


LETTER TO THE EDITOR

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Intracellular calcium signaling and phospho-antigen measurements reveal functional proximal TCR activation in lymphocytes from septic shock patients

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To the editor,

Sepsis deeply perturbs immune homeostasis by inducing a complex immune response that varies over time and associates a tremendous systemic inflammatory response to anti-inflammatory mechanisms. As a delayed consequence, some septic patients enter a state of profound immunosuppression [1]. As the latter may persist for weeks, leaving the patient at increased risk of secondary infections, immunostimulation recently appeared as a reasonable therapeutic option in patients with signs of persistent and severe immunosuppression [2].

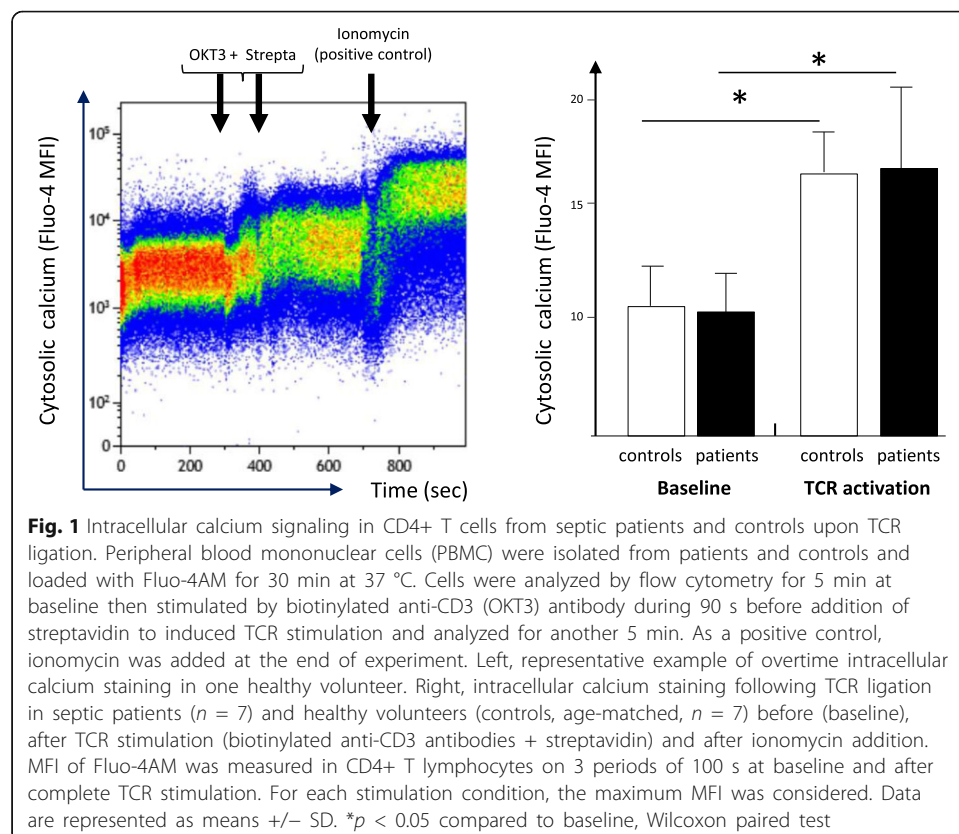
Septic patients develop marked T lymphocyte dysfunctions such as profound lymphopenia, increased expression of inhibitory co-receptor molecules, decreased repertoire diversity, and reduced functionality (proliferation and cytokine production). These alterations have been repeatedly associated with deleterious outcomes [1, 2]. However, mechanisms leading to these alterations are only partially understood. For instance, while the role of deactivated mTORC1 is established [3], the intrinsic capacity of T cell receptor (TCR) to be activated and to transduce intracellular signalling remains unexplored. Among determinants of T cell response, immediate calcium signaling following TCR ligation is of paramount importance and serves crucial effector functions. Thus, we developed a flow cytometry protocol to follow calcium flux after TCR stimulation in patients' CD4+ T lymphocytes. In addition, phosphorylation of molecules from the proximal and downstream TCR signaling cascade was analyzed (Additional file 1). We included patients with septic shock (according to SEPSIS-3 definition) presenting with features of

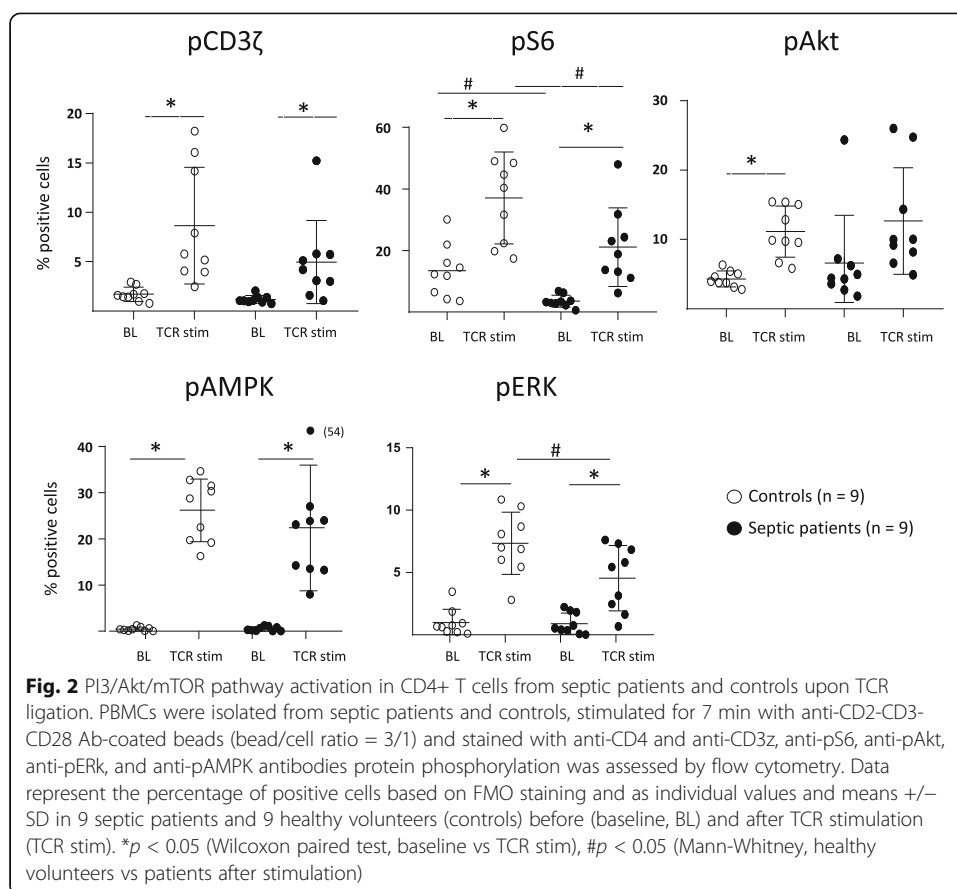
immunosuppression, i.e., decreased monocyte HLA-DR and lymphocyte count (clinical and immunological characteristics in Additional file 1: Table S1).

We show that immediate signaling downstream TCR stimulation was not altered in circulating CD4 lymphocytes from septic shock patients. Indeed, cells exhibited no deregulation of intracytoplasmic calcium influx after TCR ligation compared with healthy controls (OKT3 response, Fig. 1). In agreement, we observed a significant CD3 ζ phosphorylation (one of the first molecules to be phosphorylated after TCR engagement) after T cell stimulation in both cells from septic patients and controls (Fig. 2). This showed that immediate response after TCR activation was unaffected after sepsis.

In contrast, activation of more distal molecules in the TCR signaling cascade was impacted. For example, stimulation-induced rise of pAkt and pERK was affected leading to a limited mTORC1 activation capacity as measured by S6 phosphorylation after stimulation. In contrast, activation of AMPK, an inhibitor of mTORC1 was mostly unaltered in patients compared to controls (Fig. 2).

In conclusion, the present results obtained in septic shock patients show that proximal TCR signaling remains functional in circulating CD4⁺ T cells from septic shock patients while downstream activation of mTORC1 pathway is markedly diminished. PI3K-Akt pathway integrates signals from both co-activating/inhibitory receptors and an increased expression of such co-inhibitory receptors has been described on circulating T cells from septic patients [4]. In that respect, this suggests





that inhibitory receptors known to block downstream signaling are likely of utmost importance in sepsis-induced T lymphocyte dysfunctions. As TCR from septic lymphocytes remains actionable [5], the present results reinforce the rationale for blocking co-inhibitors (e.g., with anti-PD-1) or stimulating mTORC1 (for example with rhIL-7) as reasonable immunoadjuvant approaches to tackle sepsis-induced immunosuppression [6–8].

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s40635-019-0287-5>.

Additional file 1: Table S1. Clinical and biological data from septic shock patients.

Abbreviations

anti-PD-1: Anti-Program-Death-1; HLA-DR: Human leukocyte antigen-DR isotype; mTORC1: Mammalian target of rapamycin complex 1; OKT3: Monoclonal antibody anti-CD3 receptor (muromomab); pAkt: Phosphor-Akt (aka phospho-protein kinase B); pAMPK: Phosphor-mitogen-activated protein kinases; pERK: Phosphor-extracellular signal-regulated kinases; rhIL-7: Recombinant human interleukin-7; TCR: T cell receptor

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Not applicable

Authors' contributions

CDR, KK, MG, EP, FV, and GM designed the experiments. CDR and KK performed the experiments and the statistical analyses. TR, CM, MC, and LA included patients. All authors discussed the data, drafted or revised critically the manuscript for important intellectual content, and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Septic shock patients: this project was approved by our Institutional Review Board for ethics ("Comité de Protection des Personnes Sud-Est II", number 11236). This study is registered at the French Ministry of Research and Teaching (#DC-2008-509), at the Commission Nationale de l'Informatique et des Libertés, and on [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT02803346). Oral information and non-opposition to inclusion in the study were mandatory and recorded in patients' clinical files.

Healthy volunteers: peripheral blood from healthy volunteers was provided by the "Etablissement Français du Sang" from Lyon. According to EFS standardized procedures for blood donation and to provisions of the articles R.1243–49 and following ones of the French public health codes, a written non-opposition to the use of donated blood for research purposes was obtained from HV. The blood donors' personal data were anonymized before being transferred to our research laboratory.

Consent for publication

Not applicable.

Competing interests

EP is an employee of bioMérieux. All other authors declare that they have no competing interests.

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