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Helicobacter pylori

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INTRODUCTION

The first isolation of *Helicobacter pylori* in 1982 by Marshall and Warren (212) ushered in a new era in gastric microbiology. Although spiral organisms had been observed in the gastric mucus layer many times in the preceding century, the isolation of *H. pylori*, in conjunction with increased interest in the pathogenesis of gastroduodenal diseases, as well as the relatively frequent availability of clinical specimens via endoscopic biopsy, has led to important breakthroughs in medical care. The purpose of this paper is to critically review the now enormous literature on *H. pylori* in relation to selected aspects of clinical microbiology. We highlight advances in the detection of the presence of the organism and methods of differentiating among types of *H. pylori*, and we provide a background for appropriate chemotherapy of the infection. In addition, we will briefly discuss *H. heilmanii*, which is recognized as an infrequent cause of gastritis in humans.

DISCOVERY OF *H. PYLORI* AND HISTORICAL DEVELOPMENT OF ITS ROLE IN HUMAN DISEASE

In 1979 Robin Warren, a pathologist in Perth, Western Australia, began to notice that curved bacteria often were present in gastric biopsy specimens submitted for histological examination. These organisms were not present within the gastric mucosa but were present in the mucus layer overlying the tissue (210, 332). Warren found that similar organisms had been described by European pathologists in the late 19th century, but because they could never be isolated, they were ignored and ultimately forgotten by generations of physicians and scientists. A young trainee in internal medicine, Barry Marshall, became interested in Warren's observations, and together the two sought to isolate the organisms from biopsy specimens. Since the organisms had the appearance of curved, gram-negative rods, the investigators used methods for the isolation of *Campylobacter* species, which involved inoculating the biopsy specimens onto selective media and incubating the cultures under microaerobic conditions. Since most campylobacters grow within 48 h under such conditions, plates without visible growth were discarded within 3 days. The initial cultures from approximately 30 patients were negative, but by chance one culture was incubated for 5 days over an Easter holiday and colonies were seen (212)! Subsequently, organisms were isolated from 11 patients; the organism was characterized and called *Campylobacter pyloridis* (now known as *Helicobacter pylori*). Following publication of this seminal report, investigators all over the world rapidly confirmed the presence of these organisms in the gastric mucus (162, 192, 214). By 1984, it had become clear that *H. pylori* infection was strongly associated with the presence of inflammation in the gastric mucosa (chronic superficial gastritis), and especially with polymorphonuclear cell infiltration (chronic active gastritis), although it would be several years before there was sufficient evidence that an etiologic role could be concluded (29). A wide body of evidence now indicates that once acquired, *H. pylori* persists, usually for life, unless eradicated by antimicrobial therapy (30, 33).

Marshall and Warren noted that *H. pylori* infection was associated with duodenal ulceration (212), and this observation too was rapidly confirmed and extended to include gastric ulceration (reviewed in reference 28). By 1994, a consensus conference convened by the National Institutes of Health concluded that *H. pylori* was a major cause of peptic ulcer disease and recommended that infected individuals with ulcers be treated to eradicate the organism (244).

A wide body of evidence had indicated that chronic gastritis is linked to the development of adenocarcinoma of the stomach, the most important gastric malignancy in the world (57), but the causation of the gastritis was then unknown. In 1991, four reports first showed associations between *H. pylori* infection and the presence (310) or the development (123, 246, 256) of gastric cancer. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, reviewed the available evidence and declared that *H. pylori* was a carcinogen of humans (8).

H. pylori infection also has been associated with the development of gastric non-Hodgkin's lymphomas (257) and with another lymphoproliferative disorder, gastric mucosa-associated lymphoid tissue (MALT) lymphoma (MALToma) (106, 342). Importantly, treatment of gastric MALToma patients with antibiotics that eradicate *H. pylori* often leads to regression of the tumor (24, 331, 341). Thus, in total, *H. pylori*, a previously obscure organism, has now been associated with many of the most important diseases involving gastroduodenal tissue.

EPIDEMIOLOGY OF *H. PYLORI* INFECTION

Descriptive Epidemiology

H. pylori has been found in the stomachs of humans in all parts of the world (and is commonly isolated from nonhuman primates as well). In developing countries, 70 to 90% of the

population carries *H. pylori*; almost all of these acquire the infection before the age of 10 years (reviewed in reference 312). In developed countries, the prevalence of infection is lower, ranging from 25 to 50%. The data from developed countries also suggest that most infections are acquired in childhood (312). Consistent with this model is a wide body of evidence that the incidence of *H. pylori* infection has been declining with the changes concomitant with industrial development (22, 254, 255). Thus, in an era of "emerging" microbes, we might think of *H. pylori* as gradually "submerging" from probably near-universality several hundred years ago to the present situation when fewer than 10% of children in developed countries are becoming infected (121, 325). Most studies suggest that males and females are infected at approximately the same rates, although in at least one study, male sex was a significant risk factor for infection (282a). In developed countries, persons of higher socioeconomic status have lower infection rates, although among certain ethnic minorities, high rates persist despite economic advancement (143, 218).

Helicobacter heilmanii (*Gastrospirillum hominis*), first described by Devreker et al. (84), is a tightly spiraled, urease-producing gram-negative bacterium associated with a small subset of cases of human gastritis (81). After an extensive effort by many laboratories, an *H. heilmanii*-like organism has been isolated (5). In both adults and children, *H. heilmanii* infection is associated with active chronic gastritis which is usually milder than that associated with *H. pylori* (249). Whether infection with *H. heilmanii* is associated with peptic ulcer disease, gastric carcinoma, or gastric lymphoma in humans has not been determined. The 16S rRNA gene of *H. heilmanii* has been sequenced; the sequence shows significant homology to that of rRNA from other *Helicobacter* spp. (300). Dogs and cats may serve as a reservoir for *H. heilmanii* (302). Treatment is similar to that used for *H. pylori* (82).

Transmission

There appears to be no substantial reservoir of *H. pylori* aside from the human stomach. Other animals harbor organisms that resemble *H. pylori*, but with the exception of nonhuman primates (94) and, under particular circumstances, perhaps cats (124), none harbor *H. pylori*. Thus, the major question of transmission is how *H. pylori* travels from the stomach of one person to that of another. Three routes have been described.

The first and least common is iatrogenic, in which tubes, endoscopes, or specimens in contact with the gastric mucosa from one person are introduced to another person (1). Improved disinfection of endoscopes has reduced the incidence of transmission (168, 320). Interestingly, endoscopists, especially those who did not wear gloves during procedures, were at increased risk of becoming infected (229). Occupationally acquired infections also have been reported (296); although there does not appear to be any special risk associated with handling this organism, laboratorians should use universal precautions when handling clinical specimens and should remember that *H. pylori* strains are human pathogens.

Fecal-oral transmission is perhaps most important. Although *H. pylori* has been isolated from the feces of young children infected with the organism (314), fecal isolation is not common; this could indicate that shedding is intermittent. Fecally contaminated water may be a source of infection (173), but the organism has not been isolated from water. Food-borne transmission has not been substantiated.

Finally, oral-oral transmission has been identified in the case of African women who premasticate foods given to their infants (215). There is no identified association of infection with sexual transmission (270, 280); therefore, if it occurs, it is uncommon. Transmission via aspiration of *H. pylori* from vomitus is another possibility but has not been documented.

In summary, although *H. pylori* is present in the stomachs of about half of the world's population, we do not yet clearly understand its transmission.

Association with Particular Diseases

All *H. pylori*-infected patients develop chronic gastric inflammation (29), but this condition usually is asymptomatic. This gastritis is not a disease or illness per se. Peptic ulcer disease had been considered to be idiopathic or to be due to agents such as aspirin or nonsteroidal anti-inflammatory drugs or, rarely, to Zollinger-Ellison syndrome, Crohn's disease, and several other inflammatory disorders (271). The idiopathic form of peptic ulcer disease represents 60 to 95% of all cases (depending on the extent of nonsteroidal anti-inflammatory drug use in the population); we now know that *H. pylori* is the cause of nearly all of these cases in adults (244) and that treatment that eradicates *H. pylori* leads to cure of the ulcers. Thus, in any population, *H. pylori* causes the majority of all cases of both gastric and duodenal ulcers.

Carriage of *H. pylori* also is strongly associated with the risk of development of atrophic gastritis (29), which is a precursor lesion to gastric cancer. Thus, not surprisingly, *H. pylori* carriage also is associated with adenocarcinoma of the distal but not the proximal (cardia) stomach (246, 256, 310). Infection is associated with both the intestinal and diffuse histologic types of tumors. This association is extremely important since, in total, gastric cancer is the second leading cause of cancer death in the world (242).

H. pylori infection is associated with a rare gastric disease (Ménétrièr's disease), in which the gastric folds are hypertrophic (25, 346). Patients with a varied group of upper gastrointestinal symptoms that has been termed nonulcer dyspepsia may or may not be infected with *H. pylori*; at present, there is no generally recognized association of nonulcer dyspepsia with *H. pylori* infection (56, 159).

Although early reports suggested that *H. pylori* infection was associated with heart disease (217, 259), later studies suggested that this association may have been confounded by other factors (200, 287). *H. pylori* infection also has been associated with short stature (261), but that finding has not been confirmed. There is no recognized association of *H. pylori* infection and the chronic fatigue syndrome.

MICROBIOLOGICAL CHARACTERISTICS

Morphology

H. pylori organisms are spiral, microaerophilic, gram-negative bacteria that demonstrate bluntly rounded ends in gastric biopsy specimens (140). However, when cultured on solid medium, the bacteria assume a rod-like shape; spiral shapes are infrequent or absent (139). After prolonged culture on solid or in liquid medium, coccoid forms typically predominate (38, 245). By electron microscopy, coccoid forms appear as U-shaped bacilli with the ends of the two arms joined by a membranous structure. Coccoid forms are metabolically active; however, they cannot be cultured in vitro (38, 245). In gastric biopsy specimens, *H. pylori* organisms are 2.5 to 5.0 μm long and 0.5 to 1.0 μm wide; there are four to six unipolar sheathed flagella (139, 140), which are essential for bacterial motility. Each flagellum is approximately 30 μm long and approximately

2.5 nm thick (128, 139). Flagella exhibit a characteristic terminal bulb, which is an extension of the flagellar sheath (128, 140). The flagellar sheath exhibits the typical bilayer structure of a membrane (128).

Ultrastructurally, when tannic acid is used as a mordant, it can be seen that the outer membrane of *H. pylori* is coated with a glycocalyx-like structure; in gastric biopsy specimens, the surface of individual bacteria may be linked to gastric epithelial microvilli by thread-like extensions of the glycocalyx (140). The surface of viable *H. pylori* cells grown on agar plates is coated with 12- to 15-nm ring-shaped aggregates of urease and HspB, a homolog of the GroEL heat shock protein (19). Urease and HspB are also associated with the surface of viable *H. pylori* in vivo (96).

Genome and Plasmids

The genome size of *H. pylori* ranges from 1.6 to 1.73 Mb, with an average of 1.67 Mb (26). The G+C composition averages 35.2 mol%, with a range of 34.1 to 37.5 mol% (26). Approximately 40% of *H. pylori* isolates contain plasmids ranging in size from 1.5 to 23.3 kb, but the plasmids do not contain recognized virulence factors (172, 227). The *H. pylori* genome possesses at least two copies each of the 16S and 23S rRNA genes (311). The variable location of multiple genes in genomic maps suggests that extensive rearrangement of the *H. pylori* genome occurs (43, 161, 311). *H. pylori* exhibits significant sequence diversity in multiple genes including those that encode urease structural (125) and accessory (83) proteins, flagellin (122), vacuolating cytotoxin (69), and CagA (126, 167, 226). Examination of allelic variation in six genes by multilocus enzyme electrophoresis has confirmed the genetic diversity of *H. pylori* strains (137). *H. pylori* strains are naturally competent for DNA uptake, which, through recombination, provides a mechanism for the diversity observed. The biological significance of such diversity is not known. The anticipated publication of the *H. pylori* genome (315) should facilitate our understanding of the variation and metabolism of these microorganisms.

Respiration and Metabolism

In tests involving routine microbiological methods, *H. pylori* does not appear to utilize carbohydrates either fermentatively or oxidatively (139). However, studies by Mendz and Hazell have begun to elucidate metabolic pathways in *H. pylori.*

H. pylori isolates exhibit glucose kinase activity (220), which is associated with the bacterial cell membrane. In addition, enzyme activity characteristic of the pentose phosphate pathway has been identified (220). Thus, *H. pylori* appears to be capable of catabolizing D-glucose. *H. pylori* possess specific D-glucose transporters; some characteristics of the glucose transport system appear to be unique (219).

H. pylori exhibits urea cycle activity, which may serve as an effective mechanism to extrude excess nitrogen from bacterial cells (222). The Entner-Doudoroff pathway has been demonstrated in *H. pylori* (223). Fumarate reductase is an essential component of the metabolism of *H. pylori* and as such constitutes a possible target for therapeutic intervention (224). *H. pylori* can metabolize amino acids by fermentative pathways similar to those in anaerobic bacteria (221). Cytochromes involved in termination of the respiratory chain of *H. pylori* have been characterized (207, 240). The elevated level of CO_2 required for growth of *H. pylori* in vitro may be due in part to activity of the enzyme acetyl coenzyme A carboxylase (44).

H. pylori cells contain polyphosphate granules, which may function as a reserve energy source in bacteria associated with a degenerated epithelium, where an exogenous energy source may be absent (37, 46). Such studies of the metabolism of *H. pylori* are rapidly expanding our basic understanding of this important gastric pathogen; a practical outcome may be the identification of unique metabolic pathways which may serve as therapeutic targets with minimal effect upon the host (42).

Cell Wall and Lipopolysaccharide

A variety of putative outer membrane proteins (OMPs) have been identified. The molecular masses of these OMPs range from 31 to 80 kDa (90). Urease and HspB, a homolog of the GroEL protein of *Escherichia coli*, are abundant in OMP preparations (97). Urease and HspB are located strictly within the cytoplasm in early log phase cultures of *H. pylori* (276). However, in late-log-phase cultures, urease and HspB become associated with the bacterial surface in a novel manner: these cytoplasmic proteins are released by bacterial autolysis and become adsorbed to the surface of intact bacteria due to the unique characteristics of the outer membrane (276). Analyses of *H. pylori* in human gastric biopsy specimens demonstrate that surface adsorption of urease and HspB also occurs in vivo, presumably by a similar mechanism (96). Such "altruistic lysis," in which autolysis of a fraction of the bacterial population presumably benefits the remaining viable bacteria, appears to be essential in understanding the pathogenesis of *H. pylori* and helps to explain (i) how vaccines against an archetypal cytoplasmic protein such as urease can be effective against *Helicobacter* spp. in animal models; (ii) how the integral membrane proteins of *H. pylori* evade immune detection, thus contributing to bacterial persistence in the face of humoral and cellular immune responses; (iii) how the noninvasive bacterium *H. pylori* can present virulence factors and immunogens to the immune system; and (iv) the observed variability of *H. pylori* adhesion to eukaryotic cells in vitro (96, 276).

A family of four porin molecules, designated HopA, HopB, HopC, and HopD, with apparent molecular masses of 48 to 67 kDa, has been purified and characterized. Each of the proteins forms pores with low single-channel conductances in a planar lipid bilayer model membrane system (115). An additional porin molecule, HopE, has homology to the P2 porin of *Haemophilus influenzae* and is antigenic in infected individuals (89).

Some of the OMPs of *H. pylori* are repressible by iron; such proteins may be involved in the uptake of heme from the host (339). At least one of the iron-repressible proteins (apparent molecular mass, 77 kDa) is immunogenic in infected individuals and thus is expressed in vivo (340).

The lipopolysaccharide (LPS) of *H. pylori* has low biological activity, a property which may aid in the persistence of infection (34). The O-specific chain of *H. pylori* LPS mimics Lewis blood group antigens in structure (10, 13, 14, 291, 337). Molecular mimicry between *H. pylori* LPS and the host, based on Lewis antigens, may contribute to pathogenesis. The expression of Lewis antigens on their surface may help to camouflage the bacteria, thus aiding the survival of *H. pylori* (336). Alternatively, immune cross-reactivity between the bacteria and the gastric mucosa may play a direct role in pathogenesis (34).

Nutritional Requirements

Defined media are not available for culture of *H. pylori*; the organism requires complex basal medium (either solid or liquid) with some form of supplementation such as whole blood, heme, serum, charcoal, cornstarch, or egg yolk emulsion (144, 149). While some of these supplements may serve as nutritional substrates, a key function may be detoxification of the medium and protection of the organisms (148). In this regard,

supplementation of media with cyclodextrins supports excellent growth of *H. pylori* (209).

Fresh isolates of *H. pylori* grow best under microaerobic conditions (139). However, after laboratory passage, some strains become sufficiently aerotolerant that they can grow in 10% CO_2. *H. pylori* grows poorly, if at all, under anaerobic conditions (139). Growth in liquid media is typically enhanced by agitation and by incubation in a CO_2-rich atmosphere (60). Growth occurs at 30 to 37°C but not at 25°C. Variable growth of *Helicobacter* spp. occurs at 42°C (139).

PATHOGENESIS OF INFECTION

Evidence That Colonization with *H. pylori* Induces Gastric Inflammation

There now is extensive evidence implicating *H. pylori* in the pathogenesis of chronic superficial gastritis. Voluntary ingestion of the bacterium by two human volunteers resulted in acute or chronic gastritis (211, 234). Eradication of *H. pylori* by antimicrobial therapy clears gastritis (258, 324). Nearly every untreated individual infected with *H. pylori* shows evidence of persistent immunological response to the bacterium. The titer of *H. pylori*-specific antibodies typically decreases after eradication. Animal models of *H. pylori* infection have been developed. After experimental challenge with *H. pylori*, gnotobiotic piglets develop gastritis and a specific serological response (103). *H. pylori* exhibits specificity of tissue involvement; it is associated exclusively with gastric mucosal cells and is not associated with intestinal epithelium either in the small intestine or in intestinal metaplasia of the stomach. *H. pylori* is seen in the duodenal bulb only in association with gastric metaplasia. The organisms have been found in patients with heterotopic gastric mucosa in Meckel's diverticula and in the rectum (80, 235). In addition, *H. pylori* is associated with specific gastroduodenal pathology. Specifically, the presence of *H. pylori* is associated with chronic superficial and type B atrophic gastritis but not with type A, bile reflux, or secondary gastritis. Finally, there have been several reported outbreaks of epidemic hypochlorhydria with gastritis. Based on clinical history, histopathological examination, and serologic results, it appears that these cases were due to iatrogenic *H. pylori* infection (271).

Histopathology of *H. pylori* Infection

Classification of histologic gastritis, which previously had not been uniform from center to center, has now been standardized and updated (87). *H. pylori* is most commonly associated with chronic superficial gastritis. This pattern is characterized by mononuclear inflammatory cell infiltration associated with neutrophilic infiltration of the epithelium and is not specifically associated with metaplastic change, granuloma formation, or fundus gland atrophy (86). The amount of inflammation may be highly variable, ranging from minimal infiltration of the lamina propria with intact glandular architecture to severe dense inflammation with microabscess formation and reactive epithelial atypia (131).

Concomitantly, there often are degenerative changes of the surface epithelial cells including mucin depletion, cytoplasmic vacuolization, and disorganization of mucosal glands. After eradication of *H. pylori* infection by antimicrobial agents, most of these features disappear rapidly (86); however mononuclear cells may persist for several months.

The inflammatory response to *H. pylori* in children differs somewhat from that in adults. Endoscopy may reveal a finely granular or nodular mucosal surface, which microscopically corresponds to lymphonodular hyperplasia, especially in the antrum (133). Such lymphoid aggregates often contain activated germinal centers. In addition, the quantity of neutrophils may be smaller than that seen in adults.

TABLE 1. Putative mechanisms by which *H. pylori* alters gastric physiology

Mechanism	Reference(s)
Induction of gastric inflammation	
IL-8 secretion	48, 70, 155, 290, 319
Neutrophil adherence to endothelial cells	114
PAF	297, 298
Lipopolysaccharide	170, 233, 238, 279, 337
Urease	146, 295
Disruption of the gastric mucosal barrier	
Phospholipases (A_2 and C)	213, 252, 334
Mucinase	294
Vacuolating cytotoxin	64, 69, 150, 196, 275, 288, 313
Reactive oxygen metabolites	20, 21, 75, 76
Induction of iNOS	206, 335
Induction of apoptosis	206, 236
Altered gastrin-gastric acid homeostasis	
Decreased somatostatin release	45, 307
Hypergastrinemia	45, 51, 201
Diminished responsiveness of parietal cells to gastrin	30

Mechanisms of Tissue Injury

H. pylori is able to colonize and persist in a unique biological niche within the gastric lumen. The putative pathogenic determinants of *H. pylori* can be divided into two major groups: virulence factors, which contribute to the pathogenetic effects of the bacterium, and maintenance factors, which allow the bacterium to colonize and remain within the host. Virulence factors will be discussed as they contribute to the three major pathogenic effects of *H. pylori*: gastric inflammation, disruption of the gastric mucosal barrier, and alteration of gastric physiology. It is likely that many *H. pylori* factors function as both virulence and maintenance factors in vivo. Putative pathogenic mechanisms of *H. pylori* are outlined in Table 1; putative maintenance factors are outlined in Table 2.

Induction of gastric inflammation. Superficial gastritis, characterized by infiltration of the gastric mucosa by polymorphonuclear leukocytes and/or mononuclear cells, is invariably present in patients infected with *H. pylori*, suggesting that inflammation may be important for the survival of *H. pylori* in vivo (30).

(i) Interleukin-8. Interleukin-8 (IL-8) is a small peptide (chemokine) secreted by a variety of cell types, which serves as a potent inflammatory mediator recruiting and activating neutrophils. Several studies have demonstrated that *H. pylori* strains are capable of inducing IL-8 secretion from gastric carcinoma cells in vitro (70, 155, 290). Wild-type strains that were $VacA^+$ $CagA^+$ produced significantly more IL-8 than did wild-type $VacA^-$ $CagA^-$ strains (70, 155, 290). However, there was no decrease in IL-8 production by isogenic $VacA^-$, $CagA^-$, or $VacA^-$ $CagA^-$ mutants or by mutants lacking urease subunits (70, 155, 290). Other gram-negative bacteria that do not colonize the gastric mucosa also were able to induce IL-8 secretion under the conditions used (290). Inactivation of a gene encoding a homolog of the *Bordetella pertussis* toxin

TABLE 2. Putative factors enabling maintenance of *H. pylori* in the stomach

Factor	Reference(s)
Motility	
Spiral shape	139, 140
Flagella	104, 105, 128, 164, 251, 304, 305
Adaptive enzymes/proteins	
Urease	23, 95, 99, 153, 154, 230, 269
Catalase	247, 276
Superoxide dismutase	301
Heat shock protein homologs	98, 110, 112, 205, 306
P-type ATPase	127, 216, 239
Siderophores	157, 158
Bacterial adhesins (and cellular receptors)	11, 39, 111, 116, 138, 199, 323
Immune evasion	
Suppression of immune response	175, 194
Resistance to killing by PMNs[a]	171
Molecular mimicry—Lewis antigens	291, 336, 337

[a] PMN, polymorphonuclear leukocyte.

secretion protein, known as *picB* or *cagE*, knocks out the induction of IL-8 in gastric epithelial cells (48, 319).

(ii) Neutrophil adherence. Evans et al. have characterized a 150-kDa protein which increases the expression of neutrophil CD11b/CD18 and increases neutrophil adherence to endothelial cells (114). The protein, designated HP-NAP, is a polymer of 10 identical subunits. The gene (*napA*) shows homology to the gene encoding the bacterioferritin family of proteins (114).

(iii) Platelet-activating factor. Platelet-activating factor (PAF) is a phospholipid mediator which is recognized as a potent ulcerogenic agent. Lyso-PAF is produced by gastric mucosal cells under basal conditions and in response to gastrin in healthy persons (298). PAF stimulates gastric acid secretion via specific parietal cell receptors (297). *H. pylori* can metabolize the nonulcerogenic precursor lyso-PAF into PAF (297). Thus, through synthesis of PAF, *H. pylori* may induce mucosal injury directly or indirectly via increased acid secretion.

(iv) Lipopolysaccharide. *H. pylori* LPS disrupts the gastric mucus coat by interfering with the interaction between mucin and its mucosal receptor; the anti-ulcer agent ebrotidine counteracts this effect (279). However, the outstanding feature of the *H. pylori* LPS is its low proinflammatory activity (233, 238). This phenomenon is mediated by its unique lipid A structure and affects binding to CD-14 (170). The finding of Lewis antigens expressed on part of the polysaccharide side chains (10, 13, 14, 337) is consistent with an LPS molecule having a low profile in its specific host.

(v) Urease. *H. pylori* urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production (146). In vitro, urease activity also is toxic to human gastric epithelial cells (295). Thus, urease appears to function as both a colonization (maintenance) factor and a virulence factor.

Disruption of the gastric mucosal barrier. *H. pylori* can inhibit the secretory response of mucus cells in vitro, indicating a potential deleterious effect on this primary defense mechanism of the gastric mucosa (225).

(i) Phospholipase. *H. pylori* disrupts the protective phospholipid-rich layer at the apical membrane of mucus cells (213). Furthermore, changes can be induced in phospholipid layers in vitro by phospholipases A_2 and C expressed by *H. pylori* (252, 334); their effects can be inhibited by bismuth salts (252).

(ii) Mucinase. *H. pylori* possesses a gene that is almost identical to a mucinase gene of *Vibrio cholerae* (294). Such mucinase activity, if expressed in vivo, would probably contribute to disruption of the gastric mucosal barrier.

(iii) Vacuolating cytotoxin. Approximately one-half of *H. pylori* strains produce a vacuolating cytotoxin in vitro (196). This cytotoxin induces acidic vacuoles in the cytoplasm of eukaryotic cells; these vacuoles accumulate neutral red dye, facilitating their analysis (66, 68). The purified vacuolating cytotoxin, which is active on a wide range of eukaryotic cells, migrates as an approximately 87-kDa protein under denaturing, reducing conditions (63). The vacuolating activity of the cytotoxin is neutralized by specific rabbit antiserum; neutralizing antibodies to the cytotoxin are detectable in the sera of many *H. pylori*-infected individuals (64), including those who develop gastric carcinoma (150).

Almost simultaneously, four groups reported the cloning and sequencing of the *vacA* gene (3.9 kb in size), which encodes the vacuolating cytotoxin (69, 275, 288, 313). The *vacA* gene encodes a 139-kDa protoxin that contains a leader sequence of 33 amino acids, the cytotoxin itself (VacA), and a C-terminal fragment of approximately 50 kDa that exhibits homology to a C-terminal fragment of the IgA protease precursor of *Neisseria gonorrhoeae*. The C-terminal fragment of *N. gonorrhoeae* IgA protease is known to be involved in the translocation of the protease through the outer membrane. By analogy, the C-terminal fragment of the *H. pylori* protoxin may play a role in the secretion of cytotoxin (288). Isogenic *H. pylori* mutants with mutations in the *vacA* gene have been constructed (69, 275, 288). As expected, these mutants do not express the cytotoxin and lack vacuolating activity. The cytotoxin does not exhibit significant relatedness to other known toxins that might give clues to its mechanism of action. Cytotoxin-induced vacuoles appear to be derived from the late endosomal compartments within eukaryotic cells (253).

(iv) Reactive oxygen species. *H. pylori* induces the synthesis of reactive oxygen species (ROS) in gastric mucosa in vivo. There is a positive association between the amount of ROS present, the infective load of *H. pylori*, and the extent of gastric mucosal injury (75). Smoking, drugs, and alcohol consumption had no independent effect upon ROS production in vivo (76). There is no evidence for ROS participation in gastric mucosal injury in cases not related to *H. pylori* infection (76). Levels of 9-hydroxydeoxyguanosine, a marker for oxygen free radical-induced DNA damage, are increased in individuals infected with *H. pylori* compared with uninfected individuals (21), further demonstrating that *H. pylori* infection is associated with ROS production.

There now is direct evidence that ascorbic acid acts as a scavenger of ROS produced in human gastric mucosa (92). Many anti-ulcer drugs function as scavengers of ROS (286, 309, 347), helping to explain how they act to minimize mucosal injury induced by *H. pylori*. Taken together, available data suggest that *H. pylori* stimulates mucosal ROS production both in vitro (20) and in vivo; this phenomenon is very likely to be of pathogenic significance (75).

(v) Inducible nitric oxide synthase. High-output nitric oxide production by inducible nitric oxide synthase (iNOS) is associated with immune activation and tissue injury. *H. pylori* induces iNOS in macrophages in vitro (335). Eradication of *H. pylori* reduces iNOS in human gastric epithelial cells, suggesting that *H. pylori* also induces the activity of this enzyme in vivo (206).

(vi) Apoptosis. *H. pylori* appears to induce programmed cell death (apoptosis) of gastric epithelial cells (206, 236) and to stimulate oxidative DNA damage in infected human gastric

mucosa (21). In addition, *H. pylori* appears to inhibit gastric epithelial cell migration and proliferation (283). Thus, *H. pylori* infection can induce gastric mucosal injury both directly and indirectly.

Altered gastric homeostasis. *H. pylori* infection reversibly induces expression of the acid-stimulating peptide gastrin and suppresses expression of the acid-inhibitory hormone somatostatin (45). These effects may not be due to *H. pylori* per se but may be related to the degree of gastric inflammation present. Acid-secretory studies have demonstrated that *H. pylori* increases the duodenal acid load under some circumstances but appears to decrease acid secretion under other conditions (51, 307). When acid secretion is suppressed with omeprazole, the relative susceptibility of *H. pylori* in the antrum and corpus differs, yielding the appearance of migration of bacteria from the antrum to the corpus (201).

(i) Motility. Motility is an essential colonization factor based on the inability of aflagellate, nonmotile variants of *H. pylori* to infect gnotobiotic piglets (104). Normally, *H. pylori* possesses two to six polar, sheathed flagella, whose filaments consist of two types, encoded by the *flaA* and *flaB* genes (164, 304). These genes have been cloned; the use of induced mutations has shown that both are essential for full motility (164, 304) and for colonization of gnotobiotic piglets (105). The flagellar sheath is a membranous structure which contains proteins and LPS similar to those components in the outer membrane (128); however, the function of the flagellar sheath remains uncertain. A 29-kDa protein specific to the flagella sheath has been identified (204).

Like other bacteria, *H. pylori* and the closely related *H. mustelae*, the gastric pathogen of ferrets, possess a flagellar hook, which is essential for attachment of flagella (251). Mutants with mutations in the *flgE* gene, which encodes the flagellar hook protein, are nonmotile and aflagellate but continue to synthesize flagellins (251). A gene (*flbA*) that codes for the *H. pylori* homolog of a family of conserved proteins (LcrD-InvA-FlbF family), which is involved in the regulation of motility, has been identified and cloned (305). Mutants with mutations in the *flbA* gene are nonmotile and fail to express either flagellin or the hook protein (305).

(ii) Urease. All *H. pylori* isolates, as well as each of the gastric *Helicobacter* species identified to date, produce large quantities of the enzyme urease. The native urease of *H. pylori* has a molecular mass of approximately 540 kDa and is a nickel-containing hexameric molecule consisting of two subunits (UreA [30 kDa] and UreB [62 kDa]) in a 1:1 molar ratio (95, 99, 153). The *H. pylori* urease gene cluster contains nine genes, including the *ureA* and *ureB* structural genes, as well as regulatory genes involved in the synthesis and assembly of the holoenzyme (reviewed in reference 231).

The low Michaelis constant ($K_m \sim 0.3$ mM) of *H. pylori* urease permits this enzyme to be catalytically efficient even at submillimolar concentrations of urea (95, 153). Urease is an essential colonization factor of *Helicobacter* species. Isogenic urease-negative mutants were unable to colonize gnotobiotic piglets regardless of whether the piglets had normal acid output (100) or had been rendered achlorhydric (102). Isogenic urease-negative mutants of *H. pylori* failed to colonize the stomachs of nude mice (316), and isogenic urease-negative mutants of *H. mustelae* failed to colonize ferrets (7).

It is generally presumed that urease activity is required for production of a neutral microenvironment for the organism within the gastric lumen. There is considerable evidence that urease is associated with the outer membrane of *H. pylori* (36, 95, 147). However, some urease activity also is observed within the cytoplasm (36, 276), suggesting a role in assimilation of organic nitrogen. The association between urease and the bacterial surface is apparently stabilized by divalent cations such as Ca^{2+} and Mg^{2+}, although other cations can inhibit the activity of the enzyme (269).

Recombinant urease is optimally expressed when Ni^{2+} uptake is not inhibited in vitro and when sufficient synthesis of the urease subunits UreA and UreB is permitted (154). A high-affinity nickel transport protein (NixA), which provides Ni^{2+} transport at low metal concentrations, is necessary for full activity of recombinant *H. pylori* urease (23, 230). However, isogenic *nixA*-deficient strains of *H. pylori* exhibit urease activity, although at reduced levels compared with strains possessing functional NixA protein (230).

(iii) Catalase and superoxide dismutase. The genes encoding the superoxide dismutase and catalase enzymes of *H. pylori* demonstrate significant homology to those of intracellular pathogenic microorganisms (247, 301), suggesting a role in resistance to killing by polymorphonuclear leukocytes. Of interest, a significant fraction of catalase (276) and superoxide dismutase (301) is associated with the surface of viable *H. pylori*; whether surface association of these enzymes is essential for protection against oxygen-dependent killing of *H. pylori* by neutrophils is not known.

(iv) Heat shock protein homologs. The sequence of the gene encoding the HspB protein of *H. pylori* is highly conserved compared with those of heat shock proteins of other bacteria and humans (205). This conservation suggests that the sequence cannot be modified without affecting the function of the protein. Based on structural similarity, HspB may function as a molecular chaperone for urease (98, 110, 112). The gene (*hspB*) is part of a bicistronic operon (*hspA-hspB*), which has been cloned and sequenced (306). The *hspA* gene, which is located upstream of *hspB*, codes for the *H. pylori* homolog of the GroES heat shock protein homolog. The *H. pylori hspA* gene is unique in that it contains a nickel-binding site at its C terminus (306). Expression of the *hspA* and *hspB* heat shock proteins together with the *H. pylori* urease increases the activity of urease in functional complementation experiments (306). Thus, *hspA* may play a role in the integration of nickel into the functional urease molecule (306).

(v) P-type ATPase. ATPases are of particular interest in *H. pylori* research, because an ATPase is presumed to be the target of the bactericidal action of proton pump inhibitors, such as lansoprazole or omeprazole, on *H. pylori* (239). A P-type ATPase has been cloned and sequenced (127, 216). Isogenic mutants lacking this gene were viable; inactivation of the ATPase gene had no effect on the MIC of omeprazole, suggesting that this ATPase is not the target of the bactericidal action of omeprazole (127).

(vi) Siderophores. Iron is an essential element for bacterial growth and metabolism, but studies on iron acquisition in *H. pylori* have yielded conflicting results. Husson et al. (157) reported that *H. pylori* does not produce siderophores, (iron-scavenging proteins) under iron-limiting conditions. They reported, however, that *H. pylori* could grow in the presence of human lactoferrin and suggested that *H. pylori* has surface receptors for this iron-binding protein. In contrast, Illingworth et al. detected the production of siderophores but did not detect binding of lactoferrin to *H. pylori* proteins (158). Thus, it is not yet clear how *H. pylori* acquires iron for its growth.

Bacterial adhesins and cellular receptors. It is widely accepted that *H. pylori* adheres to receptors in the gastric epithelium by means of specific adhesins. However, definitive identification of bacterial adhesins has been complicated by the observation that several major cytoplasmic proteins tend to

TABLE 3. Selected *H. pylori* proteins that may contribute to pathogenesis

Product	Gene(s)	Suggested function(s)
Urease[a]	*ure* operon	Gastric acid neutralization, nitrogen source, mucosal toxicity
NixA	*nixA*	Ni^{2+} uptake for urease function
Flagella[a]	*flaA, flaB, flgE, flbA*	Bacterial motility
Adhesins	*hpaA* and others	Adherence to gastric epithelial cells
Superoxide dismutase	*sod*	Resistance to killing by phagocytes
Catalase	*katA*	Resistance to killing by phagocytes
HP-NAP	*napA*	Neutrophil activation
Heat shock proteins	*hspA, hspB*[b]	Molecular chaperonins, Ni^{2+} uptake (HspA)
VacA	*vacA*	Cytotoxic for gastric epithelium
CagA	*cagA*	Unknown

[a] *Helicobacter* spp. lacking this product fail to colonize animal models.
[b] Essential for bacterial survival.

become associated with the surface of *H. pylori* during culture on agar (276).

H. pylori strains agglutinate animal erythrocytes (284). The hemagglutinins produced by *H. pylori* have been investigated because they may contribute to adherence in vivo. Supporting this hypothesis, the ability of strains to adhere to different cell lines correlates with their ability to produce agglutination of erythrocytes (284). Variable expression of specific adhesins on bacterial strains may explain the variation in binding by different strains of *H. pylori* which has been observed. A sialic acid-specific adhesin with lectin-like properties was expressed in lower abundance on poorly agglutinating strains than on strongly hemagglutinating strains of *H. pylori* (195). The multiplicity of adhesins present on the surface of *H. pylori* is emphasized by the isolation of a hemagglutinin which recognized α2-3-sialyl glycoconjugates (151, 194) and has affinity for fetuin but differs from the fibrillar hemagglutinin described by Evans et al. (111). However, hemagglutinins alone may not explain in vivo adherence, since some studies have failed to demonstrate a correlation between hemagglutinin titers of *H. pylori* strains and their ability to adhere to human or animal gastric epithelial cells (53, 176).

Phosphatidylethanolamine and gangliotetraosylceramide have been identified as lipid receptors in the mucosa for *H. pylori* and *H. mustelae* (138). The bacterial adhesin of *H. pylori* which recognizes these lipids, a 63-kDa exoenzyme S-like protein, has been purified and characterized (199). However, based on N-terminal amino acid sequence, the gene encoding the exoenzyme S-like protein has been cloned; the protein encoded (catalase) was demonstrated to be cytoplasmic and thus would not be expected to function as an adhesin (247).

Cell lineage-specific binding by *H. pylori* to surface mucus cells in gastric pits appears to be mediated by fucosylated structures and glycoproteins but not by sialylated moieties (116). Boren et al. have provided extensive evidence that the $Lewis^b$ blood group antigen mediates attachment to mucus cells (39). Furthermore, the availability of such fucosylated receptors might be reduced in individuals with blood group A and B phenotypes, compared with blood group O individuals, thus explaining the long-recognized greater risk of peptic ulcer development in blood group O individuals (39).

Other potential receptors for *H. pylori* binding include extracellular matrix components which may become exposed after injury to the gastric epithelium. In vitro, *H. pylori* strains bind laminin, fibronectin, various collagens, and heparan sulfate (11, 323). Based on serologic evidence, an external polysaccharide layer present on the surface of *H. pylori* may play a role in adherence (93).

Immune evasion. Although *H. pylori* can stimulate the immune system to produce antibodies, there appears to be suppressive activity against the human cellular immune response which possibly is mediated by a protein (175). *H. pylori* exhibits at least partial resistance to killing by phagocytes, perhaps due to damage to phagosomal membranes by ammonia produced by *H. pylori* (171). Sialic acid-specific hemagglutinins on the mucosal surface may delay the adhesion and ingestion of *H. pylori* (194). As noted above, expression of Lewis antigens on the surface of *H. pylori* may help to camouflage the bacteria among gastric mucosal antigens.

Another potential mechanism of immune evasion exhibited by *H. pylori* may be modification of bacterial morphology. The bacillary forms of *H. pylori* convert to coccoid forms after prolonged culture. While the morphology, physiology, and biochemistry of coccoid forms have been described by a variety of investigators (27, 38, 47, 160, 274), their significance in infection is unknown. Such forms may represent an environmentally resistant resting or dormant phase which can be induced to revert to the virulent bacillary form in vivo; however, coccoid forms of *H. pylori* do not infect gnotobiotic piglets (101). Chan et al. have demonstrated that coccoid forms occur in the human gastric mucosa (49). Selected *H. pylori* proteins thought to contribute to pathogenesis, and the genes encoding these proteins are outlined in Table 3.

DIAGNOSIS OF INFECTION

A variety of tests are now available to diagnose *H. pylori* infection (Table 4). Histological examination of gastric tissue, bacterial culture, rapid urease testing, use of DNA probes, and PCR analysis, when used to test gastric tissue, all require endoscopy; therefore they incur expense and a risk, albeit slight, of complication due to the procedure. In contrast, breath tests, serology, gastric juice PCR, and urinary excretion of [^{15}N]ammonia are noninvasive tests that do not require endoscopy. The choice of test used for diagnosis of *H. pylori* infection will depend, in most cases, on the clinical information sought and the local availability and cost of individual tests.

Methods Requiring Endoscopy

At endoscopy, many adults with *H. pylori*-associated gastritis have normal-appearing gastric mucosa. The distribution of *H. pylori* and the associated inflammation is often patchy. The patchy nature of infection can lead to endoscopic sampling error, resulting in false-negative biopsy, culture, and rapid urease test results. At a minimum, two biopsy specimens taken

TABLE 4. Diagnostic tests for detection of *H. pylori*

Test	Sensitivity (%)	Specificity (%)	Endoscopy required?	Comments	Reference(s)
Histology	93–98	95–98	Yes	Multiple antral biopsy specimens recommended; special stains improve sensitivity	109, 130, 132, 134
Culture	77–95	100	Yes	For susceptibility testing and detailed characterization of isolates	144
Rapid urease test (e.g. CLO)	89–98	93–98	Yes	Endoscopic method of choice for diagnosis of *H. pylori* infection	73, 187, 189, 280
^{13}C-UBT	90–95	90–95	No	Assesses existing load of viable bacteria; well suited to follow-up of antimicrobial therapy	54, 243
^{14}C-UBT	90–95	90–95	No	As above for ^{13}C-UBT; low radiation exposure	274, 303
Serology	88–95	86–95	No	Not appropriate for short-term follow-up of antimicrobial therapy; excellent epidemiologic tool	4, 95, 113, 152, 268, 312
PCR methods (biopsy specimen or body fluids)	85–96	90–100	Yes (biopsy), no (saliva)	Assess DNA, not necessarily viable bacteria; useful to assess *H. pylori* strain differences including antibiotic (clarithromycin) resistance	12, 107, 197, 184

from within 5 cm of the pylorus should be obtained at endoscopy, with multiple sections being examined histologically (296). Genta and Graham, however, reported a sensitivity of 100% with biopsy specimens taken from the angularis of the stomach (130). Two conditions observable directly by the endoscopist, antral nodularity and uncomplicated duodenal ulcer disease, are almost always associated with *H. pylori* infection (186); hence, testing for *H. pylori* in these settings may not be necessary.

Culture. Culture of *H. pylori* has two major advantages. First, it allows antimicrobial susceptibility testing; second, isolates obtained by culture can be characterized in detail. Although the sensitivity of culture in experienced laboratories is greater than 95%, other methods for the diagnosis of *H. pylori* infection are simpler, prone to less variability, and more timely. Culture of gastric biopsy specimens typically provides the greatest yield of *H. pylori*. However, culture of gastric juice occasionally has been successful, and there are reports of successful culture from feces (169, 314). Only the principles of culture techniques for gastric biopsies are reviewed below.

Although preimmersion of biopsy forceps in formaldehyde does not appear to adversely influence the recoverability of organisms in culture (348), we recommend that gastric mucosal samples for culture be obtained initially (before sampling for histology or other tests). Saline is a simple acceptable short-term (<6 h) transport medium (145, 330). If the culture is to be delayed, more complex media such as Stuart's medium or supplemented brain heart infusion broth should be used (145). Media containing glycerol are suitable for long-term storage of biopsy specimens at −70°C (145), or the specimens can be immediately frozen at −70°C without a fluid medium.

A variety of selective and nonselective media are available commercially for culture of *H. pylori* (144). The use of multiple media may increase sensitivity. *H. pylori* requires a microaerobic environment, high humidity, and incubation at 35 to 37°C for a maximum of 7 to 10 days. Positive cultures are usually detected after 3 to 5 days of incubation. *H. pylori* is identified on the basis of colony morphology (translucent colonies varying in size from barely detectable with the naked eye to approximately 3 mm); colonies consist of gram-negative, curved (not usually helical) rods that are urease, catalase, and oxidase positive. The addition of tetrazolium salts aids in the identification of *H. pylori* colonies cultured on agar media (280a).

Histologic assessment. *H. pylori* can be visualized at high magnification with conventional hematoxylin and eosin (H & E)-stained sections. Bacteria are located in the mucus adherent to the surface epithelium and are often found deep within the crypts. However, H & E staining may be unreliable when few bacteria are present. In addition, luminal debris on the surface of the epithelium can be mistaken for *H. pylori* in H & E-stained sections. Histological identification of bacteria is facilitated by using special stains such as the Warthin-Starry and modified Giemsa stains (109, 130). The distribution of *H. pylori* in the stomach is not uniform, nor are organisms usually found in areas of intestinal metaplasia (132).

Histologic identification of bacteria with the characteristic morphology of *H. pylori* is, in part, observer dependent. Factors that influence the ability to correctly identify *H. pylori* include bacterial density, type of stain used, and the enthusiasm and experience of the laboratorian (6, 52, 109, 178, 188, 232).

A sensitive staining technique consisting of a combination of H & E, Steiner silver stain, and alcian blue has been developed by Genta et al. (134). This stain reportedly allows ready detection of *H. pylori* while simultaneously allowing evaluation of gastric histology, thus obviating the need for additional staining. However, the Genta stain procedure can be technically difficult, and its acceptance among gastrointestinal pathologists has not been universal (190).

Selection of cases needing special staining (if not performed routinely) may be guided by the inflammatory infiltrate of the gastric biopsy specimens. In the absence of inflammation, the likelihood of *H. pylori* infection is remote, and special stains are not indicated. However, if an active inflammatory infiltrate is present and *H. pylori* is not detected, special stains may be appropriate. In fact, the presence of histologically active gastric inflammation in a patient not previously treated for *H. pylori* infection may be pathognomonic for *H. pylori* infection (73). In summary, sole reliance on H & E staining when few *H. pylori* organisms are identified or when *H. pylori* is not detected in the presence of significant inflammation may be imprudent. Interestingly, 76% of respondents to an Internet survey ordered a special stain on receipt of a gastric biopsy specimen (129). It is likely that the additional expense of the special stain is offset by a reduction in time required for slide interpretation due to improved identification of *H. pylori.*

Immunohistochemical staining techniques also have been developed to detect *H. pylori* (12). Such techniques are usually not necessary but may prove worthwhile in cases where tinctorial stains are difficult to interpret.

Biopsy urease tests. The early observation that *H. pylori* produces large amounts of urease activity led to the development of methods for the indirect detection of the organism in

gastric biopsy tissue. The sensitivity of all urease-based tests for detection of *H. pylori* is dependent upon the bacterial load in the stomach (185).

The CLOtest (Delta West Ltd., Bentley, Australia), developed by Marshall, was the first of the commercially available biopsy urease tests designed specifically for *H. pylori* detection. It consists of an agar gel containing phenol red and urea; in the presence of urease, the urea is hydrolyzed, leading to a pH (and hence a color) change of the indicator. The test is interpreted up to 24 h after placement of the gastric biopsy sample onto the agar gel. Of the available biopsy urease tests (also known as rapid urease tests) the CLOtest has been the most widely studied (73, 185, 187).

Two other biopsy urease tests are available commercially: Hpfast, a gel test similar to CLOtest but with a different pH indicator at a lower pH, and PyloriTek, a strip test. In the latter test, in the presence of urease, ammonia is produced from urea impregnated into a pad. The diffusion of ammonia through a membrane is detected by an overlying pH indicator. A potential advantage is that interpretation requires no more than 1 h. The PyloriTek test has received a waiver under the Clinical Laboratory Improvement Amendments of 1988 (CLIA); hence, it is not under the same strict regulatory control as are many laboratory tests. Comparative studies of the sensitivity and specificity of the CLOtest, Hpfast, and PyloriTek tests have been performed (189). The overall sensitivities were equivalent (88 to 93%), and the specificities were excellent (99 to 100%). At 1 h (the end point for reading PyloriTek), the sensitivities of the gel tests were significantly lower (66 to 71%). The PyloriTek test seems to be the test of choice, if a rapid result (1 h or less) is desired. However, if rapid results are not needed, all three tests provide equivalent accuracies and the choice may be made based on cost, regulatory issues, availability, physician preference, or other factors. An important point to keep in mind is that increased incubation times may lead to improved sensitivity of biopsy urease tests but to decreased specificity of detection of *H. pylori.*

PCR. PCR offers great promise as a highly sensitive and specific technique for the detection of *H. pylori*. PCR techniques for the detection of *H. pylori* in gastric biopsy specimens have been described by a number of laboratories, although the accuracy of such techniques varies widely (12, 107). Factors affecting test accuracy include the choice of primers and target DNA, specimen preparation, bacterial density, and technical issues regarding the PCR procedure. Li et al. have developed a PCR assay which is reportedly 100% sensitive and specific for detection of *H. pylori* infection with gastric mucosal biopsy specimens (197). We (265) and others (183) have also developed PCR and reverse transcriptase PCR assays which demonstrated excellent accuracy for the detection of *H. pylori* infection in a limited number of patients. In contrast, in another study, PCR was less accurate than immunohistologic staining of bacteria (12).

A potential advantage of PCR is that it may enable the diagnosis of *H. pylori* to be made noninvasively by detecting *H. pylori* DNA in nongastric fluids such as saliva. In one study, the sensitivity of PCR for detecting *H. pylori* in saliva was 84% (197). To explain the observation that the saliva of some individuals was positive for *H. pylori* by PCR in the absence of detectable bacteria within the stomach, the authors suggest that the oral cavity is the initial site of infection; colonization of the stomach is not a necessary consequence of oral infection (197). Other investigators, however, have not been able to routinely detect *H. pylori* DNA in saliva even among patients with proven gastric *H. pylori* infection (184). The reasons for these discrepancies are not clear.

The potential for obtaining false-positive results by PCR should not be underestimated. Using a primer set which amplifies a 109-bp fragment of the 16S rRNA gene of *H. pylori*, Chong et al. (51a) found a 109-bp PCR product in a variety of tissue samples from *H. pylori*-infected and uninfected individuals. They concluded that the PCR product was amplified from the human genome (51a).

PCR techniques for the detection of *H. pylori* are still in their infancy; it is unlikely that such techniques will have widespread use in the initial detection of *H. pylori*, except in the research environment. However, PCR methods hold great promise in the detection of genetic differences between *H. pylori* strains for research and epidemiologic studies.

Nonendoscopic Methods

Antibody detection. Infection of the gastric mucosa with *H. pylori* results in systemic as well as local immune responses, including elevation of specific IgG and IgA levels in serum and elevated levels of secretory IgA and IgM in the stomach, thus allowing the development of serologic tests for detection of the bacteria. Serologic methods have proven especially valuable in screening large numbers of individuals in epidemiologic studies (268, 312). Such tests are noninvasive, relatively rapid and simple to perform, and much less expensive than tests requiring endoscopic biopsy. Further, serologic tests are less likely to be confounded by suppression of *H. pylori* infection by bismuth compounds, proton pump inhibitors, or antibiotics taken for unrelated conditions than are urease-based tests, which are dependent upon and reflect the current bacterial load. Although a wide variety of serologic methods for detection of *H. pylori* have been described in the literature, most tests available commercially are enzyme-linked immunosorbent assay methods.

The utility of any serologic test for the detection of *H. pylori*-specific antibodies is dependent on the antigen preparation used. In general, three types of antigen have been used. These include crude antigens such as whole cells and whole-cell sonicates, cell fractions such as glycine extracts and heat-stable antigens, and enriched antigens such as urease and a 120-kDa antigen (4, 95, 113, 268). The sensitivities and specificities of tests involving all three types of antigen preparation typically approach 95%. However, a recent meta-analysis of studies of 11 commercial enzyme-linked immunosorbent assay kits and one latex agglutination kit found an average sensitivity of 85% and specificity of 79% (203).

In the absence of therapeutic intervention, antibody levels remain elevated, perhaps for a lifetime, reflecting the duration of infection. After eradication of *H. pylori*, specific immunoglobulin G (IgG) and IgA levels tend to decrease, typically to approximately half of the pretreatment value within 6 months (72, 152, 179). Low levels of specific IgG tend to persist for months even after eradication of *H. pylori*; therefore, using serologic tests to assess the effects of treatment may be problematic unless the pre- and posttreatment sera can be directly compared.

In general, in the United States, the clinician should appreciate that only tests approved by the U.S. Food and Drug Administration (FDA) for detecting anti-*H. pylori* IgG antibodies should be used; results from clinical laboratories which use their own in-house assays may be suspect.

Despite their ready commercial availability, serologic tests for detection of *H. pylori* are not without their drawbacks. One of the difficulties is that since serology is a "global" method, reflecting infection anywhere in the stomach, it may be more accurate than the "gold standard" biopsy-based methods,

which are local (each biopsy specimen represents approximately 0.001% of the surface of the stomach) and subject to a variety of sampling errors. For instance, serologic tests may be positive in patients with gastric atrophy (166), in which the number of *H. pylori* organisms is so small as to be undetectable by biopsy or breath test-based methods. Presumably, some individuals with gastric atrophy continue to have serologic evidence of a prior *H. pylori* infection which has "burnt out." If the likelihood of *H. pylori* infection is low (for example, a 17-year-old Caucasian North American woman complaining of dyspepsia), the predictive value of a positive test is low. Therefore, an argument can be made that antibody tests should be used only as screening tests. A negative test predicts a low probability of infection in a patient with a low likelihood of infection, and further evaluation for infection is unnecessary. Given the same patient, however, confirmation of a positive test may be desirable. In contrast, in patients with peptic ulcer disease, a positive test does not need confirmation, but sole reliance on a negative test would be inappropriate (even more so in a patient with a bleeding ulcer), since patient management is critically dependent on accurate identification of the patient's *H. pylori* status.

Antibody tests now commercially available include not only laboratory tests but also the so-called "office-based" or "near-patient" tests, which use either serum or fingerstick whole blood. These tests are relatively new, and data on their accuracy are limited. Two such fingerstick tests, Flexsure HP and Quickview One Step, however, have received FDA approval. Their performance characteristics to date suggest that they are less accurate than are at least some laboratory-based tests (55, 285). Fingerstick tests are relatively inexpensive (<$15) and simple to use, and results are available in 5 to 15 min. The One Step test is currently CLIA waived, and the Flexsure HP will probably become waived. However, the tests may yield indeterminate or invalid results related to insufficient blood obtained at fingerstick or other procedural errors, and published results mostly relate to "per protocol" analysis. It remains controversial whether these tests should replace the more costly laboratory tests for the initial diagnosis of *H. pylori* infection. A disadvantage of the office-based tests is that they are qualitative only, precluding their use for early follow-up testing for assessment of cure after anti-*H. pylori* treatment.

Other antibody tests include the detection of IgA antibody in serum (73) and detection of anti-*H. pylori* IgG antibody in saliva (117, 260, 293) and urine (3). Currently, these tests are in research and development; the accuracy of each of these assays is lower than that of serum IgG antibody tests. Although saliva and urine tests are conceptually interesting, in part because they obviate the need for phlebotomy, at present they do not have sufficient diagnostic accuracy to endorse their use (3, 293).

Urea breath tests. The principle of urea breath tests (UBTs) for the diagnosis of *H. pylori* is similar to that of other urease-based tests. Urea is provided as a substrate which, in the case of the UBT, is ingested as either [^{13}C] or [^{14}C]urea. *H. pylori* urease hydrolyzes the ingested urea into labeled bicarbonate, which is exhaled as labeled CO_2, which is collected. The ^{14}C isotope is detected with a scintillation counter, while the ^{13}C isotope is detected by mass spectrometry most commonly, although other analytical methods have been developed (177, 289). Some microorganisms within the oropharynx also may hydrolyze urea; therefore, if urea is presented in liquid form, an early peak in labeled CO_2 may occur during the breath test. Thus, the timing of breath collection, the form of delivery of urea (liquid versus tablet form), the gastric emptying time, and other factors may influence the accuracy of the test. Overall, the performance characteristics of both ^{13}C and ^{14}C tests are similar (281).

A commercial ^{13}C-UBT has been approved by the FDA (243), and it is possible that a ^{14}C test will be approved shortly. The importance of FDA approval for these tests should not be underestimated. Field trials of the now commercially available ^{13}C Meretek UBT led to changes in the cutoff value between positive and negative results. Other refinements in packaging and quality control are apparent. Although a single breath sample after ^{13}C urea ingestion is theoretically sufficiently discriminatory for defining the *H. pylori* status, two baseline breath samples and two 30-min post-urea-ingestion samples are obtained. These paired duplicate samples should minimize inadmissible results ascribed to poor collection or inadequate transfer of breath samples to the tubes, tubes that break, faulty seals, and other factors. The accuracy of the test in the absence of treatment with agents that suppress *H. pylori* has been approximately 95% in field trials (54). Only a few patients find the pudding, which is administered as part of the Meretek test protocol to delay gastric emptying and to improve the distribution of the subsequently ingested urea in the stomach, unpalatable.

A variety of ^{14}C protocols also have been used, but the Trimed test (274) is the one most likely to gain FDA approval. In this protocol, the dose of ^{14}C is low, (1 μCi), the equivalent of less than 1 day's background radiation (303). Advantages include the delivery of ^{14}C urea in tablet form, thus avoiding the problem of detecting urease-positive oral bacteria. No meal (pudding or otherwise) is given. Since the background level of ^{14}C is minimal, in contrast to that of ^{13}C, only a single breath sample need be collected (10 min after ingestion of [^{14}C]urea). As with the ^{13}C Meretek test, sensitivity and specificity are excellent. The choice of UBT (^{13}C versus ^{14}C) will depend on availability, cost, and regulatory issues. The ^{14}C test is not recommended for use in children or pregnant women.

Other assays. Collection of blood or urine to measure substrate metabolism by *H. pylori* has been described. For instance, the concentration of labeled serum bicarbonate following administration of [^{13}C]urea, as in the UBT, may accurately reflect the *H. pylori* status of patients (237). Similarly, measurement of ^{14}C in the urine has been reported to accurately reflect *H. pylori* status (262). In the presence of urease activity, [^{15}N]urea is metabolized into [^{15}N]ammonia which is absorbed and excreted in the urine. Urinary levels of [^{15}N]ammonia (which, like [^{13}C]urea, is not radioactive) have been measured in a small number of patients, allowing excellent discrimination between infected and uninfected individuals (343). However, none of these methods has been completely standardized. Further, these methods do not confer significant advantages over previously described diagnostic methods.

Assays That Differentiate among *H. pylori* Infections

H. pylori strains are highly diverse at the genetic level (reviewed in references 31 and 202). In fact, compared with 13 other important human pathogens and commensal organisms by the technique of multilocus enzyme electrophoresis, *H. pylori* showed the most diversity of the group (137). Diversity is a fundamental characteristic of *H. pylori* and reflects point mutations, genomic rearrangements, nonconserved genes, allelic variation within conserved genes, and mobile DNA including insertion sequences, and plasmids. In several cases, there now is evidence that genetic and/or phenotypic diversity is of clinical significance. In this section, we review the clinical microbiology of these markers of virulence.

***cagA*.** In the United States and Europe, about 60% of all *H. pylori* strains possess a gene called *cagA* (62, 318), although this proportion varies in different populations (267). This gene, of unknown function, is a marker for the cag region, a pathogenicity island of about 35 kb (2, 48) composed of approximately 20 genes, including *picB*, a strong homolog of type III secretion proteins which is required for *H. pylori* to induce gastric epithelial cells to secrete IL-8 (48, 319). It now is clear that infection with a $cagA^+$ strain enhances the risk for development of duodenal ulcers and adenocarcinoma of the distal stomach compared with infection with strains lacking *cagA* (32). As such, the specific detection of infection with a $cagA^+$ strain is becoming clinically relevant. A variety of techniques may be used. Perhaps the most clinically useful is serologic detection with recombinant *cagA* antigens (35, 65, 344); the reported sensitivity of IgG serology was 94.4%, with a specificity of 92.5% (35). Since serology is a global technique, unlike biopsy methods, it is probably most accurate for detecting infection with $cagA^+$ strains. Coinfection with $cagA^+$ strains and strains lacking *cagA* has been described (326); serology will identify that a host is infected with a $cagA^+$ strain even if the sole isolate studied from a biopsy specimen lacks *cagA* (35). Biopsy-based methods include PCR (265) and RT-PCR (263), both of which are highly accurate if the biopsy specimen contains a $cagA^+$ strain. Alternatively, *H. pylori* isolates can be examined by either PCR (265) or colony hybridization (318); when properly conducted, these are highly accurate techniques.

***vacA*.** Essentially all *H. pylori* strains carry a gene called *vacA* (69, 275, 288, 313), which in certain strains allows the in vitro expression of a protein toxin that induces vacuolation in a wide variety of eukaryotic cells (67, 196). It now is clear from comparisons of *vacA* from strain to strain that this gene contains both conserved and diverse regions, forming a mosaic gene (15, 69). The diversity involves two major regions, which we have called the s (for signal sequence encoding) and m (for midregion encoding) types (15). There are three allelic s types, called s1a, s1b, and s2, and two m types, called m1 and m2. This system allows for six combinations, five of which have been observed among wild-type *H. pylori* strains (15). Interestingly, s1a strains produce the highest level of the cytotoxin, followed by s1b strains; s2 strains usually produce little or no toxin (15). Similarly, s1a strains (irrespective of the m type) are associated with the highest levels of gastric inflammation, whereas m1 strains (irrespectively of the s type) are associated with the highest levels of injury to the gastric epithelium (17). At present, these strains can be differentiated only when pure cultures of the bacterial cells are available. Such differentiation can be accomplished by PCR or by colony hybridization (15). Development of type-specific recombinant peptides for serologic studies is in progress. Studies of strains from other parts of the world suggest that the m region is more complex than studies of U.S. strains have indicated, including the finding of an m1-m2 chimera (16). Thus, while promising, this work is not yet ready for clinical use.

Lewis antigens. The Lewis antigens are polymorphic fucosylated glycoconjugates, best recognized on erythrocyte surfaces but also present in a wide variety of epithelial cells, including those in gastric mucosa. Aspinall et al. found that the LPS of several *H. pylori* strains contained Le^x and/or Le^y as part of their polysaccharide side chains (13, 14). Several groups, using monoclonal antibodies, have confirmed and extended these observations (10, 291, 292, 336). Thus far, *H. pylori* strains have been shown to express Le^a, Le^b, Le^x, Le^y, or sialo-Le^x antigens (10, 291, 337). Virtually all strains express these antigens, and they often express more than one (337). Expression of the type 2 determinants (Le^x and Le^y) is much more common than expression of type 1 determinants (Le^a and Le^b) (337), paralleling the heightened expression of the type 2 antigens by gastric epithelial cells. In general, $cagA^+$ strains show higher level Le expression than do strains lacking *cagA* (337). The isolates from Le (a+b−) hosts express Le^x more strongly than they express Le^y, isolates from Le (a−b+) hosts express more Le^y than Le^x, and for isolates from Le (a−b−) hosts, expression of Le^x and Le^y is approximately equal (336). These data are consistent with selection of *H. pylori* strains and/or phenotypic regulation in relation to the host Lewis phenotype. Using monoclonal antibodies to the Le determinants, Le typing of *H. pylori* strains can be done, but at present there is no clinical utility for such studies.

Other polymorphic loci. Peek and colleagues described a locus, *iceA*, which has two major variants, *iceA1* and *iceA2*, among diverse *H. pylori* strains (264). These studies indicated that infection with an *iceA1* strain is strongly associated with duodenal ulceration. Confirmation of this observation is needed.

Posttreatment Diagnosis

Existing antimicrobial therapy is not always completely effective against *H. pylori* infection. As a result, clinicians may wish to determine if the patient has been cured of *H. pylori* after treatment. Most of the tests used for the initial diagnosis of *H. pylori* can also be used for posttreatment diagnosis. In general, evaluating for a cure of *H. pylori* with tests that assess the actual bacterial load (UBT and biopsy methods) should not be performed less than 4 weeks after therapy (174). This is because up to 4 weeks after treatment that ultimately is shown to have failed, it may not be possible to detect *H. pylori* due to "suppression" or reduction of the bacterial load. This phenomenon has been termed "clearance." It is a phenomenon that can be appreciated only in retrospect, after "recrudescence" occurs. "Cure" (or bacterial "eradication") is used to describe the absence of detectable organisms 4 weeks or more after treatment. Performing posttreatment assays sooner than 4 weeks after completion of therapy will falsely indicate treatment efficacy in a proportion of cases, since the bacterial population, when suppressed, may be difficult to detect. A prolonged time (more than 4 weeks) is required to allow suppressed populations to regrow to their original densities in the mucosa.

Biopsy-based methods. Since the sensitivity of the CLOtest is dependent on the density of *H. pylori* (185), it is reasonable to expect that biopsy urease tests may be less sensitive in the posttreatment setting. To minimize sampling errors, especially for rapid urease testing, multiple biopsy specimens should be taken (108). A single pass of a multiple-bite forceps may prove useful in this regard, although such an approach has not yet been reported. However, even in the untreated patient, the sensitivity of the CLOtest may be increased by taking biopsy specimens from both the antrum and the body compared to taking one biopsy specimen (53a). The least sensitive site for assessing the eradication of *H. pylori* appears to be the prepyloric region, with a false-negative rate of approximately 10% (108).

Serology. (i) *H. pylori*-specific antibodies. After cure of *H. pylori* infection, anti-*H. pylori* antibody levels typically decrease, allowing quantitative serologic tests to be used for follow-up evaluation of treated patients. A 50% decline in the antibody level is reliably accurate in establishing a cure (179). Conventionally, convalescent-phase and baseline serum samples are analyzed simultaneously to minimize errors resulting from assay variation. Such variation, however, is minimal and is not likely to be consequential when evaluating for declines in

antibody level of approximately 50% (95). Therefore, provided the same assay is to be used on the convalescent-phase sample as was used on the baseline sample, it is probably not essential to store the baseline sample. Of critical importance for determination of posttreatment cure, however, is the timing of the convalescent-phase assay (152). Differences in antibody level may be observed as early as 6 weeks (72, 179), but these differences do not allow for discrimination between cure and treatment failure, since in both groups there may be an initial decline in the antibody level. Distinguishing between success and failure can be reliably accomplished in approximately 85% of patients by testing at 6 months, and, indeed, in one study, 95% of cured patients became seronegative at 1 year after treatment (179). However, in another report, only 36% of patients became seronegative at 1 year follow-up (72); this may reflect in part inadequacies of the "gold standard," as described above. It is not known whether all patients eventually become seronegative after cure (or "spontaneous" eradication) of *H. pylori* infection. Because the time frame for the antibody level to decline sufficiently (usually 20 to 50% of the original level) to assess cure is unpredictable and prolonged, *H. pylori*-specific antibody assays are considered impractical in the immediate posttreatment clinical setting.

(ii) Pepsinogens. Pepsinogens belong to two immunologically distinct types, PGI (or PGA) and PGII (or PGC). PGI is produced primarily in the oxyntic mucosa of the stomach, while PGII is produced more diffusely throughout the stomach, including the antrum. Levels of PGI and PGII are elevated in many, but not all, individuals infected with *H. pylori* (182).

Levels of PGI (156, 248), and PGII (156, 277) in serum and the PGI/PGII ratio (156, 277) change in patients cured of *H. pylori* infection. A decline typically occurs in PGII, but less reliably in PGI, while the PGI/PGII ratio rises due to the greater decrease in the PGII level (presumably related to the resolution of antral gastritis). In one study (156), a decrease of 25% in the PGII level 1 month after treatment had a sensitivity of 82% for eradication of *H. pylori*, but the specificity was only 62%. In another study, with a best cutoff value 2 months after treatment, the sensitivity in the decline of the PGII level was 59% while the specificity was 62% (277). The diagnostic accuracy for the change in PGI/PGII ratio was slightly higher than that of the other parameters but was no higher than 68%. At present, based on these results, pepsinogen levels are not useful clinically. In addition, the levels of gastrin and somatostatin in serum also change following eradication of *H. pylori*, but these changes have not proven to be of clinical utility (45).

Urea breath tests. One potential pitfall regarding UBTs in the posttherapy patient relates to the topographical distribution of *H. pylori*. Specifically, the distribution of ingested urea tends to be greater in the antrum (18). However, the bacterial load may be reduced preferentially in the antrum, especially after therapy with a proton pump inhibitor (85). It is possible, therefore, that after treatment, UBTs will provide false-negative results, due to incomplete assessment of the bacterial load in the body of the stomach. Although the current standard time for use of a UBT in the posttreatment evaluation of patients is 4 weeks after the cessation of therapy, its use at 6 or 8 weeks should decrease the incidence of false-negative results caused by suppression rather than cure of infection (273).

TREATMENT

Resistance to Antimicrobial Agents

In vitro, *H. pylori* strains are susceptible to penicillins, some (cefuroxime) but not all (cephalexin) cephalosporins, macrolides, tetracyclines, nitroimidazoles, nitrofurans, quinolones, bismuth salts, and proton pump inhibitors (PPIs) (9, 61, 163). They are intrinsically resistant to H_2-receptor blockers (e.g., cimetidine and ranitidine), polymyxin, and trimethoprim (241, 278). Many inconsistencies exist between in vitro susceptibility testing for *H. pylori* and patient response to therapy. For example, methods for determining antimicrobial susceptibility and the breakpoints for defining resistance of *H. pylori* are not standardized; there may be an apparent reversibility of metronidazole resistance in vitro (328, 333). The effects of antimicrobial resistance on the efficacy of treatment may be variable and unpredictable; the role of susceptibility testing in guiding therapy is not established. In addition, extrinsic factors such as smoking may adversely influence the results of metronidazole treatment (338).

There are a number of reports on the outcome of treatment regimens based on retrospective analysis of antibiotic resistance. In regimens involving metronidazole, efficacy has not been consistently influenced by in vitro resistance of the *H. pylori* strain to metronidazole. This variability may relate, in part, to the choice of accompanying antimicrobial and other agents. Macrolide antibiotics, particularly clarithromycin, are increasingly used in regimens against *H. pylori* and, when used in combination with metronidazole and other agents, may help to mitigate the effect of metronidazole resistance. In vitro resistance to clarithromycin, however, appears to adversely affect treatment outcome more directly (141).

The prevalence rate of metronidazole resistance among *H. pylori* strains is highly variable. In developed countries, the prevalence of resistance ranges from 11 to 70% (135, 165, 250, 282). Resistance to metronidazole is even more prevalent in developing countries (136, 329), where up to 95% of isolates may be resistant (282). In contrast, the prevalence of clarithromycin resistance generally is not more than 10% (250, 273); resistance tends to be lower in countries where clarithromycin only recently has become available and where other macrolides are not widely used. An exception has been noted in Peru, however, where 50% of *H. pylori* isolates were found to be clarithromycin resistant (329). Resistance to both metronidazole and clarithromycin has been reported (250, 282). Resistance to tetracycline has been noted in up to 6% of isolates in the United Kingdom (165). Moreover, tolerance (defined as decreased bacterial killing by growth-inhibiting concentrations of the drug) to amoxicillin, an antibiotic also frequently used in anti-*H. pylori* regimens, has been reported (90a). Practically speaking, then, for populations in which metronidazole-resistant *H. pylori* strains are common, regimens that lack metronidazole but contain amoxicillin may be preferable. Indeed, in one trial, there was significantly lower efficacy for the combination of a PPI, amoxicillin, and metronidazole compared to the combination of a PPI, amoxicillin, and clarithromycin (228). However, the combination of a PPI, bismuth, tetracycline, and metronidazole has resulted in excellent cure rates despite the presence of metronidazole-resistant strains (40).

Despite the uncertainties discussed above, it is suggested that patients known or suspected to have been exposed previously to metronidazole or clarithromycin should be offered anti-*H. pylori* therapy that does not include these antimicrobial agents.

After unsuccessful treatment of *H. pylori* infection with metronidazole and/or clarithromycin, the secondary resistance rate is higher than is the primary resistance rate (71, 77, 141, 273, 322). Thus, it could be argued that patients who have failed treatment with metronidazole and/or (especially) clarithromycin should be retreated with alternative treatments. It is likely that the nature of the particular combination of medications

(and, perhaps as importantly, compliance with the treatment regimen) influences the outcome as much as does the resistance of *H. pylori* to individual antibiotics.

Current Treatment Regimens

The aim of this section is to provide an overview of *H. pylori* treatment, reflecting bias and opinion but also mirroring uncertainty, since it would be presumptuous at this time to claim that there is one "best" treatment applicable to all patients and all situations.

FDA-approved regimens for patients with peptic ulcer disease include traditional triple therapy dispensed in a blister pack consisting of bismuth subsalicylate (two tablets; 262 mg), metronidazole (250 mg), and tetracycline (500 mg), all taken four times daily for 14 days. While the packaging promotes compliance, the cost of treatment might be cheaper if the drugs were prescribed individually. Substitution of amoxicillin for tetracycline results in a lower cure rate and is not recommended except in young children, in whom tetracyclines are contraindicated. Outside the United States, bismuth subcitrate is usually used instead of bismuth subsalicylate, and treatment for 7 days has been shown to be effective. As yet, adequate direct comparisons between 7 and 14 days of therapy with bismuth subsalicylate have not been reported (345). Because of the complexity of the regimen and the associated side effects, leading to problems with compliance, greater credence should be placed on meta-analysis or pooled-data reports (266, 321, 327) of efficacy rather than single-center reports for this regimen in particular but also for *H. pylori* regimens in general. A realistic cure rate to anticipate for triple therapy is about 85% (321).

The above regimen does not include an anti-acid secretory agent, which is highly desirable in the setting of active ulcer disease (308). In general, concomitant acid-suppressive therapy need be used for only 2 weeks. However, depending on the agent chosen and the location and size of the ulcer, acid suppression may have to be continued for 4 to 6 weeks.

"Quadruple" therapy (in which triple therapy is combined with use of a PPI) has been shown to be more efficacious and associated with fewer side effects than has routine triple therapy (78–80). Such regimens would be especially appealing if the duration of quadruple therapy necessary to accomplish cure rates of >90% proved to be very short. Indeed, a cure rate of 72% with only 1 day of treatment by a regimen that could be considered a variant of quadruple therapy has been reported (317). Studies in the Netherlands report that a 7-day regimen of quadruple therapy was highly (98%) effective (78).

Other FDA-approved regimens are the dual therapies consisting of clarithromycin (500 mg three times a day) together with either omeprazole (40 mg once daily) (cure rate, 74% [118]) or ranitidine bismuth citrate (RBC) (400 mg twice a day) (cure rate, 82% [272]) for 14 days. An earlier-touted dual-therapy regimen, omeprazole plus amoxicillin, is neither reliable (142, 191) nor FDA approved and should not be used. The American College of Gastroenterology, in recognition of the suboptimal efficacy of the approved dual-therapy regimens, has recommended the addition of other antibiotics to these regimens (299). Preliminary results of two RBC modifications (a 2-week course of RBC [400 mg twice a day], metronidazole [250 mg three times a day], and tetracycline [500 mg three times a day]; or RBC [400 mg], clarithromycin [500 mg], and amoxicillin [1 g]) show excellent promise (188).

Among the most effective but not FDA-approved regimens are "new" triple-therapy regimens that are quite unlike "traditional" triple-therapy regimens in that the new regimens do not include bismuth compounds. They are known as 3-2-1 regimens: three medicines taken twice a day for 1 week. These regimens have been best studied in the so-called Mach 1 (M, metronidazole; a, amoxicillin; c, clarithromycin; h, *H. pylori*; 1, 1 week) trial (198). Efficacy, defined per protocol but not by intention to treat, was 90 to 96% in this trial (198). In per-protocol analyses, only patients who complete the entire protocol are included. Thus, patients who drop out due to side effects or other reasons are not included in the final analysis. In contrast, in typical intention-to-treat studies, results from all patients who enter a given protocol are analyzed. Patients who drop out of intention-to-test studies are considered not to have been treated successfully. Results of intention-to-treat analyses, which are more stringent than those of per-protocol analyses, are more applicable to real-life situations. Selected treatment regimens for *H. pylori* are outlined in Table 5.

Side effects of treatment relate to the particular regimen chosen. Clarithromycin may cause taste disturbances, but this does not usually affect compliance. Metronidazole, particularly at a dose greater than 1 g/day, may be associated with side effects. Traditional triple therapy may be associated with "mild" side effects in about 50% of patients; vaginal candidiasis occurs in up to 10% of women. In general, pseudomembranous colitis occurs infrequently, although in one treatment regimen 11% of individuals developed this complication (41). Overall, discontinuation of therapy related to side effects occurs in less than 5% of patients. At present, the only clear indications for therapy are in patients with peptic ulcer disease or MALTomas.

Of interest, extracts of a variety of plants inhibit the growth of *H. pylori* in vitro (115a, 309a). Whether such extracts will prove useful in the treatment of infected patients remains to be demonstrated.

Prevention and Eradication of *H. pylori* Infection by Vaccines

Although target human populations for prevention of *H. pylori* infection have not been defined, the development of vaccines is under way. Vaccination against *Helicobacter* infection has been successful in a variety of animal models involving orogastric administration of *H. pylori* antigens in combination with mucosal adjuvants such as cholera toxin and the heat-labile enterotoxin of *Escherichia coli*. In animals, a variety of immunogens confer protection against subsequent bacterial challenge or allow eradication of preexisting *Helicobacter* infection ("therapeutic immunization"); such immunogens include bacterial sonicates (50, 74, 88, 208) purified urease holoenzyme, urease subunits or portions thereof (59, 91, 119, 193), purified VacA cytotoxin (208), and the GroEL (HspB) and GroES (HspA) heat shock protein homologs (120).

The ease of production of recombinant urease in large quantities, coupled with its efficacy in protecting against infection and curing existing infection in animal models, makes urease a promising candidate for human use. A double-blind placebo-controlled clinical trial evaluated the safety of urease in human volunteers infected naturally with *H. pylori* (180). In this phase I trial, orally administered recombinant *H. pylori* urease was well tolerated by the volunteers. However, since a mucosal adjuvant was not included, the *H. pylori* infection was not cured (180). A phase II clinical trial, designed to monitor the safety and immunogenicity of recombinant urease in the presence of heat-labile toxin as a mucosal adjuvant, is in progress (180).

While much progress toward the development of vaccines against *H. pylori* has been made, little is known about the mechanisms of mucosal immunity against *Helicobacter* infec-

TABLE 5. FDA-approved and promising therapeutic regimens for *H. pylori*

Regimen	Dosage[a]	Length of therapy	Eradication rate (%)	FDA approved?
Triple therapy containing bismuth				
Tetracycline HCl plus	500 mg q.i.d.	2 wk	85	Yes
Metronidazole plus	250 mg q.i.d.			
Bismuth subsalicylate	2 tablets q.i.d.			
Dual therapy				
Clarithromycin plus	500 mg t.i.d.	2 wk	74	Yes
Omeprazole	40 mg daily			
or				
Clarithromycin plus	500 mg t.i.d.	2 wk	82	Yes
Ranitidine bismuth citrate	400 mg b.i.d.			
Triple-therapy regimens				
Metronidazole or	500 mg b.i.d.	1 wk (2 wk may be preferred)	90	No (approval pending)
Amoxicillin plus	1 g b.i.d.			
Clarithromycin plus	500 mg b.i.d.			
Omeprazole or	20 mg b.i.d.			
Lansoprazole	30 mg b.i.d.			
Ranitidine bismuth citrate plus	400 mg b.i.d.	2 wk	90	No
Clarithromycin plus	500 mg b.i.d.			
Amoxicillin	1 g b.i.d.			

[a] b.i.d., twice daily; t.i.d., three times daily; q.i.d., four times daily.

tion. In fact, it is quite surprising that proteins (such as urease and heat shock protein homologs) which are located strictly within the cytoplasm of other enteric pathogens may serve as effective vaccines against *Helicobacter* spp. In an effort to understand this paradox, we have demonstrated that urease and HspB are located strictly within the cytoplasm of bacteria in the logarithmic growth phase; these proteins become associated with the surface of *H. pylori* only concomitant with bacterial autolysis in vitro (276). Urease and HspB are associated with the surface of *H. pylori* in human gastric biopsy specimens, suggesting that bacterial autolysis leads to surface adsorption of these proteins in vivo (96). In vitro, cytoplasmic urease alone is not sufficient to protect *H. pylori* against the deleterious effects of acid pH; only when urease becomes associated with the surface of *H. pylori* is the bacterium protected against acid (181). Thus, surface-associated urease appears to be essential for the protection of *H. pylori* against acid both in vitro and in vivo. Based on such observations, it can be predicted that urease-based vaccines in humans will be effective strictly against *H. pylori* with surface-associated urease while ignoring those bacteria that lack surface-associated urease (96).

A recent study with naked DNA vaccines has demonstrated that mice vaccinated with *H. pylori ureB* DNA developed less inflammation than did nonimmunized animals (58). DNA immunization also decreased the level of infection of *H. felis* in mice, irrespective of the titer of specific antibodies (58). Further studies are necessary to determine whether DNA immunization will represent a complement to orogastric immunization against *Helicobacter* species.

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