ONCOIMMUNOLOGY
2023, VOL. 12, NO. 1, 2224672
https://doi.org/10.1080/2162402X.2023.2224672

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EDITORIAL

Bile acids regulate MAdCAM-1 expression to link the gut microbiota to cancer immunosurveillance

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ABSTRACT

In a recent paper in *Science*, Fidelle et al. unravel a gut immune checkpoint that is subverted by antibiotic treatment. Post-antibiotic dysbiosis of the ileum causes an increase in bile acids that downregulate MAdCAM−1, thereby triggering the exodus of immunosuppressive T cells from gut-associated lymphoid tissues toward tumors.

ARTICLE HISTORY
Received 29 May 2023
Revised 9 June 2023
Accepted 9 June 2023

KEYWORDS
Cancer immunotherapy; microbiota; immunosuppression; MAdCAM-1; bile acids; *Enterocloster*; gut

Over the past few years, it has become increasingly recognized that cancer is more than a disease caused by malignant cells. Rather, cancer results from a disequilibrium of the bodywide ecosystem with its metabolic, neuroendocrine and immune circuitries that also implies the microbiota.[1] Intestinal dysbiosis is a general feature of poor health and aging.[2] Dysbiosis also participates to carcinogenesis, tumor progression and oncotherapeutic failure, not only in gastrointestinal but also in distal cancers. This latter point has been particularly well documented for immunotherapy targeting the PD−1/PD-L1 interaction, where the prolonged (≥7 days) use of broad-spectrum antibiotics during the 2 months before and 1 month after initiation of the treatment is associated with poor outcome.[3]

In mechanistic terms, gut dysbiosis may be expected to result in an increase in the abundance of noxious (immunosuppressive and proinflammatory) microbes or – alternatively or in addition – the depletion of beneficial (immunostimulatory and anti-inflammatory) microbes.[3] In a recent *Science* paper,[4] we reported the finding that treatment with broad-spectrum antibiotics, followed by their discontinuation, leads to the recolonization of the gut by harmful bacteria from the *Enterocloster* genus including *E. clostridioformis*, both in mice and in cancer patients. Indeed, oral gavage of tumor-bearing mice with *E. clostridioformis* is sufficient to block the therapeutic response to PD−1 blockade.[4] Intrigued by this finding, we engaged in a combination of hypothesis-driven and systematic studies to understand how transient treatment with antibiotics subverts therapeutically induced immunosurveillance.

We emitted the hypothesis that dysbiosis caused by antibiotics might affect the long-range trafficking of immune cells from the gut through the lymphatic and cardiovascular systems. Indeed, we observed in mice that administration of antibiotics causes an increase in the trafficking of a particular immunosuppressive T cell subpopulation from the lamina propria of the ileum through the mesenteric lymph node to the tumor microenvironment and the tumor-draining lymph node. Extensive phenotyping of this T cell subset revealed that they bear both characteristics of regulatory T cells and proinflammatory T helper 17 cells (with the simultaneous expression of two master transcription factors, Foxp3 and Rorγt) leading to their designation as $T_{reg}17$ cells. In addition, the mechanisms through which such $T_{reg}17$ cells are released from the gut was unraveled. Indeed, high endothelial cells present in the ileum normally express mucosal addressin cell adhesion molecule−1 (MAdCAM−1), which interacts with a specific integrin heterodimer (α4β7) that is expressed on some immune cell types present in the gut including $T_{reg}17$ cells, hence retaining them locally. However, dysbiosis is linked to the downregulation of MAdCAM−1, thus unleashing $\alpha4^{+}\beta7^{+}$ $T_{reg}17$ cells from their local confinement and allowing them to travel to tumors. Indeed, knockout of the genes coding for MAdCAM−1 or the integrin β7, as well antibodies blocking MAdCAM−1 or the α4β7 heterodimer, are sufficient to cause the translocation of $\alpha4^{+}\beta7^{+}$ $T_{reg}17$ cells from the gut to tumors, and to compromise the efficacy of PD−1 blockade in vivo. These observations led us to the conclusion that the downregulation of ileal MAdCAM−1 expression explains why gut dysbiosis compromises immunosurveillance. In favor of this hypothesis, oral gavage of *E. clostridioformis* caused the downregulation of ileal MAdCAM−1.[4]

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The gavage with *E. clostridioformis* was accompanied by major shifts in the ileal abundance of bile acids.[4], which collectively may affect MAdCAM−1 expression[5] and mediate systemic immunosuppression.[6] To identify which particular bile acid species regulate MAdCAM−1, we engineered two distinct mouse endothelial cell lines to express green fluorescent protein (GFP) under the control of the *Madcam1* promoter. These cell lines downregulated GFP (indicative of the inhibition of the *Madcam1* promoter) in response to *E. clostridioformis* in vitro. Moreover, they upregulated GFP in response to two inflammatory cytokines (interleukin−1β and tumor necrosis factor-α), and this upregulation was inhibited by individual bile acids, in particular lithocholic acid (LCA), and ursodeoxycholic acid (UDCA). Subsequent in vivo experiments confirmed that gavage with either LCA and UDCA is sufficient to trigger the reduction of *Madcam1* mRNA expression in vivo, in the ileum, Peyer's patches and mesenteric lymph nodes.[7] However, at this point, it remains to be clarified how these effects of LCA and UDCA are achieved at the mechanistic level, likely through an action on one or several bile acid receptors.[8] Moreover, it will be interesting to explore the possibility that dietary fibers mediate their reported immunotherapy-stimulatory effects[9] through effects on toxic bile acids.[10]

Of note among these two bile acids, UDCA is clinically used for the treatment of primary biliary cholangitis, an autoimmune disease affecting cholangiocytes.[11], as well as for the avoidance of biliary complications after liver transplantation.[12] It will be interesting to explore the possibility that this liver-specific immunosuppressive effect is achieved through the UDCA-induced downregulation of ileal MAdCAM−1, followed by the homing of $T_{reg}17$ cells from the gut-associated lymphoid tissue into the inflamed liver, where MAdCAM−1 is expressed on endothelial cells.[13] Irrespective of this conjecture, we found that transgene-enforced expression of MAdCAM−1 in the liver (mostly on hepatocytes) increased the local infiltration by $T_{reg}17$ cells in mice subjected to transient antibiotic treatment and simultaneously reduced the frequency of $T_{reg}17$ cells in the tumor bed.[7] Thus, MAdCAM−1 expression by the liver can lead to the interception of $T_{reg}17$ cells during their voyage from the gut to the cancer.

Clinically, the dosage in the serum of the soluble form of MAdCAM−1 (sMAdCAM−1) helped to evaluate its expression in the gut. Indeed, we found that patients who took antibiotics showed a significant decrease in sMAdCAM−1, in accordance with the decrease of *Madcam1* expression assessed on ileal biopsies from antibiotics-treated patients.[7] Furthermore, sMAdCAM−1 was a strong prognostic factor in cancer patients treated with immunotherapies. Low levels of sMAdCAM−1 before starting the immunotherapy were associated with poor clinical outcome, in lung, renal and bladder cancer patients.[7] Also, patients with low sMAdCAM−1 showed an enrichment of their gut microbiota with *E. clostridioformis*.[7]

Altogether, the aforementioned results plead in favor of a scenario in which gut dysbiosis causes the expansion of *E. clostridioformis*, which downregulates ileal expression of MAdCAM−1, thus allowing $\alpha4^{+}\beta7^{+}$ $T_{reg}17$ cells to travel from

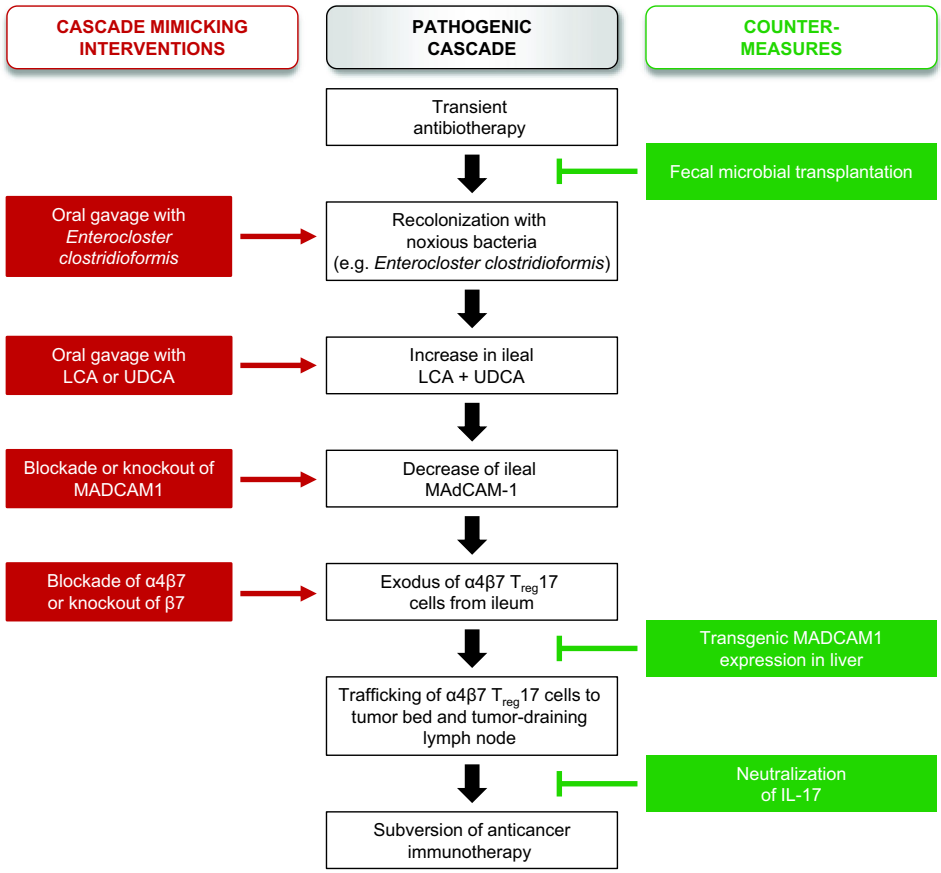

Figure 1. Summary of the pathogenic cascade linking antibiotherapy to deficient anticancer immunotherapy responses. The central pathogenic pathway can be mimicked by experimental interventions (red) or interrupted by countermeasures (green).

the gut to the mesenteric lymph node and then to tumors to cause local immunosuppression (Figure 1).

It should be noted that dysbiosis is difficult to be defined in global terms due to major geographic differences in the composition of the normal microbiota across continents, countries and regions.[14] For this reason, it would not be surprising that other bacterial species than *E. clostridioformis* would mediate similar effects as those observed in our (French and Canadian) cohorts. Indeed, it is our intuition that, cancer- and immune-relevant products of the microbiota, including metabolites and ligands of pattern recognition receptors, should be more easily discernible biomarkers of dysbiosis than shifts in the metagenomic characteristics of the microbiota. As a caveat, however, such functional products should not be investigated in feces but rather at their major site of action, most likely the ileum.

Acknowledgments

MF is supported by the Seerave Foundation and the European Union's Horizon Europe research and innovation programme under grant agreement number 101095604 - GAP-101095604 -[project acronym: PREVALUNG-EU, project title: Personalized lung cancer risk assessment leading to stratified Interception]. This work was supported by SEERAVE Foundation, European Union Horizon 2020:Project Number: 825410 and Project Acronym: ONCOBIOME, Institut National du Cancer (INCa), ANR Ileobiome - 19-CE15-0029-01, ANR RHU5 "ANR-21-5 RHUS-0017" IMMUNOLIFE", MAdCAM INCA_ 16698, Ligue contre le cancer, LABEX OncoImmunology, la direction generale de l'offre de soins (DGOS), Universite Paris-Sud, SIRIC SOCRATE (INCa/DGOS/INSERM 6043), and PACRI network. Ai-L is supported by the ANR RHU5 "ANR-21-5 RHUS-0017" IMMUNOLIFE". LZ and GK are supported by the Ligue contre le Cancer (équipes labellisées, Program "Equipe labelisée LIGUE"; no. EL2016.LNCC (VT/PLP)); Agence Nationale de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; AMMICa US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association "Le Cancer du Sein, Parlons-en!"; Cancéropôle Ile-de-France; Chancelerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssea, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; INCa; Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology (ANR-18-IDEX-0001); the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM).

Disclosure statement

LZ is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. LZ has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Pileje, Transgene, 9 m, Tusk Pharma, Merus, Roche and Innovate Pharma, and now has current research support from Biomérieux, Daiichi Sankyo, everImmune, Pilege, and 9 meters. LZ is in the SAB of Hookipa. LZ was on the Board of Directors of Transgene. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sotio, Tollys, Vascage and Vasculox/Tioma. GK has been consulting for Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. The funders had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the results.

Funding

The work was supported by the Agence Nationale de la Recherche [Projets blancs]; Association pour la Recherche sur le Cancer Cancéropôle Ile de France Fondation Leducq Fondation pour la Recherche Médicale Institut National Du Cancer Institut Universitaire de France Ligue Contre le Cancer [EL2016.LNCC (VT/PLP)]; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR) Gustave Roussy Odyssea the European Union Horizon 2020 Project Oncobiome Fondation Carrefour Inserm (HTE) the LabEx Immuno-Oncology [ANR-18-IDEX-0001]; the RHU Torino Lumière a donation by Elior Chancelerie des universités de Paris (Legs Poix) Association "Le Cancer du Sein, Parlons-en!" AMMICa US23/CNRS UMS3655 ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases Agence Nationale de la Recherche (ANR) – Projets blancs the SIRIC Cancer Research and Personalized Medicine (CARPEM) Equipe labelisée LIGUE [EL2016.LNCC (VT/PLP)]; Immunolife [ANR-21-5 RHUS-0017]; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE) Seerave Foundation.

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Data availability statement

All data that led to the conclusions in this manuscript have been referenced and all sources have been described.

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