Je imunoterapie sepse slepá ulička ?

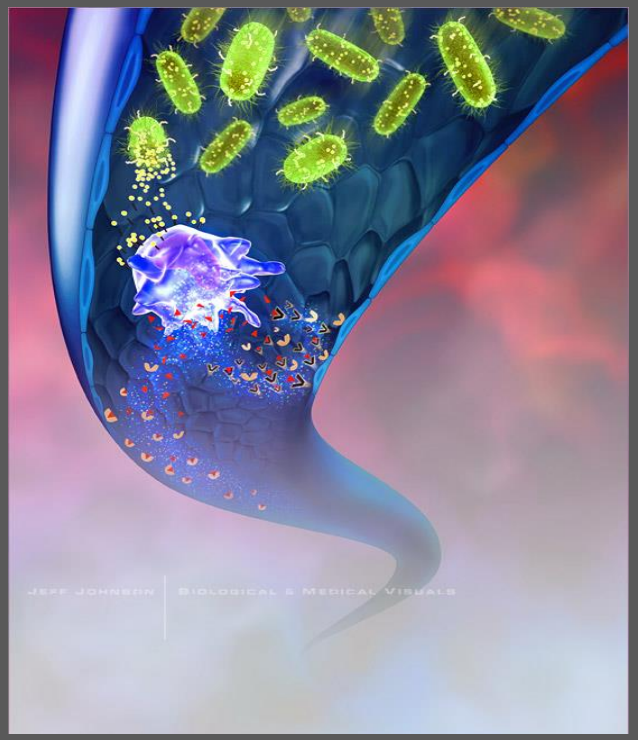
Miroslav Průcha

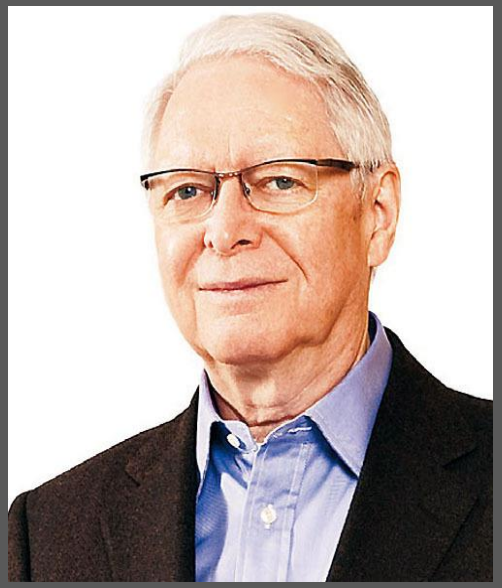
Klinická imunologie,
Nemocnice Na Homolce, Praha

Ostrava, 29. ledna 2015

Osnova přednášky

- Proč imunoterapie ?
- Patogeneze sepse
- Experimentální přístupy
- Klinické studie
- Stávající situace
- Východiska





Prof. Ján Vilcek

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Pillars of Immunology

THE JOURNAL OF IMMUNOLOGY

First Demonstration of the Role of TNF in the Pathogenesis of Disease

Jan Vilcek[1]

Národná medaila technológie a inovácie, menoval prezident Barack Obama, **2012**
Vynikajúca Američan Choice Award, Spojené štáty americké občianstvo a imigračné služby, **2012**
Stephenen K Fischel Distinguished Public Service Award (spolu s Marica Vilcek v zastúpení Vilcek nadácie), americká imigračná rada, **2012**

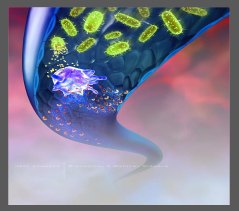
Trans Assoc Am Physicians. 1981;94:39-43.

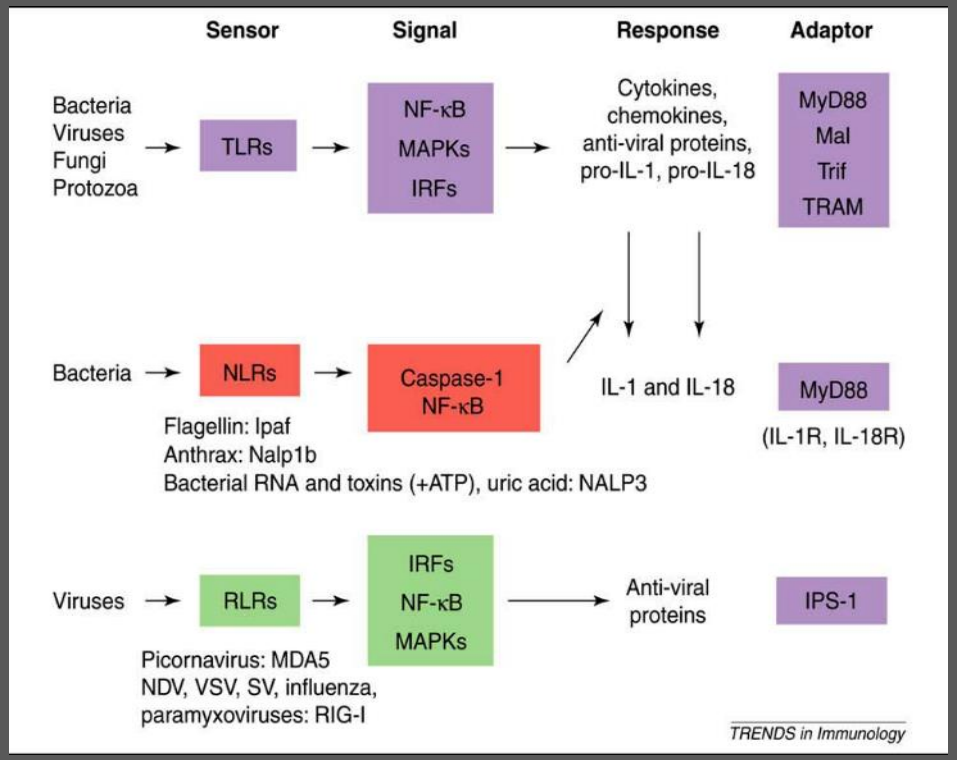
Successful treatment of human gram-negative bacteremia with antiserum against endotoxin core.

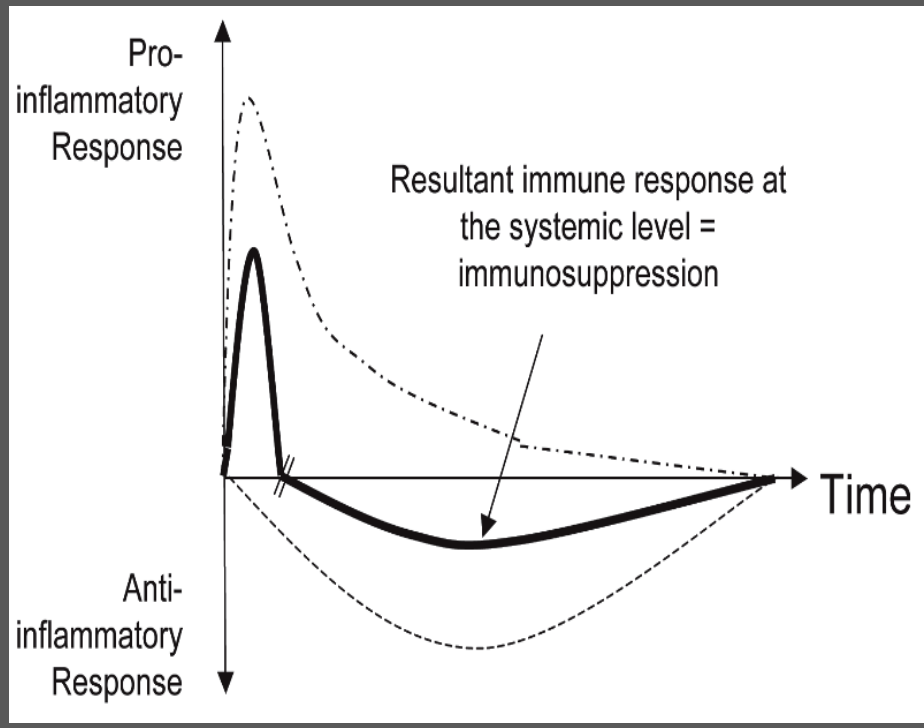
Ziegler EJ, McCutchan JA, Douglas H, Braude AI.

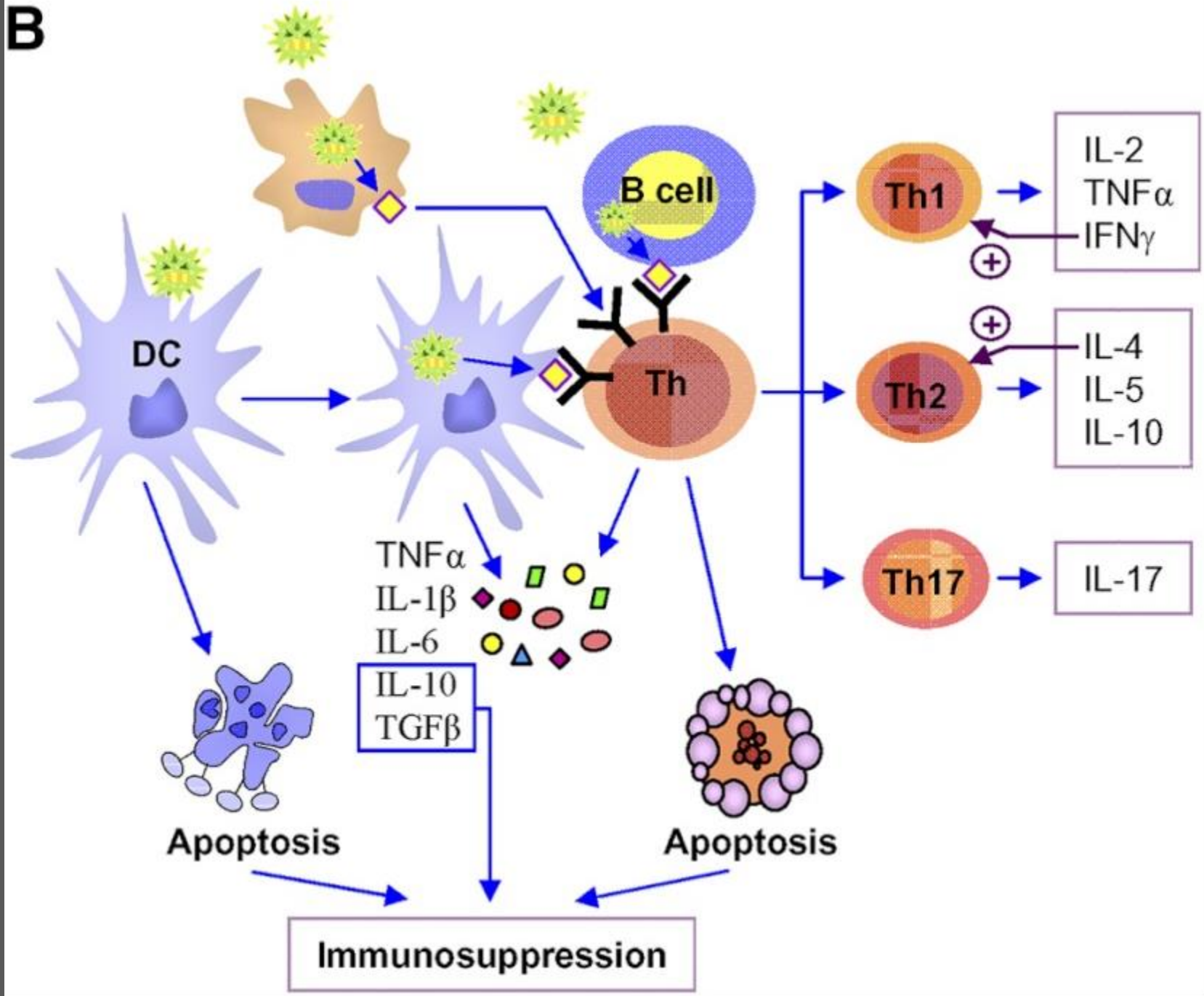
PMID: 7046193 [PubMed - indexed for MEDLINE]

Proč imunoterapie ??








B
B cell
DC
Th
Th1
Th2
Th17
IL-2
TNFα
IFNγ
IL-4
IL-5
IL-10
IL-17
TNFα
IL-1β
IL-6
IL-10
TGFβ
Apoptosis
Apoptosis
Immunosuppression

Studie o funkci imunitního systému během sepse

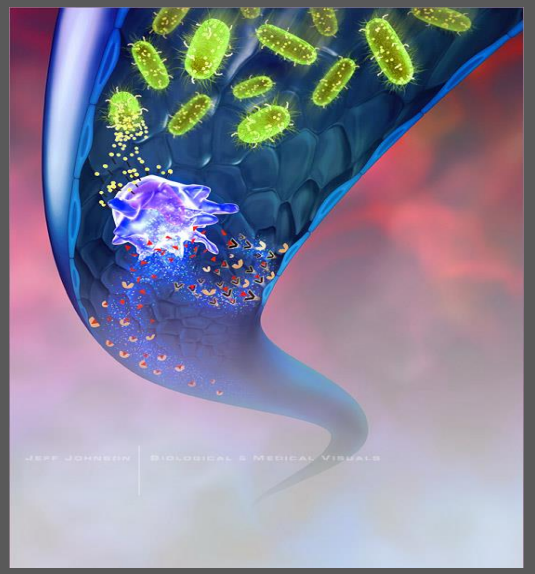
Table 2. Clinical trials for the immune system in sepsis

Identifier	Title	Study type	Sponsor
NCT01600989	Mitochondrial Function of Immune Cells in Sepsis (MitoSepsis) (2012)	Observational	University Hospital Inselspital
NCT01530932	Immune Activation, Hypoxia and Vasoreaction in Sepsis of Pulmonary Vs. Abdominal Origin (2011)	Observational	University Hospital Mannheim
NCT00187824	Regulation of Endocrine, Metabolic, Immune and Bioenergetic Responses in Sepsis (2005)	Observational	University College London Hospital
NCT01410526	Assessment of Peritoneal Immune Response in Patients with Severe Intra-abdominal Sepsis Managed with Laparostomy and Vacuum Assisted Closure (VAC) (2011)	Observational	Aristotle University of Thessaloniki
NCT01472952	System-level Monitoring of Immune Activation Concerning Susceptibility to Sepsis in Trauma Patients (2011)	Observational	University Hospital Mannheim
NCT01155674	Innate Immune Functions of Immature Neutrophils (2010)	Observational	University Hospital Geneva
NCT01766414	In Vivo Effects of C1-esteraase Inhibitor on the Innate Immune Response During Human Endotoxemia – VECTOR II (2013)	Interventional	Radboud University
NCT01649921	The Effects of Interferon-gamma on Sepsis-induced Immunoparalysis (2012)	Interventional	Radboud University
NCT00294697	Genetic Variation and Immune Response After Injury (2006)	Observational	National Institute of General Medical Sciences
NCT00638521	Immune-cell Membrane Trafficking (2008)	Observational	University of Washington
NCT01099813	Sepsis Pathophysiological and Organisational Timing (SPOT[Light]) (2010)	Observational	Intensive Care National Audit and Research Centre
NCT01756755	Endotoxin Adsorber Hemoperfusion and Microcirculation (2012)	Interventional	National Taiwan University Hospital
NCT01275976	Effect of C1-esterase Inhibitor on Systemic Inflammation in Trauma Patients with a Femur Fracture (CAESAR) (2011)	Interventional	UMC Utrecht
NCT01005589	CD64 Meaurement in Neonatal Infection and Necrotising Enterocolitis (2009)	Observational	Newcastle-upon-Tyne Hospitals NHS Trust
NCT00527384	Biomarker Analysis of Stress (2007)	Observational	National Institute of Environmental Health Sciences
NCT01397058	Reactivation of CMV Infection in Immunocompetent Patients Under Severe Stress (RECYSTRESS) (2011)	Observational	University of Athens
NCT01374711	Effects of Immunostimulation with GM-CSF or IFN-γ on Immunoparalysis Following Human Endotoxemia (2011)	Interventional	Radboud University
NCT01653665	Does GM-CSF Restore Neutrophil Phagocytosis in Critical Illness? (2012)	Interventional	Newcastle-upon-Tyne Hospitals NHS Trust

A search on ClinicalTrials.gov was performed using the search terms "Sepsis and Immune" (48 studies) or "Sepsis and Biomarkers" (74 studies) filtered by "open studies". This represents a list of the current open and enrolling clinical trials for sepsis in regards to the immune system. Interestingly, most clinical trials in sepsis have been initiated within the past 10 years as indicated by the start date of the clinical trial in parenthesis following the trial title.

CO VÍME O IMUNITNÍM SYSTÉMU U SEPSE ??

- Zvýšená apoptóza buněk vrozeného a adaptivního systému imunity
- Vyčerpání T buněčného fenotypu
- Deaktivace monocytů se snížením HLA-DR exprese
- Zvýšení T regulačních buněk
- Zvýšení negativních a snížení pozitivních kostimulačních molekul
- Přesmyk Th 1 do Th2 odpovědi
- Regulační vliv CNS na imunitní systém



Experimentální model sepse – realita???

Table 1. Advantages and disadvantages of the most commonly used experimental models for sepsis

Sepsis model	Advantages	Disadvantages
Endotoxemia model: systemic administration of LPS	Simple and reproducible	LPS-mediated signaling is strictly TLR4-dependent
	Induced response is acute	Does not reflect all complex physiological human responses
	Highly controlled and standardized model	High, rapid and transient increase in cytokines, which differs from human sepsis
		Rodents are endotoxin resistant, whereas humans are very sensitive
		Different hemodynamic response compared to human sepsis
		Variability in dose, toxin, and route of administration
Bacterial inoculum model	Presence of a bacterium allows insights into mechanisms of host response to pathogens	Growth and quantification of bacteria is needed before administration
		The single bacterium model does not reflect the diversity and combinations of infectious agents that are present in human sepsis
		Humans are normally not challenged with a massive bacterial load, but have a septic focus that intermittently and persistently challenges the body with bacteria
		High doses of bacteria induce an endotoxic instead of a septic shock, due to the presence of LPS after rapid lysis of the bacteria
		Variability in bacterial load, route of administration and bacterial strain
CLP model	Simple procedure	Abscess formation
	Presence of an infectious focus	Variability in severity due to differences in experimental procedures (Box 1)
	Polymicrobial sepsis model ?	
	using the complete spectrum of host enteric bacteria	
	Recreates human sepsis progression with similar hemodynamic and metabolic phases and the presence of both hyper- and hypoinflammatory phases	
	Prolonged and lower elevation of cytokine release, as in humans	

The disconnect between animal models of sepsis and human sepsis

Daniel Rittirsch, L. Marco Hoesel, and Peter A. Ward[1]

Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Abstract: Frequently used experimental models of sepsis include cecal ligation and puncture, ascending colon stent peritonitis, and the i.p. or i.v. injection of bacteria or bacterial products (such as LPS). Many of these models mimic the pathophysiology of human sepsis. However, identification of mediators in animals, the blockade of which has been protective, has not translated into clinical efficacy in septic humans. We describe the shortcomings of the animal models and reasons why effective therapy for human sepsis cannot be derived readily from promising findings in animal sepsis. *J. Leukoc. Biol.* 81: 137–143; 2007.

Key Words: *cecal ligation and puncture · lipopolysaccharide · rodents*

mental models used in sepsis research currently pursue two different strategies: a septic focus originating from injection or release of feces into the peritoneal cavity or injection of bacteria or microbial components (e.g., LPS) into the abdominal cavity or bloodstream. These approaches attempt to mimic pathophysiological changes typically seen in septic patients, ranging from bacteremia to SIRS to septic shock to multi-organ dysfunction and subsequently, death. Numerous therapeutic attempts have targeted proinflammatory mediators and have had promising effects when used in animal models of sepsis, but virtually all have failed to demonstrate clinical efficacy in human clinical trials [5, 6]. Although typical symptoms such as hyperthermia (progressing to hypothermia), tachycardia, and tachypnea can be observed in septic animals, other parameters such as levels of pro- and anti-inflammatory cytokines differ between animals and humans with sepsis, providing a possible explanation as to why human clinical trials based on effective

Cecal ligation and puncture: the gold standard model for polymicrobial sepsis?

Lien Dejager[1,2], Iris Pinheiro[1,2], Eline Dejonckheere[1,2] and Claude Libert[1,2]

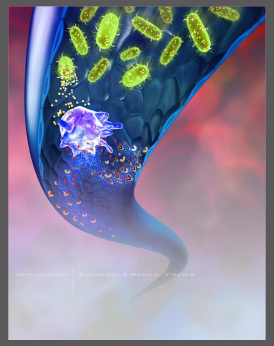
[1] Department for Molecular Biomedical Research, Flanders Institute for Biotechnology (VIB), B9052 Ghent, Belgium
[2] Department of Biomedical Molecular Biology, Ghent University, B9052 Ghent, Belgium

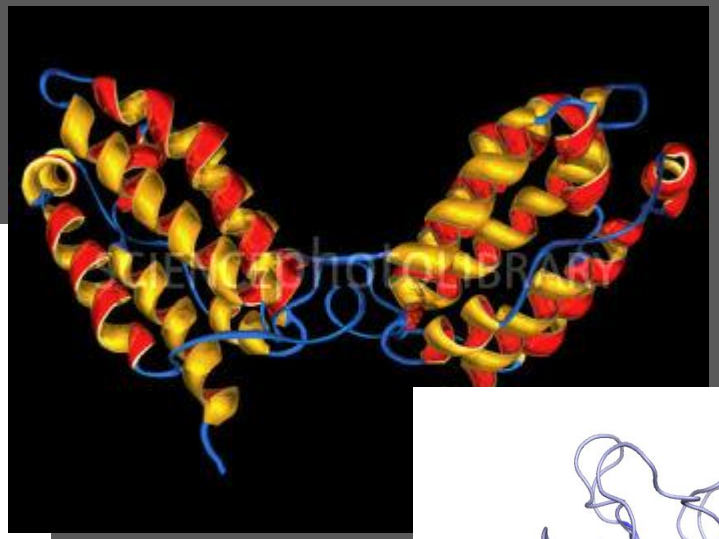
Trends in Microbiology 2012

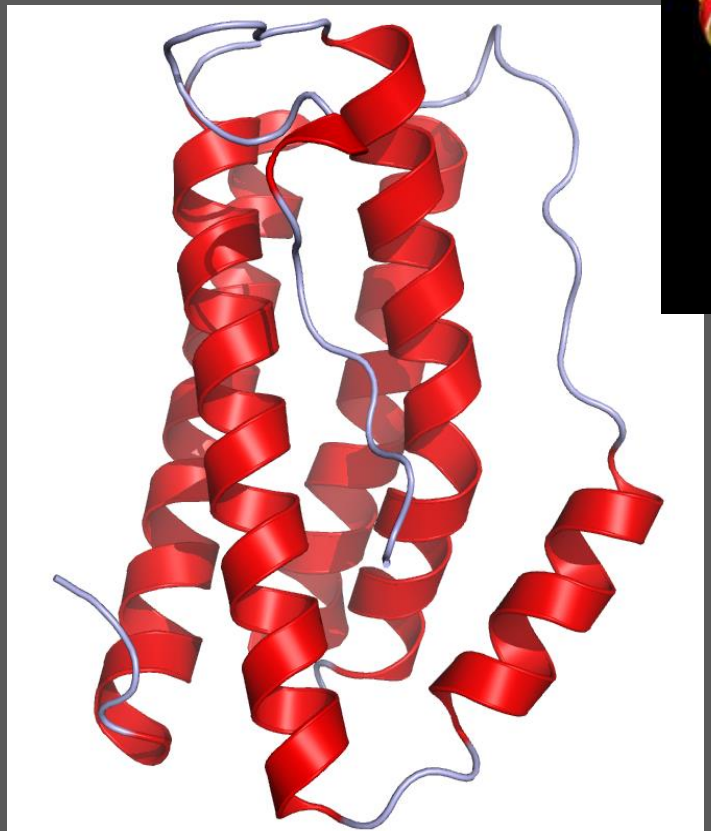
I když pomineme, že pacienti nejsou inbrední kmeny myší……

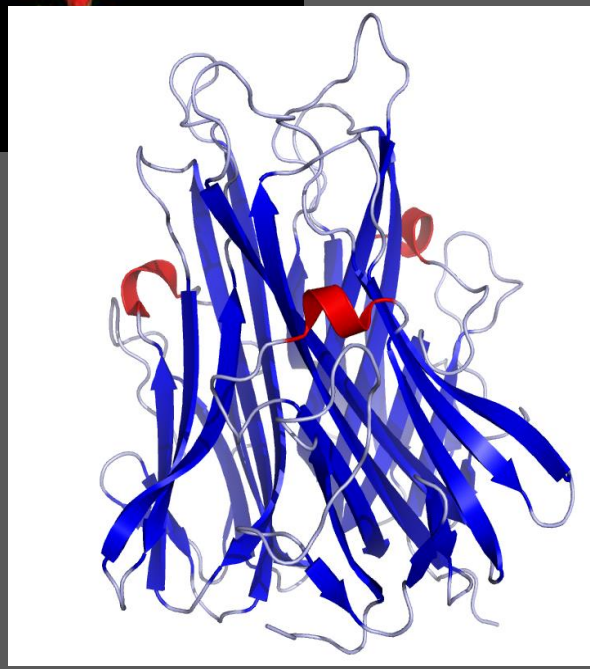
Jak je to složité……

Sepse jako mediátorové onemocnění









J Endotoxin Res. 2002;8(5):365-9.

Requirement of TNF and TNF receptor type 2 for LPS-induced protection from lethal septic peritonitis.

Echtenacher B[1], Männel DN.

Author information

Abstract

Pretreatment of mice with low quantities of LPS induces endotoxin tolerance characterized by enhanced resistance to lethal doses of LPS and to a number of infectious challenges. Mice subjected to cecal ligation and puncture (CLP) survived the ensuing septic peritonitis significantly better when they had been pretreated with LPS. This LPS-induced protection was dependent on endogenous TNF production capacity since LPS pretreatment did not protect TNF-deficient mice from death after CLP. While mice deficient in the TNF receptor type 2 (p75TNFR) were as sensitive to CLP-induced mortality as control mice, LPS pretreatment could not reduce mortality in p75TNFR-deficient mice after CLP. Therefore, activation of the TNF receptor type 2 by endogenous TNF constitutes an important interaction for the development of LPS-induced resistance to bacterial infection.

PMID: 12537695 [PubMed - indexed for MEDLINE]

Immunobiology. 2003;208(4):381-9.

Treatment of experimental sepsis-induced immunoparalysis with TNF.

Echtenacher B[1], Urbaschek R, Weigl K, Freudenberg MA, Männel DN.

Author information

Abstract

Following a severe septic abdominal infection induced by sublethal cecal ligation and puncture (CLP) in mice, a phase of depressed immune reactivity occurred two days after CLP characterized by a reduced capacity to produce TNF. To determine whether this reduced TNF production causes immunoparalysis as determined by increased susceptibility to bacterial infection and whether therapeutic TNF substitution can be beneficial during this phase, a super-infection with Salmonella enterica Serovar typhimurium or Listeria monocytogenes was induced two days after sublethal CLP. After CLP a state of true immunoparalysis developed during which Salmonella or Listeria super-infection led to increased lethality paralleled by increased bacterial numbers in spleens and livers. Injection of recombinant human TNF before or at the time of super-infection conferred protection to Salmonella but not to Listeria. In the latter case, the infection mortality was even enhanced. Thus, super-infection during the state of sepsis-induced immunoparalysis leads to increased lethality. TNF substitution during this state of immunoparalysis can be beneficial or deleterious, depending on the location of TNF activity in the animal, timing of TNF administration, or the type of super-infection. These results demonstrate that impaired TNF production capacity can account for some aspects of immunoparalysis, however, diagnostic parameters are required for a safe TNF substitution therapy.

PMID: 14748511 [PubMed - indexed for MEDLINE]

TNF

- Echtenacher B. et al: Treatment of experimental sepsis-induced immunoparalysis with TNF

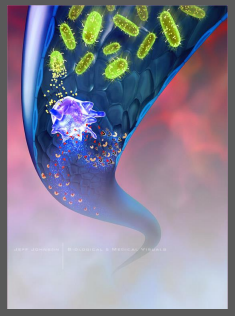
- **Přítomnost endogenního TNF-α a receptorů jsou nezbytným předpokladem pro úspěšné zvládnutí sepse!!!!**

Sepse – mediátorové onemocnění

IL – 6

IL-6 Is an Antiinflammatory Cytokine Required for Controlling Local or Systemic Acute Inflammatory Responses

Zhou Xing,* Jack Gauldie,* Gerard Cox,* Heinz Baumann,‡ Manel Jordana,* Xue-Feng Lei,* and Michelle K. Achong*
*Immunology and Infection Program, Department of Pathology, McMaster University, Hamilton, Ontario, L8N 3Z5 Canada; and
‡Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, New York 14263



J. Clin. Investig. 1998

Knock out mice for IL-6

- Endogenní IL-6 má nezastupitelnou roli v lokální i celkové protizánětlivé aktivitě a tato aktivita není nahraditelná IL-10.

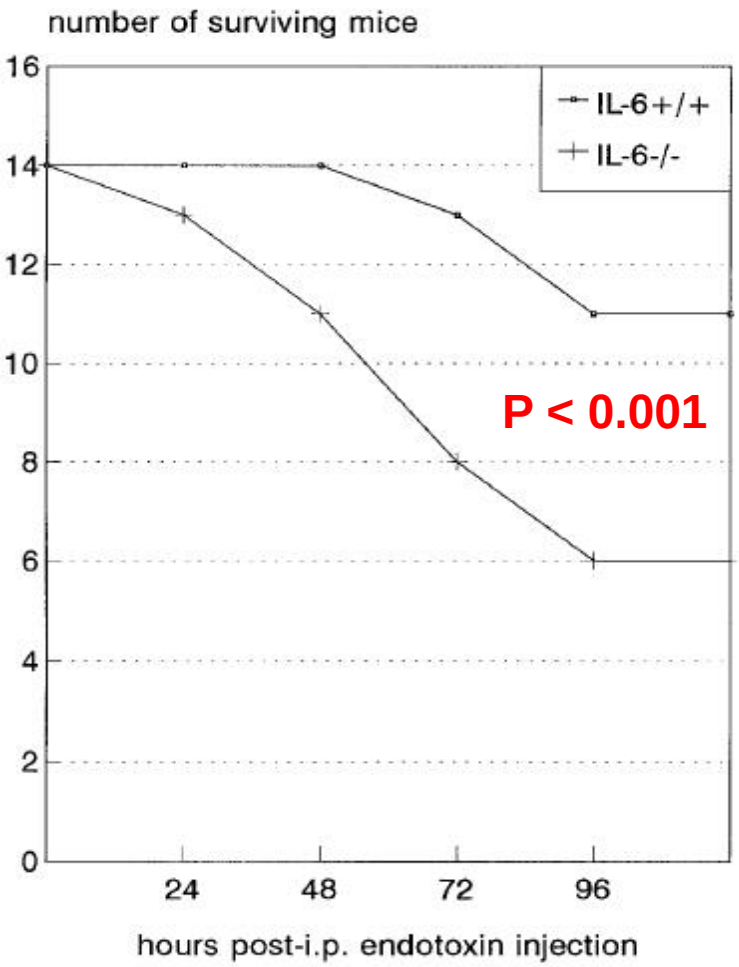


Figure 7. Mortality of IL-6+/+ and IL-6−/− mice during endotoxemia. 14 of each mice were injected intraperitoneally with 20 μg/g body weight of endotoxin and mortality was recorded in the next 5 d.

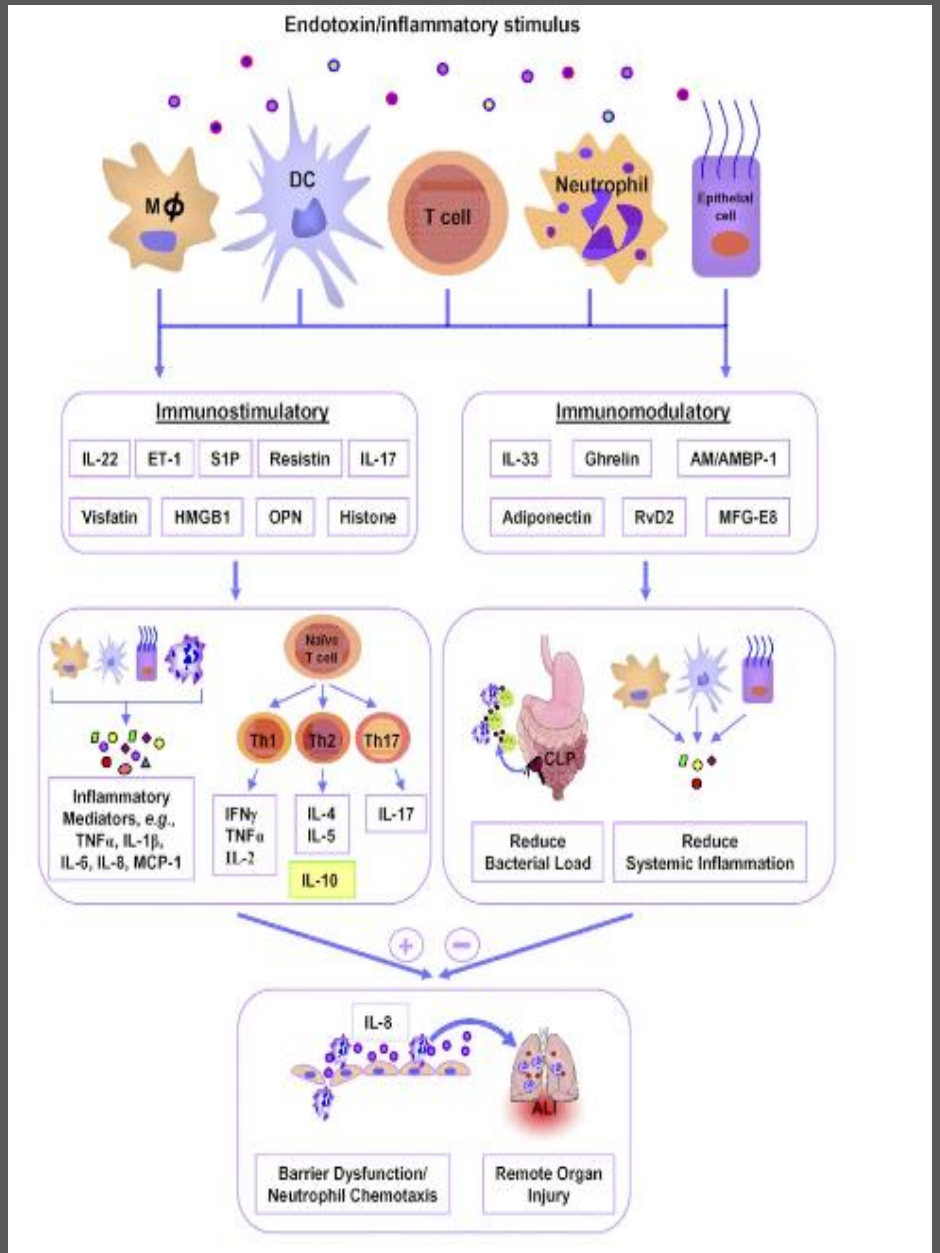
IL-10

- IL-10-Deficient Mice Demonstrate Multiple Organ Failure and Increased Mortality During Escherichia coli Peritonitis Despite an Accelerated Bacterial Clearance, The Journal of Immunology, 2001, 166:6323-6331.

- To determine the role of endogenous IL-10 in local antibacterial host defense and in the development of a systemic inflammatory response syndrome during abdominal sepsis, IL-10 gene-deficient (IL-10-/-) and wild-type (IL-10+/+) mice received an i.p. injection with Escherichia coli.

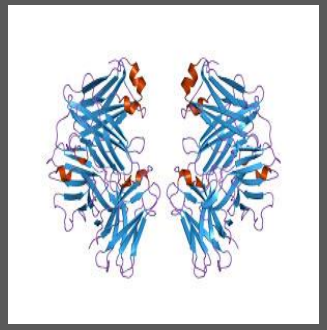
- **IL-10 chránil experimentální zvířata před rozvojem multiorgánového selhání.**

Je to stejné u G+ bakterií ??

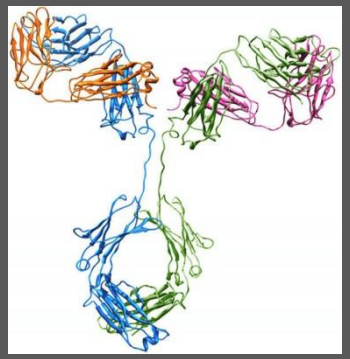


Není……

Ve smyslu signalizačních cest..



Co se dělalo……



Imunoterapii sepse můžeme rozdělit na terapii:

spojující substituční a imunomodulační účinky – představiteli jsou intravenózní imunoglobuliny(IVIG), růstové faktory a kortikosteroidy

terapii blokační – s použitím látek blokujících účinek mediátorů nebo signalizačních molekul

Hyperimunní séra, IVIG, monoklonální protilátky proti TNF, proti TLR (Eritoran)…růstové faktory – G-CSF, GM-CSF, blokace HMGB1 , p21-aktivovaná kináza

Současné názory

Immunity
Review

CellPress

Sepsis: Current Dogma and New Perspectives

Clifford S. Deutschman[1,*] and Kevin J. Tracey[2,*]
[1]Department of Anesthesiology and Critical Care and Surgery and Sepsis Research Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA
*Correspondence: deutschcl@uphs.upenn.edu (C.S.D.), kjtracey@nshs.edu (K.J.T.)
http://dx.doi.org/10.1016/j.immuni.2014.04.001

Sepsis, a clinical syndrome occurring in patients following infection or injury, is a leading cause of morbidity and mortality worldwide. Current immunological mechanisms do not explain the basis of cellular dysfunction and organ failure, the ultimate cause of death. Here we review current dogma and argue that it is time to delineate novel immunometabolic and neurophysiological mechanisms underlying the altered cellular bioenergetics and failure of epithelial and endothelial barriers that produce organ dysfunction and death. These mechanisms might hold the key to future therapeutic strategies.

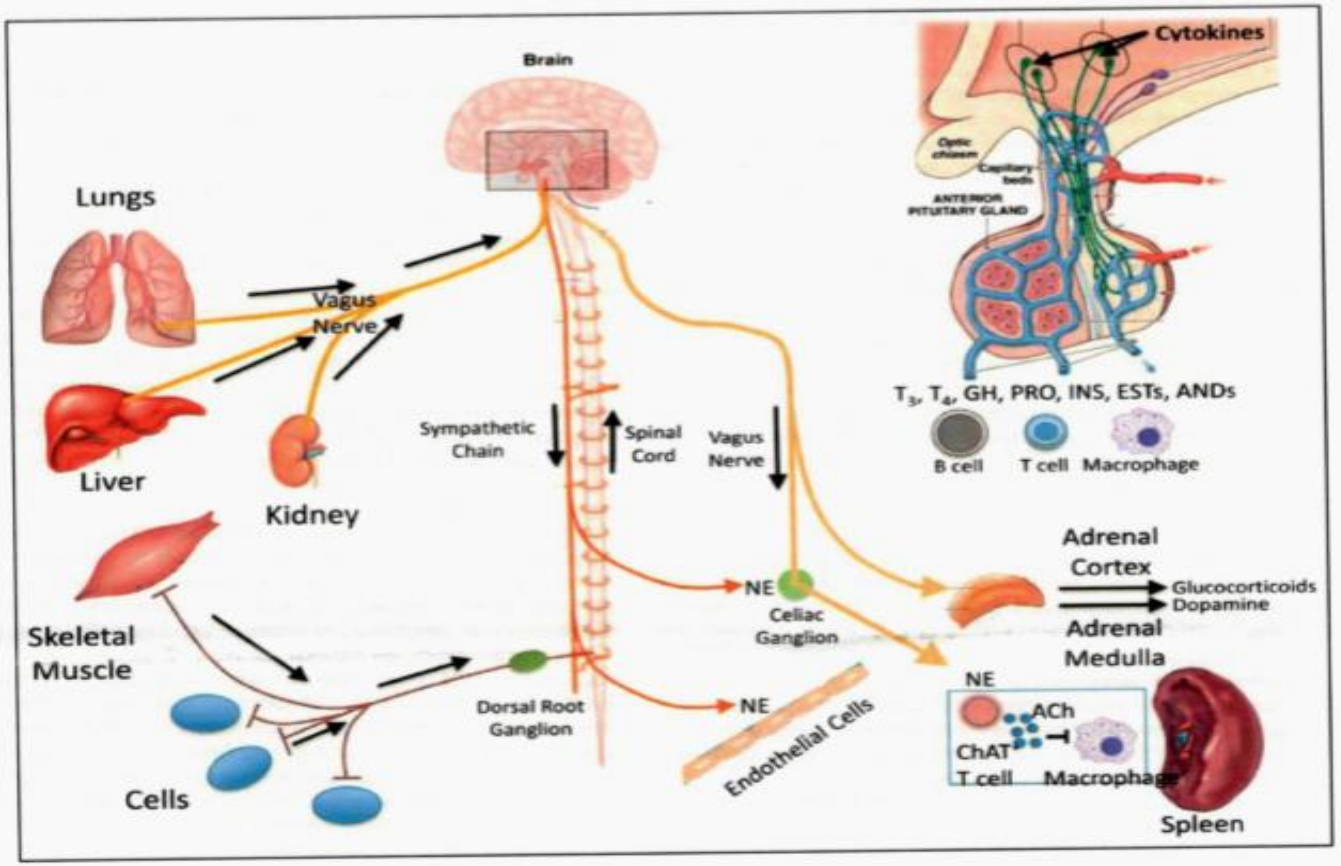


Figure 2. Neuroendocrine Afferent and Efferent Pathways Restore Homeostasis

Inflammatory signals arising in organs are conducted along the afferent vagus nerve to the nucleus tractus solitaries of the brainstem. Sensory impulses from somatic tissue generate electrical impulses that are modulated in the dorsal root ganglia (DRG) and relayed via the spinal cord to nuclei in the brainstem and hypothalamus. Descending neuronal impulses are conducted in both the efferent vagus nerve and the sympathetic chain. Vagal signals terminate in the celiac ganglion and interact with adrenergic nerve-cell bodies that project distally via the splenic nerve. The termini of these nerves in the spleen release norepinephrine (NE) that in turn stimulates T cells expressing choline acetyltransferase (ChAT), enhancing acetylcholine (Ach) biosynthesis. Ach interacts with α_7nACh receptors (α_7nAChRs) on macrophages, suppressing NF-kB activation and limiting inflammatory cytokine expression and release. Activation of the sympathetic chain leads to release of NE in target tissues. NE stimulation of alpha adrenergic receptors enhances, whereas beta-adrenergic receptor stimulation suppresses cytokine release. Vagal impulses also modulate dopamine release by the adrenal medulla. Stimulation of D1 receptors on monocytes and macrophages limit cytokine expression and/or release. Inflammatory afferents affecting the endocrine system arise from (1) signals carried via the afferent neuronal pathways, (2) cytokine transfer across the attenuated blood-brain barrier of the hypothalamic-pituitary junction, and (3) direct cytokine production by cells of the CNS. CNS factors that modulate cytokine production and release in the brain include malanocyte-stimulating hormone (MSH), thyroid stimulating hormone (TSH), glucocorticoids, leptin, ghrelin, and adrenocorticotropin (ACTH). Hypothalamic responses to cytokines alter the release of ACTH, TSH, prolactin (PRO), growth hormone (GH), and follicle stimulating hormone. Monocyte and macrophage activity, including cytokine production, and is altered by thyroid hormones (T_3, T_4), while both T and B cells function are decreased by estradiols (EST) and increased by androgens (AND). GH, prolactin, and insulin stimulate T cell activity.

3 nosologické jednotky

SEPSE ???

TĚŽKÁ SEPSE

SEPTICKÝ ŠOK

??? Vincent et al 2013

PCI persistent critical illness

Acute septic shock is an immunopathology, mediated by excessive TNF release……only small proportion of patients

Severe sepsis and PCI - are not immunopathologies but represent a failure of homeostasis that results from dysfunction of the neuroendocrine and immune systems……**????**

The major unaswered questions about the onset and persistence of cellular dysfunction and organ failure, which are the ultimate causes of death, center on the immunometabolic signaling pathways….

These mechanisms likely underlie the failure of intracellular barriers and immunity……

Hindawi Publishing Corporation
Journal of Immunology Research
Volume 2014, Article ID 164309, 8 pages
http://dx.doi.org/10.1155/2014/164309

Review Article

Powering the Immune System: Mitochondria in Immune Function and Deficiency

Melissa A. Walker,[1] Stefano Volpi,[2] Katherine B. Sims,[1] Jolan E. Walter,[3] and Elisabetta Traggiai[4]

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[2] *Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genoa, 16147 Genova, Italy*
[3] *Division of Allergy and Immunology, Department of Pediatrics, Massachusetts General Hospital for Children, Boston, MA 02114, USA*
[4] *Novartis Institute for Research in Biomedicine, Basel, Switzerland*

Correspondence should be addressed to Melissa A. Walker; walker.melissa@mgh.harvard.edu and Elisabetta Traggiai; elisabetta.traggiai@novartis.com

Received 16 June 2014; Revised 20 August 2014; Accepted 25 August 2014; Published 21 September 2014

Academic Editor: Luca Gattinoni

Mitochondria are critical subcellular organelles that are required for several metabolic processes, including oxidative phosphorylation, as well as signaling and tissue-specific processes. Current understanding of the role of mitochondria in both the innate and adaptive immune systems is expanding. Concurrently, immunodeficiencies arising from perturbation of mitochondrial elements are increasingly recognized. Recent observations of immune dysfunction and increased incidence of infection in patients with primary mitochondrial disorders further support an important role for mitochondria in the proper function of the immune system. Here we review current findings.

the latter report, decreased T, B, and natural killer (NK) cell subsets were reported in conjunction with a very high frequency of activated/memory T cells (CD45RA) and poor T cell proliferation to mitogen. Curiously, based on the reported data, these patients would score as "unlikely" affected with a primary mitochondrial disorder by Bernier criteria [62].

5. Susceptibility to Infection in Patients with Documented Mitochondrial Dysfunction

Conversely, while there are currently no primary immunodeficiencies also recognized as primary mitochondrial disorders, there is likewise limited data regarding immune

Table 1: Primary mitochondrial disorders are recognized as a group of multisystem disorders with various features and supporting laboratory findings [62]. Genetic or metabolic diagnoses—when identifiable—arise from perturbations of gene products localizing to the mitochondrion that may be nuclear or mitochondrially encoded [9] and are not necessary for a clinical diagnosis. The immunodeficiencies above have not typically been described as primary mitochondrial disorders but are linked to genetic defects of genes localizing to the mitochondrion. While published cases of Barth syndrome and Cartilage Hair Hypoplasia would be scored as "possible" or "likely affected" based on criteria by Bernier and colleagues, published cases of Omenn syndrome score as "unlikely affected" by a primary mitochondrial disorder.

Syndrome	Gene	Phenotype/immunologic phenotype	Bernier criteria classification
Barth syndrome	Tafazzin (*TAZ*)	3-Methylglutaconic aciduria, cardioskeletal myopathies/neutropenia	*Likely affected*
Omenn syndrome	*Adenylate kinase (AK) 2*	Inflammatory variant of leaky severe combined immunodeficiency (L-SCID)	*Unlikely affected*
Cartilage Hair Hypoplasia	*Mitochondrial RNA processing endoribonuclease (RMRP)*	Dwarfism/predisposition to infections, variable immune deficiency with T cell dysfunction	*Possible*

Am J Clin Exp Immunol 2013;2(1):30-54
www.ajcei.us /ISSN:2164-7712/AJCEI1211002

Review Article

Changing the energy of an immune response

Meghan M Delmastro-Greenwood[1,2], Jon D Piganelli[1,2]

[1]*Diabetes Institute, Division of Immunogenetics, Department of Pediatrics, Rangos Research Center, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Avenue, Pittsburgh, PA 15224, USA;* [2]*Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA*

Received November 13, 2012; Accepted January 17, 2013; Epub February 27, 2013; Published March 9, 2013

Abstract: The breakdown of nutrients into the critical energy source ATP is the general purpose of cellular metabolism and is essential for sustaining life. Similarly, the immune system is composed of different cell subsets that are indispensable for defending the host against pathogens and disease. The interplay between metabolic pathways and immune cells leads to a plethora of different signaling pathways as well as cellular activities. The activation of T cells via glycolysis-mediated upregulation of surface markers, for example, is necessary for an appropriate effector response against an infection. However, tight regulation of immune cell metabolism is required for protecting the host and resuming homeostasis. An imbalance of immunological metabolic function and/or metabolic byproducts (reactive oxygen species) can oftentimes lead to diseases. In the case of cancer, overactive glucose metabolism can lead to hyperproliferation of cells and subsequent decreases in cytotoxic T cell activity, which attack and destroy the tumor. For this reason and many more, targeting metabolism in immune cells may be a novel therapeutic strategy for treatment of disease. The metabolic pathways of immune cells and the possibilities of immunometabolic therapies will be discussed.

Keywords: Metabolism, immune response, aerobic glycolysis, oxidative phosphorylation

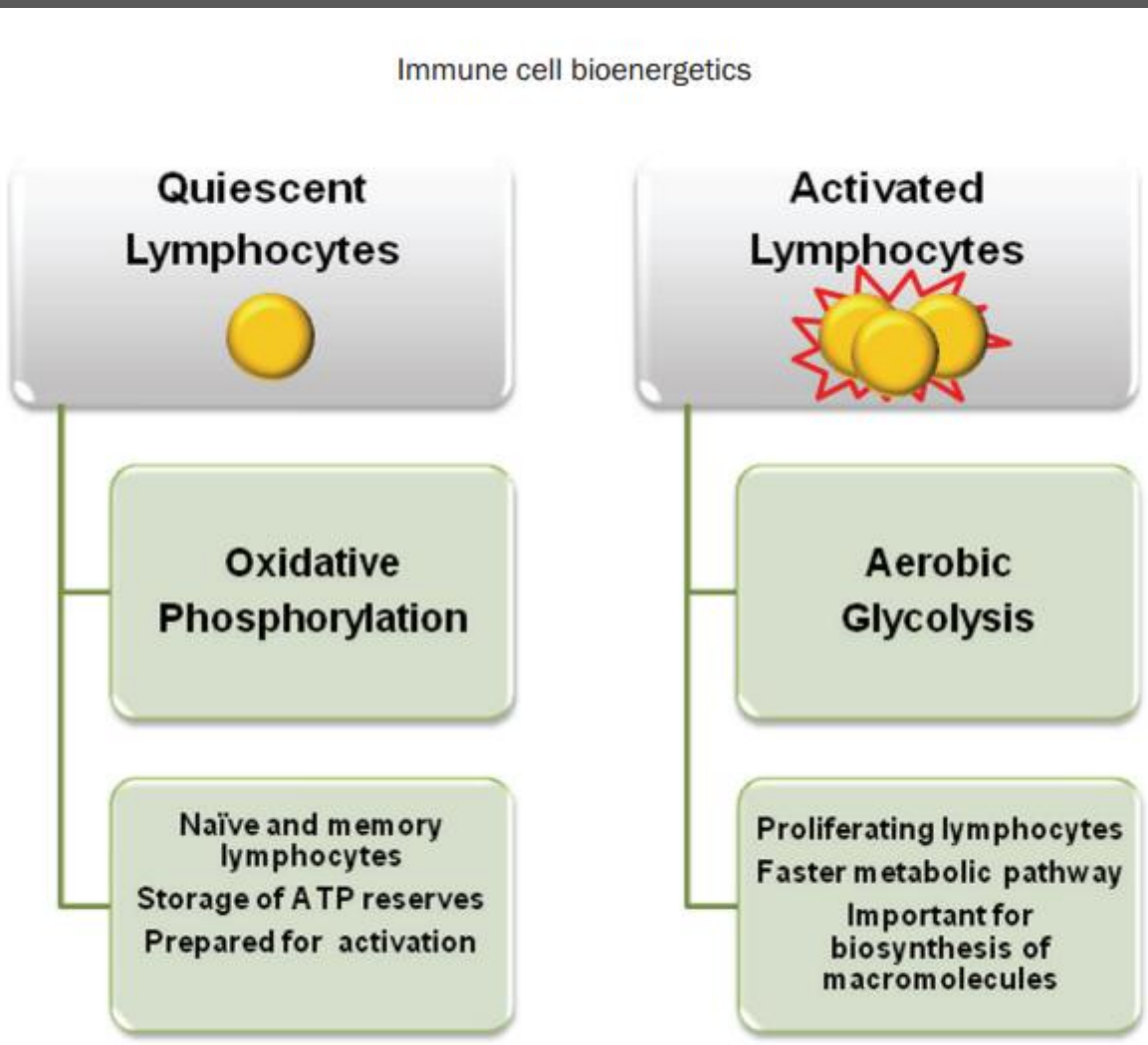


Figure 1. Lymphocyte metabolism fluctuation during resting and activated states. Quiescent, or resting, lymphocytes primarily utilize oxidative phosphorylation to build up reserves of ATP in preparation for activation. Activated, or proliferating, lymphocytes predominately use aerobic glycolysis due to its rapid speed and critical role in forming biosynthetic precursors.

Terapeutické možnosti

- Ovlivnění „specifických neurálních okruhů“
- HMGB1 – inhibice této molekuly v „recovery“ fázi u sepse

SPECIAL FOCUS REVIEW

Virulence 5:1, 1–12; January 1, 2014; © 2014 Landes Bioscience

The changing immune system in sepsis

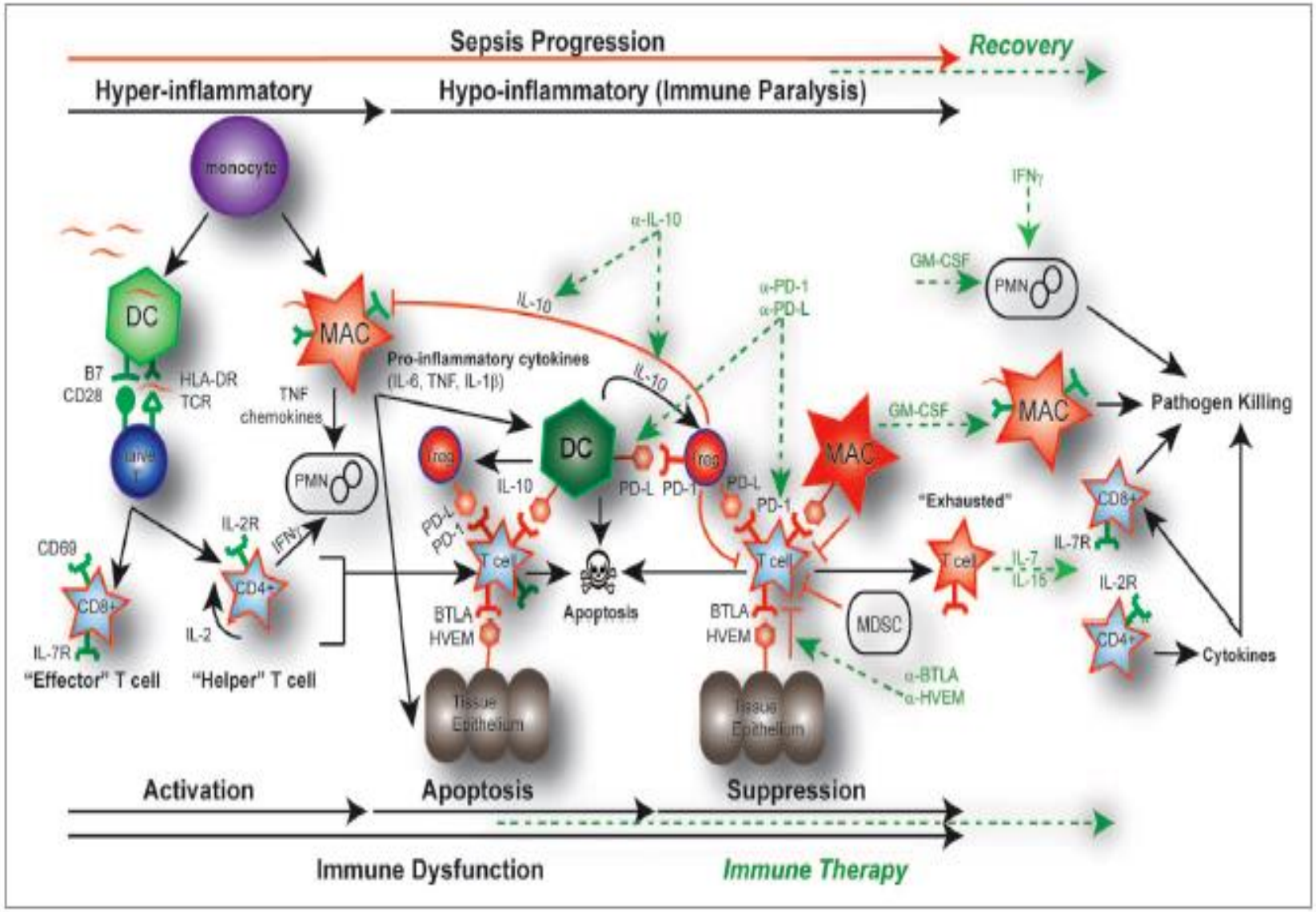
Is individualized immuno-modulatory therapy the answer?

Jonathan S Boomer[1,*], Jonathan M Green[1], and Richard S Hotchkiss[2]

[1]Department of Internal Medicine; Washington University School of Medicine; St. Louis, MO USA; [2]Department of Anesthesiology, Medicine, and Surgery; Washington University School of Medicine; St. Louis, MO USA

Keywords: sepsis, immune therapy, cell exhaustion, immune suppression, adaptive immunity

Abbreviations: MDSC, myeloid derived suppressor cells; APC, antigen presenting cells; Th1, T lymphocyte type 1; Th2, T lymphocyte type 2; Treg, regulatory T cell; SIRS, systemic inflammatory response syndrome; VAP, ventilator associated pneumonia; PD-1, programmed death receptor 1; PD-L, programmed death ligand; BTLA, B and T lymphocyte attenuator; HVEM, herpesvirus entry mediator; MODS, multi-organ dysfunction syndrome; ARDS, adult respiratory distress syndrome; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns; PRRs, pattern recognition receptors; TLR, toll-like receptor; CTL, cytotoxic T lymphocyte; DC, dendritic cell; CARS, compensatory anti-inflammatory response syndrome; PBMCs, peripheral blood mononuclear cells; LCMV, lymphocytic choriomeningitis virus; ICU, intensive care unit; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; NCI, National Cancer Institute; TIM-3, T cell membrane protein-3; LAG-3, lymphocyte-activation gene-3


Sepsis Progression
Recovery
Hyper-inflammatory
Hypo-inflammatory (Immune Paralysis)
monocyte
DC
MAC
B7
CD28
HLA-DR
TCR
naive T
TNF
chemokines
Pro-inflammatory cytokines
(IL-6, TNF, IL-1β)
α-IL-10
IL-10
α-PD-1
α-PD-L
IFNγ
GM-CSF
PMN
res
PD-L
PD-1
T cell
Apoptosis
BTLA
HVEM
Tissue
Epithelium
MDSC
"Exhausted"
CD69
CD8+
IL-7R
IL-2R
CD4+
IL-2
IFNγ
IL-7
IL-15
Pathogen Killing
Cytokines
α-BTLA
α-HVEM
"Effector" T cell
"Helper" T cell
Activation
Apoptosis
Suppression
Immune Dysfunction
Immune Therapy

Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy.
Hotchkiss RS1, Monneret G, Payen D.
Nat Rev Immunol.
2013 Dec;13(12):862-74. doi: 10.1038/nri3552. Epub 2013 Nov 15.

Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach.
Hotchkiss RS, Monneret G, Payen D.
Lancet Infect Dis. 2013 Mar;13(3):260-8. doi: 10.1016/S1473-3099(13)70001-X. Review.

Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies.
Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G.
Curr Opin Immunol. 2013 Aug;25(4):477-83. doi: 10.1016/j.coi.2013.05.006. Epub 2013 May 28. Review.

Immunotherapy - a potential new way forward in the treatment of sepsis.
Payen D, Monneret G, Hotchkiss R.
Crit Care. 2013 Feb 20;17(1):118. doi: 10.1186/cc12490.

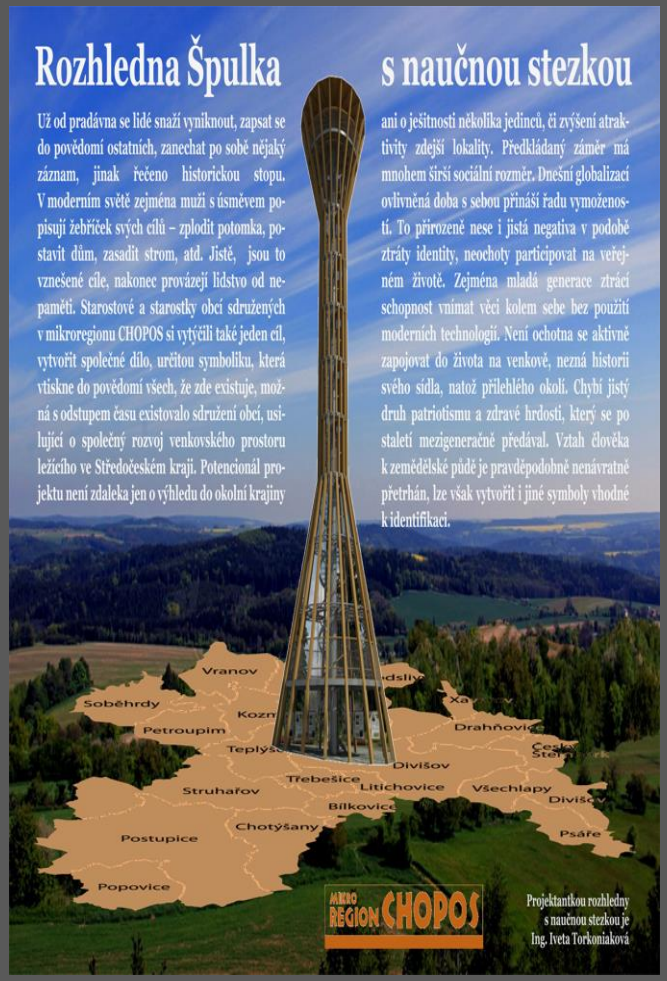
Studie s použitím imunostimulační terapie

Table 1. Immune enhancing therapy: clinical trials in sepsis

Agent	Study	Outcomes	References
G-CSF	RCT in patients with pneumonia and severe sepsis	Increased WBC counts No reduction in mortality Well tolerated	Root et al.[103]
G-CSF	RCT in patients with multilobar pneumonia	Increased WBC counts Reduction in mortality (trend) Well tolerated	Nelson et al.[101]
GM-CSF	RCT in patients with sepsis or septic shock and sepsis induced immunesuppression	Increased HLA-DR expression Restored cytokine secretion in monocytes Improved patient outcomes	Meisel et al.[105]
GM-CSF, rIFN-γ (ongoing)	Effects of immunostimulation with GM-CSF or IFN-γ on immunoparalysis following human endotoxemia	Cytokine secretion by lymphocytes HLA-DR expression Monocyte/neutrophil function Lymphocyte gene expression Volunteer responses	NCT01374711
rIFN-γ	RCT in trauma patients	Increased HLA-DR expression Decreased severe infections (trend)	Polk et al.[106]
rIFN-γ	RCT in patients with burns	No improved patient outcomes	Wasserman et al.[107]
rIFN-γ	RCT in trauma patients	Reduced infection related deaths	Dries et al.[108]
rIFN-γ (ongoing)	Effects of interferon-gamma on sepsis-induced immunoparalysis	Cytokine secretion by lymphocytes HLA-DR and receptor expression (PD-1) Lymphocyte gene expression Reversibility of monocyte dysfunction Patient outcomes	NCT01649921

Thus far, the focus of enhancing immune function in sepsis has been limited to trials for GM-CSF or G-CSF, a stimulator of the innate immune system, and IFN-γ, a stimulator of the adaptive immune system. Abbreviations: RCT, randomized controlled trial; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; rIFN-γ, recombinant interferon gamma; PD-1, programmed death receptor-1; WBC, white blood cell count; NCT, ClinicalTrials.gov identifier.

Můj pohled po 25 letech……



PubMed sepsis – immunotherapy
3000 publikací

EBioMedicine 1 (2014) 64–71

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journal homepage: www.ebiomedicine.com

Original Article

An Endotoxin Tolerance Signature Predicts Sepsis and Organ Dysfunction at Initial Clinical Presentation

Olga M. Pena [a,1], David G. Hancock [b,1], Ngan H. Lyle [a], Adam Linder [c], James A. Russell [a], Jianguo Xia [a], Christopher D. Fjell [a,c], John H. Boyd [c], Robert E.W. Hancock [a,d,*]

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Diagnosis
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Endotoxin tolerance
Signature
Immune dysfunction

ABSTRACT

Background: Sepsis involves aberrant immune responses to infection, but the exact nature of this immune dysfunction remains poorly defined. Bacterial endotoxins like lipopolysaccharide (LPS) are potent inducers of inflammation, which has been associated with the pathophysiology of sepsis, but repeated exposure can also induce a suppressive effect known as endotoxin tolerance or cellular reprogramming. It has been proposed that endotoxin tolerance might be associated with the immunosuppressive state that was primarily observed during late-stage sepsis. However, this relationship remains poorly characterised. Here we clarify the underlying mechanisms and timing of immune dysfunction in sepsis.

Methods: We defined a gene expression signature characteristic of endotoxin tolerance. Gene-set test approaches were used to correlate this signature with early sepsis, both newly and retrospectively analysing microarrays from 593 patients in 11 cohorts. Then we recruited a unique cohort of possible sepsis patients at first clinical presentation in an independent blinded controlled observational study to determine whether this signature was associated with the development of confirmed sepsis and organ dysfunction.

Findings: All sepsis patients presented an expression profile strongly associated with the endotoxin tolerance signature ($p < 0.01$; AUC 96.1%). Importantly, this signature further differentiated between suspected sepsis patients who did, or did not, go on to develop confirmed sepsis, and predicted the development of organ dysfunction.

Interpretation: Our data support an updated model of sepsis pathogenesis in which endotoxin tolerance-mediated immune dysfunction (cellular reprogramming) is present throughout the clinical course of disease and related to disease severity. Thus endotoxin tolerance might offer new insights guiding the development of new therapies and diagnostics for early sepsis.

Published in final edited form as:
Science. 2012 February 24; 335(6071): 936–941. doi:10.1126/science.1214935.

Disease Tolerance as a Defense Strategy

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[1]Howard Hughes Medical Institute, Department of Immunobiology, Yale University School of Medicine, New Haven, CT

[2]Department of Microbiology and Immunology, Stanford University, Palo Alto, CA

[3]Instituto Gulbenkian de Ciência, Oeiras, Portugal

Abstract

The immune system protects from infections primarily by detecting and eliminating the invading pathogens; however, the host organism can also protect itself from infectious diseases by reducing the negative impact of infections on host fitness. This ability to tolerate a pathogen's presence is a distinct host defense strategy, which has been largely overlooked in animal and human studies. Introduction of the notion of "disease tolerance" into the conceptual toolkit of immunology will expand our understanding of infectious diseases and host pathogen interactions. Analysis of disease tolerance mechanisms should provide new approaches for the treatment of infections and other diseases.

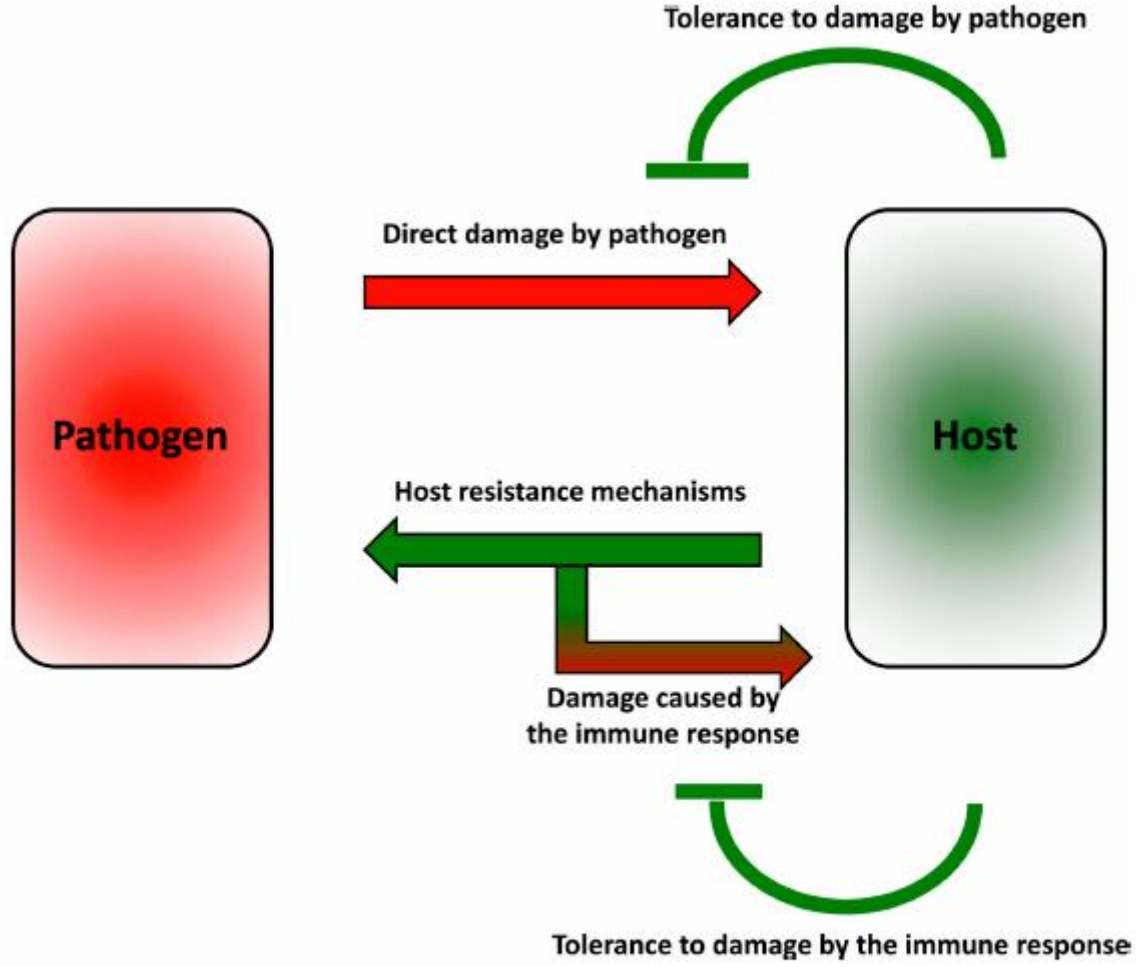


Figure 1.
Two types of fitness costs associated with infections. Pathogens can directly damage the host tissues. The immune system of the host reduces the pathogen burden through the resistance mechanism. The immune response can also damage the host tissues. The host can reduce fitness costs through tolerance mechanisms that reduce both the direct tissue damage by pathogens, and immunopathology.

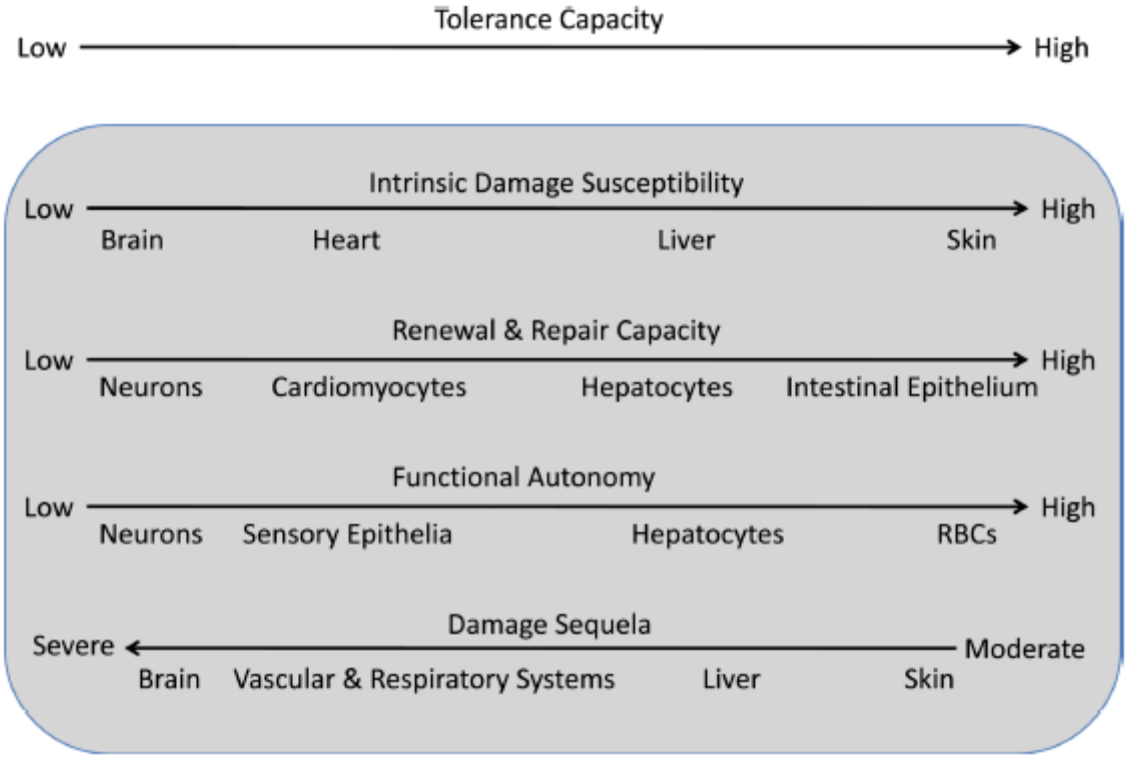


Figure 3.
Tolerance capacity is a function of intrinsic damage susceptibility, repair capacity, functional autonomy, and damage sequela of different tissues and organs. Although tissues generally tend to fall at the same ends of the four spectra, the four characteristics do not necessarily correlate with each other.

Co víme z kliniky a co je důležité?

Sepse x těžká sepse x septický šok

Rozdílné nosologické jednotky

Produkce prozánětlivých a protizánětlivých cytokinů
v detekovatelných koncentracích je kolem 50%

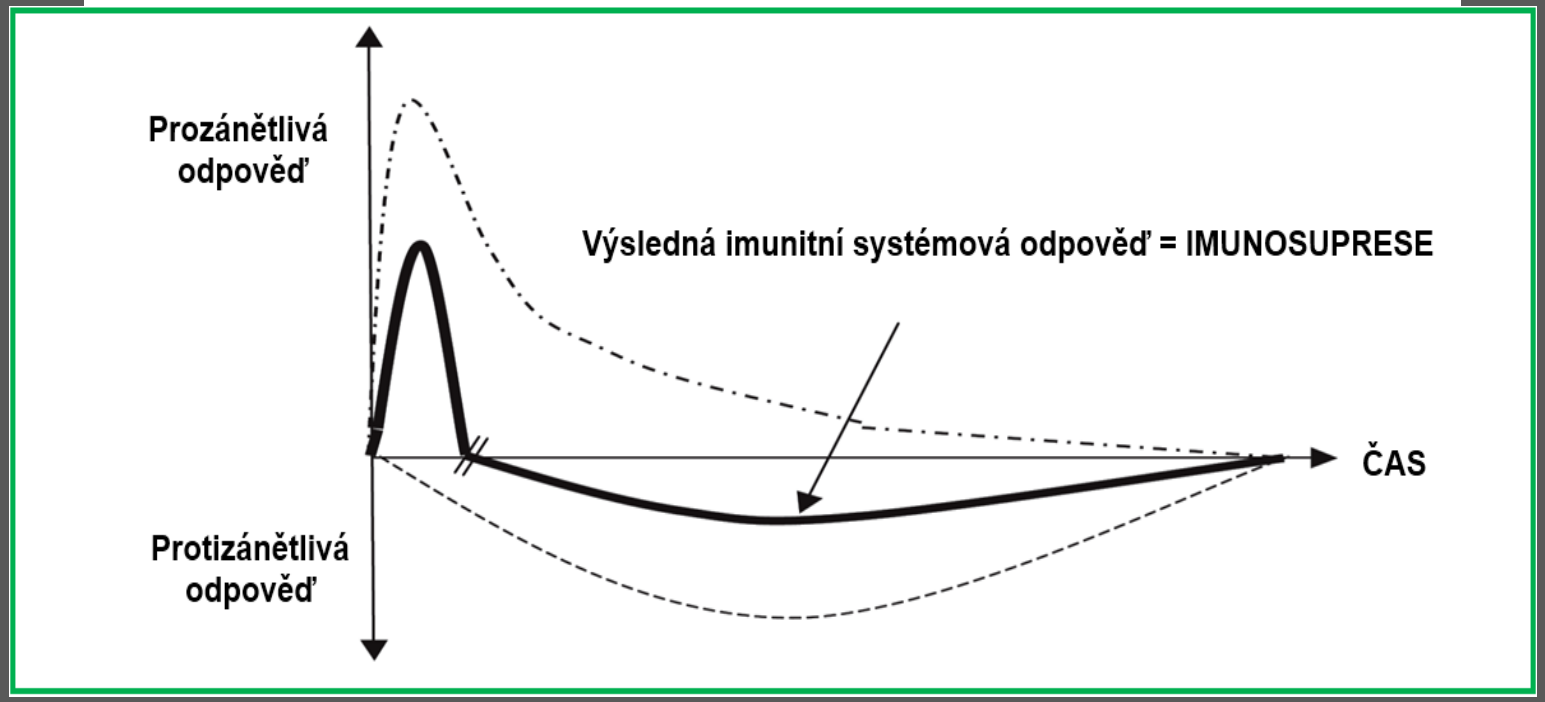
Genomická data nepodporují hypotézu dvou časových fází –
pro a protizánětlivé … a ještě téměř nic nevíme o proteomice

Existuje systémová imunosuprese

Genome-wide transcription profiling of human sepsis: a systematic review

Critical Care 2010

Benjamin M Tang[1,2*], Stephen J Huang[1], Anthony S McLean[1]



Conclusions: Sepsis related inflammatory changes are highly variable on a transcriptional level. We did not find strong genomic evidence that supports the classic two phase model of sepsis.

Co máme ? studie prokazující stav funkční imunosuprese na úrovni orgánů i celku

A prospective analysis of lymphocyte phenotype and function over the course of acute sepsis.
Boomer JS, Shuherk-Shaffer J, Hotchkiss RS, Green JM.
Crit Care. 2012 Jun 28;16(3):R112. doi: 10.1186/cc11404.

Immunosuppression in patients who die of sepsis and multiple organ failure.
Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, Srivastava A, Swanson PE, Green JM, Hotchkiss RS.
JAMA. 2011 Dec 21;306(23):2594-605. doi: 10.1001/jama.2011.1829

Reactivation of multiple viruses in patients with sepsis.
Walton AH, Muenzer JT, Rasche D, Boomer JS, Sato B, Brownstein BH, Pachot A, Brooks TL, Deych E, Shannon WD, Green JM, Storch GA, Hotchkiss RS.
PLoS One. 2014 Jun 11;9(2):e98819. doi:

Co nevíme a co je důležité…

- Příčina smrti u pacientů v sepsi
- Imunomodulační resp. imunosupresivní vliv terapie používané na ICU
- Spojení proteomiky v návaznosti na genomiku, transkriptomiku a metabolomiku a funkční stav proteinů u těchto stavů…..

The Autopsy Pathology of Sepsis-Related Death
Lucas S 2010

- There are no specific morbid anatomical features of „septicaemia“ - the historical „diffluent“ or „septic“ spleen is a debatable gross entily (Arismendi-Morrillo et al 2004)
- „shock“ lung“ and „acute tubural necrosis“ are difficult to diagnose on gross examination alone
- There are no standard histopathological features that reliably point to „septicaemia“

Goldenberg et al. Critical Care 2014, 18:540
http://ccforum.com/content/18/5/540

CRITICAL CARE

EDITORIAL

Is nosocomial infection really the major cause of death in sepsis?

Neil M Goldenberg[1], Aleksandra Leligdowicz[2], Arthur S Slutsky[2,3,4], Jan O Friedrich[2,3] and Warren L Lee[2,4*]

Introduction

Over 25 clinical trials for sepsis have failed [1,2], suggesting that our current understanding of its pathogenesis is incomplete. Deaths occur days to weeks after diagnosis and have been attributed to one of two phenomena [3]. First, a subset of patients succumbs to an overwhelming acute inflammatory response driven by the innate immune system, leading to death within days of the initial infection. However, most patients survive this phase and the repeated failure of anti-inflammatory therapies for sepsis (for example, anti-tumor necrosis factor antibodies [4], high-dose corticosteroids [5]) indicates that inflammation *per se* is unlikely to be a major cause of death. Most sepsis deaths occur later and have been associated with dysfunction of the innate and adaptive immune systems [6], characterized by decreased cytokine production and lymphocyte apoptosis [7]. These mechanisms have been postulated to cause immunosuppression [3,8,9], predisposing patients to fatal nosocomial infections. Based on this hypothesis, immunoadjuvant therapy to boost the immune system has been proposed recently as a therapeutic approach.

The second study did not report the incidence of positive cultures in patients who died from sepsis, a critical statistic for determining the contribution of nosocomial infection to mortality [1]. This study described three phases of mortality, divided into deaths occurring within hospital days 0 to 5 (phase I), days 6 to 15 (phase II) and days 16 to 150 (phase III). Despite the fact that phase III included the largest number of days by far, the mortality rate was highest in phase I, arguing against late nosocomial infection being the main cause of death.

A recent retrospective analysis in our own center has provided further evidence against this theory. We considered all patients admitted to the ICU who were screened for a sepsis study of heparin (Heparin Anticoagulation to Improve Outcomes in Septic Shock; ClinicalTrials.gov NCT 1648036) and subsequently died. From these patients, we selected those who actually had sepsis and looked for evidence of a secondary nosocomial infection, defined as a detected new microbial isolate prior to death. Of 26 consecutive patients dying of septic shock in a mixed medical–surgical ICU, only three (14 %) patients had evidence of a new infection at the time of death (Table 1). While our study is not defini-

Existuje reálná možnost snížit mortalitu u pacientů s těžkou sepsí a septickým šokem?????

Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Mortality Related to Severe Sepsis and Septic Shock Among Critically Ill Patients in Australia and New Zealand, 2000-2012

Kirsi-Maija Kaukonen, MD, PhD, EDIC; Michael Bailey, PhD; Satoshi Suzuki, MD; David Pilcher, FCICM; Rinaldo Bellomo, MD, PhD

DESIGN, SETTING, AND PARTICIPANTS Retrospective, observational study from 2000 to 2012 including 101 064 patients with severe sepsis from 171 ICUs with various patient case mix in Australia and New Zealand.

MAIN OUTCOMES AND MEASURES Hospital outcome (mortality and discharge to home, to other hospital, or to rehabilitation).

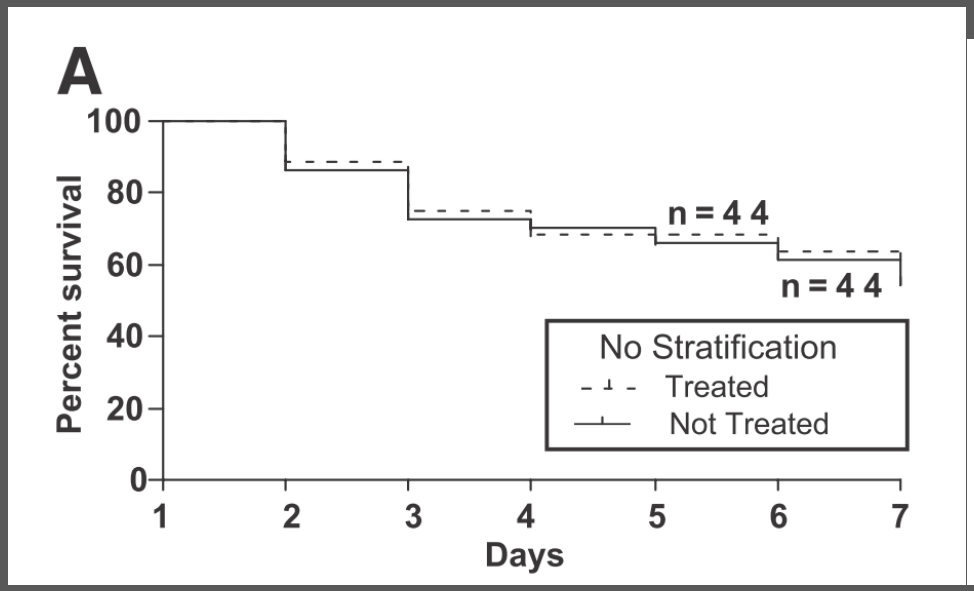
RESULTS Absolute mortality in severe sepsis decreased from 35.0% (95% CI, 33.2%-36.8%; 949/2708) to 18.4% (95% CI, 17.8%-19.0%; 2300/12 512; $P < .001$), representing an overall decrease of 16.7% (95% CI, 14.8%-18.6%), an annual rate of absolute decrease of 1.3%, and a relative risk reduction of 47.5% (95% CI, 44.1%-50.8%). After adjusted analysis, mortality decreased throughout the study period with an odds ratio (OR) of 0.49 (95% CI, 0.46-0.52) in 2012, using the year 2000 as the reference ($P < .001$). The annual decline in mortality did not differ significantly between patients with severe sepsis and those with all other diagnoses (OR, 0.94 [95% CI, 0.94-0.95] vs 0.94 [95% CI, 0.94-0.94]; $P = .37$). The annual increase in rates of discharge to home was significantly greater in patients with severe sepsis compared with all other diagnoses (OR, 1.03 [95% CI, 1.02-1.03] vs 1.01 [95% CI, 1.01-1.01]; $P < .001$). Conversely, the annual increase in the rate of patients discharged to rehabilitation facilities was significantly less in severe sepsis compared with all other diagnoses (OR, 1.08 [95% CI, 1.07-1.09] vs 1.09 [95% CI, 1.09-1.10]; $P < .001$). In the absence of comorbidities and older age, mortality was less than 5%.

CONCLUSIONS AND RELEVANCE In critically ill patients in Australia and New Zealand with severe sepsis with and without shock, there was a decrease in mortality from 2000 to 2012. These findings were accompanied by changes in the patterns of discharge to home, rehabilitation, and other hospitals.

Stratification is the key: Inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis

- Objective: This study examined the effectiveness of prospective stratification to identity and target high-dose glucocorticoid therapy for subject developing lethal sepsis.

CLP model – stratifikace na základě koncentrace IL-6 do dvou skupin – kteří přežijí a zemřou…… poté aplikován dexamethason polovině zvířat v každé skupině


A
100
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60
40
20
0
Percent survival
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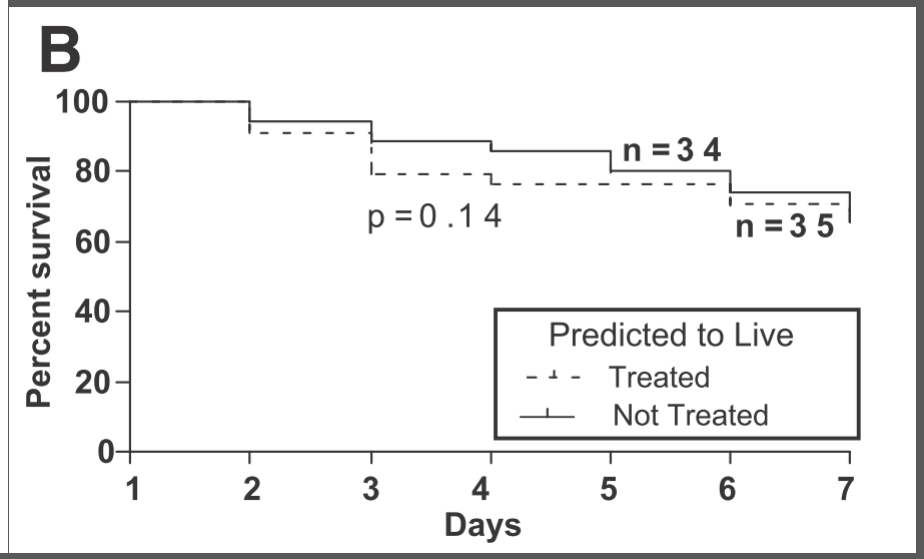
2
3
4
5
6
7
Days
n = 4 4
n = 4 4
No Stratification
Treated
Not Treated


B
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Percent survival
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n = 3 4
p = 0 .1 4
n = 3 5
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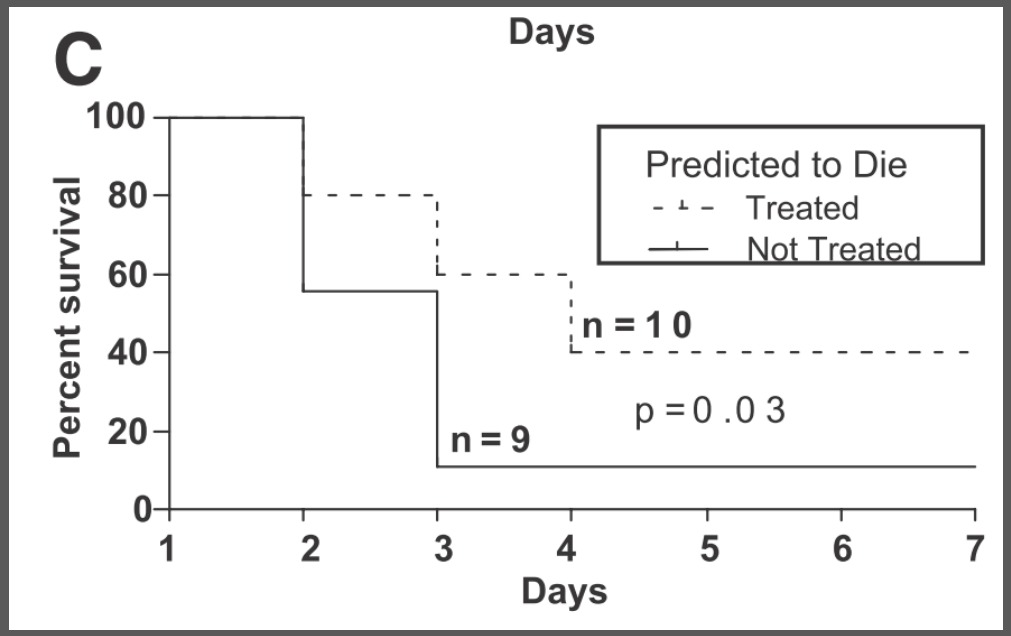
Treated
Not Treated


C
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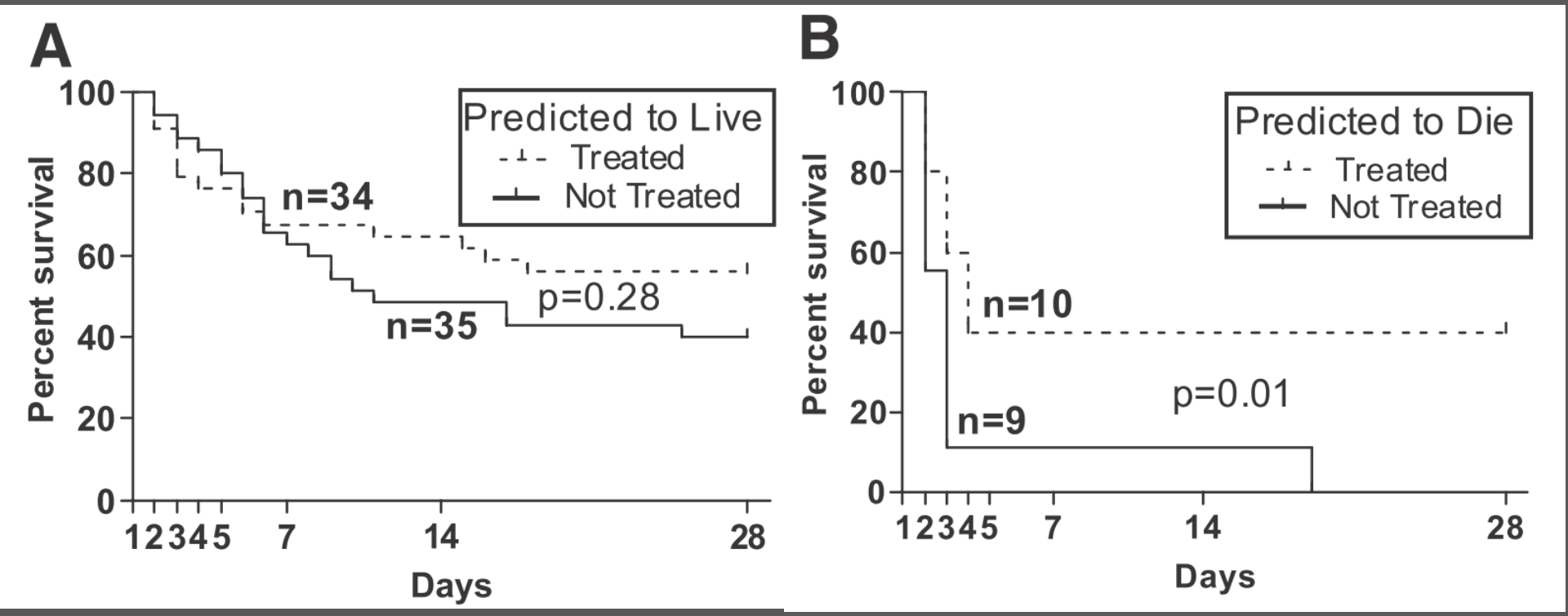
Percent survival
1
2
3
4
5
6
7
Days
Predicted to Die
Treated
Not Treated
n = 1 0
p = 0 .0 3
n = 9

Dlouhodobá mortalita



Stratification is the key……

- Conclusions: Following CLP-induced sepsis, early and accurate survival prediction allows targeted immunosuppression that improves survival. …….
- **Na základě koncentrace IL-6 byla zjištěna rozdílná mortalita zvířat při podání protizánětlivé terapie ….**
- **Protizánětlivá terapie zásadním způsobem tuto mortalitu snížila**

Osuchowski et al. Crit Care Med 2009; 37:1567-1573

Otázky k řešení – stratification is the key

- Máme parametr, který by nám odhalil pacienta vhodného pro imunologickou intervenci ?
- osobní zkušenost HLA-DR – nejvíce koreluje s mortalitou
- Jaká by měla být imunologická intervence ?
- Už dnes máme léky, které dokázaly HLA-DR zvýšit , ale bez vlivu na mortalitu
- ?????????

Východiska

- Nalezení parametrů které nám určí pacienty, kteří budou profitovat z cílené imunomodulační terapie
- Řešit problematiku těžké sepse resp. septického šoku a PCI jako rozdílných nosologických jednotek
- Bude se lišit terapie pacientů v akutní fázi (septický šok) a PCI (persistent critically illness)

Terapie biotechnologická s využitím „imunitních“ reparačních mechanismů

Nat Med. 2014 Oct;20(10):1211-6. doi: 10.1038/nm.3640. Epub 2014 Sep 14.

An extracorporeal blood-cleansing device for sepsis therapy.

Kang JH[1], Super M[2], Yung CW[3], Cooper RM[4], Domansky K[5], Graveline AR[5], Mammoto T[6], Berthet JB[5], Tobin H[6], Cartwright MJ[5], Watters AL[5], Rottman M[2], Waterhouse A[5], Mammoto A[6], Gamini N[5], Rodas MJ[5], Kole A[5], Jiang A[6], Valentin TM[5], Diaz A[5], Takahashi K[7], Ingber DE[8].

⊕ Author information

Abstract

Here we describe a blood-cleansing device for sepsis therapy inspired by the spleen, which can continuously remove pathogens and toxins from blood without first identifying the infectious agent. Blood flowing from an infected individual is mixed with magnetic nanobeads coated with an engineered human opsonin--mannose-binding lectin (MBL)--that captures a broad range of pathogens and toxins without activating complement factors or coagulation. Magnets pull the opsonin-bound pathogens and toxins from the blood; the cleansed blood is then returned back to the individual. The biospleen efficiently removes multiple Gram-negative and Gram-positive bacteria, fungi and endotoxins from whole human blood flowing through a single biospleen unit at up to 1.25 liters per h in vitro. In rats infected with Staphylococcus aureus or Escherichia coli, the biospleen cleared >90% of bacteria from blood, reduced pathogen and immune cell infiltration in multiple organs and decreased inflammatory cytokine levels. In a model of endotoxemic shock, the biospleen increased survival rates after a 5-h treatment.

PCI – Persistent critical illness

Lancet Respir Med. 2014 Dec;2(12):1016-26. doi: 10.1016/S2213-2600(14)70217-6. Epub 2014 Oct 28.

Mesenchymal stem cells: mechanisms of potential therapeutic benefit in ARDS and sepsis.

Walter J[1], Ware LB[2], Matthay MA[3].

⊕ Author information

Abstract

Multipotent mesenchymal stem (stromal) cells (MSCs) have shown promising therapeutic effects in preclinical models of both acute respiratory distress syndrome (ARDS) and sepsis. Although initial research focused on the ability of MSCs to engraft at sites of tissue injury, increasing evidence suggests that MSCs have their therapeutic effects through mechanisms unrelated to long-term incorporation into host tissue. One of the most compelling of these pathways is the ability of MSCs to interact with injured tissue through the release of soluble bioactive factors. This Review provides an overview of the general properties of MSCs, and then outlines ways in which the paracrine effects of MSCs might reduce lung injury and enhance lung repair in ARDS and sepsis. Finally, we summarise ongoing challenges in MSC research and identify areas in which the discipline might progress in the coming years.

Virulence. 2014 Jan 1;5(1):219-25. doi: 10.4161/viru.25965. Epub 2013 Aug 13.

Anti-endotoxin vaccines: back to the future.

Cross AS[1].

⊕ Author information

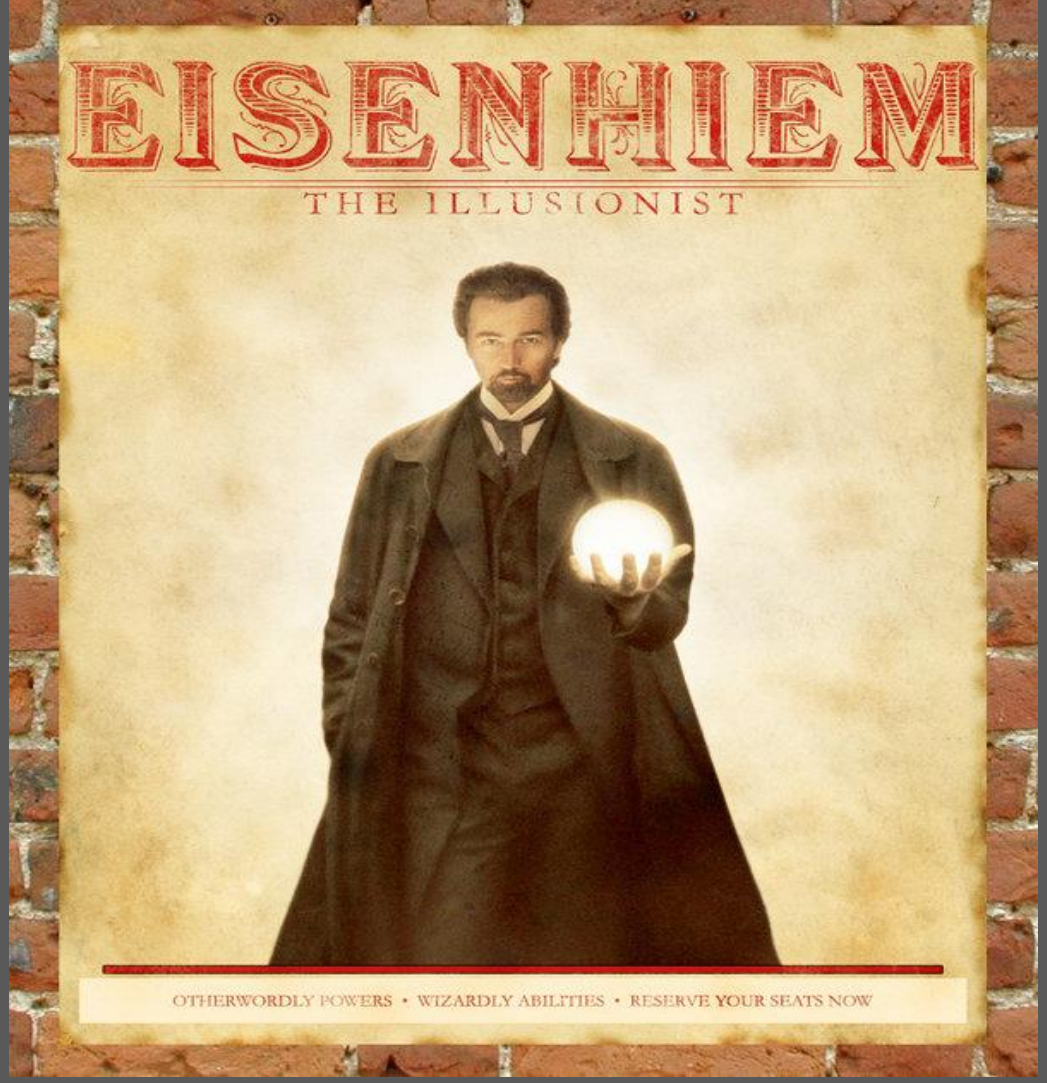
Abstract

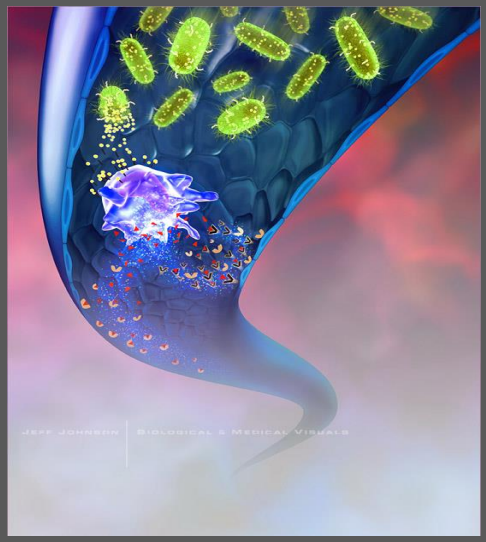
Gram-negative bacterial (GNB) infections are a leading cause of serious infections both in hospitals and the community. The mortality remains high despite potent antimicrobials and modern supportive care. In the last decade invasive GNB have become increasingly resistant to commonly used antibiotics, and attempts to intervene with novel biological therapies have been unsuccessful. Earlier studies with antibodies directed against a highly conserved core region in the GNB lipopolysaccharide (LPS, or endotoxin) suggested that this approach may have therapeutic benefit, and led to the development of a subunit vaccine that has progressed to phase 1 clinical testing. Since only a few serogroups of GNB cause bacteremia, O-specific vaccines had been developed, but these were not deployed because of the availability of other therapeutic options at the time. Given the likelihood that new antibiotics will not be soon available, the development of vaccines and antibodies directed against endotoxin, both O and core antigens, deserves a "second look".

KEYWORDS: antibody; clinical trial; core glycolipid; gram-negative bacteria; immunoglobulin; lipopolysaccharide; sepsis; vaccine

Souhrn

- Nalezení parametru/ů které určí pacienty vhodné k imunologické intervenci – nebude to velké %
- Úloha proteinomiky
- Ověření úlohy zásadních mediátorů v patogenezi těžké sepse resp. septického šoku
- Rozdílné nosologické jednotky – PCI a septický šok resp. těžká sepse se mohou lišit ve funkčním stavu imunity a proto je pravděpodobné, že se bude lišit jejich imunomodulační léčba





Jak veliké jsou naše iluze ?

Nemohou být malé, abychom mohli jít dál…