7 Summary

Effects of latent membrane protein 2A expression und constitutive nuclear factor-κB activity on B cell development in mice

Hodgkin lymphoma are among the most frequent malignant lymphoid disorders. The Hodgkin/Reed Sternberg (H/RS) cells, the malignant cells of the lymphoma, are B cell derived. The molecular pathogenesis of these cells is unknown to date. Common features of more than 50 % of H/RS cells are the constitutive activity of the transcription factor *Nuclear Factor* (NF) -κB und the expression of the Epstein-Barr virus latent membrane protein 2A (LMP2A).

Aim of this study was to establish a mouse model with constitutively active NF-κB und LMP2A expression. The effects of these factors on B-cells in mice were investigated.

To establish the model modified fetal haematopoietic stem cells were transplanted into B cell deficient recipients. The donor haematopoietic stem cells were extracted from foetuses with a knockout of the NF-κB-inhibitor IκBα. Before transplantation they were transfected with a retrovirus encoding for LMP2A. Haematopoietic stem cells that were infected with the LMP2A retrovirus from wildtype IκBα foetuses served as controls. Mice with defined genetic changes in their B cell population were analysed.

Five weeks after transplantation the recipients showed constitutive NF-κB activity und LMP2A expression in haematopoietic cells. This model enabled us to analyse effects of permanent NF-κB activity und expression of LMP2A in combination *in vivo*.

Flowcytometric analysis showed lower fractions of B cells when LMP2A was expressed. Additionally the fraction of high IgM expressing B cells decreased. Transcriptional downregulations of B cell specific differentiation factors EBF und Pax5 could be the reasons for this. In EBF und Pax5 knockout mice B cell development is blocked in the pro-B cell stage.

Additionally, proliferation assays demonstrated decreased proliferative responses of LMP2A expressing B-cells to B cell specific stimuli. The inhibition of proliferation und the down regulation of the transcription of B-cell specific transcription factors (EBF und Pax5, shown in RT-PCR analysis) revealed deregulated differentiation und proliferation of the B-cells.

Permanent NF-κB activity increased B-cell proliferation after stimulation. That means it lead to advantages in survival compared to cells with physiological NF-κB activity.

NF-κB as well as LMP2A increased transcription of the receptor und transcription factor Notch1. It was already shown that Notch1 is active in H/RS cells und provides proliferative und antiapoptotic effects. NF-κB und LMP2A might use the Notch1 signalling pathway to protect B-cells from cell death.

This mouse model enabled us to show that permanent NF-κB activity und LMP2A expression are not sufficient to turn B-cells malignant like H/RS cells. Nevertheless, analysis demonstrated strong effects on B-cells und their transformation. NF-κB increases the tumor potentials of B-cells by increasing proliferation. Both factors decrease transcription of the transcription factors EBF und Pax5.