ImmunoTools *special* Award 2013

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L-Tryptophan depletion in the post-acute phase of polymicrobial sepsis in mice

A whole-body inflammation or sepsis is caused by the highly complex response of the immune system to bacteria, viruses, fungi or other microorganisms. In the severe phase of sepsis this can lead to a potentially life-threatening condition with multiple organ failure and shock. With 60.000 deaths per year sepsis is the third common cause of death in Germany. The molecular mechanisms of sepsis are under research but not fully understood. It is known that in the first steps of sepsis immune cells of the innate immune system are activated by pathogen associated molecular patterns (PAMPs) like bacterial molecules of the membrane, exotoxins, bacterial DNA or viral RNA. Recognized by pattern recognition receptors (PRRs) of the immune cells it leads to an initial activation of monocytes/macrophages. Activated monocytes/macrophages release enormous amounts of pro-inflammatory cytokines like tumor necrosis factor alpha (TNFα) and interleukin 1(IL-1). This gives rise to the production of other cytokines and mediators like IL-6, IL-8, IL-10, or high mobility group protein B1 (HMGB-1). This initial massive release of cytokines and mediators is believed to be the reason for several complications in the course of sepsis like the multi organ dysfunction syndrome (MODS) and causes in patients who survive the first acute inflammatory response several long-term effects. The post-acute phase of sepsis is characterized by an extended immunosuppression. Especially the lymphocyte compartment (B-cells, T-cells, NK T cells) is believed to be impaired in an adequate adaptive immune response.

It is believed that the tryptophan metabolism plays a key role in the control of the balance between tolerance and immune defense. Indoleamine 2,3-dioxygenase (IDO) plays an important role in the tryptophan levels in blood and is therefore crucial in immune tolerance. IDO catalyzes the first and rate limiting step of tryptophan catabolism leading to the degradation product kynurenine which accumulation could cause additional organ damage. The tryptophan depletion is believed to have beneficial effects during infections because it´s not only essential for humans but also for pathogens. In contrast it has been shown that strong tryptophan depletion could lead to suppression of T cell proliferation and other but not fully understood consequences like the decreased synthesis of serotonin. Tryptophan is the only source to build serotonin and the following melatonin. Depression as well as changes in mood, aggression, sleep und eating behavior can occur.

This study is intended to link tryptophan depletion and accumulation of kynurenin in blood, measured with ELISA, with the immune status of mice which underwent a polymicrobial sepsis. An intraperitoneal injection of human faces causes an inflammatory response. Ten days after that insult the post-acute immune status of the mice are determined. From blood serum the levels of cytokines are analyzed. The levels of macrophages (F4/80, CD11b) and neutrophils (Gr-1, CD45R) are measured in peritoneal lavage fluid and peripheral blood by flow cytometry. Other immune cells like:

- T cells (CD2,CD3e, CD4, CD8a, CD49d)
  T cell subsets: memory T-cells (CD44, CD45RO), naïve T-cells (CD62L, CD45RA), regulatory T-cells (CD4, CD25, Foxp3)
- B cells (CD45R, CD19, CD21, CD38, CD40, CD9, CD138)
- NK cells
- granulocytes (CD16, CD18, CD11b, CD45)

are prepared and characterized by different biochemical and molecular biological methods. To investigate the activation status on T cells it is further intended to determine activation marker (CD25, CD69, CD11a, CD134). After culture in tryptophan free medium we would like to investigate proliferation as well as apoptosis (Annexin V and CD95) rates after different strong activation stimuli.

To investigate the described interaction of tryptophan depletion and the effects on the immune system some reagents from ImmunoTools would be very helpful.

ImmunoTools *special* AWARD for **Cynthia Weigel** includes 25 reagents

FITC - conjugated anti-mouse CD3e, CD4, CD44, CD45, CD45R, Gr-1, NK-cells, isotype control IgG2b,

PE - conjugated anti-mouse CD4, CD8a, CD11b, CD19, Gr-1, NK-cells, isotpye control IgG2b,

APC -conjugated anti-mouse CD3e, CD4, CD11b, CD49d, CD62L, Gr-1, isotpye control IgG2b,

DETAILS