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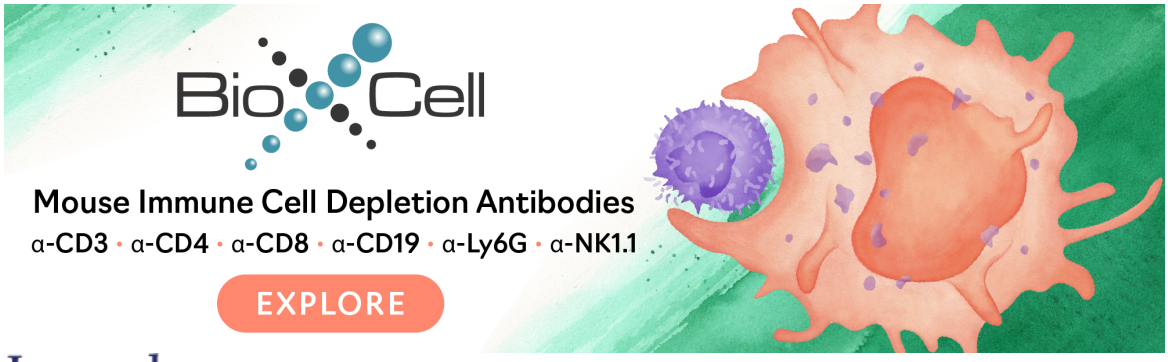

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IL-7 Restores Lymphocyte Functions in Septic Patients

Fabienne Venet,*,† Anne-Perrine Foray,* Astrid Villars-Méchin,* Christophe Malcus,* Françoise Poitevin-Later,* Alain Lepape,†,‡ and Guillaume Monneret*,†

Septic syndrome is the leading cause of mortality for critically ill patients worldwide. Patients develop lymphocyte dysfunctions associated with increased risk of death and nosocomial infections. In this study, we performed preclinical experiments testing the potential of recombinant human IL-7 (rhIL-7) as a lymphostimulating therapy in sepsis. Circulating IL-7 and soluble IL-7 receptor α-chain (soluble CD127) concentrations were measured in plasma, whereas cellular CD127 expression was evaluated on circulating CD4$^+$ and CD8$^+$ lymphocytes from septic shock patients and healthy volunteers. Lymphocyte proliferation, IFN-γ production, STAT5 phosphorylation, and B cell lymphoma 2 induction were measured ex vivo in response to T cell stimulation in the presence or not of rhIL-7. We show that IL-7 pathway (plasmatic IL-7 concentration and cellular and soluble CD127 expressions) is not overtly altered and remains activable in septic patients. Most importantly ex vivo treatment of patients' cells with rhIL-7 significantly improves lymphocyte functionality (CD4$^+$ and CD8$^+$ lymphocyte proliferations, IFN-γ production, STAT5 phosphorylation, and B cell lymphoma 2 induction after stimulation). To our knowledge, this constitutes the first report of rhIL-7 ability to restore normal lymphocyte functions in septic patients. These results support the rational for initiating a clinical trial testing rhIL-7 in septic shock. ***The Journal of Immunology*****, 2012, 189: 5073–5081.**

Septic syndrome is the leading cause of mortality for critically ill patients worldwide although an enormous effort has been expended during the past 20 y to find new therapies that decrease the mortality of this syndrome (1). It is now largely demonstrated that septic patients develop severe immune alterations (innate and adaptive), which intensity and duration are correlated with increased risk of death and nosocomial infections (1). Thus, immune-stimulating therapies are now considered in the treatment of sepsis (2).

Sepsis-induced lymphocyte alterations share many similarities with immune dysfunctions described in patients with chronic viral infections such as HIV (3–5). In particular, severe lymphopenia due to increased apoptosis, decreased T cell proliferation, and cytokine production after stimulation as well as increased percentage of CD4$^+$CD25$^+$ regulatory T cells (Treg) have been described in both clinical contexts. In HIV infection, innovative immunorestorative therapies such as recombinant human IL-7 (rhIL-7) are currently tested as adjunctive treatment in phase I and II clinical trials.

IL-7 is a 25-kDa glycoprotein secreted by stroma cells and other epithelial cells. IL-7 is a nonredundant cytokine for T and B cell development and function. IL-7 activity is mediated via its binding to a receptor (IL-7R) constituted of two chains: the α-chain (CD127) and the common cytokine receptor γ-chain. This receptor is expressed continuously on most resting human T cells with high levels on naive and central memory cells and lower levels on Treg (6). In healthy volunteers, rhIL-7 therapy induces B cell lymphoma 2 (BCL2) upregulation and sustained increased in peripheral blood CD4$^+$ and CD8$^+$ T cell numbers. Consequently, this T cell expansion caused a significant broadening of circulating TCR repertoire diversity independently of subject's age (7). Interestingly, rhIL-7 therapy is associated with a reduction in the circulating proportion of Treg (7).

In chronic viral infection, rhIL-7 treatment has proven to be safe, well tolerated and to result in potent immunorestorative effects (4). In sepsis, three studies in mice have described beneficial effects of rhIL-7 treatment on mortality and sepsis-induced immune dysfunctions (8–10).

The objective of the current study was thus to perform an ex vivo preclinical study of rhIL-7 therapy in septic shock patients. We first tested whether parameters of IL-7 pathway were altered in patients in comparison with controls. In a second set of experiments, patients' lymphocyte response to rhIL-7 treatment was tested ex vivo. These experiments should provide the preliminary results necessary to the initiation of a clinical trial testing rhIL-7 in septic shock.

Materials and Methods

Patients

Septic patients belong to a global study on intensive care unit (ICU)-induced immune dysfunctions approved by our Institutional Review Board for ethics ("Comité de Protection des Personnes") and registered at Ministère Français de la Recherche et de l'Enseignement (no. DC-2008-509). The study group consisted in 70 septic shock patients according to the diagnostic criteria of the American College of Chest Physician/Society of Critical Care Medicine (Table I) (11). The onset of septic shock was defined as the beginning of vasopressor therapy. EDTA–anticoagulated blood was collected at two time points after diagnosis: day 1–2 and day 3–4. Mortality was defined as death occurring within 28 d after the onset of

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Abbreviations used in this article: BCL2, B cell lymphoma 2; ICU, intensive care unit; mHLA-DR, HLA-DR expression on circulating monocyte; PD-1, programmed cell death-1; rhIL-7, recombinant human IL-7; sCD127, soluble CD127; Treg, regulatory T cell.

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shock. Secondary ICU-acquired infections were defined according to European definitions of European Centre for Disease Prevention and Control.

Reference values and control samples for functional assays were obtained from a cohort of 55 healthy volunteers after informed consent was given.

Reagents

Anti–CD3-CD28-CD2 coated beads (T cell activation/expansion kit human) were purchased from Miltenyi Biotec (Auburn, CA). PE and PE–cyanine7-conjugated anti-CD3, Alexa Fluor 647-conjugated anti-phosphorylated STAT5, PE-conjugated anti-BCL2, and isotypic controls as well as protein transport inhibitor (GolgiPlug) were purchased from BD Biosciences (San Jose, CA). Allophycocyanin-conjugated anti-CD8, PE-conjugated anti-CD4, allophycocyanin-conjugated anti-CD4, PE-cyanine7–conjugated anti-CD8, PE-conjugated anti–IFN-γ as well as anti-human CD127 Abs (clone R34.34) and isotypic controls were purchased from Beckman Coulter (Hialey, FL). rhIL-7 as well as goat anti-human CD127 polyclonal Abs and recombinant human CD127-Fc chimera were purchased from R&D Systems (Minneapolis, MN).

Quantification of plasma CD127 and IL-7 concentrations

A soluble CD127 (sCD127) ELISA test was designed as described previously (12). Briefly, 96-well plates were coated overnight with mouse anti-human CD127 mAb (Beckman Coulter). Plates were blocked with BSA for 1 h, washed, and then samples were incubated for 1 h. Bound sCD127 was detected following a 1-h incubation with biotinylated goat anti-human CD127 polyclonal Abs (R&D Systems) and streptavidin-HRP. Reactions were developed using the tetramethylbenzidine microwell peroxidase substrate (BioMérieux, Marcy l'étoile, France) and visualized with a plate reader at 450 nm. Sample concentrations were also extrapolated from standard curves of recombinant human CD127-Fc chimera (R&D Systems).

Plasmatic IL-7 concentration was measured using a multiplex ELISA (Bio-Rad, Hercules, CA) by using the manufacturer's recommendations.

Cell isolation and culture

PBMCs were isolated by Ficoll-Paque PREMIUM (GE Healthcare, Waukesha, WI) density gradient centrifugation. Cells were washed three times in sterile PBS (bioMérieux) and resuspended in complete culture media. (RPMI 1640 medium with HEPES [Eurobio, Courtaboeuf, France] supplemented with 2 g/l sodium bicarbonate 7.5% [Institut de biotechnologies Jacques Boy, Reims, France], 2 mM L-glutamine [Lonza, Basel, Switzerland], 10% human AB plasma [Etablissement Français du Sang, Gerland, Lyon, France], 20 U/ml penicillin and 20 μg/ml streptomycin [Sigma-Aldrich, St. Louis, MO], and 2.5 μg/ml fungizone [Bristol-Myers Squibb, Rueil-Malmaison, France].)

The number of cells per well was adjusted to 1×10^6 cells/ml. Cells were stimulated with anti–CD2-CD3-CD28 Abs coated beads (ratio beads/cells = 4:1) and/or with rhIL-7 (100 ng/ml).

Tritiated thymidine incorporation

T cells proliferation was analyzed by detecting ^{3}H-labeled methyl-thymidine incorporation (20 μCi/well) (PerkinElmer, Boston, MA). ^{3}H-labeled methyl-thymidine was added 24 h before harvesting cells on a fiberglass filter using an automated cell harvester (PerkinElmer). Incorporated radioactivity was measured in a direct beta counter (PerkinElmer). Assays were carried out in triplicate.

Flow cytometry

HLA-DR expression on circulating monocytes (mHLA-DR), percentage of Treg ($CD4^+CD25^{high}CD127^{low}$) as well as CD127 expression on $CD4^+$ and $CD8^+$ T cells were monitored as described previously (13, 14). $CD4^+$ and $CD8^+$ T cell proliferation was analyzed by monitoring decreased fluorescence after cell labeling with CFSE (Molecular Probes, Grand Island, NY) at a concentration of 10 pM and after cell culture during 5 d. STAT5 phosphorylation in T cells was analyzed by intracellular staining after overnight TCR stimulation and 15-min incubation with rhIL-7. BCL2 expression in T cells was analyzed by intracellular staining after 2-d incubation with TCR stimulation and rhIL-7. IFN-γ production by $CD8^+$ T cells was analyzed by intracellular staining after 4 h incubation with GolgiPlug, TCR stimulation and rhIL-7. The samples were run on a FACSAria II flow cytometer and analyzed using FACSDiva software (BD Biosciences).

Statistics

Results are presented as box-plots with individual values in Figs. 1–3 and as means and SEM in Figs. 4–7. Comparisons between groups were made using the nonparametric Mann–Whitney *U* test. The nonparametric Wilcoxon paired test was used to assess variations between time points in septic patients or between cell culture conditions in the ex vivo experiments. Statistical analyses were performed using Prism software (version 4.03; GraphPad Software, La Jolla, CA). A p value <0.05 was considered as statistically significant.

Results

Plasmatic IL-7 concentration is slightly increased after septic shock

Plasmatic IL-7 concentration was measured in septic shock patients at days 1–2 and 3–4 ($n = 24$) in comparison with healthy controls ($n = 26$). We observed that circulating IL-7 level was increased in septic shock patients at day 1–2 and at day 3–4 in comparison with controls (Fig. 1A). However, these levels remained relatively low (<10 pg/ml) and no significant differences were observed between survivors ($n = 15$) and nonsurvivors ($n = 9$; Fig. 1B) or between patients developing or not a nosocomial episode ($n = 4$ and $n = 20$, respectively; Fig. 1C).

This suggests that IL-7 plasmatic level may be increased in a homeostatic manner after septic shock. Because circulating IL-7 concentration is mostly regulated by its scavenging rate by T cells, such increased concentration is not surprising in markedly lymphopenic hosts such as septic shock patients (Table I) (4, 15).

CD127 expression is maintained on $CD4^+$ and $CD8^+$ lymphocytes after septic shock

A strong regulation of IL-7 receptor α-chain (CD127) has been described in various clinical conditions including HIV infection (16). We thus measured CD127 expression by flow cytometry on $CD4^+$ and $CD8^+$ T cells from 20 septic shock patients sampled at days 1–2 and 3–4 after the onset of shock in comparison with 30 healthy volunteers (Fig. 2).

No significant differences were observed between patients and controls at day 1–2 either on $CD4^+$ cells (Fig. 2A) or on $CD8^+$ cells (Fig. 2B). At day 3–4, a slightly decreased CD127 expression was measured on $CD8^+$ T cells from septic patients in comparison with healthy volunteers (Fig. 2B), but no difference was observed for $CD4^+$ cells (Fig. 2A). In paired samples, a decrease over time was observed for CD127 expression on $CD4^+$ between days 1–2 and 3–4 but not on $CD8^+$ cells. To note, absolute numbers of circulating $CD4^+CD127^+$ and $CD8^+CD127^+$ cells were calculated. As expected considering the marked lymphopenia observed after septic shock, these parameters were significantly decreased in septic patients versus healthy volunteers (data not shown).

No significant differences were observed between survivors ($n = 12$) and nonsurvivors ($n = 8$) for CD127 expression measured either on $CD4^+$ cells (Fig. 2C) or on $CD8^+$ lymphocytes (Fig. 2D). Similarly, no significant differences were observed between patients who will develop or not a nosocomial episode ($n = 4$ and $n = 16$, respectively; Fig. 2E, 2F). Overall, these results show that CD127 expression is not overtly regulated on circulating lymphocytes after septic shock.

The plasmatic concentration of the soluble form of CD127 is increased in patients with ICU-acquired nosocomial infection after septic shock

Importantly, plasmatic concentration of sCD127, known to possess in vitro antagonistic activity on IL-7 (12, 16) was measured in septic shock patients ($n = 33$) and healthy volunteers ($n = 16$).

No significant differences were observed between patients and healthy controls neither at day 1–2 nor at day 3–4. This is in contrast with the observation made in HIV infection of a significantly increased sCD127plasmatic level (12, 16). Meanwhile, a

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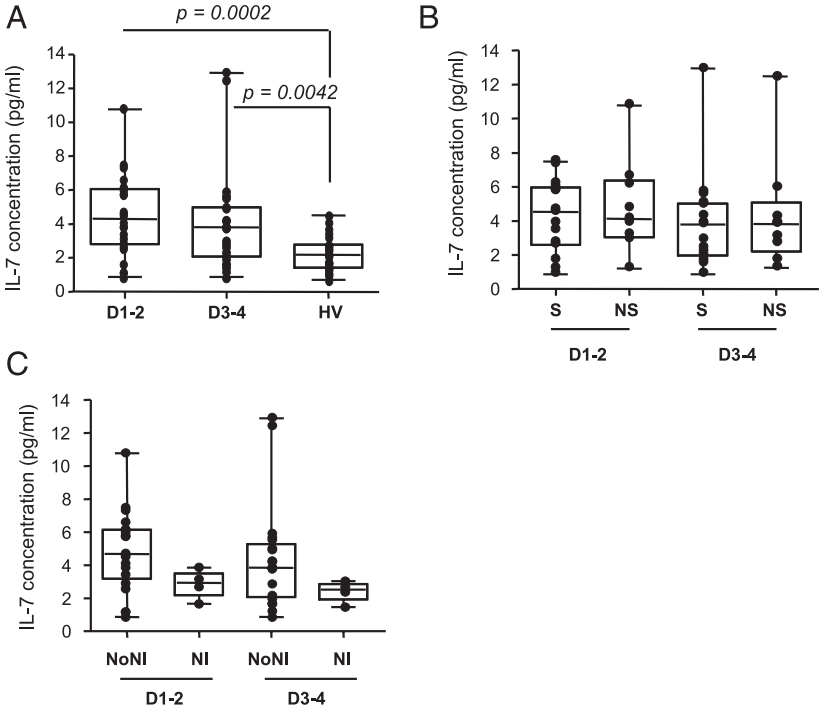

FIGURE 1. Plasmatic IL-7 concentration in septic shock patients. Plasmatic IL-7 concentration was measured in septic patients (n = 24) at day 1–2 (D1–2) and day 3–4 (D3–4) after the onset of shock in comparison with healthy volunteers (HV, n = 26) (**A**), in survivors (S, n = 15) versus nonsurvivors (NS, n = 9) (**B**), and in patients who will develop a secondary infection (NI, n = 4) versus patients remaining free of any nosocomial episode (NoNI, n = 20) (**C**). Nonparametric Mann–Whitney U test was used to compare results between groups.

significant decrease was measured between these two time points in patients (Fig. 3A).

No differences were observed between survivors (n = 20) and nonsurvivors (n = 13), although a significant decrease over time was observed in both groups of patients (Fig. 3B). Interestingly and despite the low number of patients in each group, a significantly higher concentration was measured in patients who developed a nosocomial episode (n = 5) in comparison with patients who remained free of any secondary infectious challenge (n = 28). This difference was present both at day 1–2 and at day 3–4 (Fig. 3C).

In total, this first set of observational studies shows that, in contrast with results observed during chronic viral infections, parameters of the IL-7 pathway are not markedly modified after septic shock. This suggests that this pathway remains potentially activable during sepsis and thus that rhIL-7 could restore normal lymphocyte functions in patients. To test this hypothesis, we de-

Table I. Demographic, clinical, and immunological data for septic shock patients

Clinical data		
Age at admission (y)		73 [62–80]
Gender	Male	44 (63)
SAPS II at diagnosis of shock		58 [47–67]
SOFA score		11 [8–13]
Comorbidities	0	23 (33)
	≥1	47 (67)
Initial infection	Bacilli Gram$^-$	19 (27)
	Cocci Gram$^+$	19 (27)
	Fungi	5 (7)
	Others	11 (16)
Type of infection	Community-acquired	43 (61)
	Hospital-acquired	27 (39)
Site of infection	Pulmonary	24 (34)
	Abdominal	17 (24)
	Others	29 (41)
Mortality	Nonsurvivors	24 (34)
Secondary infections	Occurrence	14 (20)
Immunological data		
CD3$^+$CD4$^+$ T lymphocytes	D3–4	355 [190–530]
Treg (%)	D3–4	10 [8–14]
mHLA-DR (%)	D3–4	41 [19–70]

Blood samples were obtained from 70 septic shock patients after the onset of shock. For clinical parameters, categorical data are presented as number of cases and percentages respective to the total population in parentheses. Continuous data as well as biological parameters are presented as medians and interquartile ranges [Q1–Q3] in brackets. The following leukocyte subsets were analyzed at day 3–4 after the onset of shock: number of circulating CD3$^+$CD4$^+$ T lymphocytes per microliter, percentage of CD4$^+$CD25^{High}CD127^{Low} Treg measured among CD3$^+$CD4$^+$ T lymphocytes and percentage of monocytes expressing HLA-DR among total monocytes (mHLA-DR). Normal values from our laboratory for age-matched individuals are 500–1250 CD4$^+$ T cells/μl, 5–7% Treg, and >90% mHLA-DR.

SAPS II, Simplified Acute Physiologic Score II calculated at inclusion in the protocol; SOFA score, Sequential Organ Failure Assessment score measured after 24 h of ICU stay.

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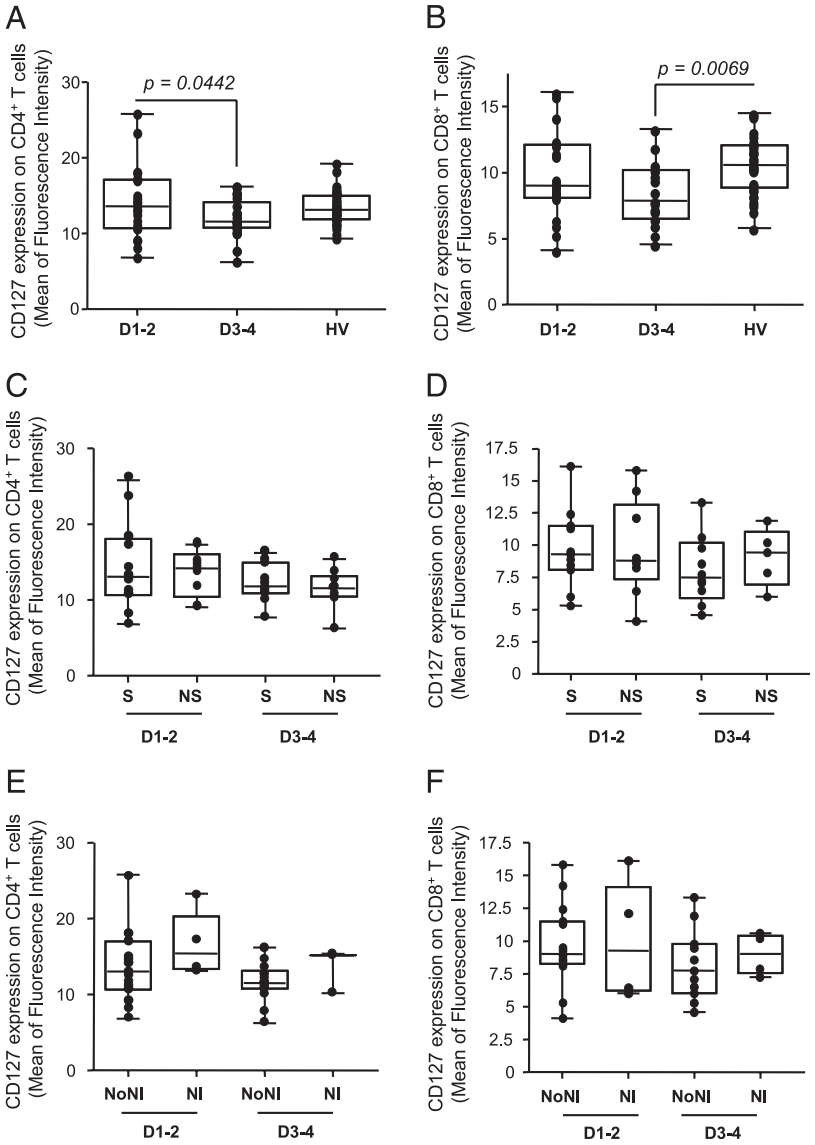

FIGURE 2. Lymphocyte expression of IL-7R α-chain in septic shock patients. Cell surface expression of IL-7R α-chain (CD127) was measured as mean of fluorescence intensity on $CD4^+$ and $CD8^+$ T cells from septic patients (n = 20) at day 1–2 (D1–2) and day 3–4 (D3–4) after the onset of shock versus healthy volunteers (HV, n = 30) (**A**, **B**), from survivors (S, n = 12) versus nonsurvivors (NS, n = 8) (**C**, **D**), and from patients who will develop a secondary infection (NI – n = 4) versus versus patients remaining free of any nosocomial episode (NoNI, n = 16) (**E**, **F**). Nonparametric Mann–Whitney U test was used to compare results between groups.

veloped ex vivo functional assays with purified mononuclear cells from septic shock patients incubated with rhIL-7.

rhIL-7 restores ex vivo sepsis-induced decreased cell proliferation

Lymphocyte proliferation was measured after TCR stimulation of PBMCs extracted from septic shock patients (n = 10) and healthy volunteers (n = 10) and cultured in presence or not of rhIL-7. Proliferation was evaluated by tritiated thymidine incorporation.

Septic patients presented with a significantly decreased proliferation in response to TCR stimulation in comparison with healthy volunteers (Fig. 4A). Importantly, cell incubation with rhIL-7 significantly increased this proliferative response in patients whereas no significant effect was measured in healthy volunteers (Fig. 4A). This is illustrated by the significant difference of rhIL-7–induced fold increase in cell proliferation observed between patients and healthy controls (Fig. 4B).

rhIL-7 restores ex vivo sepsis-induced decreased $CD4^+$ and $CD8^+$ T cell proliferations

To decipher whether this rhIL-7–induced cell proliferation was affecting selectively $CD4^+$ and/or $CD8^+$ cells, PBMCs extracted from septic patients (n = 7) and healthy volunteers (n = 4) were stained with CFSE before culture in presence of TCR stimulation and rhIL-7.

We observed that both $CD4^+$ (Fig. 5A, 5C) and $CD8^+$ (Fig. 5B) T cell proliferations were decreased in patients versus controls. This decrease was significant for $CD4^+$ T lymphocytes (Fig. 5A). Most importantly, incubation with rhIL-7 significantly increased $CD4^+$ lymphocyte proliferation to a level that was not different from that of healthy volunteers (Fig. 5A). A similar trend was observed for $CD8^+$ T cells (Fig. 5B). Interestingly, the number of $CD4^+$ cell divisions after rhIL-7 stimulation was increased in septic shock patients to a level similar to control values (Fig. 5D). Similar results were observed for $CD8^+$ cells (data not shown). Representative examples of CFSE stainings of $CD4^+$ T cells from one septic shock patient and one healthy control are shown in Fig. 5C. These results suggest that rhIL-7 is efficacious in restoring both $CD4^+$ and $CD8^+$ T cell dysfunctions after septic shock.

rhIL-7 restores ex vivo sepsis-induced decreased IFN-γ lymphocyte production

Another aspect of sepsis-induced lymphocyte dysfunctions is a reduction in IFN-γ production by lymphocytes. We thus measured by flow cytometry intracellular IFN-γ production by $CD8^+$ T cells

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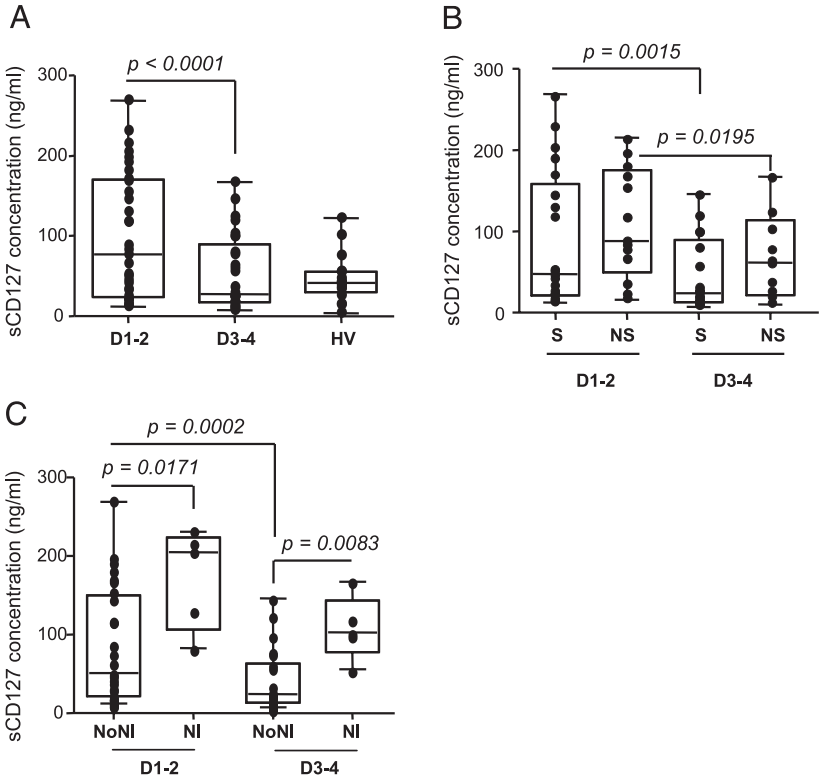

FIGURE 3. Plasmatic concentration of soluble IL-7R α-chain in septic shock patients. Plasmatic concentration of the soluble fraction of IL-7R α-chain (sCD127) was measured in septic patients (n = 33) at day 1–2 (D1–2) and day 3–4 (D3–4) after the onset of shock versus healthy volunteers (HV, n = 16) (**A**), in survivors (S, n = 20) versus nonsurvivors (NS, n = 13) (**B**), and in patients who will develop a secondary infection (NI, n = 5) versus patients remaining free of any nosocomial episode (NoNI, n = 28) (**C**). Nonparametric Mann–Whitney U test was used to compare results between groups. Nonparametric Wilcoxon paired test was used to evaluate evolution over time within a group.

in patients (n = 12) and controls (n = 10) after TCR stimulation and incubation with rhIL-7.

We observed that TCR-induced IFN-γ production was significantly reduced in patients versus controls (Fig. 6). Most importantly, incubation of patients' cells with rhIL-7 significantly increased this cytokine response to a level that was not different from healthy controls. Representative examples of flow cytometry stainings are shown in Fig. 6B. These results show that rhIL-7 is efficacious in restoring $CD8^+$ T cell dysfunctions after septic shock.

rhIL-7 efficiently activates parameters of its intracellular pathway in septic shock patients

A key signaling molecule for IL-7 is STAT5 (signal transducer and activator of transcription 5), which is phosphorylated following recruitment of CD127. Beside, one significant consequence of CD127 signaling is the maintenance of cell survival by increasing BCL2 protein expression. We thus investigated the expressions of these molecules in response to TCR stimulation and/or rhIL-7 in septic patients (n = 10) and controls (n = 10).

To our knowledge, we show for the first time that TCR stimulation was associated with a significantly decreased induction of STAT5 phosphorylation (Fig. 7A, 7B) and BCL2 expression (Fig. 7C) in patients compared with healthy volunteers. Most importantly, cell incubation with rhIL-7 was able, by itself and after TCR stimulation, to significantly increase the expression of these molecules in patients and controls (Fig. 7). This shows that IL-7 intracellular pathway is mobilizable in septic patients although its activation is not adequate after TCR stimulation alone.

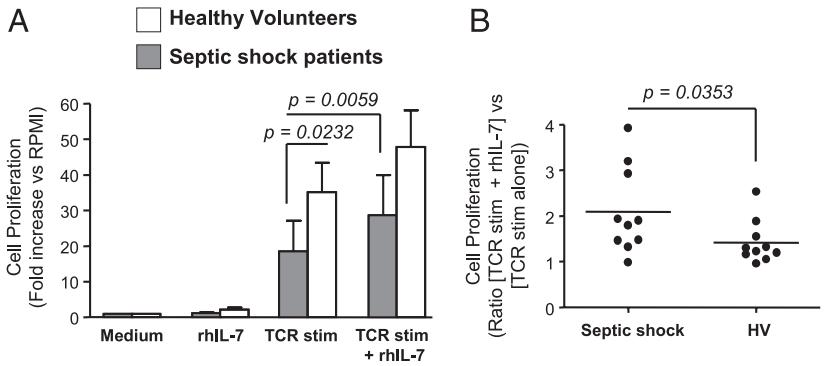

FIGURE 4. Lymphocyte proliferation is restored by rhIL-7 in septic shock patients. Mononuclear cells were isolated from peripheral blood of septic shock patients (gray columns, n = 10) and healthy volunteers (open columns, n = 10). Cells were cultured for 5 d in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads (TCR Stim, ratio of beads/cells = 4:1) and rhIL-7 (100 ng/ml). Cell proliferation was evaluated by [^{3}H]thymidine incorporation. (**A**) Results are presented as mean ± SEM of proliferative response evaluated for each cell culture condition and each experiment as the ratio versus non-stimulated cells response. (**B**) Results are presented as individual values of rhIL-7–induced proliferation evaluated for each experiment as the ratio of the proliferative response induced by TCR stimulation alone or in the presence of rhIL-7. Nonparametric Mann–Whitney U test was used to compare results between groups. Nonparametric Wilcoxon paired test was used to evaluate evolution over time within a group.

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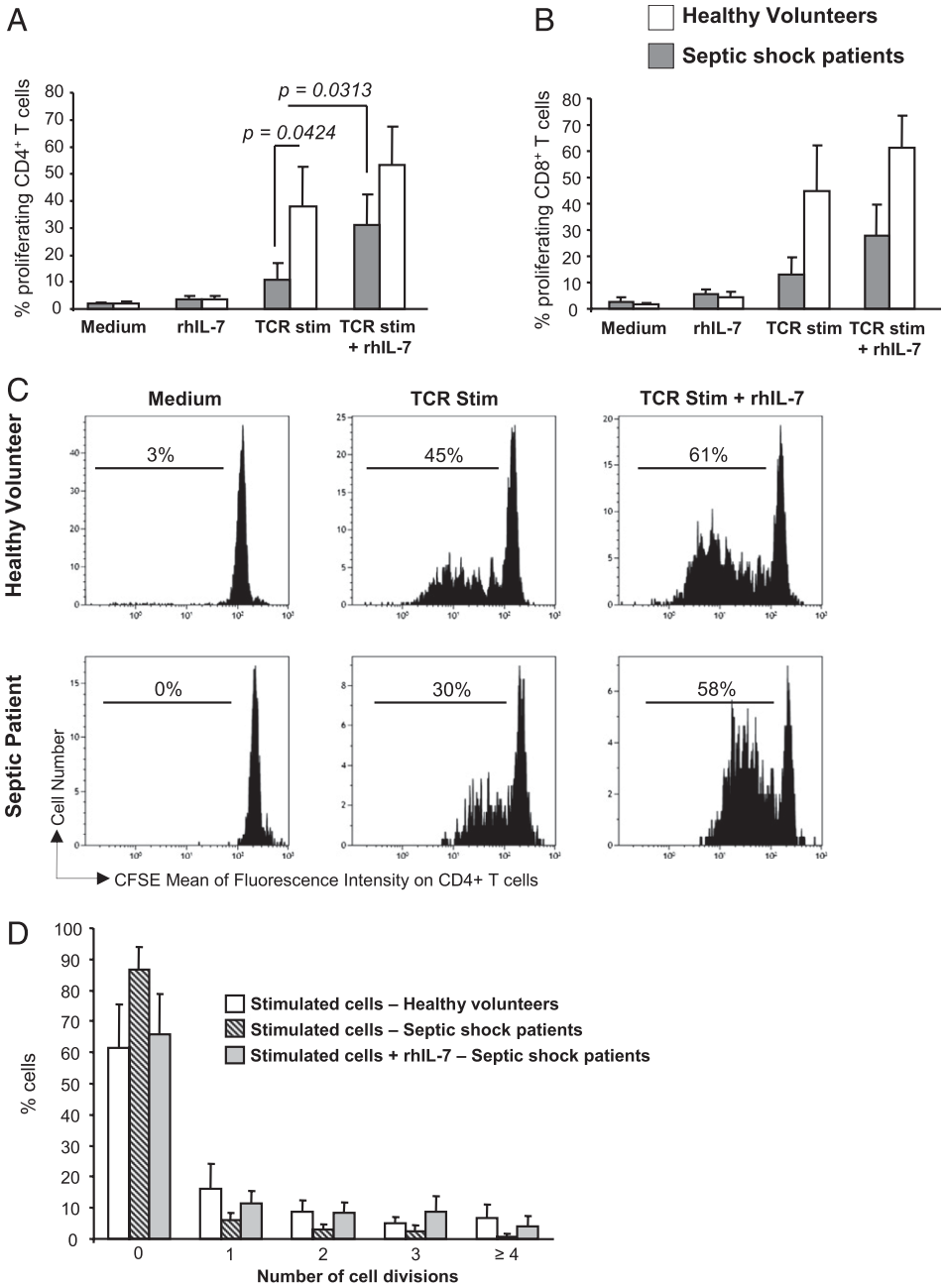

FIGURE 5. $CD4^+$ and $CD8^+$ lymphocyte proliferations are restored by rhIL-7 in septic shock patients. Mononuclear cells were isolated from peripheral blood of septic shock patients (gray columns, $n = 7$) and healthy volunteers (open columns, $n = 4$). Cells were stained with CFSE and cultured for 5 d in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads (TCR Stim, ratio of beads/cells = 4:1) and rhIL-7 (100 ng/ml). Cell proliferation was evaluated by flow cytometry (decreased CFSE fluorescence intensity). Results are presented as mean ± SEM of percentages of proliferating cells (i.e., with a decreased CFSE fluorescence intensity in comparison with nonstimulated cells). (**A**) $CD4^+$ T cell proliferation. (**B**) $CD8^+$ T cell proliferation. (**C**) One representative example of a CFSE staining after culture in one septic shock patient and one healthy volunteer is presented. Nonparametric Wilcoxon paired test was used to evaluate evolution over time within a group. (**D**) Numbers of $CD4^+$ T cell divisions in septic shock patients and healthy controls after stimulation with anti-CD2/CD3/CD28 Ab-coated beads alone (stimulated cells) or with rhIL-7 (stimulated cells + rhIL-7).

Discussion

Severe sepsis and septic shock represent the first cause of mortality in ICU (17). Septic syndromes induce alterations of the immune response that have, for long, been solely considered as an overwhelming proinflammatory syndrome. However, the failure of numerous clinical trials testing anti-inflammatory drugs to show any improvement in mortality has led to the re-evaluation of our understanding of severe sepsis and septic shock pathophysiology. In line, it is now globally accepted that, in parallel with a tremendous proinflammatory response leading to shock and organ dysfunctions, septic patients develop an immunosuppressive phase associated with immune dysfunctions (2, 3). Importantly, it has recently been shown that, in patients, these dysfunctions are not only observed at the systemic level but also locally in organs therefore illustrating the importance of this compensatory mechanism (18). In addition, observational clinical studies have linked the intensity and duration of these sepsis-induced immune dysfunctions with increased risk of death and of secondary nosocomial infections (3). Consequently, clinical trials testing immunostimulating drugs are now initiated in septic patients (2).

Sepsis-induced immune alterations affect both innate and adaptive immune responses (3, 19). So far, most work has been devoted to the study of the innate part of these sepsis-induced immune dysfunctions. Conversely, far less work has been dedicated to the study of lymphocyte alterations although a link between a reduced delayed-type hypersensitivity response (predominantly mediated by T cells and a hallmark of immune suppression) in ICU patients and increased risk of nosocomial infections and death has been described more than 30 y ago (20). Indeed, sepsis-induced lymphocyte dysfunctions make up 1) a dramatic lymphopenia affecting every lymphocyte subsets associated with major apoptosis, 2) functional alterations such as decreased proliferation and cytokine production in response to stimulation, 3) phenotypic alterations such as increased coinhibitory receptor (CTLA-4 and pro-

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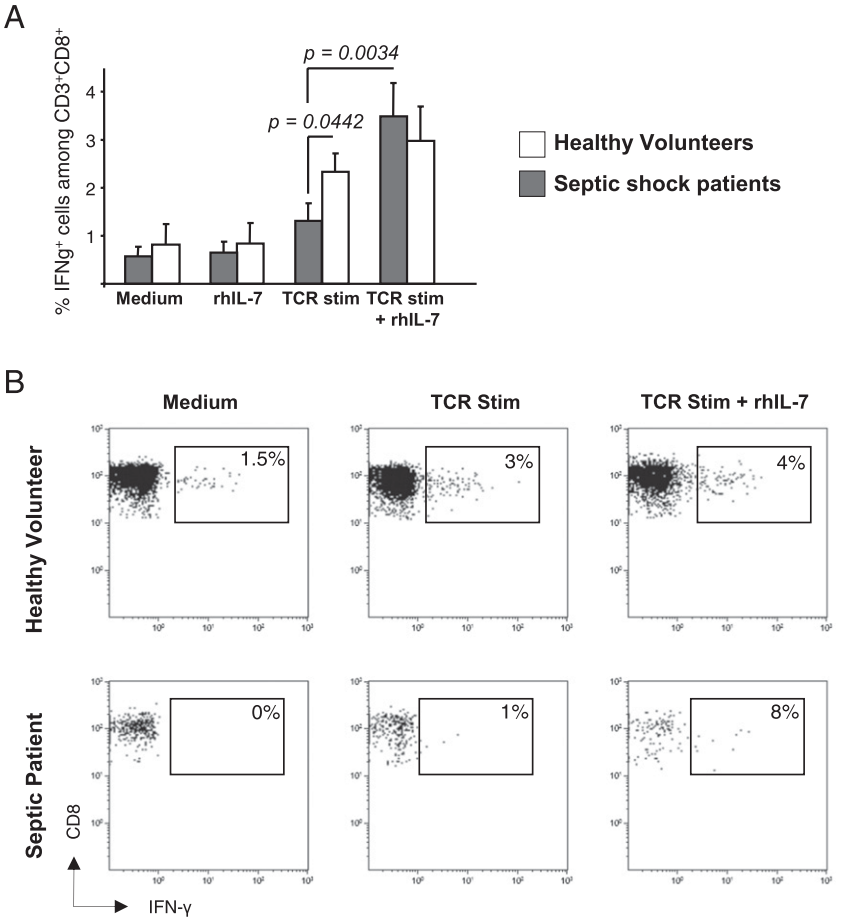

FIGURE 6. IFN-γ production by CD8$^+$ T lymphocytes is restored by rhIL-7 in septic shock patients. Mononuclear cells were isolated from peripheral blood of septic shock patients (gray columns, n = 12) and healthy volunteers (open columns, n = 10). Cells were cultured for 4 h in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads (TCR Stim, ratio of beads/cells = 4:1) and rhIL-7 (100 ng/ml). IFN-γ production by CD8$^+$ T cells was then measured by flow cytometry (intracellular staining). (**A**) Results are presented as mean ± SEM of percentages of IFN-γ–producing cells among total CD8$^+$ T lymphocyte–proliferating cells. (**B**) One representative example of an IFN-γ staining after culture in one septic shock patient and one healthy volunteer is presented. Nonparametric Wilcoxon paired test was used to evaluate evolution over time within a group.

grammed cell death-1 [PD-1]) and decreased costimulatory receptor (CD80 and CD86) and CD3 expressions, 4) decreased TCR diversity, and 5) increased percentage of circulating CD4$^+$ CD25$^+$ Treg (3, 21). These lymphocyte dysfunctions are likely illustrated in patients by viral reactivations normally solely occurring in immunocompromised hosts (CMV and herpes simplex virus). Importantly, they are associated with deleterious outcome after septic shock (22, 23). Therefore immunostimulating therapies able to restore these lymphocyte dysfunctions would represent an innovative therapeutic strategy in septic shock.

Interestingly, sepsis-induced lymphocyte dysfunctions share many similarities with cell alterations observed in patients with chronic viral infections. In particular, HIV-infected patients also present with a marked decrease in circulating CD4$^+$ T cell number, functional alterations such as decreased lymphocyte proliferation after stimulation, phenotypic alterations such as increased coinhibitory receptor expression (i.e., PD-1), decreased TCR diversity and increased percentage of circulating Treg (5). Interestingly, in this clinical context, rhIL-7 is currently tested as an adjunctive therapy in phase I and II clinical trials to restore these lymphocyte dysfunctions. As of 2011, at least 15 ongoing studies of rhIL-7 therapy were underway. Investigators are seeking to use rhIL-7 to enhance viral clearance in the setting of chronic viral infections (hepatitis B virus, hepatitis C virus, and HIV). rhIL-7 is also being tested for its capacity to augment immune reconstitution following chemotherapy and/or bone marrow transplantation and in the setting of idiopathic CD4$^+$ lymphopenia. Finally, trials have been initiated to test rhIL-7 efficacy as a vaccine adjuvant in aged populations and to determine whether this molecule will augment the efficacy of tumor vaccines and adoptive T cell therapy for cancer. Thus far, this agent has been well tolerated, inducing only mild constitutional symptoms and no evidence of the capillary leak syndrome observed with rhIL-2 therapy. In each of the studies, patients treated with rhIL-7 show diversification of their TCR repertoire and increases in circulating CD4$^+$ and CD8$^+$ T cell numbers stable for weeks to months after completion of rhIL-7 therapy. Interestingly, some of these trials showed treated patients experience substantial decreases in the relative frequency of Treg within the peripheral T cell pool (6).

We thus postulated that rhIL-7 could represent an innovative therapy in septic shock patients. Therefore, we first studied the regulation of parameters of IL-7 pathway in patients. In a second set of experiments, ex vivo functional testings were performed to evaluate rhIL-7 capacity to restore normal lymphocyte functions in septic patients.

We observed that parameters of the IL-7 pathway (plasmatic IL-7 concentration, CD127 expression on circulating lymphocytes and sCD127 level) were not markedly modified after septic shock. The only significant change was an increase in circulating IL-7 level in patients versus healthy controls. However, because an inverse relationship between serum IL-7 levels and lymphocyte counts has been constantly described in various settings of lymphocyte depletion (e.g., in HIV-infected patients), such increase in circulating IL-7 is not surprising in markedly lymphopenic hosts as septic shock patients. Moreover, this elevation is not of major importance since max concentrations measured in patients peaked at 13 pg/ml, whereas normal values for circulating human IL-7 range from 2 to 8 pg/ml (6).

Interestingly, cell surface and plasmatic concentrations of CD127 were not significantly different in septic patients versus

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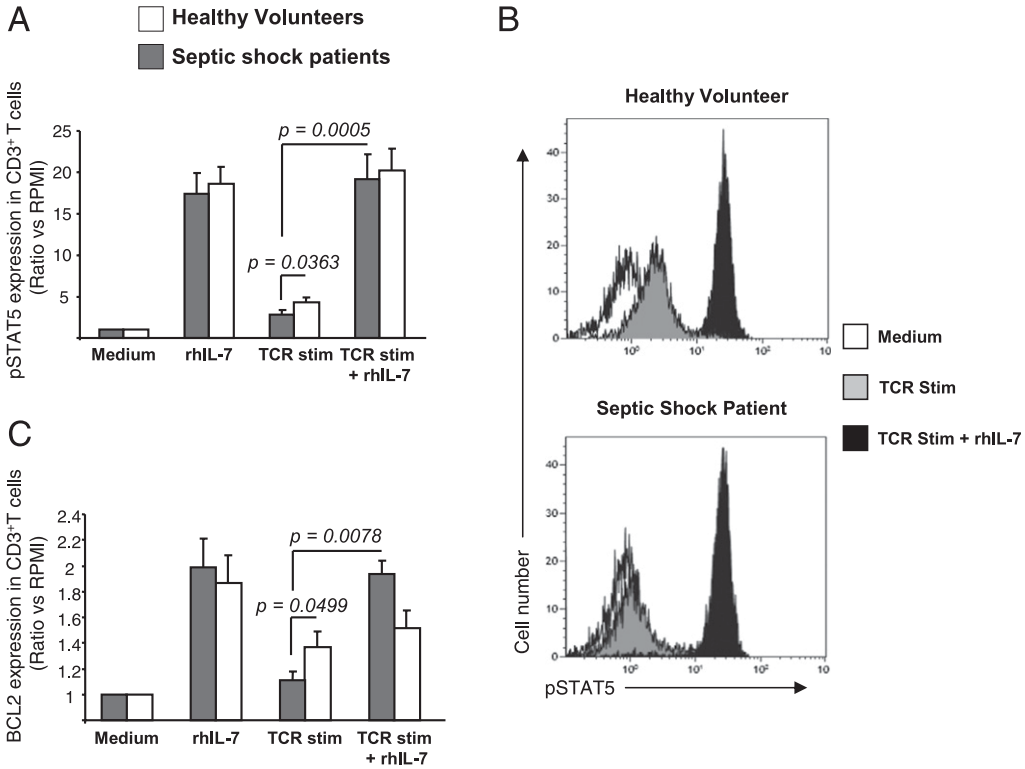

FIGURE 7. STAT5 phosphorylation and BCL2 induction in T lymphocytes are restored by rhIL-7 in septic shock patients. Mononuclear cells were isolated from peripheral blood of septic shock patients (gray columns, $n = 10$) and healthy volunteers (open columns, $n = 10$). (**A**) Cells were cultured overnight in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads (TCR Stim, ratio of beads/cells = 4:1) and 15 min in the presence or not of rhIL-7 (100 ng/ml). STAT5 phosphorylation in T cells was then measured by flow cytometry (pSTAT5, intracellular staining). For each experiment and culture condition, means of fluorescence intensity of pSTAT5 were normalized by value measured in the control well. (**B**) One representative example of a pSTAT5 staining after culture in one septic shock patient and one healthy volunteer is presented. (**C**) Cells were cultured 48 h in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads (TCR Stim, ratio of beads/cells = 4:1) and rhIL-7 (100ng/ml). BCL2 expression in T cells was then measured by flow cytometry (intracellular staining). For each experiment and culture condition, means of fluorescence intensity of BCL2 were normalized by value measured in the control well. Results are presented as mean ± SEM. Nonparametric Wilcoxon paired test was used to evaluate evolution over time within a group.

healthy controls. This is in contrast with results observed in HIV infection, as significantly decreased CD127 lymphocyte expression as well as marked increased concentration of its soluble form have been observed in patients (12, 16). Interestingly and despite these alterations, rhIL-7 is effective in restoring lymphocyte dysfunctions in HIV-infected patients (4). Thus, as we did not observe such alterations of IL-7 pathway in our cohort, we postulated that rhIL-7 should be all the more effective in restoring normal lymphocyte functions after septic shock.

To test this hypothesis, we measured ex vivo the response of septic patients' lymphocytes to stimulation in the presence or absence of rhIL-7. As previously described, we observed that patients present with significantly decreased lymphocyte proliferation (both of $CD4^+$ and $CD8^+$ T cells) in response to TCR stimulation. In addition, IFN-γ response was decreased in these patients as well. Importantly, we describe here two additional sepsis-induced lymphocyte dysfunctions. To our knowledge, we show for the first time that STAT5 phosphorylation and BCL2 induction in response to T cell stimulation are significantly altered after septic shock. This suggests that intracellular TCR activation pathway is dysfunctional in septic shock patients. Overall, our experiments recapitulate and enlarge the description of sepsis-induced lymphocyte dysfunctions in patients.

Most importantly, in these ex vivo experiments, cell incubation with rhIL-7 significantly restored every sepsis-induced lymphocyte alterations. Indeed, rhIL-7 significantly increased lymphocyte response to stimulation as measured by cell proliferation, IFN-γ production, STAT5 phosphorylation or BCL2 expression in septic patients. Importantly, this restoring effect of rhIL-7 was observed in survivors as well as nonsurvivors (data not shown). To our knowledge, these results are the first to show that septic patients' cells remain responsive to rhIL-7 treatment and that rhIL-7 is efficacious ex vivo in restoring sepsis-induced lymphocyte dysfunctions. Although performed ex vivo, they suggest that rhIL-7 could represent an innovative therapy in sepsis.

These data are very complimentary to results obtained in murine models of sepsis (8–10). Indeed, in mice, in vivo treatment with rhIL-7 was associated with significant improvement in T cell viability, trafficking and functionality after bacterial as well as fungal sepsis (8, 10). Importantly, in these experiments, survival was significantly improved as well. Interestingly and as observed in the current study, rhIL-7 treatment was associated with restoration of IFN-γ production and increased BCL2 expression. More generally, these animal studies showed that, although targeting adaptive immune cells, rhIL-7 therapy could impact every aspect of the immune response, including innate immune cells such as neutrophils (9). Although not tested in our study, this suggests that rhIL-7 therapy could efficiently restore both innate and adaptive immune dysfunctions after sepsis.

Moreover, results observed in ongoing clinical trials in other pathologies sharing similar immune dysfunctions further support the potential beneficial effect of rhIL-7 in sepsis. In particular, beside the improvement of T cell viability and functionality, this molecule has been shown to improve TCR repertoire diversifica-

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tion, whereas septic patients present with a marked decrease in TCR repertoire diversity (21). Similarly, the effect of rhIL-7 involves the downregulation of expression of immunoregulatory receptors such as PD-1, whereas PD-1 expression has been shown to be increased on circulating cells from septic patients (24). Importantly, PD-1 knockout mice have been shown to be more resistant to sepsis than wild-type animals (24). Finally, rhIL-7 therapy also leads to a relative decrease in the frequency of Treg while an increased percentage of circulating Treg has been repeatedly shown in septic patients (25).

In total, our results strongly support the hypothesis that rhIL-7 could represent an innovative therapy in the treatment of sepsis as we show that IL-7 pathway is not significantly altered and is still functional in septic shock patients and that rhIL-7 treatment significantly restores sepsis-induced lymphocyte functions to a normal response. This supports the rational for the initiation of a clinical trial testing rhIL-7 in septic shock patients, although the adequate design of such study still deserves to be precisely determined.

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Disclosures

The authors have no financial conflicts of interest.

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