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Preface to the Series

The mechanisms of disease production by infectious agents are presently the focus of an unprecedented flowering of studies. The field has undoubtedly received impetus from the considerable advances recently made in the understanding of the structure, biochemistry, and biology of viruses, bacteria, fungi, and other parasites. Another contributing factor is our improved knowledge of immune responses and other adaptive or constitutive mechanisms by which hosts react to infection. Furthermore, recombinant DNA technology, monoclonal antibodies, and other newer methodologies have provided the technical tools for examining questions previously considered too complex to be successfully tackled. The most important incentive of all is probably the regenerated idea that infection might be the initiating event in many clinical entities presently classified as idiopathic or of uncertain origin.

Infectious pathogenesis research holds great promise. As more information is uncovered, it is becoming increasingly apparent that our present knowledge of the pathogenic potential of infectious agents is often limited to the most noticeable effects, which sometimes represent only the tip of the iceberg. For example, it is now well appreciated that pathologic processes caused by infectious agents may emerge clinically after an incubation of decades and may result from genetic, immunologic, and other indirect routes more than from the infecting agent in itself. Thus, there is a general expectation that continued investigation will lead to the isolation of new agents of infection, the identification of hitherto unsuspected etiologic correlations, and, eventually, more effective approaches to prevention and therapy.

Studies on the mechanisms of disease caused by infectious agents demand a breadth of understanding across many specialized areas, as well as much cooperation between clinicians and experimentalists. The series *Infectious Agents and Pathogenesis* is intended not only to document the state of the art in this fascinating and challenging field but also to help lay bridges among diverse areas and people.

M. Bendinelli
H. Friedman

Preface

The discovery and concept that *Helicobacter pylori* is associated with gastric disease including gastric cancer which is one of the most common and frequently lethal forms of malignancy, heralded a new and rapidly expanding field recognizing the emergence of many new pathogens and disease syndromes in clinical medicine, as well as basic infectious disease research. There is now an extensive and widely known literature of how *H. pylori* is involved in a wide variety of disease syndromes. As summarized in the introductory chapter of this volume, many major advances have been made in diagnosis, both serologic and endoscopic in time of the involvement of this organism in patients with upper GI ailments as well as its presence in those who are not clinically ill. The Introduction describes the rapid development of understanding the role of this organism in disease.

The basic bacteriology of *H. pylori* is then described in the second chapter. Diagnostic tests for detecting *H. pylori* infection is then highlighted, as well as the role of such infection in gastric cancer. Current knowledge concerning risk factors and peptic ulcer pathology associated with *H. pylori* is then described. Newer information concerning therapy of *H. pylori* infection and colonization is described in a subsequent chapter as well as one concerning the effects of antibiotics on *H. pylori* infection. The extensive literature on natural substances with anti-*H. pylori* activity is then described, followed by a chapter dealing with newer knowledge concerning how this microorganism binds to gastric cells. The involvement of *H. pylori* in autoimmune diseases is then described as well as the pathogenesis of how this organism is related to diseases. The effects of toxins by the organism on host cells as well as development of animal models for *H. pylori* infection studies is described, followed by a review of development of a gerbil model for *H. pylori* infection. New information concerning development of a vaccine against *Helicobacter* is also presented in the final chapter.

It is anticipated by the editors as well as the authors of the individual chapters, who represent cutting edge laboratories from this country as well as many other countries, that this book will provide useful and valuable information

concerning the current status of immunologic, microbiologic, as well as therapeutic prevention studies against the important human pathogen *H. pylori*. The Editors thank Ms. Ilona Friedman for excellent editorial assistance in coordinating and assisting in the preparation of the manuscripts for this volume in this series.

Paul Hoffman
Yoshimasa Yamamoto
Herman Friedman

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Introduction and Perspectives

PAUL HOFFMAN

The latter quarter of the 20th century saw the emergence of many new pathogens or disease syndromes, including Legionnaires' disease, toxic shock syndrome, Lyme disease, and AIDS; but perhaps the most surprising finding was that a microbe (*Helicobacter pylori*) was associated with duodenal and gastric ulcers. Prior to the findings of Warren and Marshall in 1983¹ and until general acceptance of the finding by the 90's, most believed that ulcers were due to life style, behavior type, or diet. Interestingly, the spiral-shaped microaerobic bacteria associated with gastric biopsy material had been previously observed at the beginning of the century, but the association with gastric disease was never established. *Helicobacter pylori* became the subject of one of the most intensive investigations in the history of medicine as new knowledge revealed that half or more of all people worldwide are infected by this organism and life long infection is a proven risk-factor in development of gastric cancer, one of the most common and frequently lethal of all cancers. The pharmaceutical industry, once the benefactors of billion dollar ulcer treatment therapeutics (Zantac, Tagamet, Tums and Roloids to mention a few), now focused on development of new antimicrobials for the potentially worldwide market, while others focused on development of diagnostic tools from endoscopic devices to rapid diagnostic kits for singling out those for eradication therapy. Pharmaceutical companies provided unprecedented levels of financial support to this area, and were a driving force behind many of the new

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technologies (genomics, bioinformatics and proteomics) that have contributed to characterization of *H. pylori*. Population biology and microbial ecology have also contributed substantially to our understanding of strains and diversity. More recently we have learned that nearly all mammals harbor their own species of *Helicobacter*² and some are suggesting now that infection might be benign or even beneficial by protecting against development of esophageal reflux and cancer of the esophagus.³

Helicobacter pylori often establishes life long infections of the gastric mucosa, a site generally considered as sterile only a few decades ago, which raises many fundamental questions as to how it persists in this niche, how it survives stomach acid, and why the host immune system, while recognizing the presence of infection, seems incapable of mounting an eradicated immune response. Understanding persistence and host immune response is key to vaccine strategies, be it protective or therapeutic. Physiological studies reveal that *H. pylori* is unremarkable in its resistance to acid,⁴ being no more resistant than *Escherichia coli*. The bacteria produce a powerful urease that is regulated in response to acid,⁴ so that the ammonia and carbonate produced by this enzyme most likely create a microenvironment that is alkaline, something no other studied pathogen seems capable of doing. Further study shows that the densest colonization by *H. pylori* occurs in the antrum (lower portion of the stomach) where conditions are less acidic. As the infection becomes more pronounced, or under conditions where the antrum becomes more alkaline (e.g., acid suppressive therapeutics like proton pump inhibitors and H2-blockers), the motile bacteria migrate up into the cardia (body) of the stomach. Infection with *H. pylori* bacteria is basically in three dimensions, as these bacteria not only can move north and south in the mucosa in response to acid levels, but they are able to move freely up and down in the mucus layer that coats the gastric mucosa and provides a protective barrier against the diffusion of strong acid onto the epithelium. The notion of being “off shore” and therefore out of reach of the macrophages and cells of host immune defense may also play an important role in survival. Finally, mounting evidence suggests that *H. pylori* may control the immune response through mimicry (LPS displaying Lewis antigens) and selective release of inflammatory factors. The balance between promotion of inflammation and immune suppression are a key to persistence and an area where novel therapeutics, perhaps in combination with vaccine strategies, could be directed.

The discovery of and extreme interest in *Helicobacter*, a relative of *Campylobacter* (bacterial pathogens of the lower GI tract), fortunately coincided with the beginning of the genomics era, and is the beneficiary of two completely sequenced genomes of *H. pylori*.^{5,6} The results reveal a small genome (1.67 megabases) containing some 1553 genes encoding around 1,300 proteins. Despite possessing a limited number of genes, *H. pylori* displays auxotrophy for only a few amino acids and appears to possess most catabolic and anabolic pathways found in bacteria with larger genomes. Recent studies examining essentiality testing

on a genome scale suggested that there are few redundancies and backups in metabolic pathways and thus the percentage of *H. pylori* genes found essential may be greater than expected for organisms with larger genomes, perhaps opening a door for development of *Helicobacter* selective therapeutics.⁷ Other caveats from genome gazing is that there are few regulatory genes, particularly two component signal transduction systems, that are so typical of enteric bacteria and many pathogens, perhaps suggesting that other than acid and related stresses, *H. pylori* has little else to worry about in its gastric niche.^{8,9,10}

Major interest has focused on genes clustered in a pathogenicity island (PAI) that are associated with strains causing more pathology and gastric manifestations. These CagA positive strains (Cytotoxin associated gene) possess a 40 kilobase region encoding genes with orthologs in the type IV secretion and pathogenesis system of *Agrobacterium* and *Bordetella pertussis* to mention a few.¹¹ The CagA gene is located at one end of the PAI and serves as a marker for Cag status. Strains lacking the Cag PAI are characterized as less virulent as indicated by colonization at lower microbial densities and producing less inflammation than Cag⁺ strains. However, there is still debate over relative association with ulcers and more severe disease. Genes within the Cag PAI promote secretion of a vaculating cytotoxin protein (VacA) whose gene is located outside of the Cag locus. Recently, studies show that the Cag PAI is also responsible for delivery of the CagA protein into mammalian cells and that this protein exhibits tyrosine phosphatase activity.¹² Thus these toxins are considered important in the pathogenesis and severity of disease.

New applications of bioinformatics and proteomics have also contributed to expanded knowledge of protein interactions¹³ and assignment of function to many of the *H. pylori* genes whose function is unknown. As more microbial genomes become available and annotated, it is likely that more of the unknown genes will be assigned a biological function. Also anticipated is that selected DNA sequencing from a wide range of strains that are geographically distinct will provide information on genetic diversity of this species. Population biological studies have established genetic differences among European and Asian strains and these warrant further study as the information might be applied to selective therapeutics for strains from a particular region.¹⁴ Other areas of study that will develop in the future will include study of genes common to microaerophiles such as those common between *H. pylori* and *Campylobacter jejuni*, but unique from those of other microbes, might be exploited more generally for therapeutics or vaccines applicable to eliminating these organisms not only from man but from fowl (common reservoir for *Campylobacter*).

Advancements in diagnostics, both serologic, stool antigen test, and invasive (endoscopy) have led to sorting of infected people with upper GI ailments from those who are uninfected. Studies now show that nearly 35% of the world's population suffers from dyspepsia, but only 5 to 20% of these individuals are also infected with *H. pylori*. In western societies, even fewer are infected with more

severe CagA strains and in either case, studies have shown that eradication of *H. pylori* infection may not necessarily resolve dyspeptic symptoms.¹⁵ Thus, general practitioners tend not to investigate the basis for dyspepsia and often simply treat the symptoms with anti-acids and/or acid secretion blockers. The assumption that *H. pylori* infection is rather benign—particularly in western societies where gastric cancer is rare in the first place (10 on the list of cancers in the US for example)—seems to justify the practice. Where alarm symptoms are noted, referral to a specialist and further investigation by endoscopy is generally pursued. As mentioned earlier, the worry over cure of *H. pylori* and the subsequent development of gastric esophageal reflux disease (GERD) has raised concerns among physicians and the notion that the only good *Helicobacter* is a dead one, has been challenged. It is not clear what the future will bring, but given that 75 to 80% of North Americans are or never were infected with *H. pylori*, needs to be considered in this argument. Are they now at greater risk of developing GERD? Of the remaining 20–23% that are infected in North America, are they more likely upon cure to have a different outcome than those who have never been infected.

Much of the concern associated with whether to treat or not stems from the fact that treatment regimes have variable outcomes. While *H. pylori* is susceptible to a large variety of antimicrobials in vitro, the therapeutic activity in the gastric milieu is often nil and monotherapies have generally demonstrated poor efficacy. The most common practice is to use combinations of drugs, the so called triple or quadruple therapies containing amoxicillin, macrolides like clarithromycin, and/or nitroimidazoles like metronidazole in combination with proton pump inhibitors (omeprazole) that reduce acidity and improving therapeutic action of the antimicrobials.¹⁶ Treatments can also contain bismuth salts and tetracycline. The fact that all the current therapeutic agents are old drugs and generally of broad spectrum has alarmed many who do not understand why the pharmaceutical industry has not developed novel therapeutics specifically for treating *Helicobacter* infections. Unfortunately, despite the obvious need, the drug industry market analyses show a rather small market and one that is disappearing—reinfection rates are so low in developed countries that patients would only be treated once and the market would eventually disappear. The need for new therapeutics is still very real for developing countries where resistance to metronidazole and macrolides renders many current therapeutics nearly useless. Unfortunately, the cost of drug development could never be recouped through sales in these markets where need is greatest. In western societies, the educational initiatives associated with efforts to reduce the wide spread practice of prescribing antibiotics have succeeded in that many physicians are reluctant to prescribe first line broad spectrum antibiotics for treatment of non-life threatening infections as *H. pylori* out of fear of contributing to drug resistance.

Animal models of infection, particularly the mouse model and Mongolian gerbil have permitted investigators to follow the course of infection and to examine

antimicrobial efficacy and vaccine candidates. The distribution of cytokines and chemokines in the mouse model during infection has yielded much new information on mucosal inflammatory responses. Vaccine strategies using subunits of the urease have shown some promise, but often require toxin adjuvants that will likely never be used in humans. Animal studies with *H. felis*, which produces greater inflammation than *H. pylori* in the mouse model, has yielded much more information on inflammation and development of gastritis.¹⁷ Studies with other species of *Helicobacter* in animals implicate the microbes in other diseases including liver diseases and lower GI diseases such as inflammatory bowel disease. These models may lead to new developments in diseases other than gastritis and stomach ulcers.

The chapters that follow provide state of the art information in many areas of current interest in this exciting field. The authors accurately convey information and where controversies abound, present a balanced view. There are many areas where information is rapidly advancing in diagnostics, genomics and in disease management. There is still much to learn in areas of persistence, vaccine development, and in the basic biology of *Helicobacter* and its activities in the gastric mucosa. It is hoped that readers will be stimulated by historical aspects and as well become motivated by the many existing gaps in knowledge to join the many who are investigating this fascinating microbe.

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