Published in final edited form as:

Shock. 2016 March ; 45(3): 282–291. doi:10.1097/SHK.0000000000000495.

Immune cell phenotype and function in sepsis

Thomas Rimmelé[1], **Didier Payen**[2], **Vincenzo Cantaluppi**[3], **John Marshall**[4], **Hernando Gomez**[5], **Alonso Gomez**[7,8], **Patrick Murray**[6], and **John A. Kellum**[5] **On behalf of the ADQI XIV Workgroup**[9]

[1]Anesthesiology and Critical Care Medicine, Edouard Herriot Hospital, Hospices Civils de Lyon, University Claude Bernard Lyon 1, Lyon, France [2]Department of Anesthesiology & Critical Care and UMR INSERM 1160; Lariboisière Hospital, AP-HP and University Paris 7, Sorbonne Paris Cité, Paris, France [3]Nephrology, Dialysis and Kidney Transplantation Unit, Department of Medical Sciences, "Citta' della Salute e della Scienza di Torino- Molinette" University Hospital, Torino, Italy [4]Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, Ontario Canada [5]Center for Critical Care Nephrology; The CRISMA (Clinical Research, Investigation, and Systems Modeling of Acute Illness) Center, Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA [6]University College Dublin, Dublin, Ireland [7]Academia Colombiana de Medicina Critica (ACOMEC) [8]Division of Critical Care Medicine, Clínica Palermo, Bogotá, Colombia

Abstract

Cells of the innate and adaptive immune systems play a critical role in the host response to sepsis. Moreover, their accessibility for sampling and their capacity to respond dynamically to an acute threat increases the possibility that leukocytes might serve as a measure of a systemic state of altered responsiveness in sepsis.

The working group of the 14th Acute Dialysis Quality Initiative (ADQI) conference sought to obtain consensus on the characteristic functional and phenotypic changes in cells of the innate and adaptive immune system in the setting of sepsis. Techniques for the study of circulating leukocytes were also reviewed and the impact on cellular phenotypes and leukocyte function of non extracorporeal treatments and extracorporeal blood purification therapies proposed for sepsis was analyzed.

A large number of alterations in the expression of distinct neutrophil and monocyte surface markers have been reported in septic patients. The most consistent alteration seen in septic neutrophils is their activation of a survival program that resists apoptotic death. Reduced expression of HLA-DR is a characteristic finding on septic monocytes but monocyte antimicrobial

Corresponding Author: Thomas Rimmelé, MD, Edouard Herriot Hospital, Hospices Civils de Lyon, Place d'Arsonval, 69003 Lyon, France, th.rimmele@gmail.com.

[9]A complete list of authors is provided in Appendix 1.

Authors' contributions: TR, DP, VC, and JM created the first draft of this manuscript, develop subsequent drafts and approved the final manuscript. JK, HG, AG and PM all contributed to the pre-conference and post-conference e-mail discussions on this review and reviewed and edited the final version of this manuscript. In addition, all contributed to the group breakout sessions during the ADQI XIV conference. All authors (appendix 1) contributed to group discussion and consensus.

Declaration of interest: TR, VC, DP, JM and HG have no disclosures.

function does not appear to be significantly altered in sepsis. Regarding adaptive immunity, sepsis-induced apoptosis leads to lymphopenia in patients with septic shock and it involves all types of T cells (CD4, CD8 and Natural Killer) except T regulatory cells, thus favoring immunosuppression. Finally, numerous promising therapies targeting the host immune response to sepsis are under investigation. These potential treatments can have an effect on the number of immune cells, the proportion of cell subtypes and the cell function.

Introduction

Sepsis is a descriptive term for a common disorder characterized by a broad and complex set of cellular changes evoked in response to infection or other signatures of danger (1). These changes are elicited by the engagement of conserved pattern recognition receptors including toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I helicases, and C-type lectin receptors expressed on most cell types (2). They are ultimately effected through the expression or inhibition of a large number of immune and metabolic genes, and through post-translational changes in key intracellular proteins involved in signaling and transcriptional regulation. The extent of this response is staggering. The injection of a single bolus of endotoxin into a healthy human volunteer results in the differential expression of more than 3700 transcripts in circulating leukocytes, along with an array of changes in immune responsiveness, metabolism, and circulating inflammatory mediators (3). Moreover manipulation of any of more than 130 different molecular species can attenuate the response, and enhance survival in a murine endotoxemia model (4).

Cells of the innate and adaptive immune systems play a critical role in the host response to infection, and therefore in the syndrome of sepsis. Moreover their accessibility for sampling and their capacity to respond dynamically to an acute threat raises the possibility that leukocytes might serve as a measure of a systemic state of altered responsiveness in sepsis.

Methods

Complete methods are available in the companion article to this series. (Editor please insert appropriate citation: this is the paper entitled - Acute Dialysis Quality Initiative (ADQI) XIV Sepsis Phenotypes and Targets for Blood Purification in Sepsis: The Bogotá Consensus) Briefly, we assembled a group of international experts with distinct clinical and scientific backgrounds; this group included physicians, specialists in critical care, anesthesiology, nephrology, surgery and emergency medicine, and basic scientists with expertise in biology and physiology, who were recruited based on their expertise in sepsis and organ dysfunction. The group consisted of 23 international experts from 5 continents. A set of questions was generated through mutual agreement and we sought evidence to answer each question by searching the Cochrane Controlled Trials Register, the Cochrane Library, MEDLINE, and EMBASE from 1966 to present. Search terms for question regarding epithelial dysfunction are provided in Appendix 2. Finally we reviewed the evidence with the group and used the Delphi method to achieve consensus.

Results

Based on literature review and consensus among the workgroup members, the following key questions were considered:

1. What are the characteristic changes in neutrophil and monocyte function in sepsis?
2. What are the characteristic functional and phenotypic changes in cells of the adaptive immune system in sepsis?
3. What techniques are informative for the study of circulating cells in sepsis? Can immune cells serve as “biopsy”?
4. Can extracorporeal blood purification therapies and non extracorporeal treatments alter cellular phenotypes and/or modify leukocyte function?

What are the characteristic changes in neutrophil and monocyte function in sepsis?

The predominant circulating cells of the innate immune system – the polymorphonuclear neutrophil (PMN) and the monocyte – are phagocytic cells derived from common bone marrow precursor cells (Figure 1) (5). Their fate once in the circulation differs. Neutrophils circulate for hours to days before undergoing apoptosis and being removed by fixed tissue phagocytes (6,7). Monocytes, on the other hand, circulate for several days, then pass into the tissues where they mature into macrophages and dendritic cells (8), and so play a key role in the regulation of both innate and adaptive immunity.

Microarray studies of human volunteers challenged with intravenous endotoxin reveal differential expression of more than 2000 transcripts in the neutrophil population, with particular upregulation of genes involved in inflammation and the inhibition of apoptosis (9); the response is similar to that seen following multiple trauma (10,11). In neutrophils from septic pediatric patients, genes related to mitochondrial dysfunction and to redox pathway-related signaling are maximally upregulated (12). In contrast, peripheral blood mononuclear cells (including both monocytes and lymphocytes) reveal down-regulation of genes involved in an inflammatory response, and increased expression of genes involved in apoptosis (13). A number of alterations in the expression of distinct cell surface markers have been described in septic patients (Table 1).

While the transcriptional profile of the septic neutrophil or monocyte is fundamentally altered, the impact of these changes on functional activity is more modest.

In response to experimental inflammatory stimuli, neutrophils exhibit a pattern of responses that serve to direct the cells towards a focus of injury or infection (14). Reciprocal upregulation of β2 integrins and L-selectin on the neutrophil surface and their cognate ligands on the endothelial cell result in margination and the egress of the neutrophil from the vasculature into the tissues. Locally active antibacterial defenses that are activated include the release of granule constituents such as elastase, enhanced generation of reactive oxygen intermediates, and microbial phagocytosis and intracellular killing. In addition, the neutrophil can extrude its DNA to create neutrophil extracellular traps (NETs) that serve to enmesh bacteria and to activate local coagulation mechanisms (15). Enhanced host defense

capacity is supported by the ability of the neutrophil to subvert its constitutive tendency to undergo apoptosis, and instead to activate anti-apoptotic signaling cascades that promote sustained cell survival (16).

Although there is animal evidence to suggest impaired localization of neutrophils at the site of an inflammatory challenge, the initial capacity of these cells to migrate appropriately does not seem to be impaired. Ahmed and colleagues, for example, used skin windows in patients with sepsis to study neutrophil migration into an inflammatory focus. They reported a reduction in the capacity of the neutrophil to migrate from the circulation, but showed that phagocytic and bactericidal capacity was increased when compared to healthy controls, and that oxidative burst capacity was intact (17). Others have shown that whereas basal activation of the neutrophil is enhanced in sepsis, its capacity to respond to a de novo stimulus is blunted (18).

One of the most consistent and profound alterations seen in septic neutrophils is their activation of a survival program that resists the constitutive tendency of the neutrophil to die an apoptotic death following its release from the bone marrow. Whereas 50% of resting neutrophils will show the morphologic changes of apoptosis following 24 hours of *in vitro* culture, the corresponding rates for septic neutrophils are only 5 to 10% (19). De novo gene expression is necessary for prolonged neutrophil survival including interleukin-1β (20) and PBEF/Nampt – a protein that is the rate-limiting step in a salvage pathway of NAD biosynthesis (21).

Reduced expression of the major histocompatibility antigen HLA-DR is a characteristic finding on septic monocytes (22). Its persistence correlates with an increased risk of infectious complications and death (23), and HLA-DR levels have been promoted as a potential biomarker to guide immune-enhancing therapy in sepsis (24,25). Monocyte antimicrobial function does not appear to be significantly altered in sepsis, however monocyte survival, like that of the neutrophil, is prolonged (26).

Recent evidence suggests that the immature neutrophils seen in the circulation during sepsis can undergo differentiation to become monocytic cells (27), underlining the plasticity of myeloid cell responses to an acute threat, and the challenges of ascribing distinct biologic responses to discrete cellular phenotypes in sepsis. Two alterations in cellular phenotype merit special mention.

Myeloid-derived suppressor cells (MDSCs) are circulating $CD34^+$ $CD11b^+$ myeloid precursors that are capable of inhibiting adaptive immune responses, particularly T cell activation (28,29). Functionally they are characterized by an ability to generate reactive oxygen species and arginase. MDSCs are protective in animal models of sepsis, attenuating cytokine production, enhancing microbial clearance, and reducing mortality (30,31). Their role in human sepsis is unclear. Their presence is associated with lymphopenia and increased mortality (32).

Macrophages also comprise an heterogeneous population of cells that can become polarized into distinct subpopulations. M1 macrophages are activated and support pro-inflammatory

functions, while M2 macrophages exert anti-inflammatory and reparative activity (33). The extent to which the balance between these two populations is altered in sepsis is unknown.

Defects in PMN numbers or function result in poor clinical outcomes (34). But activated PMNs are non-selective in their targets. Bystander tissue injury secondary to PMN activation has been described in the lung, kidney, liver, and gastrointestinal tract (35) in animal models, and attributed to the release of reactive oxygen species and proteases such as elastase. It has been reported in a murine model of cecal ligation and puncture (CLP) that depletion of neutrophils prior to the infectious challenge increases mortality, whereas neutrophil depletion 12 hours after the induction of peritonitis results in enhanced bacterial clearance and improved survival (36). The induction of ischemia-reperfusion injury by temporary occlusion of the superior mesenteric artery in the rat induces massive pulmonary neutrophilia and a significant mortality. Neutrophil numbers can be reduced, and survival enhanced, by the infusion of heat-killed bacteria into the lung – a stimulus that triggers neutrophil apoptosis.

Sepsis in humans is associated with profound inhibition of rates of constitutive neutrophil apoptosis (19,37). Moreover neutrophils harvested from patients who have sustained sepsis or multiple trauma can induce the apoptotic death of other cells through the dephosphorylation of epithelial cell caspase-8 (38). Expression of PDL-1 is increased on septic neutrophils, and through interactions with lymphocyte PD-1, can induce the apoptotic death of CD4+ lymphocytes (39).

What are the characteristic functional and phenotypic changes in cells of the adaptive immune system in sepsis?

The functional and phenotypic changes of adaptive immune system in sepsis can be seen as follows: 1- the cooperation between innate and adaptive immune system; 2-the mechanism and phenotypic modifications observed over time.

The adaptive immune system is composed of T and B lymphocytes and employs antigen receptors that are not encoded in the germ line but are generated *de novo* in each organism. This system is then highly specific via the microbial pattern recognition receptors of the innate immune system (PRRs). Their contribution in activation of specific arm of adaptive immune response is not fully understood. One intrinsic innate immune recognition that links to adaptive immune cells is called “antigen presenting cell” (APC). After processing microbial antigens, APCs present the antigen to naïve T cells via the major histocompatibility complex (MHC) molecules. In addition to MHC class II molecules, T cell activation requires the co-stimulatory signals and cytokines environment. The co-engagement of the B cell receptor with one of the several innate immune signaling pathways results in a profound enhancement of antibody response.

In total, whatever the pathogen detected, the direct activation of dendritic cells (DC) by PRR activate T cell responses. Immune patterns are changing along time after the initial septic injury according to several scenarios. One of the most convincing scenarios separates the immediate from the delayed immune response. The immediate response is characterized by “an inflammatory storm” with the release of both pro- and anti-inflammatory mediators, the

cell activation and cooperation to eradicate infection. Although necessary, such a storm may overcome the tissue/organ/organism tolerance capability, which is partly dependent on the presence of co-morbidity and ageing. This "storm" may lead to organ failure and death for at least 50 % of the early death during septic shock (40,41). This early phase remains a great challenge for therapeutic innovation, with a delay from the onset of sepsis and the initiation of treatment difficult to reduce, despite the well-described basic molecular aspects (42). The adaptive immunity modifications during this phase (*i.e* 5 to 7 days) have received little attention in clinical situation. The lymphocyte count associated or not with the ratio polymorphonuclear cells/lymphocyte count had been reported. The pioneer work from Zahorec et al. had shown that both surgical stress, systemic inflammation and sepsis induced large modifications in leucocyte count, with neutrophilia associated with lymphopenia in correlation with the severity of clinical course (43). The ratio neutrophil/lymphocyte as a stress factor was used to predict the severity and/or outcome. In 2002, a marked lymphopenia at the onset of sepsis was more pronounced in survivors on days 2, 3, 5, and 7 than in non survivors (44). Compared with healthy subject, the reduction was a 50% in CD4 and CD8 T cell absolute or relative count in non-survivors and -75% in survivors. At day 1, 2, and 5, the % of both T-cell subpopulations was about twice as high in non-survivors compared to survivors with a CD4/CD8 T cell ratio significantly elevated above normal range (1.95±0.21) on day 1 and 2. This ratio went back to normal value on day 14, with no difference between survivors and non-survivors. The subsets of lymphocytes have relatively been poorly reported in human sepsis. In 32 septic patients having purulent meningitis (45), a drop in absolute number of total T-lymphocytes was observed at admission, and recovered rapidly after 7 days. This lymphopenia concerned both the CD4, CD8 and NK cells and was more pronounced with Gram + than Gram – infection. No correlation between severity scores and lymphocyte count has been observed. Compared to healthy volunteers, the circulating B lymphocyte count was not significantly modified in infected patients, but the reduced numbers of circulating lymphocyte B correlated well with the incidence of nosocomial infection. The pivotal role of B cells in both adaptive and innate immune response received recently more attention. Mice devoid of an adaptive immune system demonstrated an attenuated inflammatory response to bacterial sepsis (46), which was shown to be linked to B cell deficiency. Recently, a functional and phenotypic B lymphocytes alteration have been reported in 52 septic shock patients, suggesting strongly other B-cell functions beyond the production of antibodies (47). Marker of activation and regulation of B-cell at admission appeared associated to good outcome, with significantly lower markers of apoptosis in survivors than in non-survivors. In 50 severe sepsis or septic shock patients, 42% died within the first 72 hours. No significant changes in CD3+, CD4+, CD8+ T cell and B cell count at day 1, day 3, day 10 between survivors and non survivors were observed (48). Only NK cell were increased in non-survivors at day 1 with no mechanistic explanation.

After the early phase, the immune cellular phenotype of survivors is shifting towards immune-depression status. Several patterns of immune profile have been theorized: 1- ageing patients particularly with numerous comorbidities may develop a limited initial hyper-inflammatory phase rapidly followed by an immunosuppression profile; 2- patients having an early hyper-inflammation rapidly developing a transitory immunosuppression

until they recover; 3- some not all patients elicited a sustained immunosuppression status that exposed the patients to secondary infections, reactivation of virus multiplications, or unresolved initial infection. This 3rd scenario might have cycling phases along time between hyper-inflammatory and hypo-inflammatory states (49). Although the determinants of such different patterns have not been totally elucidated, the post-aggressive immunodepression (PAID), especially for adaptive immunity, has been well characterized in many inflammatory circumstances. It may concern trauma, ischemia-reperfusion syndrome, major surgery and acute brain injury. This evolution seems to obey to a stereotype that is more pronounced in septic patients (22). Various clinical evidence fits well with this theory: patients with sepsis and trauma had lost the delayed hypersensitivity response type, a finding that correlated with mortality (50,51); reactivation of latent viruses including cytomegalovirus and herpes simplex virus occurs in patients having sepsis (52); secondary infection with relatively avirulent pathogens can occur (22). Blood studies on patients during this late phase of sepsis show an increase in proportion of regulatory T cell (immunosuppressor) and an increase in the production of PD-1 or the ligand PD-1 L1 (53,54). Looking at the subtypes of adaptive immune cells, absolute number of all types of T cells was reduced in septic patients or septic shock, except the T regulatory cells (circulating CD4+CD25+ Treg cells) (55), which did not relate with cell activation status (53). Such observations on circulating cells have also been observed in fresh postmortem human tissue. Adaptive immune cells upregulated the expression of selected inhibitory receptors such as PD-1, with an expansion of T cell suppressor and myeloid derived suppressor cells in different organ tissues (56). The clinical consequence of this delayed and sustained PAID have recently been published in a retrospective monocentric study (57). Lymphopenia observed at the onset of sepsis was both present in 28-day survivors and in non survivors with no difference between the 2 groups. By day 4, the median absolute lymphocyte count was higher in survivors than in non-survivors and was independently associated with 28-day survival and increased development of secondary infections.

Various mechanisms may account for such adaptive immunodepression. The key discovery was the demonstration that apoptosis causes a marked depletion in CD4, CD8 T cells, B cells in various organs of patients dying from septic shock, with no difference related to age and type of microorganisms. Sepsis-induced apoptosis may result both from death receptor pathways and from metabolic pathways (49,58,59). The “immune metabolism” concerns the role of metabolic pathways that may regulate immune response, not only by substrate availability but also by signaling pathways elicited by the metabolites (60). Energy to perform immune function results from ATP production at the mitochondrial level, using two major substrates and pathways: first, the glucose via the glycolysis converting glucose to pyruvate entering the tricarboxylic acid (TCA) cycle (61,62); second, the oxidation of fatty acids as a source for specific lymphocyte T cell subsets (60). Then metabolic changes might be the cause or the consequence of changes in immune function. In the contrary to innate immune cells, T cells have the ability to extensively and rapidly proliferate upon activation, a process that engages a Warburg metabolism (high rate of aerobic glycolysis). Activated T cells engage oxidative phosphorylation and glycolysis, producing pyruvate excreted as lactate, with some pyruvate entering the TCA cycle. Metabolizing glucose in the pentose phosphate pathways can yield both nucleotides and NADPH for lipid synthesis and for

reactive oxygen species synthesis. Memory T cells and Treg cells use fatty acid oxidation for survival and to support functions (60). When a naïve T cell recognizes an antigen, it undergoes a developmental program for rapid growth, proliferation, and acquisition of specialized function that requires metabolic reprogramming. Although not demonstrated in clinical context, it is reasonable to hypothesize that changes in metabolic pathways can influence the development and activity of various T cell subsets, as suggested by the proposed tight control of glycemia (63,64).

A key question to solve is to know if such a commonly observed induced-PAID during sepsis is an adaptive "normal" response after the acute phase or if PAID is a failure of the immune system that has to be treated. The concept of a defense strategy to tolerate a pathogen's presence and to limit the negative impact of infections on host fitness has to be considered (65). As an example, exposure to low level of LPS injection can protect from lethal doses of LPS through the induction of negative regulators of LPS signaling and selective suppression, such as the LPS-inducible genes (66). However, tolerance to a pathogen could be protective for another pathogens, but can also be incompatible with the tolerance to other pathogens.

What techniques are informative for the study of circulating cells in sepsis? Can immune cells serve as "biopsy"?

Different cell and molecular biology techniques are currently available to investigate the immune status of patients with sepsis. However, previous studies focused on the role of circulating mediators as indicators of mortality and/or multiple organ failures. C Reactive Protein (CRP) and Procalcitonin (PCT) mirror both microbiological status and host response: since they are indirect biomarkers of infection, their sensitivity and specificity may vary in different disease states. In particular, PCT may be elevated in systemic inflammatory response not associated with sepsis and immediately after trauma and surgery. However, these molecules are widely used in clinical practice to monitor infection and response to antibiotic therapy (67,68).

ELISA was used to evaluate plasma levels of molecules involved in inflammation and apoptosis, describing their association with mortality and illness scores. Presepsin is cleaved from the monocyte-specific CD14 receptor complex after binding with LPS and it has been shown to discriminate sepsis severity (69). Angiopoietin (Ang)-2 is an endothelium-specific growth factor regulated by pro-inflammatory stimuli able to increase vascular leakage. Serum Ang-2 levels are increased during sepsis and associated with disease severity (70). Soluble CD40 Ligand (sCD40L) shows pro-thrombotic and pro-inflammatory properties after binding to its cell surface receptor CD40. Circulating sCD40L levels are significantly higher in septic patients than in controls and in non-survivors (71). Huttunen et al. evaluated the prognostic value of apoptosis markers such as soluble Fas (sFas), Fas ligand (FasL) and sFas/FasL ratio in patients with bacteremia, describing the direct association between these mediators and a high SOFA score (72).

Fluorescence-activated cell sorting (FACS) is a type of flow cytometry commonly used for immune status evaluation. FACS allows the simultaneous determination of several antigens stained with different fluorophores and it can be used as a first step analysis to determine the

count of specific immune cells (leukocyte typing). Multivariate Cox regression analysis in septic patients showed that CD56+ NK T cell count was associated with an increased risk of early mortality (73). Specific surface antigen staining can identify T helper 1 or 2 polarization and lymphocyte subsets. The relative increase of circulating CD4+CD25+Foxp3+ T regulatory cells (Tregs) may play a role in lymphocyte anergy and sepsis-associated immunoparalysis (55,74). Other studies showed that the decline of T cell-mediated immunity during sepsis is associated with reduced CD8+ T memory cell counts (75). FACS can also be used to evaluate the percentage of non immune cells such as CD34+CD133+KDR+ endothelial progenitor cells that are significantly higher in septic patients than in controls, identifying microvascular injury (76).

As a second step, FACS can identify the surface expression of molecules that indicate immune cell activation or anergy (Figure 2). The decrease of HLA-DR expression on monocyte surface is considered a reliable predictor for mortality in patients with severe sepsis (77,78). FACS can also identify adhesion molecules and integrins that are increased on leukocyte surface and then shed in the bloodstream as soluble isoforms. It has been described an apoptotic pathway in human neutrophils that is triggered by the surface molecule CD24 (79). Neutrophil CD64 has been shown to be a highly sensitive and specific marker for systemic infection, sepsis and tissue injury (80). A modulated expression of the inhibitory receptor programmed cell death-1 (PD-1) protein expression in lymphocytes of septic patients has been observed (81). In NK cells, FACS analysis of NKG2D is used as a marker of cell activation and CD107 expression to identify degranulation (82,83). FACS is also able to discriminate the expression of surface molecules from intracellular antigens: intracellular levels of TLR2 and TLR4 in NK cells from septic patients were increased compared to healthy subjects (79). Moreover, FACS allows the analysis of cell cycle phases and apoptosis, the programmed cell death mechanism known to play a key role in sepsis-associated tissue injury and immunoparalysis (84).

Gene profiling of blood leukocytes has been evaluated to find the relation between encoding gene expression and related protein levels during sepsis. Microarray analysis of genes involved in inflammation revealed the modulation of genes further confirmed by quantitative RT-PCR (85). RT-PCR has also been used to study the decrease of HLA-DR expression in monocytes at gene level with promising results (86). Genotyping of HLA-DRB1 identified 4 alleles associated with a lower need of renal replacement therapies in patients with sepsis-associated AKI (87). Studies examining single nucleotide polymorphisms (SNPs) in sepsis generated conflicting results. However, the functional TNF gene SNP rs1800629 was strongly associated with susceptibility to sepsis (88).

In the next years, the evolving field of “OMICS” technologies (genomics, transcriptomics, proteomics, and metabolomics) may lead to a system biology-based approach that could serve as a sort of “molecular microscope” for developing new diagnostic tools in sepsis as already reported in other clinical settings (89). Furthermore, new diagnostic strategies have been currently under evaluation for potential clinical application including measurement of mitochondrial or cell free DNA by qRT-PCR or by direct fluorescence assays (90,91). Recent studies highlighted the key role of epigenetics in sepsis-associated immune dysfunction, in particular DNA methylation and histone acetylation of inflammatory genes

(92,93). Among epigenetic mechanisms, microRNAs, small non-coding RNAs able to modulate gene expression in target cells, are known to be modulated in plasma during sepsis. MicroRNAs can be identified by microarray and confirmed by RT-PCR as described for gene expression: miR-15a, miR-16, miR-122, miR-133, miR-193, miR-223 and miR-483-5p are increased in human sepsis and predict mortality (94-96). Circulating extracellular vesicles (EVs), potential emerging disease biomarkers, are microparticles involved in cell-to-cell crosstalk through the transfer of proteins, receptors, bioactive lipids and genetic material (mRNA and microRNA) that can be detected in plasma by FACS or by specific techniques such as Nanotrack analysis (97). Circulating plasma EVs are also potential mediators of sepsis-induced endothelial injury and myocardial dysfunction (98,99).

In conclusion, the possibility of performing multiple analyses and the continuous improvement of basic sciences and *omics* technologies in the next years may lead to a better definition of immune status in septic patients, thus allowing new personalized molecular-based therapeutic approaches.

Can extracorporeal blood purification therapies and non extracorporeal treatments alter cellular phenotypes and/or modify leukocyte function?

The therapeutic modulation of the host immune response during sepsis has always been very challenging. During the last decades, several attempts have failed to demonstrate any benefit in terms of patient outcomes (100,101). To date, both increased knowledge of sepsis pathophysiology and technological progress in the field of extracorporeal therapies allow the scientific community to be slightly more optimistic for the future. Two therapeutic strategies are indeed currently proposed for the modulation of the immune response to sepsis. First, the entire panel of the extracorporeal blood purification therapies can potentially positively impact the immune response by modifying immune cells phenotype and/or their function. Second, several new molecules have recently shown very promising effects for this purpose and are also currently under clinical investigation.

Convection-based strategies such as high volume hemofiltration and its variants (pulse high volume hemofiltration and cascade hemofiltration) have been primarily focused on the possibility of removing inflammatory mediators from the blood, a phenomenon that can subsequently change leukocytes phenotype and function. In a porcine model, Yekebas et al. reported that high volume hemofiltration can prevent the *in vitro* sepsis-induced endotoxin hyporesponsiveness. In addition, CD14 expression on monocytes was improved by the blood purification technique as did the oxidative burst and the phagocytosis capacity of polymorphonuclear leukocytes (102). Besides these effects and the removal of numerous cytokines, high-volume hemofiltration could also increase monocyte HLA-DR expression in patients with severe acute pancreatitis (103). Hemoperfusion is another extracorporeal blood purification technique, based on the interaction between a sorbent and molecules targeted for removal from blood *via* adsorption. Polymyxin-B hemoperfusion targets endotoxins and was shown to have the capability to restore HLA-DR expression on monocytes and the CD16 intensity on granulocytes with a decrease of IL-10 levels (104). Although Payen et al. recently reported disappointing clinical data with this therapy (105), Kumagai et al. highlighted another interesting capability of this type of sorbent which is the selective

removal via adsorption of activated neutrophils expressing high levels of CD11b/CD64 and low levels of CXCR1/CXCR2. That specific cell removal was associated with an *ex vivo* reduction of the ability of the circulation cells to cause damage to an endothelial cell monolayer (106). Moreover, hemoperfusion with the Cytosorb technology also has this capability to adsorb activated leukocytes in addition to the ability to remove circulating cytokines (107,108). Furthermore, it is suggested that the removal of cytokines and chemokines through this hemoperfusion device modifies local chemokine gradients between the nidus of infection and the plasma and therefore steers leukocyte trafficking towards an enhanced leukocyte recruitment (109). The hybrid blood purification technique named Coupled Plasma Filtration Adsorption (CPFA) may also be able to impact the immune response due to the removal of inflammatory mediators by adsorption on a specific resin. Indeed, Ronco et al. reported that CPFA can restore leukocyte responsiveness to LPS in a prospective crossover clinical trial enrolling patients with septic shock (110). Other investigators demonstrated additional beneficial effects of septic plasma resin adsorption. Indeed, septic plasma can induce direct injury of tubular cells by favoring granulocyte adhesion, inducing cell apoptosis and altering cell polarity and function. Since these biological effects are related to the presence of circulating inflammatory mediators, their removal by resin adsorption limits tubular cell injury (111). Finally, the use of high cut-off membranes in septic patients with acute kidney injury has been shown to decrease polymorphonuclear neutrophil phagocytosis and restore peripheral blood mononuclear cell proliferation (112,113).

Molecules aiming at « reboosting » the immune system during the immunoparalysis phase of the sepsis course have exhibited even more promising results in animals and preclinical studies. In a human double blind, placebo controlled, randomized pilot study, Leentjens et al. have reported that Interferon-γ can attenuate the reduction of the LPS-induced TNF-α response and can increase monocyte HLA-DR expression (114). Meisel et al. have demonstrated in a randomized controlled study that GM-CSF also reverses monocytes deactivation (increased monocytic HLA-DR) and restores *ex vivo* TLR-2/4 induced pro-inflammatory monocytic cytokine production. Interestingly, some positive clinical effects have even been observed such as a shorter time of mechanical ventilation and ICU length of stay in the GM-CSF group (115). The *in vitro* blockade of the PD-1/PD-L1 pathway can also impact immune cells phenotype and leukocyte function with a decrease of lymphocyte apoptosis and the restoration of the ability of immune effector cells to produce cytokines such as Interferon-γ and IL-2, which are essential for host immunity (116). Finally, Venet et al. have shown that an *ex vivo* treatment with recombinant human IL-7 can improve lymphocyte functionality with an increase in $CD4^+$ and $CD8^+$ T cell proliferation, an increase in Interferon-γ lymphocyte production, an increase in the phosphorylation of the key signaling molecule named STAT5 (signal transducer and activator of transcription 5) and an increase in B cell lymphoma 2 induction (117).

In conclusion, numerous promising therapies targeting the maladaptive host immune response to sepsis are under investigation (Figure 3). Importantly, these potential treatments can have an effect on the three different levels we have discussed earlier: a) the number of immune cells; b) the proportion of cell subtypes with the modification of the leucocyte

surface markers expression; and c) the cell expression and function. Large clinical studies are now urgently warranted to assess the impact of these therapies and treatments on clinical outcomes.

Acknowledgments

Funding: ADQI XIV was funded by unrestricted educational grants from (in alphabetical order) Astute Medical Inc., Baxter Healthcare Corporation, Bellco S.R.L., Cytosorbents Inc., Fresenius Medical Care, Spectral Diagnostics Inc., Toray Medical CO LTDA.

JK has also received research grants from Alere, Astute Medical, Atox Bio, Bard, Baxter, Cytosorbents, Gambro, Grifols, Kaneka, and Spectral Diagnostics, and has licensed technologies through the University of Pittsburgh to Astute Medical, Cytosorbents and Spectral Diagnostics. AG has received consulting fees from Fresenius Medical Care. PM has received consulting fees from AM Pharma, Abbvie, FAST Diagnostics. He has also received research funding from Abbott, Alere, EKF Diagnostics. HG research funding from NHLBI (1K12HL109068-02).

Appendix 1. ADQI XIV Workgroup

Nishkantha Arulkumaran

Vincenzo Cantaluppi

Lakhmir S. Chawla

Daniel de Backer

Clifford S. Deutschman

Mitchell P. Fink

Stuart L. Goldstein

Hernando Gómez

Alonso Gómez

Glenn Hernandez

Can Ince

John A. Kellum

John C. Marshall

Philip R. Mayeux

Patrick Murray

Trung C. Nguyen

Steven M. Opal

Gustavo Ospina-Tascón

Didier Payen

Michael R. Pinsky

Thomas Rimmelé

Paul T. Schumacker

Brian S. Zuckerbraun

Appendix 2. Search Terms

Blood purification, Flow cytometry, Immune cells, Inflammation, Lymphocyte, Monocyte, Neutrophil, Sepsis.

References

1. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013; 369(9):840–851. [PubMed: 23984731]
2. Wiersinga WJ, Leopold SJ, Cranendonk DR, van der Poll T. Host innate immune responses to sepsis. Virulence. 2014; 5(1):36–44. [PubMed: 23774844]
3. Calvano SE, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ, Chen RO, Brownstein BH, Cobb JP, Tschoeke SK, et al. A network-based analysis of systemic inflammation in humans. Nature. 2005; 437(7061):1032–1037. [PubMed: 16136080]
4. Marshall JC. Why have clinical trials in sepsis failed? Trends Mol Med. 2014; 20(4):195–203. [PubMed: 24581450]
5. Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. Blood. 2008; 112(4): 935–945. [PubMed: 18684880]
6. Savill JS, Wyllie AH, Henson JE, Henson PM, Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation. J Clin Invest. 1989; 83(3):865–875. [PubMed: 2921324]
7. Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L. In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood. 2010; 116(4):625–627. [PubMed: 20410504]
8. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. Proc Natl Acad Sci U S A. 1996; 93(6):2588–2592. [PubMed: 8637918]
9. de Kleijn S, Kox M, Sama IE, Pillay J, van Diepen A, Huijnen MA, van der Hoeven JG, Ferwerda G, Hermans PW, Pickkers P. Transcriptome kinetics of circulating neutrophils during human experimental endotoxemia. PLoS One. 2012; 7(6):e38255. [PubMed: 22679495]
10. Visser T, Pillay J, Pickkers P, Leenen LP, Koenderman L. Homology in systemic neutrophil response induced by human experimental endotoxemia and by trauma. Shock. 2012; 37(2):145–151. [PubMed: 22089204]
11. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 2013; 110(9):3507–3512. [PubMed: 23401516]
12. Wong HR, Freishtat RJ, Monaco M, Odoms K, Shanley TP. Leukocyte subset-derived genomewide expression profiles in pediatric septic shock. Pediatr Crit Care Med. 2010; 11(3): 349–355. [PubMed: 20009785]
13. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC. Gene-expression profiling of peripheral blood mononuclear cells in sepsis. Crit Care Med. 2009; 37(3):882–888. [PubMed: 19237892]
14. Nauseef WM, Borregaard N. Neutrophils at work. Nat Immunol. 2014; 15(7):602–611. [PubMed: 24940954]
15. Yipp BG, Kubes P. NETosis: how vital is it? Blood. 2013; 122(16):2784–2794. [PubMed: 24009232]

16. Marshall, JC.; Malam, Z.; Jia, SH. Modulating neutrophil apoptosis. In: Novartis Foundation. , editor. Sepsis: New Insights, New Therapies. Chichester, U.K.: John Wiley & Sons; 2007. p. 53-72.
17. Ahmed NA, McGill S, Yee J, Hu F, Michel RP, Christou NV. Mechanisms for the diminished neutrophil exudation to secondary inflammatory sites in infected patients with a systemic inflammatory response (sepsis). Crit Care Med. 1999; 27(11):2459–2468. [PubMed: 10579265]
18. Romaschin AD, Foster DM, Walker PM, Marshall JC. Let the cells speak: neutrophils as biologic markers of the inflammatory response. Sepsis. 1998; 2(2):119–125.
19. Taneja R, Parodo J, Kapus A, Rotstein OD, Marshall JC. Delayed neutrophil apoptosis in sepsis is associated with maintenance of mitochondrial transmembrane potential (DYM) and reduced caspase-9 activity. Crit Care Med. 2004; 32(7):1460–1469. [PubMed: 15241089]
20. Watson RW, Rotstein OD, Parodo J, Bitar R, Marshall JC. The interleukin-1 beta converting enzyme (caspase-1) inhibits apoptosis of inflammatory neutrophils through activation of IL-1b. J Immunol. 1998; 161(2):957–962. [PubMed: 9670975]
21. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, Marshall JC. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest. 2004; 113(9):1318–1327. [PubMed: 15124023]
22. Lukaszewicz AC, Grienay M, Resche-Rigon M, Pirracchio R, Faivre V, Boval B, Payen D. Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. Crit Care Med. 2009; 37(10):2746–2752. [PubMed: 19707128]
23. Venet F, Tissot S, Debard AL, Faudot C, Crampé C, Pachot A, Ayala A, Monneret G. Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: Correlation with severity and secondary septic shock. Crit Care Med. 2007; 35(8):1910–1917. [PubMed: 17568330]
24. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, Volk HD, Kox W. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. Nature Med. 1997; 3(6):678–681. [PubMed: 9176497]
25. Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. Curr Opin Immunol. 2013; 25(4):477–483. [PubMed: 23725873]
26. Vaki I, Kranidioti H, Karagianni V, Spyridaki A, Kotsaki A, Routsi C, Giamarellos-Bourboulis EJ. An early circulating factor in severe sepsis modulates apoptosis of monocytes and lymphocytes. J Leukoc Biol. 2011; 89(3):343–349. [PubMed: 21030664]
27. Koffel R, Meshcheryakova A, Warszawska J, Hennig A, Wagner K, Jorgl A, Gubi D, Moser D, Hladik A, Hoffmann U, et al. Monocytic cell differentiation from band-stage neutrophils under inflammatory conditions via MKK6 activation. Blood. 2014; 124(17):2713–2724. [PubMed: 25214442]
28. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009; 182(8):4499–4506. [PubMed: 19342621]
29. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9(3):162–174. [PubMed: 19197294]
30. Sander LE, Sackett SD, Dierssen U, Beraza N, linke RP, Muller M, Blander JM, Tacke F, Trautwein C. Hepatic acute-phase proteins control innate immune responses during infection by promoting myeloid-derived suppressor cell function. J Exp Med. 2010; 207(7):1453–1464. [PubMed: 20530204]
31. Derive M, Bouazza Y, Alauzet C, Gibot S. Myeloid-derived suppressor cells control microbial sepsis. Intensive Care Med. 2012; 38(6):1040–1049. [PubMed: 22552586]
32. Guerin E, Orabona M, Raquil MA, Giraudeau B, Bellier R, Gibot S, Bene MC, Lacombe F, Droin N, Solary E, et al. Circulating immature granulocytes with T-cell killing functions predict sepsis deterioration*. Crit Care Med. 2014; 42(9):2007–2018. [PubMed: 24942511]
33. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity. 2005; 23(4): 344–346. [PubMed: 16226499]
34. Dinauer MC. Disorders of neutrophil function: an overview. Methods Mol Biol. 2007; 412:489–504. [PubMed: 18453130]

35. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. Lancet. 2006; 368(9530):157–169. [PubMed: 16829300]
36. Hoesel LM, Neff TA, Neff SB, Younger JG, Olle EW, Gao H, Pianko MJ, Bernacki KD, Sarma JV, Ward PA. Harmful and protective roles of neutrophils in sepsis. Shock. 2005; 24(1):40–47. [PubMed: 15988319]
37. Jimenez MF, Watson RW, Parodo J, Evans D, Foster D, Steinberg M, Rotstein OD, Marshall JC. Dysregulated expression of neutrophil apoptosis in the systemic inflammatory response syndrome. Arch Surg. 1997; 132(12):1263–1270. [PubMed: 9403528]
38. Jia SH, Parodo J, Charbonney E, Tsang JL, Jia SY, Rotstein OD, Kapus A, Marshall JC. Activated neutrophils induce epithelial cell apoptosis through oxidant-dependent tyrosine dephosphorylation of caspase-8. Am J Pathol. 2014; 184(4):1030–1040. [PubMed: 24589337]
39. Wang JF, Li JB, Zhao YJ, Yi WJ, Bian JJ, Wan XJ, Zhu KM, Deng XM. Up-regulation of Programmed Cell Death 1 Ligand 1 on Neutrophils May Be Involved in Sepsis-induced Immunosuppression: An Animal Study and a Prospective Case-control Study. Anesthesiology. 2015; 122(4):852–863. [PubMed: 25437496]
40. Wenzel RP, Edmond MB. Septic shock--evaluating another failed treatment. N Engl J Med. 2012; 366(22):2122–2124. [PubMed: 22616829]
41. Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, Kellum JA. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock. 2014; 41(1):3–11. [PubMed: 24346647]
42. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect dis. 2013; 13(3):260–268. [PubMed: 23427891]
43. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl lek listy. 2001; 102(1):5–14. [PubMed: 11723675]
44. Tschaikowsky K, Hedwig-Geissing M, Schiele A, Bremer F, Schywalsky M, Schuttler J. Coincidence of pro- and anti-inflammatory responses in the early phase of severe sepsis: Longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonin, C-reactive protein, and changes in T-cell subsets in septic and postoperative patients. Crit care med. 2002; 30(5):1015–1023. [PubMed: 12006796]
45. Holub M, Kluckova Z, Helcl M, Prihodov J, Rokyta R, Beran O. Lymphocyte subset numbers depend on the bacterial origin of sepsis. Clin Microbiol Infect. 2003; 9(3):202–211. [PubMed: 12667252]
46. Kelly-Scumpia KM, Scumpia PO, Weinstein JS, Delano MJ, Cuenca AG, Nacionales DC, Wynn JL, Lee PY, Kumagai Y, Efron PA, et al. B cells enhance early innate immune responses during bacterial sepsis. J Exp Med. 2011; 208(8):1673–1682. [PubMed: 21746813]
47. Monserrat J, de Pablo R, Diaz-Martin D, Rodriguez-Zapata M, de la Hera A, Prieto A, Alvarez-Mon M. Early alterations of B cells in patients with septic shock. Crit Care. 2013; 17(3):R105. [PubMed: 23721745]
48. Andaluz-Ojeda D, Iglesias V, Bobillo F, Almansa R, Rico L, Gandia F, Loma AM, Nieto C, Diego R, Ramos E, et al. Early natural killer cell counts in blood predict mortality in severe sepsis. Crit Care. 2011; 15(5):R243. [PubMed: 22018048]
49. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013; 13(12):862–874. [PubMed: 24232462]
50. Schneider RP, Christou NV, Meakins JL, Nohr C. Humoral immunity in surgical patients with and without trauma. Arch Surg. 1991; 126(2):143–148. [PubMed: 1992991]
51. Rode HN, Christou NV, Gordon J, Meakins JL, MacLean LD. Anergy in surgical patients: is the failure at the afferent or the efferent limb? Surg Forum. 1979; 30:41–43. [PubMed: 538650]
52. Walton AH, Muenzer JT, Rasche D, Boomer JS, Sato B, Brownstein BH, Pachot A, Brooks TL, Deych E, Shannon WD, et al. Reactivation of multiple viruses in patients with sepsis. PloS one. 2014; 9(2):e98819. [PubMed: 24919177]

53. Monneret G, Debard AL, Venet F, Bohe J, Hequet O, Bienvenu J, Lepape A. Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. Crit Care Med. 2003; 31(7):2068–2071. [PubMed: 12847405]
54. Chang KC, Burnham CA, Compton SM, Rasche DP, Mazuski RJ, McDonough JS, Unsinger J, Korman AJ, Green JM, Hotchkiss RS. Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. Crit Care. 2013; 17(3):R85. [PubMed: 23663657]
55. Venet F, Chung CS, Kherouf H, Geeraert A, Malcus C, Poitevin F, Bohe J, Lepape A, Ayala A, Monneret G. Increased circulating regulatory T cells (CD4(+)CD25 (+)CD127 (-)) contribute to lymphocyte anergy in septic shock patients. Intensive Care Med. 2009; 35(4):678–686. [PubMed: 18946659]
56. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA. 2011; 306(23):2594–2605. [PubMed: 22187279]
57. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS. Persistent lymphopenia after diagnosis of sepsis predicts mortality. Shock. 2014; 42(5):383–391. [PubMed: 25051284]
58. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003; 348(2): 138–150. [PubMed: 12519925]
59. Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, Buchman TG, Karl IE. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med. 1999; 27(7):1230–1251. [PubMed: 10446814]
60. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. Immunity. 2013; 38(4):633–643. [PubMed: 23601682]
61. Losser MR, Damoisel C, Payen D. Bench-to-bedside review: Glucose and stress conditions in the intensive care unit. Crit Care. 2010; 14(4):231. [PubMed: 20727232]
62. Losser MR, Bernard C, Beaudeux JL, Pison C, Payen D. Glucose modulates hemodynamic, metabolic, and inflammatory responses to lipopolysaccharide in rabbits. J Appl Physiol. 1997; 83(5):1566–1574. [PubMed: 9375321]
63. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. N Engl J Med. 2006; 354(5):449–461. [PubMed: 16452557]
64. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. N Engl J Med. 2001; 345(19):1359–1367. [PubMed: 11794168]
65. Medzhitov R, Schneider DS, Soares MP. Disease tolerance as a defense strategy. Science. 2012; 335(6071):936–941. [PubMed: 22363001]
66. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature. 2007; 447(7147):972–978. [PubMed: 17538624]
67. Hoeboer SH, Groeneveld AB. Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients. PLoS One. 2013; 8(6):e65564. [PubMed: 23762396]
68. Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med. 2006; 34(7):1996–2003. [PubMed: 16715031]
69. Masson S, Caironi P, Spanuth E, Thomae R, Panigada M, Sangiorgi G, Fumagalli R, Mauri T, Isgrò S, Fanizza C, et al. Presepsin (soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial. Crit Care. 2014; 18(1):R6. [PubMed: 24393424]
70. Luz Fiusa MM, Costa-Lima C, de Souza GR, Vigorito AC, Penteado Aranha FJ, Lorand-Metze I, Annichino-Bizzacchi JM, de Souza CA, De Paula EV. A high angiopoietin-2/angiopoietin-1 ratio is associated with a high risk of septic shock in patients with febrile neutropenia. Crit Care. 2013; 17(4):R169. [PubMed: 23915833]

71. Lorente L, Martín MM, Varo N, Borreguero-León JM, Solé-Violán J, Blanquer J, Labarta L, Díaz C, Jiménez A, Pastor E, et al. Association between serum soluble CD40 ligand levels and mortality in patients with severe sepsis. Crit Care. 2011; 15(2):R97. [PubMed: 21406105]

72. Huttunen R, Syrjänen J, Vuento R, Laine J, Hurme M, Aittoniemi J. Apoptosis markers soluble Fas (sFas), Fas Ligand (FasL) and sFas/FasL ratio in patients with bacteremia: a prospective cohort study. J Infect. 2012; 64(3):276–281. [PubMed: 22207003]

73. Andaluz-Ojeda D, Iglesias V, Bobillo F, Almansa R, Rico L, Gandía F, Loma AM, Nieto C, Diego R, Ramos E, et al. Early natural killer cell counts in blood predict mortality in severe sepsis. Crit Care. 2011; 15(5):R243. [PubMed: 22018048]

74. Venet F, Pachot A, Debard AL, Bohe J, Bienvenu J, Lepape A, Powell WS, Monneret G. Human CD4+CD25+ regulatory T lymphocytes inhibit lipopolysaccharide-induced monocyte survival through a Fas/Fas ligand-dependent mechanism. J Immunol. 2006; 177(9):6540–6547. [PubMed: 17056586]

75. Duong S, Condotta SA, Rai D, Martin MD, Griffith TS, Badovinac VP. Polymicrobial sepsis alters antigen-dependent and -independent memory CD8 T cell functions. J Immunol. 2014; 192(8): 3618–3625. [PubMed: 24646738]

76. Patschan SA, Patschan D, Temme J, Korsten P, Wessels JT, Koziolek M, Henze E, Müller GA. Endothelial progenitor cells (EPC) in sepsis with acute renal dysfunction (ARD). Crit Care. 2011; 15(2):R94. [PubMed: 21396100]

77. Trimmel H, Luschin U, Köhrer K, Anzur C, Vevera D, Spittler A. Clinical outcome of critically ill patients cannot be defined by cutoff values of monocyte human leukocyte antigen-DR expression. Shock. 2012; 37(2):140–144. [PubMed: 22089188]

78. Wu JF, Ma J, Chen J, Ou-Yang B, Chen MY, Li LF, Liu YJ, Lin AH, Guan XD. Changes of monocyte human leukocyte antigen-DR expression as a reliable predictor of mortality in severe sepsis. Crit Care. 2011; 15(5):R220. [PubMed: 21933399]

79. Parlato M, Souza-Fonseca-Guimaraes F, Philippart F, Misset B, Adib-Conquy M, Cavaillon JM. Captain Study Group. CD24-triggered caspase-dependent apoptosis via mitochondrial membrane depolarization and reactive oxygen species production of human neutrophils is impaired in sepsis. J Immunol. 2014; 192(5):2449–2459. [PubMed: 24501201]

80. Dimoula A, Pradier O, Kassengera Z, Dalcomune D, Turkan H, Vincent JL. Serial determinations of neutrophil CD64 expression for the diagnosis and monitoring of sepsis in critically ill patients. Clin Infect Dis. 2014; 58(6):820–829. [PubMed: 24363321]

81. Chang K, Svabek C, Vazquez-Guillamet C, Sato B, Rasche D, Wilson S, Robbins P, Ulbrandt N, Suzich J, Green J, et al. Targeting the programmed cell death 1: programmed cell death ligand 1 pathway reverses T cell exhaustion in patients with sepsis. Crit Care. 2014; 18(1):R3. [PubMed: 24387680]

82. Forel JM, Chiche L, Thomas G, Mancini J, Farnarier C, Cognet C, Guervilly C, Daumas A, Vély F, Xéridat F, et al. Phenotype and functions of natural killer cells in critically-ill septic patients. PLoS One. 2012; 7(12):e50446. [PubMed: 23236375]

83. Kjaergaard AG, Nielsen JS, Tønnesen E, Krog J. Expression of NK cell and monocyte receptors in critically ill patients potential biomarkers of sepsis. Scand J Immunol. 2015; 81(4):249–258. [PubMed: 25619264]

84. Brahmamdam P, Watanabe E, Unsinger J, Chang KC, Schierding W, Hoekzema AS, Zhou TT, McDonough JS, Holemon H, Heidel JD, et al. Targeted delivery of siRNA to cell death proteins in sepsis. Shock. 2009; 32(2):131–139. [PubMed: 19033888]

85. Payen D, Lukaszewicz AC, Belikova I, Faivre V, Gelin C, Russwurm S, Launay JM, Sevenet N. Gene profiling in human blood leucocytes during recovery from septic shock. Intensive Care Med. 2008; 34(8):1371–1376. [PubMed: 18392609]

86. Cajander S, Bäckman A, Tina E, Strålin K, Söderquist B, Källman J. Preliminary results in quantitation of HLA-DRA by real-time PCR: a promising approach to identify immunosuppression in sepsis. Crit Care. 2013; 17(5):R223. [PubMed: 24093602]

87. Payen D, Lukaszewicz AC, Legrand M, Gayat E, Faivre V, Megarbane B, Azoulay E, Fieux F, Charron D, Loiseau P, et al. A multicentre study of acute kidney injury in severe sepsis and septic

shock: association with inflammatory phenotype and HLA genotype. PLoS One. 2012; 7(6):e35838. [PubMed: 22701553]

88. Song Z, Song Y, Yin J, Shen Y, Yao C, Sun Z, Jiang J, Zhu D, Zhang Y, Shen Q, et al. Genetic variation in the TNF gene is associated with susceptibility to severe sepsis, but not with mortality. PLoS One. 2012; 7(9):e46113. [PubMed: 23029405]
89. Loupy A, Lefaucheur C, Vernerey D, Chang J, Hidalgo LG, Beuscart T, Verine J, Aubert O, Dubleumortier S, Duong van Huyen JP, et al. Molecular microscope strategy to improve risk stratification in early antibody-mediated kidney allograft rejection. J Am Soc Nephrol. 2014; 25(10):2267–2277. [PubMed: 24700874]
90. Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, Quintana C, Osorio JC, Wang Z, Zhao Y, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. PLoS Med. 2013; 10(12):e1001577. [PubMed: 24391478]
91. Avriel A, Paryente Wiessman M, Almog Y, Perl Y, Novack V, Galante O, Klein M, Pencina MJ, Douvdevani A. Admission cell free DNA levels predict 28-day mortality in patients with severe sepsis in intensive care. PLoS One. 2014; 9(6):e100514. [PubMed: 24955978]
92. Semmler A, Prost JC, Smulders Y, Smith D, Blom H, Bigler L, Linnebank M. Methylation metabolism in sepsis and systemic inflammatory response syndrome. Scand J Clin Lab Invest. 2013; 73(5):368–372. [PubMed: 23566119]
93. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H, et al. Suppression of inflammation by a synthetic histone mimic. Nature. 2010; 468(7327):1119–1123. [PubMed: 21068722]
94. Tacke F, Roderburg C, Benz F, Cardenas DV, Luedde M, Hippe HJ, Frey N, Vucur M, Gautheron J, Koch A, et al. Levels of circulating miR-133a are elevated in sepsis and predict mortality in critically ill patients. Crit Care Med. 2014; 42(5):1096–1104. [PubMed: 24413579]
95. Ma Y, Vilanova D, Atalar K, Delfour O, Edgeworth J, Ostermann M, Hernandez-Fuentes M, Razafimahatratra S, Michot B, Persing DH, et al. Genome-wide sequencing of cellular microRNAs identifies a combinatorial expression signature diagnostic of sepsis. PLoS One. 2013; 8(10):e75918. [PubMed: 24146790]
96. Wang H, Zhang P, Chen W, Feng D, Jia Y, Xie L. Serum microRNA signatures identified by Solexa sequencing predict sepsis patients' mortality: a prospective observational study. PLoS One. 2012; 7(6):e38885. [PubMed: 22719975]
97. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int. 2010; 78(9):838–848. [PubMed: 20703216]
98. Azevedo LC, Janiszewski M, Pontieri V, Pedro Mde A, Bassi E, Tucci PJ, Laurindo FR. Platelet-derived exosomes from septic shock patients induce myocardial dysfunction. Crit Care. 2007; 11(6):R120. [PubMed: 17996049]
99. Gambim MH, do Carmo Ade O, Marti L, Veríssimo-Filho S, Lopes LR, Janiszewski M. Platelet-derived exosomes induce endothelial cell apoptosis through peroxynitrite generation: experimental evidence for a novel mechanism of septic vascular dysfunction. Crit Care. 2007; 11(5):R107. [PubMed: 17894858]
100. Sprung CL, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, et al. Hydrocortisone therapy for patients with septic shock. N Engl J Med. 2008; 358(2):111–124. [PubMed: 18184957]
101. Marti-Carvajal AJ, Sola I, Lathyris D, Cardona AF. Human recombinant activated protein C for severe sepsis. Cochrane Database Syst Rev. 2012; 3:CD004388. [PubMed: 22419295]
102. Yekebas EF, Eisenberger CF, Ohnesorge H, Saalmuller A, Elsner HA, Engelhardt M, Gillesen A, Meins J, The M, Strate T, et al. Attenuation of sepsis-related immunoparalysis by continuous veno-venous hemofiltration in experimental porcine pancreatitis. Crit Care Med. 2001; 29(7): 1423–1430. [PubMed: 11445702]
103. Gong D, Zhang P, Ji D, Chen Z, Li W, Li J, Li L, Liu Z. Improvement of immune dysfunction in patients with severe acute pancreatitis by high-volume hemofiltration: a preliminary report. Int J Artif Organs. 2010; 33(1):22–29. [PubMed: 20162528]
104. Ono S, Tsujimoto H, Matsumoto A, Ikuta S, Kinoshita M, Mochizuki H. Modulation of human leukocyte antigen-DR on monocytes and CD16 on granulocytes in patients with septic shock

using hemoperfusion with polymyxin B-immobilized fiber. Am J Surg. 2004; 188(2):150–156. [PubMed: 15249240]

105. Payen DM, Guilhot J, Launey Y, Lukaszewicz AC, Kaaki M, Veber B, Pottecher J, Joannes-Boyau O, Martin-Lefevre L, Jabaudon M, et al. Early use of polymyxin B hemoperfusion in patients with septic shock due to peritonitis: a multicenter randomized controlled trial. Intensive Care Med. 2015 in press.
106. Kumagai T, Takeyama N, Yabuki T, Harada M, Miki Y, Kanou H, Inoue S, Nakagawa T, Noguchi H. Apheresis of activated leukocytes with an immobilized polymyxin B filter in patients with septic shock. Shock. 2010; 34(5):461–466. [PubMed: 20386499]
107. Rimmelé T, Kaynar AM, McLaughlin JN, Bishop JV, Fedorchak MV, Chuasuwan A, Peng Z, Singbartl K, Frederick DR, Zhu L, et al. Leukocyte capture and modulation of cell-mediated immunity during human sepsis: an ex vivo study. Crit Care. 2013; 17(2):R59. [PubMed: 23531333]
108. Peng ZY, Carter MJ, Kellum JA. Effects of hemoadsorption on cytokine removal and short-term survival in septic rats. Crit Care Med. 2008; 36(5):1573–1577. [PubMed: 18434884]
109. Peng ZY, Bishop JV, Wen XY, Elder MM, Zhou F, Chuasuwan A, Carter MJ, Devlin JE, Kaynar AM, Singbartl K, et al. Modulation of chemokine gradients by apheresis redirects leukocyte trafficking to different compartments during sepsis, studies in a rat model. Crit Care. 2014; 18(4):R141. [PubMed: 24992991]
110. Ronco C, Brendolan A, Lonnemann G, Bellomo R, Piccinni P, Digito A, Dan M, Irone M, La Greca G, Inguaggiato P, et al. A pilot study of coupled plasma filtration with adsorption in septic shock. Crit Care Med. 2002; 30(6):1250–1255. [PubMed: 12072677]
111. Cantaluppi V, Weber V, Lauritano C, Figliolini F, Beltramo S, Biancone L, De Cal M, Cruz D, Ronco C, Segoloni GP, et al. Protective effect of resin adsorption on septic plasma-induced tubular injury. Crit Care. 2010; 14(1):R4. [PubMed: 20064258]
112. Morgera S, Haase M, Rocktaschel J, Bohler T, von Heymann C, Vargas-Hein O, Krausch D, Zuckermann-Becker H, Muller JM, Kox WJ, et al. High permeability haemofiltration improves peripheral blood mononuclear cell proliferation in septic patients with acute renal failure. Nephrol Dial Transplant. 2003; 18(12):2570–2576. [PubMed: 14605279]
113. Morgera S, Haase M, Rocktaschel J, Bohler T, Vargas-Hein O, Melzer C, Krausch D, Kox WJ, Baumann G, Beck W, et al. Intermittent high-permeability hemofiltration modulates inflammatory response in septic patients with multiorgan failure. Nephron Clin Pract. 2003; 94(3):c75–80. [PubMed: 12902634]
114. Leentjens J, Kox M, Koch RM, Preijers F, Joosten LA, van der Hoeven JG, Netea MG, Pickkers P. Reversal of immunoparalysis in humans in vivo: a double-blind, placebo-controlled, randomized pilot study. Am J Respir Crit Care Med. 2012; 186(9):838–845. [PubMed: 22822024]
115. Meisel C, Schefold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, Weber-Carstens S, Hasper D, Keh D, Zuckermann H, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med. 2009; 180(7):640–648. [PubMed: 19590022]
116. Chang K, Svabek C, Vazquez-Guillamet C, Sato B, Rasche D, Wilson S, Robbins P, Ulbrandt N, Suzich J, Green J, et al. Targeting the programmed cell death 1: programmed cell death ligand 1 pathway reverses T cell exhaustion in patients with sepsis. Crit Care. 2014; 18(1):R3. [PubMed: 24387680]
117. Venet F, Foray AP, Villars-Méchin A, Malcus C, Poitevin-Later F, Lepape A, Monneret G. IL-7 restores lymphocyte functions in septic patients. J Immunol. 2012; 189(10):5073–5081. [PubMed: 23053510]

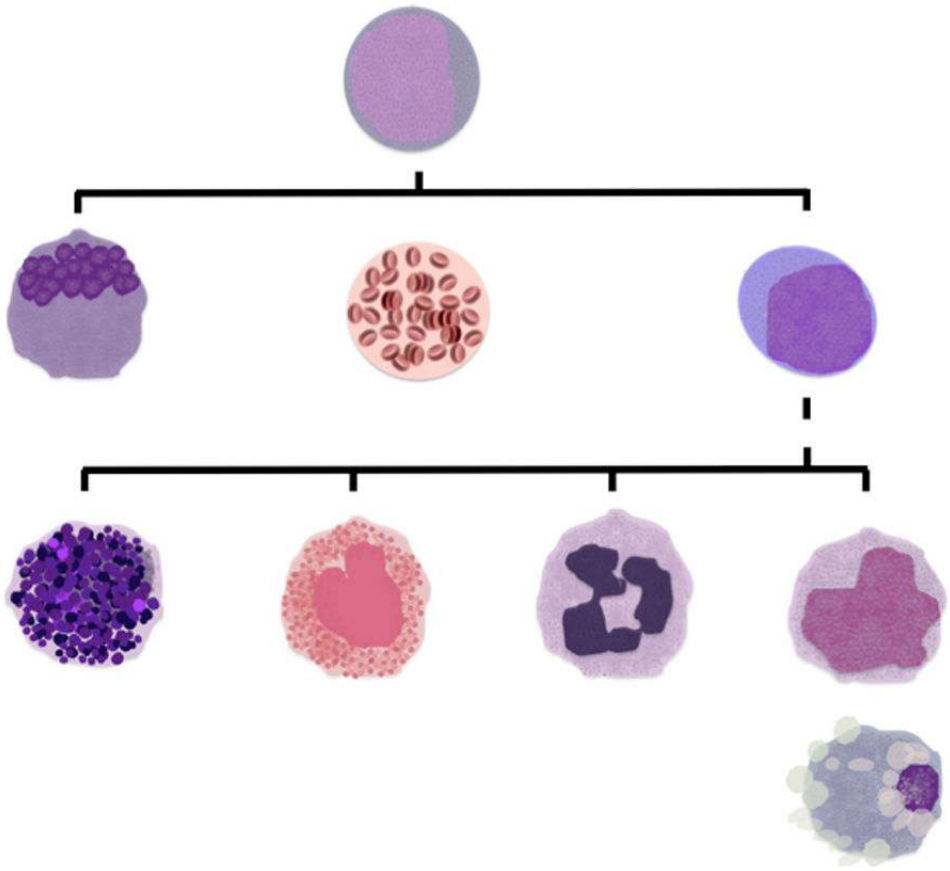

Figure 1.

The ontology of myeloid cell development in the bone marrow. Undifferentiated precursor cells give rise to precursors of myeloid and lymphoid cells. Myeloid precursors, in turn, differentiate to become megakaryocytes, erythrocytes, and myeloblasts, the latter giving rise to neutrophils and monocytes. Neutrophils undergo apoptosis within hours of their release into the circulation, whereas monocytes circulate, then exit the vasculature where they become fixed tissue macrophages.

Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission.

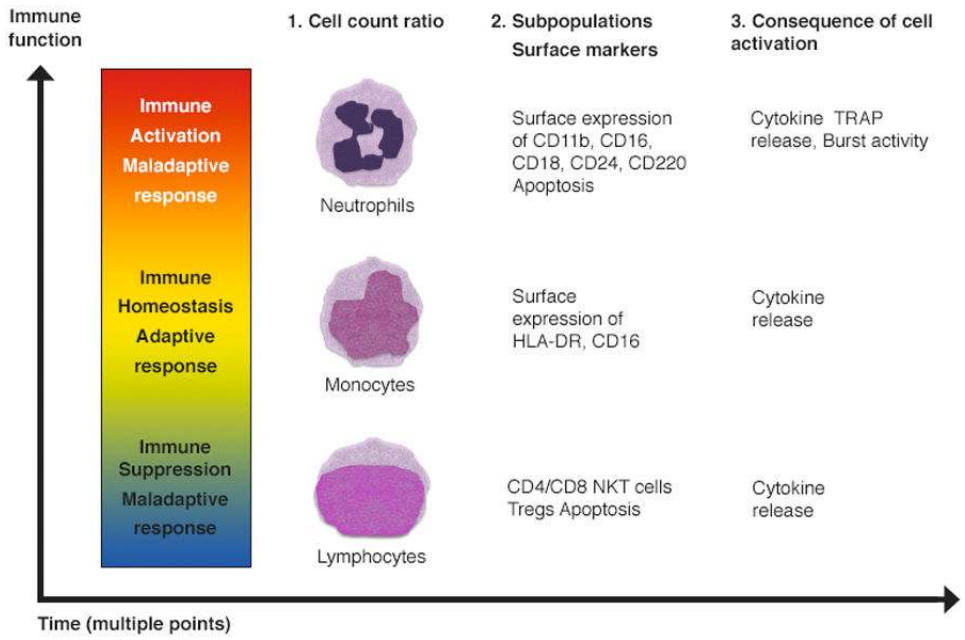

Figure 2.

Multi-step evaluation of immune cell activation, suppression and homeostasis. FACS can identify on neutrophils, monocytes and lymphocytes: 1) cell count ratio; 2) specific subpopulation surface markers; 3) biological consequences of cell activation.

Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission.

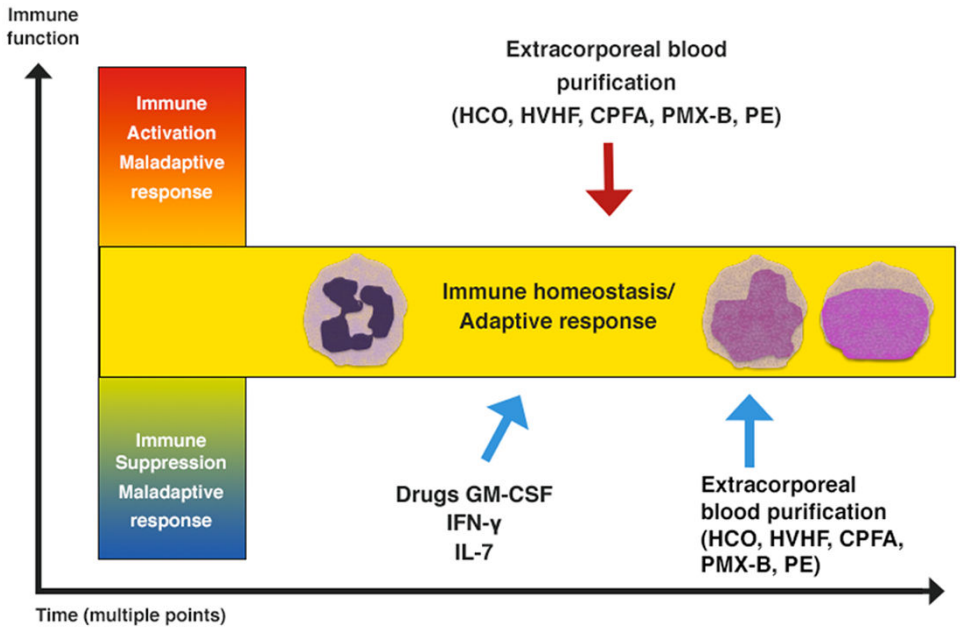

Figure 3.

Possible biological effects of different drugs and of extracorporeal blood purification therapies on immune system activation, suppression and homeostasis.

HCO: high cut-off;

HVHF: high volume hemofiltration;

CPFA: coupled plasma filtration adsorption;

PMX-B: polymyxin B hemoperfusion;

PE: plasma exchange;

GM-CSF: granulocyte-macrophage colony-stimulating factor;

IFN-γ: interferon gamma;

IL-7: interleukin 7.

Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission.

Table 1
Changes in Expression of Cell Surface Molecules Associated with Sepsis

	Expression	
Cell Type	**Increased**	**Decreased**
Neutrophils	CD11b	CD16
	CD14	CD80
	CD64	CD86
	CD66b	L-selectin
	CD163	TLR2
	TLR4	TNFr
	TLRs 1, 2, 5, 8	
	TREM-1	
	Fas	
	PD-1	
Monocytes	CD163	HLA-DR
	TREM-1	CD14
	Tissue factor	L-selectin
	TLR2	CX3CR1