TITLE: THE ROLE OF ADJUNCTIVE THERAPIES IN SEPTIC SHOCK BY GRAM NEGATIVE MDR/XDR INFECTIONS

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SUMMARY

Patients with septic shock by multidrug resistant microorganisms (MDR) are a specific sepsis population with a high mortality risk. The exposure to an initial inappropriate empiric antibiotic therapy has been considered responsible for the increased mortality, although other factors such as immune-paralysis seem to play a pivotal role. Therefore, beyond conventional early antibiotic therapy and fluid resuscitation, this population may benefit from the use of alternative strategies aimed to support the immune system. In this review we present an overview of the relationship between MDR infections and immune-response and focus on the rationale and the clinical data available on the possible adjunctive immunotherapies, including blood purification techniques and different pharmacological approaches.

INTRODUCTION

Since early 90s, the American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference has placed great emphasis to sepsis and its definition [1]. The definitions issued by 1991 Consensus Conference are the followings: sepsis was defined as an infectious insult with systemic inflammatory response syndrome (SIRS) while severe sepsis has been associated with organ dysfunction and septic shock, finally, it has been identified as hypotension or hypoperfusion refractory to adequate fluid resuscitation. More recently, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) revised the definitions emphasizing especially the role of the host response and the related pathophysiological problems [2]. In Sepsis-3 the definition of severe sepsis was eliminated as well as the role of SIRS was downgraded by defining sepsis as an organ dysfunction distant from the primary site of infection, whereas septic shock was more strictly related to increased mortality. The change of perspective from invading pathogens to the host response has radically transformed the vision of sepsis pathobiology in the last decades. The current concepts indicate that the sepsis processes develop on a double track sustained both by products of infecting microorganisms and by endogenous mediators derived from complement activation and by specific cell-surface receptors expressed on immune, epithelial and endothelial cells. In this way, a complex system of intracellular signals is created by the binding of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [3]. The development of these multiple intracellular signals leads to the expression of several common gene classes that are involved in inflammation, adaptive immunity and cellular metabolism. More specifically, the recognition of PAMPs and DAMPs produces the recruitment of pro-inflammatory intermediates that initiate the expression of early activation genes [4].

SEPSIS RELATED IMMUNE PARALYSIS

It became clear that the host response may be hyper or hypo-reactive, or both at different time, with an overwhelming inflammation associated to a boost of pro-inflammatory cytokines in the former and an immune paralysis with the prevalence of anti-inflammatory cytokines and cellular apoptosis in the latter. Although pro-inflammatory and anti-inflammatory responses may occur simultaneously, early phases of sepsis are usually characterized by hyper-inflammatory processes associated to classical clinical signs ranging from slight to severe impairment of organ

function, including shock appearance [5]. On the other hand, Immune suppressive state becomes predominant in later stages of sepsis producing the so call persistent inflammation/immunosuppression and catabolism syndrome (PICS)[6]. Despite the precise mechanisms are still unclear, the hypothesis for explaining the development and maintenance of PICS are mainly two: i) a persistent and dysregulated activity of PAMPs, DAMPs, inflammasomes and tissue "alarmins" and ii) the role of opportunistic infections (e.g. viral reactivation, infection Acinetobacter spp), changes in the host microbiota and invasive procedures performed in critically ill patients [4]. Sepsis related immunosuppression causes profound changes in both the innate and adaptive immunity [7, 8] with persistent lymphopenia and high level of immature forms of myeloid cells. The dysfunction of immune system during sepsis prone patients, particularly those with severe comorbidities or multiple surgical interventions, to colonization and infections, including breakthrough infections, by opportunistic nosocomial multi-drug resistant (MDR) bacteria. Therefore, patients carrying MDR bacteria should be considered a special population requiring specific strategies directed to supported immune system beyond the sole antibiotic therapy .

PATIENTS WITH MDR BACTERIA: WHY THEY ARE A SPECIAL POPULATION?

Sepsis and septic shock related to MDR bacteria are progressively increasing in the last decades with gram negative pathogens responsible for the majority of cases [9]. International guidelines define MDR bacteria as microorganisms non-susceptible in vitro to at least three different antimicrobial categories (previously excluding intrinsic resistance), XDR as non-susceptible to at least one agent in all but two or fewer antimicrobial categories and PDR as resistant to any agents in all antimicrobial classes tested [10]. The burden of infections sustained by MDR bacteria is variable in different areas: world data show a lower incidence in northwest of Europe, USA and Canada a higher incidence in southeast of Europe, Latin America and Asia Pacific [11]. According to recent studies, from one third to the half of intensive care unit (ICU) acquired infections are sustained by MDR bacteria and among gram negative Acinetobacter spp., Klebsiella pneumoniae, and Pseudomonas spp. are the most common isolates [12]. The ability of these bacteria to survive for prolonged time in the hospital environment, the facility of transfer among patients and healthcare staff together with the antibiotic resistance are responsible for their increasingly widespread. To note that MDR infections are progressively more common also in community acquired infections and the acquisition of these pathogens through travels in different world regions is becoming frequent.

MDR infections influences patients' outcome with higher mortality rates in metallo-β-lactamases Enterobacteriaceae and Pseudomonas aeruginosa and in carbapenem-resistent Klebsiella pneumoniae, likely due to the delay in the appropriate antimicrobial therapy [13]. The Centre for control of Diseases calculates that gram negative MDR infections are responsible for approximately 40,000 cases and more than 2,800 deaths in the United States (CDC 2013 Threat report). It is well known that a goal in the management of septic shock is the administration of intravenous antimicrobials within the first hour of diagnosis and an initial non-effective therapy is related to increase mortality [14]. In MDR infections, the choice of an appropriate antimicrobial treatment is truly more complicate. In patients with bloodstream infections sustained by ESBL producing bacteria the 3-week mortality in patients with an initial inadequate therapy was 60% compared to 19% in those receiving the appropriate one [15]. Ivady et al. observed in a pediatric population with gram-negative bloodstream infection that MDR acquisition was associated with polymicrobial infections and higher risk of evolution in septic shock and multiple organ failure [16].

The relationship between MDR infections and the host immune response is so far unclear. A recent study described the interactions between different clones and resistance phenotypes of Klebsiella pneumoniae and innate immune response. In vitro stimulation of human peripheral blood mononuclear cells (PBMCs) with different heat-killed isolates of K. pneumoniae led to different patterns of TNF-α production. In particular, the highly virulent KPC-producing isolates of the ST17 clones are associated with low release of both TNF-α and IL-17 mediated by toll like receptor 9 that may contribute to a state of immunosuppression [17]. A similar work on P. aeruginosa showed that antibiotic susceptible isolates induce a significantly higher production of IL-1β and IL-6 and by human monocytes compared to MDR ones [18]. These results suggest that multi-drug resistance could play a role in the modulation of host both innate and adaptive immune response. However, further studies are needed to better understand this complex relationships and the potential relevance of a specific immunomodulatory therapy in these infections.

THE ROLE OF IMMUNE ADJUVANT THERAPIES IN MDR INFECTIONS

As described above, immune paralysis is an important hallmark in patients colonized or infected by MDR bacteria. These observations associated with the characteristics of most patients affected by sepsis and septic shock (e.g. elderly, oncologic, with liver and/or renal chronic dysfunction, treated with immunosuppressive drugs) and with the difficulties related to the

antibiotic resistance of pathogens make attractive the development of new immune stimulatory therapies to improve the prognosis. So far, despite many treatments have been investigated on animal models, only few have been used in patients.

Extracorporeal blood purification techniques

Different extracorporeal blood purification techniques have been recently developed and tested to remove inflammatory mediators and, thus, modify immune cell functions. Among these techniques, two meta-analyses showed no benefits by the use of high volume hemofiltration appears in septic patients [19, 20], whereas cascade hemofiltration, using two different filters (the first with an elevated cut-off and the second with a low cut-off) able to remove middle molecular weight molecules such as cytokines but, after promising results in an animal model [21], seems to have no effects on the need for catecholamines as recently demonstrated in 60 patients with septic shock [22]. The use of hemoperfusion with polymyxin-B cartridge showed contrasting results in different trials [23, 24]. Plasma exchange seems to be effective in the removal of cytokines and the association between plasma filtration and adsorption (CPFA) could be even more efficient leading to an improvement of immune paralysis with an increase in HLA-DR expression on monocytes and a restored lipopolysaccharide (LPS)-induced TNF-α production [25]. Unfortunately, a recent randomized control trial in patients with septic shock did not show significant benefits by the use of CPFA. Highly adsorptive membranes and high cut-off membranes can also be used to obtain a blood purification and the progressive optimization of these techniques will lead to preservation of useful molecules and a more selective removal of inflammatory mediators [26].

Pharmacological approaches

Another way to interfere in host immune response is the use of different molecules able to modulate the immune system. Granulocytes-macrophage colony stimulating factor (GM-CSF) and interferon-γ (INF-γ) have been proposed and used because of their effects on antigen presenting cells whose function in septic shock is deeply impaired. A meta-analysis on randomized, placebo vs GM-CSF trials in septic shock showed a better infection clearance in treated patients but no improvement in 28-day mortality. To note that the trial with GM-CSF administration guided by mHLA-DR expression observed a reduced use of mechanical ventilation and a shorter ICU and hospital length of stay in treated patients [27]. INF- γ has been also administrated in subjects with trauma and burns with contradictory results. Again, it is to underline that, in burn patients with

significant reduction of HLA-DR expression on monocytes, its use concomitant to GM-CSF was able to increase HLA-DR and to restore TNF-α secretion in ex-vivo stimulated PBMCs [28].

Another potential target is the PD-1/PD-L pathway: septic patients show an increased expression of PD-1 on T cells which lead to inhibition of cell proliferation, induction of IL-10 secretion, apoptosis and anergy. Different studies have observed that block of this axis is able to improve survival in murine models of sepsis. So far antibody anti-PD-1 and anti PD-L have been tested, in humans, only to treat different types of cancer inducing a restoration of T cell activity [29]. In septic shock the PD-1 expression on T cells and/or PD-L expression on antigen presenting cells could be used as biomarkers of T cell exhaustion to drive anti-PD-1 and anti PD-L antibody administration. Other inhibitory receptors on T cell surface, such as TIM-3, LAG-3 and CTLA-4, BTLA could be used for the same purpose (biomarkers of immune dysfunction and target for neutralizing antibodies) in sepsis and septic shock but clinical trials are still lacking [30].

The use of recombinant interleukins in order to improve lymphocytes survival and function has been only experimented in humans in HIV and cancer patients, but the potential benefits of these pleiotropic molecules in septic shock have been demonstrated in animal models. IL-7 has an anti-apoptotic effect on T cell and is a crucial factor for lymphocyte production, maturation and proliferation [31]. In different murine models of sepsis, the use of IL-7 is able to restore depleted T cells in lymphoid organs, induce T cell proliferation and INF-γ secretion leading to a significant improvement in survival [32]. IL-15 appears an interesting option too: in addition to anti-apoptotic and function-enhancing properties on lymphocytes, displays also effects on innate immune cells: promotes survival of dendritic cells and contributes to natural killer-dendritic cell interactions [33].

Among the few immunomodulatory treatments experimented on human sepsis and septic shock, more data exists on the effect of intravenous immunoglobulins administration. The pleiotropic effects of these molecules resulting in a modulation of the immune response and the reduction of circulating IgG and IgM in the first days of septic shock are the rationale for their use in these pathologies [34, 35]. Two preparations obtained from plasma of healthy donors are available: polyclonal standard IgG (IVIG) and IgM-enriched (IgGAM) formulation. Both preparations are able to determine pathogen clearance but the higher killing on gram-negative bacteria is obtained with IgM-enriched immunoglobulins [36]. Trautmann et al measured a higher rate of LPS-specific antibodies in IgGAM because of the concentration of these antibodies in IgM fraction which is known to be the most relevant in the neutralization and clearance of toxins [37]. Despite the relative high number of studies evaluating this additional therapy, the scarce number

of patients and heterogeneity in terms of type of preparation, dosages and durations hinder the significance of the results observed. Nevertheless, a meta-analysis of 18 studies reveals a reduction in mortality using polyclonal Ig compared to control arm, in particular protocols with lower doses associated with longer duration of treatment reach more favorable outcome [34]. Differently from studies on GM-CSF and INF-γ, where a biomarker of immunosuppression such as HLA-DR expression has been used, in the case of polyclonal Ig administration neither patient's plasma concentration of Ig nor other immunological markers has been tested to guide the administration. The importance of this issue is underlined by the studies on GM-CSF and the observation by Berlot et al that the timing in the administration of IgGAM influences the outcome of patients: the delay in the treatment is a significant and independent predictor of the odds of dying [38]. Considering the measurement of immunoglobulin plasma levels as markers to identify patients which could take advantage in the Ig administration, recent studies have observed that a single measurement of circulating Ig at the onset of septic shock is not effective, on the contrary their kinetic during the first week seems able to differentiate survivors from non-survivors in particular regarding IgM [39]. Because of the outstanding variations in immune response of each patient the identification of useful biomarkers appears fundamental to identify those with immune dysfunction and to develop a customized immune-therapy.

CONCLUSIONS

Septic shock in patients suffering from infection supported by MDR or XDR gram negative is definitely a challenge for intensivists worldwide. Taking into account that the multi-resistant germs are spreading worldwide, the probability to face with a patients with septic shock sustained by MDR or XDR bacetria is no longer an extraordinary event. The application of the Surviving Sepsis Campaign guidelines [40] and timely administration of antibiotics are often ineffective due to the poor immunological status of these patients [41]. Therefore, the use of adjunctive supportive therapies for restoring immune function seems to be very attractive and promising. Unfortunately, to date there is no scientific sound validation on these alternative strategies and appropriate clinical trials are urgently needed.

COMPETING INTEREST

Massimo Girardis have consulted for Biotest-Germany, all the other authors declare that they have no competing interests.

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