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Summary

Chemokines are a family of small chemotactic cytokines (8–15 kDa) that direct the migration of cell types that express the corresponding receptors. Chemokines are classified into four subgroups (CXC, CC, C, and CX3C) dependent on the presence and position of conserved cysteine residues. They are best known for their functions in development and activation of the immune system. In recent years, the zebrafish embryo model has emerged as an important platform to study the function of the innate immune system in infectious diseases. The clear temporal separation of development of the innate and adaptive immune system makes the zebrafish embryo model a convenient system to study the contribution of innate immune cells to host-pathogen interactions. In this thesis, we have employed *Salmonella typhimurium* and *Mycobacterium marinum* infection models to study CXC chemokine signaling, particularly focusing on the identification of bacterial-induced chemokines and the functional analysis of a chemokine receptor gene *cxcr3.2*.

In **chapter 1**, we give a general introduction on the innate immune system of zebrafish and the tools and methods used for visualizing specific immune cell populations in embryos. The interaction of embryonic innate immune cells (macrophages and neutrophils) with infectious microbes can be excellently traced in the optically transparent zebrafish embryos. Various methods have been developed for visualizing and isolating these innate immune cells, for establishing infections by different micro-injection techniques, and for analyzing the host-microbial recognition in the innate immune response. We describe recent strategies to achieve systemic or local infection of embryos with bacterial pathogens, and we discuss quantification methods to analyze bacterial burden at low- or high-throughput levels. We also discuss microarray and deep sequencing technologies for characterizing global gene expression patterns of immune cells and responses to infections. Finally, recent functional studies of key factors in the innate immune system of zebrafish embryos are summarized in this chapter.

In **chapter 2**, we used a microarray strategy to identify genes that are expressed in embryonic immune cells and that directly or indirectly depend on Spi1, a transcription factor which is crucial for development of cells of the myeloid and lymphoid lineages. By microarray-based expression analysis we identified a group of 249 putative genes that are down-regulated in *spi1* knockdown embryos and simultaneously enriched in myeloid cells obtained from embryos of *spi1:GFP*

transgenic zebrafish by fluorescence-activated cell sorting (FACS). This gene group included many known genes with roles in hematopoiesis and immune system development as well as novel genes with immune-related functions. We demonstrated four of these genes (*cxcr3.2*, *mfap4*, *mpeg1*, and *ptpn6*) to be macrophage specific in early zebrafish embryos. Furthermore, by morpholino knockdown experiments, we showed that the function of *cxcr3.2*, the gene coding for a CXC chemokine receptor, is necessary for macrophage migration to local bacterial infection with *Salmonella typhimurium*.

In **chapter 3**, we have investigated the family of CXC chemokine genes in zebrafish and studied their phylogenetic relationships with human chemokines. Phylogenetic analysis shows a large divergence of the CXC subfamily between human and zebrafish. While zebrafish chemokines homologous to human CXCL12 and CXCL14 are the most strongly conserved, several duplications appear to have occurred for zebrafish chemokines that are related to human CXCL8 or to the human CXCL9/10/11 group. Next, we examined the expression of zebrafish CXC chemokines upon challenges with two different bacterial pathogens: *Salmonella typhimurium* and *Mycobacterium marinum*. We selected two strongly infection-inducible chemokines, Il8 and Cxcl11, for protein purification using a *Pichia pastoris* expression system and performed *in vivo* leukocyte migration studies to establish the function of these chemokines during the inflammatory response. Our data showed that Il8, one of four chemokines in zebrafish that are homologous to human IL8, has chemoattractive properties on neutrophils. It also revealed a chemoattractive function on macrophages for Cxcl11. Based on the phylogeny analysis, Cxcl11 could be a putative ligand of the zebrafish Cxcr3.2 receptor, which in chapter 2 was proposed to be involved in macrophage recruitment to local bacterial infection sites.

In **chapter 4**, we focused on the functional analysis of the Cxcr3.2 receptor. We employed a *cxcr3.2* knockout mutant to investigate the behavior of zebrafish embryonic immune cells during infections. In agreement with the morpholino knockdown results of chapter 2, we found that *cxcr3.2* knockout partially impairs bacterial-induced macrophage migration. In *S. typhimurium* and *M. marinum* infection studies, knockout of *cxcr3.2* led to insufficient macrophage recruitment to infection foci and decreased bacteria clearance. The impact of *cxcr3*.2 deficiency was most notable in local hindbrain infections with two attenuated mutant strains of *M. marinum* (ΔRD1 and FAM53), which led to a strong reduction of macrophage recruitment and extracellular growth of *M. marinum* in *cxcr3.2* mutants. In addition, local hindbrain injection of purified protein of the infection-inducible Cxcl11

chemokine resulted in the directional migration of macrophages in wild type but not in *cxcr3.2* mutant embryos. Based on these results, we could identify the Cxcl11 chemokine as a putative ligand of zebrafish chemokine receptor Cxcr3.2.

In **chapter 5**, we summarized and discussed the results and conclusions from our studies on the role of CXC chemokine-chemokine receptor interaction in bacterial-induced inflammatory responses in zebrafish embryos. An important step towards understanding the chemokine signaling network underlying innate immune responses in the zebrafish model is our identification of Cxcl11-Cxcr3.2 as the first ligand-receptor pair with a proposed function in bacterial-induced macrophage migration and control of local infections. In addition, we report in chapter 5 that a member of the CC chemokine receptor subfamily, *ccr12.3*, shows a Spi1-dependent and leukocyte-specific expression pattern in early zebrafish embryos, which is similar to that of the *cxcr3.2* gene, the main subject of this thesis. Furthermore, we obtained a broader overview of the expression of chemokine receptors and other G-protein-coupled receptors in zebrafish immune cells by investigating RNA deep sequencing data sets of macrophages, neutrophils and immature T-cells from zebrafish larvae, These RNA-seq data have provided a clear view on the primary candidate genes for functional studies of the role of chemokine receptors in coordinating immune cell responses in zebrafish embryo and larval models.