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Toll-Like Receptor–Dependent Activation of Antigen-Presenting Cells Affects Adaptive Immunity to *Helicobacter pylori*

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Background & Aims: **Recognition of infection leads to induction of adaptive immunity through activation of antigen-presenting cells (APCs). Among APCs, dendritic cells (DCs) have the unique capacity to deliver antigens from the periphery to T cells in secondary lymphoid organs.** ***Methods:*** **We analyzed molecular mechanisms of the *Helicobacter pylori*–induced APC activation in vitro and investigated the influence of Myd88 signaling on the phenotype of adaptive immunity to *H pylori* in a murine infection model.** ***Results:*** **The adaptor protein Myd88 mediates Toll-like receptor (TLR), interleukin (IL)-1, and IL-18 signaling. DCs from wild-type, IL-1R$^{-/-}$, and IL-18$^{-/-}$ mice responded to *H pylori* with secretion of proinflammatory cytokines and up-regulation of major histocompatibility complex II and costimulatory molecules. In Myd88$^{-/-}$ DCs these processes were impaired profoundly, showing that TLR-dependent *H pylori*–sensing affects DC activation. Analysis of the *H pylori*–specific DC transcriptome revealed that large parts of the bacteria-induced transcriptional changes depended on Myd88 signaling, comprising numerous genes involved in crucial steps of immune regulation, such as DC maturation/differentiation, antigen uptake/presentation, and effector cell recruitment/activation. The impaired ability of Myd88$^{-/-}$ DCs, B cells, and macrophages to mount a proinflammatory response to *H pylori* in vitro was reflected in vivo by reduced gastric inflammation and increased bacterial colonization in Myd88-deficient mice. Furthermore, *Helicobacter*-specific IgG2c/IgG1 ratios were reduced in Myd88$^{-/-}$ animals, suggesting the involvement of the Myd88-dependent pathway in the instruction of adaptive immunity toward a T helper cell type 1 phenotype.** ***Conclusions:*** **A principal pathway by which DCs sense *H pylori* and become activated is the TLR-dependent signaling cascade. In vivo, Myd88 signaling affects adaptive immunity to the bacterium.**

Since the paradigm-shifting discovery of *Helicobacter pylori* by Marshall and Warren[1] more than 20 years ago, the bacterium has emerged as the most successful bacterial pathogen worldwide.[2,3] *H pylori* infects more than 50% of the earth's population and almost invariably persists for life.[4] The consequence for the host is a chronic gastric inflammatory process, which in 10%–20% of infected persons leads to the development of gastric diseases such as gastric and duodenal ulceration, gastric carcinoma, or mucosa-associated lymphoid tissue lymphoma.[4,5] There is now compelling evidence that the host response to *H pylori* is a key component of the pathogenesis of gastroduodenal disease.[6] In the past 2 decades many aspects of the inflammatory response to *H pylori* have been dissected at the molecular level. There has been particular emphasis on describing the interaction of *H pylori* and its virulence factors with gastric epithelial cells. Numerous studies have provided comprehensive and fascinating insights into the epithelial signaling events induced by *H pylori* and have described the resulting implications for the development of innate immunity.[7–10] Less understood, however, are the cellular and molecular mechanisms that initiate adaptive immunity and instruct the phenotype of the T-cell response. As adaptive immunity develops, different T-helper (Th) cell subsets arise with characteristic patterns of cytokine secretion. Because *H pylori* is noninvasive and induces a systemic and a mucosal humoral response, a Th2 polarization would be expected. Paradoxically, however, in human beings and different mouse strains, *H pylori*–specific gastric T cells predominantly present a Th1 phenotype and induce cell-mediated immunity.[6] The bacterial and host components determining the fate of adaptive immunity to *H*

Abbreviations used in this paper: APC, antigen-presenting cell; BMDC, bone marrow–derived dendritic cell; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; GFP, green fluorescent protein; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MOI, multiplicity of infection; Myd88, myeloid differentiation factor; PCR, polymerase chain reaction; Th, T helper cell; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRIF, Toll/interleukin-1R domain–containing adapter inducing interferon-β.

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pylori as well as the underlying molecular processes are largely unknown.

Central to the induction of adaptive immunity are dendritic cells (DCs).[11] Among antigen-presenting cells (APCs), DCs have the unique capacity to capture antigen from the periphery and deliver it to secondary lymphoid organs where they activate naive T cells. DCs not only prime and sustain T-cell expansion, but also direct T-cell differentiation. Thus, DCs are regarded as choreographers of immune responses. They are capable of integrating signals from pathogens, leading to the generation of an adaptive immune response of the appropriate class.[11]

Sensing of microbial components occurs via pattern-recognition receptors that recognize conserved molecular patterns of potential pathogens. Of all cells of the immune system, DCs express the broadest repertoire of pattern-recognition receptors, including the Nod proteins, the Toll-like receptors (TLRs), and several C-type lectins, such as dendritic cell-specific intercellular-adhesion-molecule-grabbing non-tegrin or Dectin-1.[12] The best-characterized class of pattern-recognition receptors is the TLR family, which consists of 11 members.[13] TLRs 1, 2, 4, 5, and 6 seem to specialize mainly in the recognition of bacterial or yeast products such as lipopolysaccharide (LPS; detected by TLR4), bacterial lipoproteins, lipoteichoic acids, zymosan (detected by TLRs 2, 1, and 6), and flagellin (detected by TLR5).[13] TLRs 3, 7, 8, and 9 specialize in recognition of nucleic acids, such as the unmethylated CpG DNA of bacteria and viruses (detected by TLR9), double-stranded viral RNA (detected by TLR3), and single-stranded RNA (detected by TLR7/8).[13–15] Initiation of TLR signaling is controlled either by the adaptor molecule Myd88 (used by all TLRs, except TLR3) or by Toll/IL-1R domain–containing adapter inducing interferon-β (TRIF; used by TLR3 and TLR4). Myd88 ultimately controls, directly or via interleuken-1 receptor-associated kinase and TNF receptor-associated factor 6, activation of mitogen-activated protein kinases and nuclear translocation of the transcription factors nuclear factor κB, activator protein-1, and interferon regulatory factor 7. However, TRIF is believed to activate the type-1 interferon pathway via phosphorylation of interferon regulatory factor 3 in an interleuken-1 receptor-associated kinase/TNF receptor-associated factor 6-independent manner.[13] TLRs have emerged as key components of the innate immune response and recent studies have suggested that TLRs also are involved critically in the initiation of adaptive immunity through the control of multiple DC functions.[16]

The receptors involved in *H pylori* sensing have been studied mainly on epithelial cells. The mechanisms of *H pylori* sensing by DCs, however, are unknown. The molecules responsible for recognition of the bacterium, the signaling events, the transcriptional responses, and subcellular processes leading to DC activation are not understood. Here we show that many aspects of DCs activation in response to *H pylori* are controlled by signals from TLRs. TLR-dependent detection of infection triggered a multitude of antimicrobial and inflammatory responses in DCs and other APCs. In vivo, Myd88 deficiency influenced the severity of gastric inflammation. Finally, we found that a subset of Myd88 signaling is dedicated to the polarization of specific immunity toward a Th1 phenotype.

Materials and Methods

Bacteria

The mouse-adapted *H pylori* strain SS1 was used for mouse inoculation experiments. For stimulation of DCs we used the *H pylori* strains SS1, Hp76, and G27. Bacteria were grown at 37°C under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2) on Wilkins Chalgren agar plates (Oxoid, Basingstoke, England). Agar plates were supplemented with 10% horse serum (Gibco, Eggenstein, Germany) and a standard *Helicobacter*-selective antibiotic mixture (DENT supplement; Oxoid). For quantitative *H pylori* culture after recovery of bacteria from the mouse stomach, Wilkins Chalgren agar plates were supplemented further with bacitracin (200 μg/mL), nalidixic acid (10 μg/mL), and polymyxin B (3 μg/mL). For analysis of bacterial adherence and uptake, the *H pylori* G27 strain was transformed with a plasmid, encoding the green fluorescent protein (GFP) from *Aequorea victoria* under the transcriptional control of the *H pylori flaA* promoter. The vector was kindly provided by Dr Steffen Backert (University of Magdeburg, Magdeburg, Germany).

Infection of Mice

Wild-type mice were purchased from Harlan Winkelmann (Borchen, Germany). Myd88$^{-/-}$ mice were a kind gift from Dr S. Akira (Osaka University, Osaka, Japan), IL-1R$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and IL-18$^{-/-}$ mice were generated in-house.[17] Mouse strains were on a C57Bl/6 background. Only male mice were used for the isolation and generation of APCs as well as for in vivo experimentation. All animals were kept under specific pathogen-free conditions. Experiments were conducted according to the German animal protection law and were approved by the government of Upper Bavaria. Six-week-old male mice were infected with *H pylori* SS1 by gastric intubation with a feeding needle as described previously[18] (0.1 mL of a suspension at an optical density$_{590}$ of 5/mL; corresponding to $\sim 5 \times 10^8$ organisms). Infection was repeated twice on days 3 and 5. After 4 months mice were anesthetized with Ketamin (Gräub AG, Bern, Switzerland) and Xylacin (Xylacinhydrochloride, Bayer, Leverkusen, Germany) and blood was collected by intracardial injection. Subsequently, mice were killed by cervical dislocation.