Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes

Project: Transcriptom Analysis to Determine the Primary Site of Immunity and the Role of Epithelial and Hematopoietic Cells for Host Susceptibility During Infection with *Helicobacter pylori*

Michael Lohoff, Christine Rosenplänter - Phillips University, Marburg - lohoff@post.med.uni-marburg.de

Introduction

Helicobacter pylori is a gram-negative human pathogen, which colonizes the stomach of about half of the world's population. The infection with *H. pylori* is associated with the risk of different gastroduodenal diseases comprising atrophic gastritis, duodenal ulcer, gastric cancer, and MALT-lymphoma. Despite extensive investigations in 20 years, only little is known about the pathogenesis of the resulting diseases. Virulence factors (e.g. CagA and urease) as well as the immune response of the host might play a role.
Because of its flagella, *H. pylori* is able to cross the gastric mucosa and adhere to the epithelial cells. The host reacts with a local inflammation, the release of chemotactic molecules and attraction of polymorphonuclear cells. In humans as well as in the established mouse models, infection with *H. pylori* is followed by the development of a Th1 immune response (1, 2). Despite this strong immune reaction against *H. pylori*, the host is not able to clear the *H. pylori* infection.
The generated Th1 effector cells might be responsible for the *H. pylori* associated pathological alterations. As a result, *H. pylori* infected C57/BL6 mice show a high infiltration of polymorphonuclear leukocytes and lymphocytes, formation of lymphoid follicles, and metaplasia (Fig. 1A). In contrast, *Helicobacter* infected RAG-deficient and SCID mice display normal gastric mucosa (3, 4). Similarly, no gastritis was seen in infected mice deficient in the transcription factor interferon-regulatory factor-1 (IRF-1) (Fig. 1B) (5). IRF-1 is involved in the transcription of different genes, e.g. IL12, IL18, IL23 and iNOS, regulating the TH1 immune response (6).

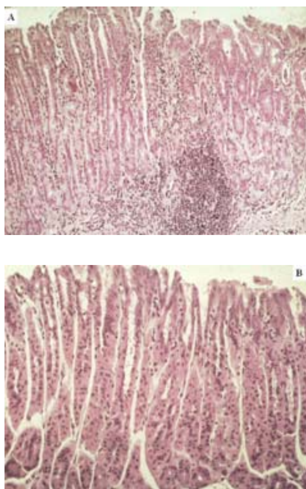

Fig 1: *Histological preparation (staining with hematoxylin-eosin) of the gastric mucosa of C57/BL6 (A) and IRF-1$^{(-/-)}$ (B) mice infected with H. pylori SS1 after 4 months (5).*

The aim of this project is to identify the primary site of immune response during a *H. pylori* infection by using transcriptom analysis. The comparison of gene expression patterns of infected wildtype and IRF-1$^{(-/-)}$ mice will shed light on marker genes for a gastritic course. Thereafter, we will create bone marrow chimeras to study which cells are responsible for the respective gene patterns in these mice. Lastly, we will compare the respective gene patterns with those observed after infection with the intestinal pathogen *Yersinia*.

Results/Project Status

The first part of the project is to determine the primary site of the immune response in *H. pylori* infected C57/BL6 wildtype mice. For the infection studies, we used the mouse-adapted *H. pylori* Sydney strain (SS1), which shows high levels of colonization and causes chronic active gastritis (7). At different time points (e.g. 12 h, 48 h, 4 d), we sacrificed the mice and determined the bacterial load of *H. pylori*. Furthermore we collected different immune organs (e.g. Peyers patches, stomach, mesenterial lymph nodes) to define the level of the proinflammatory cytokines TNFα and IL12p40 as marker cytokines for a primary immune response. One-third of the stomach was analysed by histological investigations. In addition to the early time points, we infected mice for a period of 3,5 months to verify that the used *H. pylori* strain was able to cause gastritis.
The quantification of the proinflammatory cytokines is performed by TaqMan-real-time-PCR. To get reliable and reproducible results we had to establish a protocol. Therefore, we tested different endogenous mRNA controls in the collected *H. pylori* infected immune organs in comparison to the uninfected immune organs to exclude regulation of the house-keeping gene during infection. The most promising endogenous control is HPRT (hypoxanthine phosphoribosyltransferase), because it shows minor variation between uninfected and infected conditions. To provide for reproducibility, we perform quantification using the standard curve method.
Presently, we analyse the prepared cDNA of the immune organs of the first time points. Within a short time there will be results available!

Outlook

After localization of the site of the primary immune response we will perform microarray analysis to get gene expression profiles in C57/BL6 and IRF-1$^{(-/-)}$ mice. The profiles should give information about which gene expression patterns correlate with a susceptible or resistant course of the *H. pylori* infection.
A further part is the identification of the cell populations that are responsible for mediating gastritis and an immune response against *H. pylori*. Therefore, we will generate bone marrow chimeras and reconstitute them by bone marrow of either wild-type mice or mice deficient in particular cell populations.
Finally the patterns of gene expression of *H. pylori* infected mice will be compared to the respective patterns seen in the same immune organs after infection with *Yersinia enterocolitica*.

*Lit.: **1.** D'Elios MM et al. T helper 1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease. J. Immunol. 1997; 158: 962-967. **2.** Smythies LE et al. Helicobacter pylori-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFNγ, gene-deficient mice. J. Immunol. 2000; 165: 1022-*

NGFN Nationales Genomforschungsnetz

GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

1029. ***3.*** *Roth KA et al. Cellular immune responses are essential for the development of Helicobacter felis-associated gastric pathology. J. Immunol. 1999; 163: 1490-1497.* ***4.*** *Eaton KA et al. Murine splenocytes induce severe gastritis and delayed-type hypersensitivity and suppress bacterial colonization in Helicobacter pylori-infected SCID mice. Infect. Immun. 1999; 67: 4594-4602.* ***5.*** *Sommer F et al. Lack of gastritis and of an adaptive immune response in interferon regulatory factor-1-deficient mice infected with Helicobacter pylori. Eur. J. Immunol. 2001; 31: 396-402.****6.*** *Lohoff M & Mak TW. Roles of interferon-regulatory factors in T-helper-cell differentiation. Nat. Rev. Immunol. 2005; 5: 125-135.* ***7.*** *Lee A et al. A standardized mouse model of Helicobacter pylori infection: introducing the Sydney strain. Gastroenterology 1997; 112: 1386-1397.*