Chemokine signaling in innate immunity of zebrafish embryos

Cui, C.

Chemokine Signaling in Innate Immunity of Zebrafish Embryos

Chao Cui
崔超

ISBN: 978-94-6203-205-7
Printed by Wöhrmann Print Service, Zutphen

Chemokine Signaling in Innate Immunity of Zebrafish Embryos

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 20 december 2012
klokke 15:00 uur

door

Chao Cui

Geboren te Tianjin, China
in 1982

Promotiecommissie

Promotor:	Prof. dr. H.P. Spaink
Co-promotor:	Dr. A.H. Meijer
Overige Leden:	Prof. dr. C.J. ten Cate
	Prof. dr. A.P. IJzerman
	Dr. B.E. Snaar-Jagalska
	Prof. dr. M.J. Smit (Vrije Universiteit Amsterdam)
	Dr. A.E. Proudfoot (Merck Serono)

The publication of this thesis was sponsored by ZF-screens B.V.

“If you can concentrate always on the present, you'll be a happy man...Life will be a party for you, a grand festival, because life is the moment we're living right now”

Paulo Coelho

To my dear parents and Wen
献给我亲爱的父母和妻子

Contents

Outline of the thesis

Communication between cells is essential for the proper functioning of the immune system. Chemokines, which are a group of small secreted proteins, play an important role in this communication process. Cells that express the appropriate receptors can migrate along a gradient towards a source of chemokine, a process called chemotaxis. Transparent zebrafish embryos are very suitable to visualize migration of immune cells and study the role of chemokines and their receptors in this process. The first immune cells that develop in one-day-old zebrafish embryos are the macrophages. When the embryos are injected with bacteria, these macrophages respond immediately by migrating towards the site of infection, where they will engulf the bacteria and try to eliminate them by activating anti-microbial defense mechanisms. Different steps in this infection process can be followed in detail over time by marking the embryo's immune cells and the bacteria with different fluorescent colors. Furthermore, efficient knockdown and knockout techniques can be applied in zebrafish for functional studies of genes of interest. In this thesis, the zebrafish model is used to study the function of infection-related chemokines and an early macrophage-specific chemokine receptor, Cxcr3.2.

In **chapter 1**, an introduction is given on the innate immune system of zebrafish and the tools and methods used for visualizing specific immune cell populations in embryos. We describe various 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, we review recent functional studies of key factors in the innate immune system.

In **chapter 2**, we used a microarray strategy to discover genes that are expressed in embryonic immune cells and that directly or indirectly depend on Spi1, a transcription factor required for immune cell development. We identified a gene group that was down-regulated in *spi1* knockdown embryos and simultaneously enriched in immune cells obtained by fluorescence activated cell sorting using embryos of *spi1*:*GFP* transgenic zebrafish. This gene group contains many immune-related genes, including a chemokine receptor gene, *cxcr3.2*. We demonstrate this gene to be macrophage-specific in early zebrafish embryos. Furthermore, by morpholino knockdown experiments we show that the function of

cxcr3.2 is necessary for macrophage migration to local bacterial infection with *Salmonella typhimurium*.

In **chapter 3**, we have analyzed the family of CXC chemokine genes in zebrafish and studied their phylogenetic relationships with human chemokines. We investigated the expression of CXC chemokines upon two different bacterial challenges: *S. typhimurium* and *Mycobacterium marinum*. Two chemokines genes that were strongly induced by bacterial infections were selected for protein purification using a *Pichia pastoris* expression system, and subsequently used for leukocyte migration studies in zebrafish embryos. One of the purified chemokines, a homolog of human IL8, showed chemoattractive properties on neutrophils. The second chemokine, which is more closely related to human CXCL11, was capable of attracting embryonic macrophages.

In **chapter 4**, we employed a *cxcr3.2* knockout mutant to investigate the function of *cxcr3*.2 in the behavior of zebrafish embryonic immune cells. In agreement with the morpholino knockdown results of chapter 2, we show that *cxcr3.2* knockout partially impairs bacterial-induced macrophage migration. In *S. typhimurium* and *M. marinum* infection studies this leads to insufficient macrophage recruitment to infection foci and decreased bacteria clearance. Injecting purified protein of an infection-inducible chemokine with similarity to human CXCL11 resulted in the attraction of macrophages in wild type but not in *cxcr3.2* mutant embryos. Based on these results, we could identify this chemokine as a putative ligand of Cxcr3.2 and propose that the Cxcl11-Cxcr3.2 ligand-receptor interaction is important for macrophage migration in inflammatory responses during bacterial infection.

In **chapter 5**, we discuss the results and conclusions from our studies on the role of CXC chemokine-chemokine receptor interaction in bacterial-induced inflammatory responses in zebrafish embryos. In addition, we report 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 obtain a broader overview of the expression of chemokine receptors in zebrafish immune cells by investigating RNA deep sequencing data sets of macrophages, neutrophils and immature T-cells from zebrafish larvae. Taken together, the studies in this thesis have demonstrated a function for chemokine receptor *cxcr3.2* in bacterial-induced macrophage migration and have provided a solid basis for further analysis of chemokine signaling in the immune system of zebrafish.