INFECTION AND IMMUNITY, May 2008, p. 2235–2239 Vol. 76, No. 5
0019-9567/08/$08.00+0 doi:10.1128/IAI.01481-07

Macrophages Are Mediators of Gastritis in Acute *Helicobacter pylori* Infection in C57BL/6 Mice[∇]

Maria Kaparakis,[1]† Anna K. Walduck,[1] Jason D. Price,[1,2] John S. Pedersen,[3] Nico van Rooijen,[4] Martin J. Pearse,[2,5] Odilia L. C. Wijburg,[1,2]‡* and Richard A. Strugnell[1,2]‡

Department of Microbiology and Immunology and the NHMRC Bacterial Pathogenesis Group, The University of Melbourne, Parkville, Victoria 3010, Australia[1]; CRC for Vaccine Technology[2] and Department of Anatomical Pathology, Alfred Hospital,[3] Prahran, Victoria 3181, Australia; Department of Molecular Cell Biology, Vrije Universiteit VUMC, Amsterdam, The Netherlands[4]; and CSL Limited, Poplar Rd., Parkville, Victoria 3052, Australia[5]

Received 6 November 2007/Returned for modification 2 January 2008/Accepted 26 February 2008

***Helicobacter pylori is* the etiological agent of human chronic gastritis, a condition seen as a precursor to the development of gastrointestinal ulcers or gastric cancer. This study utilized the murine model of chronic *H. pylori* infection to characterize the role of macrophages in the induction of specific immune responses and gastritis and in the control of the bacterial burden following *H. pylori* infection and vaccination. Drug-loaded liposomes were injected intravenously to deplete macrophages from C57BL/6 mice, and effective removal of CD11b$^+$ cells from the spleens and stomachs of mice was confirmed by immunofluorescence microscopy. Transient elimination of macrophages from C57BL/6 mice during the early period of infection reduced the gastric pathology induced by *H. pylori* SS1 but did not affect the bacterial load in the stomach. These data suggest that macrophages are important to the severity of gastric inflammation during *H. pylori* infection.**

Helicobacter pylori is the causative agent of human gastroduodenal disease, resulting in acute gastric inflammation (16), ulcers, gastric adenocarcinoma, or mucosa-associated lymphoid tissue lymphoma (18, 24). *H. pylori* resides extracellularly within the gastric mucus layer of the human stomach, with only 1% of the organisms attached to host epithelial cells (11). The normal gastric mucosa of *H. pylori*-negative adults and children is populated by very few macrophages (12). Macrophages and neutrophils enter gastric tissue in response to *H. pylori* infection (20) and increase in number with the severity of gastritis and the duration of infection (12). *H. pylori* can activate macrophages and elicit interleukin-1 (IL-1), tumor necrosis factor alpha, IL-6, IL-8, MIP-1α, and GRO-α production (5, 6, 13), which induce the recruitment and activation of inflammatory cells, including macrophages and T cells.

Murine *Helicobacter* infection mimics many aspects of human *H. pylori* disease, including the development of chronic inflammation characterized by an influx of T cells expressing Th1 cytokines (14). The murine model therefore provides an excellent opportunity to analyze the genesis and adaptive immune regulation of *Helicobacter*-induced chronic gastritis (19). Moreover, the murine model is seen as an important aid in understanding vaccination against *H. pylori* infection (3).

While extensive studies have been performed to define the role of the acquired immune response during *H. pylori* infection and vaccination, the role of the innate immune response is not well understood. Very few in vitro studies have investigated the role of macrophages and their interaction with *Helicobacter* (1, 8, 17). To date, there is no reported study examining in vivo interactions of macrophages as antigen-presenting cells, and the initiator of *H. pylori* inflammation, during infection or vaccination. In this study, the role of macrophages in driving the inflammatory response and in controlling levels of *H. pylori* colonization in C57BL/6 mice during infection was examined, identifying the macrophage as a key cell in the induction of gastritis during *H. pylori* infection.

MATERIALS AND METHODS

Mice. Six-week-old female C57BL/6 mice were bred and housed under specific-pathogen-free conditions at the Department of Microbiology and Immunology Animal Facility, The University of Melbourne. Mice were fed sterile food and H_2O ad libitum and euthanized by CO_2 asphyxiation. All experiments were approved by The University of Melbourne Animal Ethics Committee.

In vivo macrophage depletion. Macrophages were depleted in vivo by using the well-characterized liposome-mediated macrophage "suicide" technique (21). Mice were intravenously (i.v.) injected with 200 μl of dichloromethylene diphosphonate (Cl_2MDP)-loaded liposomes. Cl_2MDP was a kind gift from Roche Diagnostics GmbH (Mannheim, Germany). Macrophage depletion was maintained, when required, by i.v. injecting 50 μl of Cl_2MDP-loaded liposomes every 4 to 5 days for the indicated periods.

***H. pylori* culture and infection of mice.** *H. pylori* SS1 was grown in brain heart infusion broth (Oxoid, England) supplemented with 5% (vol/vol) fetal calf serum (10). Mice were anesthetized with Penthrane (Abbot Laboratories) and received approximately 10^8 bacteria in 200 μl of phosphate-buffered saline (PBS) by oral gavage. The animals were infected three times within 5 days for infection studies or challenged after prophylactic immunization with a single dose (10^8 CFU) of bacteria.

***H. pylori* colonization and gastritis levels of infected mice.** Mouse stomachs were opened along the greater curvature, washed in PBS, and cut in half to include the greater curvature. One half was used to quantify *H. pylori* colonization by determining the number of CFU per gram of stomach tissue (10). The remaining half was embedded in Tissue-tek O.C.T. compound (Sakura Finetech) and frozen over liquid nitrogen. Hematoxylin-and-eosin-stained cryosections (7 μm) were used to grade the inflammatory response as previously described (10). The following six-point scale was used to define mononuclear cell infiltration: 1, mild multifocal; 2, mild widespread or moderate multifocal; 3, mild widespread and moderate or severe multifocal; 4, moderate widespread; 5, moderate wide-

* Corresponding author. Mailing address: Department of Microbiology and Immunology, The University of Melbourne, Melbourne, VIC 3010, Australia. Phone: 61 3 8344 9919. Fax: 61 3 9347 1540. E-mail: odilia@unimelb.edu.au.

† Present address: Department of Microbiology, Monash University, Melbourne, Australia.

‡ Contributed equally as senior authors.

∇ Published ahead of print on 10 March 2008.

spread and severe multifocal; 6, severe widespread. All sections were graded blindly.

***H. pylori*-specific ELISA.** Mouse serum *H. pylori*-specific antibody levels were determined by enzyme-linked immunosorbent assay (ELISA). In brief, MaxiSorp immunoplates (NUNC, Roskilde, Denmark) were coated with 10 μg/ml *H. pylori* sonicate and diluted sera were subsequently incubated in the plates. Bound antibodies were detected with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin (Silenus Laboratories, Hawthorn, Australia). Enzymatic activity of bound antibodies was detected with Immunopure *O*-phenylenediamine (Pierce) with H_2O_2 as the substrate. Absorbances were read by determining optical density at 492 nm with a Titertek Multiskan (Titertek, Finland). Serum endpoint titers are expressed as the reciprocal of the dilution of the specific antibody that gave an optical density at 492 nm of five times the value of horseradish peroxidase-conjugated antibody alone. Positive and negative control sera with known *Helicobacter* antibody titers were included in all ELISA experiments.

Immunohistochemistry. Acetone-fixed cryosections of spleen and stomach tissues were washed in PBS and blocked for 30 min with 20% (vol/vol) goat serum–2% mouse serum–FcγRIII antibody (eBiosciences) in PBS. Sections were incubated with anti-CD11b clone M1/70 (BD Biosciences) and/or rabbit anti-*H. pylori* serum diluted in 5% (vol/vol) goat serum–PBS overnight at 4°C. Bound antibody was detected with fluorescein isothiocyanate-conjugated anti-rat antibody and/or phycoerythrin-conjugated anti-rabbit antibody (Jackson ImmunoResearch). Slides were mounted with Mowiol 4-88 (Merck, Australia) and examined with a UV epifluorescence microscope.

Statistics. The nonparametric two-tailed Mann-Whitney U test was used for statistical analysis of the results. One stomach section removed from along the greater curvature per animal was analyzed for pathology and colonization by *H. pylori* bacteria. Antibody levels below the detection limit of the assay were assigned the minimum level of detection of the assay to enable statistical analysis. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Macrophage elimination by treatment with Cl_2MDP-liposomes. The role of macrophages in the control of *H. pylori* colonization and immunopathology were examined by transiently depleting mice of macrophages with Cl_2MDP-liposomes. Mice were injected i.v. with Cl_2MDP-liposomes, and macrophage depletion was maintained by i.v. injection of Cl_2MDP-liposomes every 4 to 5 days, from 8 days prior to the first dose of infection to 10 days after the final dose of infection with *H. pylori* (a total of six injections). Control mice received PBS instead of Cl_2MDP-liposomes. Three days after the final dose of bacteria, mice were euthanized and the spleens and stomachs of the mice were frozen and used for histological analysis. Cryostat sections were stained with anti-CD11b antibody to determine if macrophages were depleted from Cl_2MDP-liposome-treated mice. Figure 1 shows that CD11b$^+$ cells were easily distinguished in the spleens of control naïve and *H. pylori*-infected animals. Cl_2MDP-liposome treatment resulted in successful elimination of CD11b$^+$ cells from the red pulp and marginal zone area of the spleens of naïve and *H. pylori*-infected animals. There were very few detectable CD11b$^+$ cells in the gastric tissue of PBS-treated mice and no detectable CD11b$^+$ cells in Cl_2MDP-liposome-treated naïve mice. Analysis of the presence of CD11b$^+$ cells (green) and *H. pylori* bacteria (red) detected in the stomachs of Cl_2MDP-liposome-treated and control *H. pylori*-infected animals demonstrated that infection with *H. pylori* resulted in an influx of CD11b$^+$ cells into the gastric tissue and that treatment with Cl_2MDP-liposomes before and during administration of the bacteria resulted in a substantial reduction in the number of detectable CD11b$^+$ cells in the stomach.

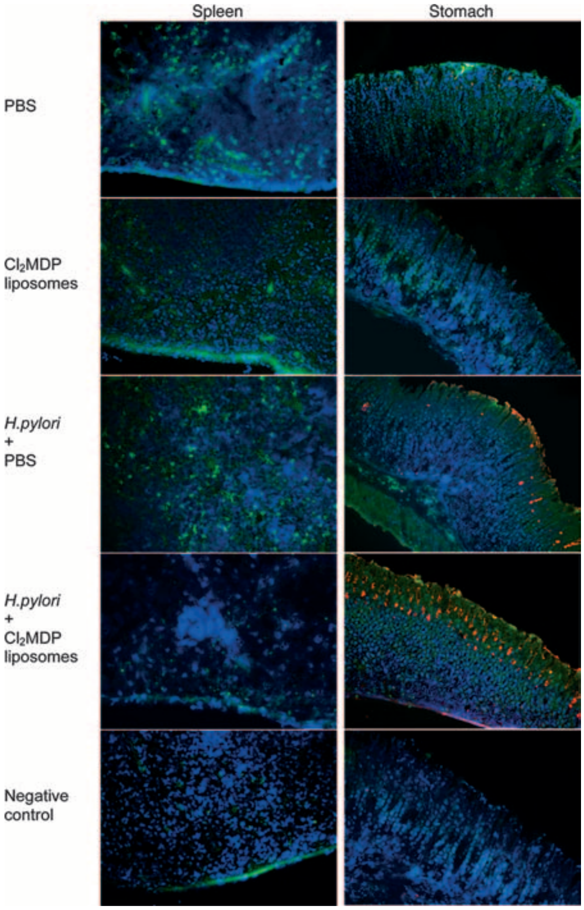

FIG. 1. Absence of CD11b$^+$ cells in the spleens and stomachs of Cl_2MDP-liposome-treated mice. C57BL/6 mice received Cl_2MDP-liposomes or PBS via i.v. injection every 4 or 5 days from 8 days prior to the first dose of *H. pylori* until 10 days after the final dose of *H. pylori*. Three days after the final dose of bacteria, mice were euthanized and their spleens and stomachs were frozen for analysis by histology. Shown are cryostat sections of the spleens and stomachs of PBS-treated or Cl_2MDP-liposome-treated, *H. pylori*-infected and naïve animals. Tissues were stained with rat anti-CD11b and anti-rat–fluorescein isothiocyanate (green) and rabbit anti-*H. pylori* antibodies and anti-rabbit–Texas Red (red, *H. pylori*-infected stomach only), and nuclei are stained with DAPI. Negative control slides were stained with fluorochrome-conjugated second antibody only. Representative sections from each treatment group are shown ($n = 5$ for each group). Data are representative of two independently performed experiments. Original magnifications: spleens, ×20; stomachs, ×10.

Cl_2MDP-liposome treatment during early *H. pylori* infection does not alter gastric colonization levels. Cl_2MDP-liposome-treated and PBS-treated control mice were euthanized 3 days, 10 days, or 3 months after the final dose of *H. pylori* infection, and gastric *H. pylori* colonization levels were determined by culture (Fig. 2A). *H. pylori* was isolated from the gastric tissue of all infected mice at all time points, and colonization levels of Cl_2MDP-liposome-treated mice were comparable to those of PBS-treated control mice during the 3-month infection period (at 3 days [data not shown; $n = 5$]; $P = 0.31$ [$n = 5$] and $P = 0.10$ [$n = 10$] at both 10 days and 3 months, respectively).

The *H. pylori*-specific antibody response is not affected by Cl_2MDP-liposome treatment. *H. pylori*-specific antibody re-

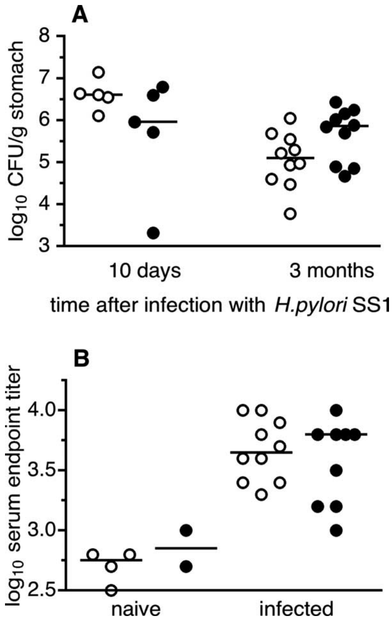

FIG. 2. Cl_2MDP-liposome treatment during *H. pylori* infection does not affect colonization levels or *H. pylori*-specific antibody responses. C57BL/6 mice were treated with either PBS (white symbols) or Cl_2MDP-liposomes (black symbols) from 8 days prior to the first dose of *H. pylori* until 10 days after the final dose of *H. pylori*. (A) At 10 days (n = 5) and 3 months (n = 10) after infection, mice were euthanized and stomach homogenates were plated onto selective media to determine the gastric colonization levels. (B) The *H. pylori*-specific serum endpoint titer was determined by ELISA 3 months after infection. The median levels of the groups in panels A and B are indicated by dashes, and each symbol represents an individual animal. Data are representative of two independently performed experiments.

sponses in the sera of all infected mice were measured by ELISA 3 months after infection (Fig. 2B). *H. pylori*-specific antibodies were detectable in the sera of all infected mice, and Cl_2MDP-liposome treatment did not affect the levels of *H. pylori*-specific antibodies in serum.

Reduced gastric pathology in *H. pylori*-infected mice as a result of treatment with Cl_2MDP liposomes. Gastric mononuclear cellular infiltrate in Cl_2MDP-liposome-treated and PBS-treated control *H. pylori*-infected mice was scored 3 months after infection. Figure 3A shows an immunohistological analysis of the inflammatory infiltrate of a representative sample from each stomach region from each group of mice. The average inflammatory score of each group of mice is shown in Fig. 3B. Overall, Cl_2MDP-liposome-treated mice had lower inflammation scores, with significantly reduced levels of chronic inflammation in the lower regions of the stomach than the PBS-treated control mice (P = 0.035 [n = 10] in the antrum and lower-body regions, respectively). The average inflammation in the mid- and upper-body regions of Cl_2MDP-liposome-treated mice (1.8 ± 0.41 [mean ± standard deviation; n = 10] and 1.3 ± 0.5 [n = 10], respectively) was reduced compared with that of PBS-treated control mice (2.4 ± 0.53 and 2.1 ± 1.0, respectively), although the differences were not statistically significant (P = 0.068 in the mid-body region and P = 0.09 in the upper-body region). Gastric tissue from all mice was

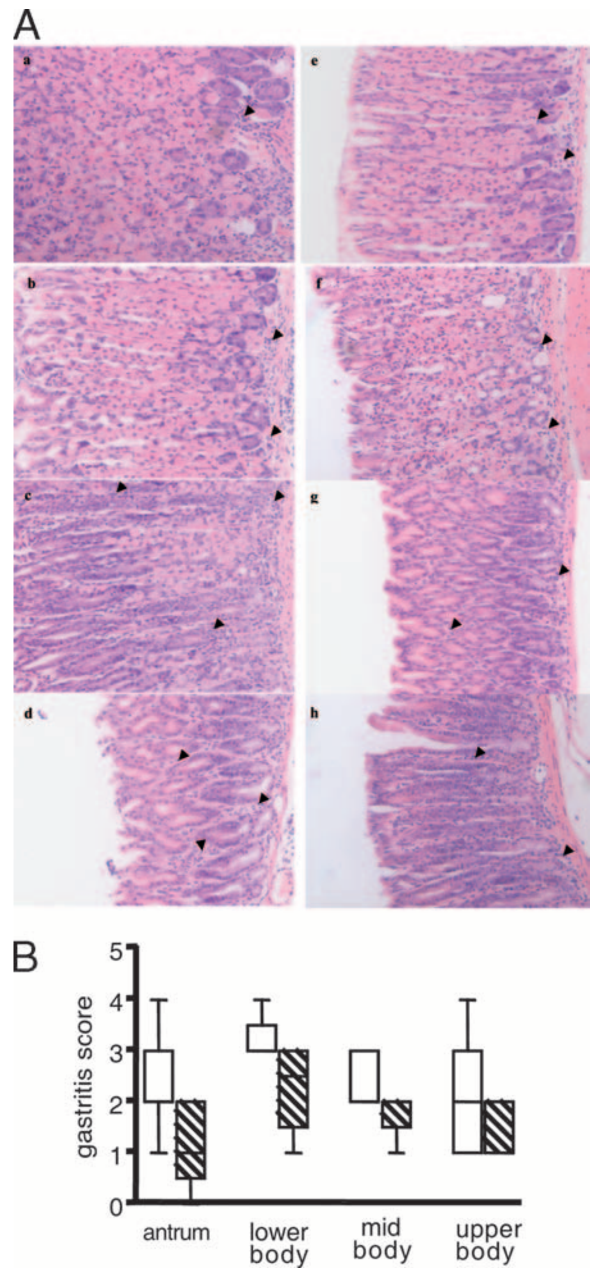

FIG. 3. Cl_2MDP-liposome treatment during *H. pylori* infection reduces gastric inflammation. C57BL/6 mice were treated with either PBS (white symbols) or Cl_2MDP-liposomes (black symbols) from 8 days prior to the first dose of *H. pylori* until 10 days after the final dose of *H. pylori*. (A) Sections of gastric tissue from mice infected *H. pylori* for 3 months were stained with hematoxylin and eosin and examined for gastritis by light microscopy. Values for mononuclear cell infiltration, described as chronic inflammation, were estimated by using a six-point scale. Shown are representative images of PBS-treated (a to d) and Cl_2MDP-liposome-treated (e to h) mice showing inflammatory infiltrate (arrows) in the upper-body (a [score = 2.5] and e [score = 1.0]), mid-body (b [score = 2.0] and f [score = 1.5]), lower-body (c [score = 4.0] and g [score = 2.5]), and antrum (d [score = 3.0] and h [score = 2.0]) regions of the stomach. Original magnification, ×20. (B) Box plot showing the gastric inflammatory scores of Cl_2MDP-liposome (n = 10, hatched boxes) and PBS-treated (n = 10, white boxes), *H. pylori*-infected animals determined by histology 3 months after infection. Data are representative of two independently performed experiments.

negative for acute gastric inflammation (influx of neutrophils) and atrophy.

DISCUSSION

Macrophages are bone marrow-derived mononuclear phagocytes that reside within tissues, act as scavengers of immunogenic debris, and eliminate foreign particles by phagocytosis (7). Macrophages are thought to have a central function in controlling and regulating the immune response; once activated, they produce proinflammatory cytokines and chemokines (7). Activated macrophages also express major histocompatibility complex class II and can therefore activate antigen-specific $CD4^+$ T cells (4).

In this study, macrophages were transiently depleted in vivo by the well-characterized liposome-mediated macrophage suicide technique (21). Van Rooijen et al. demonstrated that Cl_2MDP affects cellular metabolism once intracellular concentrations of Cl_2MDP exceed a threshold and macrophages are depleted by apoptosis. It should be noted that elimination of macrophages by Cl_2MDP-loaded liposomes is transient, as macrophage numbers return to almost normal levels in the spleen 4 weeks after Cl_2MDP-liposome treatment ceases (22).

The depletion of macrophages from the spleens of Cl_2MDP-liposome-treated C57BL/6 mice was confirmed by immunohistochemistry and reproduced previously published results (21). We also demonstrated the removal of macrophages from the stomachs of naïve animals and a substantial reduction in the number of $CD11b^+$ cells in the stomachs of Cl_2MDP-liposome-treated, *H. pylori*-infected animals.

This paper describes the effect of in vivo macrophage depletion during experimental infection of mice with *H. pylori*. Mice were injected with Cl_2MDP-liposomes prior to infection with *H. pylori* until 10 days postinfection. While the *H. pylori* colonization levels were unaltered by the liposome treatment over the 3-month period following infection, the transient elimination of macrophages from the spleen and stomach significantly reduced the *H. pylori*-induced chronic gastric inflammation in C57BL/6 mice.

There are several potential explanations for the observation that macrophages modulate *H. pylori*-mediated gastritis during the establishment of infection. The accumulation of phagocytic cells in the gastric tissue during *H. pylori* infection correlates with the development of gastritis (25). Macrophages accumulated in the gastric mucosa are stimulated by *H. pylori* proteins, which results in the production of IL-1, tumor necrosis factor alpha, IL-6, IL-8, MIP-1α, and GROα (5, 6, 13). These chemokines and inflammatory molecules produced during *H. pylori* infection may play a key role in the regulation, recruitment, and activation of inflammatory cells, such as macrophages themselves, in the gastric mucosa. For example, MIP-1α is implicated in the continuation of mucosal immune and inflammatory reactions of *H. pylori*-positive patients (13). Also, inducible nitric oxide synthase is important in the pathogenesis of *H. pylori* gastritis, as it is up-regulated in patients with chronic active gastritis or gastritis with intestinal metaplasia (23). Ihrig et al. reported that $iNOS^{-/-}$ mice infected with *H. felis* had decreased antibody levels and less gastritis than wild-type mice (9). Therefore, cytokine production by mucosal macrophages may be central to the genesis and severity of *H. pylori*-induced inflammation and malignancy.

Furthermore, macrophages express Toll-like receptors that recognize *H. pylori* antigens and induce MyD88 and NF-κB signaling pathways (15) that may aid in the development of *H. pylori*-induced gastritis. Moreover, products generated by macrophages can also have adverse effects on host tissue. Matrix metalloproteinase 9 and 2 levels are increased in the gastric mucosa of *H. pylori*-infected individuals, contributing to the destruction of gastric tissue during infection (2).

This study demonstrates that macrophages have a central role in *Helicobacter* infection-induced gastritis but do not affect *H. pylori*-specific antibody responses. This effect is most probably due to cytokine secretion and/or antigen presentation, and this hypothesis is the subject of ongoing studies. In identifying a role for macrophages in the initiation of gastritis during *H. pylori* infection, this study may assist in future studies targeting the inhibition of gastritis in the host and provide a stimulus to study the capacity of macrophage-modifying drugs to reduce the gastritis associated with *Helicobacter* disease.

ACKNOWLEDGMENTS

These studies were supported by the National Health and Medical Research Council of Australia and CSL Limited. O.L.C.W. is an NH&MRC R. D. Wright Fellow.

REFERENCES

1. **Basu, M., S. J. Czinn, and T. G. Blanchard.** 2004. Absence of catalase reduces long-term survival of *Helicobacter pylori* in macrophage phagosomes. Helicobacter **9:**211–216.
2. **Bergin, P. J., E. Anders, W. Sicheng, J. Erik, A. Jennie, L. Hans, M. Pierre, P.-H. Qiang, and Q.-J. Marianne.** 2004. Increased production of matrix metalloproteinases in *Helicobacter pylori*-associated human gastritis. Helicobacter **9:**201–210.
3. **Blanchard, T. G., S. J. Czinn, and J. G. Nedrud.** 1999. Host response and vaccine development to Helicobacter pylori infection. Curr. Top. Microbiol. Immunol. **241:**181–213.
4. **Bryant, P., and H. Ploegh.** 2004. Class II MHC peptide loading by the professionals. Curr. Opin. Immunol. **16:**96–102.
5. **Crabtree, J. E., T. M. Shallcross, R. V. Heatley, and J. I. Wyatt.** 1991. Mucosal tumour necrosis factor alpha and interleukin 6 in patients with Helicobacter pylori-associated gastritis. Gut **32:**1473–1477.
6. **Eck, M., B. Schmausser, K. Scheller, A. Toksoy, M. Kraus, T. Menzel, H. K. Muller-Hermelink, and R. Gillitzer.** 2000. CXC chemokines Groα/IL-8 and IP-10/MIG in Helicobacter pylori gastritis. Clin. Exp. Immunol. **122:**192–199.
7. **Gordon, S.** 2003. Alternative activation of macrophages. Nat. Rev. Immunol. **3:**23–35.
8. **Harris, P. R., H. L. T. Mobley, G. I. Perez Perez, M. J. Blaser, and P. D. Smith.** 1996. Helicobacter pylori urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production. Gastroenterology **111:**419–425.
9. **Ihrig, M., M. T. Whary, C. A. Dangler, and J. G. Fox.** 2005. Gastric *Helicobacter* infection induces a Th2 phenotype but does not elevate serum cholesterol in mice lacking inducible nitric oxide synthase. Infect. Immun. **73:**1664–1670.
10. **Kaparakis, M., K. L. Laurie, O. Wijburg, J. Pedersen, M. Pearse, I. R. van Driel, P. A. Gleeson, and R. A. Strugnell.** 2006. $CD4^+$ $CD25^+$ regulatory T cells modulate the T-cell and antibody responses in *Helicobacter*-infected BALB/c mice. Infect. Immun. **74:**3519–3529.
11. **Ko, G. H., S. M. Kang, Y. K. Kim, J. H. Lee, C. K. Park, H. S. Youn, S. C. Baik, M. J. Cho, W. K. Lee, and K. H. Rhee.** 1999. Invasiveness of *Helicobacter pylori* into human gastric mucosa. Helicobacter **4:**77–81.
12. **Krauss-Etschmann, S., R. Gruber, K. Plikat, I. Antoni, H. Demmelmair, D. Reinhardt, and S. Koletzko.** 2005. Increase of antigen-presenting cells in the gastric mucosa of *Helicobacter pylori*-infected children. Helicobacter **10:**214–222.
13. **Kusugami, K., T. Ando, A. Imada, K. Ina, M. Ohsuga, T. Shimizu, T. Sakai, T. Konagaya, and H. Kaneko.** 1999. Mucosal macrophage inflammatory protein-1α activity in *Helicobacter pylori* infection. J. Gastroenterol. Hepatol. **14:**20–26.
14. **Lee, A., J. O'Rourke, M. C. De Ungria, B. Robertson, G. Daskalopoulos, and**

M. F. Dixon. 1997. A standardized mouse model of Helicobacter pylori infection—introducing the Sydney strain. Gastroenterology **112:**1386–1397.
15. **Mandell, L., A. P. Moran, A. Cocchiarella, J. Houghton, N. Taylor, J. G. Fox, T. C. Wang, and E. A. Kurt-Jones.** 2004. Intact gram-negative *Helicobacter pylori*, *Helicobacter felis*, and *Helicobacter hepaticus* bacteria activate innate immunity via Toll-like receptor 2 but not Toll-like receptor 4. Infect. Immun. **72:**6446–6454.
16. **Marshall, B. J., J. A. Armstrong, D. B. McGechie, and R. J. Glancy.** 1985. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med. J. Aust. **142:**436–439.
17. **Menaker, R. J., P. J. Ceponis, and N. L. Jones.** 2004. *Helicobacter pylori* induces apoptosis of macrophages in association with alterations in the mitochondrial pathway. Infect. Immun. **72:**2889–2898.
18. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. Helicobacter pylori infection and the risk of gastric carcinoma. N. Engl. J. Med. **325:**1127–1131.
19. **Sawai, N., M. Kita, T. Kodama, T. Tanahashi, Y. Yamaoka, Y. I. Tagawa, Y. Iwakura, and J. Imanishi.** 1999. Role of gamma interferon in *Helicobacter pylori*-induced gastric inflammatory responses in a mouse model. Infect. Immun. **67:**279–285.
20. **Suzuki, T., K. Kato, S. Ohara, K. Noguchi, H. Sekine, H. Nagura, and T. Shimosegawa.** 2002. Localization of antigen-presenting cells in *Helicobacter pylori*-infected gastric mucosa. Pathol. Int. **52:**265–271.
21. **Van Rooijen, N., and A. Sanders.** 1994. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J. Immunol. Methods **174:**83–93.
22. **van Rooijen, N., A. Sanders, and T. K. van den Berg.** 1996. Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. J. Immunol. Methods **193:**93–99.
23. **Wilson, K. T., K. S. Ramanujam, H. L. T. Mobley, R. F. Musselman, S. P. James, and S. J. Meltzer.** 1996. Helicobacter pylori stimulates inducible nitric oxide synthase expression and activity in a murine macrophage cell line. Gastroenterology **111:**1524–1533.
24. **Wotherspoon, A. C., C. Ortiz-Hidalgo, M. R. Falzon, and P. G. Isaacson.** 1991. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet **338:**1175–1176.
25. **Yoshida, N., D. N. Granger, D. J. Evans, D. G. Evans, D. Y. Graham, D. C. Anderson, R. E. Wolf, and P. R. Kvietys.** 1993. Mechanisms involved in Helicobacter pylori-induced inflammation. Gastroenterology **105:**1431–1440.

Editor: F. C. Fang