Editorial

Pro-tumoral role of gut bacteria: sabotaging immune cell recruitment

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Introduction

The gut is populated by trillions of bacteria impacting on metabolism, immune system development and overall fitness (1,2). In the past five years, the role of commensal bacterial in the responsiveness to anti-cancer treatments has emerged. Initial studies have focused on the association between response to chemotherapy and gut colonization by specific bacterial strains (3,4). More recently, gut colonization by defined bacterial species has been shown to critically influence responsiveness to immunological checkpoint blockade in melanoma and epithelial cancers (5-8). Although mechanisms remain largely elusive (9), these data have emphasized the possibility of actively conditioning the intestinal flora by colonization with specific bacterial strains, as cancer treatment to complement established therapeutic protocols.

In contrast, in a recently published study, the presence of defined bacterial species in the gut microbiome has been found to inhibit tissue infiltration by immune cells, resulting in enhanced tumor progression (10).

The study by Ma *et al.*

In their study, Ma *et al.* (10) show that antibiotic treatment reduces progression of experimental primary and metastatic liver cancers. Importantly, treatment is associated with increased liver infiltration by NKT cells, in tumor-bearing and control animals. Intra-hepatic accumulation of these cells is controlled by the interaction of CXCR6 receptor with its ligand, CXCL16 (11). Indeed, NKT cells recruited upon antibiotic treatment represent a majority of liver-infiltrating CXCR6+ lymphocytes, largely express CD69 early activation marker, and produce IFN-γ in unstimulated conditions and following *in vivo* activation by α-galactosylceramide (α-GalCer). This compound typically triggers CD1d-restricted T-cell receptor activation in NKT cells.

CXCL16 is mainly produced in the liver by sinusoidal endothelial cells (LSEC), and the authors have observed a higher production of this chemokine by LSEC in antibiotic-treated animals. Intriguingly, treatment also resulted in an increase of primary bile acid tauro-β-muricholic acid (T-β-MCA) and a reduction of secondary bile acids Tω-MCA, taurodeoxycholic acid (TDCA), ω-MCA, taurolithocholic acid (TLCA) and tauroursodeoxycholic acid (TUDCA). Treatment with exogenous secondary bile acids was then shown to reduce CXCL16 production by LSEC *in vitro* and to reverse antibiotic-induced tumor inhibition *in vivo*.

Since gut bacteria regulate bile acid composition, the authors have addressed the identification of species associated with increased secondary bile acid production, reduced liver infiltration by NKT cells and enhanced tumor progression. They found that *Clostridium scindens* (*C. scindens*), a species with high bile acid metabolization potential, induced the observed events upon re-colonization of antibiotic-treated animals. Furthermore, a correlation between the primary/secondary bile acid ratio and *CXCL16* gene expression was also observed in "tumor-free" human

liver tissues from patients with hepatocellular carcinoma (HCC) and cholangiocarcinoma, the most frequent forms of liver cancer.

Taken together these data indicate that metabolic conditions associated with the presence of defined gut bacterial species may drive production of chemokines recruiting immune cells into normal and diseased tissues and suggest that appropriate modification of gut flora may impact on liver infiltration by NKT cells and primary or metastatic liver cancers.

Open questions

The recruitment of immune cells within tumor tissues represents a key step of anti-cancer immune response (12,13). Therefore, the analysis of molecular mechanisms associated to the expression and functions of chemokines and their receptors is of critical relevance in cancer immunobiology. Moreover, NKT cells continue to attract the attention of tumor immunologists (14). However, evidence of their role in immune responses against human solid tumors remains scant (15). The translational relevance of the data by Ma *et al.* should be analyzed within this framework.

Analysis of TCGA public database (16) provides interesting insights. Indeed, in human HCC and cholangiocarcinoma, the large majority of human liver cancers, low *CXCR6* gene expression is highly associated with poor outcomes. Instead, expression of *CXCL16* gene does not appear to significantly correlate with the clinical course. Furthermore, correlation between *CXCR6* and *CXCL16* expression is modest (R=0.2), and lower than that detectable in matched healthy tissues (R=0.48) (own unpublished data). These observations may suggest that chemokines other than CXCL16 could be involved in the recruitment of NKT cells into human liver. Indeed, human NKT have been reported to also express CCR2, CCR5, CXCR3, and CXCR4, in addition to CXCR6 (17). Intriguingly, *CXCR6* expression correlates with *CD8* expression in normal tissues (R=0.8) and in liver cancers (R=0.75). Nevertheless, public databases' data should be considered cautiously, due to the high heterogeneity of tumors under investigation and the relatively low number of cases included.

NKT cells, expressing NK and T-cell markers, and an invariant T-cell receptor recognize, in a CD1d-restricted manner, glycolipidic antigens. Most functional studies have been conducted using α-GalCer, a sea-sponge derived compound, with high CD1d affinity. However, products from different bacterial species, including human commensals, have also been shown to be able to stimulate NKT cells, although with lower affinity for CD1d. Furthermore, "self" lipid antigens may also be recognized in the presence of adequate co-stimulation (18). The three murine cell lines used in the study were CD1d+. Therefore, intrahepatic NKT cells could respond to the presentation of endogenously generated CD1d– restricted antigens. Importantly, the authors suggest that even for CD1d– tumor cells, cross-presentation by adequate CD1d+ APC might still result in NKT cell activation, thereby supporting a microenvironment potentially favoring the induction of "conventional" adaptive immune responses. Alternatively, indirect antitumor effects might result from the elimination of tumor associated immune-suppressive macrophages. In humans, *CD1d* gene has been reported to be overexpressed in a group of 41 HCC, as compared, to matched non-tumor tissue (19). Notably however, NKT cells are significantly (>10 fold) more abundant in murine, as compared to human livers (20).

Most importantly, *C. scindens* is a normal component of human gut flora, and has been reported to protect against *C. difficile* colonization (21), and to be associated to healthy gut tissue, as opposed to transformed colorectal mucosa (22).

Future directions

The study by Ma *et al.* (10) indicates that, by mediating metabolic effects, defined microbial species may modulate liver infiltration by specific immune cell types. These findings provide the background for different translational applications. Elimination from gut flora of *C. scindens* or bacteria with similar metabolic potential may enhance the effectiveness of natural or immunotherapy-induced anti-cancer immune responses in the liver. However, some caveats should be considered. Currently available antibiotics usually affect relatively large numbers of bacterial species. Therefore, antibiotic treatments such as those utilized in the study could also eliminate "favorable" species, or to promote colonization by "unfavorable" bacteria. A careful microbiome analysis might provide useful hints about active gut colonization with clusters of bacteria controlling bile metabolizing species.

Notably, administration of antibiotics, including those used in this study, has been shown to enhance bacterial translocation from the lumen into the mucosa, and trigger inflammation, thus increasing the risk of developing inflammatory disorders (23).

Importantly, antibiotic treatment of experimental animals inhibits the progression of liver metastases, but appears to enhance the growth of lung metastases. Thus, effects appear to be liver specific. On the other hand, as suggested (10), the use of bile metabolizing bacteria might prove important in clinical conditions where liver infiltration by NKT cells is associated with autoimmune diseases (24).

In conclusion, this work provides evidence of a key role played by the gut commensal bacteria in modulating liver cancer infiltration by immune cells endowed with anti-tumor activity. Our group has also described a link between the presence of gut commensal bacteria and immune cell infiltration in human colorectal cancer (13). This emerging evidence, together with previous reports underlining the impact of gut flora composition on responsiveness to chemo- and immunotherapy (3-8), provides a solid rationale for the therapeutic modification of the microbiome in cancer treatment.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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