ImmunoTools *special* Award 2014

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Characterisation of murine immune cells at different time points during the course of sepsis

Sepsis is a life-threatening condition that can lead to shock and multiple organ failure due to an extremely complex and dynamic systemic inflammatory response to an infection with bacteria, fungi or viruses. Today Sepsis is the leading cause of death from infection worldwide and is increasing considerably. During its course an uncontrolled pro-inflammatory immune response (SIRS) is followed or accompanied by a massive anti-inflammatory immune reaction in the post-acute phase which often leads to protracted immune paralysis. It is believed that especially the lymphocyte compartment (NK-, B- and T-cells) is impaired to form an adequate adaptive immune response. This disturbance of the immune homeostasis affects dramatically the survival of the patients and even after prolonged treatment with antibiotics many patients which succumb to sepsis still have septic foci in the body. So in spite of improved critical care and big efforts in research in the last decades the pathophysiology of sepsis remains inadequately understood and prevents effective therapies.

So far most studies concentrated on just 1 or 2 time points in which they analysed the impact of sepsis on the immune system. In this study we want to monitor and investigate the functional state of the immune system during the whole course of a sepsis infection (beginning 24h after the insult and up to 30 days) in a close-meshed scale. The focal point of the experiments will be on T-cells, but we are also looking at the other members of the lymphocyte and leucocyte compartment. To achieve consolidated findings, three different sepsis/endotoxemia mouse-models are used in our lab. The injection of LPS or CpG (each in sub lethal doses) mimics the infection with bacteria. This treatment causes a very strong systemic inflammation without an actual infection in the periteneum. As a “bona fide” model for severe sepsis the intraperitoneal injection of human faeces (PCI – peritoneal contamination and infection) is used. This leads to a polybacterial infection accompanied with a strong cytokine storm at the beginning of the illness and leads in later stages to cell- and organ-dysfunctions. To mimic the situation of human patients on intensive care units the mice are treated with antibiotics.

The aim of this study is to get a more profound and comprehensively understanding of the composition of the immune cells (neutrophils, macrophages, NK-, B- and T-cells), their capability to mount an immunological response and the release of important pro- and anti-inflammatory cytokines at several different time points after the insult in the course of sepsis/endotoxemia.

Cells will be prepared from peripheral blood, from the spleen and from the cells which have migrated into the peritoneum (sampled via peritoneal lavage). Additionally to these experiments we will take a closer look at the activation status of the cells, their ability to react to immunological stimuli with proliferation and their cytokine release in answer to different immune modulators.

Through the use of the different fluorescent-tagged antibodies from ImmunoTools we will be able to analyse in depth several important immune cell- types. For the enhancement of T-cell culture (required for the analysis of the activation status and proliferation after stimulation), the following recombinant mouse cytokines will be helpful: rm IL-2 and rm IL-7

The results from the mentioned experiments we will give us a more detailed picture of the changes in the composition and the ability of the immune cells to fight bacterial infections during a sepsis. This pilot study will deliver new hints for possible new target and approaches for more effective and comprehensive therapies for human patients.

ImmunoTools *special* AWARD for **Robert P. Requardt** includes 19 reagents

FITC - conjugated anti-mouse CD3ε, a/b TCR, g/d TCR, CD44, CD45, CD45R, isotype control IgG2b,

PE - conjugated anti-mouse CD4, CD19, CD56, Gr-1, NK-cells, isotype control IgG2b

APC -conjugated anti-mouse CD8a, CD11b, CD62L, isotype control IgG2b

recombinant human cytokines rm IL-2, rm IL-7 DETAILS