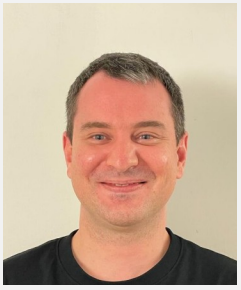
ImmunoTools *special* Award 2021



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Impact of immune cells on regeneration of skeletal muscle in age and disease

We live in an aging society, and increasing aging-associated pathologies are inevitable; thus, improving clinical treatment options is of high priority. Skeletal muscle is one of the affected tissues, making up to 40 % of the total body weight. Tissue-resident muscle stem cells (MuSCs), also called satellite cells because of their adjacent position on the myofibers, confer the remarkable ability to regenerate injured muscle tissue. However, during aging and aging-associated skeletal muscle pathologies, such as sarcopenia or cancer cachexia, there is a decline in the number and functionality of MuSCs, leading to impaired regenerative responses, which ultimately correlates with muscle loss and fragility in the elderly, thereby significantly contributing to co-morbidity.

Previous work identified cell intrinsic and extrinsic factors controlling MuSC functionality and thereby success rate of regeneration. MuSCs maintain a state of quiescence under homeostatic conditions but can rapidly activate upon stimuli, such as injury. Some activated MuSCs will self-renew to replenish the stem cell pool for future demands, whereas others start to proliferate and undergo myogenic differentiation, including fusion and formation of new myofibers to repair the damaged tissue. The process of muscle regeneration is complex and involves not only MuSC´s autonomous genetic programs but also the rich interaction with various cell types, e.g., fibro-adipogenic progenitors (FAPs) and cells of the innate and adaptive immune system. In fact, immune cell infiltration is amongst the first responses to muscle damage being essential to initiate the regenerative program. Neutrophils and macrophages are rapidly recruited to the injury site, initially secreting pro-inflammatory cytokines like IL-1β or TNF-α to attract further immune cells and helping clear cell debris, and then undergo a functional switch, secreting different factors like IL-10 to promote MuSC activation and proliferation. The function of the immune system changes in conditions such as aging and age-associated pathologies, including shifts in cell populations and their secreted factors thereby contributing to impaired regeneration under the aforementioned conditions.

Our group strives to identify novel approaches to combat the aging-associated decline of MuSC functionality in the process of muscle regeneration. In future experiments, we will focus on the intercellular communication of MuSCs with other cell types in the regenerative milieu. Therefore, we employ experimental models of myotoxin-induced muscle damage and the subsequent analysis of muscle regeneration comparing young and old mice [1], as wells as tumor-bearing cachectic mice [2]. We will use the awarded reagents from ImmunoTools to quantitatively determine the contribution and dynamics of immune cell populations ($CD45^+CD3^+CD4^+CD8^-$ CD4 T-cells, $CD45^+CD3^+CD4^-CD8^+$ CD8 T cells, $CD45^+CD11b^+F4/80^+MHCII^{hi}CD86^+$ M1 macrophages, $CD45^+CD11b^+F4/80^+CD206^{hi}$ M2 macrophages, $CD45^+CD11b^+Ly\text{-}6G^+$ neutrophils) to the muscle regeneration by flow cytometry thereby comparing different mouse ages (young, old, geriatric) and disease states (healthy versus cachectic). Moreover, isolated single myofiber-associated MuSCs [3], as well as primary myoblasts ($CD45^-CD11b^-CD31^-Sca\text{-}1^-alpha7\text{-}integrin^+$) or FAPs ($CD45^-Sca1^+CD11b^-CD31^-alpha7\text{-}integrin^-$) will be treated with ImmunoTools recombinant cytokines like TNF-α and IL-1β to investigate possible direct effects of immune cell-secreted factors on MuSC proliferation and differentiation or FAPs activation.

The ImmunoTools award will support our research by providing critical reagents to identify mediators of skeletal muscle regeneration and to identify novel strategies counteracting the age-associated decline in MuSC function.

References:

[1] Ahrens, H.E., H. Henze, S.C. Schuler, M. Schmidt, S.S. Huttner, and J. von Maltzahn. 2019. Analyzing Satellite Cell Function During Skeletal Muscle Regeneration by Cardiotoxin Injury and Injection of Self-delivering siRNA In Vivo. *J Vis Exp.*

[2] Schmidt, M., C. Poser, and J. von Maltzahn. 2020. Wnt7a Counteracts Cancer Cachexia. *Mol Ther Oncolytics*. 16:134-146.

[3] Huttner, S.S., C. Hayn, H.E. Ahrens, M. Schmidt, H. Henze, and J. von Maltzahn. 2021. Single Myofiber Culture Assay for the Assessment of Adult Muscle Stem Cell Functionality Ex Vivo. *J Vis Exp*.

ImmunoTools *special* AWARD for **Soeren Huettner** includes 10 reagents

FITC - conjugated anti-mouse CD3e, CD45, isotype control IgG2b

PE - conjugated anti-mouse CD4, isotype control IgG2b

APC - conjugated anti-mouse CD8a, CD11b, isotype control IgG2

recombinant mouse cytokines: rm IL-1β, rm TNF-α

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