

Effects of IL-7 on sepsis: a systematic review

Efeitos da IL-7 na sepse: uma revisão sistemática

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ABSTRACT: *Objective:* The aim of this systematic review to evaluate the effects and possible therapeutic relevance of interleukin-7, a novel immunoadjuvant agent, against sepsis. *Data Sources:* We have searched PubMed, Scopus, LILACS and SciELO for any study which had IL-7 as intervention for sepsis. *Study Selection:* For each source, a single author was chosen to select all studies based on title and abstract. The remaining two authors then proceeded to read the complete text for relevance assessment. *Results:* Eleven sources were retrieved, consisting of six studies performed on animals, four on *ex vivo* human cells, and a single clinical trial. Several parameters were evaluated and reported, such as improved animal survival (3 trials; n= 264); improved cell count, activation, cytokine secretion, proliferation and survival; and higher patient CD4+ and CD8+ lymphocyte count (1 trial; n= 27). Major limitations were the heterogeneity of studies and the complexity of sepsis presentation. *Data Extraction:* Any data related to IL-7 intervention in septic patients was extracted. Animal and *in vitro* data was chosen according to outcomes in either overall survival or immune response markers. The data extraction from each study was conducted by a single author and then revised by the other two. Bias was assessed by all three authors together, without any specific methodology. *Conclusions:* Although IL-7 as a potential therapy option for sepsis still requires further investigation and clinical evaluation, current studies show promising results of how the interleukin could act against the disorder.

Keywords: Sepsis; Interleukin-7; IL-7.

RESUMO: *Objetivo:* O objetivo desta revisão sistemática é avaliar os efeitos e a possível relevância terapêutica da interleucina-7, um novo agente imunoadjuvante, contra a sepse. *Fontes Dados:* As bases de dados PubMed, Scopus, LILACS e SciELO foram percorridas por qualquer estudo que tivesse IL-7 como intervenção para sepse. *Seleção do Estudo:* Para cada fonte, um único autor foi encarregado de selecionar os estudos com base no título e resumo. Em seguida, os outros dois revisores leram o texto completo para avaliação da relevância. *Resultados:* Onze estudos foram selecionados, dos quais de seis estudos foram realizados em animais, quatro em células humanas *ex vivo* e um único ensaio clínico. Diversos parâmetros foram avaliados e reportados em cada um dos estudos, como melhor sobrevida em animais (3 estudos; n= 264); melhor contagem, ativação, secreção de citocinas, proliferação e sobrevivência de células; e maior contagem de linfócitos CD4+ e CD8+ em pacientes (1 ensaio; n= 27). As principais limitações foram a heterogeneidade dos estudos, além da complexidade da apresentação da sepse. *Extração de Dados:* Foram selecionados todos os dados relacionados ao uso intervencionista da IL-7 em pacientes sépticos. Dados de estudos em animais e *in vitro* foram selecionados conforme desfechos tanto de sobrevida geral quanto em marcadores de resposta imune. A extração de dados de cada estudo foi feita por um único autor, com posterior revisão pelos demais. A análise de viés foi feita pelos três autores em conjunto, sem metodologia específica. *Conclusões:* Embora a IL-7 como uma potencial opção terapêutica para sepse ainda exija maior investigação e mais ensaios clínicos, os estudos atuais mostram resultados promissores de como a interleucina pode agir contra a doença.

Palavras-chave: Sepse; Interleucina-7; IL-7.

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INTRODUCTION

Sepsis, according to the most recent consensus, can be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection¹. Aside from antibiotic therapy and supportive care, the disorder still has no currently approved immune treatment, even though more than over 100 therapeutic clinical trials have been conducted². Such a challenge seems to be largely due to its complex clinical course. Sepsis is traditionally presented as an early hyper-inflammatory phase (SIRS) followed by an anti-inflammatory one (CARS), although recent research points to both phases happening simultaneously^{3,4}.

Most of early potential therapies targeting sepsis focused on halting its pro-inflammatory, pro-coagulative phase, such as TNF- α antagonists, Toll-like receptor 4 (TLR4) antagonists, corticosteroids, anti-lipopolysaccharide and activated protein C⁵. More recently, the anti-inflammatory aspect of the disorder has received marked attention, as studies on sepsis “immunoparalysis” mount. Sepsis has been proven to have various deleterious effects on immune cells, including apoptosis, anergy and enhanced tolerance, which lead to a persistently impaired immunity and late mortality⁶. Thus, in an attempt to reverse such profile, several immunomodulatory, pro-inflammatory molecules are currently under research, chiefly among them IFN- γ , IL-15, GM-CSF, Anti-PD-1/PD-L and IL-7⁷.

IL-7 is a cytokine produced by non-haematopoietic stromal cells that mediates anti-apoptotic and proliferative effects on T lymphocytes. The IL-7 receptor, composed of IL-7R α (also known as CD127) and the common cytokine receptor γ , signals through the activation of PI3K and the JAK-STAT pathway, the downregulation of p27 and the modulation of Bcl-2⁸. Although IL-7 treatment is still not approved for any condition, CYT107, a glycosylated recombinant human version of the cytokine, has been the intervention of choice in clinical trials for HIV, bone marrow reconstitution, idiopathic lymphopenia, cancer, and hepatitis C. These conditions, although very different from each other, all lead to common disruptions of the immune system, which could be also similar to the one caused by sepsis⁹.

The aim of this review, thus, is to systematically evaluate current literature on the effects of IL-7 in the sepsis-induced immune profile, in order to assess its potential as a novel treatment for the condition.

METHODS

We have searched for controlled trials that administered IL-7 to patients with sepsis or septic shock. Animal and *ex vivo* preclinical studies were included in order to explore the mechanisms, efficiency and limitations of the drug. There were no language or date restrictions,

although the studies on pediatric patients were excluded.

The research was made on PubMed (1966 - present), Scopus (2004 - present), LILACS (1982 - present) and SciELO (1997 - present) databases. “IL-7”, “interleukin-7” and “sepsis” were the searched terms.

For each database, one author was selected to search for studies and select them based on title and abstract, while the remaining authors read the complete article for relevance assessment. Disagreements were resolved by discussion between the three authors. The excluded articles were related to the pathophysiology of sepsis, immunodeficiency, general immunotherapy and/or reviews of new treatments, secondarily related diseases and also articles covering IL-7 and other cytokines as markers of sepsis instead of treatment options. Figure 1 depicts the flow diagram of article selection.

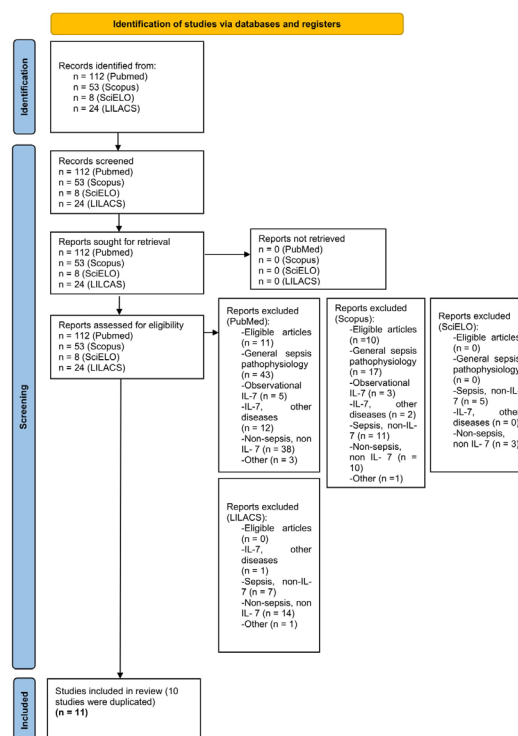


Figure 1 – Flow diagram of articles

A total of 11 articles were recovered from PubMed, 10 from Scopus and none from LILACS and SciELO. Every article selected from Scopus was also listed on PubMed. Among the final 11 studies, there were six studies performed on animals, four on *ex vivo* human cells, and a single clinical trial.

Data was extracted according to the following criteria. For the human study, all data related to the therapeutic use of IL-7 was extracted. For each animal and *ex vivo* preclinical study, a single author selected relevant

information concerning IL-7 intervention on either (1) overall survival or (2) modified immune response markers. The remaining two authors then proceeded to review the extracted data on the selected outcomes. Data related to pre-intervention immunological markers or survival prognosis based on cellular activity, for instance, was thus excluded. Data related to the effects of IL-7 on sham animals and non septic patients were also excluded from tables, but these results could still be referenced during discussions. There was no missing or unclear information in the selected studies about population, sepsis model, intervention or outcome.

For each selected study, the elected effect measure for the result report was the mean difference between interventional and control groups. Extracted data was formatted as tables according to variables specific to each study group (animal, human *ex vivo*, clinical trial). For every individual study, results were synthesized and tabulated according to the categories “assessment”, “sample”, “intervention”, “outcome” and “p-value”. There was no need for data conversion to make comparison possible. No methods were employed so as to evaluate the heterogeneity among study results. Additionally, no methods were used to assess neither the robustness of the data synthesis, nor the certainty of the evidence.

Bias assessment was made by all three authors in unison. All animal studies had control groups, but no blinding. The sepsis inducing procedure and further interventions were clearly stated in every study. *In vitro* studies did not have blinding and control groups and were conducted on either healthy or critical (but not septic) patients' cells. The clinic trial was randomized, double-blind and placebo-controlled. Definitions of sepsis were explicitly stated in every *ex vivo* and clinical trial study. After consideration, the authors came to agreement that no study was to be excluded based on possible bias risk. There were no specific methods employed to evaluate risk of bias due to missing results.

RESULTS

The retrieved studies, even after separation in either animal, human or cell-based groups, showed marked heterogeneity in regard to population, sepsis model, intervention regimen or analyzed immune function markers. Regardless, no selected study failed to fulfill the inclusion criteria. The differences between two of the groups (six animal studies and four *ex vivo* studies) were then summarized in Tables 1 and 2, respectively. The clinical trial, as it was the only one performed so far, was left out of the comparison.

Table 1: Animal studies

Source	Population	Model	Intervention	Assessments
Unsinger et al. ¹⁰ 2010	C57BL6 (inbred) and CD1 (outbred) male mice.	Cecal ligation and puncture (CLP).	Either antibody stabilized human rIL-7 (R&D Systems) and human rIL-7 (Cytheris). Various regimens: - 5 µg 90 min post surgery; - 5 µg at 90min, 24h and 48h marks - 5 µg for 3 consecutive days; - 2.5 µg 90 min postsurgery and every 48 h for two additional doses.	- Survival Protocol; - Delayed-type hypersensitivity (DTH); - Cell Count; - Cell Proliferation (Ki67); - Apoptosis (Bcl2, PUMA); - Activation (IFN-γ); - Surface Markers (LFA-1, VLA-4, CD4, CD8, IL-7R).
Kasten et al. ¹¹ 2010	C57BL/6, TCR αβ/γδ-deficient and TCR γδ-deficient mice.	Cecal ligation and puncture (CLP).	5 µg of human rIL-7 (Cytheris) post surgery.	- Survival Protocol; - Tissue Damage (AST, pulmonary MPO and intestinal dry/wet ratio); - bCell Count; - Apoptosis (Bcl2); - Activation (serum IL-6); - Surface Markers (CXCR3); - Innate Immunity (neutrophil recruitment); - Pathogen Burden (bacteremia).
Unsinger et al. ¹² 2012	C57BL6 (inbred) or CD1 (outbred) male mice.	Cecal ligation and puncture (CLP) followed by imipenem. Intravenous <i>C. albicans</i> injection followed by fluconazole.	Human IL-7 (Cytheris). Various regimens: - 2.5 µg for 5d, 6d postsurgery and 2d after secondary challenge - 2.5 µg for 4d, 5d postsurgery and 1d after secondary challenge.	- Survival Protocol; - Delayed-type hypersensitivity (DTH); - Cell Proliferation (Ki67); - Activation (CD69, IL-2, IFN-γ, TNF-α, and IL-6); - Surface Markers (LFA-1); - Pathogen Burden (Candida foci).

continue

Table 1: Animal studies

Source	Population	Model	Intervention	Assessments
Shindo et al. ¹³ 2015	C57BL/6 mice.	Cecal ligation and puncture (CLP) followed by imipenem. Intravenous <i>C. albicans</i> injection followed by fluconazole.	2.5 µg of Human IL-7 (Cytheris) for 5d, 4d postsurgery and 1d after secondary challenge. A group also received a concomitant dose of 200 µg of anti-PD1 (Bio X Cell) on both the first and the last day of treatment.	- bCell Proliferation (Ki67); - Activation (IFN-γ); - Surface Markers (LFA-1, VLA-4, CD28); - Innate Immunity (MHC II expression).
Shindo et al. ¹⁴ 2017	C57BL/6 mice.	Cecal ligation and puncture (CLP) followed by imipenem. Intratracheal <i>P. Aeruginosa</i> followed by imipenem.	2.5 µg of human rIL-7 (Revimmune) for 6d post surgery. Secondary challenge 3d post surgery.	- Survival Protocol - Cell Count (CD4, CD8, NKT and γδ T-cells); - Cell Proliferation (Ki67), - Activation (IL-17, IL-22, IFN-γ, TNF-α, NF-kB activation and phosphorylated STAT3 expression); - Innate Immunity (NK, IL2, IL3).
Kulkarni et al. ¹⁵ 2018	C57BL/6 mice.	Intraperitoneal human feces followed by meropenem.	2.5 µg of antibody stabilized human rIL-7 (R&D Systems) for 5d, 5d post surgery.	- Cell Count (IL-10+ B, DN T, MDSC); - Cell Proliferation (CD4 T cells under MDSC).

Table 2: Ex vivo studies

Sources	Population	Exclusion criteria	Blood Samples	Follow up	Assessments
Venet et al. ¹⁶ 2012	70 septic shock patients; 55 healthy volunteers.	Not reported.	Day 1–2 and day 3–4 after shock.	28 days (mortality: 24, secondary infections: 14).	- Cell Proliferation - Apoptosis (Bcl2); - Activation (IFN-γ; STAT5 phosphorylation).
Demaret et al. ¹⁷ 2015	13 septic shock patients; 10 healthy volunteers.	Younger than 18y, aplasia or immunosuppressive disease (e.g., HIV infection).	Day 3–4 after shock.	28 days (mortality: 5).	- Activation (STAT5 phosphorylation).
Venet et al. ¹⁸ 2017	75 septic shock patients; 25 healthy volunteers.	Younger than 18y, aplasia or immunosuppressive disease (e.g., HIV infection).	Day 3–4 after shock.	28 days (mortality: 18).	- Activation (metabolites concentration, metabolic pathways).
Thampy et al. ¹⁹ 2018	24 multi-drug resistant bacteria septic patients; 26 critically-ill non-septic patients.	Younger than 18 y, HIV, organ or bone marrow transplantation, high-dose corticosteroid regimens, immune-modifying biological agents, systemic autoimmune diseases.	24–48 hours after positive cultures for multi-drug resistant bacteria.	Patient survival was followed for 90 days after study entry.	- Activation (IFN-γ).

Animals Studies

Most of the animals studied were C57BL6 (inbred) or CD1 (outbred) male mice, although a single source utilized knockout mice for both αβ and γδ TCR or just γδ TCR, so as to evaluate the pathway of IL-7 action¹¹. As for the model of sepsis, two studies employed the usual colon puncture and ligation method without any other further procedure, in an attempt to assess IL-7 ability to prevent septic immunosuppression^{10,11}. Three other sources applied intravenous antibiotic treatment (imipenem) right away after the surgery, mimicking hospitalized care. The

mice were then challenged again with common nosocomial pathogens (*C. albicans* and *P. Aeruginosa*), in an effort to recreate secondary infections commonly associated with sepsis and thus to study IL-7 capabilities to reverse immunosuppression after it has been set^{12–14}. Finally, the remaining source resorted to intraperitoneal injection of a solution of human feces, followed by treatment with meropenem¹⁵. There were various drug regimens, but doses were either 2.5 mg or 5 mg of recombinant IL-7 stabilized with antibodies (R&D Systems) or rIL-7 of longer half-life (Cytheris)¹⁰.

As for the bias assessment of animal studies, all interventions had control groups, usually for both sepsis (versus sham surgery) and IL-7 administration. There was no blinding. Certainty was evaluated by the sound proposed methods, result presentation and commentary.

IL-7 improved survival

Three studies carried out sepsis survival protocols. IL-7 improved survival in both CLP mice (two or three doses of 5 µg; total n= 149; follow-up for 7 days)¹⁰ and CLP mice secondarily challenged with peritonitis (five doses of 2.5 µg, n= 75, follow-up for 14 days)¹³ and pneumonia (six doses of 5 µg; total n= 50, follow-up for 12 days)¹⁴. However, IL-7 failed to change outcomes for mice without functional T-cell receptors (TCR $\alpha\beta/\gamma\delta$ knockout)¹¹.

IL-7 improved lymphoid cell count and proliferation

Four out of the six sources on animal models performed lymphocyte proliferation test, using Ki-67 as marker. Most of them showed marked T cell proliferation for both CD4⁺ and CD8⁺ subsets (10, 12, 13), although in a single study treatment with IL-7 did not show effect on the percentage of Ki-67 displaying of CD4⁺ T cells¹⁰. Higher Ki-67 expression was also detected on naive (non septic) mice treated with IL-7¹⁴. A more evident effect was seen in peripheral lymph node cells than splenocytes in one of the peritonitis models¹³, while the pneumonia model showed similar effects to both spleen and lung lymphocyte Ki-67 expression¹⁴.

Total cell count was reported in most of the sources. IL-7 treatment had positive effect on the absolute number of various subsets of T lymphocytes, such as naive, central memory and effector memory CD4⁺ T cells¹⁰; central memory and effector memory CD8⁺ T cells¹⁰; and peritoneal $\gamma\delta$ T cells¹¹. IL-7 expanded cell numbers of other lymphoid cell lineages such as NKT cells, NK cells, ILC2 and ILC3 in naive mice¹⁴, but had no effect on the depletion of NK cells, B cells and dendritic cells of septic mice¹⁰. There was some divergence in cell counts results, as in one peritonitis study, IL-7 did not improve central memory CD4⁺ T count¹³, while in another CLP model it instead failed to increase naive CD8⁺ T cells¹⁰. On the other hand, IL-7 also induced a transient expansion of anti-inflammatory cells, such as Foxp3⁺, CD25⁺, CD4⁺ T cells, Double negative CD3⁺, CD4⁻, CD8⁻ T cells and MDSC (myeloid-derived suppressor cells)¹⁵.

IL-7 improved lymphocyte activation and functionality

Treatment with IL-7 improved IFN- γ production level in several studies, with a tendency to a more pronounced effect in CD8⁺ T cells rather than CD4⁺ T cells^{10,12-14}. However, IL-7 effects on IFN- γ seemed to be restricted to lymphocytes, as it failed to improve NK cells production of IFN- γ ¹³. The therapy also did not show

any effect on plasma IFN- γ levels¹⁴. In relation to other cytokines, IL-7 increased the number of IL-17- and TNF- α -producing ILCs and CD8 T cells in the lungs of septic mice challenged with pneumonia, and that of IL-17-, TNF- α - and IL-22-producing ILCs and T cells in their spleens¹⁴. Moreover, IL-7 treatment showed increased levels of transcription factors NF- κ B and STAT3¹⁴.

IL-7 also improved the expression of cell trafficking molecules of lymphocytes. Relative expression of integrins LFA-1 and VLA-4 on CD4⁺ and CD8⁺ were higher for both septic and naive mice treated with IL-7 (10, 12). IL-7 and combination of IL-7 and anti-PD-1 increased LFA-1 of splenic CD4 and CD8 T cells and CD4 T cells positive for VLA-4, but not CD8⁺ T cells¹³.

IL-7 treatment was responsible for a spike in serum IL-6 levels, although not lasting (effect evaluated by 6 hours, no difference at the 24h mark)¹¹. The intervention also did not affect circulating levels of other inflammatory cytokines such as IL-17, IL-22 and TNF- α ¹⁴. Supernatants of splenocytes, however, showed increased production of cytokines such as IL-2, IL-6 and TNF- α , but not of IL-10¹².

Among other markers of lymphocyte activation and functionality, IL-7 increased CD8 expression in sham, septic¹⁰ and 2-hit mice¹³, but the treatment did not expand septic mice CD4 expression¹⁰. CD69, a routinely used marker of activation, was shown to be increased by IL-7 treatment in CD8⁺ T cells, and reversed to pre-sepsis levels in CD4⁺ T cells, in one of the peritonitis model¹³.

Finally, sepsis-induced expansion of the IL-7R, as a homeostatic mechanism for replenishment of lost immune cells, is reversed by IL-7 administration, leading to receptor downregulation and probably accounting for the treatment lack of exacerbated inflammatory response¹⁰.

IL-7 prevented apoptosis

Treatment with IL-7 induced the upregulation of the anti-apoptotic protein Bcl-2 in T cells^{10,11}, and prevented the sepsis-induced upregulation of proapoptotic PUMA protein in both *in vivo* and *ex vivo* experiments¹⁰. IL-7 also showed marked reduction of both CD4⁺ and CD8⁺ T cell apoptosis as measured by the TUNNEL method¹⁰.

IL-7 improved innate immune response and pathogen clearance.

Mice treated with IL-7 showed increased cell count of a number of innate immunity cells, such as splenic NK cells, ILC2 and ILC3¹⁴. Early neutrophil recruitment was shown to be increased by IL-7 administration, although no significant difference in the local population of cells was seen at the 24h mark, nor did the treatment improve their activation, as evaluated by phagocytosis, oxidative burst and CD11b expression¹¹. Another marker of innate immune activation, class II major histocompatibility complex expression on macrophages and dendritic cells, was also not significantly increased by IL-7¹³. As for specific pathogen

burden, IL-7 administration reduced both bacteremia at the 24h mark¹¹ and the number of liver *Candida* foci¹².

IL-7 restored delayed type hypersensitivity, but caused no end-organ injury

The loss of delayed type hypersensitivity seen in septic mice was reversed by IL-7 treatment, as measured by footpad swelling to trinitrophenyl in both CPL-only¹⁰

and peritonitis models¹². Nonetheless, several markers of tissue damage, such as lung myeloperoxidase, serum aspartate aminotransferase and intestinal wet/dry ratio were not significantly increased by IL- treatment¹¹.

Individual result tables

The individual results for each study are summarized in Tables 3 to 8.

Table 3: Results from Unsinger et al.¹⁰

Assessment	Sample and Grouping	Intervention	Outcome	p-value
Overall survival.	n=14, 25 and 31 per CLP group. n=14, 24 and 31 per CLP+IL-7 group.	5 µg of rhIL-7 or saline 90 min, 24h and 48h after CLP surgery.	IL-7 treatment markedly improved survival in sepsis.	$p<0.01-0.03$
Splenic cell count.	n=11 per group (sham; sham+IL-7; CLP; CLP+IL-7).	5 µg of rhIL-7 or saline 90 min after CLP surgery. Spleens were harvested after 24h.	IL-7 treatment prevented the decrease in CD4+ and CD8+ T cells, but not in NK cells, B cells, or dendritic cells in CLP mice spleens.	$p<0.01-0.05$
Lymph node cell count.	n=6 per sham group. n=9-10 per CLP group.	5 µg of rhIL-7, 90 min after CLP surgery. Mesenteric lymph nodes were harvested after 24h.	IL-7 treatment had a significant ability to increase the absolute cell counts of all lymphocyte groups (naive, effector and memory) but naive CD8+ T cells in CLP mice lymph nodes.	$p<0.05$
Apoptosis	n=6-8 per sham group. n=11 per CLP group.	5 µg of rhIL-7, 90 min after CLP surgery. Spleens were harvested after 24h.	IL-7 treatment prevented the increase in CD4+ and CD8+ T cell apoptosis occurring during sepsis in CLP mice spleens.	$p<0.01-0.05$
Intracellular markers (Bcl-2 and PUMA).	n=6-8 per group (sham; sham+IL-7; CLP; CLP+IL-7).	Same as above.	IL-7 treatment increased Bcl-2 expression in CD4+ and CD8+ T cells in CLP mice spleens. IL-7 also prevented the increase of proapoptotic PUMA protein expression in CD4+ T cells.	$p<0.05$
Splenic T cell proliferation.	n=6-7 per group (sham; sham+IL-7; CLP; CLP+IL-7).	5 µg of rhIL-7, 90 min, 24h and 48h after CLP surgery. Spleen were harvested after 72h.	IL-7 treatment increased the proliferation of CD8+ (but not CD4+) T cells in CLP mice spleens.	$p<0.001$
Surface markers (IL-7R).	n=4 per group (sham; sham+IL-7; CLP; CLP+IL-7).	5 µg of rhIL-7 for 3 consecutive days. Spleens were harvested on the third postoperative day.	IL-7 treatment reverted the sepsis-induced increase in IL-7R expression by CD4+ and CD8+ T cells in CLP mice.	$p<0.01-0.05$
Activation (IFN-γ).	n=8-9 per group (sham; sham+IL-7; CLP; CLP+IL-7).	5 µg of rhIL-7 or saline 90 min after surgery. Spleens were harvested after 24h.	IL-7 prevented the decrease in IFN-γ production in splenic T cells (CD4+, CD8+ and CD62L+) in CLP mice.	$p<0.05$
Surface markers (LFA-1, VLA-4, CD4 and CD8).	n=10 (sham). n=9 (sham+IL-7). n=13 (CLP mice). n= 12 (CLP+IL-7).	5 µg of rhIL-7, 90 min, 24h and 48h after surgery. Spleens were harvested after 72h.	IL-7 treatment increased LFA-1, VLA-4 and CD8 expression (but not CD4 expression) in CLP mice.	$p<0.05$
Delayed-type hypersensitivity (DTH) response.	n=5 per group (sham; sham+IL-7; CLP; CLP+IL-7).	2.5 µg of rhIL-7, 90 min after surgery and 2 additional doses every 48h. Mice were sensitized four days post surgery	IL-7 treatment prevented the loss of DTH response in CLP mice.	$p<0.05$

Table 4: Results from Kasten et al.¹¹

Assessment	Sample and Grouping	Intervention	Outcome	<i>p</i> -value
Overall survival (TCR $\alpha\beta/\gamma\delta$ -deficient mice).	n=10-11 per group (WT; IL-7).	Mice were treated with either 5 μ g of rhIL-7 or vehicle at the time of CLP.	IL-7 treatment did not improve the survival of TCR $\alpha\beta/\gamma\delta$ -deficient mice.	-
Intracellular markers (Bcl-2).	n=4 per group (sham; sham+IL7; CLP; CLP+IL7).	Mice were treated with either 5 μ g of rhIL-7 or vehicle at the time of CLP. Spleens were harvested 3h after CLP.	IL-7 treatment elevates Bcl-2 expression in CD4+ naive and effector memory T cells, and CD8+ T naive cells.	<i>p</i> <0.05
Activation (IFN- γ).	n=8 per group (WT;IL-7).	Mice were treated with 5 μ g of rhIL-7 at the time of CLP. Splenocytes were obtained 3 h after CLP.	IL-7 treatment enhances IFN- γ levels in septic mice.	<i>p</i> <0.05
Humoral marker (CXCL10/IP-10).	n=4 per group (sham, sham+IL7, CLP, CLP+IL7).	Mice were treated with 5 μ g of rhIL-7 at the time of CLP. Peritoneal lavage fluid obtained 3 h after CLP.	IL-7 treatment promoted increased concentrations of peritoneal CXCL10/IP-10.	<i>p</i> <0.01
Innate immunity (neutrophil recruitment).	n=9-13 (6 h WT and IL-7). n=12-13 (24 h WT and IL-7).	Mice were treated with 5 μ g of rhIL-7 at the time of CLP. Neutrophils at the site of infection were analyzed at the indicated times following CLP.	IL-7 treatment enhances neutrophil recruitment to the peritoneum of septic mice. IL-7, however, did not increase activation or functionality of neutrophils.	<i>p</i> <0.01
Cell count ($\gamma\delta$ T cells) and Activation (IL-17).	n=4 per group (WT; IL-7).	Mice were treated with 5 μ g human of rhIL-7 at the time of CLP. Peritoneal $\gamma\delta$ T at the site of infection were analyzed 3h after CLP	IL-7 treatment increased the number of IL-17-producing peritoneal $\gamma\delta$ T cells in septic mice. IL-17 secretion was also increased by the treatment.	<i>p</i> <0.05
Humoral markers (CXCL1).	n=11-14 per group (WT; IL-7).	Mice were treated with 5 μ g human of rhIL-7 at the time of CLP. Peritoneal lavage fluid obtained 6 h after CLP.	IL-7 treatment promoted increased concentrations of peritoneal CXCL1.	<i>p</i> <0.01
Humoral markers (IL-6).	n= 8-11 per group (WT 6h, WT 24h; IL-7 6h; IL-7 24h).	Mice were treated with 5 μ g human of rhIL-7 at the time of CLP. Sera were obtained at the indicated time points.	IL-7 treatment significantly increased IL-6 serum levels at 6h, but not at 24h.	<i>p</i> <0.001
Pathogen burden	n= 8-11 per group (WT and IL-7).	Mice were treated with 5 μ g of rhIL-7 at the time of CLP. Serum bacterial loads were measured 24 h after induction of sepsis.	IL-7 treatment significantly lowered blood bacterial load.	<i>p</i> <0.05
Tissue damage (AST, pulmonary MPO and intestinal dry/wet ratio).	n= 8-11 per group (WT 6h, WT 24h; IL-7 6h; IL-7 24h).	Mice were treated with 5 μ g of rhIL-7 at the time of CLP. Serum AST levels were measured 24 h after induction of sepsis. Lungs and terminal ilea were obtained at the indicated time points.	IL-7 treatment <i>did not</i> significantly promote inflammation (as measured by AST, pulmonary MPO and intestinal dry/wet ratio) compared to controls.	-

Table 5: Results from Unsinger et al.¹²

Assessment	Sample and Grouping	Intervention	Outcome	p-value
Overall Survival.	n= 25 (control). n=30 (IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 6 post-CLP. <i>Candida</i> infection was conducted 4 days after CLP.	IL-7 treated mice had improved survival.	$p<0.02$
Delayed-type hypersensitivity (DTH) response.	n=5-9 per group (control; CLP+ <i>Candida</i> ; CLP+ <i>Candida</i> + IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 6 post-CLP. <i>Candida</i> infection was conducted 4 days after CLP. Mice were challenged with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (concentration of 10mM) on days 7 (100 µL) and 11(30 µL). Footpad was measured on day 12.	IL-7 treatment improved DTH response in septic mice.	$p<0.03$
Spleen cell count.	n= 9 (control). n=14(CL+ <i>Candida</i>). n=18(CL+ <i>Candida</i> + IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 6 post-CLP. <i>Candida</i> infection was conducted 4 days after CLP. Spleens were harvested on day 11.	IL-7 treatment improved the cell count of all groups (naive, effector memory and central memory) of CD8+ T cells. Central memory CD4+ T cell count was not significantly altered.	$p<0.05$
Cell activation (CD69) and proliferation (Ki67).	n= 5-9 per group (control, CLP+ <i>Candida</i> , CLP+ <i>Candida</i> + IL-7).	Same was above.	IL-7 treatment increased in proliferation (positivity for Ki67) and activation (positivity for CD69) in CD4+ and CD8+ T cells.	$p<0.02$
Surface markers (LFA-1).	n= 9-18 per group (control; CLP+ <i>Candida</i> ; CLP+ <i>Candida</i> + IL-7).	Same was above.	IL-7 treatment upregulated the expression of LFA-1 on CD4+ and CD8+ T cells.	$p<0.01$
Activation (IL-2, IFN-γ, TNF-α, IL-6)	n= 5-9 per group (control; CLP+ <i>Candida</i> ; CLP+ <i>Candida</i> + IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 6 post-CLP. <i>Candida</i> infection was conducted 4 days after CLP. Spleens were harvested on day 11. 10 million splenocytes were stimulated with CD3 and CD28 overnight. Supernatants were harvested at 18 hours.	IL-7 treatment increased IL-2, IFN-γ, TNF-α and IL-6 production.	$p<0.01-0.05$
Pathogen burden	n= 5-9 per group (control; CLP+ <i>Candida</i> ; CLP+ <i>Candida</i> + IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 6 post-CLP. <i>Candida</i> infection was conducted 4 days after CLP. Kidneys and livers were homogenized 3 days post- <i>Candida</i> injection.	IL-7 treatment reduced the number of <i>Candida</i> colony-forming units.	$p<0.007$

Table 6: Results from Shindo et al.¹³

Assessment	Sample and Grouping	Intervention	Outcome	p-value
Surface markers (Class II MHC).	n= 29 (control). n= 30 (IL-7).	Mice were treated with 2.5 µg of IL-7 daily for 5 days, starting on day 4 post-CLP. <i>Candida</i> infection was conducted 3 days after CLP. Spleens and peripheral lymph nodes were harvested on day 9.	Mice that received treatment with IL-7 had lower MHC II MFI (mean fluorescent intensity) compared to controls.	$p<0.01$
Surface markers (CD28).	Same as above.	Same as above.	IL-7 treatment increased the percentage of splenic CD8+ T cells positive for CD28 compared to an alternative therapy (anti-PD1).	$p<0.05$
Cell proliferation (Ki67).	Same as above.	Same as above.	IL-7 treatment increased the percentage of Ki67 positive splenic CD8+ T cells compared to an alternative therapy (anti-PD1).	$p<0.05$
Surface markers (LFA-1 and VLA-4).	Same as above.	Same as above.	IL-7 treatment increased the percentage of positivity for VLA-4 and LFA-1 compared to saline or anti-PD-1 treated mice.	$p<0.05$
Activation (IFN-γ)	Same as above.	Same as above.	IL-7 significantly increased the percentage of CD8 T cells that were positive for IFN-γ.	$p<0.05$

Table 7: Results from Shindo et al.¹⁴

Assessment	Sample and Grouping	Intervention	Outcome	p-value
Overall survival.	n= 25 per group (rhIL-7; saline).	After sepsis induction (CLP and <i>P.aeruginosa</i> pneumonia), mice received 2.5 µg of IL-7 for 6 days consecutively.	IL-7 treatment improves survival in septic animals.	p=0.006
Spleen and lung cell count.	Same as above.	Same as above.	IL-7 treatment increased the absolute number of CD8+ T cells, NKT cells, γδ T cells, ILC2 and ILC3 in either the lung or spleen of septic mice.	p<0.01-0.05
Humoral markers (IL-17, IL-22, IFN-γ, and TNF-α).	Same as above.	Same as above.	IL-7 treatment <i>did not</i> increase the levels of IL-17, IL-22, IFN-γ, and TNF-α in septic mice fluids (plasma; bronchoalveolar lavage; lung and spleen homogenate).	-
Cytokine-producing cell count (lung and spleen).	Same as above.	Same as above.	IL-7 treatment increased the number of IL-17-, IL-22-, and IFN-γ-producing CD8+ T cells; TNF-α- and IFN-γ-producing γδ T, NKT and NK cells; and IL-17-, TNF-α- and IFN-γ-producing ILC in septic mice lungs and spleen.	p<0.001-0.05
Intracellular markers (pp65 and pSTAT3).	Same as above.	Same as above.	IL-7 treatment increased pp65 and pSTAT3 protein levels in septic mice lungs.	p<0.05

Table 8: Results from Kulkarni et al.¹⁵

Assessment	Sample and Grouping	Intervention	Outcome	p-value
Regulatory cell count (IL-10+ B cells).	n= 6-10 (1 week), 9-16 (1 month), 7-16 (3.5 months) per group (sham; sepsis; sepsis+IL7).	Mice were treated with 2.5 µg of rhIL-7 from day 5 to 9 after sepsis induction. IL-10 + B cells from the spleen were analysed 1 week, 1 month and 3.5 months after sepsis induction.	IL-7 treatment increased the count of IL-10+ B cells. IL-7 increases the total B cells.	p<0.05 (1 week and 1 month) p<0.01 (3.5 months).
Regulatory cell count (Foxp3+CD4+ T cells count).	n= 7-13 (1 week), 9-16 (1 month), 5-18 (3.5 months).	Same as above.	IL-7 treatment led to a transient increase in Foxp3+ CD4+ CD25+ Treg population.	p<0.05
Regulatory cell count (Double negative CD3+, CD4-, CD8- T cells).	n= 7-13 (1 week), 5-10 (1 month), 3-6 (3.5 months).	Same as above.	IL-7 treatment led to a sustained increase in CD3+, CD4-, CD8- (DN) Treg population.	p<0.001
Regulatory cell count (myeloid-derived suppressor cells).	n= 6-9 (1 week), 5-12 (1 month), 8-20 (3.5 months).	Same as above.	IL-7 treatment led to a sustained increased MDSC population.	p<0.001
Cell proliferation (CD4+ T cells under MDSC)	n=3-4 (for all groups).	Same as above.	IL-7 treatment led to a were slightly, almost not significantly, more resistant to the effect of MDSCs.	-

1. Human *ex vivo* Studies

Human *ex vivo* studies were performed by blood sampling patients either 1-4 days after septic shock, or after 24–48 hours of cultures returning positive for multiple drug resistant bacteria. In regard to bias and certainty evaluation, *in vitro* studies had no septic control groups, but healthy or

critical controls instead. There was no blinding.

1.1 IL-7 increases intranuclear markers STAT5 and BCL2

STAT5 and BCL2 are key intracellular effectors recruited by the activation of the IL-7. IL-7 was shown to

reverse the diminished STAT5 phosphorylation and BCL2 expression in septic patients.¹⁶ The phosphorylation of the transcription factor STAT5 was recorded to be higher on effector rather than regulatory CD4 T cells of healthy patients treated with IL-7¹⁷. Finally, it was also shown to be increased in the effector cells of survivors¹⁷.

2.2 IL-7 improved IFN- γ production

IL-7, among other immunoadjuvants, was the most effective at increasing the number of IFN- γ producing T cells, raising it by at least 20% in 62.5% (15 out of 24) of septic patients and in 81.2% (13 out of 16) of critical, but not septic, patients¹⁹. Another study showed that the percentage of IFN- γ producing CD8 T cells of septic patients incubated with IL-7 were no different from that of healthy controls¹⁶.

2.3 IL-7 increases cell proliferation

Table 9: Results from Venet et al.¹⁶

Assessment	Sample and Grouping	Intervention	Outcome	<i>p</i> -value
Lymphocyte proliferation.	n=10 per group (septic and healthy).	Isolated mononuclear cells were cultured for 5 days in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads and rhIL-7 (100 ng/ml).	Cell incubation with IL-7 significantly increased proliferative response in septic shock patients. No significant effect on healthy volunteers.	$p < 0.05$
Specific CD4+ and CD8+ lymphocyte proliferation.	n=7 (septic shock) and n=4 (healthy volunteers).	Mononuclear cells stained with CFSE and cultured for 5 d in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads and rhIL-7 (100 ng/ml).	CD4 + and CD8 + lymphocyte proliferations are restored by IL-7 in septic shock patients.	$p < 0.05$
Activation (IFN- γ).	n= 12 (septic shock) and n= 10 (healthy volunteers).	Isolated mononuclear cells were cultured for 4 h in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads and rhIL-7 (100 ng/ml)	IFN- γ production by CD8 + T lymphocytes is restored by IL-7 in septic shock patients.	$p < 0.01$
Intracellular markers and apoptosis.	n=10 per group (septic and healthy).	Mononuclear cells were cultured overnight in the presence or not of anti-CD2/CD3/CD28 Ab-coated beads and 15 min in the presence or not of rhIL-7 (100 ng/ml).	STAT5 phosphorylation and BCL2 induction in T lymphocytes are restored by IL-7 in septic shock patients.	$p < 0.01$

Table 10: Results from Demaret et al.¹⁷

Assessment	Sample and Grouping	Intervention	Outcome	<i>p</i> -value
Intracellular markers (STAT5).	n=13 (septic patients) and n=10 (healthy volunteers).	CD4 + T cells were stimulated with increasing concentrations of rhIL-7 and then marked with anti-CD4, anti-pSTAT5 and anti-FOXP3.	The lowest concentrations of IL-7 (1 pg/ml) significantly increased the phosphorylation of STAT5 on T effector lymphocytes in septic patients (but not T regulatory).	$p < 0.02$
Intracellular markers (STAT5).	Same as above.	Same as above.	The lowest concentrations of IL-7 (1 pg/ml) significantly increased the phosphorylation of STAT5 of both T effector and regulatory lymphocytes in healthy controls.	$p < 0.002$

After TCR stimulation, IL-7 was able to significantly increase the proliferation count of mononuclear lymphocytes of septic patients, which included the selective expansion of CD4 and CD8 T cells in septic patients to levels similar to controls¹⁶.

2.4 IL-7 restores T cell metabolic alteration in sepsis

IL-7 treatment reversed metabolic abnormalities seen in CD4 T cells of septic patients. The activation of the mammalian target of rapamycin (mTOR), GLUT1 expression and glucose uptake were all raised in treated lymphocytes, whereas the change in Akt phosphorylation did not reach significance¹⁸.

2.5 Individual result tables

The individual results for each study are summarized in Tables 9 to 12.

Table 11: Results from Venet et al.¹⁸

Assessment	Sample and Grouping	Intervention	Outcome	<i>p</i> -value
Intracellular markers (mTOR).	n=6–19 (septic patients).	Peripheral blood mononuclear cell were isolated and allowed to rest for 1 h and then were stimulated with anti-CD2-CD3-CD28 Ab-coated beads and PHA (4 mg/ml) for 15 min or with anti-CD2-CD3-CD28 Ab-coated beads and rhIL-7 (100 ng/ml) and/or with rapamycin (10 ng/ml) for 72 h.	IL-7 treatment promoted mTOR activation (average increase = 123% versus untreated cells) in septic T cells.	<i>p</i> <0.005
Intracellular markers (GLUT1).	n=8-30 (septic patients).	Same as above.	IL-7 treatment promoted significant increase in intracellular GLUT1 expression in septic T cells.	<i>p</i> <0.005
Glucose metabolism.	n=8-31 (septic patients).	Same as above.	IL-7 treatment increased glucose entry into septic T cells.	<i>p</i> <0.005
Proliferation.	n=8-30 (septic patients).	Same as above.	IL-7 treatment improved proliferation (11±6% versus 23±7% proliferating CD4 + cells).	<i>p</i> <0.005

Table 12: Results from Thampy et al.¹⁹

Assessment	Sample and Grouping	Intervention	Outcome	<i>p</i> -value
IFN- γ secretion.	n=24 (septic patients).	Peripheral blood mononuclear cells were stimulated overnight with RPMI 1640 media supplemented with human AB serum containing anti-CD3/anti-CD28, and rhIL-7 (50 ng/ml).	15 of 24 septic patients (62.5%) responded to IL-7 treatment. Response was determined as the increase in the number of cells positive for IFN- γ immunostaining of at least 20% compared to the control.	-

3. Clinical Trial

The IRIS-7 clinical trial, registered under NCT02640807 and NCT02797431, is the first trial to date to administer IL-7 to septic patients. It is also the first clinical trial to target adaptive immunity, in contrast to previous clinical immunoadjuvant trials focused on innate immunity, such as those of IFN- γ , G-CSF and CG-CSF, or the other several past trials focusing on immunosuppression⁹.

The chosen initial dosage was 10 μ g/kg of CYT107 twice a week for the first week on 17 septic patients. Afterwards, two different dosing regimens were established: one group (n=8) was treated with 10 μ g/kg CYT107 once a week (low frequency), while the second group (n=9) was treated with CYT107 10 μ g/kg twice a week (high frequency). The total therapy time for both groups was 4 weeks.

As for bias and certainty assessment, the clinical trial was randomized, double-blind and placebo-controlled, with full description of method, selection, dropout criteria and results.

3.1 IL-7 improved lymphocyte cell count and proliferation

On average, both high and low-frequency groups

exhibited a 3.5 to 4.5 fold increase in absolute lymphocyte counts on the 8th day of therapy (three doses had been administered). Moreover, the total cell expansion lasted for 2-4 weeks after the discontinuation of treatment. Specifically, CD4 T cells were four times as numerous as before the beginning of the therapy in both groups, and CD8 cell counts were higher in the high-frequency group, but not in the low one. The low-frequency group also exhibited a significant, transient, decrease in circulating CD8 T cells, which was coincident with augmented homing molecules expression⁹.

As for proliferation, as marked by Ki67 expression, IL-7 treatment failed to show significant differences after adjustment for baseline, although CD4 and CD8 T cells positive for KI67 did exhibit a significant increase in a few selected days⁹.

3.2 IL-7 regulated surface molecules

IL-7 receptor α , known to be downregulated soon after IL-7 administration, had decreased in CD4 and CD8 T cell expression in patients treated with both low- and high-frequency treatment regimens versus placebo on day 4⁹.

As for lymphocyte activation markers, IL-7 did improve CD38 expression on CD4 T cells, but failed to

do so on CD8 T cells. Other markers, such as HLA-DR, showed significant change only on a few selected days, and there was no sufficient evidence of PD-1 expression difference⁹.

Table 13: Results from Francois et al.⁹

Assessment	Sample and Grouping	Intervention	Outcome	<i>p-value</i>
Overall survival.	n=10 (placebo). n=8 (low frequency) CYT107). n=9 (placebo).	Patients received 10 µg/kg IL-7 twice a week for the first week. Afterwards, they received 10 µg/ kg IL-7 either once (low frequency) or twice (high frequency) a week. Therapy was continued for a total of 4 weeks.	15 of 24 septic patients (62.5%) responded to IL-7 treatment. Response was determined as the increase in the number of cells positive for IFN-γ immunostaining of at least 20% compared to the control.	-
Cell count.	Same as above.	Same as above.	IL-7 treatment significantly increased the absolute lymphocyte count in both low- and high-frequency group patients. This increase in circulating lymphocytes persisted for at least 2–4 weeks.	<i>p=0.004</i>
Cell count (CD4+ and CD8+ T cells)	Same as above.	Same as above.	IL-7 treatment increased the CD4 + T cell count in both low- and high-frequency group patients. It also increased the CD8 + T cell count in the high-frequency group patients.	<i>p=0.021-0.030</i>
Cell count (CD8+ T cells)	Same as above.	Same as above.	IL-7 provoked a transient reduction in CD8+ T cell count on day 4 compared to controls. The result was only significant for the low frequency group.	<i>p=0.009</i>
Innate immunity (neutrophil count).	Same as above.	Same as above.	IL-7 treatment increased the absolute neutrophil count. The result was only significant for the high frequency group.	<i>p< 0.01</i>
Proliferation (Ki67).	Same as above.	Same as above.	IL-7 treatment increased the percentage of Ki67-positive CD4 + and CD8+ T cells in both treatment groups.	<i>p< 0.01-0.05</i>
Surface marker (IL-7Rα)	Same as above.	Same as above.	IL-7 treatment decreased IL-7 receptor α (CD127) expression in CD4+ and CD8+ T cells in both treatment groups.	<i>p<0.011-0.001</i>
Activation (CD38 and HLA-DR and PD-1).	Same as above.	Same as above.	IL-7 treatment significantly increased CD38 expression in CD4+ T cells. The result was only significant for the low frequency group.	<i>p=0.004</i>

DISCUSSION

In the light of all of the reviewed articles, IL-7 treatment seems to be a promising option to the still unaccounted burden of sepsis. In the past decade, animal and *ex vivo* studies have progressively worked on the various effects of IL-7 in restoring immune function. The IRIS-7 trial, although still insufficient as a source of evidence for the efficacy of the treatment, also demonstrated some of the previous findings on actual patients. Taking into account all the compiled experimental data, IL-7 treatment

3.3 Result table

The results from the clinical trial are summarized in Table 13.

seems to have three major features that may account for its potential to improve survival.

The first would be IL-7 effect on improving leukocyte cell number counts, whose depletion is a hallmark of sepsis and it is tied to poor outcome²⁰. The cytokine seems to increase lymphocyte count by two major pathways. One would be through increased proliferation, which was determined by several studies using enhanced Ki67 expression as a marker^{10,12-14}, or 3H-thymidine¹⁶. Another positive effect would be its antiapoptotic effect, evaluated through either the upregulation of antiapoptotic

protein Bcl-2(10, 11) or the downregulation of the proapoptotic BH3 molecules such as Bim, PUMA and BMF¹⁰. Overall, a general finding of the studies was that CD 8 T cell numbers seemed to respond with greater potency to IL-7 than CD 4 T cells.

The second proposed major impact of the cytokine on survival is related to its potential to improve lymphocyte function. Several studies evaluated such activation, pointing towards enhanced production of IFN- γ and other cytokines, upregulated expression of receptors like CD8 and cell adhesion molecules like LFA-1 and VLA-4, less deranged cell metabolism and enhanced activation of key transcription factors.

IFN- γ is a cytokine implicated in phagocyte activation, improving their own cytokine production, phagocytosis and antigen-presentation, which play an essential role in fighting opportunistic nosocomial agents such as *Candida*¹². IFN- γ also acts to promote other downstream effects in both adaptive and innate immune cells, such as the recruitment of $\gamma\delta$ T cells and neutrophils mediated through the upregulated interferon gamma inducible protein 10 (IP-10)¹¹. Finally, IL-7 therapy also had an impact on other cytokines, as it increased the number of cells producing IL-17 and TNF- α . This role of IL-7 treatment on multiple cytokines concentrations is probably related to its activation of CD4⁺ cells, which guide both adaptive and innate immune response¹¹.

Another promising marker for lymphocyte activation induced by IL-7 therapy was the reversal of sepsis caused metabolic derangements. As lymphocytes are activated, their metabolism changes from a catabolic, β oxidation focused metabolism, to an anabolic, glycolytic one (Warburg effect). Sepsis seems to interfere with this process, reverting lymphocytes from a fatty oxidation pattern characteristic of anergic cells. IL-7 treatment, however, through the activation of mTOR, was able to make the cells regain the effector, proliferative, glycolytic profile¹⁸.

Finally, NF- κ B signalling, which promotes cytokine production, and STAT3, a downstream factor of cytokine signalling, were shown to be increased by IL-7 treatment¹⁴.

Moving on from activation results, a third advantage of IL-7, especially against other pro-inflammatory cytokines, is the fact that IL-7 treatment does not lead to an exacerbated immune response. IL-7 seems to play a role in regular lymphocyte homeostasis, as it is a limited resource continually produced by stromal cells. Lymphocytes normally compete for the cytokine, and receptor activation leads to IL-7R α downregulation and increases the available pool. Similarly, in cases of lymphopenia, the concentration of IL-7 rises, which promotes the restoration of lymphocyte population⁸. IL-7 also promotes co-stimulatory effects on naive T cells, and resting memory T cells reexpress the receptor for it. In contrast to IL-2, IL-7 supports the lymphocyte population

not exposed to signs of activation. For instance, IL-7R α is expressed in recent thymic emigrants (RTEs), inducing proliferation in the absence of TCR signalling. Finally, the internalization IL-7R α after ligation may be responsible for the lack of an overstimulated inflammatory response in most experiments, except for a grade 2-3 rash observed in 4 of 17 patients in the clinical trial⁹.

The combination of several immunostimulant effects, reverting key sepsis abnormalities, tied to a controlled immune response, sets IL-7 apart from most previous anti-inflammatory drugs. In addition, IL-7 has a different profile from some of the pro-inflammatory interventions, such as IL-2, which may induce symptoms such as fever and capillary leak syndrome¹⁰. Nevertheless, IL-7 still needs further investigation on its role against sepsis, as the retrieved studies had a number of limitations.

A major limitation of the studies is related to the complexity and dynamicity of the disease course, as a single time point was usually selected¹⁹. Sepsis *per se* is a complex disease, and generalizability may always prove to be challenging. For instance, in one study later administration of IL-7 (5-9 days after CLP) was shown to increase immunoregulatory cell population¹⁵.

As for the clinical trial, a possible limitation was the lymphopenia inclusion criterion, defined as an absolute lymphocyte count of 900 lymphocytes/ μ l or lower. Lymphopenia is tied to a poor prognosis in sepsis²⁰, and it seems to be a central factor in the pathophysiology of the disease in several animal studies⁹. Even so, it should be advisable to evaluate the impact of IL-7 treatment in septic but no lymphopenic patients, or even those that show cell dysfunction rather than the low absolute count.

Another limitation is that the clinical trial did not evaluate tissue lymphocyte count, but only circulating numbers. Although IL-7 treatment was shown to increase the number of lymphocytes in spleen and diffuse lymphoid tissues in HIV²¹ and cancer²² clinical trials, it may not work in the same way in sepsis, considering the rather complex effect of the disease in multiple immune functions, as for instance leukocyte trafficking. Notwithstanding, animal models show the same initial decrease in circulating levels, followed by a rebound, after IL-7 treatment in septic patients, suggesting a homing response⁹.

High heterogeneity of samples, including comorbidities, site and type of infection and evolution of clinical status, was also a relevant factor in the trial. Some studies resorted to nonseptic, but critically-ill, controls, who could be highly different among each other for altered immune function¹⁹. Some *ex vivo* studies also did not match the age of patients and controls, even if the immune parameters analysed seemed to not be modified by it¹⁸. Specific sepsis courses may also have been responsible for unexpected results in the trial, such as better responses from low-frequency patients, or high count of neutrophils in high-frequency ones⁹.

To minimize the highly variable presentation and timing of sepsis, there is great interest in expanding markers and testing methods. For instance, immunoassay tests for low IFN- γ or other impaired T cells function markers may be used to identify possible patients for immunotherapy, including IL-7. Considering *ex vivo* tests limitations, even patients whose cells are unresponsive may benefit from treatment with IL-7, especially considering its targeting of multiple immune functions¹⁹. At least one *ex vivo* study showed different results of IL-7 treatment in surviving and non surviving patients, according to key flow cytometry results, which could anticipate responses to cytokine therapy¹⁷.

Another constraint on evaluation of the effects of IL-7 was the restricted follow-up after administration. IL-7 therapy has a still unknown impact on long term immune regulation. IL-7 seems to increase the number of regulatory T cells, regulatory IL-10+ B cells and MDSC, promoting long-term effects on still unknown impact on sepsis survival¹⁹. Nevertheless, an *ex vivo* study showed a preferred activation of STAT5 of effector T cells rather than regulatory ones¹⁷.

All the listed limitations open possibilities for further intervention and research. Thus, as for recommendation, given the complexity of sepsis, not only a larger trial is in order to assess the effectiveness of IL-7 as a treatment, but also one capable of addressing the heterogeneity of patients. Particularly, future trials should consider the timing of intervention, the biomarker profile, especially in

regard to cytokine levels and cell counts, and the number of comorbidities of patients when evaluating the eligibility, and advisability, of IL-7 treatment.

Another recommendation, in regard to future animal studies, could be a greater focus on the pathways downstream to IL-7 that could be responsible for the improved survival. Even if the cytokine has been shown to improve lymphocyte numbers and activation, mechanisms posterior to such effect are still partially unaccounted, and could answer questions raised in new clinical trials design. Another point of major interest for animal studies are the long to mid-term effects of IL-7 treatment, especially concerning its effect on regulatory immune cells.

CONCLUSION

Sepsis is but one of the several diseases currently under study as a promising target for IL-7 treatment. However, in comparison to the other disorders, sepsis still lags behind when it comes to immunotherapy, in no small part for its complicated clinical course. Nevertheless, immunostimulation shows great, if initially prospects, for long-term survival of sepsis. After reviewing the current literature, from early experiments on animal survival to *ex vivo* studies on human cells to the recent clinical trial, we can conclude IL-7 is among one of these new potential immunomodulatory drugs. The actual efficacy of IL-7 on sepsis survival, however, still requires a larger, randomized, double blind trial, which should soon be started.

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