Beta-Glucan induced immune modulation of wound healing in common carp (Cyprinus carpio) - DTU Orbit (01/06/2019)

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Immune modulators are compounds capable to interact with the immune system and to modify the host response. This interaction enhances non-specific defense mechanisms, improving health and promoting survival. β-glucans are glucose polysaccharides present in sea weed, bacteria, fungi and cereal but not in animals. β-glucans are commonly used as immune modulators, but the mechanisms through which the modulation is achieved remains to be understood. Wound healing and tissue regeneration are essential mechanisms to ensure the survival and health of any organism. Studies based in mammalian systems have shown the importance of fibroblasts, macrophages, reactive oxygen species (especially hydrogen peroxide) and certain cytokines during wound healing processes. In fish however, only a few studies have been devoted tissue regeneration and modulation of cell proliferation during wound healing, even though mechanical injury as well as numerous diseases can severely damage fish tissues. The work presented in this thesis examines for the first time the immunomodulatory effects of β-glucans during wound healing processes in common carp. First, in order to choose the most suitable methodology for the measurement of reactive oxygen species (ROS), the nitroblue tetrazolium assay (NBT) and the real time luminolenhanced chemiluminescence assay (RT-luminol assay) were compared. Both methodologies successfully detected changes in the production of reactive oxygen species. However, only the RT-luminol assay was able to measure hydrogen peroxide, and allowed the monitoring of the ROS kinetics.

Second, an in vivo study was performed to evaluate the immunomodulatory effect of β-glucan during wound healing in carp. Mechanically wounded carps were bath treated with β-glucans or left untreated. The wound healing process was monitored using image analysis and showed that β-glucan bath treatment promoted wound closure in carp. Expression of IL1β and IL6 were increased at day 3 in the site of injure of β-glucan bath treated carps. Furthermore, an increased expression of IL-8 was measured at day 3 and 14 in untreated mechanically wounded carps. Therefore, the β-glucan immune-stimulatory effect on wound healing might be due to the enhancement of an early inflammatory response, with a prompt withdraw of an elevated influx of neutrophils from the wound site. In vitro studies showed that direct stimulation with β-glucan did not have any effect in cell proliferation and wound recovery of scratch-wounded Cyprinus carpio brain (CCB) fibroblasts cultures. This observation suggests that interaction of tissue-resident leukocytes or other components of the fish immune system and fibroblasts is required to obtain the immune modulatory effect of β-glucan in wound healing observed in vivo.

Third, in vitro stimulation of carp head kidney (HK) derived macrophages showed that different ROS patterns are produced after stimulation with PAMPs (β-glucan) and DAMPs (fish fibroblast lysates). β-glucan stimulation of HK derived macrophages resulted in fast and vigorous production of reactive oxygen species, consistent with a pathogen eradication strategy. This response was highly dominated by production of superoxide anion. In contrast, DAMP stimulation led to a slow, subtle but long-lasting production of oxygen radicals dominated by hydrogen peroxide. To determine the effect of hydrogen peroxide release in fibroblast proliferation during wound healing, scratch-wounded CCB fibroblasts were stimulated with different doses of hydrogen peroxide and the wound closure was followed by image analysis. Fibroblast stimulation with low doses of hydrogen peroxide (5 μM) showed a slight increase in percentage of wound recovery, whereas high doses (300 μM) impaired wound closure and caused cell death. The results elucidated the capacity of hydrogen peroxide to influence the fate of tissue regeneration through the establishment of environments suitable for tissue regeneration or oxidative stress. To conclude, β-glucan treatment enhanced wound closure in carp, probably due to the enhancement of a localized inflammatory response. The wound healing modulatory effect of β-glucan seems to be orchestrated by the immune system, since no direct effect on fibroblast proliferation was observed. Furthermore, production of ROS such as hydrogen peroxide may influence the fate of tissue regeneration, and differences in ROS patterns could be one of the possible ways in which fish alert the immune system to drive the immune response towards pathogen eradication or tissue repair.

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