tion machinery? What is the role of the specific chaperones? What are the cues that stimulate type III secretion? How do the different effector proteins function inside the host cell? The study of these systems will continue to provide insight into the mechanisms of manipulation of host cell functions by bacterial pathogens. The presence of type III secretion systems exclusively in bacteria with pathogenic potential may provide a unique target for the development of therapeutic agents that may spare normal flora. Furthermore, harnessing the type III secretion system for the delivery of heterologous proteins may provide a valuable tool for the development of novel vaccines and therapeutic approaches.

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46. We regret that because of space constraints, we were unable to reference many important papers. We thank C. Roy, J. F. Kim, and members of the Galán and Collmer laboratories for careful reading of this manuscript; K. Eichelberg for preparing Fig. 1; and W.-L. Deng for the micrographs in Fig. 3. Work in our laboratories is supported by NIH grants GM52543 and AI30491 (to J.G.) and by NSF grant MCB-9631530 and National Research Initiative Competitive Grants Program/U.S. Department of Agriculture grant 97-35303-4488 (to A.C.). J.G. is an investigator for the American Heart Association.

REVIEW

Helicobacter pylori Virulence and Genetic Geography

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Isolated for the first time in 1982 from human gastric biopsy, *Helicobacter pylori* is responsible for gastritis, peptic ulcer, and gastric cancer. A pathogenicity island acquired by horizontal transfer, coding for a type IV secretion system, is a major determinant of virulence. The infection is now treated with antibiotics, and vaccines are in preparation. The geographic distribution suggests coevolution of man and *Helicobacter pylori*.

Human, plant, and animal diseases are often caused by infection with unrecognized or uncultivated (or both) etiologic agents. Until 1982, when it was isolated by accidental extended incubation, *Helicobacter pylori* (*Hp*) was part of the unknown microbial world (*1*). Today it is a well-recognized pathogen that chronically infects up to 50% of the world's human population. It is a Gram-negative, microaerophilic bacterial rod, associated with gastritis, peptic ulcer, and gastric cancer.

Hp lives for decades in the extreme environment of the human stomach. Like other bacteria specialized to live in a single environment, *Hp* has a small genome (1.67 megabases) containing a minimal set of metabolic genes (*2*). The mechanisms for environmental adaptation such as the stringent response and the two-component regulatory systems are absent or rare, respectively (*3*). For example, *Pseudomonas aeruginosa*, an opportunist bacterium able to survive in most environments, contains 90 two-component regulatory systems, whereas *Hp* contains only four (*3*, *4*).

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Population Genetics and Diversity

Hp isolates from unrelated individuals have totally different genetic fingerprints, so that *Hp* can be considered a "quasi-species" (*5*). However, the genome itself is not so diverse. Variability is due to changes at the third base of codons, to inversions, and to translocations, events that modify the DNA but not the protein sequences. Comparison of the genomic sequence of two independent clinical isolates has shown that they are highly conserved, with only 7% of the proteins being strain-specific (*6*).

Infection is disproportionately acquired in childhood. Once the stomach is colonized, the same organism persists for decades, if not for a lifetime. Bacteria isolated from the same patient at intervals of several years have identical DNA fingerprints, and mixed infections are uncommon (*7*). In spite of the fact that DNA fingerprints are maintained, however, continuous evolution occurs within the stomach of the infected person, because of (i) nucleotide mutations, (ii) excision of the *cag*-pathogenicity island (PAI) (see below), (iii) transposition of insertion elements, (iv) recombination with DNA from incoming strains that do not establish a chronic infection, and (v) horizontal transfer of new genes (*7*, *8*). As a consequence, bacteria isolated from adult patients, although similar in DNA fingerprint to that acquired at a younger age, have accumulated much genetic variability that can be detected as single-nucleotide polymorphism.

The family is the core unit of *Hp* transmission. Frequently children are infected by a strain with a genetic fingerprint identical to that of one of the parents. The children chronically maintain the same strain even after leaving home and establishing their own family (*9*). Hence, transmission is likely to be more frequent within the family or among infants within a community. Once infection is established, subsequent infection by other *Hp* strains appears to be rare (*7*). Husbands and wives do not exchange their strains, and infection is rarely transmitted to an uninfected partner. It is likely, however, that transiently infecting *Hp* strains, although unable to colonize, provide genetic material to the resident strain and increase its fitness.

Present Epidemiology

Although in most developing countries infection is almost universal in adults, today in northern Europe and the United States infection is less frequent, and although it is still present in 30 to 50% of adults who acquired the infection in childhood, it is rare in today's children ("birth cohort effect") (*10*). *Hp* more commonly infects people of lower socioeconomic status. Yet, even when socioeconomic status is taken into account, certain racial or ethnic groups (in the United States, blacks and Hispanics) still have higher rates of *Hp*. This may be due to genetic predispositions to infection that are as yet poorly understood. Males in many populations also appear to have 20 to 30% higher rates of infection than females (*11*).

The mode of transmission of *Hp* is unknown. The most widely held hypotheses are that the organism is transmitted directly from person to person by human feces (fecal-oral spread), gastric contents (gastric-oral spread), or improperly cleaned endoscopic equipment. The handful of documented cases of acute *Hp* infection have all suggested gastric-oral routes of transmission. *Hp* has been cultured from vomitus, saliva, and diarrheal stools (*10*). There is evidence that *Hp*, by decreasing the gastric acidity, permits acid-sensitive gastrointestinal pathogens to pass through the stomach into the intestines (*12*). By thus increasing the risk for gastroenteritis, *Hp* may be favoring its own excretion and perpetuation.

Links Between *Hp* and Disease

Hp causes acute and chronic inflammation in the stomach, although the magnitude of inflammation varies from strain to strain and from host to host. In the majority of infected humans, there are no clinical consequences to *Hp* gastritis. In 20 to 30%, however, the end result of infection can be life-threatening. Four diseases are now widely acknowledged to be caused by *Hp*: duodenal ulcer, gastric ulcers, adenocarcinoma of the distal stomach (antrum and fundus), and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Taken together, each year, at least 7 million cases of these diseases occur worldwide, resulting in hundreds of thousands of deaths (*13*). Gastric adenocarcinoma is the 14th leading cause of death in the world and, with the aging of the world's population, is expected to be the 8th leading cause of death by the year 2010.

Evidence for the association between *Hp* and the aforementioned diseases includes randomized clinical trials (duodenal and gastric ulcers), nonrandomized trials (gastric lymphoma), and large epidemiological studies (all four diseases). However, the epidemiology of duodenal ulcer disease and gastric cancer incidence do not completely parallel one another (*14*). Moreover, people with *Hp*-related ulcer disease are less likely to develop gastric adenocarcinoma than the average population (*14*). This reflects the importance of the unknown cofactors that contribute to disease outcome. Variability in host factors such as blood group antigens, human lymphocyte antigen type, age of infection, and environmental exposures has been suggested to explain the different clinical outcomes. Synergistic or antagonistic interactions between *Hp* and putative risk factors—whether they be genetic, dietary, infectious, or occupational—require rigorous investigation.

In addition to the above diseases, *Hp* has been linked to dyspepsia and to a multitude of nongastric conditions including atherosclerosis, allergic skin diseases, hepatic encephalopathy, childhood anemia, and growth retardation. It has also been reported that *Hp* infection may be beneficial and protect against reflux esophagitis and adenocarcinoma of the distal esophagus and gastric cardia (*15*). None of these associations has been consistently demonstrated.

Virulence

Many factors contribute to the virulence of *Hp* (Table 1). Expressed by all isolates are factors required for colonization and survival in the human stomach. Most notable among these factors are the urease and flagella. Urease metabolizes urea to carbon dioxide and ammonia to buffer the gastric acid. Flagella allow the bacterium to swim across the viscous gastric mucus and reach the more neutral pH below the mucus. Knockout mutants of the urease or flagellar genes are defective in colonization in a gnotobiotic piglet model of infection (*16*).

Once below the mucus, *Hp* adheres tightly

Table 1. Reported virulence factors.

Factor	Function	Distribution	Reference
Urease	Buffers stomach acid	All strains	(*51*)
Flagella	Motility	All strains	(*52*)
NAP	Neutrophil activation	All strains	(*20*)
BabA	Adhesin for Le^b	Prevalent on type I strains	(*18*)
LPS	Low toxicity	All strains	(*53*)
$Lewis^{x,y}$ antigens	Molecular mimicry	Some strains	(*19*)
IceA	Homolog of Nla III restriction endonuclease	Some strains	(*54*)
VacA	Cytotoxicity (two alleles)	All strains	(*21*,*23*)
cag PAI	31 genes coding for type IV secretion system	Type I strains	(*26*)
CagA	Immunodominant antigen (part of *cag* PAI)	Type I strains	(*33*)
PicB	Equivalent to CagE	Type I	(*26*)

to the underlying cells. Several epithelial structures have been implicated in adhesion, including lipids, gangliosides, and sulfated carbohydrates, but to date, the adhesins on the bacterial surface that bind to the epithelium are poorly understood (*17*). The best characterized *Hp* adhesin is a protein (BabA) that binds the Lewis b blood group antigens on the gastric epithelium (*18*). Lewis blood group antigens have also been implicated in another variable aspect of *Hp* pathogenesis; the lipopolysaccharide of some strains contains structures identical to the fucosylated Lewis x and Lewis y blood group antigens expressed on the gastric mucosa. This antigenic mimicry may result in immune tolerance against antigens of the pathogen or in induction of autoantibodies that recognize gastric epithelial cells, frequently observed in patients with chronic active gastritis (*19*).

Among the molecules that act directly on the surrounding tissues, the most important are the neutrophil activating protein (NAP) and the vacuolating cytotoxin (VacA). The former is an oligomeric protein made of 10 to 12 copies of a 17-kD polypeptide with homology to iron-binding proteins. This protein has the capacity to activate neutrophils and may be involved in the recruitment of these cells to the gastric mucosa and hence may contribute to the inflammatory response (*20*).

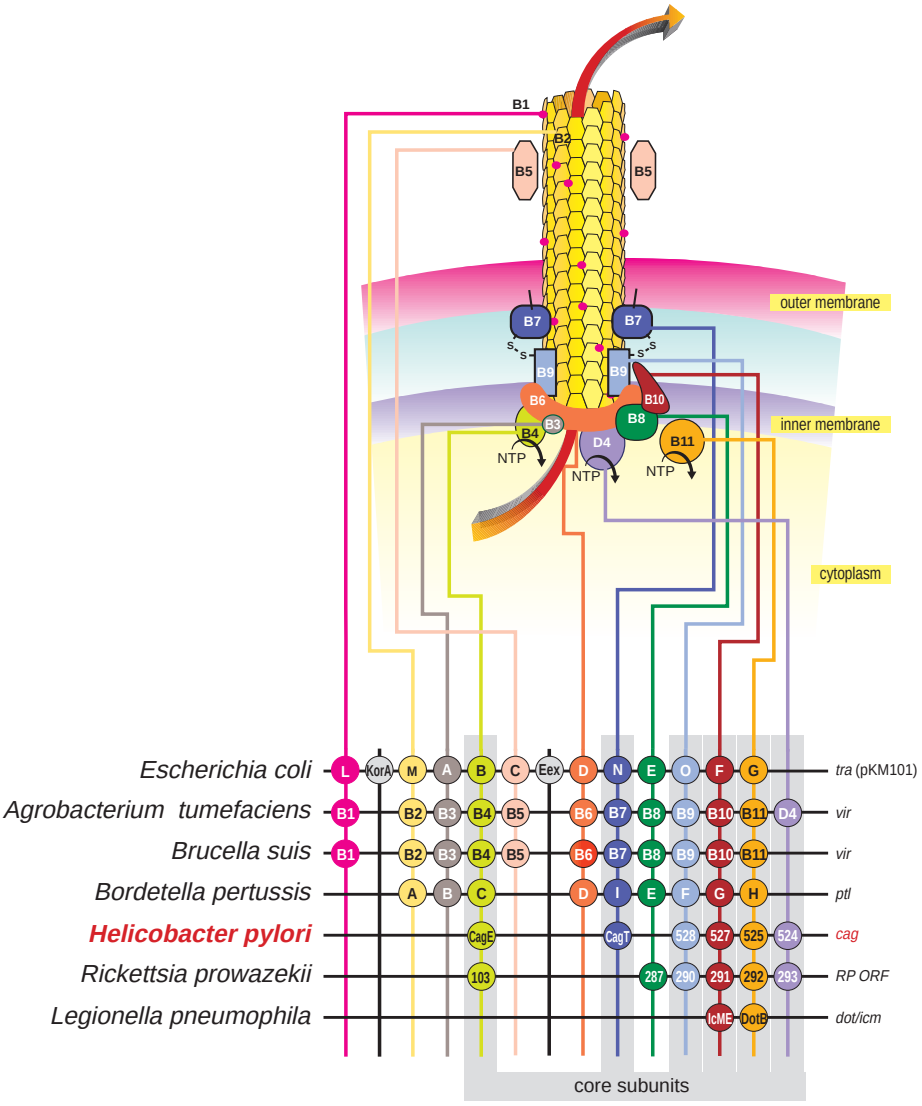

Fig. 1. Schematic representation of the putative structure (**top**) of the type IV organelle and the operons coding for it in different bacteria (**bottom**). The scheme is drawn with the conjugative pilus of *E. coli* and *A. tumefaciens* as a prototype. The product of each gene, named with the *Agrobacterium* VirB system (B indicates VirB), is wired to the corresponding protein in the organelle. NTP indicates ATPase activity. The large red arrow indicates transfer of a secreted component across the bacterial envelope. Homologous genes are indicated by the same color. For *Hp*, indicated in red, only the *cag* genes with sequence homology to the *vir* genes of *Agrobacterium* are reported. Genes boxed in gray identify the core subunits that have been proposed to be necessary for assembly of the minimal type IV structure. ORF, open reading frame.

VacA is a secreted protein toxin that is responsible for the gastric epithelial erosion observed in infected hosts. It causes vacuolar degeneration of target cells by interfering with intracellular membrane fusion. The vacuoles appear to be a hybrid between lysosomal and late endosome compartments, and their generation requires the vacuolar adenosine triphosphate–dependent proton pump and the small guanosine triphosphate–binding protein Rab7. More recently, the toxin has been shown to reduce transepithelial resistance by loosening tight junctions (*21*).

VacA is an oligomeric toxin, with flower-shaped structure and sixfold or sevenfold radial symmetry (*22*). The oligomeric toxin is inactive, and dissociation into the 90-kD monomer by treatment at low pH is required to reveal its activity. VacA associated with the surface of the bacteria is, however, active in the absence of low pH dissociation, suggesting that the toxin forms oligomers only after release from the bacteria (*21*).

There are two alleles (m1 and m2) of a 300–amino acid region containing the cell-binding domain of VacA, which have different target cell specificities. Only the m1 form is toxic on HeLa cells in the standard assay of vacuolization (*23*); however, both of them are active on primary gastric cells. It is not clear why this functional polymorphism has evolved, but it may reflect human genetic polymorphism, because the m1 form is predominant in western, Korean, and Japanese isolates, whereas the m2 form is found in 75% of Chinese isolates (*24*).

A Type IV Secretion Machinery Building a Pathogen

The major disease-associated, genetic difference in *Hp* isolates is the presence or absence of a so-called PAI, which, as in other bacterial pathogens, has been acquired by horizontal transfer of a genetic cassette. It is not known from which organism the "alien" DNA was acquired; however, this DNA has maintained a GC content that is different from that of *Hp*, a signature of the donor organism (*25*). The *Hp* PAI, named *cag*, is a 40-kb locus, containing 31 genes, inserted into the chromosomal glutamate racemase gene. At some point during evolution, IS*605*, a mobile sequence encoding two transposases, entered the *Hp* genome and in some strains interrupted, mutilated, or deleted parts of the PAI (*26*).

Six of the *cag* genes are homologous to well-known genes present in a collinear arrangement in operons of *Bordetella pertussis*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Legionella pneumophila*, *Rickettsia prowazekii*, and *Brucella suis* (Fig. 1). These operons code for type IV export machineries specialized in transfer of a variety of multimolecular com-

plexes across the bacterial membrane to the extracellular space or into other cells (*27*). The known functions of type IV secretion systems are summarized schematically in Fig. 2. In *E. coli*, the system codes for the conjugative pilus and the necessary components to transfer DNA from one bacterium to the other (1 in Fig. 2). In *A. tumefaciens*, it codes for the system that transfers the Ti plasmid DNA from the bacterium to the nucleus of the plant cell (4 in Fig. 2). In *B. pertussis*, it codes for the apparatus that allows secretion of pertussis toxin into the medium (2 in Fig. 2). In *L. pneumophila* and *R. prowazekii* (*28*), pathogens that live in intracellular vacuoles, the type IV systems are believed to export to the vacuolar membrane or to the cytoplasm of the host cell macromolecules that adapt the vacuolar environment to the bacterial needs (5 in Fig. 2).

The function and the localization of some of these homologous genes are known (Fig. 1). For instance, VirB2 is the structural subunit of the conjugative pilus, whereas VirB4, VirB9, and VirD4 have a functional adenosine triphosphatase (ATPase) activity. It has been suggested that VirB4, VirB7, VirB9, VirB10, VirB11, and VirD4 (indicated by a gray area in Fig. 1) assemble as a complex and constitute the minimum core structure necessary for type IV transporter biogenesis (*27*). VirD4 is necessary when transfer of nucleoproteins occurs, and therefore the presence of the VirD4 homolog in several type IV systems suggests that conjugative DNA transfer to mammalian cells may be possible. In summary, there are many similarities between the type IV system described here and the type III secretion system described in a separate article in this issue (*29*). Both systems evolved—possibly by gene duplication—from transmembrane structures with extracellular, tubular protrusions (the flagellus and the conjugative pilus, respectively) and mediate communication processes between cells by delivering macromolecular messengers.

In *Hp*, the *cag* PAI induces epithelial cells to secrete interleukin 8, a mediator of inflammation, by activating nuclear factor kappa B complexes; it also induces (i) remodeling of the cell surface and pedestal formation, (ii) tyrosine phosphorylation of a 145-kD host protein (*30*), (iii) activation of the transcription factor AP-1, and (iv) expression of the proto-oncogenes c-*fos* and c-*jun* by activation of the ERK/MAP kinase cascade, thus resulting in ELK-1 phosphorylation and increased c-*fos* transcription (*31*). *Hp* mutated in *cag* genes does not induce any of the above activities.

It is not known which are the effector molecules of the *cag* PAI and how the above signals are integrated to increase virulence. In the mouse model, however, cag^+ strains (also named type I) induce visible gastric damage, whereas cag^- strains (also named type II) do not induce dramatic changes and resemble commensal bacteria more than pathogens

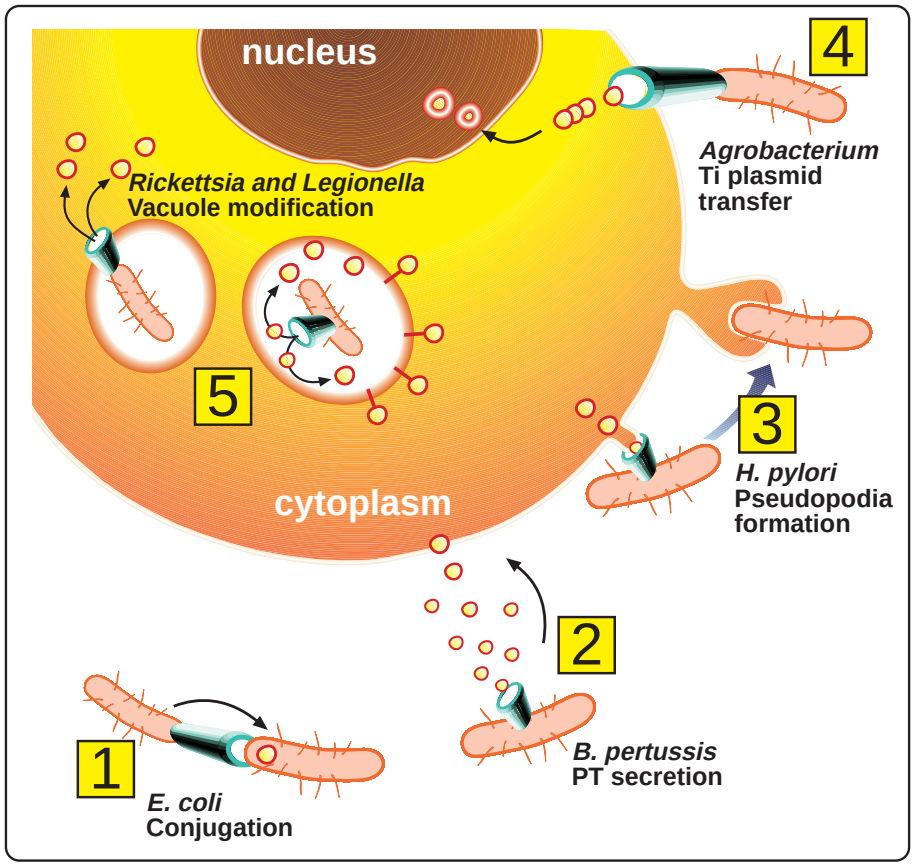

Fig. 2. Schematic representation of the functions of type IV secretion machineries. 1, *Escherichia coli* conjugative transfer of DNA. 2, Export of pertussis toxin (PT) in the extracellular medium by *Bordetella pertussis*. 3, Contact-dependent signaling of *Hp* to epithelial cells inducing pedestal formation. 4, Mobilization of Ti plasmid DNA from *Agrobacterium tumefaciens* to the plant cell nucleus. 5, *Rickettsia*, *Legionella*, and *Brucella* delivery of effector molecules to the cell cytoplasm or to the vacuolar membrane.

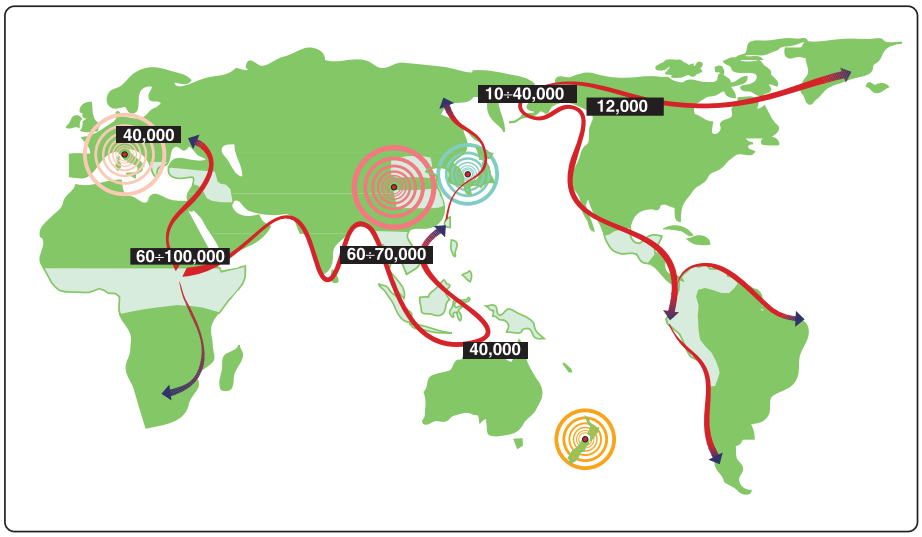

Fig. 3. World map indicating the direction of human migrations (arrows) and time range (years since migrations happened), as taken from Cavalli-Sforza (*47*) and Diamond (*48*). The geographic centers of the major *Hp* genotypes known today are indicated by concentric circles of different colors. According to the hypothesis presented here, *Hp* followed man during the migrations indicated by the arrows, giving rise to the present genotype distribution (indicated by circles). Light green areas indicate the locations where the development of agriculture and animal breeding was initially started, resulting in the expansion of the initial human populations (*46*–*48*).

(*32*). In humans, there is a strong correlation between infection with *cag*$^+$ organisms and occurrence of peptic ulcers and cancer. Most epidemiological studies have been done by reporting the isolation of a single colony from each patient. These studies indicate that 60 to 70% are *cag*$^+$ and 30 to 40% are *cag*$^-$. Notable exceptions are Korea and Japan, where *cag*$^+$ represent nearly 100% of the isolates (*5*, *33*). When multiple colonies were isolated from each patient, however, almost invariably all patients were found to be coinfected by *cag*$^+$ and *cag*$^-$strains with identical DNA fingerprints. This suggests that, during the prolonged residence in the stomach, *cag*$^+$ strains excise the *cag* PAI and generate isogenic *cag*$^-$ strains (*9*, *34*). Depending on the host conditions, the *cag*$^-$ strains may remain a minority, disappear, or outgrow momentarily the *cag*$^+$ strains. We should therefore imagine in each stomach a dynamic equilibrium of *cag*$^+$ and *cag*$^-$ strains modulated by the host, with alternating periods of disease and remission mediated by overgrowth of *cag*$^+$ and *cag*$^-$strains, respectively. Different areas of the stomach harbor bacterial populations with different *cag* status and density. This is consistent with the observed correlation between *cag*$^+$ isolates and disease. The low frequency of *cag*$^-$ isolates in Japan and Korea could be due to a decreased frequency of *cag* excision or to host factors that restrict the growth of *cag*$^-$ strains.

Vaccines May End the Coexistence of Man and *Hp*

The discovery of the infectious nature of peptic ulcer has dramatically changed the medical approach to this disease. Although combinations of antibiotics are effective in eradicating *Hp*, strains resistant to antibiotics are already emerging (*35*), decreasing the efficacy of the currently used triple therapy. Furthermore, antibiotics cannot be used to eradicate the infection from the whole population, especially in developing countries. It is therefore predictable that although antibiotics are a good solution for individual treatment of disease, they will not represent a definitive solution for society. Hence, vaccination—the most effective medical practice in controlling infectious diseases on a global scale—may represent the ultimate solution.

The challenge to develop a vaccine has been particularly successful in mouse models with either the *Hp*-related species—*Helicobacter felis* (*36*)—or the mouse-adapted *Hp* that mimics human infection (*32*, *37*). Vaccine-induced protection from infectious challenge and eradication of established infection have been proved with many antigens, including whole inactivated cells, bacterial lysates, and several purified antigens (*36*, *38*) (Table 2). The most successful approach has been mucosal immunization with adjuvants such as cholera or *E. coli* enterotoxins or the genetically detoxified derivative, LTK6, mixed with one or more of the above antigens (*39*). Recently, however, it has been reported that systemic vaccination can also induce protection in the mouse model (*40*). The question we face now is whether the promising results obtained in mice will be reproduced in man.

While clinical trials are being prepared, other animal models have been developed and tested. Among these are Mongolian gerbils, in which induction of gastric adenocarcinoma has been shown, and gnotobiotic piglets (*41*). Another model recently developed in beagle dogs permits periodic endoscopic observation of disease progression, without the need to kill the animal (*42*). Although no correlate of protection has been found, progress has been made in understanding the mechanism of protection. Experiments with B cell (antibody)–deficient mice (μMT) have shown that antibodies are not required for protection that can be mediated entirely by CD4$^+$ T cells (*43*), although a role for CD8$^+$ cells has also been evoked (*44*). Whereas CD4$^+$-mediated immunity is a common mechanism of protection against intracellular parasites, it is an unusual mechanism to induce immunity against a bacterium that remains in the extracellular environment.

A major question is why immunization would be successful if the natural immune response does not clear the infection. There is evidence showing that the majority of CD4$^+$ T cells from infected patients with peptic disease have a Th1 phenotype (*45*) and are specific for CagA. This suggests that *Hp* infection induces an interferon-γ (Th1)–mediated proinflammatory response that is not able to eliminate the bacteria. It is possible that vaccination triggers a Th2 immune response capable of mediating protection (*45*).

Animal models have their limits, however. Well-designed clinical trials are now required to answer whether the promising results obtained in animals apply to humans. If successful, vaccination may indeed be able to end the coexistence of humans and *Hp*.

Did *Hp* Coevolve with Man?

The long permanence of each strain within the same person and the family-linked mode of transmission suggest that the evolution of *Hp* is linked to the social behavior of man. For most of history, humans have been socially organized in small, isolated communities with limited genetic exchange. As a consequence, human genetic traits segregated in different villages and, on a larger scale, among cities and countries (*46*–*48*). It is

Table 2. *Helicobacter pylori* antigens, vaccine formulations, and routes of administration proven efficacious in animal models of infection. *Hp* antigens that have been shown to exhibit efficacy in animal models in preventing infection or in eradicating an already established infection with *Hp* [reviewed in (*36, 38*); for nonmurine models, see (*41, 55*)]. Urease (and its subunits) and heat shock proteins have also been tested in the murine model of infection with *H. felis*, because of the conservation of these proteins. Most of these antigens have been given mucosally, more often orally, in association with mucosal adjuvants such as CT and LT or the genetically inactivated LT mutant LTK63 (*37, 39*). More recently, other mucosal routes have been tested (*56*). Finally, the parenteral route of immunization has been shown to represent a potentially feasible approach (*40*).

Animal model	*H. pylori* antigen(s)	Adjuvant* or vector	Route†	Infection with‡
		Prophylactic vaccination		
Mice	Whole-cell lysate	CT, LT, LTK63	os	Hf, Hp
	Urease	CT, LT, LTK63	os	Hf, Hp
		CT, LT	in	Hf, Hp
		Saponin derivative	sc	Hp
	UreB subunit	CT, LT	os	Hf, Hp
		S. typhimurium	in	Hp
	HspA	CT, LT	os	Hf
	HspB	CT, LT, LTK63	os	Hf, Hp
	UreB + HspA	LT	os	Hf
	Catalase	CT, LT	os	Hp
	VacA	LT, LTK63	os	Hp
	CagA	LTK63	os	Hp
		LTK63	In	Hp
	VacA + Urease	LT, LTK63	os	Hp
Gnotobiotic piglets	Whole-cell lysate	LT	os	Hp
		Freund's	sc	Hp
		Therapeutic vaccination		
Mice	Whole-cell lysate	CT, LTK63	os	Hf, Hp
	UreB	CT	os	Hf
	VacA	LTK63	os	Hp
	CagA	LTK63	os	Hp
Ferrets	Urease	CT	os	Hm
Rhesus monkeys	UreB	LT	os	Hp

*CT, wild-type cholera toxin; LT, wild-type *E. coli* heat-labile enterotoxin; LTK63, genetically detoxified LT mutant carrying a Ser → Lys substitution at position 63 of the A subunit (*57*). †os, peroral; sc, subcutaneous; in, intranasal. ‡Hf, *Helicobacter felis*; Hp, *Helicobacter pylori*; Hm, *Helicobacter mustelae*.

likely that during the social evolution, while mutations accumulated and segregated in the human genes, a cosegregation of the genes of *Hp* occurred.

Although our knowledge of the population genetics of *Hp* is limited, on the basis of the nucleotide sequence, we can differentiate Asian strains from those isolated from the white population in Europe, North America, and South Africa (*8*, *49*), and on the basis of the frequency of the m1 and m2 alleles of *vac*A, we can differentiate between strains from northern and southern Asia (*24*). A study on strains isolated from the indigenous Maori population of New Zealand has shown that these differ from strains of late colonizers (*50*). Therefore, at least for the four groups of isolates indicated as concentric circles in Fig. 3, the genetic geography of *Hp* coincides with that described for man by Cavalli-Sforza (*47*) and Diamond (*48*). The overlap between genetically distinct human and *Hp* populations supports the hypothesis that *Helicobacter* was already established in man's stomach at least 100,000 years ago before the beginning of human migrations and followed him thereafter (Fig. 3). The hypothesis presented here suggests that we could expand our understanding of human and bacterial evolution by elucidating the genetic geography of *Hp* around the globe.

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