STUDIES ON THE RECONSTITUTION OF T CELL PRODUCTION IN ZAP-70 DEFICIENT MICE

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The ZAP-70 (70 kDa Zeta-Chain Associated Protein) kinase plays a crucial role in the signal transduction by the antigen receptor during T cell activation. It is essential in T cell differentiation as well, in its absence, T cell maturation is blocked in the double positive ($CD4^+CD8^+$) stage in the thymus, leading to the complete lack of mature αβ T cells in the periphery which results in severe immunodeficiency in both human and mice.

T cell maturation could be stably restored in ZAP-70 deficient mice by simple intraperitoneal (ip.) injection of thymocytes isolated from their wild type (ZAP-70 expressing) siblings. T cells appeared in the blood and lymphoid organs of the transferred mice and, importantly, the survival of the animals increased significantly, which clearly demonstrated the correction of the severe immunodeficiency.

In our present work investigated the characteristics and kinetics of the T cell reconstitution by monitoring the changes in the morphology and cellular composition of the thymus and peripheral lymphoid organs.

Following the thymocyte transfer of $ZAP\text{-}70^{-/-}$ mice the histology of the thymus and the appearance of T cells in the periphery were analyzed regularly. Flow cytometry revealed that 3 weeks after the ip. thymocyte transfer αβ T cells appeared in significant numbers in the blood, spleen and lymph nodes of the animals. This was preceded by the appearance of mature $CD4^+$ or $CD8^+$ cells in the thymus 2 weeks after the transfer. Using quantitative immunohistology we have found that the area of the medullary region increased after the transfer, which also indicated the normalisation of T cell maturation.

In future experiments, we plan to test the *in vivo* effects of ZAP-70 point mutations using ZAP-70 deficient mice. We will reconstitute the ZAP-70 molecule under the control of T cell specific promoter(s) into T cell precursors of ZAP-70 deficient mice. Using various mutant forms of ZAP-70 or the normal molecule we can investigate the effects on T cell development and function. As an important preliminary step, we have cloned the T cell specific proximal- and distal Lck promoters and a CD4 minimal promoter construct. The T cell-specific activity of these promoter constructs was verified on cell lines using a lentiviral expression system.

We have managed to correct the severe T cell deficiency using adoptive thymocyte transfer. According to our results the transferred T cell progenitors with normal ZAP-70 expression level can induce long-term normalisation of T cell maturation. Combined with the T cell-specific expression of ZAP-70 variants we will possess an excellent tool to study T cell differentiation and function.

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