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

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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, metaplastic 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/6J00064 (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 microisolation cages under specific 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 γ cell irradiator. Donor marrow was collected from the tibia, femur, and iliac crest of wt C57BL/6 male mice as previously outlined (37). Marrow was made by sequential 10-fold dilutions of purified 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.

Helicobacter infection. H. felis (strain 49179) was obtained from the American Type Cell Culture (Rockville, MD) and grown as recommended. Mice were infected by oral gavage (1 × 10<sup>7</sup> colony-forming units) using a 19-gauge Popper feeding tube every other day for three doses. After bacterial or mock infection, mice were randomized to predetermined, timed experiment. Four hours after irradiation, recipient mice were reconstituted with wt C57BL/6 male mice as previously outlined (37). Marrow was collected from the tibia, femur, and iliac crest of wt C57BL/6 male mice as previously outlined (37). Marrow was washed and viable cells were counted and resuspended in cold PBS for injection. Four hours after irradiation, recipient mice were reconstituted with 3 × 10<sup>6</sup> total marrow cells via a single tail injection as previously described (37). Mice were allowed to recover for 4 weeks before further experimentation.

ELISA for immunoglobulin G1 and immunoglobulin G2c antibodies against Helicobacter felis. Serum was collected at necropsy and evaluated by ELISA for immunoglobulin G (IgG) 2c (T-helper 1–promoted isotype) and IgG1 (T-helper 2–promoted isotype) responses to H. felis antigens. Briefly, outer membrane antigens of H. felis were obtained by culturing H. felis in trypticase soy broth containing 5% fetal bovine serum for 48 hours under microaerobic conditions. After three washes in PBS and examination for bacterial contaminants using Gram stain and phase microscope, the pellet was resuspended in 4 mL of 1% N-octyl-p-glucopyranoside (Sigma, St. Louis, MO) for 30 minutes at room temperature. Insoluble material was removed by ultracentrifugation at 100,000 × g for 1 hour. After dialysis against PBS for 24 hours at 4°C, supernatant protein concentration was measured by the Lowry technique (Sigma). For the ELISA, antigen was coated on plates at a concentration of 10 ng/mL for subclass isotyping of the serum IgG response. Sera were diluted 1:100 and biotinylated secondary antibodies were included for detection of IgG1 and IgG2c (monoclonal antinose antibodies produced by clones AB5-1 and 57, respectively; PharMingen, San Diego, CA). Incubation with extravidin peroxidase (Sigma) was followed by ABTS substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) for color development. Absorbance development at 405 nm was recorded by an ELISA plate reader (Dynatech MR7000, Dynatech Laboratories, Inc., Chantilly, VA). All samples were analyzed twice with good correlation between results.

Quantitative analysis of Helicobacter felis colonization. A 2 × 2-mm piece of gastric mucosa taken at the fundus/antral border was snap frozen at the time of necropsy. All samples were processed together as follows: DNA was extracted using High Pure PCR Template Preparation kit (Roche Molecular Biochemicals, Indianapolis, IN) according to the protocol of the manufacturer. Two microtubes of extracted DNA were used for real-time PCR (SmartCycler, Cepheid, Sunnyvale, CA) using QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA). Standards were made by sequential 10-fold dilutions of purified H. felis DNA producing a range from 500,000 to 5 copies per reaction. This is based on the premise that 2 fg of H. felis chromosomal DNA is equivalent to 1 copy of the H. felis genome. Each sample was analyzed in triplicate. Primer sequences for a 225-bp fragment of the flaB gene were 5'-TTCGATTGGCTCCTACCGGTCCTAGA-3' and 5'-TTCTTTGTTGA-TGACATTGACCA-CCAGA-3'. Annealing temperature was 55°C. Results are reported as bacterial copies per microgram of gastric tissue.

Genotype analysis of Fas. One half of each spleen was snap frozen at the time of necropsy and all samples were processed together. DNA was extracted using High Pure PCR Template Preparation kit (Roche Molecular Biochemicals) according to the protocol of the manufacturer. One hundred nanograms of input DNA were used for PCR of WT and lpr-specific bands using the following primer trio and conditions: forward 5'-GTAAATATTGTTGCTTCGTAC-3', WT 179-bp fragment reverse 5'-CAAATCTTAGCTTTAACAGTG-3', and lpr 217-bp fragment reverse 5'-TAAAGGTTTCCGACGGGTTGTCG-3'; 30 seconds at 94°C, 1 minute at 59°C, and 1 minute at 72°C for 35 cycles. Products were resolved on a 4% agarose gel and quantitated with densitometry.

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 Prefer fixative for 4 hours, processed by standard methods, 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; early submucosal extension; 3, coalescing mucosal inflammation with prominent multifocal submucosal extension +/+ follicle formation; 4, severe diffuse inflammation of mucosa, submucosa, +/+ deeper layers. Hyperplasia: 0, normal; 1, one and 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 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.
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 mucosae; 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 (VectorStain ABC kit, Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature followed by incubation with ABC reagent (VectorStain ABC kit, Vector Laboratories). Sections were developed with 3,3’-diaminobenzidine (DAB) by using DAKO liquid DAB Substrate-Chromogen System (DAKO Corp., Carpinteria, 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 chimera 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 *Helicobacter 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). At late stages of infection, parietal cells could be detected at the edge of neoplastic lesions in the lpr/wt mice, suggesting a mass effect redirecting parietal cell position and arguing against elimination of this lineage being the inciting factor for malignant growth.

**Mice lacking Fas antigen develop early and aggressive gastric cancer.** Inflammation was not significantly different between infected wt/wt and lpr/wt mice although it tended to be somewhat lower in lpr/wt mice. There was a marked difference in metaplasia with infected wt/wt mice developing columnar lined elongated glands and mucous cell metaplasia composed of cells with pale foamy cytoplasm and staining with Alcian blue (pH 2.5). The infected lpr/wt mice rarely showed more than a single isolated focus of metaplasia and half of the mice examined showed no evidence of metaplasia at all (Fig. 4E). At 4, 7, or 10 months, we did not detect any dysplastic cells in the infected wt/wt mice and only detected grade ≥2 (moderate to severe dysplasia or GIN) after 15 months of infection.
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 transfor-
mation (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.

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. Parietal
and chief cells were depleted in infected WT mice whereas
specialized cells were essentially preserved in lpr/wt mice.

Figure 4. Parietal cells are preserved in lpr mice. Infected wt/wt
tumors 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 turn over does not seem to increase the susceptibility to gastric cancer as the lpr/wt mouse does not develop cancer in the absence of H. felis infection.

In the wt/wt mouse, 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...
apoptosis. Under constant ligand exposure from invading immune cells, there is likely pressure to select out those cells that 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 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 that normal architecture can at least be partially restored with successful bacterial eradication (51). These findings support a role that premature death due to 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.

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References


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