6. Conclusion

6.1 α-Mannosidase I

N-linked protein glycosylation represents an important cellular process for modifying protein properties. It resembles a cascade of various enzymatic reactions, in which class I α–mannosidases play a central role. It is well established that N-glycosylation plays a major role for immune functions. Interestingly, Sawitzki and colleagues identified α-mannosidase I as being highly expressed in graft infiltrating cells of tolerance developing recipients. I now studied the expression and function of α-mannosidase I in total $CD4^+$, naïve and memory T cells by analysing α-mannosidase I transcription and activity. Alpha-mannosidase I function was altered by i) treatment with Kifunensine, a specific inhibitor for class I α-mannosidases, ii) down-regulation of α-mannosidase I gene expression using siRNA transfection, and iii) over-expression utilising retroviral gene transfer. T cell activation was evaluated studying CD69 expression, IL-2 and IFN-γ production. My results demonstrate that α-mannosidase I transcription is transiently down-regulated following T cell activation, and that α-mannosidase I exerts an inhibitory effect on T cell activation. Most interestingly, these effects were restricted to naïve $CD4^+$ T cells, while memory cells remained nearly unaffected. Thus complex N-glycans generated by enzymes such as α-mannosidase I inhibit the activation of naïve T cells. These findings could be used to improve the *ex vivo* priming of naïve T cells for adaptive T cell therapies.

6.2 Receptor for hyaluronan-mediated motility (RHAMM)

Sawitzki and colleagues identified the receptor for hyaluronan-mediated motility (RHAMM) as being highly expressed in transplantat infiltrating leucocytes of acutely rejecting recipients. I now studied the expression and function of RHAMM during T cell activation *in vitro* and *in vivo*. My results demonstrate that RHAMM transcription is highly up-regulated during T cell activation. Furthermore, inhibition of RHAMM expression utilising siRNA transfer resulted in decreased IFN-γ expression by T cells as well as diminished their migratory potential and effector capacity in a model of transfer colitis. The results also show that in the murine system more than three RHAMM isoforms are transcribed. However, their transcriptional regulation and role during T cell activation are still unknown. Using siRNA technology transcription of RHAMM can be suppressed, providing a possibility for a more detailed examination of its role during T cell activation.