Inflammation and Cancer

I. Rodent models of infectious gastrointestinal and liver cancer

Arlin B. Rogers and James G. Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Rogers, Arlin B., and James G. Fox. Inflammation and Cancer. I. Rodent models of infectious gastrointestinal and liver cancer. Am J Physiol Gastrointest Liver Physiol 286: G361–G366, 2004; 10.1152/ajpgi.00499.2003.—Chronic gastrointestinal and liver infections account for a significant percentage of human cancer deaths. Rodent models help elucidate how infection can lead to malignancy. Helicobacter pylori, the leading cause of human gastric tumors, produces similar disease in Mongolian gerbils. H. pylori, H. felis, and H. hepaticus induce stomach, lower bowel, or liver tumors in susceptible wild-type and genetically engineered mice. Immune dysregulated mice recapitulate features of inflammatory bowel disease including colon carcinoma. Hepatitis B and C virus transgenic mice provide insights into viral hepatitis and hepatocellular carcinoma. Rodent models enhance our understanding of infectious cancer pathogenesis and suggest novel targets for intervention.

digestive system neoplasms; Helicobacter; viral hepatitis

TUMORS OF THE STOMACH, LIVER, AND LOWER BOWEL

Rodents are routinely exposed to infectious agents in the gastrointestinal system, and most naturally acquired gut microflora are capable of transforming to pathogenic features. Helicobacter pylori, Helicobacter hepaticus, and Helicobacter bilis infect the enterohepatic system; thus organisms following colonization of the upper gastrointestinal system gain access to the enterohepatic system. The importance of animal husbandry and environment when designing and interpreting rodent models of infectious GI and liver cancer cannot be overstated. Unfortunately, little attention is often given to these critical issues, and still less is reported. The current authors (21) refer to this transitional zone as the squamocolumnar or forestomach/zymogenic junction. Gastric oxyntic glands in humans are only a few glands in length in rodents, and some authors use the phrase “fundic glands” as a generic term for oxyntic glands in rodents.

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initial infection are most readily demonstrated in the cardia and in the proximal antrum (21). Interestingly, in mice, severe proliferative and dysplastic lesions usually arise in the cardia and progress aborally in conjunction with oxyntic gland atrophy, although bacteria can be identified in antral glands throughout the course of infection (8, 10). The unfortunate term “atrophic gastritis,” referring to the loss of chief and parietal cells in a setting of inflammation, is enconced in the medical lexicon and has been transferred to rodent models, even though subjects with atrophic gastritis often have a markedly thickened mucosa due to reactive epithelial proliferation. When compared with humans, rodents have a much larger cecum to facilitate bacterial digestion of their herbivorous diet. The anatomy of the rodent liver parallels that of humans except that rats lack a gallbladder. However, there are key functional differences between species including high susceptibility to tumors induced by peroxisome proliferators in rodents. Species differences do not invalidate animal models of infectious GI and liver cancer, but comparative pathophysiology should be understood before extrapolating findings in rodents to equivalent human diseases.

INFECTION STOMACH CANCER

Most stomach tumors in humans are associated with chronic Helicobacter pylori infection, which colonizes half of the world’s population. Although only a small percentage of H. pylori-infected individuals develop tumors, the ubiquity of persistent colonization explains why stomach cancer follows only lung cancer in mortality prevalence. In addition to adenocarcinoma, chronic H. pylori infection induces tumorlike proliferations of mucosal-associated lymphoid tissue (MALT) lymphomas, which may regress with bacterial eradication. As with humans, Helicobacter spp. are the only proven bacterial cause of gastric tumors in rodents. Mouse strains such as C57BL, which mount strong T_{h}1 responses to Helicobacter infection, demonstrate lower colonization levels but increased gastritis and hyperplasia/dysplasia versus BALB/c and other strains with T_{h}2-predominant responses that maintain higher bacterial burdens but suffer less epithelial damage.

The murine pathogen H. felis induces severe gastritis that can lead to adenocarcinoma in C57BL mice (9). In mice with hemizygous deletion of p53, a paradoxical protective effect is observed as the progression of H. felis-induced gastric tumorigenesis, attributable in part to depressed T_{h}1 immune responses (9). As with humans, chemotherapeutic immune suppression decreases Helicobacter-induced gastric lymphoepithelial lesions in mice. Coinfection of a T_{h}2-provoking helminth parasite with H. felis ameliorates gastric disease in C57BL mice and has been offered as a potential explanation for the “African enigma,” wherein gastric adenocarcinoma is underrepresented in countries with concurrently high H. pylori and gastrointestinal parasite prevalence (4). The main differences between H. felis in mice and H. pylori in humans are that neutrophils are a less prominent feature of the murine gastritis and that H. felis lacks key pathogenicity-associated islands (PAI) found in H. pylori including cag. Nevertheless, histological progression in H. felis-infected C57BL mice closely mimics the sequence of events in human H. pylori disease including gastritis, oxyntic gland atrophy, surface epithelial proliferation, metaplasia, dysplasia, and neoplastic transformation, making this natural murine model a valuable tool in the characterization of inflammation-associated gastric carcinogenesis. Indeed, the progression from acute to chronic inflammation, epithelial proliferation, dysplasia, and, ultimately, neoplastic transformation is repeated in all murine models of Helicobacter-induced infectious GI and liver cancer (Fig. 1).

The two most commonly used rodent species to model H. pylori-associated gastritis and cancer are the mouse and the Mongolian gerbil. Mongolian gerbils chronically infected with H. pylori develop gastroduodenitis, ulcers, and antral cancer closely resembling the human disease (17). Mice have been successfully infected with several strains of H. pylori, including the mouse-adapted Sydney strain-1 (SS1), human isolate B128, and others (10). Although susceptible mice develop gastritis following H. pylori infection, WT strains (i.e., without genetically engineered mutations) have not been described that develop gastric adenocarcinoma, probably a reflection of poor host adaptation. Nevertheless, H. pylori has been shown to induce oxidative damage-induced gastric DNA mutations in C57BL Big Blue mice, which carry a lambda phage transgene that acts in vitro as a chromogenic mutation biomarker (23). Hypergastrinemic INS-GAS mice, which constitutively ex-
press humanized gastrin under an insulin promoter, acquire spontaneous gastric tumors within 2 yr and develop severe gastritis and carcinoma within months when infected with *H. felis* or *H. pylori* (10, 24). As is the case with humans, males are at increased risk (8). Rodent models provide opportunities for in vivo analysis of putative bacterial virulence factors. For example, deletion of *H. pylori cagE* (picB) dampens immune responses and retards but does not prevent tumorigenesis in INS-GAS mice (10).

Multiple *Helicobacter* spp. can induce lesions typical of MALT lymphoma in persistently colonized BALB/c mice. Microarray analysis of laser capture microdissected gastric lesions from BALB/c mice chronically infected with *H. heilmannii* demonstrates transcriptional alteration of many genes associated with immune maturation and mucosal epithelial responses, and the gene calgranulin A/Mrp-8 appears strongly associated with development of MALT lymphoma (15).

**INFLAMMATORY BOWEL DISEASE AND LOWER BOWEL CANCER**

Although only a small percentage of human colorectal carcinomas (CRC) are attributable to IBD, patients with Crohn’s disease or ulcerative colitis have significantly increased risk of developing CRC versus the general population. Although no specific infectious agent has yet been causally linked to IBD, it is generally acknowledged that intestinal bacteria initiate the cascade of events leading to chronic enterocolitis in susceptible individuals. In the broadest sense, then, IBD-associated CRC may be acknowledged as an “infectious” cancer. As our knowledge of the pathogenesis of these human diseases increases, it seems plausible that some bacteria will be identified as inducers of disease in susceptible individuals, whereas other microbes may exert a protective effect.

Although no animal model faithfully recapitulates all features of IBD and its associated cancers, rodent systems that reproduce important hallmarks of the human disease are used to investigate basic pathogenetic components of Crohn’s disease and ulcerative colitis. Studies of the roles of endogenous microflora in IBD-like disease in mice are complicated by the fact that results are dependent on the setting. Under “conventional housing conditions,” genetically engineered mice with a proinflammatory phenotype develop typhlocolitis and lower bowel tumors in some facilities but remain free of disease in others. In contrast, the murine pathogen *H. hepaticus* reliably induces disease in susceptible mouse strains in virtually all non-germ-free environments. Availability of the recently published complete genome of *H. hepaticus* should facilitate studies on the role of putative bacterial PAI on disease induction (20). Other bacteria of rodents, including *Citrobacter rodentium* and *Lawsonia intracellularis*, induce proliferative enterocolitis and can act as tumor promoters when combined with chemical mutagens or predisposing genetic mutations such as APC<sup>min</sup> (16).

With the exception of known tumor-associated genes such as APC<sup>min</sup> and Cdx2, microbial-induced inflammation appears to be a prerequisite for development of lower bowel tumors in most genetically engineered mice (GEM) with targeted mutation of Smad3, IL-2, IL-10, G0<sub>12</sub>, Muc2, and other genes.

Fig. 1. Sequential histopathology of *Helicobacter*-induced infectious murine gastrointestinal and liver cancers: *H. felis* in C57BL stomach (a), *H. hepaticus* in IL-10<sup>−/−</sup> lower bowel (b), and *H. hepaticus* in A/JCr × C57BL F1 liver (c). Left to right each row: normal tissue (1), acute inflammation (2), chronic inflammation with hyperplasia and dysplasia (3), and carcinoma (4). Hematoxylin and eosin; bar = ~300 μm.
involved in immune signaling (1). GEM on a 129/Sv strain background appear to be especially susceptible to IBD-like disease, suggesting that host genetic determinants besides the targeted gene disruption influence inflammation severity and tumor risk. Interestingly, GEM on a C57BL background are sometimes resistant to IBD-like tumorigenesis compared with equivalent “knockout mice” on other genetic backgrounds. Although NF-kB is traditionally considered a proinflammatory signaling molecule, mice with targeted disruption of p50/p65 heterodimers develop more severe colonic and concomitant inflammation (typhlocolitis) than corresponding NF-kB-viable mice, highlighting the complex regulatory functions of this key signal-transduction molecule. Results from a study using p50−/−p63−/− (“3X”) Rag2−/− mice demonstrate that NF-kB exerts influence on innate immune responses in part through modulation of IL-12 p40 expression (22).

TGF-β signaling pathways lie at the nexus of inflammation and neoplastic transformation in ulcerative colitis-associated colorectal carcinoma (1). TGF-β-deficient mice die in utero in part due to uncontrolled lymphocyte proliferation. TGF-β-deficient GEM rescued from embryonic lethality by coexpression of a lymphocyte-deficient phenotype, including SCID and Rag2−/− mice, develop typhlocolitis and tumors under “conventional” but not germ-free housing conditions (1). H. hepaticus infection has been identified as a likely causative factor for tumor development in TGF-β-deficient mice (1). However, TGF-β deficiency is not required for tumorigenesis, because 129/SvEv Rag2−/− mice with normal TGF-β expression develop severe typhlocolitis and tumors as early as 2–3 mo after inoculation with H. hepaticus (2).

Adoptive transfer of syngeneic CD4+ T lymphocytes or their subsets into immunodeficient (e.g., SCID and Rag−/−) mice confirms that CD45RBhi T effector (Teff) cells induce severe typhlocolitis and tumors, whereas CD25+ T regulatory (Treg) cells ameliorate or completely reverse the process (2). CD25, the α-subunit of the IL-2 receptor, is not exclusively expressed by Treg cells, but selection for this marker results in Treg enrichment. Absence of CD25 ligand helps explain why IL-2-deficient mice also exhibit a proinflammatory phenotype in the lower bowel. Adoptive transfer of CD25+ cells from IL-10-deficient mice fails to ameliorate typhlocolitis and tumorigenesis in these models, confirming the critical role of IL-10 for proper Treg function (3).

INFECTIOUS LIVER CANCER

Nearly 10% of people worldwide are infected with either hepatitis B virus (HBV) or hepatitis C virus (HCV). The estimated prevalence of HCV infection is 170 million individuals, or 3% of the global population, whereas the number of carriers of HBV exceeds 350 million or 6% of all people (14). More than 1 million people die from HBV-associated liver failure and cancer every year (14). About 70% of individuals exposed to HCV become chronically infected, and of those 5–10% will develop fatal cirrhosis or cancer (14). Because most HCV carriers are unaware of their infection status, the true prevalence of HCV-associated disease may exceed current estimates. Chronic viral hepatitis of either type B or C greatly increases the risk of hepatocellular carcinoma (HCC). Indeed, the vast majority of HCC diagnoses worldwide are made in people seropositive for HBV, HCV, or both. As with other causes of HCC, males are at 3–10 times greater risk for cancer than females. Importantly, this male-predominant tumor risk is recapitulated in rodents, making these animal models a valuable resource to investigate the role of sex hormones in hepatocellular carcinogenesis.

Rodents cannot be infected with HBV or HCV. However, studies using HBV- and HCV-transgenic mice clearly demonstrate that viral gene products can induce tumors a priori (12). Although results have been variable, several mouse models transgenic for one or more HBV or HCV genes develop HCC, usually after 1 yr (reviewed in Refs. 12 and 19). Some HCV transgenic mice also develop the steatosis characteristic of human hepatitis C (12). Viral transgenes are often placed under the control of the albumin promoter to limit expression to the liver or under the broadly transactivating HBVX promoter in the case of some HCV models (12). Although HBVX is a known tumor promoter and tumorigenic incompetence of avian hepatitisviruses is attributed to the absence of a corresponding X gene, mice that produce only surface and large envelope proteins in high quantity also develop HCC (19). As is the case in humans, transgenic mouse models of HBV and HCV demonstrate higher quantities of virus production and greater risk of HCC in males as well as accelerated tumor development when exposed to mutagenic chemicals.

The main drawback to mouse HBV and HCV models is that transgenes expressed during embryogenesis induce immunotolerance, and carriers fail to develop hepatitis. Investigators have applied a number of strategies to address this shortcoming including adoptive transfer of cytotoxic T lymphocytes (CTL) from antigen-primed syngeneic WT mice, intrahepatic viral DNA injection, and splenocyte adoptive transfer into transgenic SCID mice (19). These strategies have recapitulated hepatocellular damage accrued during acute or fulminant hepatitis, highlighted the importance of interferon-mediated hepatocyte-sparing virus clearance orchestrated by CTL, and produced a low-grade chronic inflammatory state, which, in some instances, has contributed to tumorigenesis (19). A second strategy to overcome immune tolerance in transgenic mice is placement of the viral transgenes under an inducible promoter such as the Cre/lox system in which lox P inhibits transgene expression until Cre recombinase is separately administered; however, mice thus far generated using this strategy have only developed mild hepatitis.

To date, the only proven nonviral infectious causes of human liver tumors are biliary trematodes, including Opisthorchis viverrini and Clonorchis sinensis, which increase the risk of cholangiocarcinoma (11). Although rats harboring Cysticercus fasciolaris (the larval form of the tapeworm Taenia taeniformis) are susceptible to hepatic tumors, these sarcosomes are presumed to arise from chronic granulomatous inflammation directed against the parasite capsule, likely representing a disease process significantly different from human fluke-associated biliary carcinomas. A better model is infection of Syrian hamsters with O. viverrini. Infected hamsters develop cholangitis, biliary dysplasia, and oxidative DNA damage; however, an additional mutagen such as dimethylnitrosamine is required for cholangiocarcinoma development (11). Hepatic schistosomiasis appears to increase the risk of cholangiocarcinoma in humans, although the link is less clear than for the
flukes described above. Mice and/or hamsters infected with Schistosoma japonicum exhibit oxidative cholangiohepatic DNA damage but require additional mutagens for tumor development (11).

In the early 1990s, H. hepaticus was isolated from the livers of untreated male A/JCr mice with unusually high cancer and hepatitis prevalence in a 2-yr National Toxicology Program carcinogenesis study (25). H. hepaticus was the first member of the genus shown to persistently colonize the lower intestinal tract and migrate to the liver, making it the prototype enterohepatic Helicobacter species (6). Such organisms are now known to commonly infect humans and a wide array of animals (13). An association has been proven between H. hepaticus infection and hepatocellular tumors in susceptible strains of mice (7). To date, enterohepatic Helicobacter spp. are the only natural murine infectious pathogens known to induce HCC. Thus H. hepaticus infection of mice provides a uniquely valuable animal model for exploring basic mechanisms underlying infectious liver cancer. A/JCr mice are classically used as a valuable animal model for exploring basic mechanisms under-


