Metabolism and Gut Microbiota in Cancer Immunooediting, CD8/Treg Ratios, Immune Cell Homeostasis, and Cancer (Immuno)Therapy: Concise Review

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Key Words. Immunometabolism • Immunooediting • Gut microbiota • Homeostasis • Combination therapies

ABSTRACT

The concept of immunooediting, a process whereby the immune system eliminates immunogenic cancer cell clones, allowing the remaining cells to progress and form a tumor, has evolved with growing appreciation of the importance of cancer ecology on tumor progression. As cancer cells grow and modify their environment, they create spatial and nutrient constraints that may affect not only immune cell function but also differentiation, tipping the balance between cytotoxic and regulatory immunity to facilitate tumor growth. Here, we review how immunometabolism may contribute to cancer escape from the immune system, as well as highlight an emerging role of gut microbiota, its effects on the immune system and on response to immunotherapy. We conclude with a discussion of how these pieces can be integrated to devise better combination therapies and highlight the role of computational approaches as a potential tool to aid in combination therapy design.

SIGNIFICANCE STATEMENT

Expansion of the concept of immunooediting to include not only phenotypic but also metabolic heterogeneity of immune cells, the effect of gut microbiota, as well as the impact thereof on immune cell composition in tumor microenvironment, can help improve immune-mediated therapeutic approaches that can turn “cold” tumors “hot.”

INTRODUCTION

Cancer cells are heterogeneous with respect to numerous aspects, ranging from growth and death rates, to modes of nutrient metabolism, to their ability to elicit an immune response. Cancer immunooediting is a process whereby immunogenic cancer cells are selectively killed off by cytotoxic lymphocytes [1–3], leaving behind cells that are more resistant to the immune system, a process akin to natural manifestation of therapeutic resistance.

Our understanding of immunooediting has been evolving over time from its initial conception as a three-phase process of elimination, where immune system manages to clear out all the cancer cells, to equilibrium, where cancer cell growth is halted, preventing tumor formation, to escape, where nonimmunogenic cancer cells of the “edited” tumor circumvent the immune response and succeed in growing in an uncontrolable fashion [2, 3]. It is becoming increasingly recognized that there exist numerous aspects of cancer ecology—the aggregate effect of the constantly changing tumor microenvironment (TME)—that go beyond genetic heterogeneity and that contribute to tumor escape from the immune system [4–6]. Here, we review several mechanisms that may contribute to tumor escape, with a particular focus on altered homeostasis of immune cell composition in the TME, the impact of nutrient metabolism on various subsets of immune cells, and the effect of the gut microbiota on immune cell activity and response to therapy. We will conclude with a discussion of how therapeutic interventions might take into account some of the complex and multifaceted aspects of tumor-immune interactions.

CD8/TREG RATIOS AND IMMUNE CELL HOMEOSTASIS

Homeostatic balance between regulatory and effector cells of the immune system is critical for both maintaining an adequate immune response to fight disease, such as cancer, and to concurrently prevent damage to the healthy tissue. This
balance is maintained by a mutually regulatory relationship between effector cells, such as CD8+ T cells, and regulatory T cells, or Tregs.

Tregs are CD25+CD4+ cells, whose expression of nuclear transcription factor Forkhead box P3 (FoxP3) is crucial for maintaining suppression of the immune system [7]. Deficiency or dysfunction of CD4+CD25+FoxP3+ Tregs alone is sufficient to result in low self-tolerance, as demonstrated by spontaneous development of autoimmune disease in animals with depleted Tregs [8], as well as severe autoimmune disease in individuals with mutations in FoxP3 [9].

There are several mechanisms whereby Tregs perform their function. They can suppress proliferation of CD4+ and CD8+ cells [8, 10], or suppress production of cytokines, such as interleukin-2 (IL-2), by CD8+ and CD4+ cells, thereby affecting effector survival and function [10]. Tregs can also kill responder T cells via both granzyme and perforin-dependent mechanisms [11, 12]. Other mechanisms include inhibition of T-cell proliferation via upregulation of cyclic adenosine monophosphate [13], or interacting with CD80/86 receptors on effector cells, promoting T-cell exhaustion [14].

In cancer, the normal homeostatic ratio of effector to regulatory T cells is altered. This is confirmed by numerous observations suggesting that low CD8+/Treg ratios are associated with poor prognosis. For instance, in [15], the authors looked at the association between tumor-infiltrating triple positive Tregs (CD4 +CD25+FOXP3+) and clinical outcome in advanced ovarian cancer patients, including 31 patients with good survival and 21 with poor survival of under 18 months. They observed that while the total numbers of Tregs were not significantly different between the two outcome groups, the ratios of CD8+/Treg cells in the group with a better outcome were significantly higher, suggesting that effector-suppressor ratio can potentially be a more informative predictor of clinical outcome than individual cell count. Specifically, the authors reported average CD8/Treg ratios of 0.98 versus 0.32 for the two patient groups.

Similar results were observed in patients with muscle invasive urothelial carcinoma of the bladder [16]. The authors showed that while neither CD8 nor Treg density in the tumor was associated with response to neoadjuvant chemotherapy, the ratio of CD8 to Treg cells did show statistically significant association. The importance of CD8/Treg ratio as a predictor of clinical outcome was also observed in squamous cell carcinoma of the cervix [17], colorectal cancer [18], and breast cancer [19]. The CD8/Treg ratio was also observed as a potential predictor of response to immunotherapy in mouse models of pancreatic cancer [20], further solidifying its potential as an important biomarker and maybe even driver of response to therapy.

TMEs are known to be immune suppressive [4, 21]; however, the suppression can come from different mechanisms: it can occur via direct suppression of effector cells, such as through interfering with activation of antigen-presenting cells [22], or through increase in regulatory T cells that can then interfere with the activity of cytotoxic lymphocytes.

Like with many mechanisms of tumor-mediated immune suppression, it is useful to first consider the nature of normal physiological mechanisms that in a tumor have acquired pathological characteristics. Transient immune suppression is normal during wound healing, when injured tissue needs to be protected from additional damage by the body’s own immune system. Low blood flow that results from compromised blood vessels in the damaged tissue creates a temporary hypoxic environment, which can act as a trigger for immune suppression [23]. Additional normal immune suppressive signals include increase in extracellular adenosine [24], vascular endothelial growth factor upregulation [25, 26], increase in IL-2, IL-10, tumor growth factor (TGF)-β, among others [27, 28], all of which are frequently overexpressed in tumors. Furthermore, Nosbaum et al. [29] showed that Tregs can accumulate in the skin shortly after wounding, suggesting that transient Treg accumulation may be a normal physiological process during wound healing. Since tumors are characterized by abnormal wound healing mechanisms [23, 27, 30–32], upregulation of Tregs may be yet another mechanism whereby a tumor can escape the immune system.

**IMMUNOMETABOLISM**

The effects of metabolism on T-cell function have been studied extensively. It has been shown that cytotoxic effector cells in an activated state preferentially use glycolysis for glucose metabolism, increasing their nutrient demands compared with the resting state [33–35]. It has further been shown that competition for shared resources in the TME can result in loss of ability of activated cytotoxic lymphocytes to either proliferate or perform their function, with immune cells thus becoming incapacitated by cancer cells through competitive inhibition [36–38]. The emerging field of immunometabolism is providing additional insights into the impact of nutrient consumption on tumor escape from the immune system.

In [39], the authors review the six major metabolic pathways that shape immune cell responses. These include glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway, fatty acid oxidation, fatty acid synthesis, and amino acid metabolism. Different metabolic pathways can affect function and survival of different immune cell subsets. For instance, rapidly proliferating effector T cells (including CD8+ T cells, Th17, and Th1 cells) use glycolysis and fatty acid synthesis to promote proliferation and cytokine production, while amino acid metabolism additionally supports effector cell differentiation. Regulatory T cells rely on the TCA cycle and fatty acid oxidation to promote generation of Tregs and to increase tolerogenic stimuli. Furthermore, amino acid metabolism plays an important role in immune cell function; and, as is now becoming increasingly recognized, it can affect not only cell functionality but also cell fate.

In [40], the authors study two aspects of glutamine metabolism on immune cells: the effects of glutamine deprivation, and the effects of inhibition of the enzyme glutaminase, which is necessary to convert glutamine to glutamate, making it a critical metabolite. Through a series of in vivo and in vitro experiments, the authors showed that absence of glutamine prevented production of cytokines and interfered with proliferation of Th1 and Th17 cells, while promoting generation of Tregs. Acute glutaminase inhibition interfered with T-cell respiration; this diminished contribution of glutamine to the TCA, increasing glucose contribution to cell metabolism. Interestingly, in the presence of IL-2, both CD4 and CD8 T cells were able to acquire a glutaminase-independent Th1 phenotype, whereas Th17 cells consistently remained glutaminase-dependent. In sum, the authors showed that glutamine metabolic pathway serves not only as a source of energy but is also involved in cell signaling and gene expression, which can affect differentiation and proliferation of various...
subtypes of immune cells, sometimes with opposite functions. It has been further shown that genetic loss in T cells of transporter ASCT2, which is responsible for uptake of amino acids such as glutamine, resulted in impaired function of Th1 and Th17 cells, while leaving Treg production unaffected [40, 41].

This can have important implications in the context of the competition-driven immunoediting in the TME. As glucose becomes depleted in the TME due to consumption by both cancer and cytotoxic immune cells, alternative sources of fuel become necessary. If cancer cells additionally start depleting shared glutamine, as has been proposed in [42], then glutamine deprivation can interfere with production of cytokines and Th1 cell proliferation, while simultaneously promoting Tregs and further affecting the CD8/Treg ratio.

Furthermore, as tumors outgrow their blood supply and form hypoxic regions, reliance on glycolysis as a primary mode of glucose metabolism leads to microenvironment acidification through accumulation of lactic acid. Acidic TME affects immune cell polarization, decreasing antitumor immunity, disrupting T-cell motility, and causing loss of cytotoxic function in CD8+T cells [43–48]. Furthermore, lactate metabolism in oxygenated cancer cells has been shown to increase glutaminolysis [42], further promoting competition for resources between cancer and immune cells, the outcome of which can determine not only the function but also composition of immune cells in the TME. It is possible that competition for other amino acids that affect cytotoxic immune cell function, such as arginine and tryptophan [39], can have similar effects, an area that remains to be investigated.

Finally, biomechanical forces in the TME can affect the ability of cancer cells to escape the immune system. It has been suggested that higher interstitial flow, stiffer extracellular matrix and increase in compression stress and fluid pressure can contribute to change in behavior of immune cells [49], and can promote acquisition of invasive phenotype by cancer cells [50].

Notably, while the effect of glutamine metabolism on Th17 polarization has been identified [40,51], the impact of Th17 cells on tumor progression is not yet clear. In [1], the authors highlighted that while Th17 signature was found to be beneficial in some cancers, such as gastric [52] and esophagean [53], it was found to be correlated with shorter survival in other cases, such as non-small cell lung cancer [54] and colorectal cancer [55]. Furthermore, the authors noted that an inverse effect is observed for Th2 signature as well: when Th2 signature was correlated with favorable disease outcome, Th17 signature was correlated with the opposite [1], and vice versa, suggesting the need to untangle the other underlying mechanisms of tumor-immune interactions. A summary of the key aspects of the effects of metabolic competition on immune cell function and composition is given in Figure 1.

Figure 1. Key mechanisms that may propel the escape phase of immunoediting, including effects of immunometabolism on balance and functionality of cytotoxic and regulatory immunity.

Cancer Stem Cells

The discussion of cancer cell metabolism would not be complete without that of cancer stem cells (CSCs), a subpopulation of self-sustaining cells within a tumor that are characterized by unlimited replicative capacity [56–58]. CSCs have been implicated in driving tumor progression, and have repeatedly been demonstrated to exhibit resistance to various forms of therapeutic interventions [59–63]. However, there is still a lack of consensus on the preferential mode of nutrient metabolism by CSCs. Pastò et al. [64] demonstrated that CSCs from patients with epithelial ovarian cancer overexpress genes associated with glucose uptake and oxidative phosphorylation; CSCs showed higher mitochondrial reactive oxygen species and resisted both in vitro and in vivo glucose deprivation, suggesting preference for oxidative phosphorylation over glycolysis. Vlach et al. [65] showed that glioma stem cells (GSCs) are less glycolytic compared with differentiated glioma cells; however, when challenged, these cells could use alternative metabolic pathways. GSCs also exhibited high levels of radioresistance, prompting the authors to suggest that targeting glycolysis may in fact spare these cells. Viale et al. [66] showed that pancreatic cancer cells that are responsible for tumor relapse after oncogene ablation exhibit CSC-like properties and rely preferentially on aerobic metabolism.

In contrast, Feng et al. [67] showed that breast CSCs preferentially use glycolysis over oxidative phosphorylation compared with nonstem cancer cells. Gordon et al. [68] showed that the largest CSC subpopulation in breast cancer exhibited a glycolytic gene expression profile even in the presence of ample oxygen, suggesting preferential reliance on glycolysis. Li et al. [69] showed additionally dependence of pancreatic cancer stem cells (PCSCs) on glutamine; the authors showed that glutamine...
metabolism and the gut microbiome in cancer therapy. The impact of the gut microbiome on immune responses; tumor-bearing mice that were treated with antibiotics to eliminate these Gram-positive bacteria developed resistance to cph. Gut microbiota has also recently been implicated in drug metabolism, thereby affecting interpersonal sensitivity to therapeutic interventions.

In sum, gut microbiota may be an important player in modulating "hot" versus "cold" TME, ultimately affecting treatment efficacy. Some of the key mechanisms described above are summarized in Figure 2.

**Therapeutic Interventions**

The key role of immune cell composition in cancer has been recently emphasized in a seminal study by Thorsson et al. [80], where the authors performed extensive analysis of immunological data available through The Cancer Genome Atlas of over 10,000 tumors of 33 diverse cancer types. The authors identified six stable and reproducible immune subtypes that represent features of the TME and tumor-immune interactions that cut across traditional cancer classifications. These include: (a) wound healing, (b) interferon (IFN)-γ dominant, (c) inflammatory, (d) lymphocyte depleted, (e) immunologically quiet, and (f) TGFB-β dominant types. The differences were expressed through macrophage and lymphocyte signatures, Th1:Th2 ratios, extent of intratumoral heterogeneity and antigen load, aneuploidy, as well as expression of immunomodulatory genes and prognosis. Incorporating this analysis can provide a more guided approach for devising therapeutic approaches, including revealing mechanistic underpinnings for current failures in immunotherapy administration, and ways for improvement.

Given the complexity of various aspects of tumor-immune interactions, it is most likely that the most successful future strategies for therapeutic intervention will involve combinations of therapies that complement each other. For instance, some chemotherapeutic drugs have immunogenic potential (for excellent reviews of the topic see [81–83]). This makes them prime candidates for combination with immunotherapy, where the activity of a chemotherapeutic agent, such as cyclophosphamide or doxorubicin [81, 84] provides immunostimulatory effect, while an immunotherapeutic agent, such as a checkpoint inhibitor, would alleviate immune cell exhaustion, augmenting therapeutic
efficacy. Within the context of cancer ecology, such a combination can have an additional effect of reducing competition for nutrients between cancer and immune cells through eliminating some of the cancer competitors, giving the combination an additional boost [85].

The timing and dosing of drug administration in combinations, however, needs to be carefully evaluated to prevent drug interference. For instance, cyclophosphamide can additively be toxic to both natural killer cells and CD8+T cells [86–88], necessitating its administration at a lower dose and higher frequency schedule rather than maximum tolerated dose to mediate this effect [87–92]. It is therefore possible that administration of an immunotherapeutic agent, such as a checkpoint inhibitor, before CTLs had time to recover may minimize the efficacy of immunotherapy, a hypothesis that needs to be evaluated experimentally for various combinations.

Similarly to chemotherapy, radiotherapy (RT) has been shown to exhibit both immunostimulatory and immunosuppressive effects [93–96]. In addition to creating DNA damage and promoting cell death, RT exhibits indirect effects on various subpopulations of immune cells, including CD8+T cells and Tregs. Qu et al. [97] evaluated the effect of gamma radiation on lymphocyte subsets in C57BL/6 mice, revealing significantly enhanced ratios of functional CD4+CD25hiFoxp3+ Treg cells in blood, spleen and lymph nodes of irradiated mice. The authors showed that irradiation can significantly alter the balance of effector to regulatory cells in mice [98].

Balogh et al. [99] showed in a similar experiment that Tregs are less prone to radiation-induced apoptosis and have faster repopulation kinetics compared with other immune cell types; notably, both authors show that irradiated Tregs are functional but have reduced immunosuppressive capacity. In a later study, Muroyama et al. [100] showed increased proportion and number of tumor infiltrating Tregs with equal or enhanced suppressed capacity. Liu et al. [101], however, reported decreased percentage and absolute counts of Tregs following low dose whole body irradiation, resulting in decreased tumor burden, suggesting the need for further studies to fully understand the effects of RT on Tregs.

Dose can additionally influence the effect of RT on different immune cell subsets. Cao et al. [102] showed that high dose irradiation (30 Gy) abolished Treg suppression, with no effect on Tregs following low dose irradiation. The authors also showed that irradiated Tregs decreased expression of TGF-β, contributing to decreased Treg functionality. Battaglia et al. [103] showed that dose changes can affect CD8/Treg ratios following RT, which may in turn potentially affect patient outcomes.

Given the evidence that Tregs may modulate the therapeutic efficacy of RT, it is rational to combine it with Treg suppressive agents. Kachikwu et al. [104] demonstrated that Tregs were increased in mice following whole-body irradiation, indicating higher radioresistance compared with other lymphocytes; however, systemic ablation of Tregs with an anti-CD25 antibody increased radiation-induced tumor regression. Similar results were obtained by Oweida et al. [105] in a mouse model of head and neck cancer. The authors showed that combining RT with an anti-CD25 antibody led to tumor eradication and enhanced immune response compared with RT alone. Twyman-Saint et al. [106] recently showed that combination of RT with anti-CTLA4 antibody can increase the CD8/Treg ratio, improving therapeutic response in a subset of patients and suggesting that combinations that appropriately modulate different subsets of the immune system are likely to be necessary to improve patient outcomes.

In devising approaches to improve therapeutic efficacy, one also needs to take into account the effects of drugs that have anti-angiogenic potential. Decrease in tumor vascularization, while starving out cancer cells, additionally restricts nutrient access of cytotoxic lymphocytes, instigating immune evasion mechanisms described above. Additionally, loss of vascularization decreases access of therapeutic agents, thus limiting their efficacy [5, 107–110]; therefore, the positive effects of anti-angiogenic drugs on starving cancer cells need to be counterbalanced with the drawbacks caused by concurrent starvation of immune cells, as well as limited drug access.

Finally, gut microbiota can impact responsiveness to immunotherapy, as has been briefly reviewed above. Administration of antibiotics to treat any infections that arise during cancer treatment may alter gut microbiota, rendering further cancer treatment less beneficial, an effect that in mice has been shown to be reversible [73]. Microbiota management could potentially serve as an important complement to devising appropriate therapy combinations.

The order of combination of several immunotherapeutic agents also appears to be important, at least in some cases. For instance, in [111], the authors showed that concurrent administration of immunostimulatory OX40 agonist with a PD-1 checkpoint inhibitor negated the effects of OX40, causing diminished infiltration of CD8+T cells and an overall lower antitumor immune response. However, sequential combination of the two agents,
where OX40 administration was followed by anti-PD-1 therapy, but not in the reverse order, resulted in significantly improved therapeutic efficacy [112]. It is possible that similar effects could be observed with other drug combinations. These considerations are summarized in Figure 3.

One way to approach finding appropriate doses and treatment schedules for drug combinations is through the use of mathematical modeling, where the pharmacokinetic (the dynamics of the drug as it passes through the body) and pharmacodynamic (the effects of the drug on its target) properties can be described using well developed mathematical tools, typically systems of ordinary differential equations. Such models can be coupled with semi-mechanistic models of tumor-immune dynamics, where the effect of the drug on both its intended target (i.e., increased cancer cell death) and the drug’s off-target effects (i.e., cytotoxic effects of the drug on some of the immune cells) can be simulated. A promising approach to then optimize drug regimens is a technique called optimal control, described in more detail in this context by [113], where one can calculate an optimal treatment solution subject to predefined constraints (such as drug toxicities) that can then be tested against standard treatment regimens. Application of this technique, just as any other modeling approach, has to be done on a case-by-case basis and requires an understanding of the key properties of underlying mechanisms to be as thorough as possible; in such a case, mathematical modeling has the potential to be an indispensable complementary tool in devising combination therapies.

ACKNOWLEDGMENTS

This research was supported by EMD Serono, a U.S. subsidiary of Merck, KGaA. I thank two anonymous reviewers for their very helpful comments and suggestions, as well as Gideon Coltof and Meryll Pray for their help with figures.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

I.K. is an employee of EMD Serono, a U.S. subsidiary of Merck, KGaA.

REFERENCES


