The β_1-adrenergic receptor contributes to sepsis-induced immunosuppression through modulation of regulatory T cell inhibitory function

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Key words: sepsis; adrenoreceptor; beta blocker; lymphocytes; immunosuppression

Abstract

Objective: Although cardiovascular benefits of β_1-adrenoreceptor blockade have been described in sepsis, little is known about its impact on the adaptive immune response, specifically CD4 T cells. Herein we study the effects of β_1-adrenoreceptor modulation on CD4 T cell function in a murine model of sepsis.

Design: Experimental study.

Setting: University laboratory

Subjects: C57BL/6 mice

Interventions: High-grade sepsis was induced by caecal ligation and puncture (CLP) in wild-type mice ($\beta_1^{+/+}$) with or without esmolol (a selective β_1-adrenoreceptor blocker), or in β_1-adrenoreceptor knockout mice ($\beta_1^{-/-}$). At 18 hours after surgery echocardiography was performed and blood and spleen were collected to analyze the lymphocyte function.

Measurements and Main Results: At 18 hours, $\beta_1^{+/+}$ CLP mice exhibited characteristics of high-grade sepsis and three surrogate markers of immunosuppression, namely decreased splenic CD4 T cells, reduced CD4 T cell proliferation and increased Treg cell proportions. Pharmacologic and genetic β_1-adrenoreceptor blockade reversed the impact of sepsis on CD4 T and Treg proportions and maintained CD4 T cell proliferative capacity. β_1-adrenoreceptor blocked CLP mice also exhibited a global decrease in both pro- and anti-inflammatory mediators and improved *in-vivo* cardiovascular efficiency with a maintained cardiac power index despite the expected decrease in heart rate.

Conclusion: β_1-adrenoreceptor activation enhances Treg inhibitory function and thus contributes to sepsis-induced immunosuppression. This can be attenuated by β_1-

adrenoreceptor blockade, suggesting a potential immunoregulatory role for this therapy in the treatment of sepsis.

Abreviations:

AR: adrenergic receptor

CD: cluster of differentiation

CI: cardiac index

CLP: caecal ligation and puncture

CO: cardiac output

CPI: cardiac power index

ELISA: enzyme-linked immunosorbent assay

FBS: fetal bovine serum

IL: interleukin

MAP: mean arterial pressure

PBS: phosphate-buffered saline

PHA: phytohemagglutinin

PMA: phorbol myristate acetate

RPMI: Roswell Park Memorial Institute

SNS: sympathetic nervous system

SV: stroke volume

Th: T helper cell

TNF: tumor necrosis factor

Treg: regulatory T lymphocyte

Introduction

Sepsis and septic shock are a leading cause of intensive care unit admission and recognized by the World Health Organization as a major healthcare issue(1). Unfortunately, despite significant effort, all therapeutic research conducted over the last four decades has systematically failed(2).

Sepsis is the consequence of a highly complex and dysregulated inflammatory host response to infection leading to life-threatening organ dysfunction (3). After initial activation, the immune system is downregulated, leading to immunosuppression that predispose the patient to secondary infection. A disproportionate and prolonged activation of the sympathetic nervous system (dysautonomia) also occurs, characterized by loss of cardiovascular variability, and excessive release of catecholamines(4). These hormones, mainly possessing α_1 and β_1 adrenergic effects, are crucial factors underlying immunosuppression(5). Sepsis-induced immunosuppression is characterized by increase in regulatory T cells (Treg cells) leading to a decreased ability to respond to subsequent infectious insults(6).

Beta-1 adrenoreceptor (β_1-AR) blockade is a therapeutic approach aiming at downregulating the excessive adrenergic stimulation induced by sepsis. Initial single-center studies in human septic shock have mainly reported hemodynamic benefits with improvements in cardiac efficiency, with one also finding survival benefit (7-9). Some experimental studies have investigated the inflammatory effects of β_1-AR blockade in sepsis and demonstrated a global decrease in both pro- and anti-inflammatory plasma cytokines(10-12). However, little is known about the effects of β_1-AR blockade on the early adaptive immune response, specifically on CD4 T cells(13, 14). We hypothesized

that, during sepsis, β_1-AR blockade plays a crucial role in the early adaptive immune response and allow a decrease of sepsis-induced immunosuppression. Thus, the present study was designed to assess the effects of β_1-AR blockade on CD4 T cells in a murine model of severe sepsis.

Materials and Methods

Sepsis model

β_1-AR (C57BL/6J, DBA/2, 129Sv) knockout mice ($\beta_1^{-/-}$) and wild type littermates ($\beta_1^{+/+}$), obtained free of charge from the Institute of Pharmacology and Toxicology of Munich, Germany, were used for this study. The French Animal Care Committee, in accordance with European regulations, approved all protocols (n°APAFIS#4636). Sepsis was induced by caecal ligation and puncture (CLP) in both male and female mice aged 12-16 weeks, weighing 22-32 grams. Mice received a subcutaneous fluid bolus with physiological saline solution (0.9% NaCl; 5ml/100g; Baxter, Guyancourt, France). Full description is provided in **Supplemental Digital Content – Methods**. An incision was performed between the scapulae in all mice and a mini-osmotic pump (2001D, Alzet) implanted subcutaneously in animals to be treated with the selective β_1-AR blocker, esmolol (Esmocard®, Amomed). In non-treated groups, animals only underwent a skin incision without implantation of the mini-osmotic pump. Esmolol dosage was chosen according to literature and preliminary experiments to reducing heart rate (HR) by 20% (**Supplemental Digital Content – Figure 1)**.

Experimental design

$\beta_1^{+/+}$ mice were randomly assigned to four groups: sham, sham + esmolol, CLP or CLP + esmolol. Esmolol was infused at 18 mg/kg/h (8 µl/h, 120 µl over the 15 hours of intervention) via a subcutaneous mini-osmotic pump which was activated 3 hours post-insertion. $\beta_1^{-/-}$ mice were randomly assigned to two groups: sham or CLP. The number of mice used and reasons for failed measurements and for exclusion are provided in **Supplemental Digital Content – Methods and Table 1**.

Immunophenotyping

Splenocyte were isolated according to conventional methods**.** Splenocyte Fc receptors were blocked (CD16/CD32-purified antibodies, eBioscience™, ThermoFisher Scientific) and cell surface markers used to identify total T cells (CD3+), CD4 T cells (CD3+ CD4+), CD8 T cells (CD3+ CD4-) and Treg cells (CD3+ CD4+ $CD25^{hi}$ $CD127^{low}$, Foxp3) in viable cells. Th1 (INF-γ+), Th2 (IL-4+) and Th17 (IL-17+) status was also characterized by intracellular staining of CD4 T cells. Full description is provided in **Supplemental Digital Content – Methods, and Treg cells gating strategy in the Supplemental Digital Content – Figure 2.**

Proliferation assay

Splenocytes were stained with Tag-it Violet™ Proliferation and Cell Tracking Dye (5 μM/10^6 cells; Biolegend) according to manufacturer's instructions and stimulated with phytohemagglutinin (PHA; 4μg/10^6 cells; Remel, ThermoFisher Scientific) for 72 hours at 37°C. Tag-it Violet signal was assessed in live CD4 T cells by flow cytometry. Full description is provided in **Supplemental Digital Content – Methods.**

Purification of CD4+ CD25- T cells and CD4+ CD25+ Treg cells

CD4 T and Treg cells were isolated in a two-step procedure following the manufacturer's instructions (CD4+ CD25+ Regulatory T Cell Isolation Kit, MiltenyiBiotec). Full description is provided in **Supplemental Digital Content – Methods and Supplemental Digital Content – Figure 3 panel A and B.**

Co-culture of CD4 T cells with catecholamine-stimulated Treg cells

Treg cells were stimulated with 1 μM dobutamine (a β_1-AR agonist), norepinephrine (α_1- and β_1-AR agonist), phenylephrine (α_1-AR agonist); (all from Sigma-Aldrich) or

PBS for 30 minutes then washed twice to remove the catecholamines. Pre-treated Treg cells were co-cultured with CD4 T cells at a 1:4 ratio. Cells were activated with anti-CD3/CD28 beads and proliferation was assessed by taking pictures every 2 hours for 5 days (37 °C, 5% CO2) using the 20× objective of the IncuCyte® S3 Live-Cell Analysis System (Sartorius). Analysis was performed using IncuCyte™ Basic Software. Full description is provided in **Supplemental Digital Content – Methods and Supplemental Digital Content-Figure 3 panel C.**

Echocardiography

Transthoracic echocardiography was performed 18 hours after CLP with a VEVO770 (FUJIFILM VisualSonics) and a 30 MHz mechanical probe. Full description is provided in **Supplemental Digital Content – Methods.** Mean arterial pressure (MAP) was measured by a blood pressure transducer (Emka) inserted through the left carotid artery.

Blood counts

In 5–6 mice from each group, complete blood counts were measured (Micros 60 ABX model).

Quantification of inflammatory mediators

In 5–6 mice from each group, the plasma levels of 46 inflammatory mediators were measured in duplicate using the Magnetic Luminex® Performance Assay – Mouse XL Discovery Premixed kit (Bio-Techne) according to the manufacturer's instructions.

Survival study

Survival was studied in $\beta_1^{+/+}$ CLP mice, $\beta_1^{+/+}$ CLP + esmolol (18 mg/kg/h) mice, and $\beta_1^{-/-}$ CLP mice. Census of animals was undertaken twice a day for 5 days. Administration of esmolol (Esmocard®, Amomed) was started 3 hours post-CLP and continued for a total infusion period of 24 hours.

Statistical analysis

Results are expressed as mean and standard deviation (mean ± SD). Results in $\beta_1^{-/-}$ mice were compared using a Mann-Whitney test while the Kruskal-Wallis test was used to compare $\beta_1^{+/+}$ groups. When the Kruskal-Wallis test was significant, *post hoc* comparisons were performed between sham, CLP, and CLP + esmolol groups using Dunn's multiple comparisons and a Bonferroni correction.

Variations were calculated as follows: 100-((mean group 1/mean group 2)x100).

To analyze the effect of catecholamines on Treg cells co-cultured with CD4 T cells, we firstly assessed the interaction between mouse at each time point. Considering the mouse has a random effect, the p-value of the test for the treatment effect was calculated. Only the last p-value (obtained at 132 hours) is shown.

For measurement of plasma inflammatory mediators, groups were compared using the Mann-Whitney test. A Benjamini-Hochberg adjustment was performed for multiple comparisons.

Kaplan–Meier curves were drawn. Significance was assessed using the log-rank test. P values <0.05 were considered statistically significant. Statistical analysis was performed using R, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria), using Graph-Pad Software 6.0, (San Diego, CA) and using NCSS Statistical Software 9 (East Kaysville, UT).

Results

β_1-AR contributes to post-septic immunosuppressive phenotype.

Male and female mice were used in equal numbers for all experiments (sex ratio=1.02). Eighteen hours after surgery, septic mice present a decrease in white blood cells and in total blood lymphocytes (-72% *vs.* sham, p=0.05 and -79% *vs.* sham, **Figure 1, panel A and B**). **Figure 1, panel C** shows that in the spleen, $\beta_1^{+/+}$ mice, sepsis induced a significant decrease in CD4 T cell proportions (-34% *vs.* sham, p<0.001) counteracted by β_1-AR blockade (+37% *vs.* CLP, p=0.03). In $\beta_1^{-/-}$ mice, sepsis significantly decreased the proportion of CD4 T cells (-17% *vs.* sham, p=0.02), however no change in CD8 T cells proportion was observed between groups (**Figure 1, panel D**).

With respect to Treg cells ($CD25^{high}$ $CD127^{low}$ Foxp3+), in $\beta_1^{+/+}$ mice, CLP induced an increase in Treg cells proportion (+65% *vs.* sham, p <0.001 **Figure 1, panel E**) and a trend increase in Treg cells count in the spleen (**Supplemental Digital Content – Figure 4**), inhibited by β_1-AR blockade (-19% *vs.* CLP, p=0.04).

In $\beta_1^{-/-}$ septic mice, the proportion of Treg cells was unchanged (+7% *vs.* sham, p=0.68). Of note, β_1-AR blockade in $\beta_1^{+/+}$ sham mice had no impact on CD4 T cell and Treg cell proportions (**Supplemental Digital Content – Figure 5)**. No modification was observed in other CD4 T cells subsets (Th1 (INF-γ+), Th2 (IL-4+) and Th17 (IL-17+)) in these experimental groups (**Supplemental Digital Content – Figure 6)**. These findings were not caused by a difference in cell viability among groups (**Supplemental Digital Content – Figure 7)**.

β_1-AR activation induces a decrease in CD4 T cell proliferation during sepsis.

After culturing splenocytes for 72 hours with PHA stimulation, the proliferative response of CD4 T cells was measured to assess sepsis-induced immune suppression. In $\beta_1^{+/+}$ mice, sepsis reduced the proliferative capacity of CD4 T cells (-15% *vs.* sham, p=0.04). However, administration of a β_1-AR blocker limited this effect (-8% *vs.* sham, p=0.38). In $\beta_1^{-/-}$ mice, sepsis did not decrease the proliferative capacities of CD4 T cells (-4% *vs.* sham, p=0.71) (**Figure 1, panel F**). These results were not caused by variations in cell viability among groups (**Supplemental Digital Content – Figure 8).** Of note, β_1-AR blockade in $\beta_1^{+/+}$ sham mice had no impact on CD4 T cell proliferation (**Supplemental Digital Content – Figure 5)**.

β_1-AR stimulation of Treg cells increases their immunosuppressive capacities.

The direct effect of β_1-AR stimulation on the immunosuppressive functions of Treg cells was assessed in vitro. CD4 T cell counts tended to decrease after five days of co-culture with Treg cells pre-treated with β_1-AR agonist dobutamine, in comparison to untreated Treg cells (p=0.08, **Figure 2, panel A**). This lower cell count, noted when co-culture was performed with dobutamine pre-treated Treg cells, was not caused by an increase in cell death (**Figure 2, panel B**). Similar results were found when co-culture assays of CD4 T cells were performed in the presence of norepinephrine and phenylephrine pre-treated Treg cells (**Supplemental Digital Content – Figure 9)**.

β_1-AR blockade, despite a reduced heart rate, did not alter hemodynamic characteristics in sepsis.

Hemodynamic parameters were assessed by echocardiography (**Supplemental Digital Content – Table 2)**. In $\beta_1^{+/+}$ septic mice, administration of a β_1-AR blocker

induced a decrease in HR (-24% *vs.* sham, p<0.001; -14% *vs.* CLP, p=0.07). In $\beta_1^{-/-}$ mice, sepsis did not affect HR (+9% *vs.* sham, p=0.24).

Compared to $\beta_1^{+/+}$ sham, high-grade sepsis induced a decrease in MAP (-27% *vs.* sham, p<0.001), SV (-40% *vs.* sham, 0.004), CI (-49% *vs.* sham, p<0.001), and CPI (-64% *vs.* sham, p<0.001). These parameters remained unaltered by the administration of a β_1-AR blocker (MAP: +8% *vs.* CLP, p=0.51; SV: +6% *vs.* CLP, p=0.99; CI: +2% *vs.* CLP, p=0.99; CPI: +25% *vs.* CLP, p=0.99). In $\beta_1^{-/-}$ mice, sepsis induced a decrease in MAP (-19% *vs.* sham, p=0.04), SV (-47% *vs.* sham, p<0.001), CI (-37% *vs.* sham, p=0.002) and CPI (-50% *vs.* sham, p<0.001).

β_1-AR modulates the concentration of both pro- and anti-inflammatory circulatory mediators.

The plasma levels of 46 inflammatory mediators were measured. Briefly, all inflammatory mediators were increased in $\beta_1^{+/+}$ CLP mice compared to sham controls (**Supplemental Digital Content – Table 3)**. Compared to $\beta_1^{+/+}$ CLP mice, esmolol-treated CLP mice showed an overall decrease in plasma concentrations of 58% (18/31) and 60% (9/15) for pro- and anti-inflammatory mediators, respectively **(Figure 3)**. For instance, compared to $\beta_1^{+/+}$ CLP mice, administration of a β_1-AR blocker reduced plasma TNF-α by 66% *vs.* CLP (p=0.02), and IL-10 by 82% *vs.* CLP (p=0.01). In $\beta_1^{-/-}$ mice, sepsis induced an overall increase in both pro- and anti-inflammatory mediators compared to sham animals (**Supplemental Digital Content – Table 3)**.

β_1-AR modulates survival during sepsis.

The survival rate was documented in $\beta_1^{+/+}$ CLP, $\beta_1^{+/+}$ CLP + esmolol and $\beta_1^{-/-}$ CLP mice **(Figure 4).** Survival at 110 hours decreased from 60% in $\beta_1^{-/-}$ CLP mice, to 37% in $\beta_1^{+/+}$ CLP + esmolol mice and 35% in $\beta_1^{+/+}$ CLP mice (log rank p=0.13).

Sex does not affect the β_1-AR effect.

Sensitivity analyses were performed on results obtained for major hemodynamic parameters (**Supplemental Digital Content – Figure 10)** and immune (**Supplemental Digital Content – Figure 11**) effects according to sex. No differences were found between male and female mice.

Discussion

In the present study, we confirm that experimental high-grade sepsis induces three hallmark features of sepsis-induced immunosuppression: a significant decrease in splenic CD4 T cells, an increase in splenic Treg cells, and a decrease in CD4 T cell proliferation capacity(6). A notable finding was the marked increase in Treg cells in the spleen of septic mice, which could be counteracted by pharmacologically or genetically blocking β_1-AR signaling. This observed decrease in Treg cells in treated animals was associated with a simultaneous increase in CD4 T cells and a global decrease in circulating pro- and anti-inflammatory mediators, indicating the involvement of β_1-AR in the septic-immune phenotype. These results also translated into an *in vivo* global improvement in cardiac efficiency.

To investigate the immune and cardiovascular roles of β_1-AR in sepsis, we deliberately chose only a fluid-resuscitated murine model of CLP to avoid any interference between exogenous catecholamine administration and the immune system. This model depicts all hemodynamic and survival characteristics of a typical high-grade sepsis, with an increase in both plasma pro- and anti-inflammatory mediators and a decrease in CD4 T cell proportion(15-19). Analysis of CD4 T subtypes revealed that early after onset of sepsis, the percentages of Th1, Th2 and Th17 cells were unchanged. Many studies usually report a switch from Th1 to Th2 phenotypes, and a decrease in Th17 cell percentages(20-23). However, other studies found that sepsis could also disrupt in a time-dependent manner both the representation and the function of CD4 T cell subsets, including Th1, Th2, Th17(24-27). Unlike other lymphocyte populations, Treg cells are increased during trauma in both animals and humans, as well as during sepsis and septic shock (as seen in our study) (25, 26). This increase in Treg cells contributes

to lymphocyte paralysis and, more broadly, enhances the post-septic immunosuppressive process (28).

ß-adrenergic stimulation contributes to CD4 T cell dysfunction

In both innate and adaptive immune cells, adrenergic receptors are widely expressed though not well characterized, particularly for the β subtype(29). Numerous studies have demonstrated the presence of β-AR on almost all lymphocyte subtypes(30-32). However, until recently, only β_2-AR but not β_1-AR subtypes were found on T cells(33, 34). As a consequence, β_1-AR stimulation was not considered to have a major role in inflammation(35). β_1-AR were found to be widely expressed on splenic CD4 T cells only recently. In cirrhotic mice, the β_1-AR agonist dobutamine, could re-activate inhibited Treg cells, suggesting the presence of β_1-AR on their surface(36). In accordance, we too found that *in vitro* CD4 T cell proliferation tended to be reduced by Treg cells pre-treated with dobutamine. Apart from its β_1-AR activity, dobutamine also has activity on the β_2-AR. This is known to exert some anti-inflammatory effects when stimulating Treg cells and which may have mitigated the β_1 effects (31). In addition to CD4 T cell dysfunction, stimulation of β-ARs in innate and adaptive cells has recently been confirmed as a crucial factor involved in immunoparalysis. We have summarized the effects of β-AR agonists on innate and adaptive immunity in Supplemental Digital Content – Figure 12 (5).

ß-adrenoreceptor blockade reversed sepsis-induced dysautonomia

In numerous conditions, such as acute heart failure, burn injuries, and septic shock, β_1-AR blockade has been consistently associated with protective effects on inflammation and hemodynamics(11, 37, 38). As others, we too found a global

decrease in pro- and anti-inflammatory mediators in animals with high-grade sepsis treated by β_1-AR blockade(11, 39, 40). Underlying mechanisms are still not elucidated. In *Listeria monocytogenes* infected mice acute cold or restraint stress (surrogates of sympathetic adrenergic stimulation) inhibited the host T cell response and increased cytokine production(41). In this model, pharmacological blockade of the β_1-AR but not β_2-AR could downregulate this pro-inflammatory state and shorten time to bacterial clearance(42).

In β_1-AR blocked animals with high-grade sepsis, we found the decrease in Treg cells in the spleen was associated with a concomitant increase in CD4 T cells, as reported previously(43). In addition, we found that proliferative capacity of splenic CD4 T cells in septic animals was maintained when the β_1-AR was blocked either pharmacologically or genetically. This suggests that β_1-AR activation contributes to sepsis-induced immunosuppression, which could be reversed by β_1-blockade. By comparison, volunteers challenged with lipopolysaccharide, and then being infused with norepinephrine for 5 hours showed a shift to an anti-inflammatory state (decreased TNF-α/IL-10 ratio) which could be counterbalanced by β-AR blockade (increased TNF-α/IL-10 ratio)(44). β_1-AR blockade in our septic animals reduced the inflammatory burden and, taken as a whole, helped to shift from an immunosuppressive state to a state of immune homeostasis.

Beside this immune rebalancing in experimental high-grade sepsis, it is important to reiterate that β_1-AR blockade also has major cardiovascular effects. In agreement with most other experimental or clinical studies, septic mice receiving β-AR blockade showed no alteration in cardiac power, despite a decrease in HR, suggesting improved cardiac efficiency(9, 11, 39, 45). However, it should be borne in mind that animals only

received an initial subcutaneous bolus of fluid resuscitation and then no fluids nor catecholamines thereafter. While the association between an increased Treg cell percentage during sepsis and an immunosuppressive state is well proven, effects on survival remain inconclusive(46, 47). The benefits of β_1-AR blockade on immune and cardiovascular function should improve the survival rate. However, in the present study, and without any treatment (antibiotics, fluid or vasopressors), no improvement was seen in survival. In a similar CLP surgery model, Ackland *et al*. also found that metoprolol infusion, commenced 6 hours after the onset of fecal peritonitis, did not confer any benefit in improving survival whereas pretreatment was effective(40). A dose effect may be relevant as the β_1-AR blocker was infused only during the first 24 hours after CLP surgery. In a single-center study prolonged administration of esmolol in septic shock patients, titrated to maintain heart rate between 80-94 bpm, was associated with increased survival(9).

Perspectives and limitations

β_1-AR and β_2-AR are widely expressed on both innate and adaptive immune cells. Specifically, it has been reported that naïve CD4 T cell subtypes Th1, Th17 and Treg cells, but not Th2, express β_2-AR(30, 48). β_2-AR stimulation promotes a shift from Th1 to Th2 anti-inflammatory patterns and could activate Treg suppressive functions(31, 49). β_2-AR stimulation may enhance the macrophage M2 anti-inflammatory phenotype, favoring IL-10 production by CD4 T cells(32). Beneficial effects of β_2-AR modulation remain unclear during sepsis. In a murine endotoxemia model, β_2-AR blockade reduced survival while, in septic pigs, it amplified sepsis-induced hepatic injury(32, 50, 51). In this study, we only investigated the impact of β_1-AR blockade on alteration of CD4 T cell patterns. The impact of selective β_2-AR modulation as well as non-specific

β-AR blockade on CD4 T cells during sepsis merits investigation. Finally, well-known limitations of murine CLP models (young age, similar infectious insult and severity, similar time-interval from CLP induction to evaluation) preclude full comparison with human sepsis which is far more heterogenous in nature. Nonetheless, such models remain useful for mechanistic understanding and assessing the impact of interventions. Moreover, measurements were only performed at one timepoint: 18 hours post-CLP. Serial assessments, both earlier and later, in a less severe model could help to clarify the evolving inflammatory profile of this disease.

In summary, in the murine model tested, the β_1-AR contributes to sepsis-induced immunosuppression through modulation of regulatory T cell inhibitory functions. β_1-AR blockade, while restoring CD4 T cell homeostasis, also decreased global systemic inflammation and improved cardiac efficiency **(summary Figure 5)**. Thus, instead of considering β_1-AR blockade as only a hemodynamic treatment, we suggest an immunoregulatory mechanism by which β_1-AR blockers may also be beneficial in sepsis.

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Authors' contributions.

MD, AK: study concept and design. MD, HL: Experiments (MD: involved in all experiments, HL involved in flow cytometry supervision. CL, AK with the help of François Husson: statistical analysis, AK, MD, PA, JPF, MS, BGC: Drafting of the manuscript. All authors were involved in critical revision of the manuscript. All authors read and approved the final manuscript.

Availability of supporting data.

Raw data and statistical analyses of all presented results are available at the following: https://osf.io/nz5u9/ or DOI 10.17605/OSF.IO/NZ5U9.

MD takes the responsibility of the integrity of the data.

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Figures Legends

Figure 1: β_1-AR contributes to the post-septic immunosuppressive phenotype, which is reversed by b_1-AR blockade. The counts of white blood cells (A) and lymphocytes (B) in whole blood were determined in each group. Splenocytes were immunophenotyped 18 hours after sepsis induction. The proportion of CD4 T cells among live cells (C), the proportion of CD8 T cells among live cells (D), and the proportion of Treg cells ($CD25^{high}$ $CD127^{low}$) among live CD4 T cells (E) were assessed. (F) Proliferation of CD4 T cells according to cell tracking dye staining after 72 hours of culture with a mitogen (PHA). Data are expressed as mean ± SD; each point represent a mouse. * p<0.05; ** p<0.01; *** p<0.001.

Figure 2: In vitro β_1-AR stimulation of Treg cells increases their immunosuppressive capacities. Treg cells isolated from $\beta_1^{+/+}$ mice were treated with dobutamine or PBS for 30 minutes. Pre-treated Treg cells were then co-cultured with CD4 T cells at a 1:4 ratio in the presence of anti-CD3 and anti-CD28 beads for 5 days. A fluorescent green reagent was added for counting dead cells. (A) shows the real-time cell count normalized to the first count at H0, and (B) shows the dead cell count as a percentage of total cells. Data shown are the means of 4 images per well in duplicate with 4 mice per time point ± SEM (total 32 images/time/group). Images were taken at 20× magnification on the IncuCyte® imager.

Figure 3: β_1-AR modulates the level of both pro- and anti-inflammatory circulatory mediators. Plasma levels of 46 inflammatory mediators were measured in 5–6 animals per group. Data are expressed as Log10(mean).

Figure 4: β_1-AR blockade modulates survival during sepsis. Survival curve of $\beta_1^{+/+}$ CLP mice, $\beta_1^{+/+}$ CLP mice receiving esmolol for 24 hours, and $\beta_1^{-/-}$ CLP mice. The one

553 animal that died before the first count was considered a technical failure and excluded
554 from the study.
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556 **Figure 5:** Summary of the findings
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