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Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies

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Abstract

Preliminary studies suggest that a subgroup of septic patients with severe immune alterations is at high risk of death or nosocomial infection and therefore could benefit from adjunctive immune stimulating therapies. There is thus an urgent need for robust biomarkers usable in routine conditions evaluating rapidly evolving immune status in patients. Although functional testing remains a gold standard, its standardisation remains challenging. Therefore, surrogate markers such as monocyte HLA-DR expression, are being developed. Such biomarkers of immune functionality will enable a novel approach in the design of clinical trials evaluating immunostimulating therapies in sepsis at the right time and in the right patient.

Introduction

Sepsis has been called a hidden public disaster. In 2012, over 20 million patients world-wide are estimated to be afflicted by sepsis annually and recent epidemiological analyses showed that mortality from severe sepsis and septic shock is still elevated – around 30% both in Europe and USA [1]. Both pro and anti-inflammatory responses are initially induced in septic shock patients with the secondary occurrence of sepsis-induced immunosuppression [2]. Importantly, the intensity and duration of sepsis-induced immune alterations have been associated with increased risk of deleterious events in patients, while over 70% of total mortality after septic shock occurs in a delayed fashion (i.e., after the first 3 days). This constitutes the rationale for the initiation of innovative clinical trials testing adjunctive immunostimulating drugs in sepsis [2].

However, as there is no clinical sign of immunosuppression, there is an urgent need for rapid, sensitive and specific biomarkers of the robustness of patients' immune status. This

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ability to semi-quantitate patient immune status will contribute to the success of future immune targeted clinical trials based on the inclusion of appropriately stratified patients. The review will focus on recent advances in aspects of immunomonitoring in septic patients with the perspective of a use as stratification tools in immunostimulating clinical trials.

The concept of sepsis-induced immunosuppression

Several lines of clinical evidence suggest that septic patients who survive the initial few days of the disorder acquired various defects in immunity. This is mainly illustrated by their decreased capacity to overcome initial or secondary infectious challenges. Indeed, a recent post-mortem study showed that approximately 80% septic patients had unresolved septic foci at time of death [3]. Similarly, many pathogens responsible for nosocomial sepsis in intensive care unit [ICU] (*Acinetobacter*, *Candida*...) are weakly virulent opportunistic germs usually isolated from severely immunodepressed patients [4]. This is consistent with the high incidence in septic patients of reactivation of latent viruses (cytomegalovirus, herpes simplex virus) that are normally held in abeyance by host immunity [5–7]. Finally, clinical studies showed a link between altered immune functions and patients' susceptibility to secondary infections [8–11]. This phenomenon of ICU-acquired immune dysfunctions has been called compensatory anti-inflammatory response syndrome, sepsis-induced immunosuppression, or cellular reprogramming [2,12].

Although not exhaustively described, pathophysiologic mechanisms at play have begun to be understood (Table 1). In particular, epigenetic regulation, energetic failure, central and endocrine regulations, increased apoptosis or endotoxin tolerance have been shown to be playing a role both in clinical observations and animal models of sepsis [13–16]. Such immune alterations affect both the innate and adaptive parts of the immune response, occur rapidly after patients' admission, and are present both peripherally in circulating blood cells but also locally in organs [17]. They thus represent a very profound and systemic process. Importantly, a number of clinical observations showed that the intensity and duration of these sepsis-induced immune dysfunctions (evaluated by different approaches [18]) are associated with increased risk for deleterious outcomes (death or secondary ICU-acquired infections).

Monitoring innate immune alterations in sepsis and related therapies

Innate immune cells represent the first line of defence after an infection and thus play a central role in the control of pathogens and initiation of adaptive immune responses. Animal models of sepsis and experimental studies in patients depicted altered innate immune cell functions [13]. However, except for monocytes, few studies identified a link between these alterations and clinical outcomes in patients. In particular, recent studies showed that, after sepsis, circulating neutrophils have altered functions such as impaired bacterial clearance, abnormal radical oxygen species production, and decreased recruitment to infected tissues [19,20]. Similarly, recent experimental studies proposed a role for late onset myeloid-derived suppressor cells (MDSC) in sepsis pathophysiology [21,22]. However, to date, no clinical studies showed a link between these parameters and clinical outcomes in patients. Regarding MDSC, it might be due to the complexity of their immunophenotyping and to the absence of any consensually accepted phenotype in humans. Interestingly, recent studies described NK cell functional alterations (reduced IFN γ secretion) and decreased cell number in sepsis [23–25]. However clinical studies formally identifying a link between these alterations and clinical outcomes are still lacking. Moreover, the transfer of such functional approaches (in vitro cytokine release [26,27] or granulomatous response [25]) in clinical practice remains very complex.

Dendritic cells (DC), the professional antigen presenting cells, are sentinels in tissues and play an important role in initiation and modulation of T cell responses. DCs in sepsis exhibited altered phenotype and functions with, in particular, a decreased expression of Human Leukocyte Antigen-DR (HLA-DR) and reduced secretion of pro-inflammatory cytokines upon stimulation by bacterial products [11,28]. Interestingly, in septic patients, the peripheral DC count is low and this decrease has been shown to be associated with worsened clinical outcomes in sepsis including death and nosocomial infections [11,29].

Besides the previously mentioned clinical studies, many studies have described altered monocyte phenotype and functions in sepsis [30–32]. In particular, decreased expression of HLA-DR (mHLA-DR) on circulating monocytes has been observed and is proposed as a surrogate marker of immune failure [33]. Increased circulating IL-10 production has been postulated to play a role in this process and studies showed the association between increased plasmatic IL-10 concentration and clinical outcomes such as nosocomial infections [34,35]. mHLA-DR downregulation has been shown to be predictive of complications in numerous clinical conditions (Table 2) and is applicable for clinical practice with standardized tests [36,37]. Importantly, studies show an association between the lack of recovery of mHLA-DR expression overtime and secondary infections in sepsis [9,10]. This finding highlights the importance of the timing of immunomonitoring in regard to the expected homeostatic response and the phase of sepsis. In other words, immune enhancing therapy needs to be applied during the immunosuppressive phase of the disorder as indicated by decreased mHLA-DR expression. Therapeutic protocols with GM-CSF, G-CSF and IFN γ have been conducted in sepsis with the aims to stimulate innate immune function, improve myelopoiesis and limit lymphocyte apoptosis. Small clinical studies in ICU patients with sepsis or trauma showed no effect to decrease mortality but there was a beneficial effect on illness severity and rate of pathogen clearance [38–42]. Interestingly, no study detected any adverse events. The lack of overall benefit on mortality in these pilot studies might be related to the failure to select patients based upon their respective immune status, e.g. using monocyte mHLA-DR expression. In those clinical studies in which mHLA-DR was measured, immune-adjuvant therapy lead to increased mHLA-DR expression [10,39,43]. Along with the recovery of mHLA-DR expression, stimulated whole blood TNF α release was restored in response to immunotherapy [39,42] thereby suggesting that mHLA-DR expression might be clinically useful as a biomarker for identifying patients who would be appropriate candidates for immunotherapy and to monitoring responsiveness to treatment.

Monitoring adaptive immune alterations in sepsis and related therapies

Since the seminal description of a loss of DTH response in surgical patients associated with increased risk of nosocomial infections more than 20 years ago, T lymphocyte anergy has been shown to be a hallmark of sepsis-induced immune dysfunctions [8,44]. Numerous aspects of this dysfunction have been described both in animal models of sepsis and in clinic (Figure 1). In particular, marked decreased cell number linked with increased apoptosis was repeatedly described in patients [45,46]. Functional alterations such as reduced *ex vivo* proliferation, decreased pro-inflammatory (IL-2, IFN γ , IL-17) and increased anti-inflammatory (IL-10) cytokine productions were observed [17,47,48]. Phenotypic alterations such as reduced co-activating receptor (CD28), increased co-inhibitory receptor (PD-1, CTLA4) expressions or decreased T cell receptor repertoire diversity [17,49–51] also represent characteristics of septic T lymphocytes. Finally, an increased percentage of circulating CD4⁺CD25⁺ regulatory T cells has been repeatedly observed in septic patients [52]. In some studies, a link between the intensity and duration of these alterations and subsequent mortality/ nosocomial infections risk was observed [48,50,51].

However, as opposed to mHLA-DR measurement, to date, no consensually defined marker of sepsis-induced lymphocyte alterations has emerged in the literature. So far, the best available test for assessment of lymphocyte functionality remains the measurement of proliferation in response to recall antigens or mitogen stimulations. However, limitations inherent to such measurement (tritiated thymidine uptake, several days-incubation time) preclude its use in large clinical studies. Other parameters of lymphocyte functionality such as expression of positive or negative co-inhibitory receptors, percentage of T regulatory cells, quantitation of lymphocyte ATP, etc. suffer from a lack of standardization and paucity of clinical studies in sepsis. Thus, there is no “gold standard” method for evaluating lymphocyte function that would enable investigators to link cell function with sepsis outcome. Therefore, a major effort is needed to develop robust biological tests of lymphocyte function that are suitable for routine clinical laboratory and which correlate with objective outcomes such as risk of nosocomial infection or sepsis survival.

Recognizing the pivotal role of lymphocytes (mainly CD4⁺ T cells) in orchestrating immune responses, one may expect that, by restoring their function, it might be possible to generate a positive global effect on the overall immune response, both mediated by lymphocytes and innate immune cells. Promising immunotherapeutic agents include recombinant human IL-7 (rhIL-7) and anti-programmed cell death-1 (anti-PD-1) antibody [2,53]. Inhibitory therapy of PD-1 is currently being examined in phase III clinical trials in oncology [54]. Preliminary results showed its efficacy in inducing durable tumor regression and prolonged stabilization of disease in patients with advanced cancers [55]. In sepsis, such therapy might be administered to patients after stratification based on PD-1 expression [50]. rhIL-7 is also currently being tested in phase I and II clinical trials in chronic viral infections, following chemotherapy and/or bone marrow transplantation, and in idiopathic CD4⁺ lymphopenia [56]. In murine models of polymicrobial sepsis, rhIL-7 restored DTH response, decreased sepsis-induced lymphocyte apoptosis, reversed sepsis-induced depression of IFN- γ production and improved survival [57,58]. We recently demonstrated rhIL-7's ability to reverse sepsis-induced T cell alterations (decreased CD4⁺ and CD8⁺ T cell proliferation, IFN- γ production, STAT5 phosphorylation and Bcl-2 induction) in septic shock patients *ex vivo* [47].

Conclusion

Immune monitoring after sepsis enables the identification of a subgroup of patients at high risk of death or nosocomial infection who could benefit the most from adjunctive immune stimulating therapies. It is estimated that over half of patients with sepsis develop immunosuppression and would benefit from therapies that boost immunity (Figure 2). The failure of many therapies in sepsis is likely related to the lack of stratification of patients based upon their particular immune functional status. Furthermore beyond variability in the immune response, septic syndromes also represent a heterogeneous group of diseases (different co-morbidities, types and sites of infections, initial severities, community-acquired or nosocomial infections). Therefore inclusion of such patients without prior biomarker-based stratification might have largely participated in the failure of previous clinical trials in the field [59]. Thus, if we want to partially decrease this heterogeneity, future clinical trials of immune-adjuvant therapies in sepsis must be based on patients' stratification using immune status biomarkers that reflect both innate and adaptive immune function. Potential markers include some currently available measures such as mHLA-DR, absolute CD4⁺ T cell count, circulating IL-10, and regulatory T cells measurements. Among these, some are already currently available in routine labs (absolute CD4⁺ T cell count, plasmatic IL10 concentration). Others, such as regulatory T cell evaluation, should benefit from recent technical development before becoming available in routine practice (e.g. one-step intracellular FOXP3 staining). Monocyte HLA-DR expression, in contrary, has already been

tested in inter-laboratory experiments and is ready for prime time [36,37]. A recent study by Meisel et al. used these principles of patient selection based upon immune status [39]. In a prospective, randomized, double-blind, placebo-controlled trial, 38 patients with severe sepsis or septic shock were treated with GM-CSF or placebo. Inclusions were restricted to patients presenting with sepsis-associated immunosuppression as defined by a decreased mHLA-DR (< 8,000 monoclonal antibodies bound per cell) for 2 consecutive days. Results showed that biomarker-guided GM-CSF therapy in sepsis was safe, effective in restoring monocytic immunocompetence cytokine production of TNF- α , and, most importantly, shorten the time of mechanical ventilation and hospital/intensive care unit stay. These very promising preliminary results should now be validated in a large multi-center clinical trial using similar biomarker-guided stratification. We strongly believe that such biomarker-guided strategy can be safely applied and will offer an exciting new approach to this highly lethal disease.

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Highlights

- Immunomonitoring identifies septic patients who could benefit from immunotherapy
- Immune stratification should be considered in clinical trials design in sepsis
- Functional tests remain a gold standard but surrogate markers are being identified
- Robust biomarkers of immune status in sepsis need to be further developed

Figure 1. T Lymphocyte alterations in sepsis and link with outcomes
TCR: T Cell Receptor, Ag: Antigen, MHC II: Major Histocompatibility Complex Class II, PD-1: Programmed cell Death-1, PD-L1: Programmed cell death ligand 1, CTLA4: Cytotoxic T-Lymphocyte Antigen 4, HAI: Hospital-Acquired Infection.

Figure 2. Septic shock patients stratification based on a biomarker: one virtual cohort based on real data

Based on personal data, one hundred septic shock patients were included in a virtual clinical study and monitored at Day 1-2, Day 3-5 and Day 7-10 for Human Leukocyte Antigen-DR expression on circulating monocytes [9,32]. Results are expressed as number of anti-HLA-DR antibodies bound per monocytes (AB/C). Patients are stratified based on mHLA-DR measured at Day3-5 (mHLA-DR threshold = 8 000 AB/C [39]). Twenty-eight day mortality is 34% (i.e. $n = 34$ non-survivors in total). Only 8 deaths occur within the first 3 days. Ninety-eight patients are still alive at Day 4 and could be stratified. Decreased mHLA-DR is observed in 55 patients. In this subgroup, 22 deaths occur (i.e. 65% of total mortality). This virtual cohort based on real observations illustrates that a decreased mHLA-DR identifies a subgroup of septic shock patients at high risk of death or secondary infection. In this subpopulation, there is a strong rational for the initiation of innovative immune stimulating therapies.

Table 1

Sepsis-induced immune dysfunctions: pathophysiology at a glance

Mechanisms	Features of sepsis-induced immune alterations
Endotoxin tolerance	↓ pro-inflammatory ↑ anti-inflammatory cytokine production ↓ Ag presentation capacity
Apoptosis	↓ cell number Cell anergy
Energetic failure	Cell anergy Apoptosis Mitochondrial dysfunction
Anti-inflammatory mediators	↓ activating co-receptor expressions ↑ inhibitory co-receptor expressions Cell anergy Endotoxin tolerance
Epigenetic regulation	↓ pro-inflammatory gene expressions Cellular reprogramming
Central and endocrine Regulations	↓ pro-inflammatory cytokine production

Table 2
Recent clinical studies evaluating mHLA-DR predictive value regarding outcome in injured patients.

1 st author, Journal, Year	Nbr of patients	Pathology	Evaluation	Results	Other inflammatory parameters
Monneret, Intensive Care Med, 2006	93	septic shock	outcome	No association of initial mHLA-DR with outcome Low mHLA at day3-4 associated with poor outcome	No
Venet, Crit Care Med, 2007	14	severe burns	outcome and infection	Low HLA-DR associated with outcome and infection	Decreased plasma IL-6 and TNF α , but not IL-10
Lukaszewicz, Crit Care Med, 2009	283	ICU patients	outcome and infection	No association of initial mHLA-DR with outcome Variation of mHLA-DR over time associated with infection	No
Berres, Liver Int, 2009	38	decompensated liver cirrhosis	outcome	Association of initial low mHLA-DR with poor outcome mHLA-DR decreased (day0 to day 3) in NS	Plasma IFN γ , <i>ex vivo</i> stimulated TNF α and IL-6
Landelle, Intensive Care Med, 2010	209	septic shock	outcome and infection	No association of initial mHLA-DR with outcome Day3-4 and day 9-11 mHLA-DR associated with infection	No
Chéron, Crit Care, 2010	105	trauma	Infection and sepsis	No association of initial mHLA-DR with outcome Low mHLA-DR at day3-4 associated with infection	No
Wu, Crit Care, 2011	79	severe sepsis	outcome	mHLA-DR associated with outcome	No
Wu, Crit Care, 2011	35	severe sepsis	outcome	mHLA-DR increase associated with outcome	Stimulated IL-6 and IL-12
Zhang, Eur J Neurol, 2011	53	neurologic patients	infection	Day 2 mHLA-DR predictive for infection Persistent low mHLA-DR associated with outcome	No
Lin, Blood, 2011	40	B cell non-Hodgkin lymphoma	outcome	High ratio of HLA-DR low monocytes is related to more aggressive disease	Low TNF α receptor II Decrease production of IFN γ and proliferation
Berry, Intensive Care Med, 2011	100	cirrhosis	outcome	In 33 ICU patients, decrease in mHLA-DR associated with poor outcome	<i>Ex vivo</i> stimulated IL-10, IFN γ , TNF α
Gouel-Chéron, PLoS One, 2012	100	trauma	Infection and sepsis	Variation of mHLA-DR over time associated with infection	Plasma IL-6
Trimmel, Shock, 2012	413	ICU patients	outcome	Minimal value of HLA-DR associated with outcome	No

Only studies published in or after 2006 were considered. NS: non survivors, ICU: intensive care unit, mHLA-DR: monocyte Human Leukocyte Antigen-DR, IFN: interferon, IL: interleukin, TNF: tumor necrosis factor, LPS: lipopolysaccharide