

# Hematopoietic stem cell culture in crayfish as a model for studies of hemocyte differentiation and immunity

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Hematopoiesis is the process by which blood cells (hemocytes) mature and subsequently enter the circulation and we have developed a new technique to culture the hematopoietic progenitor cells *in vitro*. The reason for the successful culture was the isolation of a plasma protein that turned out to be a novel cytokine, astakine 1 (Ast1) containing a domain present in several vertebrates, so-called prokineticins. Now we have detected several astakines from other invertebrate species. Depending on our discovery of the cytokine Ast1 we have an opportunity to study in detail the differentiation of cells in the hematopoietic tissue (Hpt) of a crustacean, a tissue of evolutionary interest for studies of the connection between the vascular system and the nervous system. We have been able to isolate the entire hematopoietic tissue and for the first time detected a link between this tissue and the brain. We have further localized a proliferation center (APC) in the tissue and characterized its different parts. We have also used this system to isolate a new hematopoietic factor CHF, a small cysteine rich protein with high similarity to the N-terminal region of vertebrate CRIM1 that is important in the crossroad between apoptosis and hemocyte differentiation. The formation and development of hemocytes involve proliferation, commitment and differentiation from undifferentiated hematopoietic cells. Our technique for culture of crayfish hematopoietic stem cells provides a simple tool for studying the mechanism of astakine induced hematopoiesis, but also enables detailed studies of immune defense reactions. Further, the culture system has been used for studies of viral defense and the system is suitable for gene silencing which allow functional characterization of different molecules involved in host defense as well as in hemocyte differentiation.