UltraRapid Communication

Influence of Helicobacter pylori Infection During Atherogenesis In Vivo in Mice

François Mach, Galina K. Sukhova, Murielle Michetti, Peter Libby, Pierre Michetti

Abstract—Inflammatory diseases may have a role in the pathogenesis of atherosclerosis. Several epidemiological and clinical studies have explored the possible association between Helicobacter pylori seropositivity, cardiovascular risk factors, and ischemic heart disease. The contradictory results of these studies have fueled a debate regarding the link between H pylori infection and atherogenesis. This study tested the hypothesis that H pylori infection might influence atherosclerosis in vivo in mice. Male wild-type C57/Bl6 mice and LDL-receptor deficient congenic mice were randomly assigned for infection with H pylori. All animals were fed a high-cholesterol diet (1.25%) for 6 or 12 weeks. At autopsy, we compared aortic atherosclerotic lesion formation and lipid deposition. H pylori infection influenced neither the progression of atherosclerotic lesions nor lipid deposition. Moreover, the cellularity of atherosclerotic lesions (macrophages and T cells) did not differ between mice infected or not infected with H pylori. This in vivo study performed in a mouse model of atherosclerosis revealed no indication that H pylori infection might contribute to the development of atherosclerotic lesion formation. The full text of this article is available at http://www.circresaha.org.

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Key Words: Helicobacter pylori ■ atherosclerosis ■ inflammation

Helicobacter pylori represents one of the most widespread human infectious diseases. Almost half of the adult population has serological evidence of infection, and numerous recent reports support a causal link between this infection and the majority of upper gastrointestinal diseases.1 Recently, a number of epidemiological studies have highlighted a possible relation between ischemic heart disease and several infectious disorders, such as chronic dental infections, cytomegalovirus, Chlamydia, or H pylori.2 Several epidemiological and clinical studies have suggested an association between H pylori seropositivity, cardiovascular risk factors, and ischemic heart disease. Since the first report of a high incidence of H pylori seropositivity in patients with coronary heart disease,3 various observational studies have explored an association between H pylori infection and ischemic heart disease.4-7 Recently, Danesh et al8,9 presented a meta-analysis of 18 epidemiological studies involving more than 10 000 patients that measured serum antibody titers to H pylori and risk factors for coronary heart disease. They concluded that evidence for correlations between H pylori seropositivity and vascular risk factors were weak and largely or wholly due to chance or the preferential publication of positive results. The contradictory results of these studies have fueled the debate regarding the role of inflammation and infection in the development of ischemic heart disease. Very recently, several reports have demonstrated the presence of H pylori within atherosclerotic lesions in situ,10,11 although even these findings have proven controversial.12,13 Present within the arterial wall or not, this infection might still promote vessel-wall cell activation and atherosclerotic lesions indirectly.14 To seek direct evidence for a role of H pylori infection in atherogenesis, we examined the effect of this infection in atherosclerosis-prone mice.

Materials and Methods

Animals and Study Design
Male wild-type C57/Bl6 mice and LDL-receptor deficient syngeneic mice (LDLR−/−), all from Jackson Laboratory, Bar Harbor, Maine, were randomly assigned to be infected (orally inoculated)15,16 with the H pylori strain SS1, a mouse-adapted cag+ strain,17 or sham-infected (n=8 per group). Animals were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School. All animals were fed a high-cholesterol diet (Research Diets, Inc, product No. D12108, 1.25% cholesterol, 0% cholate), as described previously.18,19 After 6 or 12 weeks, mice (n=8 per group) were euthanized and atherosclerotic lesion formation as well as lipid deposition measured using a quantitative computer-assisted image analysis, as described previously.19 Briefly, arch and abdominal portion of the aorta were separated. Aortic arches were perfused with PBS, snap-frozen in OCT (OCT compound, Tissue-Tek). To measure plaque size and wall thickness, longitudinal sections (~30 per mouse) of the aortic arch were analyzed microscopically for all mice. For each mouse, the section with
maximal area and thickness of the inner aortic wall was identified and the average per group calculated. The presence of macrophages and T lymphocytes was determined for all mice within the aortic arch area defined above by cell-specific immunostaining (anti-Mac3 for macrophages and anti-CD3 for T lymphocytes). Areas that stained positively for macrophages were measured using computer-assisted image quantification (Optimas 5.2, Optimas Corp), and T lymphocytes identified by anti-CD3 staining were counted microscopically by two blinded observers. The abdominal aortas were fixed with 10% buffered formalin and stained for lipid deposition with Sudan IV (Fischer Scientific). The aortas were then opened longitudinally to the iliac bifurcation and pinned out on a black wax surface using 0.2-mm steel pins. The percentage of stain deposition was calculated within the total surface using computer analysis. \(^{18}\) \(H\) pylori infection was confirmed by rapid urease test and by histology, both on gastric tissues. In the infected group, 31 of the 32 mice (97%) were positive, and none in the sham-infected group were positive. In the infected group, only the confirmed infected mice were analyzed. For plasma analysis, cholesterol was determined by spectrophotometry (Sigma) and fibrinogen by a clot-rate assay as described. \(^{20}\)

**Results**

*Helicobacter pylori* Infection Does Not Influence Aortic Atherosclerotic Lesions or Aortic Lipid Deposition In Vivo

Aortic arch lesions were minimal in wild-type mice regardless of *H pylori* status after 6 or 12 weeks of a high-cholesterol diet (Figures 1A and 1B). In contrast, LDLR\(^{-/-}\) mice showed extensive atherosclerotic lesions, as defined by aortic wall area and thickness (\(P<0.001\) for both parameters versus wild-type values; Wilcoxon rank-sum test), but *H pylori* infection did not affect lesion severity (Figure 2). Lipid deposition was minimal in infected (2.5±1.0% of abdominal aortic surface area) and noninfected (2.5±0.9) wild-type mice after 6 or 12 weeks of a high-cholesterol diet (Figure 1C). In LDLR\(^{-/-}\) mice, however, lipid deposition was 21±3% at 12 weeks (\(P<0.001\) versus wild-type) in noninfected animals but was not modified by *H pylori* infection (22±4%). The measurements of aortic wall area, thickness, and lipid deposition in LDLR\(^{-/-}\) mice were similar to those previously published by our group and others. \(^{18,19}\) *H pylori* infection did not influence the cellularity of atheroma. Within atherosclerotic lesions, infected or noninfected animals exhibited the same macrophage-specific staining, and no difference was found in T-lymphocyte count (data not shown).

As with controls, serum lipid profiles (total cholesterol, LDL, and triglycerides), circulating leukocytes, hematocrit, fibrinogen, and body weight did not differ (\(P<0.05\)) among infected or uninfected animals in all groups (Table).

**Discussion**

Atherosclerosis displays many features of a chronic inflammatory process. \(^{21-23}\) The notion that chronic infection represents a possible contributor to the development of atherosclerosis and its clinical complications of unstable angina, myocardial infarction, and stroke has engendered considerable interest. \(^3\) Thus far, seroepidemiological or pathological

**Figure 1.** Measurement of atherosclerotic lesions in aorta of wild-type or LDL-receptor knockout mice (LDLR\(^{-/-}\)), with or without *H pylori* infection after 6 or 12 weeks of a high-cholesterol diet. The maximal aortic arch wall area (A) and the maximal thickness (B) of the inner aortic arch wall for each mouse were measured as described in Materials and Methods. The percentage of lipid-deposition area (C) was calculated as described in Materials and Methods. Data represent mean±SEM (n=8 per group). Open bars indicate uninfected controls; shaded bars, *H pylori*-infected mice. *\(P<0.001\) for both parameters vs wild-type values.

**Figure 2.** Photomicrographs show aortic arch atherosclerotic lesions from LDLR\(^{-/-}\) mice (fed a high-cholesterol diet for 12 weeks) not infected (left) or infected (right) with *H pylori*. The carotid arteries are at the top and the aortic sinus is at the left of the figures. The ostia of the innominate artery (IA) and the left common carotid artery (LCCA) are indicated. Representative specimens from both groups are shown.
evidence has implicated one virus (cytomegalovirus) and two bacteria (Chlamydia pneumoniae and H pylori) in human atherosclerosis. These associations remain controversial. In the case of cytomegalovirus or C pneumoniae, most have envisaged direct vessel wall infection with local inflammatory activation of atheroma-associated cells, such as endothelial cells, smooth muscle cells, and macrophages. In contrast, H pylori might affect atherosclerosis indirectly due to a remote infection, for example, in the gastrointestinal tract as modeled here. Indeed, several epidemiological and clinical studies have investigated a possible association between H pylori seropositivity, cardiovascular risk factors, and the prevalence of ischemic heart disease. The recent negative meta-analysis of 20 studies published by Danesh and coworkers, as well as the prospective case-control from the Physicians’ Health Study, has intensified the debate regarding H pylori infection and the development of ischemic heart disease. To provide direct data regarding this controversy, we studied the influence of H pylori in a well-established mouse model of atherosclerosis.

We found that H pylori neither induces atherosclerosis in wild-type animals nor influences the progression of atherosclerotic lesion formation in hypercholesterolemic mice prone to develop atherosclerosis. Furthermore, we found no effect of H pylori infection on traditional risk factors of atherosclerosis, such as lipid profile or fibrinogen. We concluded that under the conditions of this experiment, H pylori infection does not contribute to the development of atherosclerotic lesion formation.

Limitations of the present study include its inability to distinguish whether H pylori infection may influence atherosclerotic lesion stability and thereby trigger plaque disruption with thrombosis, which commonly causes acute coronary syndromes. We also cannot exclude that in a longer time course, H pylori may induce a more pronounced inflammatory state that could participate in either atherosogenesis or even plaque rupture. In the LDLR−/− mouse, hyperlipidemia causes the atherosclerosis and may minimize modulatory effects of other potential risk factors. Extrapolation of animal experiments to humans always requires due caution. Furthermore, our results do not exclude participation of other infectious agents in atherosogenesis. In conclusion, although many currently consider atherosclerosis an inflammatory process, these data do not support a role for H pylori infection as a contributor to the development of this common disease.

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