JCI The Journal of Clinical Investigation

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J Clin Invest. 1991;87(3):894-900. https://doi.org/10.1172/JCI115095.

Research Article

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Soluble Surface Proteins from *Helicobacter pylori* Activate Monocytes/Macrophages by Lipopolysaccharide-independent Mechanism

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Abstract

The inflammatory lesions associated with *Helicobacter pylori* gastritis and duodenitis contain large numbers of mononuclear cells. The close proximity of *H. pylori* to gastric mucosa suggests that the organism interacts with mononuclear cells, thereby modulating the inflammatory response. To investigate the role of monocytes/macrophages in this response, we examined the effect of whole *H. pylori* bacteria, *H. pylori* surface proteins, and *H. pylori* lipopolysaccharide (LPS) on purified human monocytes. Whole *H. pylori* and the extracted LPS induced expression of the monocyte surface antigen HLA-DR and interleukin-2 receptors, production of the inflammatory cytokines interleukin 1 and tumor necrosis factor (peptide and messenger RNA), and secretion of the reactive oxygen intermediate superoxide anion. Since *H. pylori* in vivo does not invade mucosal tissue, we determined whether soluble constituents of the bacteria could activate monocytes. Soluble *H. pylori* surface proteins, which are enriched for urease and do not contain LPS, stimulated phenotypic, transcriptional, and functional changes consistent with highly activated monocytes.

These findings indicate that *H. pylori* is capable of activating human monocytes by an LPS-independent as well as an LPS-dependent mechanism. *H. pylori* activation of resident lamina propria macrophages and monocytes trafficking through the mucosa, leading to the secretion of increased amounts of inflammatory cytokines and reactive oxygen intermediates, could play an important role in mediating the inflammatory response associated with *H. pylori* gastritis and duodenitis. (*J. Clin. Invest.* 1991. 87:894–900.) Key words: *Helicobacter pylori* • monocyte/macrophage • surface proteins • activation • lipopolysaccharide

Introduction

The presence of *Helicobacter pylori*, formerly called *Campylobacter pylori*, in the gastric antrum of humans is associated with chronic type B gastritis and peptic ulcer disease (1–5), and an increasing body of evidence indicates an etiologic role for this organism (6). In infected persons, the bacteria are present within or beneath the antral mucus and in close association with the epithelium (7). Although invasion of the epithelium appears not to occur, the presence of *H. pylori* in the gastric antrum is associated with a mucosal inflammatory reaction that consists of large numbers of polymorphonuclear and mononuclear inflammatory cells (8, 9). The pathogenesis of this inflammatory response is unknown. However, the presence of inflammatory cells in the mucosa during infection with *H. pylori* suggests that the organism interacts in some way with these cells. In addition, the presence of high titers of circulating (10–12) and local (13) antibodies to *H. pylori* in persons with *H. pylori*–associated antral gastritis indicates that the bacteria are capable of eliciting an immune response. Such a response implicates interaction between monocytes/macrophages and *H. pylori* since these cells are required for antigen presentation.

To elucidate the potential role of these cells in the inflammatory response to *H. pylori*, we investigated the interaction between whole *H. pylori*, as well as certain of its constituents, and purified human monocytes. We found that *H. pylori* activates human monocytes by both lipopolysaccharide (LPS)-dependent and LPS-independent mechanisms. The activation of circulating monocytes trafficking through the mucosa and/or local tissue macrophages may play an important role in the pathogenesis of the inflammatory response to *H. pylori*.

Methods

H. pylori strains. The *H. pylori* strain used to investigate the interaction between whole microorganisms and monocytes was from the Collection Institut Pasteur, Paris (CIP 101260) and was grown in liquid media containing brain heart infusion broth (Difco Laboratories, Inc., Detroit, MI) supplemented with 10% fetal calf serum (FCS) (Whittaker M. A. Bioproducts, Walkersville, MD) and 0.25% yeast extract (Difco Laboratories, Inc.) in a microaerobic, humidified atmosphere at 37°C (14). A second strain (84-183), obtained from the culture collection of the Denver Veterans Administration Medical Center *Campylobacter* Laboratory (15), was used to confirm the experimental observations with whole organisms and to examine the interaction between *H. pylori* constituents and monocytes. Whole *H. pylori* were harvested, lyophilized, and reconstituted in the media required for the specific assay.

Preparation of H. pylori constituents. H. pylori strain 84-183 was grown on trypticase soy agar (Difco Laboratories, Inc.) with 5% sheep erythrocytes (Remel, Lenexa, KS) for 48–72 h in a microaerobic, humidified atmosphere at 37°C. Cells were harvested in 0.15 M NaCl and centrifuged at 3,000 *g* for 25 min at 25°C. The cell pellet was resuspended in an equal volume of sterile distilled water, vortex-mixed for 45 s, and again centrifuged at 3,000 *g*. The supernatant (water-extracted surface proteins), which is enriched for urease activity (16), was stored at −20°C. LPS was prepared from the extracted cells by the hot phenol-water method of Westphal (17), and after lyophilization stored at room temperature. For the whole bacterial cells, water extract, and LPS, we determined the amount of endotoxin activity by *Limulus* assay (18), protein content by modification of the Lowry method (16), ketodeoxyoctonate (KDO)[1] content by the thiobarbituric acid method (17), and urease activity by enzymatic hydrolysis of urea (16).

This study was presented in part at the annual meeting of the American Gastroenterological Association, San Antonio, TX, 15–17 May 1990.

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Received for publication 15 March 1990 and in revised form 27 August 1990.

1. *Abbreviations used in this paper:* IL-2R, interleukin-2 receptor(s); KDO, ketodeoxyoctonate; SOD, superoxide dismutase; TNF, tumor necrosis factor.

Monocyte isolation. Healthy adult donors were leukapheresed and their mononuclear leukocytes separated into highly purified populations of monocytes and lymphocytes by countercurrent centrifugal elutriation (19), except that pyrogen-free phosphate-buffered saline without Ca^{++} and Mg^{++} was used for the elutriation procedure (20). Cells were suspended in Dulbecco's modified Eagle's medium (DME, Quality Biologicals, Gaithersburg, MD) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine and enumerated by Coulter counter and channelyzer (Coulter Electronics, Inc., Hialeah, FL) analysis. Cell purity was confirmed by morphology (98% monocytes), phenotypic analysis (> 98% Leu M3–positive, < 2% Leu 1–positive) and esterase staining (> 95%–positive) (19).

Incubation of monocytes with H. pylori preparations. Freshly elutriated monocytes were incubated in suspension (1×10^6/ml) in DME with either whole *H. pylori,* extracted *H. pylori* surface proteins (water extract), or *H. pylori* LPS in 17 × 100-mm polypropylene tubes (Becton, Dickinson & Co., Lincoln Park, NJ) for 24 h at 37°C. Monocyte viability after the 24-h incubation as determined by trypan blue exclusion always exceeded 95%. The monocytes and culture supernatants were harvested and assayed for the activities described below.

Monocyte surface antigen expression. Staining and analysis of surface antigens were performed as previously described (21). After a 24-h incubation of monocytes with whole *H. pylori* or *H. pylori* constituents, single-cell suspensions of elutriated monocytes (5×10^5/ml) were incubated at 4°C for 30 min with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies directed against HLA-DR and interleukin-2 receptors (IL-2R) (Becton-Dickinson Monoclonal Center, Inc., Mountain View, CA). Human type AB serum was added to the cells 10 min before incubation with the monoclonal antibodies to prevent nonspecific binding to Fc receptors. After staining, the cells were washed, resuspended in 0.5 ml 2% paraformaldehyde, and stored at 4°C until analysis by flow cytometry with a fluorescence-activated cell scanner (FACScan®, Becton, Dickinson & Co., Salt Lake City, UT). Relative mean fluorescence intensity (mean fluorescence minus background fluorescence) is a relative measure of antigen density per cell.

Measurement of interleukin 1 (IL-1) and tumor necrosis factor (TNF) activity. The activity of IL-1 in units per milliliter in the monocyte culture supernatants was determined by their ability to enhance C3H/HeJ murine thymocyte incorporation of [^{3}H]thymidine in the presence of a suboptimal concentration of phytohemagglutinin (1 μg/ml) in comparison to the activity of the IL-1 standard (Ultrapure IL-1, Genzyme, Boston, MA) (22). TNF activity in the supernatants was determined by the lysis of actinomycin D–treated L929 fibroblasts and is reported in units per milliliter, which corresponds to the reciprocal of the dilution of supernatant that resulted in 50% lysis (23).

Northern blot analysis. To investigate whether *H. pylori* constituents induce TNF and IL-1 gene expression, monocytes at a concentration of 20×10^6 cells/ml were incubated with *H. pylori* constituents (10 μg/ml) for 6 h, and the expression of IL-1 and TNFα mRNA was analyzed by Northern blot hybridization. Total cellular RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction (24), electrophoresed (5.0 μg/lane) on 1% agarose-formaldehyde gels, and transferred to nitrocellulose filters (25) for hybridization with cDNA (TNFα) and oligonucleotide (IL-1β) probes. The TNFα probe consisted of a 1.6-kb BamHI fragment of the TNFα cDNA provided by Drs. A. M. Wang and L. S. Lin (26), and the IL-1β probe was a 33-base oligonucleotide complementary to the RNA sequence of the 3′ coding region of the IL-1β gene provided by Dr. D. Carter (Immunex Corp., Seattle, WA) (27).

Superoxide anion (O_2^-) production. Monocyte production of O_2^- was measured by the reduction of ferricytochrome *c* (28). Briefly, monocytes were washed, suspended in cytochrome *c* 160 μM, and then added at a concentration of 1×10^5/100 μl to flat-bottomed, 96-well tissue culture plates (Costar Data Packaging, Cambridge, MA). To each of quadruplicate wells, 10 μl of cytochrome *c* was added either alone (background control), containing varying amounts of *H. pylori* preparations, or containing phorbol myristate acetate (PMA, Sigma Chemical Co., St. Louis, MO) 10 ng/ml (positive control). When whole bacteria were used to stimulate monocyte O_2^- production, additional wells containing whole bacteria without monocytes were included for control. The production of O_2^- by monocytes stimulated with *H. pylori* was determined by comparing the amount of O_2^- produced by stimulated monocytes with and without superoxide dismutase (SOD) to the amount produced by control monocytes alone with and without SOD (routinely less than 0.2 nmoles). Because whole *H. pylori* caused an increase in optical density, *H. pylori* alone with and without SOD was also used as a control for the experiments examining whole *H. pylori* stimulation of monocyte O_2^- production. The reduction of cytochrome *c* at an absorbance of 550 nm was measured during an 8-h period, and the amount of O_2^- was calculated by the formula: nanomoles O_2^- per well = (absorbance at 550 nm × 100)/6.3 (29).

Statistics. Data are expressed as mean±standard error of the mean (SEM), and levels of significance were determined by Student's *t* test. The level of significance for increasing antigen expression on monocytes induced by each stimulus was determined by nonparametric tests for trend (Mann test) (30), and the overall level of significance for all three stimuli was determined by combining the *P* values (Omnibus test) (31).

Results

H. pylori activation of monocytes. We first examined the effect of *H. pylori* on monocyte phenotypic markers by determining the level of expression of class II molecules and IL-2R on monocytes that had been incubated for 24 h with and without whole *H. pylori* bacteria. Because > 80% of the purified monocytes expressed HLA-DR before incubation, the effect of the bacterial cells on the density of HLA-DR on HLA-DR–positive monocytes was determined by flow cytometry. As shown in the representative fluorescence profile in Fig. 1, HLA-DR–positive monocytes that had been incubated with *H. pylori* exhibited a shift in the fluorescence intensity of FITC anti–HLA-DR staining, indicating an increase in the density of surface HLA-DR. Since we (32, 33) and others (34) have shown that activated, but not resting, monocytes express IL-2R, we also analyzed monocytes incubated with *H. pylori* for the expression of this receptor. In parallel with the ability of the bacterium to increase the expression of surface HLA-DR, *H. pylori* induced an increase in the number of cells expressing IL-2R (Fig. 1). Thus, incubation of whole *H. pylori* bacteria with human monocytes stimulated increases in the expression of HLA-DR and IL-2R, phenotypic changes consistent with monocyte activation.

Since mononuclear cells are present in the inflammatory

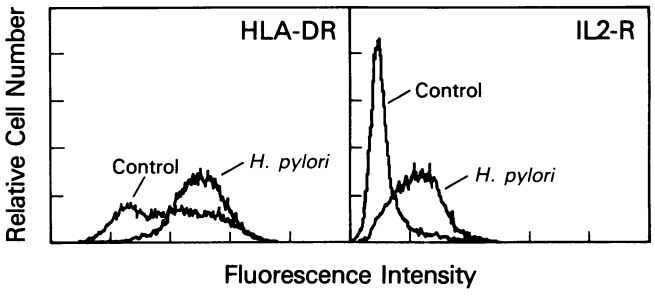

Figure 1. Induction of monocyte phenotypic changes by *H. pylori* strain CIP 101260. Human monocytes (1×10^6/ml) purified by counterflow centrifugal elutriation (98% Leu M3-positive) were incubated for 24 h with whole *H. pylori* bacteria and then analyzed by flow cytometry for the density of HLA-DR and the percent monocytes that expressed IL-2R. Results are from a single analysis representative of three experiments.

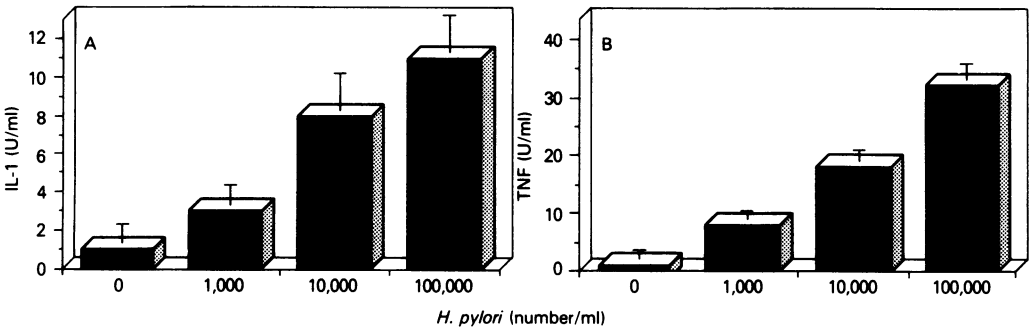

Figure 2. Induction of IL-1 and TNF production by *H. pylori* strain CIP 101260. Culture supernatants from monocytes incubated 24 h in the presence of increasing numbers of *H. pylori* cells were assayed for IL-1 (*A*) and TNF (*B*) activity as described in the Methods. Each point represents the mean (±SEM) for three determinations from three separate experiments. Comparisons between doses and between each dose and control were significant ($P < 0.05$ and $P < 0.01$, respectively).

infiltrate associated with *H. pylori* infection (8, 9), and since cytokines such as IL-1 and TNF play important roles in regulating inflammation, we also examined the ability of whole *H. pylori* to stimulate monocyte production of IL-1 and TNF. As shown in Fig. 2, increasing numbers of *H. pylori* caused a dose-dependent release of IL-1 and TNF by a constant number of monocytes ($P < 0.05$ for differences between doses; $P < 0.01$ for differences between each dose and control). Moreover, as few as 1×10^3 bacteria per ml stimulated the production of each cytokine.

In addition to IL-1 and TNF, reactive oxygen intermediates play an important role in inflammation, including that involving the gastrointestinal mucosa (35). Therefore, the effect of whole *H. pylori* on monocyte oxidative metabolism was determined. Similar to the ability of low numbers of the bacteria to induce phenotypic changes and cytokine production, incubating low numbers of *H. pylori* with monocytes induced a dose-dependent increase in the production of O_2^- by the monocytes (Fig. 3). Taken together, the ability of whole *H. pylori* bacteria to stimulate increased HLA-DR and IL-2R expression, cytokine production, and O_2^- secretion indicate that *H. pylori* is a potent activator of human monocytes.

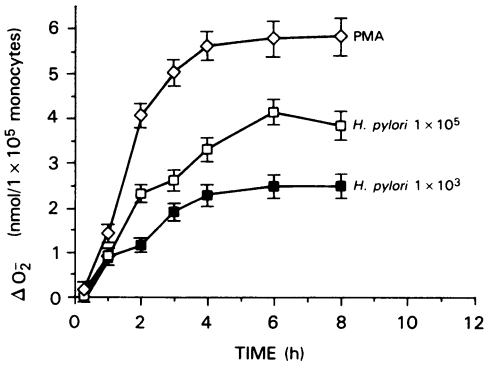

Figure 3. Stimulation of monocyte secretion of O_2^- by *H. pylori* strain CIP 101260. Purified monocytes (1×10^5/100 μl per well) incubated with increasing numbers of *H. pylori* were analyzed over 8 h for O_2^- production by the reduction of cytochrome *c*. The mean optical density of stimulated monocytes minus that of monocytes alone and *H. pylori* alone, each with and without SOD, was determined and the O_2^- calculated as described in the Methods section. Results are from a representative experiment ($n = 3$).

Effect of H. pylori constituents on monocyte activation markers. The next series of experiments focused on determining which constituent(s) of *H. pylori* was responsible for activating monocytes. For these experiments, strain 84-183 was used since the constituents of this strain have been previously characterized (15, 16). (Whole *H. pylori* from strain 84-183 also was tested in these experiments in order to confirm whether the activation of monocytes by whole *H. pylori* strain CIP 101260 [see above] was strain-specific.)

The water-extracted surface proteins had essentially no contamination by LPS as evidenced by the lack of detection of KDO (Table I), a component of the core oligosaccharide moiety of *Campylobacter* (15) and *Helicobacter* LPS (14). The *Limulus* assay results confirmed that the extracted surface proteins contained insignificant levels of endotoxin activity (Table I). Consistent with previous work (16), the major component of the surface protein was urease, indicated by the marked increase in urease activity compared with whole bacteria, despite the extraction procedure. The protein contamination of the LPS preparation was < 3% and the ratio (17) of KDO to protein for the LPS preparation divided by the value for the whole cells was 37.7, indicating extensive purification of the LPS (17) (Table I).

Table I. Biochemical Characteristics and Endotoxin Content of H. pylori (Strain 84-183) Whole Bacteria, Extracted Surface Proteins, and LPS

	Whole bacteria	Extracted surface proteins	LPS
Protein (*mg/ml*)	4.40	2.57	0.12
KDO (*mg/ml*)	0.0155	0.000	0.0156
KDO/protein ratio × 10^{-4}	35	0.0	1,320
Urease activity (*units/ml*)	38.3	98.3	ND‡
Endotoxin content* (*ng/ml*)	20.0	0.0125	>6.25 × 10^3

* Endotoxin content was determined by *Limulus* amebocyte lysate assay; lower limit of sensitivity was 0.00625 ng/ml (18).
‡ ND, not determined.

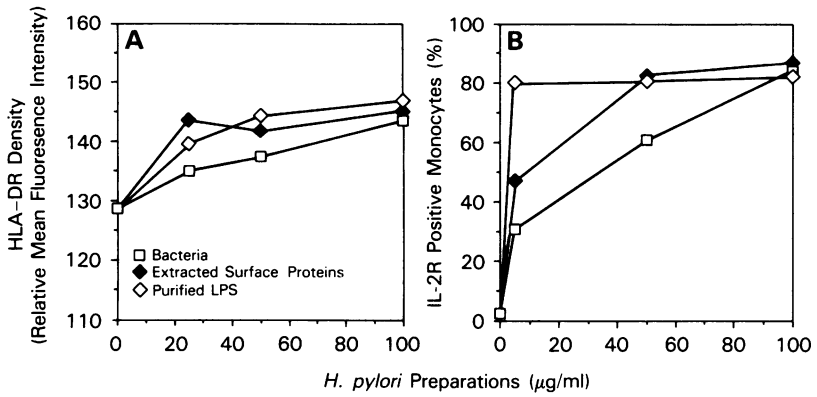

Figure 4. Induction of monocyte antigen expression by whole cells or constituents of *H. pylori* strain 84-183. Purified monocytes (1×10^6/ml) were incubated for 24 h with increasing concentrations of whole *H. pylori* cells, extracted *H. pylori* surface proteins (measured as micrograms of protein per ml), and purified *H. pylori* LPS (μg LPS/ml) and then analyzed for the expression of (*A*) HLA-DR and (*B*) the percent cells positive for IL-2R as in Fig. 1. A Mann test for trend (31) showed that increasing concentrations of each stimulus individually caused a consistent increase in HLA-DR and IL-2R expression ($P < 0.05$), except for surface proteins stimulation of HLA-DR expression ($P = 0.167$); the Omnibus test for overall level of significance (32) for *H. pylori* cells, LPS and surface proteins stimulation of HLA-DR and IL-2R expression were $P \doteq 0.01$ and $P < 0.01$, respectively. Results are from a single analysis representative of three experiments.

As expected, the *H. pylori* preparations that contained LPS, including the whole bacteria and purified *H. pylori* LPS, induced a significant increase in the density of HLA-DR on HLA-DR–positive monocytes (Fig. 4 *A*). These findings are consistent with the well-established phenomenon of LPS activation of monocytes/macrophages. However, the water-extracted surface proteins, which did not contain LPS, unexpectedly also induced HLA-DR expression. Furthermore, the expression induced by this non-LPS constituent was as great or greater than that induced by the whole bacteria. In parallel experiments, the water-extracted surface proteins, as well as the LPS-containing preparations, induced marked increases in the percent of monocytes expressing IL-2R (Fig. 4 *B*). Each preparation caused these phenotypic changes at low concentrations (< 5 μg/ml). Thus, in addition to whole bacterial cells which are potent stimulators of HLA-DR and IL-2R expression, *H. pylori* constituents, including LPS and surface proteins, also have a profound effect on monocyte activation markers.

Effect of H. pylori constituents on IL-1 and TNF gene and peptide expression. We next examined the ability of these *H. pylori* constituents to induce changes in monocyte function, such as increased production of cytokines. In parallel with their ability to induce surface antigen changes, the *H. pylori* LPS and water-extracted surface proteins both served as potent inducers of both IL-1 (Fig. 5 *A*) and TNF (Fig. 5 *B*) secretion. This induction was of the same magnitude as that caused by whole bacterial cells.

To determine the mechanism of the increased production of cytokine peptides, we analyzed monocytes incubated with *H. pylori* cells and constituents for cytokine-specific mRNA synthesis. After a 6-h stimulation, *H. pylori* surface proteins and LPS induced the expression of both IL-1β (Fig. 5 *A*) and TNFα (Fig. 5 *B*) mRNA. Thus, *H. pylori* stimulation of IL-1 and TNFα peptide secretion appears to be regulated at the level of gene transcription.

Effect of H. pylori constituents on monocyte production of O_2^-. To determine whether non-LPS-containing constituents of *H. pylori* stimulate monocyte functions other than cytokine production, we quantitated monocyte production of O_2^- during incubation of the cells with water-extracted *H. pylori* surface proteins and LPS, as well as whole bacteria. As shown in Fig. 6, each preparation of *H. pylori* stimulated O_2^- production, although the surface proteins induced less O_2^- than whole *H. pylori* or *H. pylori* LPS.

Discussion

The results presented here with two strains of *H. pylori* provide the first evidence that these bacterial cells are capable of activating human monocytes. This activation is reflected in the increases in expression of the surface molecules HLA-DR and IL-2R, enhanced synthesis of IL-1 and TNF mRNA and peptide, and increased secretion of the reactive oxygen intermediate O_2^-. Moreover, both non-LPS-containing surface constituents and purified LPS from *H. pylori* stimulated phenotypic, transcriptional, and functional changes in monocytes. Taken together, these results indicate that *H. pylori* activates monocytes by both an LPS-dependent and an LPS-independent mechanism.

For the studies presented here, we utilized water extracted surface proteins consisting largely of urease, rather than purified urease, for three reasons. First, since surface proteins are by definition secreted or shed from the bacterial cell, we probed the response of monocytes to a material that is at least theoretically similar to that which is released by *H. pylori* in vivo. Secondly, studies of partially or totally purified urease indicate that it is less antigenic to infected persons than is the water extract (16). Thirdly, we are not certain whether or not urease is the relevant activation factor in this system. Purification of the *H. pylori* protein(s) that serve as the monocyte activator will be of great interest.

Campylobacter species (36, 37) possess a complex mixture of extractable, biologically active proteins external to the outer membrane. Our findings that extractable surface proteins from *H. pylori* stimulate human monocytes to produce IL-1, TNF, and O_2^- represent the first observation that non-LPS-containing surface protein from a Gram-negative bacterium activates monocytes/macrophages. Although cell wall proteins from the outer membrane of other Gram-negative bacteria (*Salmonella* species and *Escherichia coli*) are capable of activating lymphocytes (38–40), this material is usually heavily contaminated with endotoxin components including lipid A. The surface protein(s) from *H. pylori*, which we show are capable of activat-

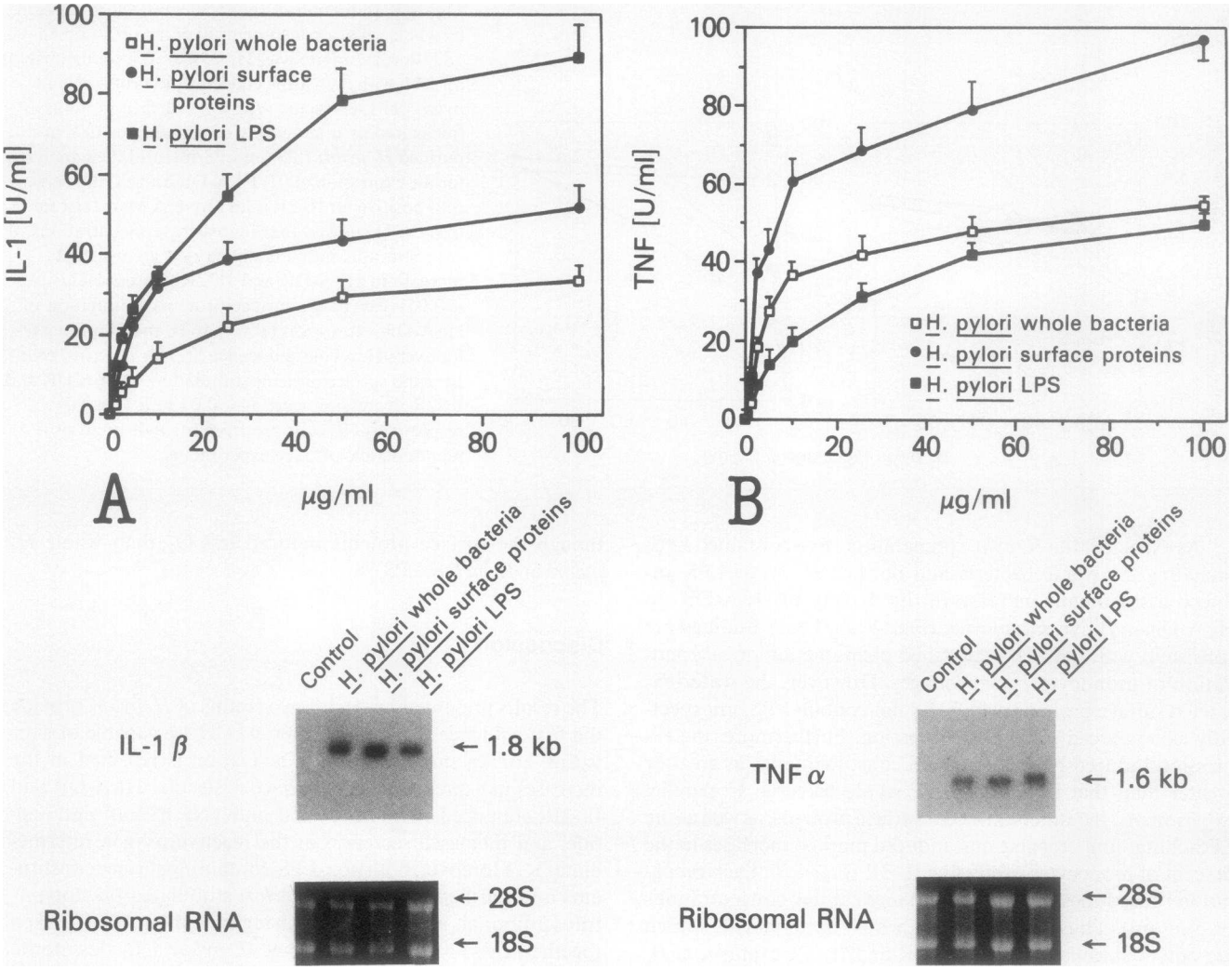

Figure 5. Stimulation of monocyte production of IL-1 and TNF by whole cells or constituents of *H. pylori* strain 84-183 (*upper panels*). Purified monocytes were incubated with *H. pylori* cells or constituents as described for Fig. 4, after which the culture supernatants were harvested and assayed for (*A*) IL-1 and (*B*) TNF activity. Each point is the mean (±SEM) of three determinations from three separate experiments. IL-1β and TNFα gene expression in monocytes stimulated by whole cells and constituents of *H. pylori* strain 84-183 (*middle panels*). RNA from monocytes (20×10^6) incubated for 6 h with media (control) and *H. pylori* whole cells (10 μg of protein/ml), purified LPS (10 μg/ml) or surface proteins (10 μg/ml) was analyzed by Northern blot as described in the Methods. Ethidium bromide staining of ribosomal RNA applied to each lane is shown in the lower panels.

ing monocytes, were free of endotoxin as determined by both the *Limulus* assay and analysis of KDO content. In contrast, Gram-positive bacteria have been reported to secrete protein(s) (exotoxins) capable of activating leukocytes. For example, staphylococcal (41, 42) and streptococcal (43) exotoxins may have a pyrogenic effect, likely through their ability to stimulate production of IL-1 and TNF by monocytes (44–46). To our knowledge, LPS-free surface proteins from a Gram-negative bacterium that are capable of activating human monocytes/macrophages has not been previously reported.

The presence of *H. pylori* in the gastric antrum is specifically associated with both acute and chronic gastritis (1–5), suggesting that the bacteria stimulate an active response by mucosal inflammatory cells. Although neutrophils predominate in acute gastritis, increased numbers of mononuclear cells are the hallmark of chronic *H. pylori*-associated gastritis (47–49). Our observation that whole *H. pylori* cells are capable of activating monocytes in vitro suggests the possibility that in vivo *H. pylori* may activate resident macrophages in the lamina propria or monocytes trafficking through the mucosa. Since *H. pylori* is usually noninvasive, the absorption of secreted soluble surface (non-LPS-containing) proteins could provide an important mechanism for the activation of these cells.

As activated cells within the mucosa, monocytes and macrophages may play critical roles in the host response to *H. pylori*. For example, since *H. pylori* can induce HLA-DR expression by monocytes *in vitro*, it is possible that the organism can stimulate HLA-DR expression on mucosal monocytes *in vivo* similar to its apparent ability to stimulate HLA-DR expression on gastric epithelial cells (50). In view of the requirement that antigens be presented to lymphocytes in the context of class II molecules, increased HLA-DR expression may facilitate presentation of the relevant *H. pylori* antigens, a critical step in the immune response to *H. pylori* and the subsequent production

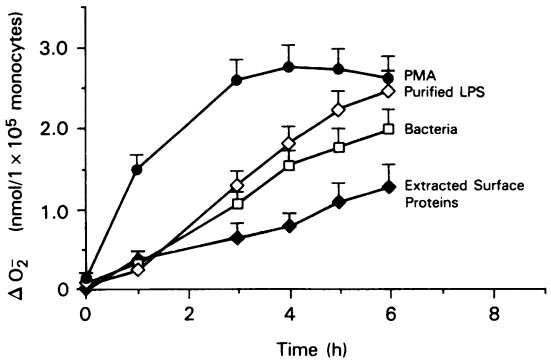

Figure 6. Augmentation of monocyte production of O_2^- by whole cells or constituents of *H. pylori* strain 84-183. Whole *H. pylori* cells or *H. pylori* constituents (50 μg of protein/ml or LPS/ml) were incubated with monocytes (1×10^5/100 μl/well) for 6 h and the amount of O_2^- released was quantitated as described for Fig. 3. Results are from a representative experiment ($n = 3$).

of *H. pylori*-specific antibodies. Also, increased IL-2R expression, which we have shown contributes to monocyte microbicidal activity (32), could facilitate host defense against *H. pylori*. Efficient monocyte microbicidal activity towards *H. pylori*, along with neutrophil microbicidal activity (51, 52), might contribute to the apparent absence of tissue invasion by the organism.

IL-1 and TNF, also shown in this report to be produced by *H. pylori*-activated monocytes, are important mediators of inflammation, including mucosal destruction. In this regard, TNF has been shown to mediate the mucosal lesion in graft-vs.-host disease (53) and the inflammation and ulceration in cytomegalovirus colitis (S. S. Saini and P. D. Smith, manuscript submitted). Similarly, local TNF production may contribute to the pathogenesis of benign gastric ulcer which has been associated with chronic gastritis (54–56) and the presence of *H. pylori* in the antrum (4). In this regard, the recent observation by Crabtree et al. (57) that cultures of gastric mucosal biopsies from *H. pylori*-infected persons release significantly greater amounts of TNFα into culture supernatants than do biopsies from uninfected control subjects supports our observations. Damage to mucosal glands and diminished mucus production are hallmarks of *H. pylori* gastritis (56). A parallel mechanism could contribute to the pathogenesis of duodenal ulceration as well, given the striking association between the presence of *H. pylori* in the duodenum and duodenal ulcer (58). Finally, *H. pylori*-induced secretion of O_2^-, a reactive oxygen intermediate that is capable of inducing cell and mucosal injury (31) and serves as the precursor for other toxic oxygen species, also may contribute to the mucosal inflammation associated with the presence of *H. pylori*. The difference in the magnitude of O_2^- production among the experiments reported here likely reflects donor variability and the use of different amounts and strains of *H. pylori*.

Thus, interaction of *H. pylori* and its constituents with monocytes causes a wide spectrum of phenotypic and functional changes that are consistent with cell activation. Once activated, tissue monocytes and macrophages likely play an important role in the host immune and inflammatory responses to *H. pylori*.

Acknowledgments

The authors thank Dr. H. Donald Hochstein, Food and Drug Administration, for performing the *Limulus* amebocyte assay and Dr. David W. Alling for assistance in the statistical analysis.

This study was supported in part by the Medical Research Service of the Veterans Administration.

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