Gut Microbiota and the Paradox of Cancer Immunotherapy

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.3389/fimmu.2014.00157">http://dx.doi.org/10.3389/fimmu.2014.00157</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>Frontiers Research Foundation</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Sat Dec 08 01:21:41 EST 2018</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/88032">http://hdl.handle.net/1721.1/88032</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Gut microbiota and the paradox of cancer immunotherapy

Theofilos Poutahidis1,2, Markus Kleinewietfeld3,4,5 and Susan E. Erdman1*

1 Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA, USA
2 Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
3 Departments of Neurology and Immunobiology, Yale School of Medicine, New Haven, CT, USA
4 Broad Institute, Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA
5 Faculty of Medicine, Dresden University of Technology (TUD), Dresden, Germany

EDITED BY:
Fang-Ping Huang, Imperial College London, UK

REVIEWED BY:
Graham Robert Leggatt, University of Queensland, Australia
Yong Lu, Cleveland Clinic Foundation, USA

*Correspondence:
Susan E. Erdman, Massachusetts Institute of Technology, 77 Mass Avenue, Cambridge, MA 02139, USA
e-mail: serdnan@mit.edu

INTRODUCTION

The neoplastic process is characterized by overwhelming complexity. Cancer is comprised of a genetically unstable population of cells that proliferate at an extraordinarily high rate. Millions of cancer deaths each year make it obvious that the battle against cancer is asymmetric, with humankind often being the weaker element (1). To date, cancer research efforts directly confront malignancy by targeting properties of individual cancer cells. In 2000, Hanahan and Weinberg described that most of the research on origins and treatment of cancer had just contributed toward “adding further layers of complexity to a scientific literature that is already complex almost beyond measure” (2).

In the same landmark paper, however, the authors were optimistic enough to predict groundbreaking upcoming advances in the conceptual rather than the technical level (3). They were proven right. One such advancement was the increased awareness for the importance of the tumor microenvironment in the etiopathogenesis of neoplasia (3, 4). We now know that initially transformed cells are much less autonomous in their growth than previously thought (5, 6). Among the microenvironment elements, immune cells and factors have emerged as fundamental players (4–6).

It is recently shown that beneficial environmental microbes stimulate integrated immune and neuroendocrine factors throughout the body, consequently modulating regulatory T-lymphocyte phenotypes, maintaining systemic immune balance, and determining the fate of preneoplastic lesions toward regression while sustaining whole body good health. Stimulated by a gut microbiota-centric systemic homeostasis hypothesis, we set out to explore the influence of the gut microbiome to explain the paradoxical roles of regulatory T-lymphocytes in cancer development and growth. This paradigm shift places cancer prevention and treatment into a new broader context of holobiont engineering to cultivate a tumor-suppressive macroenvironment.

Keywords: tumor macroenvironment, regulatory T-cells, cancer immunotherapy, inflammation and cancer, probiotic bacteria

T REG ARE CENTRAL IN PRESERVING SYSTEMIC IMMUNE HOMEOSTASIS AND GOOD HEALTH

FOXP3+ CD4+ CD25+high T REG are dominant cellular elements of the professional suppressor arm of the immune system and are important for orchestrating the control of peripheral immunological tolerance (18). The transcription factor FOXP3 is a fundamental regulator of T REG function in rodents and humans, and so far the most reliable phenotypic indicator of their identity. Recent studies on human T REG subpopulations, however, revealed that low but discernible levels of FOXP3 expression could be detected in non-suppressive T REG or even in activated effector T-cells. It is probable that this finding reflects the inherent plasticity of T REG: FOXP3+ cells co-expressing effector T-cell phenotypic markers or cytokines may be in stages of a progressive, epigenetically regulated, phenotypical, and functional shift process (14, 16, 19–22), ultimately favorable for healthful recovery of the host after environmental challenges. The role of T REG is central in preserving immune system homeostasis for health and the balance of beneficial inflammatory responses during infections while minimizing collateral tissue damage. In cancer, however, roles of T REG are traditionally considered to be negative (14–16, 23).

T REG GATHER NEAR TUMORS AND FAVOR CANCER SURVIVAL

A large body of data suggest that T REG gather near tumors and suppress the anti-tumor inflammatory response, thus favoring cancer
As the results of these trials are anticipated, the literature reveals clinical and experimental data suggest that tumor-associated T<sub>REG</sub> recognize both self and neoantigens expressed by tumor cells, counteracting antigen-specific effector T-cell responses. Consequently, immunotherapy strategies based on the vaccination with tumor-associated antigens fail to evoke an effective response against cancer cells due to the activation and expansion of tumor antigen-specific T<sub>REG</sub> (14–16). This potential interplay of T<sub>REG</sub> within tumors has been reviewed in detail elsewhere (12–16), and has led to the proposal of several anti-T<sub>REG</sub> regimens for cancer immunotherapy. These regimens aim to deplete T<sub>REG</sub> to prevent their suppressor function, prevent their homing into tumor sites, or block their differentiation/proliferation (12–16).

Several of these T<sub>REG</sub>-targeting modalities have already been tested in the clinic, with mixed results (13, 16). Blocking T<sub>REG</sub> function by depleting the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) appears promising (24), due to the depletion of T<sub>REG</sub> from tumor tissues (25, 26). However, a similar regimen could lead to an opposite effect with the accumulation of T<sub>REG</sub> and CD8<sup>+</sup> T-cells in tumors (27, 28). A phase III study of melanoma patients using a gp100 peptide vaccine with interleukin (IL)-2 administration led to equally promising results with discovery of T<sub>REG</sub> expansion in responding patients (29).

**GUT MICROBIOTA INDUCE POTENT T<sub>REG</sub> WITH SYSTEMIC ANTI-NEOPLASTIC PROPERTIES**

As the results of these trials are anticipated, the literature reveals contradictory evidence. Indeed, the studies associating high densities of tumor-associated cells expressing T<sub>REG</sub> markers including FOXP3 with a poor prognosis in several types of human cancers are now challenged by similar studies on the very same types of cancer showing the opposite outcome (30–34). The different CD8<sup>+</sup>:T<sub>REG</sub> ratios and the presence of FOXP3<sup>+</sup> cell subsets of undetermined identity in the tumor microenvironment have been proposed as probable explanations (16). Indeed, data from animal models show under certain conditions of microbial priming that T<sub>REG</sub> not only protect but also alter the tumor microenvironment to induce remission of already established intestinal, mammary, and prostate cancers (35–41). The hypothesis that the composition of the different subsets of FOXP3<sup>+</sup>, which may include effector Foxp3<sup>+</sup> cells, is intriguing (16). Indeed, it was previously shown that IFN-γ levels were increased during T<sub>REG</sub>-mediated tumor regression in mice (37). Further, feeding of probiotic microbes to mice induces systemic oxytocin secretion that shifts immunity toward IFN-γ and CD25 for improved wound healing capacity and systemic good health (42). A question subsequently arising is whether gut microbiota may be engineered to harness an anti-neoplastic FOXP3<sup>+</sup> cell milieu (5, 10, 41).

**GUT-CENTRIC HYPOTHESIS: PRIOR EXPOSURES TO MICROBES EXPLAIN BENEFICIAL ROLES OF T<sub>REG</sub>**

Stimulated by a gut-centric homeostasis hypothesis, we set out to explore and explain the paradoxical roles of T<sub>REG</sub> in cancer using several different mouse models of cancer and adoptive cell transfer methodologies (10). We found that T<sub>REG</sub> may suppress, promote, or have no effect in carcinogenesis depending upon their timing and prior exposure to gut bacterial antigens and presence of IL-10 (35–39, 41, 43, 44). Under some conditions, adoptive transfer of T<sub>REG</sub> rapidly led to apoptosis of emerging tumor cells (37, 45). Using as a model organism an opportunistic pathogen, *Helicobacter hepaticus*, commonly residing in the lower bowel of mice, we have shown in Rag2-deficient mice (otherwise lacking lymphocytes) that gut microbiota modulate inflammatory bowel disease and inflammation-associated colon cancer, a cancer process inhibited by properly functioning IL-10-dependent T<sub>REG</sub> (35, 36). Subsequently, by introducing *H. hepaticus* into the large bowel flora of mice lacking the APC tumor suppressor gene (*Apc<sup>Min<sup>+</sup></sup>*)

A potent treatment to counteract these local and systemic *H. hepaticus*-induced tumorigenic events was supplementation with T<sub>REG</sub> in an IL-10-dependent manner (10, 36, 38–40, 44, 46, 48). Purified T<sub>REG</sub> exhibited greatest anti-cancer potency when taken from donor mice previously colonized with *H. hepaticus*. By contrast, T<sub>REG</sub> taken from donor mice without prior *H. hepaticus* exposure were ineffective, and in some cases actually enhanced tumorigenesis (10). Based on these results, we theorize that the tumor microenvironment is subject to systemic inflammatory events arising from environmental exposures in the gastrointestinal tract (Figure 1). This microbi-cducible pro-inflammatory condition contributes to tumor trophic signaling. Interestingly, bacterial antigen triggered IL-10-dependent activities in the GIT tract impart sustained protection from the aforementioned events, resulting in immune cell recruitment, including T<sub>REG</sub>, which, by being more potent in their anti-inflammatory roles, work locally and systemically to suppress sepsis, myeloid precursor mobilization, and inflammatory signaling important in extra-intestinal cancer evolution (10, 43). These systemic events comprise the tumor microenvironment.

The roles of intestinal microflora in promoting cancer development within the bowel have been well established (35, 49–52). Linking gut microbial flora and local and systemic effects that promote (38) or suppress (45) tumors throughout the body, expands this paradigm in a challenging manner. Recent findings show that gut flora imbalances considerably undermine the response to both immune (53, 54) and non-immune chemotherapeutic regimens, such as cisplatin and oxaliplatin (53).

**A WEAKENED T<sub>REG</sub> FEEDBACK LOOP UNIFIES AUTOIMMUNE DISEASES AND CANCER**

These gut microbe-centric findings in mice are consistent with the “hygiene hypothesis,” according to which insufficient microbial exposures earlier in life predispose to allergies, autoimmunity disorders, and uncontrollable inflammation-associated pathologies later in life. We have shown that the basic principles of this
unstable resting peripheral T cells, which display inherent plasticity (10). Hygienic individuals with a weakened immune system status may determine the risk of developing sporadic cancer in epithelia throughout the body. Further, we found that consuming beneficial probiotic bacteria led to the expansion of a Foxp3+ cell population in the periphery (42, 45, 57) conferring protection to diet-related and genetic predisposition to mammary cancer (45). Targeted oral challenge with such probiotic bacteria resulted in the activation of interrelated systemic inflammatory and metabolic pathways, either through blood circulation or via the vagus nerve (Figure 1). Consequently, there was an upregulation of systemic hormone levels, such as oxytocin, testosterone, and thyroxin. Oxytocin serves to sustain immune and integumentary homeostasis, biasing the immune system toward IL-10 and IFN-γ, without anergy, subsequently minimizing the deleterious systemic effects of IL-17 (57). This altered immune system and metabolic profile of mice imparted healthful phenotypes including shiny fur and youthful hair follicle cycling, accelerated skin wound healing capacity, and resistance to diet-induced obesity and senility (42, 47, 57, 58). Through tightly regulated immune activities, competent TREG permit brief beneficial host inflammatory responses to eliminate invading pathogens, and later inhibit chronic deleterious inflammatory tissue damage (43). The results of our wound healing assays further suggest that the probiotic microbe-induced enhancement of the TREG-dominated arm of the immune system did not compromise the ability of mice to respond to invading pathogens (42).

**BENEFICIAL SYSTEMIC EFFECTS OF GUT MICROBES ARE TRANSPALNTABLE VIA FOXP3+ TREG INTO NAÏVE HOSTS**

Adaptive cell transfer models offer mechanistic insight as these beneficial effects were isolated to bacteria-primed TREG (42, 47, 57–59). In fact, healthful phenotypes were entirely reproducible in naive recipient mice by the adoptive transfer of highly purified TREG derived from probiotic-fed cell donors (42, 57, 59). These results suggest gut microbe-induced crosstalk with the host in a continuous and reciprocal manner. The fate of preneoplastic and neoplastic lesions arising in epithelia throughout the body depends upon this macroenvironment at the whole organism level. Consequently, the tumor macroenvironment is defined as the “holobiont,” i.e., the mammalian organism plus the microbial symbionts it bears. The TREG population is a central player of the tumor macroenvironment connecting gut bacteria with reproductive fitness, youthful phenotypes, and anti-neoplastic properties.

**MICROBIAL ENGINEERING OFFERS NEW STRATEGIES FOR PUBLIC HEALTH**

Taken together, microbial engineering strategies using food-grade bacteria highlight alternative directions in cancer immunotherapy. Modulating beneficial TREG via diet is a biologically safe and efficient approach, originating from genetic programs that have been shaped during the millions of years of co-evolution of mammals with their gut bacteria symbionts. These attributes remain largely inactive in individuals with a modern lifestyle, Westernized dietary habits, and stringent hygiene practices. Awakening these latent TREG-mediated capabilities may provide an alternative avenue to reduce cancer risk at a population level for public health. The perspectives presented here should be considered as an alternative paradigm – not only for fighting cancer – but also for promoting overall good health and longevity.
AUTHOR CONTRIBUTIONS
Theofilos Poutahidis, Markus Kleineiewietfeld, and Susan E. Erdman wrote the paper.

ACKNOWLEDGMENTS
This work was supported by National Institutes of Health grants P30-ES002109 (pilot project award to Susan E. Erdman), U01 CA164337 (to Susan E. Erdman), and RO1 CA108854 (to Susan E. Erdman).

REFERENCES

Poutahidis et al.

Gut bacteria modulate the tumor microenvironment

April 2014 | Volume 5 | Article 157 | 4


