

REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY

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Carcinogenesis of *Helicobacter pylori*

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***Helicobacter* infection is the leading cause of gastric cancer worldwide. Infection with this ubiquitous bacterium incites a chronic active immune response that persists for the life of the host, in the absence of antibiotic-induced eradication. It is the combination of bacterial factors, environmental insults, and the host immune response that drives the initiation and progression of mucosal atrophy, metaplasia, and dysplasia toward gastric cancer. Although it may seem intuitively obvious that removing the offending organism would negate the cancer risk, this approach is neither feasible (half of the world harbors this infection) nor is it straightforward. Most patients are infected in childhood, and present with various degrees of mucosal damage before any therapy. This review outlines the histologic progression of human *Helicobacter* infection from the early stages of inflammation through the development of metaplasia, dysplasia, and, finally, cancer. The effects of dietary and bacterial eradication therapy on disease progression and lesion reversibility are reviewed within the context of population studies and compared between study designs and populations tested. Eradication studies in the mouse model of infection prevents the formation of gastric cancer, and allows regression of established lesions, providing a useful model to study interaction between bacterium, environment, and host, without the difficulties inherent in human population studies. Recent advances in identifying the bone marrow-derived stem cell as the cell of origin of *Helicobacter*-induced gastric cancer in the murine model are discussed and interpreted in the context of human disease, and implications for future treatment are discussed.**

Before the association between *Helicobacter pylori* and gastric cancer was brought to the attention of the medical community, the pathology of the neoplasia and its precancerous lesions was well established. Although

several proposals for the classification of gastric carcinoma have been offered, the most widely accepted is the one developed by the Finnish pathologists Jarvi and Lauren. They pioneered the idea that gastric carcinoma could originate from islands of intestinal epithelium found in the gastric mucosa, proposed as far back as 1912.^{1,2} They concluded that the heterotopic islands in the gastric mucosa arose in a background of chronic gastritis and suggested that “prophylaxis should obviously be directed against gastritis.” The idea was further elaborated by other European pathologists who proposed the name “intestinal type of carcinoma.”³ The Finnish pathologists then reviewed 1344 cases of gastric carcinoma from their files. They were able to classify 915 as intestinal type. In 441 cases, they observed a different “manner of growth” and coined the term “diffuse carcinoma.” In 188 cases, they were unable to classify the tumors into those 2 categories, either because the tumors displayed mixed patterns or because they were composed of different cell types.² They further reported that compared to diffuse-type carcinomas, intestinal-type carcinomas were more common among men (male:female ratio 1.8:1), older patients (average age 50.4 years for men and 47.7 years for women), and had a relatively better prognosis (3-year survival rate 43% versus 35%). They also reported that intestinal-type carcinomas were surrounded by intestinalized mucosa more frequently than diffuse carcinomas. These original observations have been confirmed by investigators in other countries.

Precancerous Cascade

The presence of gastric lesions outside the tumor was described first in gastrectomy specimens and was later examined more in detail when biopsies taken by flexible gastroscopes became generally available. It then became possible to develop a model of gastric carcino-

Table 1. Rate of Transition in Gastric Pathology to All Lower or Higher Stages Between First and Second Biopsy/100 Person-Years

Progression	Rate/100 person-years	Regression	Rate/100 person-years
NAG → MAG	7.5	MAG → NAG	1.7
MAG → IM	6.7	IM → MAG	4.4
IM → Dysplasia	3.2	Dysplasia → IM	5.7

NAG, nonatrophic gastritis; MAG, multifocal atrophic gastritis; IM, intestinal metaplasia.

Modified from Correa P, Haenszel W, Cuello C, et al. Gastric precancerous process in a high risk population: cohort follow-up. *Cancer Res* 1990;50:4737–4740.

genesis that has been generally accepted.⁴ Long-term follow-up of cohorts in high-risk populations has documented the dynamics of the precancerous process^{5,6} (Table 1). The basic components of the process are chronic active nonatrophic gastritis → multifocal atrophy → intestinal metaplasia (first complete, then incomplete) → dysplasia → invasive carcinoma. These lesions are well-characterized histopathologically. However, it is well recognized that each represents a continuum of changes depicting multiple events that increase in intensity and extension with time. Histopathology scores have been developed that take into account the degree and extent of each change.⁷ The categories are described and illustrated herein.

Chronic Active Nonatrophic Gastritis

This lesion is characterized by diffuse infiltration of the gastric mucosa by white blood cells representing chronic inflammation, namely, lymphocytes, plasma cells, and macrophages. Additionally, scattered eosinophils and mast cells can be observed. The gastritis is called “active” when polymorphonuclear neutrophils are found, representing acute inflammation. They may form small aggregates either in the stroma or the epithelial layer. They are frequently prominent in the glandular necks and sometimes form intraglandular microabscesses. This phase of the precancerous process does not show loss of glands (atrophy) and is called “nonatrophic gastritis” in the updated Sydney classification of gastritis, adopted by most pathologists⁸ (Figure 1A).

In most cases, nonatrophic gastritis is localized predominantly or exclusively in the antrum. In infected patients receiving antacid medication, especially proton pump inhibitors, active nonatrophic gastritis becomes prominent in the corpus (oxyntic) mucosa. Antral predominant nonatrophic gastritis is regularly found in patients with duodenal peptic ulcer. For a reason not clearly understood, duodenal ulcer patients are not at increased risk for gastric cancer. Their form of gastritis does not usually lead to atrophy or metaplasia.⁹

Multifocal Atrophic Gastritis

In populations at high risk for gastric cancer, the precancerous cascade advances slowly and steadily. Focal loss of glands (atrophy) takes place first in the antrum–corpus junction, especially around the incisura angularis. The mechanism of cell loss appears directly related to effects of bacterial products and the cytokine milieu within the gastric mucosa. More virulent bacterial strains and a permissive host immune response are strongly associated with atrophy and progression to severe disease. With time, fibrous tissue fills the vacuum left by the lost glands (Figure 1B). It is the loss of cell–cell cross-talk, and the introduction of fibrous stromal tissue, which orchestrates the influx of blood borne stem cells responsible for subsequent tissue changes leading to cancer (discussed in detail in the latter half of this review).

Intestinal Metaplasia

At this stage of the gastric precancerous process, the original glands and the foveolar epithelium are re-

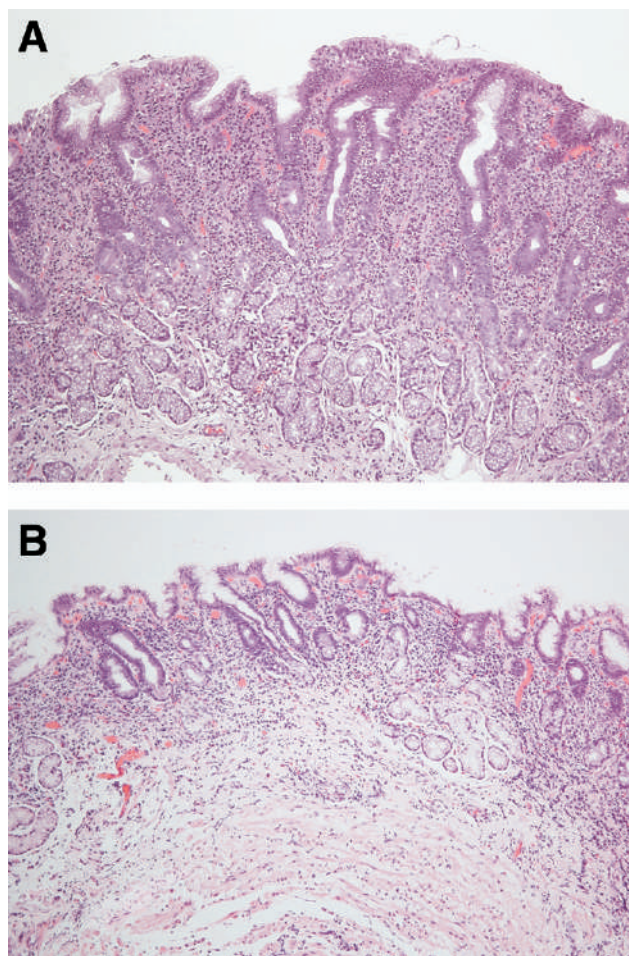


Figure 1. (A) Nonatrophic gastritis. Antral gastric mucosa with diffuse mononuclear leukocytic infiltration and well-preserved glands. (B) Multifocal atrophic gastritis. The antral glands have disappeared from the center of the field and are replaced by fibrous tissue. Diffuse mononuclear leukocytic infiltrate is also observed in the lamina propria.

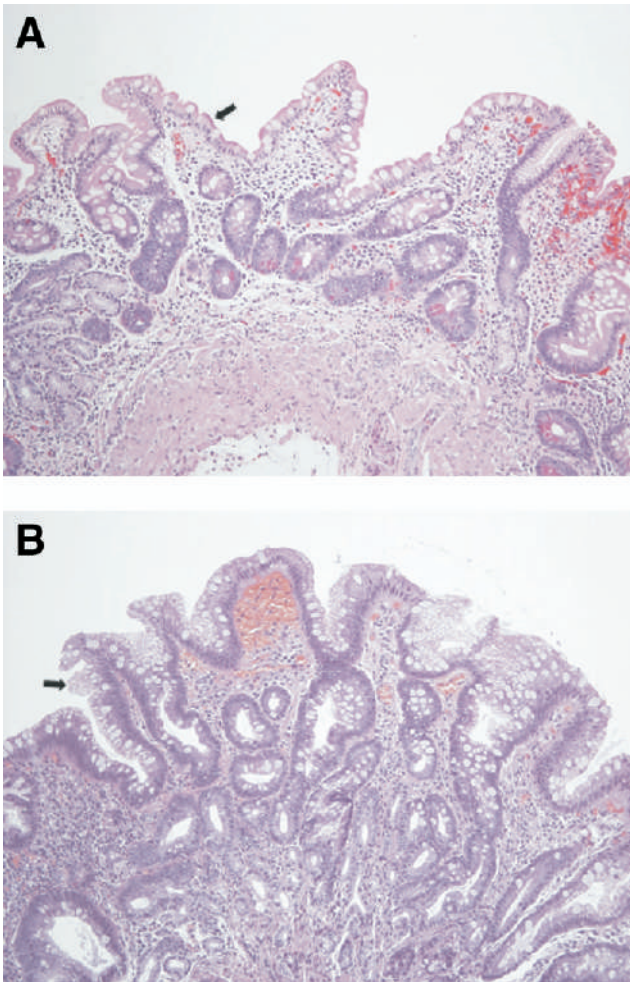


Figure 2. (A) Multifocal atrophic gastritis with complete intestinal metaplasia. The *arrow* points to absorptive enterocytes with a brush border, markers of complete metaplasia. The original glands have disappeared from the center of the field and are replaced with metaplastic cells, extending to the surface. The metaplastic epithelium consists of eosinophilic absorptive enterocytes with a well-defined brush border. Well-developed goblet cells are seen at regular intervals, and Paneth cells in the deep glands. (B) Multifocal atrophic gastritis with incomplete intestinal metaplasia. The epithelium consists of multiple, irregular goblet cells and absence of brush border. The *arrow* points to goblet cells with several irregular vacuoles and without a brush border, markers of incomplete metaplasia.

placed by cells with intestinal phenotype. The metaplastic intestinal cells in the initial phases of the process resemble the small intestinal mucosa: lined by eosinophilic absorptive enterocytes with a well-developed “brush border” composed of myriads of microvilli. Alternating with these cells at regular intervals, mucin-filled goblet cells are found (Figure 2A). This type of metaplasia has received various names: small intestinal type, based on its morphologic structure; type I, based on mucin histochemistry¹⁰; and “complete,” reflecting the fact that it secretes the normal set of digestive enzymes such as sucrase, trehalase, and alkaline phosphatase.¹¹ At later stages, the metaplastic cells lose their small intestinal

phenotypes, acquire morphologic features of the large intestine, and are lined only by goblet cells of different sizes and shapes (Figure 2B). This type is called “incomplete” or colonic metaplasia, and includes types II and III.⁸ Immunohistochemical stains for mucins show that complete metaplasia harbors the intestinal type of acid mucins, which stain with Alcian blue at pH 2.5, and are negative for sulfomucins with high iron diamine (HID) stain (Figure 3A). The mucin histochemistry reveals sulfated HID-positive mucins (Figure 3B). Molecular markers show that the typical intestinal mucin MUC2 (absent in normal gastric mucosa) is positive in goblet cells in both complete and incomplete intestinal metaplasia. MUC5AC and MUC6 are mucins normally present in gastric mucosa. MUC5AC is absent in complete intestinal metaplasia and present in the incomplete type (Figure 4A). MUC6 is absent in both types of intestinal metaplasia. Also positive in incomplete intestinal metaplasia is the large intestine marker Das-1¹² (Figure 4B).

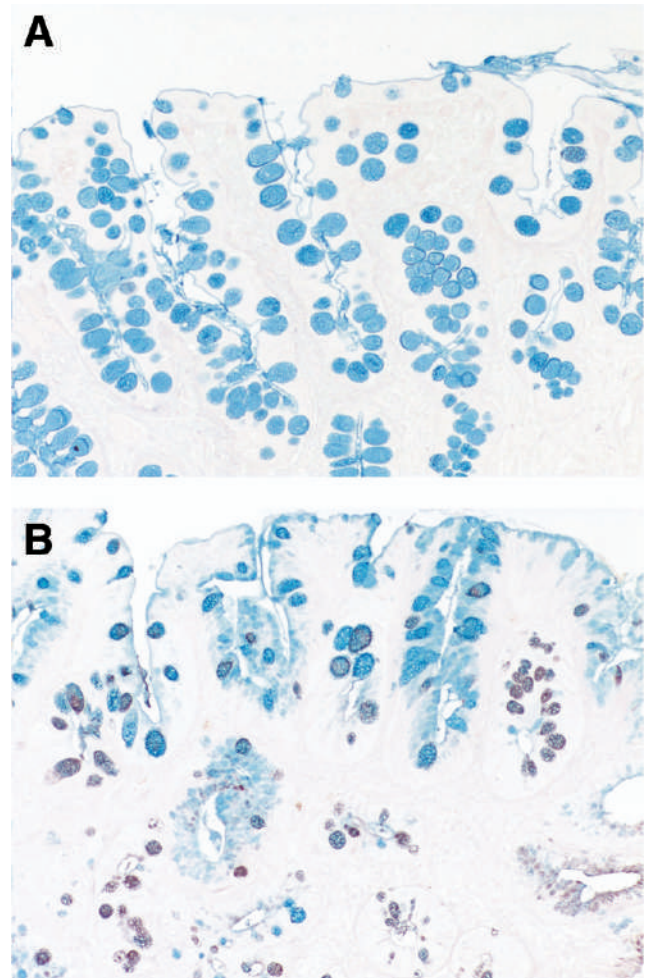


Figure 3. Alcian blue/HID staining for characterization of acid mucins. (A) Complete intestinal metaplasia displays sialomucins (blue) in goblet cells. (B) Incomplete intestinal metaplasia showing sialomucins and sulfomucins (brown) in goblet and columnar cells.

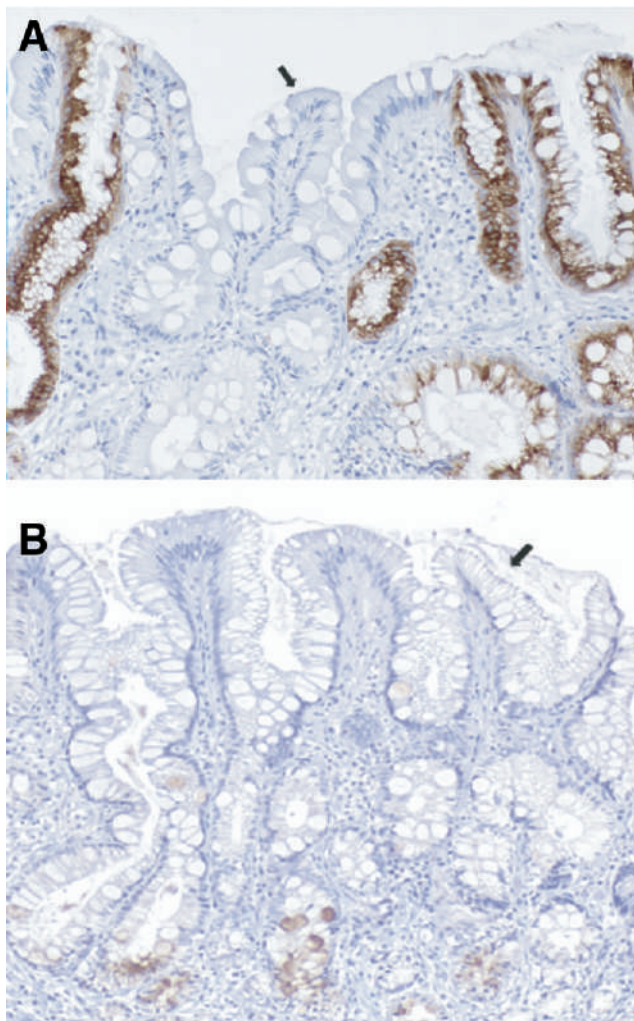


Figure 4. Immunohistochemical stainings. (A) MUC5AC. In this mixed complete and incomplete intestinal metaplasia, MUC5AC is present in the incomplete (periphery) and absent in the complete type (central). The arrow points to absorptive enterocytes with a brush border, markers of complete metaplasia. (B) Das-1. Incomplete intestinal metaplasia showing positive Das-1 staining (a marker of colonic differentiation) in deep glands. The arrow points to goblet cells with several irregular vacuoles and without a brush border, markers of incomplete metaplasia.

In younger subjects with limited multifocal disease, the complete type of metaplasia predominates. Older patients tend to also have foci of incomplete metaplasia. This tendency becomes more accentuated with age, and the extent of the metaplastic changes becomes greater. Patients with small (“early”) carcinoma frequently have areas of incomplete metaplasia around the tumor. Such is not the case with large, often ulcerated, carcinomas. Instead, invasive carcinomas tend to replace preexisting areas of metaplasia in their vicinity.

Up to this point in the cascade, the epithelium in the atrophic and metaplastic lesions remains well differentiated, with normal nuclear-cytoplasmic ratio, normal nuclear morphology, and normal tissue architecture. The

dynamics of the precancerous process to this point shows a gradual phenotypic transformation from normal epithelium to metaplastic cells with small intestinal morphology and then to cells resembling colonic mucosa, additionally expressing gastric and colonic mucins. This process usually takes decades and is progressive, supporting the notion that although environmental alterations (bacterial factors and cytokine environment, loss of cell signaling) may have initially driven differentiation decisions, with time, permanent changes in the stem cell compartment have occurred. In some patients with incomplete metaplasia, a mild degree of nuclear atypia and architectural distortion is observed, leading some investigators to consider incomplete metaplasia as a mild form of dysplasia.¹⁰

SPEM in Humans

The presence of glands with gastric antrum phenotype in the oxyntic mucosa has long been recognized and described as “antralization” of the corpus or “pseudopyloric metaplasia.”¹¹ It has received recent attention because this type of metaplasia is the predominant precancerous lesion in several animal models^{13,14} (discussed later in this review). It has been well studied in gastrectomy specimens and gastric biopsies from patients in whom a remnant gastric carcinoma was diagnosed after a previous gastrectomy several years before.¹⁵ A series from Japan studied patients who developed remnant carcinoma 16–20 years after a previous gastrectomy. In 9 patients, the stomach was previously resected for peptic ulcer disease and in 10 patients for adenocarcinoma. The glands with antral morphology present in the corpus expressed spasmolytic polypeptide, a trefoil peptide expressed in the normal intestinal mucosa. The spasmolytic polypeptide expressing metaplasia (SPEM) was ubiquitous in the mucosa surrounding the remnant carcinomas. Spasmolytic polypeptide was also detected in dysplastic and neoplastic cells. Classical intestinal metaplasia with absorptive enterocytes and goblet cells was found in 44% of stomachs with remnant carcinomas and previous history of peptic ulcer disease and in 66% of those with previous resection for carcinoma.¹⁵ SPEM has been recognized as a cancer precursor and is associated with *H pylori* infection. A systematic study of 16 gastrectomy specimens from Japanese patients with “early” carcinoma reported antral metaplasia as a form of atrophy of the oxyntic mucosa. It extended as a continuous sheet and was abundantly present around the “early” carcinomas.¹⁵ Classic intestinal metaplasia in such specimens was seen as independent foci arising within the sheet of antral metaplasia of the oxyntic mucosa or replacing extensive areas of the antral mucosa.

Dysplasia

Dysplasia is characterized by atypical changes in nuclear morphology and tissue architecture. The nuclei

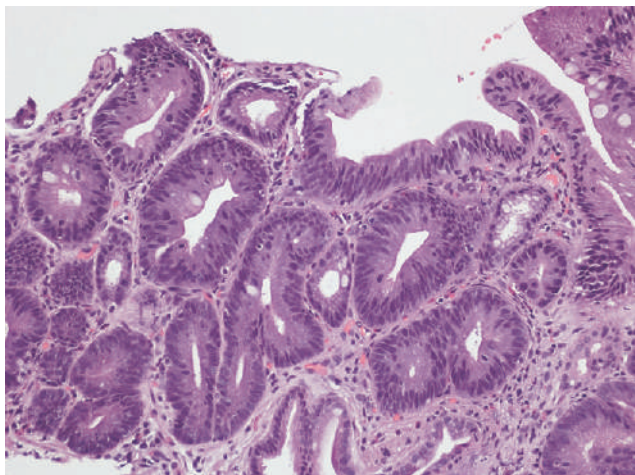


Figure 5. Low-grade gastric dysplasia. Abnormal presence of irregular glands lined by epithelial cells with crowded, enlarged, hyperchromatic, pseudostratified nuclei, and frequent mitosis. The dysplastic changes extend to the surface epithelium.

of the dysplastic epithelium are enlarged, hyperchromatic, irregular in shape, and devoid of polarity. The architecture is irregular, frequently forming closely packed tubular structures (adenomas) with irregular lumens. The atypical changes are not limited to the deeper glands; they are also seen in the surface epithelium (Figure 5). Dysplasias are classified as low- or high-grade, depending on the degree of nuclear atypia and architectural distortion. All of the atypical cells, however, are confined within the tubular structure. If they go through the basal membrane, they become invasive carcinomas. There is general agreement that the dysplastic epithelium is neoplastic, therefore, dysplasia is also called intraepithelial neoplasia.¹⁶

Dysplasias are uncommon in populations at low cancer risk, but their frequency increases with cancer risk. Their management is problematic. Borderline and mild dysplasias are regularly monitored endoscopically. But for high-grade dysplasias, there is general agreement that they should be resected, either surgically or endoscopically. Their progression to invasive carcinoma has been reported from 60% to 85% in different series.^{16–18}

Natural History of Precursor Lesions

Follow-up of patients with precursor lesions in populations at high cancer risk has thrown light on the dynamics of the process. The progression of these lesions follows a pattern of steady state, with episodes of progression to more advanced lesions and episodes of regression to less advanced lesions. Table 1 is based on the experience of a cohort of 1422 subjects followed up for an average of 5 years in the high-risk region of Narino, Colombia.⁵ Although there may be sampling errors owing to the multifocal nature of the lesions, the message is that the complex dynamic flow of precancerous lesions is

that of a slow forward movement, but the speed of such movement is not the same in all individuals. The same study shows that the changes are accelerated in older individuals. The rate of progression from metaplasia to dysplasia, per 100 person-years, was 2.1 for subjects <40 years old, compared with 4.0 for older persons. Repeated biopsies in that cohort show that in 7 patients an original diagnosis of dysplasia was not seen in the following biopsy, but was confirmed in a new biopsy months later.

The clear message of these studies is that an initial diagnosis of dysplasia should be confirmed some months later, especially after treating the apparent cause of gastritis and regenerative hyperplasia, such as *Helicobacter* infection, alcohol, or other irritants of the gastric mucosa. It is also advisable that more than one gastrointestinal pathologist confirm the diagnosis of dysplasia.

Prevention Trials

The slow progression of precancerous lesions has stimulated cancer prevention trials in several countries. These trials have addressed etiologic factors identified in epidemiologic studies, namely, the deficient intake of antioxidants and the infection with *H pylori*.

Two trials, one in a population from Venezuela¹⁹ and the other in a population from Finland²⁰ who used antioxidants in the form of food supplements, have reported negative results. These trials suffered from a number of problems making it difficult to interpret the results. A number of later studies have shown positive results. One trial, carried out in Linxian, China, provided β -carotene, vitamin E, and selenium supplements for 5 years and reported a statistically significant reduction in the incidence of gastric cancer (odds ratio, 0.71; 95% confidence interval [CI], 0.64–0.99).²¹ A trial in Colombia provided vitamin C and/or β -carotene supplements for 6 years and reported a statistically significant regression of precancerous lesions. In subjects receiving β -carotene, the relative risks of regression of multifocal nonmetaplastic atrophy and intestinal metaplasia were 5.1 (95% CI, 1.7–15.0) and 3.4 (95% CI, 1.1–9.8), respectively.²² In that study, supplements were discontinued after 6 years. At 12 years of follow-up, the beneficial effect of the antioxidants was not detected, suggesting that the effects of antioxidants last only as long as the supplements are being provided.

Several trials have addressed the issue of cancer prevention after curing *H pylori* infection. Some trials were randomized and some were nonrandomized. An outstanding example of nonrandomized trials was reported by Uemura et al from Japan.²³ They followed a cohort of 1526 patients with repeated gastric biopsies for 7.8 years. No cases of cancer developed in 280 patients negative for *H pylori* infection or in 253 in whom the infection was cured, or in 275 infected patients with duodenal ulcer. In 971 infected patients with other diagnoses (gastritis, gastric ulcer, or hyperplastic polyps), 36 cases of cancer were

detected during the follow-up period. A multicenter study in Japan examined the outcomes of the infection in 1233 patients followed for 7.7 years. One percent of patients in whom the infection was eradicated developed cancer, compared with 4% in those whose infection persisted.²⁴

Several randomized trials have also been reported. Studies in Mexico, Colombia, and China have reported regression of precancerous lesions after successful eradication.^{22,25–28} Only one study in China has reported results based on a cancer endpoint. After 7.5 years of follow-up, 7 cases of cancer developed in 804 patients receiving eradication treatment, compared with 11 cases in 794 patients on placebo. This reduction in incidence was not statistically significant. When the comparison was limited to patients who did not have atrophy or metaplasia at the start of the trial, no cases of cancer were found in 485 subjects treated for the infection, compared with 6 cases in 503 subjects receiving placebo. The difference was statistically significant ($P = .02$). It does appear that the earlier the intervention takes place, the better the chances of protecting against cancer development. The number of cancer cases ($n = 6$) was small; results from larger series are expected from ongoing trials.²⁶ It has been shown that the process of cancer prevention follows a quadratic sigmoid curve, which parallels in reverse the process of carcinogenesis.²⁹ In that model, the first years after intervention represent the initial segment of the sigmoid curve with little (statistically not significant) decline in prevention markers. It should be expected that longer observation of the Wong cohort will show the preventive effect represented in a steeper decline in cancer risk.

The previously mentioned randomized trial in Colombia reported results after 12 years of follow-up and provided a glimpse at the natural history of the prevention process. In this study, the authors used a sensitive histopathology score that took into account the lesion itself, its intensity and extension, as well as the phenotype of the metaplastic cells. Examination of subjects who had been cured of infection, and who remained free of infection for the 12 years of the study, showed that the healing effect (represented in the reduction of the score value) followed a sigmoid curve. The score was a function of the square of the time free of infection. During the first 3 years follow-up after cure of the *H pylori* infection, the initial phase of the sigmoid curve showed a minimal and not significant decline. After that time, the decline became steeper. At 6 years of follow-up, the score was significantly lower, but less than the expected 50% of the decline observed after 12 years of follow-up.⁷ The quadratic nature of the effect of the duration of carcinogen-free interval parallels in reverse the effect of exposure to a carcinogen as demonstrated by Doll and Peto³⁰ in their study of lung cancer incidence as related to tobacco use in British doctors. In that study, the sigmoid

curve of cancer incidence was a function of the duration of smoking to the 4.5 exponent.³⁰ An important lesson from these studies is that the results of chemoprevention trials can be adequately evaluated only after several years (~ 4) of the initial intervention to suppress a carcinogen.

Mechanisms of *H pylori* Carcinogenesis

After the International Agency for Research on Cancer (IARC) classification of *H pylori* infection as a class I carcinogen, a considerable amount of confirmatory evidence has accumulated. The 1994 IARC report was based entirely on epidemiologic evidence.³¹ It explicitly stated that experimental evidence was needed. The mechanism of the initial insult to DNA molecules is unknown; however, the leading hypothesis is that the neoplastic outcome is related to oxidative stress, as represented by the expression of inducible nitric oxide synthase brought about by the infection.^{32–34} (The subject is not covered in this review, but the reader is referred to the review by Wilson and Crabtree in this series [Gastroenterology 2007;133:288–308]).

To fully address the bacterial-host interactions, immune response to infection, and natural progression of disease, several animal models of *Helicobacter* infection have been developed. The first successful experiment of cancer induction by *H pylori* was done in Mongolian gerbils.³⁵ The importance of the virulence of the infecting strain has been demonstrated, especially by obtaining more virulent strains after passage through susceptible rodents.³⁶ The experimental infection in mice with *Helicobacter felis* has shed considerable light on the old mystery of the cell of origin of gastric cancer and provides for us a very useful model to study *Helicobacter* infection in ways that can not be ethically studied in humans.

Animal Models for Studying *Helicobacter*-Induced Gastric Mucosal Changes

The human studies outlined raise the important question: At what point are mucosal lesions reversible, and what is the “point of no return”—when they are irreversible and/or progressive? Studies in humans aimed at addressing the role of eradication therapy in the regression of gastric lesions and the prevention of gastric cancer are problematic for multiple reasons, including variations in patient populations, the inability to determine the length of time patients were infected, and the lack of detail regarding the extent of mucosal involvement at the time therapy is given. For these reasons, animal models have become very useful.

The C57BL/6 Mouse Model to Study the Natural History of *Helicobacter* Infection and the Effects of Eradication Therapy

Helicobacter felis infection in the C57BL/6 mouse model reproducibly results in the classic sequence of

histologic changes seen in human infection; chronic gastritis, atrophy, metaplasia, dysplasia, and adenocarcinoma, with adenocarcinoma occurring in 100% of mice that were infected for 15 months.³⁷⁻³⁹ All strains of mice appear susceptible to infection; however, strains vary dramatically in their susceptibility to mucosal damage. This inherent susceptibility or resistance is based on the host immune response, such that mice responding with a Th1 cytokine pattern are susceptible to mucosal damage, whereas those responding with a Th2 cytokine pattern are resistant to atrophy and cancer formation.⁴⁰⁻⁴⁴ Consistent with these findings, mice intermediate in their response are at variable risk of mucosal disease. The C57BL/6 model most closely recapitulates human disease, and has proven a very useful animal model. Although subtle differences in histology exist between the mouse and human models, the main features of disease are retained. In these models, the classical intestinal metaplasia with absorptive enterocytes and goblet cells seen in humans is not observed; however, the progression of atrophy to SPEM to dysplasia and cancer is consistent with the human model. There is a clear systematic progression from parietal and chief cell loss to hypertrophy of glands accompanied by the emergence of a mucus cell metaplasia and varying degrees of antralization within the fundic mucosa. SPEM has been reported in several experimental models,^{13,14} and several mouse models have been designed to explore the effects of oxyntic atrophy,⁴⁵ which occurs during long standing *Helicobacter* infection; a candidate precursor lesion to adenocarcinoma. During *Helicobacter* species infection, C57BL/6 mice developed parietal cell loss followed by highly proliferative SPEM.¹³ Similar findings have been reported in other mouse models and in gerbils. Chronic inflammation is a critical component of *Helicobacter*-induced SPEM and is required for its progression to dysplasia and neoplasia. Oxyntic atrophy can also be induced in transgenic mice. Overexpression or knock-out of key regulators may lead to alterations in cytokines and growth factors that normally maintain appropriate cellular differentiation. Also, toxic agents that destroy parietal cells, such as DMP777 induce SPEM. Interestingly, in models where chronic inflammation is not present, SPEM cells are not hyperproliferative and do not progress to dysplasia or neoplasia, stressing that the SPEM phenotype per se may not be premalignant in the absence of inflammation.

Using the C57BL/6 mouse model, it has been shown that the mucosal changes are reversible with early bacterial eradication.⁴⁶ Furthermore, if given early and at the midpoint of infection, bacterial eradication therapy completely prevents the progression to gastric cancer.⁴⁶ In this mouse model, therapy given at advanced stages of infection, a time point that may represent a substantial portion of our patients at risk for gastric cancer, prevented progression of the established lesions, and in some cases, allowed regression of lesions. Mice treated

after 1 year of infection and followed up for 1 additional year did not die of gastric cancer, which was the inevitable outcome in mice that remained infected. This protection from death due to gastric outlet obstruction occurred despite the fact that microscopic gastric adenocarcinomas were present in the majority of cases.⁴⁶ Therefore, the evidence from mouse models of gastric cancer support the human epidemiologic evidence that bacterial eradication, even in long-standing, established *Helicobacter* infection is of great benefit to the host, and decreases the incidence of cancer and deaths due to gastric cancer.

The Mouse Model Is a Useful Tool to Define Signaling Events Underlying Histologic Alterations

The reversibility of the metaplastic and dysplastic lesions after bacterial eradication suggests that cellular differentiation in the gastric mucosa depends on the local environment and may not be due to genetic alterations per se early on. Important environmental conditions include bacterial factors as well as components of the host immune environment (reviewed by Jean Crabtree and Keith Wilson [Gastroenterology 2007;133:288-308]). Indeed, the pattern of inflammatory cytokines within the gastric mucosa in both humans and the mouse model⁴⁷⁻⁵⁴ dictate the extent of mucosal injury, repair, and disease, with the specialized cells of the mucosa particularly prone to cytokine-induced Fas-mediated apoptosis,^{47,53} leading to sustained depletion of this cell population. One proposed cellular mechanism for the initiation of the metaplasia/dysplasia/carcinoma sequence is the loss of specialized cells, especially the parietal cells, resulting in loss or alterations in crucial cellular cross-talk pathways. Removal or derangement in cellular cross-talk leads to disturbed differentiation patterns; parietal cells are crucial in maturation decisions of gastric epithelial precursor cells and appear to coordinate cell migration within the gastric pits.⁴⁵ In humans as well as the mouse model of *Helicobacter* infection, loss of parietal cells precedes the development of metaplasia and dysplasia and experimental ablation of parietal cells is associated with metaplastic alterations,⁴⁵ stressing the importance of the presence of parietal cells above and beyond the effects of the inflammatory environment that lead to their depletion.

Helicobacter infection increases proliferation within the gastric mucosa. Increased proliferation is a hallmark of cancer in other organs, and current dogma suggests that a return of normal proliferation is needed to eliminate cancer risk. In the mouse model, eradication of *Helicobacter* infection early on has the potential to completely restore proliferation rates to normal.⁴⁶ Eradication of bacteria after long-standing infection decreases the proliferation rate, but does not return it to normal. This decrease in proliferation, although substantial, remains

significantly higher than basal levels, suggesting a continued cancer risk exists.⁴⁶ It is unclear whether the cells within the gastric mucosa are altered, or the environment remains abnormal, driving the proliferation. Indeed, the complex events leading up to the initiation and progression of gastric cancer is only beginning to be unraveled. If we evaluate what is known about cancer and the cell type involved in cancer initiation in general, we can then explain pertinent observations in the gastric cancer model and interpret these findings in the context of what we have learned about human disease.

Cancer Stem Cells

In general, cancer can be thought of as an abnormal organ, composed of multiple diverse cell types in various stages of differentiation and with different proliferative capacities. Recently, a population of cancer cells within tumors has been identified that serves to provide all of the cancer cells of the tumor, termed the “cancer stem cell.”^{55,56} The identity of the cancer stem cell has remained relatively elusive until recently, when several groups prospectively identified the cancer stem cell from tumors of different organs.^{57–64} These rare cells have several characteristics of peripheral stem cells, leading to the suggestion that peripheral stem cells may be the source of cancer stem cells.

Indeed, peripheral stem cells are a very attractive candidate cell in that they possess several important growth features. Peripheral stem cells have the ability to temporarily bypass normal growth control programs, allowing proliferation for tissue replacement and wound healing. However, it is well recognized that this ability to proliferate under a broader range of conditions, and within the setting of conflicting signaling, may lead to the accumulation of mutations. Additionally, apoptotic programs can be suppressed during healing, allowing cells that would normally be deleted because of damage to be inappropriately retained. Because the process of wound healing is usually short lived, this is not usually a relevant issue. However, under conditions of sustained inflammation and chronic injury, the relentless stimulus to divide coupled with a bypass of apoptotic programs can predispose to the accumulation of mutations and transformation. Despite the compelling growth properties of peripheral stem cells, one must also consider properties of peripheral stem cells, which are at odds with their functioning as a cancer stem cell.

Prospective identification of most peripheral stem cells has not been successful. The location of the gastric stem cell niche has been investigated using cell proliferation mapping and radiation regeneration/clonogenic assays. Unfortunately, there are not defined markers to identify this cell type, and its location has only been implied. Within the fundus, the stem cell is purported to reside in the mid-crypt zone, and at the base of antral glands.^{65,66} These putative stem cells slowly divide, giving rise to one

daughter stem cell and one more rapidly proliferating daughter cell, which comprises the “BrdU-positive” population routinely evaluated during studies of proliferation. Historically, the “stem cell zone” of the stomach has been defined as the area of highest density of BrdU-positive cells, which are thought to contain both the true stem cell (low proliferation) and the first few generations of rapidly proliferating daughter cells (also known as “transit amplifying cells”). If we consider the peripheral stem cell as the origin of cancer, we must reconcile the observation that this presumptive stem cell zone is the compartment most often damaged and depleted by agents thought to be carcinogens,⁶⁶ thus removing the cell type that is predicted to transform. Indeed, a common outcome of chronic inflammation of many organs, not just the stomach, is atrophy and specialized cell loss, and is an early stage in the progression to invasive cancer in humans. Atrophy and cell loss are the tangible effects of peripheral stem cell injury and loss. It is because of these data that an additional source of stem cells has been speculated to serve as the cancer stem cell—a bone-marrow derived cell (BMDC).

Bone Marrow-Derived Cells as a Source of the Cancer Stem Cell

Indeed, there is increasing support of the BMDC as a cancer stem cell beyond the theoretical and circumstantial. If one looks closely at the phenotype and surface marker profile of identified cancer stem cells and compares these to markers found on BMDC, there are very interesting similarities. Both cell types may express CD44^{67,68} and the ABC transporter Bcrp1/ABCG2^{69,70} on the cell surface, endowing both cell types with the stem cell-side population phenotype.^{69–72} Also, similar pathways for chemotaxis and metastasis are used, further tightening the connection.

But why would a BMDC be in a position to act as a cancer stem cell? Cancer arises in the context of abnormal tissue environments. Within the gastric mucosa, infection with *Helicobacter* results in ongoing tissue injury and peripheral stem cell failure with atrophic changes and loss of specialized cells. This pattern of inflammation, chronic injury, and atrophy is a common finding in tissue at risk for cancer. It may be that the loss or damage to peripheral stem cells allows BMDC to engraft within the stem cell niche and assume the stem cell function. BMDC can differentiate in vitro and in vivo along multiple diverse lineage pathways and acquire characteristics of mesoderm, ectoderm, and endoderm in an environment-dependent context.^{73–86} Under usual (noninflamed) conditions, rare BMDC engraft and assume a phenotype of terminally differentiated cells, suggesting they may serve a temporary function in tissues. However, with damage and inflammation, BMDC are increasingly found in the peripheral stem cell niche where they clonally expand and contribute to regions of involved organs.^{39,87} Based on

the present data, it is unlikely that BMDC contribute to organ maintenance under normal conditions, but may serve as a backup or "second line of defense" in situations of severe injury and repair. Once within the tissue stem cell niche, if the inflammatory/injury environment persists, the BMDC is exposed to this abnormal environment of damage, inflammation, inappropriate cell-cell contacts, and an ongoing stimulus to proliferate. This combination is likely to drive transformation of BMDCs,^{39,87} which have been shown in vitro to be inherently mutagenic.⁸⁸⁻⁹¹

Evidence for a BMDC as the Cancer Stem Cell in *Helicobacter*-Induced Gastric Cancer

Chronic inflammation is a key factor in the pathogenesis of gastric cancer, providing a useful model system for determining if a BMDC, as the ultimate uncommitted adult stem cell, could function as the cell for malignant transformation. For these experiments, the well-described C57BL/6 mouse model of *Helicobacter*-induced gastric cancer,³⁷ which closely mimics human infection and cancer formation, was used. As in humans, gastric cancer in the mouse rarely is encountered in the absence of *Helicobacter* infection, and long-standing infection carries a significant risk of gastric cancer.

Host bone marrow was ablated via irradiation and mice were transplanted with gender-mismatched bone marrow. To further facilitate in vivo and ex vivo tracking of the marrow-derived cells, BMDC carried a nonmammalian β -galactosidase enzyme [C57BL/6J*Gtrosa26* (ROSA 26)] or enhanced green fluorescent protein [C57BL/6J- *β -actin-EGFP* (GFP)]. After recovery of immune function, mice were infected with *H felis* and after varying lengths of time, BMDCs' engraftment into the gastric mucosa was evaluated by several independent methods. Enzyme activity was evaluated using the x-gal reagent, which renders all cells carrying the β -galactosidase enzyme blue, bacterial-specific β -galactosidase immunohistochemistry and detection of LacZNeo fusion gene sequence by polymerase chain reaction within β -galactosidase-positive gastric glands that had been isolated by laser capture microscopy in those mice transplanted with ROSA26 marrow. In mice transplanted with GFP, GFP was detected by specific GFP immunohistochemistry of tissue sections, and FACS analysis of GFP-positive cells from single-cell preparations derived from the infected stomach. GFP-positive BMDC were confirmed to be cytokeratin positive (carried an epithelial marker), CD45 negative (lacked a leukocyte marker), and contain a single X and Y chromosome (Y chromosome derived from the male donor), firmly establishing the donor origin of these epithelial cells. X and Y fluorescent in situ hybridization (X and Y-FISH) of tissue sections confirmed the Y-chromosome within gastric mucosal cells, allowing the deter-

mination of their position within the architecture of the gastric mucosa.³⁹

The current literature suggests that engraftment of BMDC in peripheral tissue is of a low abundance, and in the absence of inflammation, BMDC are rarely seen in the stem cell niche. It becomes important then to evaluate and interpret these findings in light of the available body of literature. Is the stomach unique in its ability to recruit and retain BMDC, or is the injured/inflammatory gastric mucosal environment a unique situation? First, it was determined what happens to BMDC within the stomach under various physiologic conditions, some of which are related to gastric cancer and some of which are not. For example, what would happen in a situation of severe gastric injury, where the insult was short lived? One could reason that the stimulus for BMDC engraftment would be increased because of cell loss and altered cellular cross-talk. On the other hand, acute injury of the stomach per se is not related to an increased risk of gastric cancer, so if the BMDC were to engraft, one would predict normal cellular differentiation without the sequence of metaplasia/dysplasia/carcinoma seen within gastric mucosa at risk for gastric cancer. The behavior of BMDCs in acute injury was evaluated using the marked marrow transplant model with cryoinjury or acetic acid-induced gastric ulcers. These agents induce acute ulceration, which heals completely upon removal of the offending agent. Examination of the area of ulceration was examined acutely, during healing, and after healing for evidence of BMDCs' engraftment. In addition, selective but reversible ablation of parietal cells allows repopulation to occur and offers the scenario to evaluate for the presence of BMDCs within the healed tissue, especially within the parietal cell lineage. Examination of the tissue revealed a massive influx of BMDC within the healing ulcers. Cells were phenotypically leukocytes or fibroblasts within the granulation tissue at the ulcer base; occasionally, bone marrow-derived endothelial cells, or in the case of parietal cell ablation, rare fibroblast-like cells in the submucosa. No gastric epithelial cells were identified as bone marrow derived. From these studies, it can be concluded that neither acute ulceration nor selective parietal cell ablation required BMDCs for repair³⁹ and neither condition was associated with any evidence of marrow engraftment as gastric epithelium.

Long-standing inflammation and inflammatory-mediated damage was next tested for an association with BMDC engraftment, an environment strongly linked to the development of cancer in many settings. In the *Helicobacter* gastric cancer mouse model, inflammation is an early event, and appears most intense early in infection, with a plateau at approximately 8 weeks. After this time, the number of recoverable organisms gradually declines, and the level of inflammation decreases despite ongoing progressive tissue damage and specialized cell loss. In the marked marrow transplant model,

early influx of marked bone marrow-derived inflammatory cells was prominent³⁹; however, despite abundant BMDC in the tissue, there was no evidence of engraftment of BMDC within the mucosa as gastric mucosal cells. It was not until 20 weeks of infection that BMDC could be detected within the mucosa, suggesting tissue damage may be needed in addition to inflammation to drive engraftment. Though slow in onset, once engraftment begins, the number of bone marrow derived glands increases substantially over the ensuing weeks suggesting a threshold for recruitment needs to be reached.³⁹ The gastric mucosa derived from bone marrow cells appears either metaplastic or dysplastic suggesting the environment driving differentiation of these cells is responsible for driving incomplete differentiation programs or conversely, the cells are not incapable of fully acquiring gastric mucosal cell phenotypes.³⁹ Parietal or chief cells derived from bone marrow cells were never seen. At later times of infection, BMDC engraftment became more pronounced such that by 30 weeks, antralized glands and metaplastic cells at the squamocolumnar junction were entirely replaced by marrow-derived cells. Additionally, the severity of intraepithelial dysplasia increased over time, and by one year of infection, most mice developed invasive neoplastic glands. All of the intraepithelial neoplasia in mice infected for 12–16 months arose from donor marrow cells. This finding strongly suggests this population of cells may be inherently more vulnerable to malignant progression.

In addition to transformed epithelial cells, BMDCs are found within the tumor stroma and within seemingly uninvolved epithelium and subepithelial spaces adjacent to the tumors. Adipocytes, fibroblasts, endothelial cells, and myofibroblasts⁹² derived from bone marrow precursors can be isolated from areas adjacent to dysplasia and neoplasia.

The data from these experiments suggest that chronic tissue inflammation leads to tissue injury, and with time, to tissue stem cell failure. Peripheral stem cell failure in turn leads to recruitment and permanent engraftment of BMDCs into the tissue stem cell niche where the BMDCs essentially take over the function of the tissue stem cell. With ongoing inflammation and injury, BMDC are exposed to an abnormal tissue environment characterized by elevated cytokine and growth factor levels and lacking chief and parietal cells. It is likely that in this abnormal environment, the BMDCs are able to initiate differentiation, but fail to regulate growth programs appropriately and progress instead through stages of metaplasia and dysplasia (Figure 6). One can envision that the environment of chronic repair and healing may enable these stem cells to retain activity of cell growth programs that normally would be shut down upon differentiation, thus setting the scenario for replication-induced mutations. Indeed, mesenchymal stem cells, a population of BMDC,

have been shown to undergo mutations at a high rate when forced to replicate repeatedly.^{88,89}

The mechanism by which these marrow-derived cells acquire a gastric cell phenotype is not known. These experiments did not demonstrate any stable fusion events between a BMDC and a peripheral cell suggesting that stable fusion was unlikely; however, the presence of fusion followed by a reductive division was not completely explored and cannot be ruled out as a mechanism of transdifferentiation. At present, there is ongoing controversy regarding the role of fusion between the BMDCs and a peripheral stem cell or peripheral differentiated cell and future experiments will likely shed light on the role each of these mechanisms play in the initiation and progression of gastric carcinogenesis. If similar mechanisms take place in human disease is an area of ongoing intense research.

The association between *Helicobacter* infection and gastric cancer is clear. The cellular target of transformation is now recognized and will shape the way gastric cancer prevention and treatment is approached. Viewing gastric cancer initiation and progression from the perspective of a BMDC disease, one can explain several behaviors of cancer cells as fundamental to the cell of origin, rather than traits acquired. These behaviors include cancer cell resistance to apoptosis, their unlimited growth potential and ability for local spread, and distant metastasis. Regardless of whether fusion takes place or not, there are several fundamental questions that remain to be addressed, including how the environment delivers homing signals, what these signals are, the role of inflammation and injury in modulating these signals, and, perhaps most important, how these signals can be manipulated for therapeutic benefit.

Summary and Future Directions

Human and experimental evidences coincide in pointing to a lengthy gastric precancerous process with sequential stages of chronic inflammation, atrophy, metaplasia, and dysplasia. The initial stages of inflammation and atrophy create an abnormal microenvironment favoring engraftment of BMDC. These cells do not enter in the pathway of complete differentiation and follow a program of uncontrolled replication, progressive loss of differentiation, and eventual neoplastic invasive behavior.

These changes are induced and sustained by persistent *Helicobacter* infection. Curing the infection interferes with the precancerous cascade if accomplished early in the process, and can prevent cancer development. Because the prognosis for invasive carcinoma is very poor, prevention is the most promising strategy for cancer control. Given that approximately 50% of humans are infected with *Helicobacter*, that only a very small fraction of infected subjects ever develop cancer, and that eradication therapy may lead to activation of antibiotic-resistant strains of other pathogens, massive

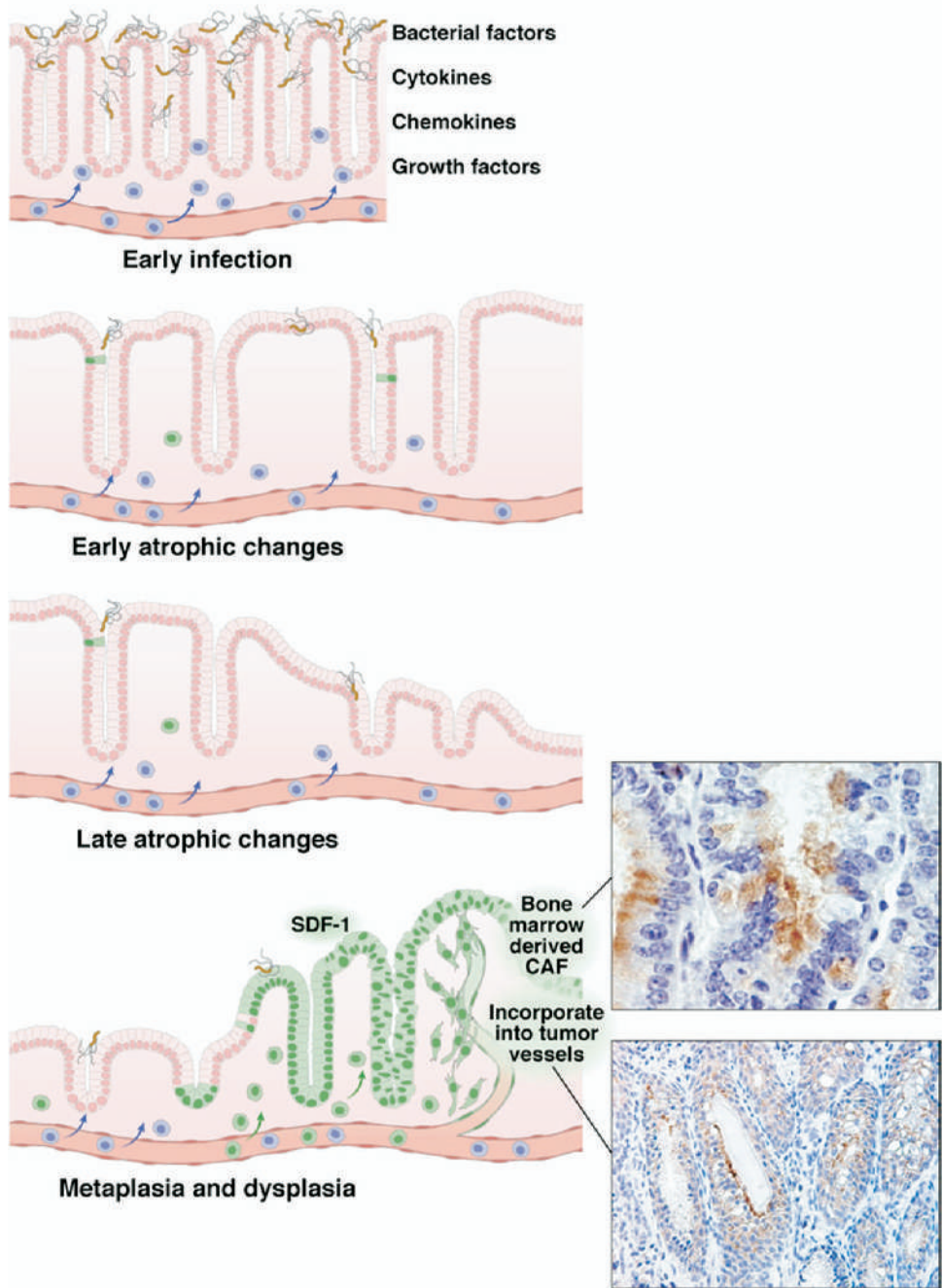


Figure 6. The role of BMDC in mucosal disease and repair. Bacterial factors and host factors recruit inflammatory cells to the gastric mucosa. Lone BMDC rarely engraft as differentiated cells (shown here marked in green) in early infection when cell loss is minimal. With ongoing inflammation and damage, the mucosa becomes atrophic and peripheral stem cell loss is evident. With time, chemokines such as SDF-1 recruit BMDC into the stem cell niche (shown here for an antral gland). These stem cells divide and repopulate entire gastric units. With time, BMDC acquire mutations and transform. BMDC also contribute to cancer-associated fibroblasts and incorporate into tumor vasculature. The illustration on the left depicts these events. The photomicrographs on the right demonstrate β -galactosidase-labeled BMDC (brown cytoplasmic staining), which are dysplastic, and comprise entire glandular units.

eradication is not feasible or advisable. There is, therefore, a need to identify subjects at the highest cancer risk because of their genetic susceptibility and their infection with *Helicobacter* genotypes of greater carcinogenic potential. These subjects are dealt with in other reviews of this series (Wilson and Crabtree [Gastroenterology 2007;133:288–308]). A complimentary approach to targeted eradication therapy can be taken from the Japanese experience where identification and resection of early cancer and dysplasia has shown effectiveness in lowering mortality rates.

The lessons learned from studies of gastric carcinogenesis and BMDC engraftment may allow unique targets of

the cancer stem cell to be identified and exploited for therapeutic intervention, and may be useful in exploring the pathogenesis of other neoplasms, especially those associated with infectious agents or chronic inflammatory situations, such as those of cervix, liver, prostate, and lung.

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