GENERAL REVIEWS

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Programmed cell death in sepsis in Balkan nephropathy

Programirana ćelijska smrt kod sepse u balkanskoj nefropatiji

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Introduction

Sepsis is defined as a suspected or proven infection in the systemic inflammatory response syndrome (SIRS) [1]. From the beginning of a systemic infection and over sepsis peaks of immune-mediators characteristic of SIRS and for the compensatory anti-inflammatory response syndrome (CARS) may be seen in sequence or in parallel [2] that enlighten the reason why broad investigation of inflammatory biomarkers during the last decade, including members of cytokine network versus sepsis outcome or patient survival did not satisfactorily pass the validation tests.

Inflammation biomarkers

Inflammation biomarkers were not more efficient than standard clinical parameters in the intensive care patients. Pierrakos and Vincent [3] displayed the results of a total of 3,370 studies that assessed 178 different biomarkers in sepsis, among them apoptotic related biomarkers and sepsis outcome. A relation between inflammation control and programmed cell death (PCD) – apoptosis type I both of immunocytes and parenchymal cells in sepsis development and regulation has been recognized. There are numerous examples of the dualism in activity of a stimulus in cell fate. Several proinflammatory cytokines (TNF, IL-6, IL-18) may trigger apoptosis through several caspases activation rather than inflammation stimulation. Conversely, caspases, as classical mediators of cell death may trigger apoptosis pathway or upregulate some (proinflammatory) cytokines that in turn induce cell survival and proliferation. Oligomerization of cell surface death receptor Fas, a member of TNF receptor family, by their cognate ligands results in formation of death-inducing signaling complex (DISC), additionally including adapter protein Fas associated death domain receptor (FADD) and caspase-8. The death domain (DD) in FADD interacts with DD in the cytoplasmic tail of the Fas, while the death effector domain (DED) in FADD binds to a DED within the prodomain of caspase-8. This promotes the autocatalytic activation of caspase-8, which then cleaves downstream effector caspases that eventually will induce TUNEL+ DNA fragmentation in apoptosis [4]. Caspases may signalize the pathway of antigen activation of immunocytes, when another adapter molecule named FLICE inhibitory protein (FLIP), which is the Fas inhibitory protein, be incorporated into the DISC. In that case caspase-8 may promote lymphocyte activation and proliferation [5, 6]. Cells have given alternative splicing of the *flip* gene with the possibility for the FLIPs short protein and the FLIPl long protein production. The FLIPl contains two DED domains and caspase-8 like p20 and p10 domains without enzymatic activity, so that the accumulation of FLIPl in DISC prevents recruitment of caspase-8 [7]. Newton and Strasser [4] proposed that FLIPl may act as a scaffold protein; and gathering of high amount of FLIPl and FLIPs to Fas may inhibit apoptosis, low level of FLIPl facilitates apoptosis, enabling FADD to assist in caspase-8 activation. Caspase-1 may activate caspase-3 and triggers cascade activation of enzymes that will lead to DNA fragmentation. Caspase-1 may purposely induce synthesis of IL-1 beta and IL-18 cytokines in activated monocytes in sepsis. IL-18 is a factor of potent IFN gamma induction in Th1 lymphocytes, regarding it stimulates them together with IL-12 to clonal expansion, promoting inflammation. TNF not only induces apoptosis by activating caspase-8 and -10, but can also inhibit apoptosis signaling through NF-kappa B stimulation, which induces the expression of IAP, an inhibitor of caspases-3, -7 and -9. In patients in septic shock serum caspase-1 is significantly increased [8] that may be the biomarker of dramatically

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amplified apoptosis, regarding the finding that an early apoptotic marker anexin V binding was also importantly higher than in control animals [9]. It would seem that widespread TUNEL+ apoptosis of immunocytes may be deleterious during sepsis.

Mitochondrial function

Adrie et al. [10] have shown failing mitochondrial function in circulating monocytes from 18 patients with severe sepsis. Opening of permeability transition pores in the mitochondrial inner membrane is followed by the change in mitochondrial transmembranes potential. The subsequent release of mitochondrial intermembrane proteins (cytochrome c, apoptosis-inducing factor -AIF) into the cytosol may activate caspases [11]. However, mitochondrial membrane alterations may also lead to ATP synthesis arrest with subsequent cell necrosis [12]. T lymphocyte mitochondrial alterations have also been described in septic mice [13]. Fas dead receptors may transmit proapoptotic signal into the cell, after oligomerization with soluble Fas ligands (sFasL), while soluble Fas (sFas) inhibits it with sFasL binding outside the cell. Doughty et al. [14] have shown that severe pediatric sepsis with poor survival is coincided with the rise of sFas blood levels in correlation with IL-6 and IL-10. sFasL does not increase. Pursuant to these results, the link of apoptosis prevention with sFas and systemic inflammation in severe sepsis with multiple organ failure syndrome (MOFS) has been proposed. The same has been noted in adult patients, as well [15]. Nevertheless, other investigators have shown that the increased sFas, which correlates with nitric oxide and circulating nitrates, does not suggest reduced apoptosis of blood mononuclear cells (MNC). On the contrary, a completely different expression of Fas and FasL on blood MNC has been noted suggesting high apoptosis rate of MNC in severe sepsis. Thus, correlations of raised blood IL-6 and TNF alpha with sFas level in these patients may be a reliable prognostic marker of poorer survival, but it does not imply infrequent lymphocyte and monocyte apoptosis. Instead, apoptosis is increased in sepsis [16].

Immune response

Non-survivors have shown increased number of peripheral monocytes with depolarized mitochondria prone to apoptosis. During the first days of sepsis anti-apoptotic Bcl-2 monocyte expression decreases *ex vivo* [10]. Apoptosis of monocytes manages complex immunomodulation in sepsis, and this may compromise host defense against microbes. Namely, stimulation of Th1 or Th2 rules out each other's response. Lymphocytes may exchange their roles and acquire or renew characteristics of either Th1 or Th2 cells, after antigen (re)stimulation and depending on the cytokine and co-stimulatory molecules from monocytes and dendritic cells, as professional antigen-presenting cells (APC), or under the influence of surrounding accessible cytokines. Mature dendritic cell may provide signals positive for the production of Th1 and other signals negative for the production of Th2 cells, following TLR activation on dendritic cell [17, 18]. Signals from APC influence whether the tolarogen or an active immune response would occur in lymphocytes to a particular antigen. Dendritic cell uptake of apoptotic cells in the absence of maturation signals induces tolerance [19]. Namely, type of lymphocytes death triggered by the pathogen is one of the leading mechanisms to define the immune response for inflammation or immune suppression in the development of sepsis. Macrophages and dendritic cells that phagocyte necrotic cells start inflammation by stimulation of mainly Th1 cell response, while macrophages and dendritic cells after phagocyting apoptotic cells stimulate preferentially the Th2 response. One has to bear in mind that necrotic debris may stimulate TLR and innate immunity, while apoptotic cells avoid TLR signaling and do not initiate innate immune response, which is in turn essential for adaptive immune response to microbes, consequently both would be silenced. Under certain conditions, proinflammatory cytokines may induce apoptosis of immunocytes, as it has been already explained for IL-18 [20]. Apoptosis may not potentiate synergistic stimulation between innate and adaptive pro-inflammatory response. This will support Th2 cell prevalence. Th2 anti-inflammatory cytokines may suppress and extinguish further function of antigen-presenting cells or induce their apoptosis. All these events lead to the so-called 'inflammatory immune suppression', and finally to anergy that happens in (lethal) sepsis [20]. The net result is a severely compromised innate and adaptive immune system with poorly functional "exhausted" CD8 and anergic CD4 T cells. *Post mortem* immunohistologic findings in septic patients reveal vast apoptosis of immune system cells, particularly B and CD4+ lymphocytes, as well as follicular dendritic cells [21, 22]. The finding of "waste spleen" is conspicuous, while natural killer (NK) cells and CD8+ lymphocytes are spared. This also implies systemic immune suppression when the immune cells die, instead of expected clonal expansion. Lymphocytopenia is evidenced. Also, massive apoptosis of intestinal epithelial cells and vascular endothelial cells is noted. It is present also in the kidneys, heart and liver. Apoptosis of non-immune cells also may induce hyporeactivity of monocytes or other APC following uptake of apoptotic bodies. Prevention of lymphocyte apoptosis in an experimental model of sepsis improves animal survival [22–25]. Intervention to suppress apoptosis with rIL-7 treatment may have influence on better severe sepsis outcome; however it is still an experimental effort [26].

Sepsis in patients with kidney disease

The site of prime infection, such as urosepsis seems to be also important for specific immune system modulations including apoptosis rate of monocytes, as APC, and a biomarker behavior in the progression to severe sepsis. In the field of acute pyelonephritis the expression of HLA-DR on monocytes, the rate of apoptosis of monocytes and the rate of apoptosis of NK cells decreased first 24 h of severe urosepsis/septic chock calculating in 42 patients, 9.3% with chronic renal failure (CRF), quite different from abdominal sepsis with decreasing CD8 count and apoptosis score [27]. In patients with kidney disease at least two additional factors potentially influence the sepsis course and outcome. These are the nature of underlying kidney dieses and the chronic renal fail-

ure. All these factors should be calculated to decide what a biomarker does say to us about the sepsis state and sepsis severity. In patients with CRF sepsis may be prolonged with predominant immunosupression reaction from the beginning of the sepsis. CRF is a state of chronic inflammation with remarkably deregulated monocyte function. The co-stimulation impairment for T and B lymphocytes acts together with monocyte aberrant cytokine secretion. Monocytes release more proinflammatory cytokines, and blood levels of TNF alpha, IL-1 beta, IL-6, IL-12 and IL-18 are progressively increased [28–30]. However, some lymphokines secreted by activated T cells, e.g. IFN gamma, are missing hypothetically due to poor lymphocyte function. Quite the opposite, when exposed to signals from normal APC (monocyte), isolated T and B cells from the blood of CRF or uremic patients are directed to function normally [31]. Blood cells of these patients stimulated by *Staphylococcus epidermidis* in culture realize significantly lower IFN-gamma synthesis than cells of healthy subjects [32]. It has been concluded that the link between innate and adaptive immunity is impaired in patients with CRF, resembling endotoxin tolerance.

Increased rate of monocyte and Th1 lymphocyte apoptosis in CRF is another important disorder affecting the immune response dysfunction in sepsis in these patients. Plasma of CRF patients has increased the pro-apoptotic potential to U937 monocytes in culture, correlating with TNF plasma levels and independently of IL-1, IL-2 or IL-10 [33, 34]. In CRF patients, inflammatory cytokine IL-18 may also participate in increased apoptosis rate of Th1, monocytes or parenchymal cells, via Fas system [35].

Sepsis in patients with Balkan endemic nepharopathy and associated upper-urothelial carcinoma

Especially intriguing is the occurrence of post-operation sepsis in the patients with Balkan endemic nephropathy (BEN) and associated upper urothelial carcinoma (UEM), which is highly prevalent malignancy in endemic areas [36, 37]. BEN is slowly progressive tubulointerstitial disease, now regarded as toxic (possibly aristolochic acid) nephropathy. Low pro-inflammatory immune response may explain almost acellular foci of interstitial fibrosis that surround progressive tubule atrophy. Savin et al. [38] discovered considerable tubule cell apoptosis in BEN that is greatly important in disease development and one may describe pathogenesis of BEN as a human apoptotic model of kidney injury [39]. In addition, half of BEN patients may develop CRF, which additionally manages apoptosis increase in sepsis. Petkovic [40] originally displayed endemic appearance of upper-UEM in Serbia, and noticed an extraordinary favorable outcome of these patients after nephroureterectomy, for even 20 years, and a 5-year survival rate was 72% for the conservative kidney operation in the Urology Clinic, Clinical Centre of Serbia, Belgrade. The same survival trend has been shown by a more detailed epidemiological investigation of the endemic village Petka in Serbia by Radovanović et al. [36]. Later on, Petronić et al. [37] suggested slow growth of these tumors in BEN patients on hemodialysis; a new or recurring urothelial carcinoma has been evidenced in 20% of patients for 5–12 years, and that indirectly imply a long survival of patients from BEN regions.

Pyelonephritis is common in patients with BEN and urothelial carcinoma, practically the same as in the patients with upper-UEM outside endemic regions. It is rational expecting greater incidence of postsurgical (uro)sepsis in BEN patients with upper-UEM and worse outcome of BEN patients in sepsis in the setting of chronic exposure to environmental toxin attacks that induce apoptotic injury of the kidney, as well. Surprisingly, by our pilot study sepsis following surgical removal of the kidney similarly occurred in patients with BEN from affected households, as in upper-UEM patients without BEN outside endemic regions (27.3% and 30%, respectively), regardless more advanced azotemia detected in BEN patients in sepsis ($p = 0.008$). Furthermore, analysis of the patient survival *vs.* sepsis after total nephrouterectomy due to upper-UEM (n = 37) denied an influence of added deleterious factors – BEN or chronic renal insufficiency in poor outcome, excepting unfavorable long-lasting effect on chronic hemodialysis, and great apoptosis in tumor before sepsis in BEN patients [41]. A possible explanation is that TUNEL+ apoptosis (PCD type I) is not the only apoptotic form in BEN, as concomitant autophagy (PCD type II) may play a protective role against toxic (kidney) injury in these patients, at least on glomerular cells.

It would be of interest to analyze the influence of those "chronic" apoptosis attacks of renal tubular cells, such as in BEN, and sepsis outcome initiated from different localization of primary infection, outside the urinary tract that may open a new approach to patients with particular tumor origin and sepsis [42].

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