ImmunoTools *special* Award 2018

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Effect of low dose anthracyclines on neutrophils under inflammatory conditions

Sepsis is the leading causes of death in intensive care units worldwide. It is defined as a life threatening organ dysfunction of a host as a consequence of a systemic inflammatory response to an infection. The pathophysiology of sepsis is under intensive research, but some mechanisms are still unclear.

The innate immune response is one of the first host defense lines against an infection. Important cellular players of that are neutrophils, which recognize pathogens via pattern recognition receptors. They are responsible for elimination of pathogens through different mechanisms, like phagocytosis, degranulation or NETosis. Apart from that they are involved in various inflammatory functions like the recruitment of further neutrophils, monocytes, dendritic cells or T-cells, which supports the eradication of pathogens and the return of the host to homeostatic conditions. Under septic conditions these host responses are dysregulated. A common sign during sepsis is the uncontrolled inflammatory response syndrome which could end up in multi organ failure. The high inflammatory response is encouraged by increased activation of neutrophils and monocytes/macrophages. Interestingly, under septic conditions neutrophils show an increased lifespan, release of cytokines and the ability to form NETs. Additionally the release of immature neutrophils, named bands, from bone marrow is increased. Furthermore studies showed a relationship between a higher amount of neutrophils and increased tissue injury which could end up in organ failure.

In experimental sepsis it was shown that low dose anthracyclines increase the survival rate of septic mice through a DNA damage response mediated mechanism, which leads to tissue tolerance. Mice showed a higher survival rate for five days after sepsis induction. The role of neutrophils after low dose anthracycline treatment in septic mice remains unclear.

In this study I want to identify the role of neutrophils in low dose anthracycline treated septic mice. Levels of immune cells, especially neutrophils (CD11b, Gr-1, Ly-6G) and macrophages (CD11b, Gr-1, F4/80) are measured in peritoneal lavage fluid and peripheral blood by flow cytometry. Additionally cytokines from blood serum are analyzed with ELISA (TNF-α, IL-6, IL-10, GM-CSF, SCF, IL-1β).

To determine the influence of low dose anthracyclines bone marrow derived myeloid cells will be analyzed from murine bones. For that hematopoietic stem cells are stimulated with SCF and IL-3 to initiate the proliferative phase of myeloid progenitors (CD45 positive cells). To drive them finally in the lineage of neutrophils SCF, IL-3, G-CSF are added and for macrophages only GM-CSF and M-CSF are supplied to the cells. Fully differentiated neutrophils (CD11b, Gr-1, Ly-6G) and macrophages (CD11b, Gr-1, F4/80) are identified via flow cytometry. The lifespan of those myeloid cells under certain conditions will be analyzed by an apoptosis assay with Annexin V. Furthermore the potential effect on the differentiation of neutrophils and macrophages is examined. A differentiation protocol combined with or without low dose anthracycline treatment will be performed. Finally functional assays for phagocytosis, migration (CXCL1, CXCL2) and cytokine release (TNFα, IL-6, IL-10, GM-CSF, SCF, IL1-β) are carried out.

ImmunoTools *special* AWARD for **Nathalie Schröder** includes 20 reagents

FITC - conjugated anti-mouse CD11b, CD45, Gr-1, isotype control IgG2b, Annexin-V

PE - conjugated anti-mouse CD11b, Erythroid cells, Gr-1, isotype control IgG2b, Annexin-V

APC - conjugated anti-mouse CD11b, CD45, Gr-1, isotype control IgG2b, Annexin-V

recombinant mouse cytokines: rm G-CSF, rm GM-CSF, rm GRO-b/CXCL2, rm IL-3, rm M-CSF, rm SCF

DETAILS more AWARDS