Overcoming Fas-Mediated Apoptosis Accelerates Helicobacter-Induced Gastric Cancer in Mice

Xun Cai, Calin Stoicoiu, Hanchen Li, Jane Carlson, Mark Whary, James G. Fox, and JeanMarie Houghton

Abstract

The initiating molecular events in Helicobacter-induced gastric carcinogenesis are not known. Early in infection, Fas antigen–mediated apoptosis depletes parietal and chief cell populations, leading to architectural distortion. As infection progresses, metaplastic and dysplastic glands appear, which are resistant to Fas-mediated apoptosis. These abnormal lineages precede, and are thought to be the precursor lesions of, gastric cancer. Acquisition of an antiapoptotic phenotype before transformation of cells suggests that loss of Fas sensitivity may be an early required trait for gastric cancer. We reasoned that forced Fas-apoptosis resistance would result in earlier and more aggressive gastric cancer in our mouse model. Fas antigen–deficient (lpr) mice or C57BL/6 wild-type mice were irradiated and reconstituted with C57BL/6 marrow forming partial lpr/wt chimera or wt/wt control mice, extending the life span of the lpr and ensuring a competent immune response to Helicobacter felis infection. Infected lpr/wt mice developed gastric cancer as early as 7 months after infection (compared with 15 months in wt/wt mice). At 10 months (90%) and 15 months (100%), mice developed aggressive invasive lesions. This earlier onset and more aggressive histology strongly argues that Fas-apoptosis resistance is an early and important feature of gastric cancer formation. (Cancer Res 2005; 65(23): 10912-20)

Introduction

Gastric cancer is the seventh leading cause of cancer mortality in the United States and the second leading cause of cancer deaths worldwide. Helicobacter infection has been identified as the most significant cause of gastric cancer with both bacterial factors and host immune response patterns contributing to cancer initiation. The genetic changes responsible for transformation, however, are not known. In general, genetic alterations underlying the development of cancer can be divided into six categories essential for initiating and maintaining malignant growth. These include self-sufficiency in growth signals, insensitivity to growth inhibitory signals, limitless replicative potential, sustained angiogenesis, capacity for tissue invasion, and metastasis and the avoidance of apoptosis (or programmed cell death; ref. 1). Of these features, the ability to avoid apoptosis is of paramount importance as the ability of a mass to expand is determined not only by the proliferative capacity of the tumor but also by the rate of cell attrition. Genetic damage triggers apoptotic programs, making apoptosis the major source of cellular attrition within a tumor. Therefore, apoptosis needs to be overcome to ensure continued expansion and survival of the tumor mass and avoidance of apoptosis must be a relatively early event in transformation to ensure accumulation of the mutations necessary for successful malignant growth.

Resistance to apoptosis can be acquired by cancer cells through a number of strategies. These strategies seem to be distinct to specific cancer types such that within cancers of a given organ, there tends to be a preferred or at least predominant mechanism of apoptosis avoidance (2–9). Additionally, there may be more than one antiapoptotic pathway present. Alterations in the p53 tumor suppressor gene through mutation or gene methylation are found in many, but not all, human malignancies and p53 is the most commonly lost regulator of apoptosis in human tumors (10–15). Other antiapoptotic strategies include regulation of surface apoptotic-receptor expression (16–20), modulation of downstream apoptotic machinery (21), and acquisition of inhibitor of apoptosis proteins, such as FLICE inhibitory protein (22–26) and survivin (8, 9). The mechanism by which gastric mucosal cells avoid apoptosis during the transition to cancer is not known. Studies examining genetic alterations within gastric premalignant, metaplastic, and dysplastic cells, compared with the genetic changes in established tumors, have not uncovered a pattern of alterations needed for malignant transformation (27). Many of the genetic alterations (such as p53 alterations) detected in gastric cancers are late events and are acquired after tumors are established. Although these alterations are unlikely to contribute to the initial malignant phenotype (27), they may impart growth advantage once tumors are established. For example, p53 abnormalities are common in many types of human as well as murine cancers with the p53 hemizygous and p53 homozygous mice susceptible to a number of tumors (28). However, patients harboring a germ line mutation in p53 (Li-Fraumeni syndrome) or mice carrying p53 deletions do not have a greater incidence of spontaneous gastric cancer or Helicobacter-induced gastric cancer (29). It is presently unclear what mechanism(s) gastric mucosal cells use to avoid apoptosis in the initial stages of cancer initiation and progression.

Apoptosis mediated through the Fas antigen surface receptor pathway plays a central role in Helicobacter-induced gastric mucosal disease in which Fas signaling facilitates parietal and chief cell depletion early in infection (30–32). Surface Fas antigen expression is markedly up-regulated in the gastric mucosa during infection (33) and is expressed at the highest levels on chief and parietal cell populations. Apoptosis of parietal and chief cells precedes architectural distortion and metaplasia/dysplasia, which are felt to be premalignant lesions. It is not clear, however, if parietal and chief cell loss is necessary for development of metaplasia and dysplasia or if this loss is a marker of an environment conducive to...
the development of neoplastic lesions. Interestingly, metaphasic and dysplastic cells are Fas resistant as evidenced by surface Fas antigen expression and a low incidence of apoptosis despite ample Fas ligand present on the invading immune cells. As infection continues, gastrointestinal intraepithelial neoplasia (GIN) develops and progresses with time to more invasive lesions. GIN and invasive gastric cancer express Fas antigen (34, 35) but these cells are resistant to apoptosis, strongly suggesting that avoidance of Fas-mediated apoptosis is an important event in gastric carcinogenesis. Alterations of Fas signaling in gastric cancer have also been shown in vitro as most cell lines derived from human gastric cancers show some degree of Fas insensitivity (36).

Our study was designed to address if avoidance of Fas-mediated apoptosis is an early event in Helicobacter-induced gastric carcinogenesis. Mice lacking Fas antigen (lpr) have a limited life span, precluding evaluation of long-term Helicobacter infection (31). Additionally, the immune response, which is vital to the development of gastric cancer, is altered in the lpr mouse. To circumvent these challenges, we created irradiation lpr/C57BL/6 chimera mice (lpr/wt) by reconstituting partially marrow-ablated lpr mice with wild-type (wt) C57BL/6 bone marrow. These mice showed a normal wt immune response to Helicobacter infection and an extended life span comparable to wt mice. After infection with Helicobacter felis, mice were followed for up to 15 months for the development of cancer. Uninfected lpr/wt mice did not differ from wt mice and did not develop gastric tumors; however, infection with H. felis induced early and aggressive gastric cancer lesions which were locally invasive. These findings strongly support that overcoming Fas-mediated apoptosis is an important and early event in Helicobacter-induced gastric carcinogenesis.

Materials and Methods

Animals. All work was done at the University of Massachusetts Medical School. Approval was obtained from the Institution Animal Care and Use Committee before the initiation of the study. C57BL/6J mice (C57BL/6) mice and Fas-deficient B6.MRL-Fas<sup>−/−</sup> (lpr) mice in the C57BL/6 background, which were viral antibody-free, parasite-free, and bacterial pathogen-free, inclusive of Helicobacter species, were purchased from The Jackson Laboratory (Bar Harbor, ME), housed in microisolator cages under pathogen-free conditions, fed standard chow, and allowed free access to water.

Creation of irradiation chimeras. Six- to eight-week-old male lpr mice or wt C57BL/6 recipient mice were irradiated with 900 rad from a 137Cs source to wipe C57BL/6 recipient mice with 900 rad from a 137Cs source. Necropsy and histology. Mice were euthanized by CO2 inhalation and cervical dislocation and the stomach removed, opened longitudinally along the greater curvature, and gently washed with PBS. Strips of gastric tissue along the lesser curvature from the squamocolumnar junction through the pylorus were taken, fixed in 10% neutral buffered formalin or ethanol, dehydrated, and embedded in paraffin, and cut into 5-μm sections. Histology was scored on H&E-stained sections for parietal cell loss, atrophy, inflammation, metaplasia, dysplasia, and carcinoma using a 1 to 4 numerical scale. Gastrointestinal lesion scoring criteria used were as follows. Inflammation: 0, normal; 1, small multifocal leukocyte accumulations in mucosa; 2, coalescing mucosal inflammation; 3, submucosal extension; 4, coalescing mucosal inflammation with prominent multifocal submucosal extension; +/− follicle formation; 4, severe diffuse inflammation of mucosa, submucosa, +/− deeper layers. Hyperplasia: 0, normal; 1, one to a half times normal thickness; 2, two times normal thickness with mitotic figures 1/3 way up to surface; 3, three times thickness with mitotic figures 1/2 way up to surface; 4, four times normal thickness or greater with mitotic figures >1/2 way up to the surface. Parietal and chief cell loss: 0, no substantial alterations; 1, <25% loss of parietal cells; 2, <50% loss of parietal cells; 3, <75% loss of parietal cells; 4, complete absence of parietal cells. Mucous
cell hypertrophy and metaplasia: 0, no substantial alterations; 1, <5% alteration; 2, 25% to 50% alteration; 3, 50% to 75% alteration; 4, >75% alteration. Dysplasia: 0, within normal limits; 1, mild to moderate changes including gland irregularities and mild cell atypia; 2, moderate dysplasia or low-grade gastrointestinal epithelial neoplasia (GIN; ref. 38); moderate to severe gland distortion, branching, piling, cellular, and nuclear atypia; 3, severe dysplasia or high-grade GIN (+/- carcinoma in situ); encompasses changes of moderate dysplasia listed above plus multifocal complete loss of columnar orientation and some bizarre mitoses; 3.5, above findings plus invasion into the muscularis mucosa; 4, above findings plus invasion into the submucosa or deeper structures including vascular and/or lymphatic invasion and metastasis.

Immunohistochemistry. The avidin-biotin peroxidase complex (ABC) technique was used for immunohistochemical studies. Briefly, paraffin-embedded tissue was cut into 5-μm sections, deparaffinized, rehydrated, and washed with water. Slides were treated with 3% hydrogen peroxide in methanol for 20 minutes to inhibit endogenous peroxidase and subsequently treated with citrate buffer (0.1 mol/L sodium citrate, pH 6.0) for antigen retrieval. After blocking with 5% normal goat serum, slides were incubated with primary rabbit antibodies [anti–proliferating cell nuclear antigen (PCNA; 1:200; Santa Cruz Biotechnology, Santa Cruz, CA), anti–H−K+−ATPase (1:500; EMD Biosciences, Inc., San Diego, CA), anti–Fas antigen (1:70; Santa Cruz Biotechnology), and anti–cleaved caspase 3 (1:100; Cell Signaling, Beverly, MA)] overnight, washed, and incubated with the appropriate biotinylated secondary antibody (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature followed by incubation with ABC reagent (Vectastain ABC kit, Vector Laboratories). Sections were developed with 3,3′-diaminobenzidine (DAB) by using DAKO liquid DAB Substrate-Chromogen System (DAKO Corp., Carpiniteria, CA) and counterstained with hematoxylin (Fisher Chemicals, Fairlawn, NJ).

Results

Fas expression and apoptosis in wild-type and lpr mice. In the absence of Helicobacter infection, wt C57BL/6 mice express minimal Fas antigen in the gastric mucosa (Fig. 1A). Occasional intraepithelial leukocytes express both cytoplasmic and surface Fas antigens (Fig. 1B, arrow) whereas parietal cells do not have detectable Fas antigen (Fig. 1A and B). After infection with H. felis, Fas antigen is markedly up-regulated (Fig. 1C and D) and can be seen in a bandlike distribution most prominent in the middle portion of the mucosa. Higher-power view shows this expression to be predominantly in the chief and parietal cell compartments (Fig. 1D, arrows) with markedly less expression in mucous cells and surface epithelial cells. Within the parietal cells, expression is both cytoplasmic and membranous with membrane-bound receptor more abundant as time of infection progresses and expression level increases (data not shown).

Early mucosal changes in the C57BL/6 mouse are believed to result from Fas-mediated apoptosis of parietal and chief cells with compensatory expansion of other lineages. Concomitant with elevated Fas antigen expression in parietal cells, levels of apoptosis increased as evidenced by activated caspase-3 staining (Fig. 1E and F, arrows). Apoptosis secondary to Helicobacter infection peaks at about 4 weeks (31) and then declines toward, but never reaches, basal levels as parietal and chief cell populations are depleted.

As expected, lpr mice do not express Fas antigen under noninfected conditions (data not shown) and do not increase Fas antigen expression with Helicobacter infection (Fig. 1G and H). As predicted, lpr mice do not have an elevation in activated caspase-3 staining (data not shown) and maintain parietal and chief cell mass during the early stages of Helicobacter infection (31).

After 30 weeks of Helicobacter infection in C57BL/6 mice, metaplastic and dysplastic glands become prominent. These cells express both cytoplasmic and surface Fas antigens (Fig. 1I and J) and yet seem resistant to Fas-mediated apoptosis as they are not eliminated despite Fas ligand–bearing leukocytes infiltrating the area. This resistance to Fas-mediated apoptosis is presumably via the acquisition of antiapoptotic pathways other than modulation of surface receptor expression.

We predicted that forced inhibition of Fas-mediated apoptosis would lead to earlier and more aggressive gastric cancer in the mouse model. However, as the lpr mouse does not have the longevity to sustain long-term infection, it is not a suitable model. To circumvent these problems, we created irradiation chimeric mice which had 50% to 75% of their bone marrow replaced with C57BL/6 marrow, thereby reconstituting the immune response and effectively preventing premature death. The chimeric lpr/wt mice were not different from the C57BL/6 or wt/wt transplanted mice with respect to body weight or general health, and similar numbers in each group survived for the duration of the study. The gastric mucosa of uninfected C57BL/6 mice and wt/wt transplanted mice (Fig. 2A) were indistinguishable from each other. Likewise, the gastric mucosa of uninfected nontransplanted lpr mice (Fig. 2B) and uninfected lpr/wt chimeric mice (Fig. 2C) could not be distinguished from each other. The lpr and lpr/wt had a normal distribution of specialized cells within the gastric fundus; however, there was a modest but consistent 25% to 30% increase in thickness of the fundic mucosa compared with the C57BL/6 mice, which persisted throughout the life span.

All C57BL/6 and wt/wt transplanted mice were genotypically wt by PCR (one representative mouse shown as control, Fig. 3A, lane W). Lpr/wt transplanted mice were 50% to 75% chimeric for wt immune cells (representative gel shown in Fig. 2A), supporting reconstitution of an adequate pool of cells for an effective immune response. We tested a cohort of chimeric mice (n = 5) and wt mice (n = 5) immediately after recovery from bone marrow transplantation and at 4, 7, 10, and 15 months of infection. Analysis of Fas antigen expression in peripheral blood showed no difference between sample times within a single animal and no variation between the infected and noninfected mice. These results suggest that infection did not result in expansion or loss of lpr cells. The remainder of the mice were evaluated for levels of chimerism using PCR analysis of the spleen at necropsy. In addition to donor-derived immune cells, the spleen contains host-derived mesothelial cells, endothelial cells of the arterial and venous channels, smooth muscle cells, and macrophages. Inclusion of these cells in the PCR reaction most likely underestimates the level of engraftment. We next tested the immune response to H. felis infection. Mice were examined after 10 months of infection or after mock infection (control mice). Bacterial colonization, quantitated by detection of H. felis–specific flaB DNA, was not different between wt/wt and lpr/wt mice. No bacterial DNA was detected in the mock-infected mice (Fig. 3B).

We next assessed the immune response to H. felis using a battery of criteria including H. felis–specific IgG isotype production, total antibody production, and tissue inflammation scores. On quantification of total serum IgG1 and IgG2c, values for the lpr/wt mice fell into the reference range for serum antibody with a wide range of values (data not shown) showing that the irradiation chimeras are fully capable of an antibody response. When individual subclass analysis was done, the wt/wt and the...
lpr/wt had statistically the same relative T-helper 1 to T-helper 2 response (Fig. 3C) although the response was somewhat blunted in the lpr/wt.

Parietal cells are lost early in infection in C57BL/6 mice but preserved in Fas antigen–deficient mice. Parietal cell loss is characteristic of *Helicobacter* infection in the C57BL/6 model and parallels the appearance of metaplasia and dysplasia. Wt/wt mice infected with *Heliocbacter felis* for 15 months develop GIN (Fig. 4A, large submucosal dilated gland) concomitant with a complete depletion of parietal cells. High-power view of sections stained for H^+-K^+-ATPase confirms a complete loss of parietal cells (Fig. 4B). Mice lacking Fas antigen (lpr/wt) develop GIN as well as more aggressive invasive lesions in areas of preserved parietal cell mass. Immunohistochemistry for H^+-K^+-ATPase confirms a complete loss of parietal cells (Fig. 4B). Mice lacking Fas antigen (lpr/wt) develop GIN as well as more aggressive invasive lesions in areas of preserved parietal cell mass. Immunohistochemistry for H^+-K^+-ATPase confirms a complete loss of parietal cells (Fig. 4B).

Figure 1. *Helicobacter* infection increases gastric mucosal Fas antigen expression in C57BL/6 mice but not in lpr mice. A, C57BL/6 mice under control (noninfected) conditions do not express Fas antigen (brown staining) in gastric epithelial cells. B, higher-power view of (A). Fas antigen expression confined to rare infiltrating leukocytes. C and D, early (2-4 weeks) *Helicobacter* infection. Fas antigen expressed in parietal cells (arrows) in the cytoplasm and on the cell surface. E and F, activated caspase 3 (arrows) detected in parietal cells of *Helicobacter*-infected C57BL/6 mice. G and H, anti-Fas antigen immunohistochemistry. Lpr mouse infected with *H. felis* for 4 weeks. I and J, resurgence of Fas antigen expression in areas of dysplasia in infected C57BL6 mouse. A, C, E, and G, ×40. B, D, F, H, and J, ×600; I, ×100. Counterstain: hematoxylin.
infection when all of the mice developed GIN secondary to *Helicobacter* infection (Figs. 4E and 5A). In sharp contrast, lpr/wt mice developed dysplasia early on, with mice infected for 7 months developing GIN. By 10 months, 90% developed severe dysplasia with invasion into the muscularis and extension to the serosa (Figs. 4E and 5A). In addition to earlier onset of dysplasia and GIN, there were notable histologic differences between wt/wt and lpr/wt lesions. Wt/wt mice predominantly developed large cystically dilated glands, first in the mucosa, then extending into the submucosa. Lining cells were cuboidal or columnar and nuclei retained basal polarity (Fig. 5B and C). In contrast, lesions in lpr/wt mice were of two distinct types. The majority of lesions consisted of irregular congeries of malignant glands with atypical architectural features including micro-papillary tufts and cribriform spaces, appearance of sheets of malignant cells without definable gland architecture, and isolated nests of malignant cells within the submucosal area. Atypical cytologic features include mucin depletion, nucleomegaly with hyperchromasia, and prominence of nucleoli, best seen under high-power view (Fig. 5D and E). The minority of invasive glands resembled those in wt/wt mice with uniform low columnar/
cuboidal cells lining invading glands. However, even in these glands, nuclear stratification and cellular atypia were greater than those seen in infected wt/wt mice. PCNA staining as a marker of proliferation was not different between wt/wt and lpr/wt mice at any point of infection (Fig. 6).

**Discussion**

Gastric cancer is the second most common cause of cancer-related mortality worldwide (39) and is largely attributed to infection with *Helicobacter pylori* (40–42). A great deal has been learned about the role for bacterial interaction with the host (for a complete review, see ref. 43) and the host immune response (for review, see refs. 44, 45) in the pathogenesis of gastric cancer; however, the early genetic alterations within the transformed cell are largely unknown. There is not a clear-cut pattern of mutations or gene alterations in the progression of gastric cancers and most mutations studied to
date accumulate once the cell has undergone malignant transformation (27), making the role these changes play in cancer initiation unclear.

The notion that resistance to apoptosis serves as a barrier to cancer was first described in 1972 after the observation of massive apoptosis of hormone-dependent tumors on hormone withdrawal (46). Indeed, a cell apoptotic program can be triggered by oncogene overexpression or altered growth programs (47, 48), and elimination of these abnormal cells via apoptosis seems to be the primary manner in which mutated cells are removed from the body. Because apoptosis removes cells at an early stage of gene alteration, it follows that overcoming apoptosis is a requisite early change in cancer initiation and likely needs to be maintained for continued survival of a cell with increasingly abnormal gene expression. Strategies for acquiring apoptosis resistance are varied with the most common being alterations in p53. Whereas p53 mutations are commonly found in gastric cancer, this mutation does not seem to be an early event. Patients with germ line p53 mutations (Li Fraumeni syndrome) have higher incidence of several types of cancer but not spontaneous gastric cancer or Helicobacter-induced gastric cancer. Another prominently used pathway for apoptosis is the Fas antigen pathway. Germ line mutations in the Fas gene are associated with an increase in several forms of cancer, including gastric, colorectal, and other epithelial tumors (49), and somatic mutations in Fas antigen have been found in malignant melanoma (50) although causality has not been shown. We investigated the role of the Fas antigen pathway in gastric cancer initiation and progression and showed that unlike the p53 mutant, mice deficient for Fas signaling have markedly accelerated Helicobacter-induced gastric cancer without an increase in spontaneous cancer. Abrogation of Fas antigen–induced apoptosis substantially shortened the time necessary for Helicobacter-induced gastric cancer and lesions that form in the lpr/wt model were more aggressive with marked architectural distortion and invasion of lesions through the serosa. In addition, the progression of mucosal alterations differed between wt and lpr chimera. Infected wt mice progress from chronic inflammation through an ordered sequence of changes, including metaplasia, atrophy, and dysplasia, followed by early intraepithelial neoplasia, and later by lesions which invade below the muscularis mucosa. The lpr chimera showed similar patterns of inflammation but, surprisingly, developed much milder metaplasia in half the mice and the remaining half developed no detectable metaplasia. Dysplasia was an early lesion in the lpr mouse with 90% of mice

Figure 4. Parietal cells are preserved in lpr mice. Infected wt/wt mice with H. felis lose parietal cell mass early in infection. A, wt/wt at 15 months of infection. Anti–H+-K+-ATPase immunohistochemistry (brown staining); parietal cells cannot be shown in long-term infection (>100). B, higher-power view (>600). C, lpr/wt at 15 months of infection. Mice have abundant parietal cells concurrent with severe dysplasia and invasive carcinoma (100×). D, higher-power view; arrows, individual parietal cells (>400). E, histology scores for wt/wt (○) and lpr/wt (●) at 10 months of infection. Inflammation scores were not significantly different. Metaplasia was markedly increased in wt/wt mice relative to lpr/wt mice. In contrast, at 10 months, there was no dysplasia in WT and 90% of lpr/wt mice had progressed to grade 4 dysplasia with lesions invading into the submucosa and in some cases through the serosa. Parietal and chief cells were depleted in infected WT mice whereas specialized cells were essentially preserved in lpr/wt mice. A, B, and D, anti–H+-K+-ATPase immunohistochemistry; hematoxylin counterstain. C, H&E stain.
infected for 10 months developing severe dysplasia/gastrointestinal intraepithelial neoplasia compared with 0% of the infected wt. The significance of the discrepancy between metaplasia and dysplasia between these mice is not clear but the absence of metaplasia in the lpr mouse may reflect the rapid progression of this cell type to dysplasia.

In the absence of infection, Fas deficiency in the gastric mucosa does not increase the incidence of gastric cancer. This is consistent with what would be expected based on the expression pattern of Fas antigen under normal conditions and with infection. Data from mouse models of gastric cancer as well as data derived from biopsy specimens taken from infected humans confirm that Fas antigen expression is negligible in the absence of infection. As Fas signaling does not seem to play a prominent role in the normal homeostasis of the stomach, it is not surprising that knocking out this receptor has little effect on the gastric mucosa under normal conditions. The lpr mouse has a modest increase (25%) in the height of the oxyntic mucosa without alterations in cell distribution. PCNA staining suggests that this is not due to an alteration in proliferation and it may possibly be due to impaired elimination of cells by apoptosis. This impairment of physiologic cell turnover does not seem to increase the susceptibility to gastric cancer as the lpr/wt mouse does not develop cancer in the absence of \textit{H. felis} infection.

In the wt/wt mouse, \textit{Helicobacter} infection induces robust surface receptor expression followed by the elimination of parietal and chief cells, which express the highest levels of surface receptor and seem to be particularly sensitive to Fas-induced apoptosis (51). In both mouse and human systems, metaplasia and dysplasia are considered premalignant lesions. Interestingly, these aberrant cell lineages express cell-surface Fas antigen and yet are resistant to

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\caption{Loss of Fas antigen leads to earlier and more aggressive gastric cancer due to \textit{H. felis}. A, wt/wt (black bar) mice develop invasive gastric cancer by 15 months of infection. Lpr/wt mice develop GIN by 7 months of infection. At 10 months, 90% of mice had GIN and by 15 months, 100% of infected lpr/wt mice had aggressive invasive gastric cancer. B, at 15 months of infection, lesions in wt/wt mice consisted predominantly of large dilated cystic glandlike structures invading beneath the muscularis mucosae into the submucosa (C). Under higher power, these glands (arrow) are seen lined by uniform cuboidal-columnar cells with little stratification of the nuclei. D and E, infected lpr/wt mice develop large invasive and histologically aggressive lesions by 15 months of infection. Dilated cystic glands similar to those seen in wt/wt mice [boxed area (D) and top arrow (E)] were found within sheets of malignant cells and poorly organized glands. Cellular pleomorphism and nuclear atypia are best seen under higher power (E). H&E staining. B and D, \texttimes100; C and E, \texttimes400.}
\end{figure}
apoptosis. Under constant ligand exposure from invading immune cells, there is likely pressure to select out those cells which are Fas resistant. It is not clear if metaplastic and dysplastic cells are inherently Fas resistant or cells with the highest resistance selectively survive. Also unclear is the mechanism by which these cells avoid Fas-mediated apoptosis, but extrapolating from data derived from established cancers, mechanisms may include alterations in caspase expression (21), increased expression of survivin (8), and/or mutations in the death domain of the Fas receptor (20). Although genetic alterations are possible, it is likely that early avoidance of apoptosis involves reversible mechanisms. This is based on data from human studies which suggest that metaplasia and possibly early dysplasia are reversible lesions and normal architecture can at least be partially restored with successful bacterial eradication (51). These findings support a role for environmental factors, such as inflammatory cytokines and bacterial products, rather than permanent genetic alterations driving the cellular phenotype. Work in the mouse model supports these findings as Helicobacter eradication in the C57BL/6 mouse model early in the time course of infection restores normal architecture whereas eradication late in infection, after the onset of severe dysplasia, halts progression of lesions, partially restores architectural integrity, and dramatically reduces death due to gastric cancer (51).

Taken together, these data suggest that avoidance of Fas-mediated apoptosis is an early event in gastric cancer and contributes to an aggressive phenotype. Strategies targeted at restoring Fas-mediated apoptosis may offer novel approaches to gastric cancer which remains resistant to conventional therapy.

Acknowledgments

References


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