Biological markers of injury-induced immunosuppression

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ABSTRACT

Severe injuries, such as severe sepsis, burn, trauma and major surgery, lead to an overlapping development of pro- and anti-inflammatory responses. It is now well established that these injuries are associated with the secondary development of immune suppression, which results in significant morbidity and mortality. Recent data suggest that immunostimulatory drugs might prevent these complications. However, intensive care patients are heterogeneous, making patient stratification essential for a targeted treatment. In the present review, we discuss potential biomarkers of injury-induced immunosuppression, mainly focusing on those that have been associated with poor outcome in various clinical settings. We namely present clinical data on monocyte human leukocyte antigen DR, lymphopenia, PD-1/PD-L1 and transcriptomic approach.

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The immune system response to major injuries often leads, after an initial hyperactivation, to an incompetent and hyporeactive immune status (Figure 1). This injury-induced immunosuppression has been described in the past decades in various clinical settings, such as septic shock, severe trauma, severe burns, or major surgery. When persistent or intense, immune paralysis is associated with the development of secondary infections and pejorative outcomes. Despite recent advances in critical care medicine, mortality remains high and often not related to the initial injury, but rather to secondary events — especially infections — that occur during the immunosuppression period. Mechanisms of this immunoparalysis and therapeutic opportunities have been recently published in the particular field of sepsis. In the present review, we will focus on cellular biomarkers of injury-induced immunosuppression resulting from various forms of severe injuries. Unless specified, all studies cited in the present review involved intensive care patients.
BIOLOGICAL MARKERS OF INJURY-INDUCED IMMUNOSUPPRESSION

ROUGET

Innate immunity

Neutrophils and immature granulocytes

Neutrophils represent the first line of innate immune defense against infection and are the most abundant leukocyte subset. Paradoxically, data about injury-induced neutrophil alterations are scarce. Sepsis leads to the massive recruitment of immature neutrophils. The immature granulocyte fraction can be assessed by a blood cell analyzer, expressing the result in Delta neutrophil index (Dn), which independently correlates with 28-day mortality in patients with sepsis. Consistently, Mare et al. found a significantly lower immature granulocyte count in the >4-week survival group compared with the patients who died within the first week of sampling.

We recently studied functional neutrophil alterations during sepsis-induced immunosuppression (day 3-4 and day 6-8), on 43 septic shock patients and 23 healthy volunteers. A high immature granulocyte count was associated with mortality. In addition, we performed a global evaluation of the neutrophil alterations in septic shock, and highlighted a markedly altered neutrophil chemotaxis (reduced integrin, selectin, and chemokine receptor expression with altered in vivo migration in response to different chemo attractants) and oxidative burst, also associated with mortality. Conversely, and in accordance with the work of Drifte et al., phagocytose and activation capacities were

Figure 1.—Simplified description of immune response to injury: a complex balance. After injury, inflammatory and immunosuppressive processes coexist (orange and red dotted lines), with an usual initial pro-inflammatory and lately anti-inflammatory resultant (green continuous line). Severe injuries lead to a biphasic situation, with a long lasting immunoparalysis following the initial inflammatory burst. Both extreme pro- and anti-inflammatory states lead to unfavorable outcome (dashed lines).

Need for biomarkers

Even considering the same diagnosis, there is a huge clinical and biological diversity resulting from the complex and highly dynamic interaction between host and injury. The panel of disease severity and patients’ comorbidities increases even more this heterogeneity. This has been suggested to participate in the failure of clinical trials in septic shock. In line, ICU patients’ immune status is heterogeneous but cannot be determined based on clinical signs. Biomarkers of patients’ immune status could help patient stratification such as proposed in the PIRO (predisposition, insult, response, and organ dysfunction) Score.

Reversing and/or shortening the immunoparalysis phase might be of crucial interest to improve patients’ outcomes. For instance, one of these promising immunoadjuvant therapies is interferon-gamma (IFNγ). It has been shown to restore the expression of transcripts associated with the development of immunosuppression in an ex vivo monocyte endotoxin tolerance model. IFNγ also partially reversed sepsis-induced immunoparalysis in a human in vivo model of Gram negative sepsis. We can also refer to the work of Meisel et al., who have shown that mHLA-DR guided GM-CSF (granulocyte–macrophage colony-stimulating factor) therapy enables to shorten the time of mechanical ventilation and hospital/intensive care unit stay. Because of the aforementioned heterogeneity, critically ill patients may not all benefit from immunostimulatory drugs. Therefore, clinicians need to know the particular immune status of each patient in real time to propose the best personalized care. Finally, markers of patient immune status could be used to monitor treatment efficacy or side effects.

Biomarkers of injury-induced immunosuppression
preserved, indicating that neutrophil function is reconfigured rather than globally suppressed. Impaired neutrophil chemotaxis, altered phagocytosis and bacterial killing were also described in burn 20-23 and trauma patients.24 However, the possible association between neutrophils and mortality/morbidity has only been investigated in sepsis.17, 18

MONOCYTES

Monocytes are myeloid cells which play a central role in innate immunity. These circulating cells are also able to release pro and anti-inflammatory molecules. After injury, many aspects of monocyte physiology have been studied.

HLA-DR

One of the most promising biomarkers of injury-induced immunoparalysis is monocyte human leukocyte antigen DR (mHLA-DR). As developed elsewhere, monocytes from septic patients display an altered antigen presentation, related to lower expression of major histocompatibility (MHC) class II system. This decrease in cell-surface expression of mHLA-DR is a marker of monocyte dysfunction, objectified by lower synthesis of pro-inflammatory cytokines in response to bacterial challenges, lower proliferation rate, and interestingly recovery of monocyte functions after restoration of normal mHLA-DR levels after treatment ex vivo and in vivo.25 Low mHLA-DR expression has been associated to both mortality and secondary infections in various clinical backgrounds: septic shock, severe burn, trauma, pancreatitis, cirrhosis etc. For instance, Gouel-Chéron et al. observed that after severe trauma, a ratio of mHLA-DR expression between day 3 and day 2 below 1.2 is an independent predictor of septic complication with an odd ratio of 5 (95% CI [1.6; 19], P=0.008) after multivariate analysis.26 Similarly, Venet et al. reported an association between mHLA-DR decrease and severity as well as secondary septic shock after severe burn injury.6

Overall, mHLA-DR is a robust prognostic marker of mortality and secondary infections in a broad variety of injury contexts. Multi-center clinical trials are still needed to define precise cut-off values, as they may depend on the type of injury.

IL10 and IL10/TNF ratio

Interleukin 10 (IL10) is a powerful anti-inflammatory mediator, which namely suppresses dendritic cell and macrophage functions. Many different cell populations may be involved in its synthesis, such as monocytes, and its regulation is complex.27 In a septic shock population, Monneret et al. showed that IL10 correlates with HLA-DR expression and that values were significantly higher among non-survivors.28 Interestingly, IL10 elevation was already significantly associated to mortality at time of admission and IL10 remained higher during 15 days in the non-survivor group. These results were consistent with previous literature regarding IL10 dosage 29-31 or IL10 mRNA measurement.32-34 The association between IL10 and septic complication or mortality was also described in burned patients35, 36 and in trauma patients.37 IL10 may therefore constitute an early marker of forthcoming immunoparalysis. Some authors also evaluated IL10/TNF ratio, which reflects the pro/anti-inflammatory balance and also correlates with unfavorable outcome.28-30

Other monocyte-related markers

During sepsis, monocytes show not only impaired antigen presentation, but also decreased pro-inflammatory response, as assessed by the down-regulation of surface markers such as CD10, CD86, CD14, GM CSF receptor, or CX3CR1.38-43 For all these markers, a reduced expression was associated with mortality. To our knowledge, none of them has been associated with poor prognosis in surgical, burn or trauma context.

Another interesting monocyte surface protein is PD-L1. This marker will be discussed
in a following subsection, together with the protein it binds.

CD163 is a scavenger receptor expressed on monocytes, macrophages, and dendritic cells. It is also known as haptoglobin-hemoglobin receptor, and also exists as a soluble form, sCD163, which is shed into plasma after stimulation by mediators of inflammation. Three clinical studies in septic populations showed that monocyte expression of CD163, or sCD163 level, were higher in non-survivors, even at early time points. Nevertheless, CD163 has not been evaluated in severe burn, surgery or trauma.

Functional testing: endotoxin tolerance

Functional approaches may be interesting, for they give an insight into in vivo cell response to an immune challenge. Monocytes from septic patients have been particularly studied; they display a predominantly anti-inflammatory phenotype, this observation leads to the concept of leukocyte reprogramming. Indeed, these cells release less pro-inflammatory mediators (such as TNF, IL6 or IL12) and as much or even slightly more anti-inflammatory molecules (IL10, IL1ra). This reduced responsiveness after LPS stimulation has been described as Endotoxin Tolerance. Hence, this method may reveal immune paralysis. Endotoxin tolerance has also been described in severe trauma as well as in major surgery.

Clinical impact of this reprogramming has been recently studied. In a heterogeneous pediatric population with multiple organ failure syndrome, Hall et al. found an association between a reduced ex vivo LPS-induced TNF response (in whole blood, on day 7 after admission) and increased nosocomial infection (RR 3.3 [1.8-6.0]) and mortality (RR 5.8 [2.1-16]). Conversely, Van Vught et al. observed an endotoxin tolerant phenotype on septic patient monocytes, but it was not associated with the development of nosocomial infections. As suggested by the authors, this negative result may be related to the early time point that was chosen (day 0). Further multicentric clinical trials are warranted to confirm the prognostic value of this functional approach in different injury contexts, and to determine its optimal timing.

Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells (APCs), playing a key role in linking innate and adaptive immune systems. DCs capture and process antigens, before migrating to lymph nodes, where they present antigens to lymphocytes. Literature provides strong evidences for both quantitative and qualitative DC alterations after injury. Hotchkiss et al. showed a dramatic reduction in the number of follicular DCs in septic patients compared with controls. MHc class II expression by DCs was also lower in septic patients. This is consistent with the observation that circulating DC count is lower in septic shock patients than in controls (shocks other than septic) as early as day 1, and persistent low circulating myeloid DC count is associated with nosocomial infection. Furthermore, DC loss in septic patients is higher in non-survivor septic patients than in survivors and is inversely correlated with severity scores.

After surgery, DC count increases acutely, and then drops below preoperative levels on days 2-3. Surgery-induced DC depletion is associated with IL12 — a pro-inflammatory cytokine — deficiency. DC functional impairment has also been observed in trauma patients with an in vitro reduced differentiation into DCs, a lower IL12 production, and an impaired T cell proliferation. Furthermore, life span and signaling function of DCs may be altered by trauma. Consistently, severe burn patients displayed early low DC count. In addition DCs from burn patients had impaired reactivity, an anti-inflammatory phenotype, and dysfunctional T cell-priming ability.

To sum up, functional and quantitative impairments of DCs have been observed in many...
different injury situations, and may be key players of injury-induced immune paralysis. As murine administration of purified DCs\textsuperscript{64, 65} and of DC growth factor (Fms-related tyrosine kinase 3 ligand, FLT3-L)\textsuperscript{66, 67} reversed mortality and secondary sepsis, monitoring and targeting DCs represent a promising therapeutic strategy.

Adaptive immunity: T and B cells

Lymphocytes are central actors in immunity, acting as both effectors and regulators. Not only are they quantitatively altered during injury, but they also display functional anomalies.

Cell counts

Lymphopenia.—Apoptosis-related loss of immune cells is an important feature of injury-induced immune paralysis. This cell loss affects both T cells (except for Treg cells) and B cells. Lymphopenia is a hallmark of SIRS and septic syndrome, and has also been described in trauma, burn and surgical patients. At day 4 of sepsis, lymphopenia was independently associated with 28-day and 1-year survival and severe lymphopenia was associated with increased development of secondary infections.\textsuperscript{68} In this study, lymphopenia at admission was not associated with mortality, contrary to the results presented by Chung et al.\textsuperscript{69} Consistently, in emergency general surgical patients, lymphopenia was independently associated with increased in-hospital mortality (OR 3.5 [1.7-7.3]).\textsuperscript{70} In trauma as well, failure to normalize lymphopenia in severely injured patients is associated with significantly higher mortality,\textsuperscript{71} and with higher rate of nosocomial infections after multivariate analysis (personal data). Moreover, lymphocyte count is cheap and included in routine biology. However, most of the severely injured patients display lymphopenia, which may limit its discriminating power. Effective stratification strategies including lymphopenia are still to be determined.

Neutrophil-to-lymphocyte ratio.—Another way to take into consideration lymphopenia is to use the neutrophil-to-lymphocyte ratio (NTL).\textsuperscript{72} From a pathophysiological perspective, NTL reflects the complex balance between inflammatory insult and immune suppression. In clinical setting, NTL was first investigated in abdominal surgery. Increased NLR after digestive surgery was associated with an increased risk of complications or death.\textsuperscript{73-75} Moreover, time points and cut-offs were very heterogeneous among these studies, and cancer in itself may interfere with NTL.

Three clinical studies recently assessed NTL in the ICU. In cirrhotic patients with acute complication, high NLR at day 1 was associated with mortality in a multivariate logistic regression.\textsuperscript{76} A retrospective study suggested that NTL at admission is associated with 28-day mortality in unselected critically ill patients, however this association was not observed in the septic patient subgroup.\textsuperscript{77} Riché et al.\textsuperscript{78} refined this result in a septic shock cohort. They highlighted a reversed NTL evolution according to the timing of death: early death was associated with low NTL at admission, whereas late death (≥day 5) was associated with high NTL between day 1 and day 5.

All in all, NTL is an easy and affordable biomarker which may be of interest to detect immune failure, yet the best timing and cut-offs are still to be determined, and, like mHLA-DR, these parameters may depend on the clinical situation.

Regulatory T cells

Regulatory T cells (Tregs) are a very specific subset of T cells, crucial for immune tolerance and homeostasis. Indeed, they inhibit the activation and proliferation of most of the other immune cells, by directly killing cytotoxic cells, inhibiting their cytotoxic production, and secreting immunomodulatory cytokines (TGFβ, IL10).\textsuperscript{79} As presented recently, the percentage of Tregs increases after the onset of septic shock,\textsuperscript{80} burn and trauma. The enhancement of their suppressive functions and the relative and
absolute elevation in their count were associated with mortality in burn.\textsuperscript{81} Chen \textit{et al.} also described a significant association between Treg count increase at day 7 and mortality in ICU patients with sepsis.\textsuperscript{82} CD39\textsuperscript{+} Treg cells form a specific subset displaying increased suppressive skills. In septic patients, increased expression of CD39\textsuperscript{+} Tregs was associated with mortality and severity of sepsis.\textsuperscript{83}

Conversely, after traumatic brain injury, the level of circulating Tregs was positively associated with neurologic recovery and lower hospital mortality.\textsuperscript{84} Indeed, Treg action is thought to be neuroprotective because, in this context, a down-regulation of inflammation may promote cell survival.

As a conclusion, Tregs may play a role in injury-induced immunosuppression, given their important regulatory properties.

**INCREASED CO-INHIBITORY RECEPTORS**

\textit{CTLA-4, BTLA.}—After antigen recognition, T cell full activation depends on the balance between co-stimulatory and co-inhibitory signals. CTLA4 — cytotoxic T lymphocyte-associated antigen 4 — is expressed in T cells and delivers a negative signal to the primed lymphocyte,\textsuperscript{85} directly antagonizing the co-stimulatory receptor CD28, and participating in T cell tolerance. CTLA-4 expression is increased after burn injury,\textsuperscript{86} after trauma-hemorrhage,\textsuperscript{87, 88} and in sepsis, and inhibits immune cell functions.\textsuperscript{89} Inoue \textit{et al.} showed a dose-dependent effect of anti-CTLA-4 on survival in a rodent model of sepsis,\textsuperscript{90} with decreased CD8\textsuperscript{+} and CD4\textsuperscript{+} lymphocyte apoptosis. Consistently, blockade of CTLA-4 improved survival in rodent fungal sepsis.\textsuperscript{89} To date, no data is available in human clinical setting regarding its prognostic value.

B and T lymphocyte attenuator (BTLA) is also a lymphocyte inhibitory receptor, expressed in a wide range of cells. BTLA decreases cytokine production and inhibits survival signaling in CD4\textsuperscript{+} lymphocytes.\textsuperscript{91} Consistently with previous literature,\textsuperscript{92} Shao \textit{et al.} showed that lower percentage of BTLA\textsuperscript{+}/CD4\textsuperscript{+} T cells during the early stage of sepsis was associated with severity and 28-day mortality.\textsuperscript{93}

CTLA-4 and BTLA might constitute interesting biomarkers for injury-induced immune paralysis, but data are still scarce. To address this question, more clinical studies are needed, including the monitoring of nosocomial infections, and exploring a wider range of injuries. Other negative immune regulators have been proposed, such as Lymphocyte-activation gene 3 (LAG-3) or T-cell immunoglobulin and mucin domain 3 (HAVCR2, also known as TIM3), as their expression is increased in septic patients presenting an exhausted T cell phenotype.\textsuperscript{94}

\textit{PD-1/PD-L1.}—Another promising biomarker is PD-1 (for Programmed Death One, official symbol PDCD1). The protein is expressed on the cell membrane of lymphocytes, myeloid and dendritic cells. It binds two ligands: PD-L1 (CD274, also known as B7-H1), PD-L2 (PDCD1LG2, also known as B7-DC or CD273). Both belong to the B7:CD28 family,\textsuperscript{95} and are expressed by epithelial, endothelial and antigen presenting cells. This complex pathway is involved in co-ligation with T cell receptor and leads to lower cytokine production and to an inhibition of cellular proliferation, negatively controlling the immune response.

A murine PD-1 knock-out model exposed to sepsis showed lower mortality and decreased bacterial burden and inflammatory response, in link with a macrophage dysfunction.\textsuperscript{96} In clinical setting, Zhang \textit{et al.} provided evidence for an up-regulation of PD-1 on T cells and PD-L1 on monocytes of 19 septic shock patients. Interestingly, PD-L1 blockade leads to a decreased apoptosis of T cells after TNF stimulation or T cell receptor ligation.\textsuperscript{97} Guignant \textit{et al.} found consistent results, and highlighted a correlation between increased PD-1, mortality and nosocomial infections after septic shock.\textsuperscript{98} In this study, trauma patients did not express increased PD1 or PD-L1, contrary to the results presented elsewhere.\textsuperscript{87}
PD-1/PD-L1 expression on immune cells was also increased after surgery, and correlates with the severity of surgical trauma. Similarly, T cell apoptosis was partially reversed by anti-PD-1 antibody. Overall, these data indicate that PD-1 might be an attracting biomarker for injury-induced immunosuppression.

**CD127**

Interleukin 7 (IL7) is an essential cytokine involved in survival, development and maturation of T and B cells. It binds to a heterodimeric receptor, consisting of an alpha chain (IL7Rα, also known as CD127) and a gamma chain (CD132), the latter is common to several interleukin receptors. Administration of IL7 restored lymphocyte functions in septic patients. IL7 level and CD127 were not associated with mortality in this study. Interestingly, a high level of soluble fraction of CD127 (sCD127) was significantly associated with the development of nosocomial infection. Recently, in a larger cohort of septic shock patients, Demaret et al. found a significant association between increased plasmatic sCD127 (at day 1 and day 3) and mortality. However, the function and the regulation of sCD127 and of CD127 are still widely unknown. Soluble CD127 could be an interesting biomarker for identification of a group of patients presenting with higher risk of secondary infections or mortality, but it is unclear whether it is involved in immune paralysis pathophysiology.

**FUNCTIONAL TESTING**

**T cell proliferation.**—Another hallmark of sepsis is the decreased lymphocyte proliferation in response to stimulation. Yet, lymphocyte proliferation is a fundamental part of immune response. Its decrease has been described in sepsis, trauma, severe burn, and after major surgery. It has been associated with nosocomial infections, poor outcome and multiorgan failure syndrome. Recent data suggested that reduced lymphocyte proliferation may be driven by an indirect mechanism: in an *ex vivo* human model derived from endotoxin tolerance, Poujol *et al.* suggested that LPS priming indirectly impairs lymphocyte functions by reducing APCs capacity to activate T cells. Nevertheless, such functional approaches are time-consuming, as proliferation requires several days of incubation. This precludes most applications in clinical settings.

**STAT5 phosphorylation.**—STAT5 (Signal Transducer and Activator of Transcription 5) is a key molecule for the response to IL7. Its phosphorylation occurs in response to the recruitment of IL7R and reflects the activated status of the cell. STAT5 phosphorylation (pSTAT5) is enhanced by *ex vivo* rhIL7 (recombinant human IL7) in septic shock patients. Low doses of rhIL7 preferentially sustain effector T cells activation, whereas rhIL2 activates regulatory T cells. A pilot study from our team suggested that pSTAT5 in septic shock patients was higher in survivors. Non-survivors failed to phosphorylate STAT5 in effector T cells in response to rhIL7. Interestingly, pSTAT5 can be assessed by flow cytometry on whole blood samples, making it suitable for daily clinical practice.

**B CELLS**

Regarding B cells, human data are limited. B lymphocytes are a heterogeneous cell population; they can not only differentiate into immunoglobulin secreting plasma cells, but they also produce cytokines and present antigens. However, their role in the pathogenesis of injury-induced immunosuppression has not been firmly established. Sepsis-induced cell depletion also deeply affects B cells, but this has not been specifically associated with mortality. Interestingly, patterns in B subset distribution and activation were different between survivors and non-survivors. At time of admission, a high percentage of CD23+ (a marker of activation and regulation) B cells appeared to be associated with good outcome, whereas CD80+ (a T cell co-stimulation marker) and CD95+ (a
marker of apoptosis susceptibility) B cell percentages were associated with increased 28-day mortality. The reason of this observation remains to be elucidated. Recently, only B cell and CD16+ monocyte counts were associated with increased mortality.\textsuperscript{107, 108} Consequently we may hypothesize that, similarly to other immune cells, B cell functional and quantitative alterations may play a role in injury-induced immune dysfunction. Thus, it might be interesting to shed light on B cell involvement and to develop B cell markers.

Both innate and adaptive immunity: transcriptomic approach

The host response to injury involves numerous intricate pathways (e.g., immune, neurologic and endocrine systems).\textsuperscript{2} Given this complexity, rather than focusing on very specific markers, some researchers have recently aimed for a systemic perspective. Genomics, transcriptomics and proteomics have expanded rapidly within the past years. Transcriptomic approach evaluates the transcripts (messenger RNA, also known as mRNA) for many genes. Assuming that certain genes may be over- or under-expressed in reaction to injury, researchers expect to find or confirm diagnostic, prognostic and follow-up markers, and to progress in pathophysiological understanding. Being able to guide therapeutic strategies disclosing patient immune profile is a seducing concept, however actual scientific knowledge is far from achieving this goal.\textsuperscript{108} Still, literature dealing with injury-induced immunoparalysis and transcriptome is abundant (Table I).

### General clinical data

As reported by Xiao \textit{et al.}, leukocytes of injured patients (in this study, severe trauma and burns) responded to stress by a global response pattern. The reason of this observation remains to be elucidated. Recently, only B cell and CD16+ monocyte counts were associated with increased mortality.\textsuperscript{107, 108} Consequently we may hypothesize that, similarly to other immune cells, B cell functional and quantitative alterations may play a role in injury-induced immune dysfunction. Thus, it might be interesting to shed light on B cell involvement and to develop B cell markers.

#### Table I.—Biomarkers: feasibility and clinical evidence.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Associated clinical outcome</th>
<th>Laboratory technique</th>
<th>Approximate minimal turnaround time</th>
<th>Possible in routine?</th>
</tr>
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<tbody>
<tr>
<td>Innate immunity</td>
<td></td>
<td></td>
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<tr>
<td>PNN</td>
<td>↑ immature forms</td>
<td>Death</td>
<td>FC, hematological analyzers</td>
<td>1h30</td>
</tr>
<tr>
<td>Monocytes</td>
<td>↓ mHLA-DR</td>
<td>Death, HAI</td>
<td>FC, IHC, ELISA</td>
<td>1h30</td>
</tr>
<tr>
<td>Endotoxin tolerance</td>
<td>↑ PD-L1</td>
<td>Few data, Death?</td>
<td>cell culture + ELISA</td>
<td>3 days</td>
</tr>
<tr>
<td>IL10 ad IL10/TNF</td>
<td>IL-12 synthesis, proliferation</td>
<td>HAI</td>
<td>FC, IHC</td>
<td>1h30</td>
</tr>
</tbody>
</table>

| Adaptive immunity | | | |
| All lymphocytes | Lymphopenia | Death | FC, hematological analyzers | 30 min | Yes |
| NTL | Death | FC, hematological analyzers | 30 min | Yes |
| T cells | ↑ CTLA4, BTLA, PD-1 | Few data | FC, IHC | 1h30 | Yes |
| CD127 | Death, HAI | Death | FC, IHC | 1h30 | No |
| Tregs | Proliferation | Death | cell culture + FC | 3 days | No |

| Both | Transcriptional | Few data, Death? | qRT PCR | 3h* | Possibly |

HAI: healthcare-associated infections; MOF: multi organ failure; PNN: polymuclear neutrophils; DC: dendritic cells; Tregs: regulator T cells; mHLA-DR: monocyte human leukocyte antigen DR; PD-1: programmed death one; PD-L1: programmed death ligand one; IL: interleukin; TNFα: tumor necrosis factor alpha; NLT: neutrophil to lymphocyte ratio; CTLA4: cytotoxic T lymphocyte-associated antigen 4; BTLA: B and T lymphocyte attenuator; FC: flow cytometry; IHC: immunohistochemistry; ELISA: Enzyme-Linked ImmunoSorbent Assay.

* if a unitized industrial method were developed.
oritization affecting >80% of the cellular functions and pathways. Authors refer to this phenomenon as “genomic storm”. Researchers observed simultaneous increase in expression of innate immune genes and suppression of adaptive immunity, in both injury situations. This supports the hypothesis of a common behavior, which would not rely on the stress type. This novel paradigm has also been described after severe blunt trauma and in sepsis.

Xiao et al. studied differentially expressed genes in complicated (recovery >14 days, no recovery, or death) versus uncomplicated clinical recovery (recovery in <5 days). Interestingly, authors highlighted an up-regulation of pro-inflammatory pathways in the complicated group, associated with down-regulated antigen presentation and T cell regulation.

To date, the literature gives many other examples of the ability of the transcriptomic approach to analyze the patient’s immune status in a global way. As proposed by Rittirsch et al. in trauma patients, the combination of clinical and transcriptomic markers may represent an even more interesting tool. Further studies are needed to develop predefined set of markers and to evaluate their predictive value regarding the diagnosis and prognosis of immune dysfunction.

Examples of transcriptomic-discovered markers: CD74, CX3CR1

Cazalis et al. used transcriptomic approach in a more targeted manner. In a prospective septic shock cohort, they investigated the link between MHC class II-related gene expression (by qRT-PCR) and mortality at day 28. Among these, low CD74 at day 3 after the onset of shock was associated with 28-day mortality after multivariate logistic regression analysis (OR 3.4 [1.2 to 9.8], P=0.026). CD74, also called HLA-DR antigen-associated invariant chain, is a protein involved in MHC II heterodimer synthesis and export towards the cell surface. CD74 still needs validation in a larger multicenter study.

CX3CR1 is a chemokine receptor expressed on monocytes, NK cells, and some lymphocyte subpopulations. It is involved in adhesion and migration of leukocytes and is thought to amplify pro-inflammatory immune response. In a microarray study, we observed an 8-fold increase of CX3CR1 in survivors compared with non survivors, which corresponded to the highest factor of change. We later reported that CX3CR1 (mRNA and protein) was strongly down-regulated in monocytes. Consistently, this down-regulation was associated with mortality.

Although these data are preliminary, both examples suggest that transcriptomic approach may allow to discover and to evaluate new biomarkers of immunoparalysis.

Conclusions

In parallel with explosive inflammatory processes, injury causes major immune dysfunctions leading to a significant morbi-mortality. Innate and adaptive immunity may both be severely compromised, in different extents depending on the patient immune profile. Being able to detect and follow immunoparalyzed patients and dysfunction subtypes is an important issue, for a targeted immunostimulating treatment may improve greatly their outcome. To date, available markers have a prognostic value, but researchers now aim to develop diagnostic tools. Such biomarkers could help targeting diagnosis procedures, allowing the clinician to define patients at risk to develop opportunistic infections. The most promising markers — i.e. markers both associated with clinical outcome in several types of injury and performable on routine basis — are decreased mHLA-DR and lymphopenia. In the light of the complexity of injury-induced immune response, combinations of biomarkers may represent an interesting line of investigation.

This review outlines current knowledge on biomarkers of injury-induced immunosuppression. It was not intended to be an in-depth survey of the whole subject and does not represent an exhaustive listing of studies in the field. We sincerely apologize for works not cited in this manuscript.
Key messages

- Injury-induced immunosuppression is a healthcare burden; patients suffering from it may benefit from immunostimulating treatments.
- Biomarkers of injury-induced immunosuppression are needed to specifically identify those patients that may benefit from immunoadjuvant therapies.
- For now, the most promising biomarkers are low mHLA-DR, lymphopenia and increased PD-1/PD-L1 pathway.

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Conflicts of interest.—Christelle Rouget And Julien Textoris are employees of Biomérieux SA. Thibaut Girardot, Julien Textoris, Guillame Monneret, Thomas Rimmé and Fabienne Venet are employees of the Hospices Civils De Lyon. Fabienne Venet and Guillaume Monneret are the inventors of patent families covering markers cited in this paper. This does not alter the authors’ adherence to all the journal policies on sharing data and materials.