ImmunoTools IT-Box-Cy55M-Award 2013

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Immunoparalysis in sepsis

Sepsis or systemic inflammation is a complex and highly dynamic systemic inflammatory response to an infection with bacteria, viruses, fungi or other microorganisms. It is a potentially life-threatening condition that can lead to multiple organ failure and shock. Despite of improved critical care, the mortality rate exceeds 25 % killing an average of 150 patients each day in Germany. Sepsis is the leading cause of death from infection around the world with increasing incidence.

Patients who survived the acute excessive inflammatory response of the innate immune system enter a post-acute stage of sepsis which is characterised by a protracted suppression of the innate and adaptive immune system. It is generally assumed that in particular, the lymphocyte compartment (B-cells, T-cells, NK T-cells) is comprised in its ability to mount an adequate adaptive immune response and therefore, contributes to immunosuppression. Beside of the functional impairment of the adaptive immune system, it has been shown that also activated immune cells from the innate immune response (e.g. macrophages, dendritic cells, NK-cells) become inactivated in later stages of sepsis. As a result, these cells lose their antibacterial activity. But there is a lack of detailed knowledge about immune paralysis in sepsis and how immune cell function affects outcome of septic patients.

Cytokines, chemokines and other immune modulator molecules play a crucial role in the regulation of pro-inflammatory and anti-inflammatory processes. Sepsis / systemic inflammation is driven by a “cytokine storm” caused by literally all types of immune cells and other cells types (endothelial cells, fibroblasts, …) in response to a septic trigger. The composition of the cytokine storm determines whether an immune response shifts towards pro-inflammatory or anti-inflammatory responses. If and how a cytokine storm in sepsis contributes to immunoparalysis is unclear.

The present study investigates the functional state and cytokine responses of immune cells during the post-acute stage of sepsis / systemic inflammation using two different mouse models. Intraperitoneal injection of sublethal doses of LPS / endotoxin causes a systemic pro-inflammatory immune response characterised by a rapid cytokine storm (e.g. IL-1, IL-6, TNFα) without an infection. Intraperitoneal injection of human faeces represents a “sepsis-

like" inflammatory response that comprises a cytokine storm, polymicrobial infection and organ / cell dysfunction.

Immune cells (e.g. T-cells, B-cells, neutrophils, macrophages) are prepared ten days after the septic insult. The immune cells are studied using different molecular biological and biochemical methods, including *ex-vivo* stimulation / treatment with different immune modulators. The *ImmunoTools IT-Box-Cy55M* includes many interesting cytokines, chemokines and growth factors that regulate immune cell functions.

One example how cytokines from the *ImmunoTools IT-Box-Cy55M* are intended to be used is the investigation of Th0 / Th1 /Th2 / Th17 responses and differentiation of $CD4^+$ T-helper cells. We hypothesize that the balance between subpopulations of T-helper cells is altered in late stages of sepsis. It is believed that in the post-acute phase T-helper cell response shifts from Th1 to Th2-response with balance to anti-inflammatory cytokines. In order to investigate whether $CD4^+$ T-cells from septic animals (day = 10) still can differentiate into Th1 T-cells and mount Th1 immunity, we want to treat naïve Th0 $CD4^+$ T-cells with **rm IL-2**, **rm IL-4**, **rm IL-6** and T-cell receptor agonist *ex-vivo* and measure Th1 response. Furthermore, we want to treat naïve $CD4^+$ T-cells with **rm IL-2**, **rm IFNγ**, rm IL-12 and TCR agonists to promote Th2 differentiation. Treatment with **rm IL-17A**, **rm IL-17C** and **rm IL-21** can lead to Th17 $CD4^+$ differentiation.

A Second example how cytokines from the *ImmunoTools IT-Box-Cy55M* are intended to be used are macrophage activation studies. Isolated macrophages from septic and control animals (day = 10) are stimulated with **rm IL-6**, **rm CD40L (CD154), rm TNFα** and bacterial compounds *ex-vivo* followed by analysis of macrophage activity.

We will also investigate cytokine responses of other immune cells (e.g. B-cells, granulocytes) therefore, the *ImmunoTools IT-Box-Cy55M* offers an interesting set of recombinant cytokines and other signal molecules.

ImmunoTools IT-Box-Cy55M for Robby Markwart

includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF