



HAL
open science

Biological markers of injury-induced immunosuppression

Christelle Rouget, Thibaut Girardot, Julien Textoris, Guillaume Monneret,
Thomas Rimmelé, Fabienne Venet

► To cite this version:

Christelle Rouget, Thibaut Girardot, Julien Textoris, Guillaume Monneret, Thomas Rimmelé, et al.. Biological markers of injury-induced immunosuppression. *Minerva Anestesiologica*, 2023, 83 (3), 10.23736/S0375-9393.16.11268-4 . hal-04064730

HAL Id: hal-04064730

<https://univ-lyon1.hal.science/hal-04064730>

Submitted on 11 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

MINERVA

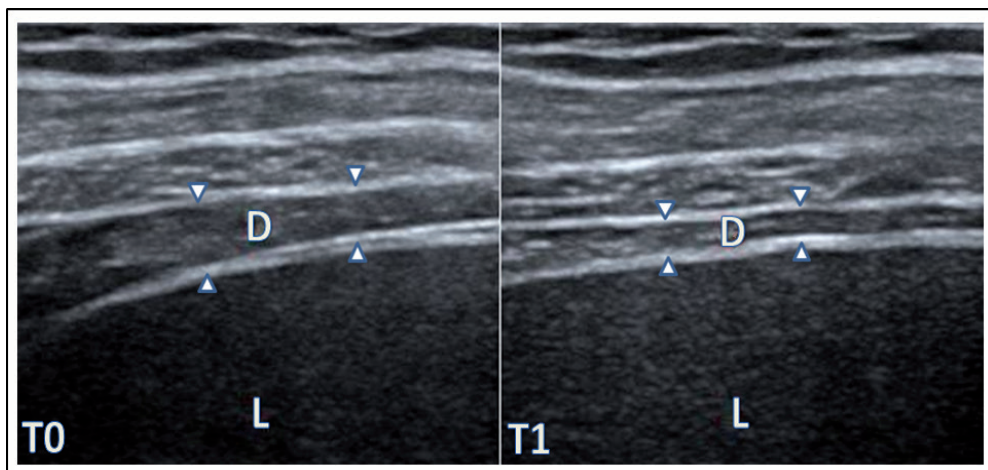
ANESTESIOLOGICA

OFFICIAL JOURNAL OF ITALIAN SOCIETY OF ANESTHESIOLOGY, ANALGESIA, RESUSCITATION AND INTENSIVE CARE (SIAARTI)



SIAARTI
PRO VITA CONTRA DOLOREM SEMPER

VOLUME 83 · No. 3 · MARCH 2017



E D I Z I O N I · M I N E R V A · M E D I C A

REVIEW

Biological markers of injury-induced immunosuppression

Christelle ROUGET¹⁻³, Thibaut GIRARDOT¹⁻³, Julien TEXTORIS¹⁻³,
Guillaume MONNERET^{1, 2, 4}, Thomas RIMMELÉ¹⁻³, Fabienne VENET^{1, 2, 4 *}

¹Joint Research Unit, Hospices Civils de Lyon, BioMérieux, Edouard Herriot Hospital, Lyon, France; ²EAM Pathophysiology of Injury-Induced Immunosuppression, UCBL1-HCL-BioMérieux, Lyon, France; ³Anesthesiology and Intensive Care Department, Hospices Civils de Lyon, Edouard Herriot Hospital, Lyon, France; ⁴Immunology Laboratory, Hospices Civils de Lyon, Edouard Herriot Hospital, Lyon, France

*Corresponding author: Fabienne Venet, Laboratoire Commun de Recherche HCL-BioMérieux, Hôpital Edouard Herriot, Pavillon P, 5 place d'Arsonval, 69437 Lyon Cedex 3, France. E-mail: fabienne.venet@chu-lyon.fr

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 immunoparalysis, mainly focusing on these 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.

(Cite this article as: Rouget C, Girardot T, Textoris J, Monneret G, Rimmelé T, Venet F. Biological markers of injury-induced immunosuppression. *Minerva Anesthesiol* 2017;83:302-14. DOI: 10.23736/S0375-9393.16.11268-4)

Key words: Wounds and injuries - Sepsis - Immunosuppression - Biomarkers - Monitoring, physiologic.

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,¹ severe trauma,² severe burns,³ or major surgery.⁴ When persistent or intense, immune paralysis is associated with the development of secondary infections and pejorative outcomes.⁵⁻⁸ Despite recent advances in criti-

cal 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.⁹ Mechanisms of this immunoparalysis and therapeutic opportunities have been recently published in the particular field of sepsis.¹⁰ 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.

Comment in p. 246.

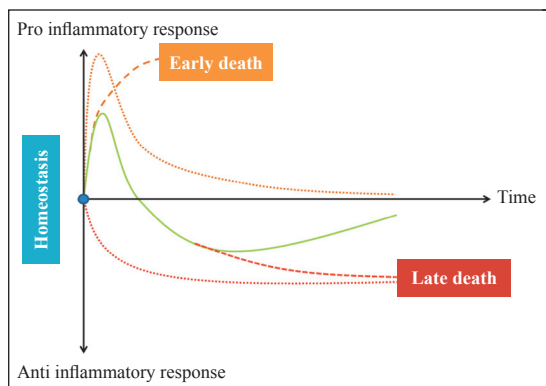


Figure 1.—Simplified description of immune response to injury: a complex balance.

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.¹¹

Reversing and/or shortening the immunoparalysis phase might be of crucial interest to improve patients' outcomes.¹² For instance, one of these promising immunoadjuvant therapies is interferon-gamma (IFN γ). It has been shown to restore the expression of transcripts associated with the development of immunosuppression in an *ex vivo* monocyte endotoxin tolerance model.¹³ IFN γ also partially reversed sepsis-induced immunoparalysis in a human *in vivo* model of Gram negative sepsis.¹⁴ 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.¹⁵ 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

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.¹⁶ 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.¹⁷

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.¹⁸ 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 *ex 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.*,¹⁹ phagocytosis and activation capacities were

preserved, indicating that neutrophil function is reconfigured rather than globally suppressed.

Impaired neutrophil chemotaxis, altered phagocytosis and bacterial killing were also described in burn²⁰⁻²³ 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 dosage²⁹⁻³¹ or IL10 mRNA measurement.³²⁻³⁴ The association between IL10 and septic complication or mortality was also described in burned patients^{35, 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.⁴⁴ Three clinical studies in septic populations showed that monocyte expression of CD163, or sCD163 level, were higher in non-survivors,⁴⁵⁻⁴⁷ 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.⁴⁸ 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).⁴⁸ 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^{49, 50} as well as in major surgery.^{51, 52}

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]).⁵³ 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.⁵⁴ 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.⁵⁵ 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.⁵⁶ Furthermore, DC loss in septic patients is higher in non-survivor septic patients than in survivors and is inversely correlated with severity scores.⁵⁷

After surgery, DC count increases acutely, and then drops below preoperative levels on days 2-3.⁵⁸ Surgery-induced DC depletion is associated with IL12 — a pro-inflammatory cytokine — deficiency.⁵⁹ 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.⁶⁰ Furthermore, life span and signaling function of DCs may be altered by trauma.⁶¹ Consistently, severe burn patients displayed early low DC count.⁶² In addition DCs from burn patients had impaired reactivity, an anti-inflammatory phenotype, and dysfunctional T cell-priming ability.⁶³

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^{64, 65} and of DC growth factor (Fms-related tyrosine kinase 3 ligand, FLT3-L)^{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.⁶⁸ In this study, lymphopenia at admission was not associated with mortality, contrary to the results presented by Chung *et al.*⁶⁹ Consistently, in emergency general surgical patients, lymphopenia was independently associated with increased in-hospital mortality (OR 3.5 [1.7-7.3]).⁷⁰ In trauma as well, failure to normalize lymphopenia in severely injured patients is associated with significantly higher mortality,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 (NLR).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.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.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.⁷⁷ Riché *et al.* refined this result in a septic shock cohort. They highlighted a reversed NTL evolution according to the timing of death:⁷⁸ early death was associated with low NTL at admission, whereas late death (\geq 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 (TGF β , IL10).⁷⁹ As presented recently, the percentage of Tregs increases after the onset of septic shock,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.⁸¹ Chen *et al.* also described a significant association between Treg count increase at day 7 and mortality in ICU patients with sepsis.⁸² CD39⁺ Treg cells form a specific subset displaying increased suppressive skills. In septic patients, increased expression of CD39⁺ Tregs was associated with mortality and severity of sepsis.⁸³

Conversely, after traumatic brain injury, the level of circulating Tregs was positively associated with neurologic recovery and lower hospital mortality.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

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,⁸⁵ directly antagonizing the co-stimulatory receptor CD28, and participating in T cell tolerance. CTLA-4 expression is increased after burn injury,⁸⁶ after trauma-hemorrhage,^{87,88} and in sepsis, and inhibits immune cell functions.⁸⁹ Inoue *et al.* showed a dose-dependent effect of anti-CTLA-4 on survival in a rodent model of sepsis,⁹⁰ with decreased CD8⁺ and CD4⁺ lymphocyte apoptosis. Consistently, blockade of CTLA-4 improved survival in rodent fungal sepsis.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⁺ lymphocytes.⁹¹ Consistently with previous literature,⁹² Shao *et al.* showed that lower percentage of BTLA⁺/

CD4⁺ T cells during the early stage of sepsis was associated with severity and 28-day mortality.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.⁹⁴

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,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.⁹⁶ In clinical setting, Zhang *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.⁹⁷ Guignant *et al.* found consistent results, and highlighted a correlation between increased PD-1, mortality and nosocomial infections after septic shock.⁹⁸ In this study, trauma patients did not express increased PD1 or PD-L1, contrary to the results presented elsewhere.⁸⁷

PD-1/PD-L1 expression on immune cells was also increased after surgery, and correlates with the severity of surgical trauma.⁹⁹ 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 (IL7R α , 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.¹⁰⁰ 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.¹⁰¹ 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.²⁵ 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.^{102, 103} 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.¹⁰⁰ Low doses of rhIL7 preferentially sustain effector T cells activation, whereas rhIL2 activates regulatory T cells.¹⁰⁴ 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 rhIL7.¹⁰⁵ 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.¹⁰⁶ 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.^{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).² Given this complexity, rather than focusing on very specific markers, some researchers have recently aimed for a systemic perspective. Ge-

nomics, 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 pathophysiologic 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.¹⁰⁸ Still, literature dealing with injury-induced immunoparalysis and transcriptome is abundant (Table I).

General clinical data

As reported by Xiao *et al.*, leukocytes of injured patients (in this study, severe trauma and burns) responded to stress by a global reprim-

TABLE I.—*Biomarkers: feasibility and clinical evidence.*

		Biomarker	Associated clinical outcome	Laboratory technique	Approximate minimal turn-around time	Possible in routine?
Innate immunity	PNN	↑ immature forms	Death	FC, hematological analyzers	1h30	Yes
	Monocytes	↓ mHLA-DR	Death, HAI	FC, IHC, PCR	1h30	Yes
		sCD127	Few data. Death?	ELISA	5h	No
		Endotoxin tolerance	Conflicting data	cell culture + ELISA	3 days	No
	DC	↑ PD-L1	HAI	FC, IHC	1h30	Not yet
		IL10 ad IL10/TNF	Death	ELISA or comparable method	5h	No
↓ DC count		Death, HAI	FC	1h30	Not yet	
	Function : IL-12 synthesis, proliferation	No	cell culture, ELISA	3 days	No	
Adaptive immunity	All lymphocytes	Lymphopenia	Death	FC, hematological analyzers	30 min	Yes
		NLT	Death	FC, hematological analyzers	30 min	Yes
		↑ CTLA4, BTLA	Few data	FC, IHC	1h30	Not yet
	T cells	↑ PD-1	Death	FC, IHC	1h30	Not yet
		CD127	Death, HAI	FC, IHC	1h30	Not yet
		Proliferation	Death, HAI, MOF	cell culture + FC	3 days	No
Tregs	↑ Treg percentage	Death	FC	1h30	Not yet	
Both	Transcriptomic	CD74, CX3CR1	Few data. Death?	qRT PCR microarray	3h* 3 days	Possibly No

HAI: healthcare-associated infections; MOF: multi organ failure; PNN: polynuclear 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.¹⁰⁹ 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 trauma¹¹⁰ and in sepsis.¹¹¹

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.¹¹² 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 28.¹¹³ 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 lympho-

cyte 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.¹¹⁴ We later reported that CX3CR1 (mRNA and protein) was strongly down-regulated in monocytes.⁴³ 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.

References

- Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* 2013;13:862-74.
- Lord JM, Midwinter MJ, Chen Y-F, Belli A, Brohi K, Kovacs EJ, *et al.* The systemic immune response to trauma: an overview of pathophysiology and treatment. *The Lancet* 2014;384:1455-65.
- Szellig L, Rendeki S, Foldi V, Lantos J, Bogar L, Csontos C. Time course of CD marker expression in patients with burns and its prognostic value. *Burns J Int Soc Burn Inj* 2014;40:575-82.
- Albertsmeier M, Quaiser D, von Dossow-Hanfstingl V, Winter H, Faist E, Angele MK. Major surgical trauma differentially affects T-cells and APC. *Innate Immunity* 2015;21:55-64.
- Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, *et al.* Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intensive Care Med* 2006;32:1175-83.
- Venet F, Tissot S, Debard A-L, Faudot C, Crampé C, Pachot A, *et al.* Decreased monocyte human leukocyte antigen-DR expression after severe burn injury: Correlation with severity and secondary septic shock. *Crit Care Med* 2007;35:1910-7.
- Chéron A, Monneret G, Landelle C, Floccard B, Allaouchiche B. [Low monocytic HLA-DR expression and risk of secondary infection]. *Ann Fr Anesthésie Réanimation* 2010;29:368-76. [Article in French]
- Lukaszewicz A-C, Griénay M, Resche-Rigon M, Pirracchio R, Faivre V, Boval B, *et al.* Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. *Crit Care Med* 2009;37:2746-52.
- Venet F, Lukaszewicz A-C, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. *Curr Opin Immunol* 2013;25:477-83.
- Hamers L, Kox M, Pickkers P. Sepsis-induced immunoparalysis: mechanisms, markers, and treatment options. *Minerva Anestesiologica* 2015;81:426-39.
- Marshall JC. The PIRO (predisposition, insult, response, organ dysfunction) model. *Virulence* 2014;5:27-35.
- Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *J Clin Invest* 2016;126:23-31.
- Allantaz-Frager F, Turrel-Davin F, Venet F, Monnin C, De Saint Jean A, Barbalat V, *et al.* Identification of Biomarkers of Response to IFN γ during Endotoxin Tolerance: Application to Septic Shock. *PLoS ONE* 2013;8:e68218.
- Leentjens J, Kox M, Koch RM, Preijers F, Joosten LAB, van der Hoeven JG, *et al.* Reversal of immunoparalysis in humans in vivo: a double-blind, placebo-controlled, randomized pilot study. *Am J Respir Crit Care Med* 2012;186:838-45.
- Meisel C, Scheffold JC, Pischowski R, Baumann T, Hetzger K, Gregor J, *et al.* Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. *Am J Respir Crit Care Med* 2009;180:640-8.
- Seok Y, Choi JR, Kim J, Kim YK, Lee J, Song J, *et al.* Delta neutrophil index: a promising diagnostic and prognostic marker for sepsis. *Shock* 2012;37:242-6.
- Mare TA, Treacher DF, Shankar-Hari M, Beale R, Lewis SM, Chambers DJ, *et al.* The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. *Crit Care* 2015;19:57.
- Demaret J, Venet F, Friggeri A, Cazalis M-A, Plassais J, Jallades L, *et al.* Marked alterations of neutrophil functions during sepsis-induced immunosuppression. *J Leukoc Biol* 2015;:jlb.4A0415-168RR.
- Drifte G, Dunn-Siegrist I, Tissières P, Pugin J. Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. *Crit Care Med* 2013;41:820-32.
- Fikrig SM, Karl SC, Suntharalingam K. Neutrophil chemotaxis in patients with burns. *Ann Surg* 1977;186:746-8.
- Brom J, Köller M, Schönfeld W, Knöller J, Erbs G, Müller FE, *et al.* Decreased expression of leukotriene B4 receptor sites on polymorphonuclear granulocytes of severely burned patients. *Prostaglandins Leukot Essent Fatty Acids* 1988;34:153-9.
- Brom J, Köller M, Müller-Lange P, Steinau HU, König W. GTP-binding proteins in polymorphonuclear granulocytes of severely burned patients. *J Leukoc Biol* 1993;53:268-72.
- Arturson G. Neutrophil granulocyte functions in severely burned patients. *Burns* 1985;11:309-19.
- Hazeldine J, Hampson P, Lord JM. The impact of trauma on neutrophil function. *Injury* 2014;45:1824-33.
- Monneret G, Venet F, Pachot A, Lepape A. Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. *Mol Med Camb Mass* 2008;14:64-78.
- Gouel-Chéron A, Allaouchiche B, Floccard B, Rimmelé T, Monneret G. Early daily mHLA-DR monitoring predicts forthcoming sepsis in severe trauma patients. *Intensive Care Med* 2015;41:2229-30.
- Couper KN, Blount DG, Riley EM. IL-10: The Master Regulator of Immunity to Infection. *J Immunol* 2008;180:5771-7.
- Monneret G, Finck M-E, Venet F, Debard A-L, Bohé J, Bienvenu J, *et al.* The anti-inflammatory response dominates after septic shock: association of low monocyte HLA-DR expression and high interleukin-10 concentration. *Immunol Lett* 2004;95:193-8.
- van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, Frölich M. Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet* 1998;351:950-3.
- Gogos CA, Drosou E, Bassaris HP, Skoutelis A. Pro-versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. *J Infect Dis* 2000;181:176-80.
- Heper Y, Akalin EH, Mistik R, Akgöz S, Töre O, Göral

- G, *et al.* Evaluation of serum C-reactive protein, procalcitonin, tumor necrosis factor alpha, and interleukin-10 levels as diagnostic and prognostic parameters in patients with community-acquired sepsis, severe sepsis, and septic shock. *Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol* 2006;25:481-91.
32. Lekkou A, Karakantza M, Mouzaki A, Kalfarentzos F, Gogos CA. Cytokine production and monocyte HLA-DR expression as predictors of outcome for patients with community-acquired severe infections. *Clin Diagn Lab Immunol* 2004;11:161-7.
 33. Pachot A, Monneret G, Voirin N, Leissner P, Venet F, Bohé J, *et al.* Longitudinal study of cytokine and immune transcription factor mRNA expression in septic shock. *Clin Immunol* 2005;114:61-9.
 34. Abe R, Hirasawa H, Oda S, Sadahiro T, Nakamura M, Watanabe E, *et al.* Up-Regulation of Interleukin-10 mRNA Expression in Peripheral Leukocytes Predicts Poor Outcome and Diminished Human Leukocyte Antigen-DR Expression on Monocytes in Septic Patients. *J Surg Res* 2008;147:1-8.
 35. Pileri D, Accardo Palombo A, D'Amelio L, D'Arpa N, Amato G, Masellis A, *et al.* Concentrations of cytokines IL-6 and IL-10 in plasma of burn patients: their relationship to sepsis and outcome. *Ann Burns Fire Disasters* 2008;21:182-5.
 36. Csontos C, Foldi V, Pálincas L, Bogar L, Róth E, Weber G, *et al.* Time course of pro- and anti-inflammatory cytokine levels in patients with burns--prognostic value of interleukin-10. *Burns* 2010;36:483-94.
 37. Stensballe J, Christiansen M, Tønnesen E, Espersen K, Lippert FK, Rasmussen LS. The early IL-6 and IL-10 response in trauma is correlated with injury severity and mortality. *Acta Anaesthesiol Scand* 2009;53:515-21.
 38. Sugimoto K, Galle C, Preiser J-C, Creteur J, Vincent JL, Pradier O. Monocyte CD40 expression in severe sepsis. *Shock* 2003;19:24-7.
 39. Wolk K, Höflich C, Zuckermann-Becker H, Döcke W-D, Volk H-D, Sabat R. Reduced monocyte CD86 expression in postinflammatory immunodeficiency. *Crit Care Med* 2007;35:458-67.
 40. Heinzelmann M, Mercer-Jones M, Cheadle WG, Polk HC. CD14 expression in injured patients correlates with outcome. *Ann Surg* 1996;224:91-6.
 41. Aalto H, Takala A, Kautiainen H, Siitonen S, Repo H. Monocyte CD14 and soluble CD14 in predicting mortality of patients with severe community acquired infection. *Scand J Infect Dis* 2007;39:596-603.
 42. Pangault C, Le Tulzo Y, Tattevin P, Guilloux V, Bescher N, Drénou B. Down-modulation of granulocyte macrophage-colony stimulating factor receptor on monocytes during human septic shock. *Crit Care Med* 2006;34:1193-201.
 43. Pachot A, Cazalis M-A, Venet F, Turrel F, Faudot C, Voirin N, *et al.* Decreased expression of the fractalkine receptor CX3CR1 on circulating monocytes as new feature of sepsis-induced immunosuppression. *J Immunol* 1950 2008;180:6421-9.
 44. Droste A, Sorg C, Högger P. Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. *Biochem Biophys Res Commun* 1999;256:110-3.
 45. Feng L, Zhou X, Su L-X, Feng D, Jia YH, Xie LX. Clinical Significance of Soluble Hemoglobin Scavenger Receptor CD163 (sCD163) in Sepsis, a Prospective Study. *PLoS ONE* 2012;7:e38400.
 46. Su L, Feng L, Song Q, Kang H, Zhang X, Liang Z, *et al.* Diagnostic Value of Dynamics Serum sCD163, sTREM-1, PCT, and CRP in Differentiating Sepsis, Severity Assessment, and Prognostic Prediction. *Mediators Inflamm* 2013;2013:969875.
 47. Kjærgaard AG, Rødgaard-Hansen S, Dige A, Krog J, Møller HJ, Tønnesen E. Monocyte Expression and Soluble Levels of the Haemoglobin Receptor (CD163/sCD163) and the Mannose Receptor (MR/sMR) in Septic and Critically Ill Non-Septic ICU Patients. *PLoS ONE* 2014;9:e38400.
 48. Cavaillon J-M, Adib-Conquy M. Bench-to bedside review: Endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit Care* 2006;10:233.
 49. Keel M, Schregerberger N, Steckholzer U, Ungethüm U, Kenney J, Trentz O, *et al.* Endotoxin tolerance after severe injury and its regulatory mechanisms. *J Trauma* 1996;41:430-7; discussion 437-438.
 50. West SD, Mold C. Monocyte deactivation correlates with injury severity score, but not with heme oxygenase-1 levels in trauma patients. *J Surg Res* 2012;172:5-10.
 51. Ogata M, Okamoto K, Kohriyama K, Kawasaki T, Itoh H, Shigematsu A. Role of interleukin-10 on hyporesponsiveness of endotoxin during surgery. *Crit Care Med* 2000;28:3166-70.
 52. Flohé S, Lendemans S, Schade F-U, Kreuzfelder E, Waydhas C. Influence of surgical intervention in the immune response of severely injured patients. *Intensive Care Med* 2004;30:96-102.
 53. Hall MW, Knatz NL, Vetterly C, Tomarello S, Wewers MD, Volk HD, *et al.* Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. *Intensive Care Med* 2011;37:525-32.
 54. van Vught LA, Wiewel MA, Hoogendijk AJ, Scicluna BP, Belkasim-Bohoudi H, Horn J, *et al.* Reduced Responsiveness of Blood Leukocytes to Lipopolysaccharide does not Predict Nosocomial Infections in Critically Ill Patients. *Shock* 2015;44:110-4.
 55. Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH, *et al.* Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J Immunol Baltim Md 1950* 2002;168:2493-500.
 56. Grimaldi D, Louis S, Pène F, Sirgo G, Rousseau C, Claessens YE, *et al.* Profound and persistent decrease of circulating dendritic cells is associated with ICU-acquired infection in patients with septic shock. *Intensive Care Med* 2011;37:1438-46.
 57. Guisset O, Dilhuydy M-S, Thiébaud R, Lefèvre J, Camou F, Sarrat A, *et al.* Decrease in circulating dendritic cells predicts fatal outcome in septic shock. *Intensive Care Med* 2007;33:148-52.
 58. Ho CSK, López JA, Vuckovic S, Pyke CM, Hockey RL, Hart DNJ. Surgical and physical stress increases circulating blood dendritic cell counts independently of monocyte counts. *Blood* 2001;98:140-5.
 59. Yadavalli GK, Chien JW, Wener KM, Deveccio JL, Gupta S, Salata RA, *et al.* Interleukin 12 and interferon-gamma synthetic deficiency is associated with dendritic cell cytopenia after cardiac surgery. *Shock* 2005;24:26-33.
 60. De AK, Laudanski K, Miller-Graziano CL. Failure of monocytes of trauma patients to convert to immature dendritic cells is related to preferential macrophage-colony-stimulating factor-driven macrophage differentiation. *J Immunol* 1950 2003;170:6355-62.
 61. Maier M, Wutzler S, Bauer M, Trendafilov P, Henrich D, Marzi I. Altered gene expression patterns in dendritic cells after severe trauma: implications for systemic inflammation and organ injury. *Shock* 2008;30:344-51.
 62. D'Arpa A, Accardo-Palumbo A, Amato G, D'Amelio L, Napoli B, Pileri D, *et al.* Decrease of Circulating Dendritic Cells in Burn Patients. *Ann Burns Fire Disasters* 2007;20:199-202.
 63. Shen H, de Almeida PE, Kang KH, Yao P, Chan CW. Burn Injury Triggered Dysfunction in Dendritic Cell Re-

- sponse to TLR9 Activation and Resulted in Skewed T Cell Functions. *PLoS ONE* 2012;7:e50238.
64. Benjamim CF, Lundy SK, Lukacs NW, Hogaboam CM, Kunkel SL. Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood* 2005;105:3588-95.
 65. Wang HW, Yang W, Gao L, Kang JR, Qin JJ, Liu YP, *et al.* Adoptive transfer of bone marrow-derived dendritic cells decreases inhibitory and regulatory T-cell differentiation and improves survival in murine polymicrobial sepsis. *Immunology* 2015;145:50-9.
 66. Toliver-Kinsky TE, Cui W, Murphey ED, Lin C, Sherwood ER. Enhancement of dendritic cell production by fms-like tyrosine kinase-3 ligand increases the resistance of mice to a burn wound infection. *J Immunol* 1950 2005;174:404-10.
 67. Wysocka M, Montaner LJ, Karp CL. Flt3 Ligand Treatment Reverses Endotoxin Tolerance-Related Immunoparalysis. *J Immunol* 2005;174:7398-402.
 68. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock* 2014;42:383-91.
 69. Chung KP, Chang HT, Lo SC, Chang LY, Lin SY, Cheng A, *et al.* Severe lymphopenia is associated with elevated plasma interleukin-15 levels and increased mortality during severe sepsis. *Shock* 2015;43:569-75.
 70. Vulliamy PE, Perkins ZB, Brohi K, Manson J. Persistent lymphopenia is an independent predictor of mortality in critically ill emergency general surgical patients. *Eur J Trauma Emerg Surg* 2016;42:755-60.
 71. Heffernan DS, Monaghan SF, Thakkar RK, Machan JT, Cioffi WG, Ayala A. Failure to normalize lymphopenia following trauma is associated with increased mortality, independent of the leukocytosis pattern. *Crit Care* 2012;16:R12.
 72. Zahorec R. Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. *Bratisl Lekárske Listy* 2001;102:5-14.
 73. Cook EJ, Walsh SR, Farooq N, Alberts JC, Justin TA, Keeling NJ. Post-operative neutrophil-lymphocyte ratio predicts complications following colorectal surgery. *Int J Surg* 2007;5:27-30.
 74. Forget P, Dinant V, De Kock M. Is the Neutrophil-to-Lymphocyte Ratio more correlated than C-reactive protein with postoperative complications after major abdominal surgery? *PeerJ* 2015;3:e713.
 75. Jaramillo-Reta KY, Velázquez-Dohorn ME, Medina-Franco H. Neutrophil to lymphocyte ratio as predictor of surgical mortality and survival in complex surgery of the upper gastrointestinal tract. *Rev Investig Clínica Organo Hosp Enfermedades Nutr* 2015;67:117-21.
 76. Moreau N, Forget P, Wittebole X, Laterre P, Castanares-Zapatero D. Neutrophil-to-lymphocyte ratio is associated with mortality in critically-ill cirrhotic patients. *Intensive Care Med* 2015;3:A688.
 77. Saliccioli JD, Marshall DC, Pimentel M, Santos MD, Pollard T, Celi L, *et al.* The association between the neutrophil-to-lymphocyte ratio and mortality in critical illness: an observational cohort study. *Crit Care* 2015;19:13.
 78. Riché F, Gayat E, Barthélémy R, Le Dorze M, Matéo J, Payen D. Reversal of neutrophil-to-lymphocyte count ratio in early versus late death from septic shock. *Crit Care* 2015;19:439.
 79. Kessel A, Bamberger E, Masalha M, Toubi E. The role of T regulatory cells in human sepsis. *J Autoimmun* 2009;32:211-5.
 80. Venet F, Chung C-S, Kherouf H, Geeraert A, Malcus C, Poitevin F, *et al.* Increased circulating regulatory T cells (CD4+CD25+CD127-) contribute to lymphocyte anergy in septic shock patients. *Intensive Care Med* 2009;35:678-86.
 81. Huang LF, Yao YM, Dong N, Yu Y, He LX, Sheng ZY. Association between regulatory T cell activity and sepsis and outcome of severely burned patients: a prospective, observational study. *Crit Care* 2010;14:R3.
 82. Chen K, Zhou QX, Shan HW, Li WF, Lin ZF. Prognostic value of CD4(+)CD25(+) Tregs as a valuable biomarker for patients with sepsis in ICU. *World J Emerg Med* 2015;6:40-3.
 83. Huang H, Xu R, Lin F, Bao C, Wang S, Ji C, *et al.* High circulating CD39(+) regulatory T cells predict poor survival for sepsis patients. *Int J Infect Dis* 2015;30:57-63.
 84. Li M, Lin YP, Chen JL, Li H, Jiang RC, Zhang JN, *et al.* Role of Regulatory T cell in Clinical Outcome of Traumatic Brain Injury. *Chin Med J* 2015;128:1072.
 85. Walker LSK, Sansom DM. Confusing signals: Recent progress in CTLA-4 biology. *Trends Immunol* 2015;36:63-70.
 86. Fazal N, Shelip A, Alzahrani AJ. Burn-injury affects gut-associated lymphoid tissues derived CD4+ T cells. *Results Immunol* 2013;3:85-94.
 87. Bandyopadhyay G, De A, Laudanski K, Li F, Lentz C, Bankey P, *et al.* Negative signaling contributes to T-cell anergy in trauma patients. *Crit Care Med* 2007;35:794-801.
 88. Hsieh CH, Hsu JT, Hsieh YC, Frink M, Raju R, Hubbard WJ, *et al.* Suppression of Activation and Costimulatory Signaling in Splenic CD4+ T Cells after Trauma-Hemorrhage Reduces T-Cell Function. *Am J Pathol* 2009;175:1504-14.
 89. Chang KC, Burnham CA, Compton SM, Rasche DP, Mazuski RJ, McDonough JS, *et al.* Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. *Crit Care* 2013;17:R85.
 90. Inoue S, Bo L, Bian J, Unsinger J, Chang K, Hotchkiss RS. Dose-Dependent Effect of Anti-CTLA-4 on Survival in Sepsis. *Shock* 2011;36:38-44.
 91. Hurchla MA, Sedy JR, Gavrielli M, Drake CG, Murphy TL, Murphy KM. B and T Lymphocyte Attenuator Exhibits Structural and Expression Polymorphisms and Is Highly Induced in Anergic CD4+ T Cells. *J Immunol* 2005;174:3377-85.
 92. Shubin NJ, Monaghan SF, Heffernan DS, Chung CS, Ayala A. B and T lymphocyte attenuator expression on CD4+ T-cells associates with sepsis and subsequent infections in ICU patients. *Crit Care* 2013;17:R276.
 93. Shao R, Li CS, Fang Y, Zhao L, Hang C. Low B and T lymphocyte attenuator expression on CD4(+) T cells in the early stage of sepsis is associated with the severity and mortality of septic patients: a prospective cohort study. *Crit Care* 2015;19:308.
 94. Boomer JS, Shuherk-Shaffer J, Hotchkiss RS, Green JM. A prospective analysis of lymphocyte phenotype and function over the course of acute sepsis. *Crit Care* 2012;16:R112.
 95. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 2008;26:677-704.
 96. Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, *et al.* PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci* 2009;106:6303-8.
 97. Zhang Y, Li J, Lou J, Zhou Y, Bo L, Zhu J, *et al.* Up-regulation of programmed death-1 on T cells and programmed death ligand-1 on monocytes in septic shock patients. *Crit Care* 2011;15:R70.
 98. Guignant C, Lepape A, Huang X, Kherouf H, Denis L, Poitevin F, *et al.* Programmed death-1 levels correlate

- with increased mortality, nosocomial infection and immune dysfunctions in septic shock patients. *Crit Care* 2011;15:R99.
99. Xu P, Zhang P, Sun Z, Wang Y, Chen J, Miao C. Surgical trauma induces postoperative T-cell dysfunction in lung cancer patients through the programmed death-1 pathway. *Cancer Immunol Immunother* 2015;64:1383-92.
 100. Venet F, Foray A-P, Villars-Méchin A, Malcus C, Poitevin-Later F, Lepape A, *et al.* IL-7 restores lymphocyte functions in septic patients. *J Immunol* 1950 2012;189:5073-81.
 101. Demaret J, Villars-Méchin A, Lepape A, Plassais J, Vallin H, Malcus C, *et al.* Elevated plasmatic level of soluble IL-7 receptor is associated with increased mortality in septic shock patients. *Intensive Care Med* 2014;40:1089-96.
 102. Wolk K, Döcke WD, Baehr V von, Volk H-D, Sabat R. Impaired antigen presentation by human monocytes during endotoxin tolerance. *Blood* 2000;96:218-23.
 103. Poujol F, Monneret G, Pachot A, Textoris J, Venet F. Altered T Lymphocyte Proliferation upon Lipopolysaccharide Challenge Ex Vivo. *PLoS ONE* 2015;10:e0144375.
 104. Dupont G, Demaret J, Venet F, Malergue F, Malcus C, Poitevin-Later F, *et al.* Comparative dose-responses of recombinant human IL-2 and IL-7 on STAT5 phosphorylation in CD4+FOXP3- cells versus regulatory T cells: a whole blood perspective. *Cytokine* 2014;69:146-9.
 105. Demaret J, Dupont G, Venet F, Friggeri A, Lepape A, Rimmelé T, *et al.* STAT5 phosphorylation in T cell subsets from septic patients in response to recombinant human interleukin-7: a pilot study. *J Leukoc Biol* 2015;97:791-6.
 106. Monserrat J, de Pablo R, Diaz-Martín D, Rodríguez-Zapata M, de la Hera A, Prieto A, *et al.* Early alterations of B cells in patients with septic shock. *Crit Care* 2013;17:R105.
 107. Park SH, Park BG, Park CJ, Kim S, Kim DH, Jang S, *et al.* An extended leukocyte differential count (16 types of circulating leukocytes) using the cytodiiff flow cytometric system can provide informations for the discrimination of sepsis severity and prediction of outcome in sepsis patients. *Cytometry B Clin Cytom* 2013 [Epub ahead of print].
 108. Amit I, Regev A, Hacohen N. Strategies to discover regulatory circuits of the mammalian immune system. *Nat Rev Immunol* 2011;11:873-80.
 109. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, *et al.* A genomic storm in critically injured humans. *J Exp Med* 2011;208:2581-90.
 110. Vanzant EL, Lopez CM, Ozrazgat-Baslanti T, Ungaro R, Davis R, Cuenca AG, *et al.* Persistent Inflammation, Immunosuppression and Catabolism Syndrome after Severe Blunt Trauma. *J Trauma Acute Care Surg* 2014;76:21-30.
 111. Cazalis M-A, Lepape A, Venet F, Frager F, Mouglin B, Vallin H, *et al.* Early and dynamic changes in gene expression in septic shock patients: a genome-wide approach. *Intensive Care Med Exp* 2014;2:20.
 112. Rittirsch D, Schoenborn V, Lindig S, Wanner E, Sprengel K, Günkel S, *et al.* Improvement of prognostic performance in severely injured patients by integrated clinico-transcriptomics: a translational approach. *Crit Care* 2015;19:414.
 113. Cazalis MA, Friggeri A, Cavé L, Demaret J, Barbalat V, Cerrato E, *et al.* Decreased HLA-DR antigen-associated invariant chain (CD74) mRNA expression predicts mortality after septic shock. *Crit Care* 2013;17:R287.
 114. Pachot A, Lepape A, Vey S, Bienvenu J, Mouglin B, Monneret G. Systemic transcriptional analysis in survivor and non-survivor septic shock patients: A preliminary study. *Immunol Lett* 2006;106:63-71.

Conflicts of interest.—Christelle Rouget And Julien Textoris are employees of Biomérieux SA. Thibaut Girardot, Julien Textoris, Guillaume Monneret, Thomas Rimmelé 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.

Article first published online: June 17, 2016. - Manuscript accepted: June 15, 2016. - Manuscript revised: May 4, 2016. - Manuscript received: February 12, 2016.