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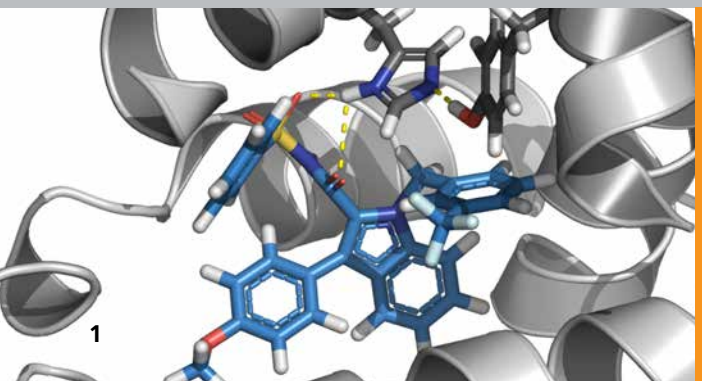
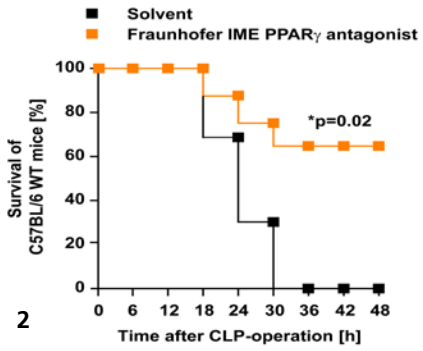
FRAUNHOFER INSTITUTE FOR MOLECULAR BIOLOGY AND APPLIED ECOLOGY IME





1 *Computational docking of Fraunhofer IME PPARγ antagonist to the PPARγ ligand binding domain © Fraunhofer IME / Thales Kronenberger, Tilo Knape.*

2 *Survival of mice after CLP-operation and treatment with Fraunhofer IME PPARγ antagonist © Fraunhofer IME / Tilo Knape.*

Fraunhofer Institute for Molecular Biology and Applied Ecology IME
Branch for Translational Medicine and Pharmacology
Theodor-Stern-Kai 7
60596 Frankfurt am Main

Contact:
Dr. Tilo Knape
Phone: +49 69 8700-25072
tilo.knape@ime.fraunhofer.de

Prof. Dr. Andreas von Knethen
Phone: +49 69 6301-6989
andreas.von-knethen@ime.fraunhofer.de

Dr. Volker Laux
Phone +49 69 8700-25076
volker.laux@ime.fraunhofer.de

www.ime.fraunhofer.de/en/TMP

RESEARCH SEPSIS AT FRAUNHOFER IME

Sepsis is a life-threatening complication of the body's response to infection, responsible for about 60,000 deaths annually and rising, making it the third most common cause of death in Germany. Normally, the immune system mounts a prompt, protective response to microorganism invasion. However, deficient immunological defense may allow the infection to expand. Additionally, a poorly regulated immune response may harm the host through excessive release of inflammatory mediators. Sepsis is thus characterized by partially overlapping phases of initial hyper-inflammation and subsequent hypo-inflammation or "immune paralysis". The immune paralysis is critical for patients, as T cells of the adaptive immune system undergo apoptosis and patients thereby often succumb to secondary infections. Sepsis-induced death is often the result of multiple organ failure (MOF) resulting from attack on organs by immunocytotoxic mechanisms, including cytotoxic T (Tc) cells.

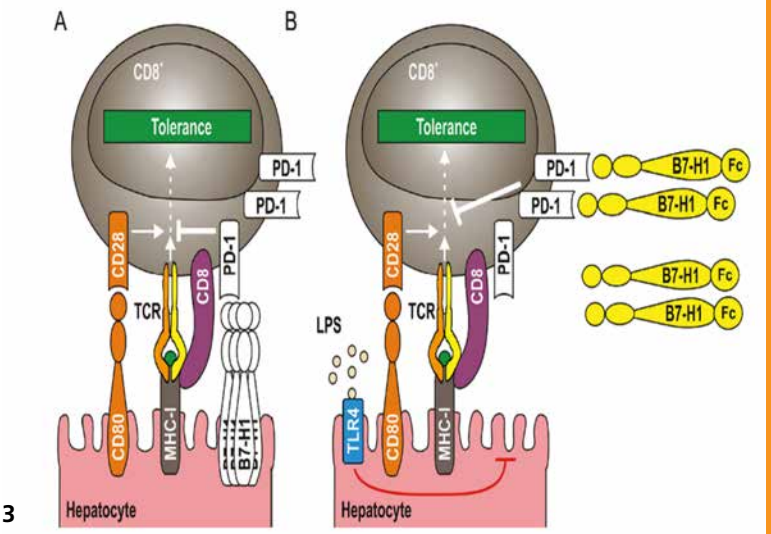
Current approaches to treatment of sepsis focus on life support systems, drug inhibition of the hyper-inflammation of sepsis and cytotoxic mechanisms in order to suppress disease progression. However, despite recent major advances, the incidence of and mortality due to sepsis continue to increase. There is, therefore, a need for new methods for diagnosis, prevention and treatment of sepsis.

Prevent T cell depletion

During the hypo-inflammation of sepsis the activation of the peroxisome-proliferator-activated receptor gamma (PPARγ) causes T cell depletion. Fraunhofer IME is developing novel PPARγ antagonists (Fig. 1) to prevent T cell depletion as a new therapy approach for sepsis (Fig. 2).

Prevent multiple organ failure

The invention relates to a fusion protein of the extracelluar part of an immunoinhibitory receptor and human immunoglobulin to



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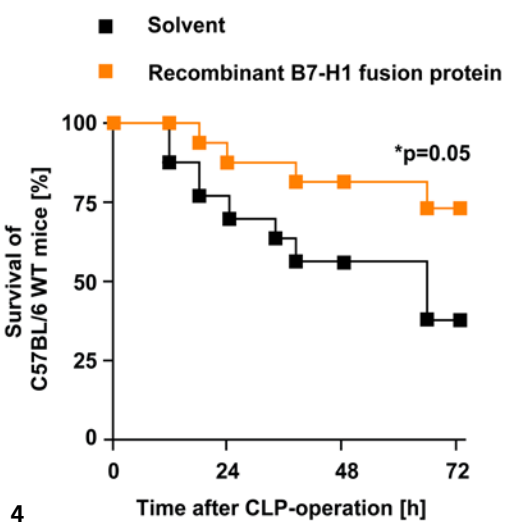

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be used for therapy of sepsis. The inhibitory receptor binds to a ligand on Tc cells and the specificity of the fusion protein can be enhanced by incorporating a binding peptide selective for Tc cells (Fig. 3). The major advantage of this fusion protein is that it replaces inhibitory B7-H1 molecules on organs lost during sepsis and induces prolonged tolerance rather than just inhibition of Tc cells (Fig. 4). The therapeutic protein thus offers more than just acute inhibition of inflammation. Similar proteins are used for other indications, but their use for sepsis has not been reported previously.

Biomarker identification and characterization

Fraunhofer IME proposed that the level of PPARγ expression in T cells from septic patients and the T cell count correlates with clinical outcome. Preliminary data suggest that both high PPARγ expression in T cells and low absolute T cell number in blood of septic patients may have potential as a new prognostic marker for a poor sepsis outcome.

Approaches

Fraunhofer IME has access to the latest in vitro technologies and animal models and is developing specific new approaches for sepsis diagnosis, prevention and treatment.

Endpoints

- Organ damage
- Defined time
- Survival
- Physiological parameters

Readout parameters

- Post mortem blood and organs are recovered for further analysis by various methods such as flow cytometry, cell sorting, FRET-based assays, fluorescence microscopy, reporter gene assays, cytotoxicity assays, proliferation assays, migration assays, mass spectrometry, next-generation sequencing, inducible cell systems western blot, immunohistochemistry, quantitative real-time polymerase chain reaction, cytometric bead array or enzyme-linked immunosorbent assay and high-performance liquid chromatography
- In vivo measuring of blood pressure, electrocardiography, heart rate, temperature and physical activity

Animal models of sepsis

- Cecal ligation and puncture (CLP) model
- Cecal slurry (CS) model
- Lipopolysaccharide (LPS) model

Selected publications

- Trümper V, von Knethen A, Preuß A, Ermilov E, Hackbarth S, Kuchler L, et al. Flow cytometry-based FRET identifies binding intensities in PPARγ1 protein-protein interactions in living cells. Theranostics 2019; 9(19): 5444-5463.
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- Netzer C, Knape T, Kuchler L, Weigert A, Zacharowski K, Pfeilschifter W, et al. Apoptotic Diminution of Immature Single and Double Positive Thymocyte Subpopulations Contributes to Thymus Involution During Murine Polymicrobial Sepsis. Shock 2017;48(2):215-226.
- Brenneis M, Aghajaanpour, Knape T, Sha LK, Neb H, Meybohm P, et al. PPARγ Expression in T Cells as a Prognostic Marker of Sepsis. Shock 2016;45(6):591-7.
- Knape T, Flesch D, Kuchler L, Sha LK, Giegerich AK, Labocha S, et al. Identification and characterisation of a prototype for a new class of competitive PPARγ antagonists. Eur J Pharmacol 2015;755:16-26.

3 *Autoactivation of Tcs towards tissue antigenes during sepsis contributes to liver- (organ-) failure. In the control situation (A) B7-H1, a co-inhibitory protein which is expressed on the patocytes, blocks Tc-dependent autoimmune activation. Addition of a recombinant B7-H1 fusion protein significantly reduces Tc-dependent cytotoxicity in vitro (B) © Goethe University Frankfurt, Fraunhofer IME / Andreas von Knethen.*

4 *Survival of mice after CLP-operation and treatment with recombinant B7-H1 fusion protein © Goethe University Frankfurt, Fraunhofer IME / Andreas von Knethen.*