ImmunoTools *special* Award 2017

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Immunodetection of key cell immuno-markers characterizing the regenerating tail versus the scarring limb and role of Growth Factors in the lizard model of tail-limb regeneration

The research involves the study on the regenerating tissues of lizards, an Amniote model gifted with unique antimicrobial and regenerative ability. While the tail regenerates, at the same time and in the same animal, the limb does not *(Alibardi L, 2010 Morphological.... mammals. Anat Embr Cell Biol 207: 1-112; Alibardi L, 2014 Histochemical.....regeneration. Progr Histoch Cytoch 48: 143-244)*.

Recent studies also show the presence of numerous antimicrobial peptides during wound healing *(Dalla Valle et al., 2012, Bioinformatic...Anolis carolinensis. Dev Comp Immun 36: 222-229; Alibardi et al., 2012, Wounding...antimicrobial barrier. Dev Comp Immun 36: 557-565)* that likely limits inflammatory reactions in the injured tissues and facilitate the massive tissue regeneration of the tail in these amniotes. Also a broad regenerative capacity of the spinal cord and the cartilaginous tissue, particularly of the articular cartilage of the knee, have been reported *(Alibardi L, 2014b. Observations on lumbar ...injured cord. J Dev Biol 2: 210-229; Alibardi L, 2015. Regeneration of the epiphyses.... Podarcis muralis. J Dev Biol 3: 71-92)*.

The understanding of these phenomena in lizards, amniotes like mammals, makes lizards in a unique position as experimental vertebrates, as recent Transcriptome studies on genes activated during tail regeneration vs limb scarring have been reported *(Vitulo N et al.. 2016. Transcriptome analysis ...regeneration in amniotes. Dev Dyn 246: 116-134, Vitulo N et al., 2017. Down-regulation of lizard immuno-genes....immuno-privileged organ. Protoplasma DOI 10.1007/s00709-017-1107-y.)*. The studies, based on the comparison between tail and limb tenascriptomes, have indicated the key genes stimulating organ regeneration in amniotes, including mammals, and have shown that the lizard blastema is an immunodepressed and tumor-like organ that is capable of auto-regulation.

So the study of the lizard blastema is a unique system also to analyze genes and proteins involved in oncogenesis. The lizard transcriptomes on genes involved in tissue regeneration have allowed to identify genes up-regulated or down-regulated during the process, but their localization-expression remains to be analyzed.

The project plans to study by immunocytochemistry, electrophoresis and western blotting proteins that are highly up-regulated during tissue regeneration in lizard (tail in comparison to the limb, including also the study on lympopoietic organs), as resulted from the recent transcriptome analysis *(Vitulo et al., 2017a,b)*. In particular snoRNAs, Wnt signaling factors, Growth Factors and their receptors, stem cell markers, and markers of immune cells, lymphocytes and macrophages in particular. The latter will be the main focus of the research, since the lizard blastema appears to be an immuno-evasive organ while the limb elicits a strong inflammation that hampers regeneration.

The detection and localization of antigens involved in immuno-evasion/immuno-differentiation under normal and experimental conditions will allow to address the process that impedes rejection of the regenerative blastema. I have evaluated the cross-reactivity of the available antibodies from ImmunoTools with the lizard antigens, where these are known from the database, in order to select those more likely cross-reactive, and potentially implicated in the maturation of B- and T- cells. Another aspect of the research will deal with the evaluation of the effect of growth factors and cytokines in stimulating limb regeneration in relation to inflammation. In particualr FGF but also EGF, BMP, TNF, their receptors etc, appear the main factors stimulating tail and limb regeneration, and cartilage formation in lizards *(Alibardi L, 2017. Review...regeneration in amniotes. J Exp Zool B, submitted)*.

Therefore the antibodies, cytokines and growth factors will be utilized to analyze the tissue localization of these proteins and will be administred to lizards to check for their effect during regeneration in the tail or scarring in the limb.