and easy test, which therefore may be helpful in the introduction of

Table 1 Summary of the technical opportunities for monitoring the immune status of the septic patient

Marker	Comments	Technique	References
TNF	Primary inflammatory mediator	ELISA	Oberholzer et al. (2005)
IL-6	Marker of inflammation	ELISA, some automatic analyzers	Oberholzer et al. (2005), Schefold et al. (2008)
IL-10	Major anti-inflammatory cytokine	ELISA	Monneret et al. (2004)
PCT	Marker of the bacterial infection, probably anti-inflammatory mediator	Immunoluminometry	Bouadma et al. (2010), Harbarth et al. (2001), Nobre et al. (2008), Tang et al. (2007)
HMGB1	Activates TLRs, marker of inflammation	ELISA	Sundén-Cullberg et al. (2005)
HLA-DR (monocytic)	Antigen presentation, reflects immunoparalysis	Flow cytometry	Döcke et al. (2005), Monneret et al. (2006, 2010)
Lymphocytes apoptosis	Reflects immunoparalysis better than lymphocyte count only	Flow cytometry	Taneja et al. (2008)
CD64 (neutrophils, monocytes)	IgGfC receptor, correlates with phagocytic function	Flow cytometry	Danikas et al. (2008)
Phagocytosis assay	Important information about the major antibacterial regimen	Flow cytometry	Danikas et al. (2008)

Flow cytometry is the most universal analytical technique and can be used for the analysis of a single probe. The results can be obtained in a very short time which makes it a suitable technique in the ICU settings. As for now, there are no reliable tests which would allow measuring cytokines concentration in a single probe (aside from IL-6). Commercially available ELISA tests which require performing standard curve each time, are too expensive for analyzing single probes. Only PCT concentrations can be easily measured and this marker should be used to guide the time of the antibiotic therapy

HMGB1 high-mobility group box 1

immunostimulatory treatment (Schefold et al. 2008). Although huge efforts have been made, the concentration of no single inflammatory mediator has been identified as a reliable marker of the patient clinical state. This may seem quite obvious as hundreds of mediators participate in the complicated pathogenesis of sepsis. However, some correlations were found between the prognosis of sepsis and the elevated levels of such proinflammatory cytokines as TNF, IL-6, IL-8 or granulocyte–macrophage colony-stimulating factor (GM-CSF) (Oberholzer et al. 2005; Schefold et al. 2008; Waage et al. 1989). Concentrations of these cytokines increase shortly after LPS stimulation (Ploder et al. 2006). An interesting cytokine that occurs in the circulation in the later phase (12–18 h) of sepsis is a high-mobility group box 1 protein. This ligand is released by the necrotic cells and activated monocytes, as well. Through TLR2 and TLR4 it stimulates other immune cells to produce TNF, activates the endothelium and injures the epithelial cells (Sundén-Cullberg et al. 2005; Wang et al. 1999). There is also some evidence of impaired production of proinflammatory prostaglandins in sepsis (Baenkler et al. 2006). On the other hand, some strong correlations were found between the anti-inflammatory cytokines and the outcome of sepsis. High level of serum IL-10 and the IL-10/TNF ratio indicate high risk of mortality (Schefold et al. 2008). IL-10 is an effective inhibitor of the synthesis of many proinflammatory cytokines and its prolonged massive release may lead to

immunoparalysis and secondary infections development (Monneret et al. 2004; Oberholzer et al. 2002). Another important agent in the generation of the immunoparalysis, including monocyte toleration of LPS, is the transforming growth factor β (TGF- β). However, available research did not reveal correlations of TGF- β concentration with clinical data (Oberholzer et al. 2002). Aside from the anti-inflammatory cytokines, many other immunosuppressive mediators were found to be activated in the septic shock, i.e. soluble HLA-G, prostaglandin E2, adrenomedullin, vasoactive intestinal factor, cortisol, norepinephrine and acetylcholine (Gonzalez-Rey et al. 2007; Monneret et al. 2007; Tracey 2007).

Procalcitonin

Procalcitonin (PCT) is a precursor peptide of the hormone calcitonin which is produced in a steady state by C cells of the thyroid and its serum level is very low (Becker et al. 2004). However, it significantly rises during bacteria-caused inflammation, but not in viral or noninfectious inflammation (Harbarth et al. 2001). Many groups have provided evidences for its diagnostic and prognostic utility in sepsis, severe sepsis and septic shock (Fioretto et al. 2008; Meynaar et al. 2011; Tang et al. 2007). PCT is commonly perceived as a marker of a bacterial infection, but it is also a mediator molecule in the sepsis cascade.

Although little is known about the biological role of PCT, its synthesis is up-regulated by TNF, a primary sepsis inflammatory mediator (Kettelhack et al. 2000). Peak level of the serum PCT follows the peak of TNF and IL-6 (Dandona et al. 1994). Polymorphonuclear blood cells stimulated by *Staphylococcus aureus* showed an increased expression of PCT which could be inhibited by the addition of anti-TNF neutralizing antibody (Balog et al. 2002). On the other hand, using a whole blood model, Monneret et al. (2000) observed that the addition of PCT can decrease LPS-induced TNF production. The concentration of PCT, contrary to TNF, remains elevated for much longer after the onset of an infection and it was observed in one study that it peaks a few days before death in non-survivors (Dahaba et al. 2006). It can be hypothesized that the deterioration of the serum PCT before death may reflect the developing immunoparalysis. Monitoring PCT levels is readily available with a commercial immunoluminometric test and for this reason it is a very attractive biomarker, but more studies are needed to prove its utility in monitoring of immunological disturbances. However, the utility of monitoring PCT concentration in sepsis patients has been proved in several randomized clinical trials (Bouadma et al. 2010; Hochreiter et al. 2009; Jensen et al. 2008, 2011; Nobre et al. 2008; Schroeder et al. 2009; Stocker et al. 2010). It allows to optimize and reduce antibiotic use in these patients which contributes to the prevention of selection of drug-resistant pathogens. The decrease of serum PCT concentration indicates the time to cease antibiotic therapy; however, the reduction of time of antibiotic therapy varies in different centers. It should be noted that PCT concentration is an additional parameter and should always be interpreted with the overall clinical status of the patient in mind.

Disruptions in Monocyte Function

It was suggested that the total number of monocytes in peripheral blood of septic patients may be decreased due to common apoptosis of these cells. It may correlate with a favorable prognosis (Giamarellos-Bourboulis et al. 2006). Ex vivo stimulation of monocytes from septic patients by LPS, TLR agonists or whole bacteria results in endotoxin tolerance. This phenomenon was observed in humans ex vivo as a decreased secondary inflammatory response to LPS stimulation after the primary inflammatory response (Granowitz et al. 1993). Moreover, monocytes of septic patients are characterized by a down-regulation of proinflammatory cytokines, like TNF, IL-1, IL-6, IL-12 (Munoz et al. 1991), accompanied by a simultaneous increase in the production of the anti-inflammatory cytokines such as IL-10. All together, those observations suggest that in sepsis, monocytes undergo activation, but the cellular

pathways are redirected to the synthesis of anti-inflammatory mediators (Cavaillon and Adib-Conquy 2007).

Another interesting feature of monocytes from septic patients is a decreased surface expression of the HLA-DR molecule, which is a member of the major histocompatibility complex II family (Monneret et al. 2004). Low expression of this molecule indicates the immunosuppressive phase of sepsis. It is the only parameter affected by multiple sepsis mediators, and may be accepted as a highly valuable diagnostic tool. This is reflected by the correlations of HLA-DR expression and plasma concentration of IL-10, IFN- γ , glucocorticosteroids and catecholamines (Le Tulzo et al. 2004; Monneret et al. 2004). Decreased expression of HLA-DR on monocytes predicts severe course of sepsis, occurrence of secondary infections and poor prognosis in critically ill patients (Döcke et al. 2005; Monneret et al. 2006, 2008). In recent years, a growing number of studies have shown the clinical utility of this parameter, which is increasingly popular due to the simplicity of its immunocytochemical staining protocol followed by a flow cytometric analysis (Monneret et al. 2010).

Disruptions in Lymphocytes Functions

T cell lymphocytes play a key role in the anti-infectious defense as both regulators and effectors. It is not surprising that sepsis is related with several profound dysfunctions of these cells. Lymphocytes from septic patients were found to be anergic, i.e. lacking the late-type hyperreactivity in the intradermal tests. This well-known phenomenon is related with the increased vulnerability to secondary infections and mortality (Christou et al. 1995). The depressed functioning of T cells is also revealed in in vitro tests, which demonstrate a low proliferation rate following either specific (anti-CD3 antibody) or non-specific stimulation (phytohemagglutinin) (Lederer et al. 1999). Another feature of lymphocytes in septic immunoparalysis is a series of changes in the expression of surface molecules, which includes up-regulation of proteins responsible for negative signaling in the immune system, such as programmed death 1, CD47 and cytotoxic T lymphocyte antigen 4 (Bandyopadhyay et al. 2007). Accompanying down-regulation of the surface molecule expression, members of stimulatory receptors like CD3 and CD28 is also observed (Hotchkiss and Karl 2003; Venet et al. 2005).

The attention was recently directed to the role of the regulatory CD4⁺CD25⁺FoxP3⁺ T cells (Tregs) in the course of sepsis (Monneret et al. 2003). This specialized subset of helper T cells is capable of modulating the immune response by the following mechanisms: direct induction of apoptosis of cytotoxic lymphocytes (e.g. via Fas/Fas ligand), inhibition of IL-2 and TNF- α release by

other cells, and by production of anti-inflammatory cytokines, mainly IL-10 and TGF- β (Jiang and Chess 2004; Venet et al. 2008). Monneret et al. (2003) described that sepsis non-survivors had significantly increased percentage of peripheral blood CD4⁺CD25⁺ cells at 7–10 days of the disease. Authors suggested that those T cells are responsible for the development of the immunoparalysis in sepsis (Monneret et al. 2003). Later, the same group proved that the increase of the Tregs percentage is due to the decrease of the other T cells (CD4⁺CD25⁻) rather than Tregs proliferation (Venet et al. 2004).

T cells are not the only lymphocytes affected in sepsis, as the count of B cells is also reduced. Interestingly, the number of cytotoxic CD8⁺ lymphocytes and the natural killer cells was initially not reported to decrease (Hotchkiss et al. 2001). However, some later studies revealed also decrease of the CD8⁺ cytotoxic T lymphocytes (Gogos et al. 2010; Monserrat et al. 2009). The decreased number of circulating CD8⁺ T cells was correlated with the survival of sepsis. Loss of lymphocyte subpopulations in the course of sepsis occurs via programmed mechanisms of apoptosis, which can be shown by Annexin V staining (Greineder et al. 2007). According to post-mortem studies, a massive immune cell death takes place in the blood, spleen and lymphoid gut tissue (Hotchkiss et al. 2001).

Changes in Neutrophil Profile

Patients with active infectious process, especially of bacterial etiology, have increased number of immature myeloid lineage cells in their peripheral blood. However, their functional activity is not fully efficient (Taneja et al. 2008). It is worth mentioning their low expression of the chemokine receptors (e.g. CXCR2) and disturbed production of cytokines in vitro (Cummings et al. 1999; Marie et al. 1998). In contrast to other leukocytes, neutrophils are less susceptible to apoptosis leading to their accumulation. Recently, a novel mechanism of neutrophil antibacterial action by formation of neutrophil extracellular traps (NETs) was described (Fuchs et al. 2007). These traps are composed of chromatin and disintegrated granules and it would be of interest to evaluate the NETs formation during sepsis. Mobilization of active neutrophils into the circulation is helpful in eradication of pathogens; however, it contributes to the damage of tissues by the release of enzymes like proteases (Adib-Conquy and Cavaillon 2009).

Disturbances in Phagocytosis

The success of the host defense against bacterial infections is dependent on the effective phagocytosis, which forms a part

of the cellular immunity. Phagocytosis is performed mainly by neutrophils and monocytes, but the impairment of phagocytosis during sepsis is actually related to the dysfunctions in neutrophils. The study by Danikas et al. (2008) has elegantly showed that the phagocytic activity of polymorphonuclear cells (PMNs) measured at admission in severe sepsis patients who died is significantly lower than in healthy controls. However, PMNs phagocytic activity in severe sepsis patients who survived is similar to that of the corresponding healthy controls (Danikas et al. 2008). Interestingly, the phagocytic activity of the monocytes measured in the same way in both patients and healthy controls did not differ. Although the role of neutrophils in the pathogenesis of sepsis is not well known, several studies have shown the correlation between the expression of CD64, the high affinity receptor to Fc fragment of IgG, on monocytes and PMNs and the phagocytic activity (Schiff et al. 1997; Wallace et al. 1997). This might prove the close biological relation between those parameters. Analysis of the CD64 expression on monocytes by flow cytometry may be another marker of prognosis and immunological status of sepsis patients.

Coagulation and Immune Disturbances

The relations between coagulation system and the inflammatory response during sepsis are numerous and complicated. As a consequence, activation of platelets, enhancement of plasma procoagulatory activity and attenuation of anticoagulatory activity are all present in septic syndromes. During SIRS endothelium, monocytes and neutrophils are stimulated by TNF and IL-1 to produce the tissue factor (TF). The TF conjugates with coagulation factor VIIa making a TF–FVIIa complex, which activates factor X to the factor Xa. The latter converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin. Thrombin activates other coagulatory factors and the inflammatory cascade as well (Zeerleder et al. 2005). Under normal conditions, thrombin converts the protein C produced in the liver into the activated protein C (APC). This protein binds the endothelium, making a complex with its receptor and thrombomodulin. Together with its cofactor protein S, it inactivates FVa and FIIIa, thus blocking the coagulation cascade. Moreover, APC has anti-inflammatory properties. It inhibits the intracellular NF- κ B signal transduction in monocytes, production of inflammatory cytokines, neutrophils recruitment and the mast cells degranulation (Slofstra et al. 2006). Also, APC protects from endothelium “leakage” and migration of the neutrophils (Elphick et al. 2009). Septic patients are deficient of APC and its regulatory mechanisms are disturbed. It has been proved that low level of the serum APC is related with the poorer outcome (Zeng et al. 2004).

Selected Available Modulating Therapies and Potential Interventions in the Immune System

Glucocorticoids

As mentioned at the beginning, the glucocorticoids were thought to be potentially beneficial in the treatment of sepsis because of their immunosuppressive features. When SIRS was thought to be the main reason for the deaths in sepsis, it was tempting to make use of the anti-inflammatory potential of steroids. After conducting several studies, it has been definitely established that the high, immunosuppressive doses of glucocorticoids, e.g. 30 mg of methylprednisolone, do not improve the outcome in sepsis. In fact, it can worsen the prognosis by increasing susceptibility to the secondary infections (Annane et al. 2002). In spite of these results, successive studies with small dose of glucocorticoids in patients requiring catecholamine treatment were conducted. The drug of choice was hydrocortisone at a dose of 200–300 mg/24 h resulting in reduction of 28-day mortality was observed. This outcome was explained by the supplementary role of this treatment in the septic adrenal gland insufficiency (Sprung et al. 2008). However, these encouraging results were unfortunately not confirmed in a recent, big and randomized CORTICUS study. This study enrolled 500 of 800 planned patients in septic shock who required vasopressors for at least 1 h. Patients were randomized to obtain 200 mg/24 h of hydrocortisone for 5 days or placebo for 72 h since the septic shock diagnosis. No survival improvement was confirmed. The only beneficial effect of hydrocortisone treatment was a shortening of the shock phase. According to this study, small doses of glucocorticoids were recommended only in selected cases of septic shock resistant to treatment (Sprung et al. 2008).

Intravenous Immunoglobulins

Most sepsis patients have hypo-gamma-globulinemia mainly due to the low concentration of IgG and these patients were found to have worse survival prognosis (Taccone et al. 2009). Intravenous immunoglobulins (IVIg) act by neutralizing endotoxins, boosting phagocytosis and reducing the cytokine production by mononuclear cells (Negi et al. 2007). Furthermore, new mechanisms of immunomodulation by IVIg are continuously discovered, like the recent effect on the expansion of regulatory T cells (Maddur et al. 2010). Bearing in mind those features, a few studies with IVIg were performed and a little beneficial effect of IVIg on mortality could be observed (Werdan et al. 2007). Unfortunately, most of those trials involved a small group of patients and were not randomized, making them methodologically dubious. It should be noted that in

the meta-analysis IgA and IgM IVIg seemed to be more effective (Kreymann et al. 2007). Currently, there is not enough evidence to recommend the wide use of IVIg in the treatment of sepsis in adult patients (Kreymann et al. 2007; Turgeon et al. 2007).

Heparin

Apart from the anticoagulatory effects, heparin acts as an anti-inflammatory agent as well. Not only does it inhibit the release of proinflammatory cytokines, but also stops activation, adhesion and migration of leukocytes. Animal studies have proved its beneficial role in sepsis (Cornet et al. 2007). Therapeutic role of heparin in the clinical setting has been well established in the anticoagulatory prophylaxis. The anti-inflammatory action of heparin has been recently evaluated in a randomized, one-center HETRASE study that enrolled 390 sepsis patients who received 500 U/h of heparin or placebo for 7 days. Although the safety of the unfractionated heparin in sepsis patients was confirmed, the study failed to show the reduction of either 28-day mortality or the length of hospital stay (Jaimes et al. 2009).

Recombinant Human APC

The clinical trials and introduction of the human recombinant APC (rhAPC) into general ICU practice was initially based on the idea of its supplementation due to reduced APC concentration in sepsis. First, the positive effects of rhAPC were assigned mostly to its anticoagulant activities. However, growing body of evidence supports the role of APC as an immunomodulating agent. As we mentioned earlier, APC has an anti-inflammatory potential as it reduces thrombin generation which has strong proinflammatory properties via the protease-activated receptors (PAR), especially PAR-1 and PAR-2 on platelets and endothelium (Ramachandran and Hollenberg 2008). Activation of PAR-1 induces production of chemokines and adhesion molecules (Neyrinck et al. 2009). Another mechanism of an anti-inflammatory action of APC is mediated by the endothelial protein C receptor (EPCR) expressed by leukocytes (monocytes, NK cells, neutrophils) (Joyce et al. 2004) and endothelium (Feistritzer et al. 2006). Ligation of the EPCR on neutrophils and eosinophils decreases chemotactic response and infiltration of tissues (Galley et al. 2008). Also, APC inhibits production of proinflammatory cytokines (TNF, IL-1 β , IL-6, IL-8) by LPS-stimulated monocytes (Okajima 2001). APC has additionally anti-apoptotic properties. In the presence of the EPCR, the PAR-1 activation on monocytes inhibits their apoptosis. It was shown that monocytes from rhAPC-treated patients had low Bax/Bcl-2 proteins ratio, which is

responsible for the anti-apoptotic state (Bilbault et al. 2007). This effect may support the host response and be beneficial as monocytes in septic patients show high apoptosis rate what contributes to the immunoparalysis, as discussed earlier.

APC have made huge, but not long-lasting career as the first US Food and Drug Administration approved biological drug in sepsis. This protein was the only anti-inflammatory agent that seemed to prove its effectiveness in the treatment of septic shock (Dellinger et al. 2008). A double-blind, randomized, multi-center study evaluated the efficiency of rhAPC in severe sepsis patients. The 96-h-long continuous infusion of rhAPC at a dose of 24 µg/kg showed to be the most effective in decreasing the concentration of D-dimers and IL-6 (Bernard et al. 2001a). Following the study, a third-phase PROWESS trial including 1,690 patients successfully proved the reduction of relative and absolute death risk by 6.1 and 19.4%, respectively (Bernard et al. 2001b). Patients who received rhAPC showed a faster hemodynamic and respiratory improvement with fewer hematological complications. However, patients with low APACHE II score and only one organ dysfunction did not benefit from the treatment (Hodder et al. 2009). Despite these encouraging results, the most recent PROWESS-SHOCK trial, which was a continuation of the PROWESS study, did not confirm the positive therapeutic effects on the morality of sepsis patients. Due to the negative results of the risk–benefit analysis (28-day mortality in rhAPC group was 26.4 vs. 24.2% in the placebo group; Mullard 2011), the company decided to withdraw the rhAPC from market. Whether this potentially immunomodulating agent will receive another chance in immune-status-guided studies remains a question.

GM-CSF and Other Cytokines

In light of the recent research results indicating depression of immune mechanisms, it might be advantageous to apply growth factors in order to restore the host immune functions. In fact, some trials to restore monocyte functions with proteins like IFN- γ , granulocyte colony-stimulating factor (G-CSF), GM-CSF were conducted. Randomized studies on IFN- γ levels in critically ill patients have revealed reduction of both secondary infections and mortality, but only in certain subgroups (Dries et al. 1994). Later study by Döcke et al. (1997) proved positive effects of treatment with IFN- γ on monocyte HLA-DR expression and recovery from infection by septic patients. Studies with GM-CSF in ten patients with acute respiratory distress syndrome (ARDS) have showed improvement of gas

exchange parameters, faster ARDS symptoms regression and recovery of alveolar leukocytes (Presneill et al. 2002). Other second-phase GM-CSF study has revealed an increase in leukocyte numbers and HLA-DR expression on monocytes, as well as more effective anti-infectious defense (Rosenbloom et al. 2005). Above studies have not shown an impact of GM-CSF on mortality, but it should be mentioned that immune system status was not evaluated at the time of the growth factor implementation. Similar results were obtained for G-CSF. In vitro and animal studies have encouraged researches to develop clinical studies. Early study on a group of ten patients with ventilator-dependent pneumonia has resulted in mortality rate lower than predicted (Hustinx et al. 1998). Unfortunately, a randomized double-blind, placebo controlled study has not shown any improvements of outcome in septic shock patients (Stephend et al. 2008). A recent meta-analysis of studies with both G-CSF and GM-CSF did not reveal reduction of mortality of septic patients either. However, studies that were analyzed were not coherent and their methodology was not satisfactory (Bo et al. 2011). Stratification of patients by the immune disturbances seems to be crucial to the introduction of specific immunomodulatory therapies.

Statins

Statins are well-known, popular and widely used inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme-A reductase, the key enzyme of the mevalonate pathway, which in turn inhibits early biosynthesis of cholesterol. In numerous trials statins have shown hypolipidemic activity and reduction of serum C-reactive protein (Arnaud et al. 2005; Ridker et al. 1999). These results resulted in additional research on the impact of statins on immune system modulation. As a consequence, statins were found to be able to inhibit migration and activation of neutrophils and monocytes, as well as capable of reducing the synthesis of protein and cytokines in the acute phase (Cortellare et al. 2002; Krysiak et al. 2003). Furthermore, they improve platelet functions, promote fibrinolysis, induce endothelial NOS expression and increase the number of circulating endothelial progenitors (Laufs et al. 1998; Llevadot et al. 2001). Altogether, these pleiotropic features seem to be a good reason for trials of the application of statins in sepsis. Thus, after interesting outcomes of animal studies (Ando et al. 2000; Yasuda et al. 2006), clinical studies have been performed. Prospective observational study of 11,490 patients with arteriosclerosis subject to either statin therapy or no treatment showed a decreased mortality caused by infections in the statin group (4.1 vs. 0.9%) (Almog et al. 2007). In another study on 361 patients hospitalized due to

infection, severe sepsis developed in 19% of non-statin patients, while only 2.4% of those administered statin developed severe sepsis (Almog et al. 2004). Among 2,036 patients admitted with suspicion of infection, the mortality in statin group was 1.9 and 4.4% in the non-statin one (Donnino et al. 2007). Recently, a meta-analysis of patients with sepsis or severe infections also showed reduction of the mortality among patients who were receiving statins (Janda et al. 2010). Although the quoted studies are highly encouraging, we are far from the intentional use of statins in the therapy of sepsis. Some problems may arise from the adverse effects of these drugs, including hepatotoxicity and myolysis. Further clinical studies testing safety and efficiency of statins are necessary. At present, the only recommendation can be no interference in statin therapy if the patient received it before developing sepsis (Gao et al. 2008), although this strategy may not bring benefit in survival (Terblanche et al. 2001).

Macrolides

Macrolides are old and well-known group of antibacterial agents with activity against Gram-positive and atypical bacteria. However, observations on patients with chronic inflammatory lung diseases like diffuse panbronchitis and cystic fibrosis suggested additional mechanism of their action which is not related to antimicrobial activity (Schultz 2005). Thus, immunomodulatory mechanisms have been suggested. Indeed macrolides were shown to modulate both innate and adaptive immunity. Patients with community-acquired pneumonia treated with clarithromycin had decreased serum concentration of IL-6, but levels of IL-10 and IFN- γ were elevated (Demartini et al. 2004). In vitro experiments with stimulated monocytes revealed that inhibition of NF- κ B pathway is responsible for the changes of cytokines production (Kikuchi et al. 2002). Clarithromycin also affects T lymphocytes leading to a shift towards Th₁ response (Williams et al. 2005). In the light of these findings it was tempting to evaluate the effect of macrolides on the course of sepsis. A retrospective-cohort study on 237 patients with sepsis showed that macrolide use was associated with significant reduction of 90-day mortality (Restrepo et al. 2009). Noteworthy, most of the pathogens were not macrolide-susceptible, what supports the immunomodulatory hypothesis. Also, results of the only one available prospective study with clarithromycin in patients with sepsis or ventilator-associated pneumonia are encouraging. Patients who received intravenously clarithromycin presented better resolution of infection and shorter time of weaning from mechanical ventilation (Giamarellos-Bourboulis et al. 2008). These results seem encouraging and require further evaluation in clinical trials and basic studies.

High-Volume Hemofiltration in Sepsis

The most recent trials of therapeutic blocking of a single inflammatory mediator in the developed septic shock have not brought desired effects (Honore et al. 2000). High-volume hemofiltration (HVHF) is a procedure that can be applied in the treatment of septic shock and its use is justified by a growing body of evidence. Early studies on HVHF performed on septic shock patients with acute kidney injury brought significant reduction of mortality among patients who underwent such renal replacement therapy (Cole et al. 2001). This beneficial effect of hemofiltration on the course and outcome of sepsis treatment is related to partial elimination of some shock mediators, such as cytokines (IL-10, IL-6, G-CSF), complement components (C3a, C5a) and other non-protein mediators like leukotrienes and bacterial toxins. Studies have showed the advantage of using the replacement dose of 6 l/h in HVHF over the classic dose of 1 l/h (Ronco et al. 2000). Reduction of plasma concentration of cytokines (C3a, C5a, IL-10), hemodynamic improvement, reduced need for catecholamines and decreased mortality have all been observed. Cole et al. (2001) have showed that performing HVHF with the filtration dose of 35 ml/kg/h resulted in 20% increase in survival. Lately, the strategy of pulse HVHF was tested with promising results (Peng et al. 2010). It is conducted in cycles of 6 h of 80 ml/kg/h of HVHF followed by 18 h of the standard filtration. This preliminary study showed clearance of some sepsis mediators like TNF- α , IL-1, IL-4, IL-6 and IL-10. It has been suggested that the elimination of circulating mediators is an effect of both filtration and absorption to the filter surface (Ronco et al. 2002, 2003). Three theories were proposed to explain the effectiveness of HVHF. According to the first one, the reduction in the highest peak blood concentration of cytokines in the early phase of sepsis can inhibit the inflammatory cascade and the homeostasis disturbance (Honore et al. 2007). Second, the concept of the immunomodulatory threshold assumes that removal of blood cytokines leads to the shift of tissue-concentrated cytokines into the circulation (Di Carlo and Alexander 2005). This may explain why not all studies have been able to show changes in the circulating levels of cytokines, although obtaining clinical improvement. The last hypothesis is based on the observation that HVHF can increase the lymphatic plasma flow even 40-fold, causing removal of large amount of cytokines from tissues (Di Carlo and Alexander 2005). Despite promising results, insufficient evidence is available concerning adequate strategies, doses and best time to start HVHF to recommend it in septic patients without septic acute kidney injury. However, HVHF at a dose of 35 ml/kg/h should be applied in septic patient requiring renal replacement therapies (Honore et al. 2009).

Conclusion

Currently, the new strategies reviewed above (except rhAPC for a few years) are not systematically used in sepsis treatment on a large scale. The emphasis is routinely put on the early diagnosis of sepsis, especially outside ICU, as it enables implementation of the recommended treatment, eventually reducing morality. Releasing and introducing international guidelines for the diagnosis and treatment of severe sepsis in 2004 has led to marked improvements in the outcomes and overall lower mortality. After 4 years the guidelines were updated, but no new drugs or procedures have been introduced. Changes concern more precise distinction between “recommendations” and “suggestions” based on the “risk–benefit analysis”.

Based on the previously released “ventilatory bundles” and their beneficial impact on the course of mechanically ventilated patients, septic bundles were introduced. “Sepsis bundle” is a set of treatment procedures, which implemented together are more effective than in separation. The choice of the elements of the bundles is based on strong evidence and the implementation of the bundles should be generally accepted. In the treatment of sepsis, two bundles have been set: the Sepsis Resuscitation Bundle and the Sepsis Management Bundle (Townsend et al. 2008) (Table 2).

Although the immune reaction in sepsis is generalized and clinically similar in most patients, some papers bring evidence that sepsis immunopathology differs between patients. It was observed that infections by Gram-positive pathogens lead to higher decrease in CD4⁺, CD8⁺, NK cells and total T lymphocytes than Gram-negative sepsis. Also, the pattern of immune recovery in Gram-positive sepsis was different and slower than in Gram-negative infections (Holub et al. 2003). On the other hand, a recent study on 505 septic patients showed differences in the circulating immune cells depending on the type of the primary infection (Gogos et al. 2010). Main differences were found by comparing sepsis severity in these infections, i.e. septic shock due to pneumonia was characterized by lower counts of NK cells, CD4⁺, CD8⁺ and B cells than sepsis due to intraabdominal infections. Septic shock due to intraabdominal infection caused lower counts of HLA-DR⁺ monocytes, CD8⁺ and CD4⁺ lymphocytes than sepsis of the same origin.

This heterogeneity of the immune system response during sepsis provides an additional argument for careful monitoring of the immune response during therapy. It can also provide a possible explanation why the implementation of the resuscitation bundles, but not the therapeutic bundles is an independent predictor of survival (Castellanos-Ortega et al. 2010). The resuscitation bundles are beneficial in each type of sepsis, as they are aimed at maintaining perfusion and early antibacterial therapy, while the therapeutic bundles involve the use of

Table 2 The elements of sepsis bundles according to the Survive Sepsis Campaign 2008 (Townsend et al. 2008)

The Sepsis Resuscitation Bundle should be started immediately and done within 6 h. It includes:
Measure serum lactate
Obtain blood cultures prior to antibiotic administration
Administer broad-spectrum antibiotic <i>within 3 h of ICU admission and within 1 h of non-ICU admission</i>
In the event of hypotension and/or serum lactate > 4 mmol/l:
(a) Deliver an initial minimum of 20 ml/kg of crystalloid or an equivalent
(b) Apply vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure >65 mmHg
In the event of persistent hypotension despite fluid resuscitation (septic shock) and/or lactate >4 mmol/L:
(a) Achieve a central venous pressure of ≥8 mmHg
(b) Achieve a central venous oxygen saturation (ScvO ₂) ≥70% or mixed venous oxygen saturation (SvO ₂) ≥65%
Sepsis management bundle should be started and done within the first 24 h:
Administer low-dose steroids for septic shock in accordance with a standardized ICU policy. If not administered, document why the patient did not qualify for low-dose steroids based upon the standardized protocol
Administer rhAPC in accordance with a standardized ICU policy. If not administered, document why the patient did not qualify for rhAPC
Maintain glucose control ≥70, but ≤150 mg/dl
Maintain a median inspiratory plateau pressure <30 cm H ₂ O for mechanically ventilated patients
Recommendation for rhAPC will be invalid, due to its withdrawal from the clinical use

immunomodulating agents, glucocorticosteroids and APC which might be helpful only in selected patients.

The implementation of the new immunomodulatory therapies should also be supported by strong-evidence results. However, in special cases, some of the reviewed treatment possibilities can be used as the salvage therapy. Clearly, continuous efforts should be made in the field of sepsis pathophysiology and the development of new therapeutic strategies.

In this review we have analyzed the studies of human sepsis. Although many interesting results have been obtained from animal models of sepsis, there are many differences between animal and human sepsis and the transition of some therapeutic regimens from murine models to clinical setting have failed. Therefore, we chose to focus on the results of the research on human sepsis and clinical studies.

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