Summary

Among other cells, macrophages, neutrophils and mast cells provide immediate host defence against invading pathogens. Upon infection with the Gram-positive bacterium *L. monocytogenes* (*L.m.*), they initiate extracellular or cytoplasmic inflammatory signalling, leading to transcription of cytokines and expression of microRNAs (miRNAs). Tight regulation of the inflammatory response and successful elicitation of adaptive immunity is necessary for bacterial clearance and survival of the host. MiRNAs represent an important mechanism of post-transcriptional gene regulation in inflammation. Canonical miRNA biogenesis is dependent on the action of RNaseIII endonucleases in the nucleus and the cytoplasm, namely Drosha/DGCR8 complex and Dicer, respectively. In this thesis, we analysed how Dicer or DGCR8 regulate myeloid cell development and function in infection, using new transgenic mouse models with conditional (LysMcre, Mcpt5cre) or interferon-inducible (Mx1cre) deletion of Dicer or DGCR8.

Our findings showed, although there were no alterations in monocyte, macrophage and neutrophil populations *in vivo* upon LysMcre-mediated deletion of Dicer (Dicer^{MYEL}KO), in Dicer$^{fl/fl}$Mx-cre mice significant reduction in peritoneal macrophages and peripheral blood neutrophils compared to wild-type littermates. *In vitro*, we found that BMDMs could differentiate in the absence of Dicer, mediated either by LysMcre or Mx1cre, but cell numbers were lower than wild-type cultures in both models, suggesting that miRNAs are important for development or cell survival.

Upon *L.m.* infection in Dicer$^{fl/fl}$Mx-cre mice, we saw a significant reduction in peritoneal macrophages and an increase in monocyte recruitment compared to wild-type littermates. To validate that these effects are due to impaired miRNA biogenesis, and not due to the absence of other Dicer-dependent non-coding RNAs, we analysed the response of DGCR8$^{fl/fl}$Mx-cre mice, in which primary miRNA transcripts are not processed in the nucleus after administration of polyI:C. Our preliminary data confirmed that Mx1cre-mediated DGCR8 deletion results in ablation of peritoneal macrophages upon *L.m.* infection, supporting the evidence that miRNAs regulate myeloid cell development and/or survival.

Interestingly, Dicer deletion led to significant increase in the production of cytokines analysed in serum of infected Dicer^{MYEL}KO compared to infected controls, indicating that Dicer-dependent miRNA biogenesis may be necessary to negatively regulate the inflammatory response against *Listeria*. Furthermore, Dicer deletion mediated by either of the mentioned Cre recombinases caused significantly higher bacterial burden in spleens and livers upon infection. However, unlike Dicer^{MYEL}KO, Dicer$^{fl/fl}$Mx-cre mice could not clear systemic infection, suggesting that miRNA biogenesis in interferon-responsive haematopoietic cells is vital for host survival. In line with this finding, we could also demonstrate that Dicer expression regulates monocyte recruitment to the peritoneum of *L.m.* infected mice, which may in turn regulate inflammatory cytokine production and elicitation of adaptive immunity necessary for clearance of infection.

Finally, we studied the role of Dicer-dependent miRNA biogenesis in mast cells upon Mcpt5-mediated Dicer deletion *in vivo* in collaboration with the group of Prof. K. Hartmann and found that Dicer$^{fl/fl}$Mcpt5-cre mice are almost completely ablated of connective tissue mast cells. Our data showed that Dicer is important regulator of mast cell development (Förster et al., 2015). Moreover, the new specific mast-cell deficient mouse model, which emerged from this project was used in this study to investigate how absence of mast cells affects host-pathogen interactions. Our results suggest that mast cells are dispensable for *Listeria* clearance *in vivo*.

Taken together, our results suggest that canonical miRNA-biogenesis is necessary for myeloid cell development and function, disruption of which compromises homeostasis and immunity.