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Abstracts
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Results: LPS tolerant mice survived significantly longer than control animals (154 ± 13h vs. 76 ± 8h, $p < 0.001$). Viable bacteria were significantly reduced in blood, peritoneal lavage and organ homogenates of LPS tolerant mice already 1h after infection. The levels of different cytokines (TNFα, IFNγ, G-CSF) in plasma and liver homogenates of tolerant mice were decreased significantly compared to control animals. The early inactivation of bacteria in LPS tolerant animals was dependent on the accumulation of neutrophilic granulocytes in the peritoneal cavivity prior to infection. However, prevention of leukocyte accumulation in the peritoneum by injection of LPS intravenously did not abrogate reduction of *Salmonella* in the late phase of infection (48h). LPS tolerant mice cleared bacteria injected via the tail vene much more efficiently than control animals (3 ± 2 % vs 39 ± 2 % of infectious dose left in blood 5 min after injection). In contrast, they had increased bacterial numbers in the liver (37 ± 7.5% of infectious dose vs 13 ± 1.3%, $p < 0.05$) at 20 min after infection. This effect was associated with increased numbers of Kupffer cells in tolerant mice.

Conclusions: Improved initial inactivation of Salmonella in the peritoneal cavity by accumulated PMN and increased hepatic uptake of *Salmonella typhimurium* in LPS tolerant mice in the initial stage of infection account for the improved clearance of bacteria from the circulation and the prolongal of survival associated with LPS tolerance.

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Improved Host Defense Against Polymicrobial Sepsis Induced by Endotoxin Tolerance

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Background: The innate immune system recognizes molecular patterns of microbes such as endotoxin, lipoteichoic acid, and proteoglycans that have potent immunostimulatory capacity. Endotoxin tolerance has been defined as state of low responsiveness to endotoxin following a primary low dose stimulus. Endotoxin tolerance was proposed to occur during human polymicrobial sepsis resulting in monocyte deactivation and immune paralysis. In the present study, the consequences of endotoxin priming for the host defense against polymicrobial sepsis was investigated using the colon ascendens stent peritonitis (CASP) model. **Results:** Mice were injected i.p. with low dose LPS 4 d before CASP. Endotoxin pretreatment significantly improved survival and diminished the numbers of viable bacteria in peripheral organs. Induction of inflammatory cytokines by CASP was reduced in endotoxin tolerant animals. Despite low levels of cytokines, the local inflammatory response was strongly augmented in endotoxin tolerant mice. Following CASP, neutrophil numbers in the peritoneal cavity of endotoxin tolerant mice were increased by up to 10-fold in endotoxin tolerant mice as compared to controls. Increased accumulation of neutrophils was related to a reduced rate of apoptosis but not to alterations of cell recruitment, peripheral leukocyte counts, or state of neutrophil differentiation. **Conclusions:** Endotoxin tolerance does not result in immune paralysis but rather improves pathogen clearance and survival of sepsis. The protective effect is related to an extended survival of neutrophils at the primary site of infection.

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Plasma Antibodies against Fecal Endotoxin of Type IgA, but not IgG are Elevated in Patients with Alcohol-Induced Liver Disease

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Introduction: Endotoxin (Lipopolysaccharide, LPS) concentrations were found to be increased in plasma of patients with alcohol-induced liver diseases. As LPS are known to stimulate both, inflammation and B-lymphocyte activation, we investigated the concentration of immunoglobulins (IgA and IgG) against gut-derived LPS in patients suffering from different stages of alcohol-induced liver damage.

Patients and Methods: Antibody concentrations were measured in 10 patients with alcohol-induced fatty liver (AF), in 11 patients with alcohol-associated hepatitis (AH) and in 9 patients with alcoholic cirrhosis (AC) and compared to that of 10 healthy controls (C) and that of 6 patients with non-alcoholic cirrhosis (NAC) by an ELISA technique. Microtiter plates were coated with a mixture of a polymyxin-LPS complex [c(LPS):20 µg/ml]. LPS was extracted from a 24h-culture, which was raised from a mixed (n=3) faecal flora. A pool plasma from 4000 individuals (c(IgA/IgG): 8 median units) served as standard.

Results: IgA concentrations (median units, MU; means±SD) in individuals with alcohol induced liver diseases were found to be all increased when compared to the control group, but not in the group with non-alcoholic cirrhosis (Tab.1). No differences were found in IgG levels (see Tab.).

	C	NAC	AF	AH	AC
c(IgG) [MU]	113±74	88±51 n.s.	67±50 n.s.	67±50 n.s.	75±41 n.s.
c(IgA) [MU]	118±93	174±77 n.s.	202±91 p<0.025	259±132 p<0.005	260±106 p<0.002

Conclusions: The appearance of alcohol-induced liver diseases is associated with a higher concentration of IgA antibodies against fecal endotoxins in the plasma of the concerned subjects. This finding underscores the importance of bacterial toxins of intestinal origin and the involvement of the immune system in the development of alcohol-induced liver diseases. Endotoxins may play a special role in this context, as they meet two essential peculiarities for active immunoglobuline production: they posess Lipid A, a strong adjuvans and a polysaccharide chain, an excellent antigen.

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A glycoconjugate of vegetal origin that protects mice from endotoxemia

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Abstract:

Purpose of study: TNF-α is a pleiotropic cytokine with diverse biological actions. TNF-α acts as a systemic mediator of endotoxemic shock. Gram-negative infections and consequent endotoxin shock forms are serious problems of medicine. TNF-α antagonists are emerging as a new group of therapeutic agents that includes molecules such as pentoxyphilline, Rollipram or IL10. Our lab have studied the TNF-α antagonist activity of a group of glycoconjugates obtained after conjugation of non-lectin proteins of vegetal origin with a glucomannan formed by disaccharidic repetitive unit having mono and diphosphoester structures.

Methods: We have performed an *in vivo* murine model of LPS-induced TNF-α. In a second approach TNF-α was also induced with LPS after priming with monocyte-macrophage system blockers (MMB) or stimulants (MMS). To test the immunomodulator activity of different components of the glycoconjugates we used an *in vitro* model with LPS-treated-PMA diferenciated THP-1 cells.

Exact Data: An inhibition of levels of TNF-α in serum from 65 to 90% was produced by a glycoconjugate in a dose dependent manner. We found 5-fold increase in the percentage of inhibition of TNF-α levels in mice primed with MMS with respect to that found in mice unprimed or primed with MMB. Both polysaccharide and proteins showed inhibitory capacity of TNF-α levels in the *in vitro* THP-1 model in a dose dependent manner. Highest inhibition was 70%.

Summary of results: Glycoconjugate is effective and safety in our model of endotoxemia by modulating serum-TNF-α levels.

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Implication of STAT1 and STAT3 transcription factors in response to supernatigens and LPS.

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LPS and superantigens have been identified as potent inducers of lethal shock because of their capacity to activate the immune system resulting in the secretion of large amounts of cytokines. To improve our knowledge of the role played by cytokines in septic shock, we analyzed the activation of STAT1 and STAT3 in response to superantigens and LPS in liver and spleen of Balb/c mice. The intraperitoneal injection of the superantigen SEB activated STAT1 and STAT3 in both liver and spleen. Nevertheless, activated STAT1 was not detected 24 hours later while that activated STAT3 was detected for more than 48 hours