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REVIEW

Apoptosis-induced lymphopenia in sepsis and other severe injuries

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Abstract Sepsis and other acute injuries such as severe trauma, extensive burns, or major surgeries, are usually followed by a period of marked immunosuppression. In particular, while lymphocytes play a pivotal role in immune response, their functions and numbers are profoundly altered after severe injuries. Apoptosis plays a central role in this process by affecting immune response at various levels. Indeed, apoptosis-induced lymphopenia duration and depth have been associated with higher risk of infection and mortality in various clinical settings. Therapies modulating apoptosis represent an interesting approach to restore immune competence after acute injury, although their use in clinical practice still presents several limitations. After briefly describing the apoptosis process in physiology and during severe injuries, we will explore the immunological consequences of injury-induced lymphocyte apoptosis, and describe associations with clinically relevant outcomes in patients. Therapeutic perspectives targeting apoptosis will also be discussed.

Keywords Apoptosis · Lymphocytes · Injury · Sepsis · Burns · Surgery · Trauma

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Abbreviations

7-AAD	7-Aminoactinomycine D
Bak	Bcl-2 antagonist/killer
Bax	Bcl-2 associated X protein
Bcl-2	B cell lymphoma 2
Bcl-xl	B cell lymphoma-extra-large
Bid	Bcl-2 homology domain 3 interacting-domain death agonist
Bim	Bcl-2-like protein 11
CD	Cluster of differentiation
CLP	Cecal ligature and punction
CTLA-4	Cytotoxic T lymphocyte associated protein 4
ELISA	Enzyme-linked immunosorbent assay
FADD	Fas-associated protein with death domain
FoxP3	Forkhead box P3
HAI	Healthcare-associated infections
HIV-1 TAT	Human immunodeficiency virus-1 trans-activator of transcription
HMGβ1	High-mobility group box 1
ICU	Intensive care unit
IFN-γ	Interferon-gamma
IL	Interleukin
ISS	Injury severity score
MyD88	Myeloid differentiation primary response gene 88
NFκB	Nuclear factor, kappa B
NK	Natural killer
PD-1	Programmed cell death
PD-L1	PD-1 ligand
Rag1	Recombination-activating gene 1
rhIL-7	Recombinant IL-7
siRNA	Small interfering RNAs
Smac	Second mitochondrial activator of caspases
TAT-BH4	Trans-activator of transcription-Bcl-2 homology domain 4

TGF-β1	Transforming growth factor, beta 1
Th2	T helper 2
TNF-α	Tumor necrosis factor-alpha
Tregs	Regulatory T cells
TUNEL	Terminal-deoxynucleotidyl-transferase-mediated dUTP nick-end labeling
z-VAD-FMK	Carbobenzoxy-valyl-alanyl-aspartyl-[*O*-ethyl]-fluoromethylketone

Introduction

Severe injuries, i.e. mainly sepsis, trauma, burns, and major surgeries, represent a real public health challenge. For example, sepsis, recently redefined as a life-threatening organ dysfunction caused by the dysregulation of the host response to an infectious process [1], represents a burden of more than 5 million deaths per year in the world [2]. In high-income countries, its incidence is estimated to 437 per 100.000 person-years in the last decade, and related hospital mortality to 17%. The most severe form of sepsis, septic shock, affects 270 per 100.000 person-years, of which more than 25% will die [2]. Healthcare-associated infections (HAI) develop in 13% of septic patients and are responsible for 11% of the observed mortality [3].

Relative to severe traumas (violence, traffic accidents), extensive burns are less frequent, with an incidence of 10 per 100.000 person-years in France in 2011, relative mortality is as high as 50% [4]. Severely burn patients are particularly susceptible to HAI, which occurrence also increases length of stay and associated costs [5, 6].

Advances in anesthesia and critical care medicine have led to the development of increasingly invasive surgical procedures. Surgeries aiming at removing invading tumors—for example esophagectomy, cystectomy, gastrectomy, pancreaticoduodenectomy—are common nowadays in high-income countries. Almost one-third of patients undergoing such highly invasive procedures develop postoperative sepsis [7]. Moreover, this complication is independently associated with long-term mortality [7]. In addition, development of infection after pancreaticoduodenectomy results in a 32% increase in total hospital costs [8]. After spine surgery, postoperative deep wound infections causes more than a doubling of related costs [9].

Although these severe injuries represent a large number of patients worldwide at high risk of death and/or HAI, the burden of injury-induced immunosuppression occurring in these patients has largely been under-recognized. Indeed, it is now well established that these severe injuries induce marked immune dysfunctions [10]. Such immune alterations have been extensively explored within the last decade in severe septic syndromes. However, it is now clear that they also occur after other acute aggressions. The resulting immunosuppression is believed to be responsible for a large part of delayed deaths after injury, as it favors viral reactivations and HAI [11, 12]. The pathophysiology of the so-called "post-aggressive immunosuppression" involves both innate and adaptive immune responses [10]. 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 and other injuries [13–15]. This review focuses on the consequences of lymphocyte apoptosis in critically ill patients after injuries and examines future therapeutic opportunities.

Apoptosis in sepsis

Apoptosis as an immunosuppressive process

Apoptosis is hypothesized to participate in the immunosuppressive process, details of which will be discussed below. Apoptosis is known to play a major role in physiology, from the embryo implantation and development to the turnover of adult tissues, as well as the selection of T cells during their maturation [16–18]. Apoptosis in cells can be induced through two main pathways. The extrinsic pathway is triggered by the binding of inducers to cell surface receptors, such as Fas, leading to subsequent caspase-8 activation. The intrinsic "mitochondrial" pathway is driven by upsetting the delicate balance between the products of pro- and anti-apoptotic genes of the Bcl-2 superfamily (B cell lymphoma 2), and by stress-induced noxious stimuli. This latter pathway is a known activator of caspase-9. Both pathways involve a common end-point, leading to caspase-3 activation and DNA fragmentation. Cross-talks exist between these two pathways, primarily through tBid, the truncated form of Bid (Bcl-2 homology domain 3 interacting-domain death agonist), causing the release of cytochrome c, endonuclease G, and Smac (second mitochondrial activator of caspases) [19, 20]. Both pathways are involved in sepsis-induced apoptosis [21–23].

Apoptosis in sepsis and immunological consequences

Apoptosis affects multiple cell types during sepsis [15]. Clinical evidences of apoptosis in splenocytes and intestinal epithelial cells were first observed in an autopsy study of adult patients who died from sepsis [13]. Similar results were later obtained in a pediatric cohort of sepsis-induced multiple organ failure [24]. Finally, the almost ubiquity of apoptosis process during sepsis was demonstrated in murine models [25, 26]. Neutrophils represent an exception, as their apoptosis is transitory decreased after septic

shock, which could participate in the major neutrophilia observed in septic patients [27].

It is now admitted that some innate immune cells such as dendritic cells, as well as cells from the adaptive immune system, especially lymphocytes, are depleted due to apoptosis early after sepsis onset [28–30]. In addition, many studies both in mice and patients showed that sepsis-induced apoptosis is driven either by extrinsic or intrinsic pathways [21–23]. Finally, post-mortem studies in septic shock patients showed that such apoptotic process occurs not only in circulating cells, but also in solid organs [14].

There are two major consequences of the apoptotic process during sepsis. First, as mentioned before, apoptotic cells present anti-inflammatory properties and induce immunological tolerance [31, 32]. For example, it has been shown that their phagocytosis inhibits pro-inflammatory cytokines production by macrophages, while increasing anti-inflammatory factors release [33]. This is concordant with observations made in sepsis, since a shift from pro- to anti-inflammatory cytokine production has been observed in monocytes from septic patients versus healthy donors [34]. Similarly, the interaction between IL-10-producing apoptotic cells and antigen-presenting cells promotes a shift towards a Th2 polarization of the immune response [35, 36]. Such shift towards Th2 cytokine production has been described after septic shock and is predictive of fatal outcome [37, 38]. Induction of FoxP3+ (forkhead box P3) regulatory T cells (Tregs) also contributes to the depressed immune response induced by apoptotic elements [39]. In elderly patients suffering from sepsis, Inoue et al. demonstrated a decrease in immunocompetent CD4+ CD28+ T cells, whereas regulatory CD4+ CD25+ FoxP3+ and immunosuppressive PD-1+ (programmed cell death 1) T cells were increased [40]. Likewise, work from our lab revealed that septic shock patients present an increased percentage of regulatory T cells, the magnitude of which seems to be associated with more intense immunoparalysis and poorer outcomes [41]. This was not due to Tregs proliferation, but rather related to the lowering of effector CD4+ T cells numeration, suggesting that Tregs may not be susceptible to apoptosis during sepsis [42]. In addition, recent data indicate that Tregs could induce CD4+ effector T cells apoptosis through a TGF-β1 (transforming growth factor, beta 1) signal, therefore increasing the anti-inflammatory response in a negative feedback loop [43].

The anti-inflammatory effect of apoptosis alters clinical outcomes. A study from Prof. Hotchkiss' team showed that, in rats, administration of apoptotic splenocytes before induction of peritonitis decreased interferon-gamma (IFN-γ) production and worsened animal survival, as compared to the transfer of necrotic splenocytes [44].

The second major consequence of apoptosis on the immune response after sepsis is the resultant massive cell loss observed in patients. While it has been described for other cell types such as dendritic cells [29], this phenomenon represents the principle cause of the severe sepsis-induced lymphopenia. This is illustrated by a study conducted in a University Hospital, which showed that, among all causes of admission, sepsis was the first etiology responsible for lymphopenia in all-cause hospitalized patients [45]. Such apoptosis-induced lymphopenia is pronounced in sepsis, since most septic patients present with <0.8 G/L absolute lymphocyte count and <0.3 G/L CD4+ lymphocytes [46]. Apoptosis-induced lymphopenia will be detailed in the next subchapter.

Apoptosis-induced lymphopenia in sepsis

Clinical demonstration

Numerous studies have investigated the occurrence of apoptosis in lymphocytes after sepsis by using different technical approaches. First, cell morphology is modified during apoptosis. This can be assessed by microscopy, showing fragmented and compacted nuclei. Cell shrinkage can also be revealed by a decrease in side and forward scatter on flow cytometric analyses, although this is largely nonspecific and rarely used in clinical studies [15]. The apoptotic signal is normally too low to be detected in healthy cells. In damaged cells, such signal is increased and might be detected and quantified by forward scatter analysis [47, 48]. Second, markers such as annexin-V, 7-AAD (7-aminoactinomycine D), APO2.7 (a protein confined to the mitochondrial membrane) [49], caspases [50, 51], among others, can be stained to assess apoptosis by flow cytometry. For example, Le Tulzo et al. showed that annexin-V staining was increased on circulating lymphocytes of sepsis and septic shock patients [52]. Similarly, Inoue et al. showed that polymicrobial sepsis induced higher percentage of caspase-3-positive splenocytes in rapidly-aging than in wild type mice [51].

Western-blot can also be used to detect caspases [53], Bid, FADD (Fas-associated protein with death domain) [54], Bcl-2, Bax (Bcl-2 associated X protein) [55], HMGβ1 (high-mobility group box 1), NFκB (nuclear factor, kappa B) [56], as well as ELISA to measure cytochrome-c concentrations [57, 58]. Such techniques have been used by Tinsley et al. and Guo et al. to demonstrate the increased thymocyte apoptosis in mice upon cecal ligature and puncture-induced sepsis [53, 57].

DNA fragmentation occurring during the late stages of apoptosis can be detected by the TUNEL (terminal-deoxynucleotidyl-transferasemediated dUTP nick-end labeling) assay [59, 60]. When apoptotic, cells are detected as TUNEL-positive by flow cytometry. In mice after cecal

ligature and punction (CLP), Unsinger et al. showed an increased percentage of TUNEL-positive CD3+ splenocytes [61], while Zou et al. found TUNEL-positive cells in thymus, spleen, and liver [50].

Studies of mRNA expression emphasized the intense modulation of gene expression during sepsis, particularly those of Bcl-2 superfamily genes. For example, the pro-apoptotic genes Bim (Bcl-2-like protein 11), Bak (Bcl-2 antagonist/killer), Bid and Fas were upregulated in septic shock patients [62, 63]. On the contrary, anti-apoptotic genes like Bcl-2 and Bcl-xl (B cell lymphoma-extra-large) were downregulated during severe sepsis [62]. These modulations in apoptosis-regulating genes may be cell-specific [64].

Overall, Le Tulzo et al. showed that lymphocyte apoptosis was increased by fivefold in septic shock patients versus controls, and by more than twofold versus other critically ill patients [52]. This major apoptotic process leads to severe lymphopenia (absolute lymphocyte count <0.5 G/L), which affects 25–63% of septic shock patients [65].

Although apoptosis results in a major decrease of lymphocyte absolute count right after the onset of septic shock [65], these alterations have been suspected to be specific to some lymphocyte subtypes. Especially, while apoptosis of CD4+ effector T cells has repeatedly been showed to be increased during sepsis not only in circulating blood cells but also in lymphoid organs, data concerning CD8+ T cells are still a subject of debate. For example, Hotchkiss et al. showed a caspase-9 mediated deletion of B and CD4+ T cells but not CD8+ or NK cells in septic patients [28]. Conversely, in a study by Le Tulzo et al. lymphopenia affected both CD4+ and CD8+ T cells subsets [52]. This is consistent with observations by Roger et al. of an enhanced apoptosis in CD4+ and CD8+ T cells during the early phase of human sepsis [66]. Poles apart, studies described that Tregs are resistant to sepsis-induced apoptosis and present pro-apoptotic effects on effector cells [67]. Overall, this strongly suggests that apoptosis favors sepsis-induced immunosuppression (Fig. 1).

Association with clinical outcomes

Numerous observational studies in clinical practice identified an association between sepsis-induced lymphopenia and poor outcomes in septic patients. For example, Chung et al. observed that the presence of severe lymphopenia at intensive care unit (ICU) admission of septic shock patients was associated with a 3.5-fold increased risk of death at day 28 [65]. In addition, persistence of this severe lymphopenia over time was also associated with poor prognosis. Drewry et al. showed that persistent lymphopenia on day 4 after the onset of sepsis predicted 28-day and 1-year survival [68]. Similarly, in children with multiple organ failure, prolonged lymphopenia for >7 days was also independently associated with an increased risk of death [24]. In fact, failure to recover a normal non-apoptotic lymphocyte count seems to be associated with higher mortality in septic shock patients [52].

Such association with increased mortality was also observed for other apoptosis markers. For example, in severe sepsis patients, caspase-cleaved cytokeratin-18 fragments concentration was higher in non-survivors than in survivors. In addition, in patients with sepsis-induced hepatic dysfunction, a higher level of total cytokeratin-18

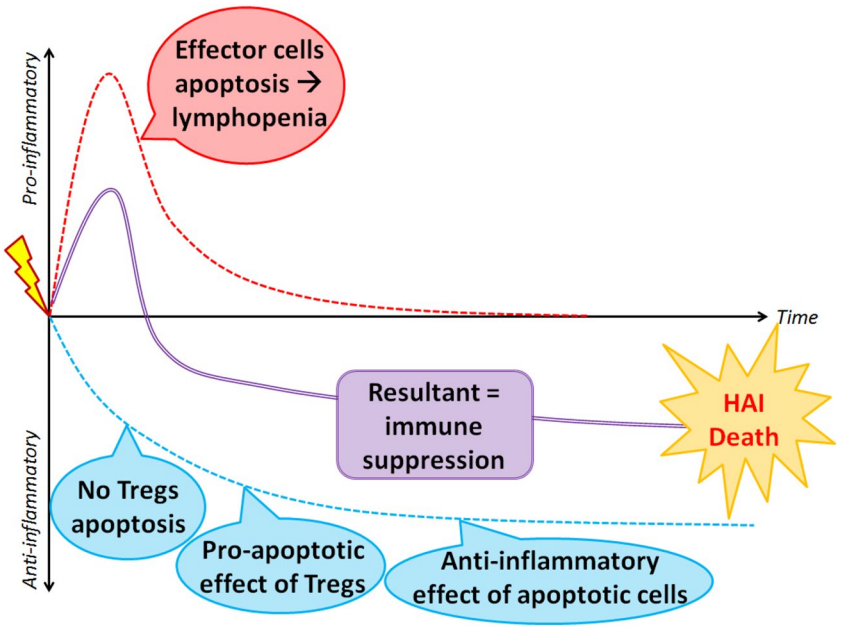

Fig. 1 Role of lymphocyte apoptosis in injury-induced immunosuppression. The initial acute injury (lightning) triggers complex pro and anti-inflammatory concomitant immune responses. Imbalance between both sides results in immunosuppression, leading to unfavorable outcomes. Lymphocyte apoptosis limits pro-inflammatory response and favors the global anti-inflammatory resultant. *HAI* healthcare-associated infections, *Tregs* regulatory T lymphocytes

at the onset of septic syndrome was also predictive for survival [69]. In a cohort of 87 septic patients, non-survivors of septic shock also presented higher levels of apoptosis markers (APO2.7 on leukocytes and lymphocytes, and 7-AAD and annexin V on monocytes) at admission to the emergency department, compared to survivors [49]. Moreover, proportion of APO2.7-positive lymphocytes predicted mortality with the same performances than lactacidemia [49].

Similar associations were observed between prolonged lymphopenia and increased risk of subsequent nosocomial infections after severe sepsis in children and adult patients [24, 68]. A pro-apoptotic gene expression pattern, with increased Bid and decreased Bcl-2 mRNA, was identified in children to predict the development of HAI after pediatric ICU admission. This was accompanied by an upregulation of anti-inflammatory cytokines gene expression [70].

Apoptosis-induced lymphopenia in other, non-septic, acute injuries

In other clinical settings and in unselected critical care patients, severe lymphopenia has been frequently observed in association with the development of injury-induced immunoparalysis and HAI [71].

Trauma

Lymphocyte apoptosis occurs early after severe trauma and is usually highest at day 3 after the injury [30, 72]. In addition, patients with higher injury severity score (ISS) exhibit more pronounced lymphopenia than less severely injured patients [30]. Lymphocyte subsets are differentially affected—CD4+ and NK T lymphocytes number is reduced, whereas CD8+ T cell number does not seem to exhibit important variations [73]. Severity of lymphopenia is associated with an increased risk of subsequent major infection or death [72]. Gouel-Chéron et al. showed that the CD4+ lymphocytes count at day 3 was lower in those patients who developed subsequent sepsis, relative to those who remained uninfected [74]. This was also observed by Heffernan et al. in a cohort of >2400 severely injured (ISS >15) patients. In this study, persistent lymphopenia over the first 4 days was independently associated with a higher risk of mortality and with an increased hospital length of stay [75].

Surgery

Major surgical procedures also affect adaptive immune response. First, various hypnotic drugs, either intravenous agents or inhaled halogenated gases, depressed the suppressor/cytotoxic lymphocyte ratio in mice, and decreased survival in a CLP model of sepsis [76]. In dogs, lymphopenia could be observed after induction of general anesthesia with thiopental, and a higher percentage of apoptotic cells was measured in animals undergoing laparotomy compared to those anesthetized but not operated [77]. In humans, pre-operative lymphopenia was associated with a 50% rise in post-operative infections [78]. Post-operative lymphocyte apoptosis observed after esophagectomy could have been induced by monocytes [79]. The intensity of lymphocyte apoptosis in the post-operative period is associated with the invasiveness of the surgical procedure. For example, surgery-induced lymphopenia was more pronounced after esophagectomy than after colorectal cancer surgery [80], whereas lymphopenia did not occur after minor surgical procedures [81]. Post-operative lymphopenia was also more common after open surgery than after laparoscopy [80]. Moreover, lymphocytes from pre-operatively lymphopenic patients exhibited increased expressions of apoptosis markers such as annexin-V and caspase-3, compared with non-lymphopenic patients [78]. T cell co-stimulatory receptor CD28 expression on T cells was markedly suppressed after major scheduled abdominal surgery, while Tregs were not affected [82]. Considering emergency intra-abdominal surgery, the lymphocyte count was shown to be decreased during the first week in non-survivors, and persistent lymphopenia was independently associated with a 3.5 fold increase in hospital mortality [83].

Burns

Following extensive burns, the total number of T lymphocytes is also drastically reduced [84]. In a murine model, depletion of lymphoid organs lasted over 2 months post-burn injury [85]. In a rat model of burns and subsequent infection, helper T cells appeared to be selectively depleted in infected animals compared to burn but non-infected ones [86]. In burn patients, a profound lymphopenia has been observed as well. While CD4+ lymphocytes are systematically affected, CD8+ count is not always reduced following thermal injury [87, 88]. Again, in this clinical context, Tregs are elevated versus controls. Moreover, their percentage is higher in non-survivors and in patients who will develop nosocomial sepsis [89].

Therapeutic perspectives

Considering the major links between injury-induced lymphocyte apoptosis and deleterious clinical outcomes, numerous therapeutic strategies aiming at restoring normal lymphocyte numbers and decreasing apoptosis have been

evaluated both in animal models and ex vivo in patients' cells.

Animal models

Many therapies have been proposed to limit lymphocyte apoptosis and its consequences during sepsis. Most of them have shown promising results, mainly in rodents. In a CLP model of polymicrobial sepsis, transgenic mice with overexpression of Bcl-2 in T lymphocytes were totally resistant to T cell apoptosis in thymus and spleen, but also developed less B cell apoptosis [90]. Moreover, mice with Bcl-2 overexpression had a lower mortality after sepsis [90, 91]. Similarly, overexpression of Bcl-xl, another anti-apoptotic gene of the Bcl-2 superfamily, reduced thymus and spleen T and B cell apoptosis as well and improved survival of septic mice [92]. As apoptosis involves many genes and pathways, they represent as many targets for gene expression modulation and putative therapeutic interventions. For example, overexpression of Akt in thymocytes and peripheral T cells of transgenic mice also decreased CD4+ T cell apoptosis via a non-Bcl-2-mediated mechanism, and improved survival in a CLP model [93]. However, to date, gene therapy appears difficult to implement in patients, and these reports rather demonstrate the interest of apoptosis modulation in sepsis than represent real ready-to-use therapies.

The balance in apoptosis regulation by Bcl-2 genes superfamily can also be modulated by antiapoptotic peptides, conjugated to permeation peptides derived from HIV-1 TAT (human immunodeficiency virus-1 trans-activator of transcription) basic domain to allow cell penetration. In vivo administration of TAT-BH4 (Bcl-2 homology domain 4) to septic mice decreased T and B cell apoptosis in splenocytes, and seemed to prevent also thymus and circulating blood T cell apoptosis [92].

Modulation can also take place at the translational level with small interfering RNAs (siRNA). Pre-treatment of mice with siRNA targeting Bim, a pro-apoptotic member of the Bcl-2 superfamily, resulted in a decrease in B and T cell apoptosis after CLP. It also conferred survival advantage to sepsis to pre-treated animals [94]. One major limitation of pre-treatment is its non-feasibility in clinical settings. However, post-exposure treatment with Fas and caspase-8 siRNA also suppressed sepsis-induced apoptosis in spleen and liver and improved mice survival after CLP [95].

Caspases are involved in both extrinsic and intrinsic apoptosis pathways. Anti-caspase therapies may therefore block sepsis-induced lymphocyte apoptosis. The broad-spectrum caspase inhibitor z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[*O*-ethyl]-fluoromethylketone) demonstrated its capacity to prevent lymphocyte apoptosis in spleen and thymus in a dose-dependent manner, and prevented mice death in a CLP model [91]. Another broad-spectrum caspase inhibitor, VX-166, lowered lymphocyte apoptosis and improved CLP mice survival [96]. Similar beneficial results on CD4+ and CD8+ T lymphocyte apoptosis and animal survival were obtained with the polycaspase inhibitor M-920 and the selective caspase-3 inhibitor M-791 [97].

An elegant therapeutic perspective is represented by protease inhibitors initially designed against HIV. Indeed, this class of antiretroviral agents is known to decrease HIV-induced CD4+ and CD8+ T cell apoptosis [98]. In a mice CLP model, pre-treatment with nelfinavir boosted by ritonavir prevented splenic and thymic lymphocyte apoptosis, increased tumor necrosis factor-alpha (TNF-α) and decreased IL-6 and IL-10 production. Moreover, it improved mice survival to sepsis, and this beneficial effect was preserved, although diminished, in case of post-CLP introduction of protease inhibitors [99]. Nelfinavir/ritonavir combination also showed its capacity to reduce apoptosis of multiple cell types in various diseases rodent models. Its *modus operandi* is located at the mitochondrial membrane level—inhibiting the loss of mitochondrial transmembrane potential by preventing the mitochondrial permeability transition pore complex adenine nucleotide translocator subunit pore function [100]. It should by highlighted that the beneficial effects of protease and caspase inhibitors are clearly mediated by lymphocytes, as Rag1−/− mice (recombination-activating gene 1), animals lacking functional lymphocytes, did not benefit these therapies [97, 99].

Post-aggressive immunoparalysis is characterized by an increased expression of co-inhibitory receptors on immune cells surface. Blockade of these receptors improves survival in experimental fungal sepsis [101], and is already used in human cancer, a condition with sepsis-like immunological defects [102]. In a mice CLP model, anti-PD-1 antibody inhibited apoptosis, thus preventing sepsis-induced lymphocyte depletion, and improved animal survival [103]. Similarly, blockade of PD-1 ligand by anti-PD-L1 antibody prevented T and B lymphocyte apoptosis in blood, spleen and thymus, enhanced bacterial clearance, and decreased mortality [104]. Inhibition of CTLA-4 (cytotoxic T lymphocyte associated protein 4), another co-inhibitory receptor, also decreased splenic T lymphocyte apoptosis in experimental polymicrobial sepsis [105].

In a murine peritonitis model, recombinant IL-7 (rhIL-7) increased splenic T lymphocyte count, probably due to the blockade of CD4+ and CD8+ spleen T cell apoptosis induced by rhIL-7 [106]. The same team observed similar increases in splenocytes count in rhIL-7-treated mice (versus septic untreated animals) after a double-hit model of sepsis, with a CLP followed by *Candida albicans* injection 4 days later. In this last study, rhIL-7 also restored IFN-γ production by CD8+ splenocytes [107]. In another study using a classic CLP model, rhIL-7 resulted in increased

Bcl-2 expression in naive and effector memory CD4+ and CD8+ splenocytes as early as 3 h after treatment onset [108]. Taken together, these results suggest that the above described effects of rhIL-7 could be related to its anti-apoptotic effects.

Other therapies, such as IL-15 [109], IL-33 [110], and agonistic anti-CD40 receptor antibody [111] have shown benefits on lymphocyte apoptosis and survival in mice models of sepsis.

Efficacy on human blood ex vivo

Encouraging results have been obtained in mice, but human cells may react in a slightly different manner than predicted by animal experiments. Evidence of anti-apoptotic effects of the previously mentioned treatments in human cells is still scarce. In human T lymphocytes co-cultured in vitro with *Escherichia coli*, apoptosis was reduced by anti-apoptotic peptides TAT-Bcl-xl and TAT-BH4 in a dose-dependent manner [92].

In human lung epithelial cells, which are also subject to sepsis-induced apoptosis, kallistatin attenuated apoptosis via down-regulating Fas/Fas-ligand signaling, reactive oxygen species formation and subsequent NF-κB activation [112]. Limiting lung cell apoptosis may prevent sepsis-induced acute lung injury and acute respiratory distress syndrome.

Co-inhibitory receptors blockade also demonstrated interesting ex vivo results in septic shock patients' blood. Chang et al. showed that anti-PD-1 and anti-PD-L1 antibodies decreased lymphocyte apoptosis and increased IFN-γ and IL-2 production [113].

Finally, work from our lab revealed that rhIL-7 was able to restore normal functions of lymphocytes purified from septic shock patients' blood. Sepsis-induced decreases in lymphocyte proliferation and IFN-γ production were corrected by rhIL-7. Furthermore, rhIL-7 increased Bcl-2 protein expression, suggesting that the induced improvements observed might be, at least partially, related to an anti-apoptotic effect [114].

Limitations

Although attractive, therapeutic strategies aiming to modulate or block apoptosis may present several limitations in patients. First, apoptosis is a physiological process that is necessary for cell homeostasis [115]. It may also be a physiological response to the initial hyper-inflammation in sepsis, counterbalancing it by its anti-inflammatory consequences. Moreover, caspases also have other non-apoptotic functions, especially on cell proliferation and differentiation of B and T cells [15]. Therefore, blockade of apoptosis could induce serious adverse events. Consistently, a study in mice with cancer showed that prevention of apoptosis by overexpression of Bcl-2 increases mortality in a pneumonia model [116]. In another study, mice deficient for MyD88—an important element in response to pathogens, linking cell surface receptors to intracellular signaling pathways—had a worsened survival to CLP despite lower sepsis-induced apoptosis [117].

Moreover, lymphocyte apoptosis and apoptosis-induced lymphopenia occur quickly after the onset of sepsis [49]. Consequently, it may not be possible in clinical settings to initiate apoptosis blockade in such a short time-frame. Therefore, treating the consequences of lymphocyte apoptosis rather than apoptosis itself may represent an interesting and feasible alternative. As mentioned previously, one of the most promising therapies to boost lymphocyte number and functions in sepsis is rhIL-7 [114]. This therapy restores T cell functions, trafficking capacities to infected *loci*, and improves survival in experimental sepsis [106]. Recently, the complementary effects of rhIL-7 and anti-PD-1 therapies in septic mice have highlighted the possible benefit of a combined treatment in septic patients [118]. Two clinical trials testing the capacity of rhIL-7 to restore immune functions of septic patients—IRIS-7A (NCT02797431) and IRIS-7B (NCT02640807)—are currently recruiting.

Conclusion

Apoptosis-induced lymphopenia is a common process during sepsis and severe injuries, such as burns, major surgeries, and trauma. This process begins early after the onset of the causative injury. Lymphopenia severity and duration are associated with poor clinical outcomes, i.e. subsequent infections and higher mortality rates. Apoptosis is one of the leading causes of injury-associated lymphopenia, and participates directly and indirectly in injury-induced immunoparalysis. Many therapies targeting apoptosis have shown promising effects on immunological parameters and survival in animal models of severe injury. Apoptosis modulation represents an exciting field for critically ill patients' treatment research, although the consequences of apoptosis blockade in patients deserve additional investigations. To date, recombinant human IL-7 represents an interesting therapy, as it may counterbalance the consequences of lymphocyte apoptosis in severely injured patients.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest related to this manuscript. All authors are employees of Hospices Civils de Lyon and Lyon 1 University (Lyon, France).

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