8 Summary

8.1 Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells

During antigenic stimulation at non-mucosal sites, the formation of plasma cells takes place in secondary lymphoid tissues. Later, a fraction of these cells can accumulate in bone marrow or inflamed tissues. In mice expression of CXCR4 is important for plasma cell accumulation in the bone marrow. Ligands for CXCR3 are expressed in inflamed tissues and can mediate plasma cell homing to those sites.

In this study the expression and regulation of the chemokine receptors CXCR3 and CXCR4 during terminal differentiation of human memory B cells into plasma cells was analyzed.

B cells were defined as CD19 positive cells. Memory B cells could be distinguished from naïve B cells by the expression of CD27. Plasmablast and plasma cells are CD38(++)/CD20(-). By flow cytometry it could be shown in more than ten experiments that CXCR3 is absent on naive B cells, but is expressed on a fraction of memory B cells. CXCR4 is expressed by the great majority of all B cells. To specify the CXCR3 expressing memory B cells in more than 10 experiments a significant correlation of the co-expression of CXCR3 and IgG1 could be shown which was absent in the case of CXCR4. In six samples the greater part of bone marrow plasmablasts expressed CXCR4, whereas CXCR3 was mainly expressed on plasmablasts of blood of eleven donors and inflamed secondary lymphoid organs like in seven tonsils and three samples of inflamed mucosa of the gut. These expression pattern leads to the assumption that in humans CXCR4 and CXCR3 are also involved in homing into the bone marrow or inflamed tissue, respectively. To analyse the regulation of the expression of CXCR3 and CXCR4 during differentiation of memory B cells, human B cells were T dependently and T independently activated into plasma blasts with various methods. Therefore purified B cells were co-cultured with the constitutively CD40L expressing cell line EL4B5 in the presence of IL-2 and IL-10. After three days the EL4B5 cells were depleted and the B cells further cultured for five more days with IL-2 and IL-10 by reaching the plasma cell phenotype CD38(++)/CD20(-). Secretion of antibodies was tested in ELISpot assay. The expression of CXCR3 and CXCR4 was studied on day 3 and day 8. B cells were activated T independently by Staphylococcus aureus Cowan I or CpG 2006 by triggering the B cell receptor or toll-like receptor 9, respectively. For the analysis of the regulation of CXCR3 inflammatory as well as Th1 and Th2 derived cytokines were added at various time points. In

13 experiments it could be shown that B cells up-regulated CXCR3 when co-stimulated with the Th1 cytokine IFN-γ during the first three days of culture. In the course of an immune response *in vivo*, before day 3 after antigenic challenge, B cells are found in the lymph nodes adjacent to T helper cells. The addition of the Th2 cytokine IL-4 as well as various inflammatory cytokines did not alter the frequency of CXCR3 expressing cells. In correlation IFN-γ could also enhance the frequency of migrating plasma blast towards the CXCR3 ligand CXCL9 shown insix chemotaxis assays . During their differentiation into plasma cells, five experiments showed that CXCR3(+) and CXCR3(-) memory B cells remained stable in the expression of this receptor. Indicating that once induced in memory B cells, CXCR3-Expression remains part of the individual cellular memory. The results presented in this study suggest that the following scenario is at least one mechanism leading to the accumulation of plasma cells within inflamed tissues. Th1 cells can stimulate activated B cells specific for the same antigen to express CXCR3, thus supporting their accumulation within the adjacent inflamed tissue (Fig.24). Plasma cell homing to bone marrow is a specific feature of T-dependent immune responses. As it could be shown in more than three experiments that differentiation of CXCR4(-) B cells is generally accompanied with the expression of this chemokine receptor, the expression of CXCR4 is not a T dependent phenomenon. Although CXCR4 is crucially involved in accumulation of plasma blasts in the bone marrow, these results indicate that the accumulation is not modulated about CXCR4, exclusively. Whereas there was no regulatory factor found for the expression of CXCR4, CXCR3-Expression was induced, precisely. The identification of IFN-γ as an inducing factor for expression of CXCR3 and it's ligands leads to better understanding of the accumulation of plasma cells in chronically inflamed tissue. On the long run this migration could be inhibited and function as therapy for autoimmune diseases as systemic lupus erythematosus or rheumatoid arthritis**.**