IN THE SPOTLIGHT

T Regulatory Cells Gone Bad: An Oncogenic Immune Response against Enterotoxigenic B. fragilis Infection Leads to Colon Cancer

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Summary: T regulatory cells trigger an oncogenic immune response against enterotoxigenic B. fragilis infection. The implications of an overall shift in the colonic homeostasis are discussed. Cancer Discov; 5(10): 1021–3. ©2015 AACR.

See related article by Geis and colleagues, p. 1098 (6).

Colorectal cancer is one of the leading causes of cancer-related death in the western world; its etiology has been linked to genetic mutations, dietary products, inflammatory processes, and specific members of the gut microbiota (1, 2). Although most colorectal cancers develop sporadically, approximately 2% are linked to an underlying inflammatory process, such as in the case of ulcerative colitis patients, in which chronic inflammation of the colon is associated with an increase risk of colitis-associated cancer (CAC). During colorectal cancer development, several genetic lesions arise, typically within the adenomatous polyposis coli (APC) gene, leading to activation of β-catenin, followed by mutations in the KRAS, TP53, and PIK3CA genes (1). Although there is significant evidence for a role of inflammation in cancer initiation through the induction of mutations and modulation of gene expression (1), it is unclear how the adaptive immune system, particularly T cells, contributes at this stage of cancer development.

Approximately 10^{13} commensal bacteria colonize the colon, and it is well established that they are one of the main drivers of inflammation in the colon contributing to colorectal cancer development (1). However, the exact mechanisms by which these microbes lead to colorectal cancer are not fully understood, and might be purely dependent on the nature of the inflammatory response that is initiated by specific members of the microbial community. In this regard, because certain human enteric bacteria induce colitis, there is increasing interest in delineating the mechanisms by which infection-induced inflammatory responses can promote colorectal cancer.

Specifically, the symbiont Bacteroides fragilis, which colonize most humans, have been the focus of recent research involving CAC models (3). There are two classes of B. fragilis: nontoxigenic B. fragilis (NTBF), which do not secrete B. fragilis toxin, and enterotoxigenic B. fragilis (ETBF), which secrete B. fragilis toxin and have been epidemiologically linked to colorectal cancer (4). In 2009, Sears and colleagues suggested a direct role for endogenous T-cell immunity in ETBF-induced colorectal cancer by demonstrating that ETBF promotes tumor development in mice harboring mutations in the Apcm-/+ (multiple intestinal neoplasia) gene via activation of TH17 helper type 17 cells (TH17; ref. 5). Moreover, they showed that B. fragilis toxin was responsible for the oncogenic effects of ETBF because NBTB was unable to induce either TH17 mucosal immune responses or tumor development in Apcm-/+ mice.

In this issue, Geis and colleagues further characterize the mechanism by which ETBF regulates procarcinogenic IL17 responses in the colon (6). Their first findings demonstrated that ETBF infection resulted in increased TH17 and T regulatory cells (Treg). Because Tregs are able to control inflammation, it was initially hypothesized that reduced Tregs in this infection-induced colorectal cancer model would lead to more inflammation and, therefore, induction of colorectal cancer. Surprisingly, Tregs were necessary for the early stages of tumor development in ETBF-infected Apcmin/+ mice, despite their suppression of acute IL17-mediated colonic inflammation. Specifically, they found that Tregs limited the availability of IL2 to uncommitted T cells in ETBF-infected mice, allowing the TH17 polarization that was necessary to promote colonic neoplasia (Fig. 1, left).

This research brings forth several interesting findings, raising new questions about the unique environment reached in the colon after ETBF infection (6). First, the authors dissociated colitis from colon carcinogenesis in a clinically relevant CAC mouse model. The early time points at which the experiments were performed were crucial for making this distinction. Soon after infection with ETBF, the colonic tissue had similar inflammatory scores irrespective of the presence of Tregs. However, the type of inflammation differed. Without Tregs, colitis characterized by an increase in IL17 shifted to IFNγ-mediated colitis (Fig. 1, right). Furthermore, although IFNγ is associated with antitumor responses, Geis and colleagues demonstrated that IFNγ-mediated colitis was not responsible for reduced tumorigenesis in ETBF-infected Apcm-/+ mice, suggesting a direct role for IL17 in early cell transformation. Although it is known that TH17 cells promote tumorigenesis (1, 7) and that the presence of TH17 cells in stage I/II colorectal cancer is associated with a reduction in disease-free survival (8), this research does not show that...
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Figure 1. Is the oncogenic Treg response against ETBF infection breaking the intestinal homeostasis, thereby potentiating colorectal cancer? Left, during ETBF infection, Tregs limit the availability of IL2 to uncommitted T cells, allowing the Th17 polarization necessary to promote the early stages of colonic neoplasia. Right, in the absence of Tregs, there is more IL2 available, which leads to a Th1 polarization and IFNγ-mediated colitis. Mechanisms that remain undefined are highlighted with question marks. Illustration by Rodrigo Irrazabal.

an IL17-induced gene expression signature is a tumor-elicited response, but rather that it is a consequence of a tumor inducer molecule that could lead to tumor formation in a genetically susceptible model of colorectal cancer. Given that mutations in the APC gene are among the most common and earliest genetic lesions in colorectal cancer (1), elucidating the mechanisms by which IL17 potentiates colon epithelial cell transformation in cells harboring mutations in the APC gene will open new therapeutic approaches for this disease.

Second, an unexpected mechanism for Tregs in IL17-mediated colon epithelial cell transformation in ETBF-infected mice was uncovered. Tregs are crucial for maintenance of gut mucosal homeostasis by suppressing strong immune responses against dietary antigens and the microbiota, but the constant suppression of the immune system can counteract cancer immune surveillance (7). This effect of Tregs on cancer has been widely established in sporadic cancer, but the role of Tregs in immunosurveillance during CAC is poorly understood. Recently, another group found that after induction of CAC using a chemically induced murine colitis model (AOM/DSS), the frequency of highly activated Tregs in colonic tumors increased (9). Pastille and colleagues attributed a tumor-promoting role to Tregs because transient ablation of these cells during the late phase of inflammation led to antitumor responses. Although the authors concluded that Tregs are required for controlling inflammation during acute colitis, they were unable to identify evidence of early Treg-mediated induction of tumorigenesis. This might be due to the nature of the chemical-induced carcinogenesis model used (AOM/DSS) or might suggest a unique interaction between ETBF and Tregs. Therefore, it will be necessary to analyze the specific populations of colonic Tregs that increased after ETBF infection, which will help determine whether these Tregs possess a different suppressive capacity and/or whether there is a shift to certain Treg subpopulations that could be specifically promoting CAC.

Third, because Tregs help to maintain the gut microbiota homeostasis, it would be very relevant to find out whether the increased Treg population in the colon leads to an overall shift in the microbiota or dysbiosis after ETBF infection. Abnormal bacterial communities could lead to changes in the microbial metabolites released into the colonic lumen,
which, in turn, could potentiate CAC (1, 2). Furthermore, it is unknown whether ETBF infection is associated with an increase in other putative carcinogenic bacteria in the colon (e.g., *Fusobacterium, Escherichia coli*; ref. 3). The implications of these possible changes in the colonic environment, before and after cancer development, warrant more attention.

Finally, the findings presented by Geis and colleagues suggest that controlling Tregs could serve as a therapeutic approach for people with a genetic susceptibility for colorectal cancer. But first it would be necessary to investigate the mechanism by which ETBF leads to increased Tregs. *B. fragilis* toxin is the only virulence factor known for ETBF, and NTBF do not lead to an increase in tumorigenesis in *A. pseudominosa* mice (5), suggesting that the induction of Tregs in the colon is mediated by *B. fragilis* toxin. Binding of *B. fragilis* toxin to an uncharacterized colon epithelial cell receptor (3) leads to E-cadherin cleavage, Wnt signaling activation, secretion of proinflammatory cytokines, increased barrier permeability, reactive oxygen species (ROS) production, and DNA damage, creating an environment suitable for colon carcinogenesis (3). Geis and colleagues postulate that after *B. fragilis* toxin induces E-cadherin cleavage, epithelial-derived signals recruit immune cells, like Tregs, that allow IL17 polarization necessary for early colonic transformation (6). However, it is interesting that despite all of the aforementioned tumor-promoting mechanisms of *B. fragilis* toxin, in this ETBF-induced carcinogenic model, the simple depletion of Tregs protects against early IL17-mediated tumor induction. Together, these data suggest that either an IL17 signature plays a major role in inducing initial colon epithelial cell transformation, and—at early stages—this signature is more relevant than any other of the carcinogenic effects attributed to *B. fragilis* toxin, or the inflammatory environment created after ETBF infection is indispensable for the action of *B. fragilis* toxin. To address this possibility, it will be necessary to find out whether any other of the *B. fragilis* toxin’s mechanisms of action, such as increased intestinal permeability, DNA damage, and ROS production, are affected after Treg depletion. Colorectal cancer has not been linked to a single bacterium, suggesting that changes in bacterial communities are responsible for colorectal cancer. The shift in the colonic immune infiltrates after ETBF infection appears to be one of the earliest processes; however, it is expected that this alteration in the inflammatory environment would lead to a shift in the microbial community, their associated genome (microbiome), and also in the bacterial metabolites released into the colonic lumen. Future studies will be necessary to define how all these factors interact to lead to colon epithelial cell transformation, because *B. fragilis* toxin, the only ETBF virulence factor, has now been found to be unable to induce the initial steps that promote colorectal cancer in the absence of this unique environment characterized by increased Treg and Th17 populations.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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