Summary of the Dissertation

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The transmembrane adaptor protein SIT regulates T-cell development and homeostasis

In this study, I characterised mice lacking the transmembrane adaptor protein SIT. I found that the loss of SIT resulted in altered T-cell development. Indeed, thymocytes deficient for SIT displayed a more activated phenotype and an enhanced positive selection that was even partially converted to negative selection in HY and P14 TCR transgenic mice. However, $SIT^{-/-}$ mice expressing a TCR with high affinity, such as OT-I, did not show any alteration of T-cell development. This indicates that the ability of SIT to regulate thymic selection processes is dependent on TCR affinity. Moreover, SIT knockout mice displayed a decreased number of SP thymocytes as well as a decreased proportion of recent thymic emigrants in the periphery. In addition, further investigation revealed that $SIT^{-/-}$ mice have strongly reduced number of naïve $CD8^+$ T cells. We showed that the loss of naïve $CD8^+$ T cells is neither due to a survival defect nor to enhanced apoptosis but it is partially caused by a reduction in the generation of T-cell precursors in the thymus. Moreover, SIT-deficient mice progressively accumulate $CD44^{hi}CD8^+$ cells in the periphery. A more detailed characterisation of those cells revealed that they closely resemble lymphocytes undergoing homeostatic proliferation. To directly test the role of SIT in T-cell homeostasis, I performed adoptive transfer experiments by injecting CFSE labelled lymph node cells from MHC-I restricted transgenic mice with low (HY), intermediate (P14) and with high (OT-I) TCR affinity into lymphopenic hosts. Strikingly, loss of SIT results in homeostatic expansion of HY transgenic T cells that normally do not undergo homeostatic proliferation in lymphopenic hosts. Similarly, $SIT^{-/-}$P14 T cells showed a more accelerated homeostatic expansion upon adoptive transfer into lymphopenic recipients as compared to $SIT^{+/+}$P14 cells. Similar to T-cell development, lymphopenia-induced proliferation of OT-I $CD8^+$ T cells was not affected by the loss of SIT. These results clearly indicate that SIT is a potent negative regulator of both T-cell development and homeostasis. Our data also demonstrate that the reduced number of naïve $CD8^+$ T cells in SIT-deficient mice is likely due to an accelerated homeostatic expansion. During this process, $CD8^+$ T cells acquire $CD44^{hi}$ expression. In addition to defective T-cell development and homeostasis, $SIT^{-/-}$ T cells are hyperreactive to TCR-mediated stimuli *in vitro* and develop more severe EAE. Collectively, these observations clearly indicate that loss of SIT results in a lowered threshold of activation in both thymocytes and T cells. Despite the fact that SIT-deficient mice displayed a perturbed T-cell homeostasis, loss of SIT did not result in any obvious sign of altered immune function in young mice. Thus, we hypothesised

a presence of a compensatory mechanism(s) that would prevent SIT deficient T cells from becoming fully activated.

Indeed, I showed that $SIT^{-/-}$ $CD8^{+}$ T cells develop sensory adaptation by adjusting coreceptor and CD5 expression. Moreover, additional data from our laboratory showed that in vitro Zap-70 activity is severely impaired in SIT deficient T cells. It is likely that compensatory mechanism(s) present in $SIT^{-/-}$ T cells is (are) sufficient to prevent T cells from becoming fully activated. However, this mechanism fails to prevent altered T-cell development, lowered threshold of activation as well as altered peripheral T-cell homeostasis.

Collectively, I showed that SIT acts as a negative regulator of T-cell development and homeostasis by modulating TCR signalling threshold.