Sepsis

Chapter 3

Apoptotic, Anti-apoptotic and Immune System Crosstalk in Sepsis

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Abstract

Sepsis is a dysregulated host response to infection related to devastating outcomes. Despite advances, sepsis care remains a crucial challenge for the scientific community. Activated apoptotic pathways have been suggested to participate in the immunosuppressive processes in the course of sepsis, affecting multiple immune cell types. The apoptotic response during the septic process is primarily characterized by a dramatic upregulation of caspases, which are cysteine proteases, linked to cell death. The apoptotic cascades are regulated by a number of antiapoptotic proteins, such as survivin. Moreover, a variable interplay between inflammatory mediators, such as cytokines or heat shock proteins, and apoptotic coordinators, such as caspases, or antiapoptotic molecules, such as survivin, could probably be expected in sepsis. These complex interactions could also account for the pathophysiologic processes occurring in sepsis, such as oxidative stress, immunoparalysis, and autophagy. Although these

responses are important for host defense against invading bacteria, their uncontrolled and excessive activation ultimately contributes to multiple organ injury during the septic process.

Keywords: Apoptosis, Sepsis, Intensive care, Caspases, Survivin

Abbreviations: ALI: Acute Lung Injury; APAF1: Apoptotic Protease-Activating Factor 1; ARDS: Acute Respiratory Distress Syndrome; ATP: Adenosine-5-Triphospate; BIR: Baculovirus Inhibitor Of Apoptosis Repeat Domain; CMV: Cytomegalovirus; DAMPs: Danger-Associated Molecular Patterns; DNA: Deoxyribonucleic Acid; eHSP: Extracellular Heat Shock Protein; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; HDL: High-Density Lipoprotein; hGRa: Human Glucocorticoid Receptor A; HLA: Human Leukocyte Antigen; HS: Heat Shock; HSF1: Heat Shock Factor 1; HSP: Heat Shock Protein; IAPs: Inhibitor Of Apoptosis Proteins; ICU: Intensive Care Unit; IFN: Interferon-γ; IL: Interleukin; iHSP: Intracellular HSP; JDP: J-domain Protein; LDL: Low-Density Lipoprotein; LPS: Bacterial Lipopolysacharide; MHC: Major Histocompatibility Complex; MODS: Multiple Organ Dysfunction Syndrome; mRNA: Messenger Ribonucleic Acid; NF-κB: Nuclear Factor Kappa-B; PAMPs: Pathogen-Associated Molecular Patterns; PAID: Post-Aggressive Immunodepression State; PICS: Persistent Inflammation/Immunosuppression And Catabolism Syndrome; SIRS: Systemic Inflammatory Response Syndrome; SNPs: Single Nucleotide Polymorphisms; TGF: Tissue Growth Factor; TLR: Toll Like Receptor; TNF-a: Tumor Necrosis Factor Alpha

1. Introduction

Severe sepsis and septic shock are the leading causes of death in adult and pediatric intensive care units (ICU) worldwide. Despite efforts in understanding the septic pathophysiology and implementing effective treatment, its annual incidence has been projected to increase by 1,5% per year [1]. Sepsis is a life-threatening multiple organ dysfunction process caused by a dysregulated, generalized inflammatory, and procoagulant host response to infectious stimulants, possibly followed by immune suppression. This heterogenous pathophysiological interplay vary in degree among patients, depending on many factors, such as the pathogen's load or virulence, host's co-morbidities or genetic factors. As a common and life-threatening infectious syndrome, sepsis contributes significantly to morbidity and mortality in clinical settings through a wide array of manifestations including septic shock, adult respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS) [2].

Sepsis may be elicited through either pathogen-associated molecular patterns (PAMPs) or danger-associated (in the absence of infection) molecular patterns (DAMPs), acting as a systemic pro-inflammatory response syndrome (SIRS) at least in the early stages of the disease [3]. Upon this triggering the progression of sepsis is very complex, since a wide variety of responses are elicited, including activation of the inner and adaptive immunity, acute inflammatory response, and bioenergetics failure. Recent studies in sepsis focus less on the pathogenicity of the invading pathogen, but more on the host's immune response upon microbial or endogenous cellular products of tissue injury, acting as "alarmins" and leading to the recruitment of pro-inflammatory intermediates, according to the "danger hypothesis" [4]. Current research has shown that mortality is associated either with periods of an overexuberant

immune response mediated by an excessive proinflammatory “cytokine storm”, or with the contrasting theory of a continuous immunosuppressive state, in which patients succumb to secondary infections within a few weeks or months due to post-sepsis “immune paralysis” [5]. Genome-wide transcription profiling has indicated that patients may cycle through each phase multiple times over the course of sepsis [6], while both pro- and anti-inflammatory cytokine genes seem to be upregulated simultaneously [7]. Recent findings also suggest that patients who survive the early stages of sepsis enter into complex clinical paths, through the development of a persistent inflammation/immunosuppression and catabolism syndrome (PICS), driven either by alarmins of injured organs, opportunistic infections or mechanical injuries secondary to therapeutic decisions [4].

Activated apoptotic pathways have been suggested to participate in the immuno-suppressive processes early in the course of sepsis, affecting multiple cell types of the innate and adaptive immunity, in an effort to dampen the initial hyper-inflammatory response. The inability of the host to restore immune homeostasis at this stage might lead to an uncontrolled hypo-inflammatory phase, possibly due to a dysfunctional adaptive immune response [8]. Alongside this theory, multiple studies have shown that the activation of a variety of pro-inflammatory cytokines could act synergistically and ultimately induce cell death [9,10]. A cytokine secretion defect in septic patients late in the course of sepsis indicates an apoptotic tendency affecting proper functioning of both the innate and adaptive immune system. The degree of lymphocyte apoptosis has been correlated with the severity of the septic process, predicting mortality, whereas apoptotic pathways upregulation seems to further expand immunoparalysis, through complex interactions with other cells of the adaptive immunity. Therefore, the outcome of critically ill septic patients might be determined by the delicate balance between pro- and anti-apoptotic mechanisms.

Personalized medicine approaches require a better understanding of the primary immune endotype, based on whether the patient is experiencing a hyperinflammatory or an immunosuppressive course of the disease, which would be instrumental in guiding therapeutic decisions [11]. Also, the efficacy of immunoadjuvant therapies or cell-survival studies currently under investigation might provide promising novel therapeutic opportunities [12,13]. Multiple studies on polymorphisms and genetic markers are also currently in progress, in an effort to provide more insight in the complex pathways of sepsis and eventually create important predictive and prognostic algorithms in the future [14]. Considering the complex immune response of each host, the development of predictive tools based on proteomics, metabolomics, genomics, cellular assays and bioinformatics could give critical answers on septic riddles and move our medical practices onwards [15].

2. Apoptotic and Antiapoptotic Pathways in Sepsis

Cell death has traditionally been linked to apoptosis, pyroptosis or necrosis processes, depending on the mechanistic pathways activated following an insult [16]. It affects multiple cell types during the septic process, and it can be triggered in a cell through either extrinsic or intrinsic stimuli. The apoptotic process has major consequences for the outcome, since apoptotic cells present anti-inflammatory properties and induce immunological tolerance. Besides, old studies have already demonstrated that the phagocytosis of apoptotic cells leads to active inhibition of pro-inflammatory cytokine production by macrophages, and to the resolution of inflammation through the release of anti-inflammatory mediators. These findings have led to the current understanding of apoptotic cells as being generally anti-inflammatory (inflammosuppressive) and also immunosuppressive [17].

2.1. Apoptosis

In early-onset sepsis, innate immune cells, such as dendritic cells, and cells from the adaptive immune system, especially lymphocytes, splenocytes, and intestinal epithelial cells are depleted through apoptotic processes, mainly driven by certain protein molecules called caspases (8). Caspases are cysteine proteases that cleave their substrates on the C-terminal side of aspartate, leading to DNA fragmentation, membrane blebbing, phosphatidylserine exposure at the cell surface, and apoptotic vesicles formation [18]. Caspases may hold key roles in sepsis in terms of apoptosis, pyroptosis, necroptosis and inflammation [19-21]. However, besides their crucial biological functions linked to cell death, caspases also have central roles during the regulation of non-cell death cascades, such as dendrite trimming, cell differentiation, migration, or milder inflammation states [21]. In this context, caspases could act both as “inflammatory” or “apoptotic” critical coordinators in the integration of various signaling cascades during normal development and homeostasis restoration, or during serious diseases and critical illness. Their complex functions highlight the tight intertwinement between the inflammatory and cell death processes, as well as their important role in regulating autophagy. Autophagy, which is a catabolic process that involves lysosomal degradation and recycling of cytoplasmic constituents, has emerged as a novel mediator of cell death and has also been linked to caspases in a cross-talk with apoptotic cascades [22].

Caspases activation follows two distinct pathways. The extrinsic apoptotic pathway (mitochondria-independent) is activated through the binding of a ligand to a death receptor at the cell surface, for instance, the TRAIL-bound TNF receptor, which leads to the activation of caspase-8, the major mediator of this apoptotic cascade [19,23]. The intrinsic (mitochondrial) pathway is activated through various endogenous cellular stress signals or irradiation, leading to loss of the mitochondrial outer membrane integrity and to the release of apoptotic factors sequestered by the mitochondria, such as cytochrome c, resulting in the activation of the

initiator caspase, caspase-9 [24]. Both intrinsic (mitochondria-mediated) and extrinsic (death-receptor-mediated) apoptotic processes are activated during sepsis. Mitochondrial oxidative stress plays an important role in both pathways but especially in the intrinsic one. The final step in this process is the formation of the "apoptosome", which reacts with caspases initiating the apoptotic pathway, via deoxyribonucleic acid (DNA) fragmentation and chromatic condensation [25]. Caspases-3 and -7 are the terminal effector executioner proteases in both pathways of apoptosis, leading to disruption of the nuclear envelope and breakdown of genomic DNA, via cleavage of structural proteins. On the other side of the coin, low level of caspase-3 activity has been advocated to be necessary for critical developmental processes in several cell types [26].

2.2. Pyroptosis

A caspase-dependent programmed neutrophil death (pyroptosis) associated with inflammation in sepsis is initiated by inflammasomes in innate immunity. In general, pyroptosis protects the host against invading microorganisms, but also causes severe sepsis or septic shock when overactivated. Pyroptosis seems to be mechanistically distinct from other types of cell death. In general, apoptosis seems to be driven through the activation of caspases-8 or -9, without the initiation of inflammation, whereas pyroptosis is mainly driven through caspase-1 and/or -11, with the activation of IL-1β and IL-18 [16,27]. In an inflammasome-related process, caspase-1 activation is identified as the primary step for pyroptosis, facilitating the activation of inflammatory pathways. Caspase-1 is activated by ligands of typical inflammasomes, while other inflammatory caspases, such as caspase-11, directly recognize LPS [28]. Published studies, have also revealed the importance of caspase-1 as a potential marker for predicting the development of sepsis, whereas caspase-1 mRNA levels have been associated with the severity of sepsis caused by cytomegalovirus (CMV) [29]. An association of caspases concentrations with sepsis severity, degree of apoptosis, and mortality in septic patients has already been reported [30], whereas apoptotic deficiency seems to play an important role in the survival of lymphocytes, leading to autoimmunity.

2.3. Inhibitors of apoptosis proteins

Inhibitors of apoptosis proteins (IAPs), including Survivin protein, seem to restrain the downstream components of caspase-activation pathways and play important roles in regulating the progress of apoptosis, but their role in sepsis has not been elucidated so far. Recent studies reveal that survivin partially interacts with multiple inflammatory mediators (such as nuclear factor-kappa-B, NF-κB), regulating LPS induced cytotoxicity and might be closely linked to worse prognosis [31]. Inclusion in the IAP family is based on the presence of at least one baculovirus inhibitor of apoptosis repeat domain (BIR) in gene encodement, a globular fold that has originally been found in insect viruses. Humans have eight IAPs, of which survivin is the

smallest protein, expressed during development and not expressed in most differentiated adult tissues. Survivin is an evolutionarily conserved eukaryotic protein (BIRC5) that is expressed in actively proliferating cells, playing a crucial role in cell division by inhibiting apoptosis and regulating the process of mitosis in embryonic and cancer cells [32]. Survivin wild type (WT) seems to be essential for the maintenance of mitochondrial integrity and function [33], by inhibiting caspases-3 and -7 [34]. Initially, survivin was thought to bind to and inhibit caspase activity directly, but current knowledge confirms that only the canonical member of this family, XIAP, can efficiently and directly inhibit caspases. XIAP then interacts with other IAPs, including survivin, in a way that improves stability and augments the inhibitory effect of XIAP. Upstream factors such as Smac/Diablo, which is released from the mitochondria upon apoptotic stimulation, can inhibit IAPs by binding to the BIR domain. Survivin may also prevent the release of apoptotic protease-activating factor 1 (APAF1) from the mitochondria, or sequester the main IAP inhibitor (Smac/Diablo) away from other IAPs [35]. Survivin's upregulation in malignancies has extensively been reported [36], while accumulated evidence shows that it exerts cell-protection in non-malignant conditions as well [37]. Survivin is also considered to be a regulator in the injury of several organs including the lung, the kidney or the liver [31]. Given that survivin is essential for mitosis, in maintaining homeostasis of the immune system, and able to inhibit apoptosis [35], piled evidence favors the involvement of survivin dysregulation in the development of inflammatory disorders [38].

2.4. Survivin splice variants

In addition to the survivin-WT transcript, several transcript variants, generated by alternative splicing of the human survivin gene (BIRC5), have been identified: survivin-ΔEx3 (a splice variant lacking exon 3), -2B (one variant retaining a part of intron 2 as a cryptic exon), and -3B (a novel exon 3B derived from a portion of intron 3) are currently well studied [39], whereas other isoforms are not yet satisfactorily characterized [40]. Multiple studies have already investigated the expression and function of survivin in different autoimmune diseases, and also elevated protein expression levels of survivin splice variants have been identified in rheumatoid arthritis tissues, confirmed by specific antibodies against survivin's different splice variants [41,42]. Several different types of cancers have been shown to express the survivin-WT, -ΔEx3, -2B, -3B splice variants, with no expression in the adjoining normal tissues [43]. Also, serum levels of survivin-WT in active rheumatoid arthritis patients and healthy controls were similar in CD4+ and CD19+ cells, while survivin-2B and -ΔEx3 were significantly higher in CD19+ B cells [44]. The expression levels and subcellular localization patterns of each isoform are associated with different functional properties, mainly studied in cancer patients. Survivin-WT, -ΔEx3 and -3B splice variants seem to have antiapoptotic properties, whereas other studies in autoimmune diseases question the antiapoptotic potential of survivin-3B, and more importantly of survivin-2B and -2α variants. Also, survivin-2B presents its proapoptotic

functions by dimerizing with the wild-type, thus reducing its antiapoptotic effects [23,45].

Multiple studies of mRNA expression have demonstrated the intense modulation of gene expression during sepsis, particularly emphasizing on the upregulation of pro-apoptotic genes and on the downregulation of anti-apoptotic transcriptive activity [8]. The importance of apoptotic/antiapoptotic balance is revealed by complex regulatory cascades involving multiple factors, such as the CD40 ligand or members of the Bcl-2 family, which suppress apoptosis, whereas Fas ligand, tumor necrosis factor-α (TNFα) and cytokines shift the balance toward pro-apoptotic signaling [46]. This delicate balance might be significantly impaired in sepsis and may represent a new target for future scientific exploration [47]. A recent study in a septic group of patients showed for the first time that the upregulation of apoptotic caspases, including active and cleaved forms, is followed by an anti-apoptotic hyperexpression, indicated by increased survivin serum levels, along with increased intracellular survivin transcript variants concentrations in early-onset sepsis [48]. The same study also showed that survivin is correlated with IL-8 and caspase-9, achieving the best sepsis discrimination and outcome predictive ability. Among critically-ill non-survivors, increased survivin serum protein levels were recorded, along with survivin wild-type, -2B and ΔEx3 splice variants gene expression upregulation [48].

3. The "Cytokine Storm" and the Pro-homeostatic Nature of Apoptosis

The underlying pathobiology of sepsis is a variable cytokine release by activated leukocytes and the development of systemic inflammation. Multiple studies indicate that there is an extensive cross-talk between apoptosis and cytokine production during various infectious processes [49-51]. It is increasingly becoming clear that sepsis is a dynamic disorder, resulting from imbalances in the immune response and inflammatory network, which is characterized as a "cytokine storm", through pyroptosis-mediated rupture of plasma membrane, resulting in excessive release of inflammatory mediators. A published study has focused on the important role of neutrophil pyroptosis as an inflammatory signal amplifier in the recruitment of immune cells, through the release of IL-1β and IL-18, which have been shown to be the most important cytokines during pyroptosis. On the other hand, some subsets of neutrophils in septic patients secrete large amounts of IL-10, which lead to the suppression of T lymphocyte proliferation and to immunosuppression [27]. An immunoinflammatory response, when moderately regulated, can provide an effective defense against the invading pathogen. A severe immunoinflammatory dysfunction, expressed by excessive pyroptotic activation or immunosuppression, commonly leads to multi-organ failure and poor outcome. Moreover, accumulating evidence suggests that the inflammasome, an intracellular multiprotein complex, is involved in the pathogenesis of sepsis, and triggers pyroptosis in a caspase-1-dependent manner [28]. This process is triggered by reactive oxygen and nitrogen-oxygen species, leading to mitochondrial dysfunction, tissue injury, organ failure and death. The initial hyperinflammatory phase may be followed

by later stages of immunosuppression and apoptosis in the course of the septic process. The pro-homeostatic nature of apoptotic cell interaction with the immune system has recently been described and is illustrated in known apoptotic cell signaling events in macrophages and dendritic cells, mainly related to toll-like receptors (TLRs) or nuclear factor κB (NF-κB). Therefore, apoptotic cells seem to have a beneficial effect on cytokine storms, ultimately leading to both anti- and pro-inflammatory cytokine signaling suppression [52].

4. The Anti-apoptotic Effects of the Heat Shock Protein Family

The heat shock response is a highly conserved cellular mechanism that protects against injury and environmental stresses. Heat shock proteins (HSPs) are molecular chaperokines that exhibit sophisticated protection mechanisms, acting as alarmins and cellular housekeepers during homeostasis. Intracellular HSPs govern protein assembly, folding, or transport and act as anti-apoptotic regulators of cell signaling pathways leading to cell death. Thereby, the expression of HSPs seems to promote cell survival upon exposure to different stressors. The most characteristic model for this response is in sepsis or inflammation-induced conditions, resulting from complex interactions between host and infectious agents. Many studies have reported the extracellular release of HSPs (eHSPs) in severe sepsis and the simultaneous reduction of intracellular HSPs (iHSPs). However, in patients with traumatic inflammation (systemic inflammatory response syndrome - SIRS), iHSPs were found increased compared to healthy controls, indicating a protective function of iHSPs during the acute phase of stress. On the other hand, the downregulation of iHSPs in severe sepsis seems to be a result of adaptation (or maladaptation) to the severity of illness [53]. Through complicated cascades these molecules can initiate both innate and adaptive immune responses and aid in immune surveillance via cytokine and chemokine production, although HSPs' upregulation has also been linked to worse outcomes and mortality.

The main HSP representatives in sepsis are Heat Shock Proteins-70 and -90 (HSP70 and 90), which have been identified on the surface of human macrophages, acting as ubiquitous chaperones with anti-apoptotic and immunomodulatory functions. Recent studies have already identified that HSPs seem to be key determinants of cell survival and can modulate apoptosis by directly interacting with components of the apoptotic machinery [54]. The most widely studied chaperone, the intracellular HSP90 (iHSP90), has been shown to stimulate apoptotic cascades through caspase-3 activation in septic models [55]. Acting as a TLR agonist, extracellular HSP70 seems to have immunomodulatory effects, since it is responsible for early immune hyperactivation and subsequent immunosuppression in the course of severe sepsis, through TLR agonists tolerance induction [56]. Intracellular HSP70, on the other hand, exerts anti-apoptotic effects, protecting the structural integrity of cells and prolonging leukocyte survival [57]. Current research has showed that extracellular HSP90 and, to a lesser extent, HSP72 are markedly elevated in children with severe sepsis compared to non-infectious SIRS or controls,

and independently related to severity of illness scores and risk of mortality. This process probably concerns the patients that might enter a state of profound immunosuppression or persistent activation of innate immunity, with intractable outcomes and high risk of mortality [58]. The immunomodulatory in-vitro effects of the heat shock response on apoptotic regulation have been recently demonstrated. HSP90 inhibition significantly reduced CD4 protein expression on T lymphocytes at the cell surface and intracellular level and downregulated key activating receptors on CD3(+) and CD8(+) T cell subsets and NK cells, disrupting their cellular activation, proliferation, and IFN-γ production [59].

5. Oxidative Stress and Apoptotic Signaling Cascades

Infection triggers the release of pro-inflammatory cytokines and production of reactive oxygen species, increasing oxidative stress and mitochondrial damage. This ultimately leads to the release of cytochrome c into the cytosol and to the initiation of autophagic activities in various cell types during the hyperdynamic phase of sepsis. Loss of mitochondrial membrane potential accompanied by the release of hydrogen peroxide and mitochondrial DNA release into systemic circulation seem to be closely connected to apoptosis. In this regard, mitophagy is thought to play an important role in eliminating defective mitochondria and, thus, protecting the host against oxidative injury and inflammatory hyperresponsiveness. The association between mitochondrial dysfunction and sepsis severity has been addressed in several studies. Dysregulated reactions, taking place in mitochondria during sepsis, such as disruption of intracellular redox homeostasis and loss of mitochondrial integrity, are closely interconnected with uncontrolled cytokine release and apoptotic signaling cascades [7,60,61]. In this context, a growing body of evidence suggests that the inability of cells to consume oxygen may play a crucial role for sepsis pathogenesis. Oxidative stress is common in critical illness, as a result of the generation of oxygen and nitrogen-derived free radicals and disruption of redox signaling or antioxidant control systems, including the decline in key antioxidant compounds [62]. According to the “redox hypothesis” the oxidation of intra- or extracellular thiols, along with radical-derived, non-radical reactive species, play an important role in the development of oxidative stress, which is hazardous for living organisms. Critically ill patients suffer from oxidative stress due to systemic activation of immune response, repression of innate immunity, and hypoperfusion/reperfusion injury. The consequences of oxidative stress include protein oxidation, lipid peroxidation and DNA damage, which may damage all major cellular constituents and contribute to cellular and organ dysfunction in ICU patients [63]. Taken together, reactive oxygen species, and the resulting redox change could be a part of signal transduction pathway during apoptosis [64].

6. Immunoparalysis through Uncontrolled Apoptosis in Sepsis

Sepsis is a struggle between pathogen-associated molecules and the host innate and

acquired immunity. Proper immunologic balance between pro- and anti-inflammatory pathways is necessary for recovery. A state of acquired immunodeficiency, with persistence of marked compensatory anti-inflammatory immune responses in sepsis, is referred to the process of immunoparalysis (endotoxin tolerance phenomenon). This is an immune cell alteration induced by uncontrolled apoptosis, which is indicated by depressed monocyte biomarker levels. Immune cell apoptosis is being increasingly recognized as a key factor in the pathophysiology of septic complications, acting either concomitantly with the initial inflammation or delayed [5,22]. Apart from the usual scenario of an early hyper-inflammation followed by a transitory immunosuppressive state until complete recovery, currently, another post-aggressive immunodepression state (PAID) has been well characterized and is being thought to account for increased mortality. This category includes those patients who survive the acute phase of sepsis but display long-term impairments in immune function due to quantitative and qualitative alterations of many immune cell populations, leading to increased susceptibility to secondary infections, viral reactivations and decreased survival [65,66]. As the host recovers from the initial septic event, the immune system becomes hyporesponsive, resulting in a long-lasting immunosuppressive state with increased mortality rates. This is probably the reason why the acute cytokine storm is responsible for only about 30% of the sepsis-related mortality [67]. Recruitment and migration of neutrophils to infectious foci is diminished and neutrophil function is significantly affected during sepsis, representing a loss of a crucial arm of the innate immune response to infection [68]. Among various alterations, lymphocyte loss is a major cause for immunosuppression, and apoptosis has been proposed as the main mechanism for lymphocyte death in sepsis. Particularly, apoptosis of lymphocytes that results in impaired innate and adaptive immune response is associated with a higher risk of poor outcomes. In addition, persistence of this severe lymphopenia over time has also been associated with an increased risk of death [8,69]. Therefore, therapeutic interventions targeting the inhibition of apoptotic molecules might be promising for an optimal outcome of septic patients. Although circulating lymphocytes undergo significant apoptosis, no apparent apoptosis in the heart, kidneys, lungs or other substantive organs occurs during the septic course [5]. Apart from sepsis, PAID and severe apoptosis-induced lymphopenia may concern trauma, ischemia-reperfusion syndrome, major surgery or acute brain injury. A rough quantification of immunoparalysis can be made through the measurement of monocyte cell-surface HLA-DR expression. HLA-DR has been shown to be negatively influenced by the presence of septic shock and to correlate with sepsis mortality. In fact, a threshold of <30% HLA-DR+ monocytes has become an accepted definition of immunoparalysis [70]. Additionally, decreased expression of HLA-DR could reduce the ability of monocytes/macrophages to present antigens and prime B and T cell responses. Similar decreasing trends in the expression of CD14/HDL-DR were associated with repressed intracellular HSP70 and HSP90 levels in patients with sepsis compared to SIRS and healthy controls. These early-onset changes in HSP70 and HSP90 monocyte and neutrophil expressions are associated either with increased cytokine levels, such as IL-6 and

IL-10, or with decreased IL-17 and IFN-γ levels in patients with sepsis [71]. Quantification of the capacity of whole blood to produce the proinflammatory cytokine tumor necrosis factor (TNF)-α, IL-1 and IL-6 after stimulation with lipopolysaccharide (LPS) has been used as a second biomarker of monocyte function and of immunoparalysis in critical illness. This is because stimulation of the Toll-like receptor (TLR)-4 complex on monocytes normally induce rapid TNFα production to more than 200pg/mL [72]. Besides the reduced TNF production and endotoxin tolerance, monocytes also exhibit an increased phagocytic ability with a conserved capacity to kill internalized pathogens, but with impaired antigen-presentation and chemotaxis capacities. They also tend to be reprogrammed towards the production of immunosuppressive molecules, such as IL-10 [22].

Recent important findings also suggest that autophagy plays a protective role in early stages of sepsis partially preserving mitochondrial integrity and halting apoptotic cascades, while autophagy suppression at late septic stages might be ultimately leading to pyroptosis and multi-organ failure [7]. Numerous promising targets, aiming at "reboosting" the immune system during the immunoparalysis phase of sepsis, are currently under investigation. In recent studies, Interferon-γ and GM-CSF therapeutic protocols attenuated the reduction of the LPS-induced TNF-α response and increased monocyte HLA-DR expression, confirming beneficial immunostimulatory effects of these agents [66].

7. Hormonal, Metabolic and Immune System Interactions Through Apoptotic Regulation

Sepsis is accompanied by major hormonal, metabolic and immune system alterations. Crosstalk between the immune and neuroendocrine networks is essential to mount an adequate response to sepsis. In this respect, upregulated inflammatory cytokines, especially IL-6, TNF-α, and IL-1, not only act as potent pyrogens to induce fever, but also stimulate neurohumolar organs signaling the invasion of pathogens [73]. Heat shock proteins seem to reflect hormonal changes taking place during an inflammatory or septic process, presenting close correlations with the activation of the hypothalamic-pituitary-adrenal axis and cortisol upregulation [53]. The acute neuroendocrine changes are probably directed toward restoring homeostasis and limiting unnecessary energy consumption. Moreover, a controlled apoptotic response during sepsis seems to promote homeostasis, since recent studies have announced that apoptotic cell uptake increases glycolysis within phagocytes, contributing to lactate release and to the reinstatement of an anti-inflammatory tissue environment [52]. Critical illness is characterized by dysregulation of the hypothalamopituitary axis, and in particular by hypometabolism, protein breakdown, hyperglycemia and altered serum lipid profile. Therefore, although the initial neurohormonal changes aim at restoring homeostasis and limiting unnecessary energy consumption, maladaptive alterations seem to prevail in the prolonged phase of critical illness, which is characterized by a central suppression of the neuroendocrine axis [73,74].

8. Conclusions

Sepsis is associated with activation of multiple proinflammatory mediators, through the transcriptional activation of interleukins, HSPs, or oxidative stress factors, which are closely interconnected with apoptotic effectors stimulation. Although these responses are important for host defense against invading bacteria, their uncontrollable and excessive reprogramming ultimately contributes to multiple organ injury. Combined apoptotic, anti-apoptotic, inflammatory, and oxidative mediators, might prove to be critical biomarkers for the prognostication of sepsis. Regarding the apoptotic/antiapoptotic dysregulation in severe sepsis, caspases inhibition or reinforcement of survivin protein signals, might open-up new therapeutic pathways in order to achieve better outcomes for septic patients. Likely, more research in the future might lead to a more individualized approach to the treatment of severe sepsis/septic shock, taking into account genetic signatures, along with all the damaged metabolic and molecular pathways. This could be crucial towards the clinical efforts to restore the imbalance of the apoptotic/anti-apoptotic response during the different hyperinflammatory or hypoinflammatory stages in the course of sepsis.

9. References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001 Jul;29(7):1303–10.

2. Boomer JS, Green JM, Hotchkiss RS. The changing immune system in sepsis: is individualized immuno-modulatory therapy the answer? Virulence. 2014 Jan 1;5(1):45–56.

3. Dhar A, Castillo L. Insulin resistance in critical illness. Curr Opin Pediatr. 2011 Jun;23(3):269–74.

4. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent J-L. Sepsis and septic shock. Nat Rev Dis Primers. 2016 Jun 30;2:16045.

5. Cao C, Yu M, Chai Y. Pathological alteration and therapeutic implications of sepsis-induced immune cell apoptosis. Cell Death Dis. 2019 Oct 14;10(10):782.

6. Tang BM, Huang SJ, McLean AS. Genome-wide transcription profiling of human sepsis: a systematic review. Crit Care. 2010;14(6):R237.

7. Ho J, Yu J, Wong SH, Zhang L, Liu X, Wong WT, et al. Autophagy in sepsis: Degradation into exhaustion? Autophagy. 2016 Jul 2;12(7):1073–82.

8. Girardot T, Rimmelé T, Venet F, Monneret G. Apoptosis-induced lymphopenia in sepsis and other severe injuries. Apoptosis. 2017 Feb;22(2):295–305.

9. Peterson LW, Philip NH, Dillon CP, Bertin J, Gough PJ, Green DR, et al. Cell-Extrinsic TNF Collaborates with TRIF Signaling To Promote Yersinia-Induced Apoptosis. J Immunol. 2016 Nov 15;197(10):4110–7.

10. Jarrah AA, Schwarskopf M, Wang ER, LaRocca T, Dhume A, Zhang S, et al. SDF-1 induces TNF-mediated apoptosis in cardiac myocytes. Apoptosis. 2018 Jan;23(1):79–91.

11. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. JCI Insight. 2020 Sep 3;5(17).

12. Nežić L, Amidžić L, Škrbić R, Gajanin R, Nepovimova E, Vališ M, et al. Simvastatin Inhibits Endotoxin-Induced Apoptosis in Liver and Spleen Through Up-Regulation of Survivin/NF-κB/p65 Expression. Front Pharmacol. 2019;10:54.

13. Shinozaki S, Inoue Y, Yang W, Fukaya M, Carter EA, Yu Y-M, et al. Farnesyltransferase inhibitor improved survival following endotoxin challenge in mice. Biochem Biophys Res Commun. 2010 Jan 15;391(3):1459–64.

14. Briassoulis G, Galani A. Prognostic markers of pediatric meningococcal sepsis. Expert Rev Anti Infect Ther. 2014 Sep;12(9):1017–20.

15. Ilia S, Briassoulis G. Sepsis is change and flows ever onwards. Expert Rev Anti Infect Ther. 2017 Jun;15(6):515–7.

16. Creagh EM. Caspase crosstalk: integration of apoptotic and innate immune signalling pathways. Trends Immunol. 2014 Dec;35(12):631–40.

17. Henson PM, Bratton DL. Antiinflammatory effects of apoptotic cells. J Clin Invest. 2013 Jul;123(7):2773–4.

18. Green DR. Apoptotic pathways: the roads to ruin. Cell. 1998 Sep 18;94(6):695–8.

19. Man SM, Karki R, Kanneganti T-D. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. Immunol Rev. 2017;277(1):61–75.

20. Jorgensen I, Miao EA. Pyroptotic cell death defends against intracellular pathogens. Immunol Rev. 2015 May;265(1):130–42.

21. Aziz M, Jacob A, Wang P. Revisiting caspases in sepsis. Cell Death Dis. 2014 Nov 20;5:e1526.

22. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nat Rev Nephrol. 2018 Feb;14(2):121–37.

23. Pavlidou A, Kroupis C, Goutas N, Dalamaga M, Dimas K. Validation of a real-time quantitative polymerase chain reaction method for the quantification of 3 survivin transcripts and evaluation in breast cancer tissues. Clin Breast Cancer. 2014 Apr;14(2):122–31.

24. Bok K, Prikhodko VG, Green KY, Sosnovtsev SV. Apoptosis in murine norovirus-infected RAW264.7 cells is associated with downregulation of survivin. J Virol. 2009 Apr;83(8):3647–56.

25. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol. 2007;47:143–83.

26. Herr AB. Evolution of an allosteric “off switch” in apoptotic caspases. J Biol Chem. 2018 Apr 13;293(15):5462–3.

27. Liu L, Sun B. Neutrophil pyroptosis: new perspectives on sepsis. Cell Mol Life Sci. 2019 Jun;76(11):2031–42.

28. Gao Y-L, Zhai J-H, Chai Y-F. Recent Advances in the Molecular Mechanisms Underlying Pyroptosis in Sepsis. Mediators Inflamm. 2018;2018:5823823.

29. Singh N, Inoue M, Osawa R, Wagener MM, Shinohara ML. Inflammasome expression and cytomegalovirus viremia in critically ill patients with sepsis. J Clin Virol. 2017 Aug;93:8–14.

30. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, López RO, Ferreres J, et al. Sustained high serum caspase-3 concentrations and mortality in septic patients. Eur J Clin Microbiol Infect Dis. 2018 Feb;37(2):281–8.

31. Zhang Y, Feng Q, Zhou S, Chen H. Downregulation of serum survivin correlates with increased inflammation, enhanced disease severity and worse prognosis in sepsis patients. Medicine (Baltimore). 2020 Jul 10;99(28):e20272.

32. Filipchiuk C, Laganà AS, Beteli R, Ponce TG, Christofolini DM, Martins Trevisan C, et al. BIRC5/Survivin Expression as a Non-Invasive Biomarker of Endometriosis. Diagnostics (Basel). 2020 Jul 30;10(8).

33. Liu Y, Lear T, Iannone O, Shiva S, Corey C, Rajbhandari S, et al. The Proapoptotic F-box Protein Fbxl7 Regulates Mitochondrial Function by Mediating the Ubiquitylation and Proteasomal Degradation of Survivin. J Biol Chem. 2015 May 8;290(19):11843–52.

34. Pavlyukov MS, Antipova NV, Balashova MV, Vinogradova TV, Kopantzev EP, Shakhparonov MI. Survivin Monomer Plays an Essential Role in Apoptosis Regulation. J Biol Chem. 2011 Jul 1;286(26):23296–307.

35. Wheatley SP, Altieri DC. Survivin at a glance. J Cell Sci. 2019 Apr 4;132(7).

36. Gu J, Ji Z, Li D, Dong Q. Proliferation inhibition and apoptosis promotion by dual silencing of VEGF and Survivin in human osteosarcoma. Acta Biochim Biophys Sin (Shanghai). 2019 Jan 1;51(1):59–67.

37. Gravina G, Wasén C, Garcia-Bonete MJ, Turkkila M, Erlandsson MC, Töyrä Silfverswärd S, et al. Survivin in autoimmune diseases. Autoimmun Rev. 2017 Aug;16(8):845–55.

38. Ebrahimiyan H, Aslani S, Rezaei N, Jamshidi A, Mahmoudi M. Survivin and autoimmunity; the ins and outs. Immunol Lett. 2018 Jan;193:14–24.

39. Moniri Javadhesari S, Gharechahi J, Hosseinpour Feizi MA, Montazeri V, Halimi M. Transcriptional expression analysis of survivin splice variants reveals differential expression of survivin-3α in breast cancer. Genet Test Mol Biomarkers. 2013 Apr;17(4):314–20.

40. Sah NK, Seniya C. Survivin splice variants and their diagnostic significance. Tumour Biol. 2015 Sep;36(9):6623–31.

41. Mokuda S, Miyazaki T, Ito Y, Yamasaki S, Inoue H, Guo Y, et al. The proto-oncogene survivin splice variant 2B is induced by PDGF and leads to cell proliferation in rheumatoid arthritis fibroblast-like synoviocytes. Sci Rep. 2015 May 22;5:9795.

42. Mokuda S, Miyazaki T, Ubara Y, Kanno M, Sugiyama E, Takasugi K, et al. CD1a+ survivin+ dendritic cell infiltration in dermal lesions of systemic sclerosis. Arthritis Res Ther. 2015 Sep 30;17:275.

43. Vivas-Mejia PE, Rodriguez-Aguayo C, Han H-D, Shahzad MMK, Valiyeva F, Shibayama M, et al. Silencing survivin splice variant 2B leads to antitumor activity in taxane--resistant ovarian cancer. Clin Cancer Res. 2011 Jun 1;17(11):3716–26.

44. Turkkila M, Andersson KME, Amu S, Brisslert M, Erlandsson MC, Silfverswärd S, et al. Suppressed diversity of survivin splicing in active rheumatoid arthritis. Arthritis Res Ther. 2015 Jul 10;17:175.

45. Knauer SK, Bier C, Schlag P, Fritzmann J, Dietmaier W, Rödel F, et al. The survivin isoform survivin-3B is cytoprotective and can function as a chromosomal passenger complex protein. Cell Cycle. 2007 Jun 15;6(12):1502–9.

46. Vetvicka V, Laganà AS, Salmeri FM, Triolo O, Palmara VI, Vitale SG, et al. Regulation of apoptotic pathways during endometriosis: from the molecular basis to the future perspectives. Arch Gynecol Obstet. 2016 Nov;294(5):897–904.

47. Hotchkiss RS, Colston E, Yende S, Angus DC, Moldawer LL, Crouser ED, et al. Immune Checkpoint Inhibition in Sepsis: A Phase 1b Randomized, Placebo-Controlled, Single Ascending Dose Study of Antiprogrammed Cell Death-Ligand 1 Antibody (BMS-936559). Crit Care Med. 2019 May;47(5):632–42.

48. Miliaraki M, Briassoulis P, Ilia S, Polonifi A, Mantzourani M, Briassouli E, et al. Survivin and caspases serum protein levels and survivin variants mRNA expression in sepsis. Sci Rep. 2021 Jan 13;11(1):1049.

49. DosReis GA, Ribeiro-Gomes FL, Guillermo LVC, Lopes MF. Cross-talk between apoptosis and cytokines in the regulation of parasitic infection. Cytokine Growth Factor Rev. 2007 Apr;18(1–2):97–105.

50. Morrow KN, Coopersmith CM, Ford ML. IL-17, IL-27, and IL-33: A Novel Axis Linked to Immunological Dysfunction During Sepsis. Front Immunol. 2019;10:1982.

51. Sharma R, Anker SD. Cytokines, apoptosis and cachexia: the potential for TNF antagonism. Int J Cardiol. 2002 Sep;85(1):161–71.

52. Karbian N, Abutbul A, El-Amore R, Eliaz R, Beeri R, Reicher B, et al. Apoptotic cell therapy for cytokine storm associated with acute severe sepsis. Cell Death Dis. 2020 Jul 15;11(7):535.

53. Vardas K, Apostolou K, Briassouli E, Goukos D, Psarra K, Botoula E, et al. Early response roles for prolactin cortisol and circulating and cellular levels of heat shock proteins 72 and 90α in severe sepsis and SIRS. Biomed Res Int. 2014;2014:803561.

54. Kennedy D, Jäger R, Mosser DD, Samali A. Regulation of apoptosis by heat shock proteins. IUBMB Life. 2014 May;66(5):327–38.

55. Li X, Luo R, Jiang R, Meng X, Wu X, Zhang S, et al. The role of the Hsp90/Akt pathway in myocardial calpain-induced caspase-3 activation and apoptosis during sepsis. BMC Cardiovasc Disord. 2013 Feb 20;13:8.

56. Tulapurkar ME, Ramarathnam A, Hasday JD, Singh IS. Bacterial lipopolysaccharide augments febrile-range hyperthermia-induced heat shock protein 70 expression and extracellular release in human THP1 cells. PLoS One. 2015;10(2):e0118010.

57. Gupta A, Cooper ZA, Tulapurkar ME, Potla R, Maity T, Hasday JD, et al. Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release. J Biol Chem. 2013 Jan 25;288(4):2756–66.

58. Fitrolaki M-D, Dimitriou H, Venihaki M, Katrinaki M, Ilia S, Briassoulis G. Increased extracellular heat shock protein 90α in severe sepsis and SIRS associated with multiple organ failure and related to acute inflammatory-metabolic stress response in children. Medicine (Baltimore). 2016 Aug;95(35):e4651.

59. Bae J, Munshi A, Li C, Samur M, Prabhala R, Mitsiades C, et al. Heat shock protein 90 is critical for regulation of phenotype and functional activity of human T lymphocytes and NK cells. J Immunol. 2013 Feb 1;190(3):1360–71.

60. Mantzarlis K, Tsolaki V, Zakynthinos E. Role of Oxidative Stress and Mitochondrial Dysfunction in Sepsis and Potential Therapies. Oxid Med Cell Longev. 2017;2017:5985209.

61. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med. 2010 Mar 15;48(6):749–62.

62. Kumar S, Gupta E, Kaushik S, Kumar Srivastava V, Mehta SK, Jyoti A. Evaluation of oxidative stress and antioxidant status: Correlation with the severity of sepsis. Scand J Immunol. 2018 Apr;87(4):e12653.

63. Goodyear-Bruch C, Pierce JD. Oxidative stress in critically ill patients. Am J Crit Care. 2002 Nov;11(6):543–51; quiz 552–3.

64. Kannan null, Jain null. Oxidative stress and apoptosis. Pathophysiology. 2000 Sep;7(3):153–63.

65. Martin MD, Badovinac VP, Griffith TS. CD4 T Cell Responses and the Sepsis-Induced Immunoparalysis State. Front Immunol. 2020;11:1364.

66. Rimmelé T, Payen D, Cantaluppi V, Marshall J, Gomez H, Gomez A, et al. IMMUNE CELL PHENOTYPE AND FUNCTION IN SEPSIS. Shock. 2016 Mar;45(3):282–91.

67. Sjaastad FV, Condotta SA, Kotov JA, Pape KA, Dail C, Danahy DB, et al. Polymicrobial Sepsis Chronic Immunoparalysis Is Defined by Diminished Ag-Specific T Cell-Dependent B Cell Responses. Front Immunol. 2018;9:2532.

68. Kovach MA, Standiford TJ. The function of neutrophils in sepsis. Curr Opin Infect Dis. 2012 Jun;25(3):321–7.

69. Jiang W, Zhong W, Deng Y, Chen C, Wang Q, Zhou M, et al. Evaluation of a combination “lymphocyte apoptosis

model" to predict survival of sepsis patients in an intensive care unit. BMC Anesthesiol. 2018 Jul 18;18(1):89.

70. Frazier WJ, Hall MW. Immunoparalysis and adverse outcomes from critical illness. Pediatr Clin North Am. 2008 Jun;55(3):647–68, xi.

71. Papadopoulos P, Pistiki A, Theodorakopoulou M, Christodoulopoulou T, Damoraki G, Goukos D, et al. Immunoparalysis: Clinical and immunological associations in SIRS and severe sepsis patients. Cytokine. 2017 Apr;92:83–92.

72. Hall MW, Knatz NL, Vetterly C, Tomarello S, Wewers MD, Volk HD, et al. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. Intensive Care Med. 2011 Mar;37(3):525–32.

73. Ingels C, Gunst J, Van den Berghe G. Endocrine and Metabolic Alterations in Sepsis and Implications for Treatment. Crit Care Clin. 2018 Jan;34(1):81–96.

74. Briassoulis G, Venkataraman S, Thompson A. Cytokines and metabolic patterns in pediatric patients with critical illness. Clin Dev Immunol. 2010;2010:354047.