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The tumor organismal environment: role in tumor development and cancer immunotherapy

Lothar C. Dieterich^a and Andreas Bikfalvi^{b,c}

^a Institute of Pharmaceutical Sciences, Swiss Federal Institute (ETH) Zurich, Vladimir-Prelog-Weg 1-5/10, 8093 Zurich, Switzerland

^b INSERM U1029, Allée Geoffroy St Hilaire, 33615 Pessac, France

^c University Bordeaux, Allée Geoffroy St Hilaire, 33615 Pessac, France

Correspondence:

Andreas Bikfalvi

INSERM U1029

Allée Geoffroy St Hilaire,

33615 Pessac, France

Tel.: +33 5 40 00 87 03

Fax.: +33 5 40 00 87 05

e-Mail: andreas.bikfalvi@u-bordeaux.fr

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Abstract

Tumor immunotherapy has resulted in dramatic effects in some cancer types, including curing of previously untreatable patients. However, the response rates are typically very heterogenous, with some patients showing dramatic responses whereas others do not or only barely respond. Consequently, there has been an ever-increasing research effort to better understand the factors that govern immunotherapy responsiveness and efficiency in order to identify predictive biomarkers and novel therapeutic targets. Clearly, traits of the tumor cells as well as aspects of the tumor microenvironment (TME) play an important role in this regard. However, a growing tumor not only interacts with cells in its immediate vicinity, but also reciprocally communicates with the entire host organism (and its microbiota). Thus, systemic influences on tumor growth and progression are likely to be similarly important as the microenvironment. In this review, we focus on various aspects of the "tumor organismal environment" (TOE), namely the lymphatic, the hematopoietic, the microbial, the neurogenic and the metabolic environment, and discuss their impact on tumor growth and immunotherapy.

Key words:

Tumor microenvironment, tumor organismal environment, macroenvironment, immune system, immunotherapy

1. Introduction

The classical hallmarks of cancer include sustained proliferative signaling, evasion of growth suppressors, activation of invasion and metastasis, replicative immortality, resistance to cell death and angiogenesis (1). More recently, emerging hallmarks including deregulation of cellular energetics, genome instability, tumor promoting inflammation and avoidance of immune-destruction have been recognized (2). Evidently, many elements of the tumor microenvironment (TME) are considered important hallmarks of cancer and have become a subject of intense research since many years.

Historically, there are two opposing views for the development of cancer. The first is a tumor cell-centric view in which a cancer originates from genetic or epigenetic alterations within single cells that deregulate intracellular signaling pathways ultimately leading to abnormal growth, survival and invasion (3). Thus, cancer development may not only include somatic mutations, but also alterations within the tumor cell epigenome. The competing view that the tumor microenvironment plays a critical role in tumor progression stems from research conducted in three different fields, namely vascular biology, developmental and matrix biology as well as immunology. This has been theorized by some as the tissue organization field theory (TOFT) (4) where the primary causal event is the destabilization of cellular/tissue interactions leading, as a consequence, to abnormal signaling in tumor cells due to increase in genetic or epigenetic alterations. Through the seminal work of Judah Folkman, Mina Bissell and many others, research within this paradigm has become an important theme in cancer research and has led to the development of targeted therapies that are successfully applied to various cancers. However, the tumor microenvironment (TME) is a notion that has a number of understandings and is often applied with a variety of meanings. Thus, a clarification of the multiple characteristics of the TME is needed.

In this article, we will first review briefly the new classification of the tumor environment (TE) introduced recently (5) and then discuss several aspects related to the cellular and molecular interactions regarding a specific subtype of the TE called the Tumor Organismal Environment (TOE). We will discuss five different TOE subtypes in this context and emphasize their relation to the responsiveness towards immunotherapy.

2. The different layers of the tumor environment - the TOE

One of the authors of this article has previously introduced the notion that the TE consists of multiple layers, only one of which is represented by the "classical" TME. Six layers of environmental interactions within the tumor were distinguished that are organized similar like a Russian doll (5). These layers are: the tumor cell to tumor cell environment (TCTCE), the surrounding niche, the confined TE (which represents in fact the "classical" TME), the proximal TE, the peripheral TE and the tumor organismal environment (TOE). This stratification, even somewhat arbitrary, seems the most reasonable since it implies sets of

distinct spatial interactions, although, from a mechanistic point of view, there is porosity between the different layers.

The TOE is spatially the largest layer and is defined as all the components of the organism not located in the tumor or in its vicinity but which nevertheless affect cancer development. Thus, the TOE stands for involvement of the organism as a whole in cancer progression and includes entities such as the tumor metabolic environment, the tumor-dependent endocrine environment, the tumor-induced systemic environment (6), and the tumor immune microenvironment (7). Some have called the TOE also "tumor macroenvironment" (8). However, we would like to emphasize that "tumor macroenvironment" does not reflect the implication of the whole organism in the interaction with the tumor and may also include tissues located in the vicinity of the tumor. Furthermore, the term "systemic environment" does not fully account for the involvement of distant organs, which, on the contrary, is the case for the TOE. Thus, the recently introduced terminology "TOE" (5, 9) seems more appropriate to us and should be adopted by the scientific community.

TOE-tumor interactions may be bi-directional in such a way that the tumor may modify the TOE (as observed for instance in priming of the premetastatic niche) or, on the contrary, that the TOE directly impacts on the tumor development and spread (as observed for gut microbiota). Tumor-TOE communication is mediated by soluble factors, microparticles/exosomes or cells themselves and involves several regulatory systems such as the immune system, the blood vascular and lymphatic system, the endocrine system and the nervous system.

We will discuss herein five types of TOE, namely the lymphatic TOE, the hematopoietic and immunologic TOE, the microbiotic TOE, the neurogenic TOE and the metabolic TOE (Figure 1). We will highlight, in particular, the relation of these TOE subtypes to immunity and immunotherapy responses in preclinical models and cancer patients.

3. Distinct Types of the Tumor-Organismal Environment (TOE)

3.1 The lymphatic TOE in tumor-host communication

At least in the case of solid tumors, the growing tumor mass is embedded in a host tissue or organ from which it arose or to which it metastasized. The tumor-surrounding tissue contributes many cellular and molecular components to the microenvironment of the tumor, and has a direct physical interface with the tumor cells along the tumor margins. At the same time, the tumor mass is also directly communicating with the remaining host organism, most importantly via the body's two vascular systems, the blood and the lymphatic system. While blood vessels primarily function to deliver nutrients, biomolecules and cells from the central circulation to the body periphery, the lymphatic system, comparable to a sewer, drains the vast majority of the interstitial fluid (including any solutes, proteins etc. within) from peripheral tissues and returns it to the blood circulation. Lymphatic capillaries are specialized, blind-ended vessels with discontinuous junctions that are particularly apt to take up fluid,

macromolecules and entire cells from surrounding tissues. Fluid entering these vessels (called "lymph") is then rapidly returned to the jugular veins via collecting lymphatic vessels, typically passing through one or several lymph nodes along its way (10).

As tumors frequently have an increased blood vessel density, leakage rate and therefore interstitial fluid pressure (11), the demand for efficient drainage is high. Therefore, it is not surprising that in the majority of tumor types, the lymphatic system reacts to this demand by expanding locally (around and within the tumor), regionally (in the draining lymph node(s)), and distantly at metastatic sites, for example in melanoma metastasis in the lung (12-14). Molecularly, the most prominent driver of lymphatic expansion is vascular endothelial growth factor C (VEGF-C), but other angiogenic and inflammatory factors may also be involved (13, 15). Furthermore, tumor growth can stimulate lymphangiogenesis even systemically, presumably at sites of future metastatic colonization (16). Tumor-associated lymphatic vessels represent a dissemination route for metastatic tumor cells to draining lymph nodes. Consequently, the extent of tumor-induced lymphatic expansion correlates with lymph node metastasis and clinical outcome in many cancer types, and the status of the sentinel lymph node is an important prognostic indicator, for instance in malignant melanoma (13). Importantly, the lymphatic system should also be regarded as a molecular communication highway between the tumor and the systemic circulation of the host, and therefore plays a pivotal role in shaping of the entire TOE in which the tumor develops. Together with the interstitial fluid, lymphatic vessels take up a large amount of tumor-derived molecules, including metabolites, cytokines and growth factors, etc., and distribute them regionally (i.e. to draining lymph nodes) and systemically. With regards to tumor immunity, lymphatic transport of tumor cell-derived antigens to tumor-draining lymph nodes is essential for any kind of adaptive immune response against the tumor. Consequently, reduced lymphatic transport has been shown to impair antigen presentation in draining lymph nodes, inflammatory cell infiltration and cytokine expression in the B16 mouse melanoma model (17, 18).

In addition, lymphatic vessels also transport larger objects derived from the tumor microenvironment. For example, tumor-derived extracellular vesicles (EVs), comprising exosomes and other classes of vesicles released by tumor cells, are efficiently taken up by lymphatic capillaries, and are transported to draining lymph nodes (19-21). These vesicles play an important role in tumor-to-host communication, and can carry molecular information from one cell to another over large distances within the body. Tumor-derived EVs exert a large variety of tumor growth-promoting functions, locally and systemically, inducing tumor cell invasiveness, angiogenesis, metastasis etc. (reviewed in (22, 23)). In addition, tumor-derived EVs have also been found to contain miRNAs and proteins that may render them immune-inhibitory (24). Thus, lymphatic transport of EVs to regional lymph nodes and beyond is likely to have a major impact on the TOE.

Finally, lymphatic vessels also facilitate the re-circulation of immune cells from the tumor microenvironment to draining lymph nodes. Although some T cell priming may occur directly

in the tumor microenvironment (25), migration of antigen presenting cells (APCs) through the lymphatic system is considered a crucial prerequisite for the efficient generation of tumor immunity. Interestingly, a recent study using a photoconvertible transgenic mouse showed that besides APCs, large numbers of T lymphocytes, including memory and regulatory T cells, egress from tumors via the lymphatic system (26). These cells accumulate in tumor-draining lymph nodes, but can also disseminate systemically, and likely affect the host immune system as well as immunotherapeutic interventions.

Next to their function as transport routes, lymphatic endothelial cells (LECs) may also regulate tumor immunity directly through interactions and crosstalk with immune cells. For example, inflamed peripheral lymphatic vessels have been found to reduce the maturation of dendritic cells on their way to draining lymph nodes via LFA1/Mac1-dependent adhesive interactions and release of prostacyclin (27, 28). Within the lymph node, resident LECs present various self-antigens on MHC-I, which is reminiscent of the thymus stroma. At the same time, these cells constitutively express high levels of PD-L1 and other immune-inhibitory molecules, which has been suggested to contribute to the maintenance of steady-state self-tolerance (29, 30). Notably, peripheral and lymph node LECs have also been reported to present exogenous antigens derived from an upstream tumor (31), possibly due to uptake, processing and cross-presentation of lymph-borne proteins (32). At the same time, tumor-associated LECs upregulate PD-L1 expression in various tumor models (33, 34), suggesting that they are directly inhibitory for tumor-specific T cells exiting the tumor. Thus, it appears that at least in some cases, tumors may adapt to induce and exploit the inherent tolerogenicity of LECs to protect themselves from the immune system.

3.2 The hematopoietic and immunological TOE and its effects on tumor immunity

Tumor growth and progression are commonly associated with alterations in the hematopoietic system of the host organism, not only in case of hematological malignancies that may directly affect the bone marrow niche and the pool of hematopoietic stem and progenitor cells, but also in case of solid tumors developing in the body periphery. Bone marrow responses to growing tumors bear many similarities with those observed in chronic inflammatory conditions, and may result in pronounced changes in the composition of circulating and tissue-infiltrating cell types. For example, up to 40 % of cancer patients present with some degree of anemia, even before initiation of any anti-neoplastic treatment (35). Cancer-related anemia may have several causes, but inflammatory cytokines, such as IL-1, TNF- α and IL-6 clearly play an important role in this process. These cytokines are released by tumor cells or host cells within the tumor microenvironment, but act systemically on erythroid progenitors in the bone marrow, reducing their proliferation and responsiveness to erythropoietin (reviewed in (36)). As a result, blood and tissue oxygen levels may be reduced, and thus, tumor hypoxia aggravated, which in turn is likely to inhibit tumor-

infiltrating lymphocytes and immunotherapy responsiveness, as discussed below (Section 4.2).

Tumor-induced changes to the hematopoietic system can also affect the host immune system directly, deviating the generation and maturation of various leukocyte subtypes. This has been studied most intensely with regards to the myeloid lineage. During acute systemic inflammatory conditions, the bone marrow can react quickly and transiently, increasing the output of myeloid immune cells such as granulocytes and monocytes to meet an exceptional demand for such cells. This process, often referred to as “emergency myelopoiesis”, is driven by inflammatory cytokines and prostaglandins acting on hematopoietic precursors and by direct recognition of danger signals in the bone marrow (37). In chronic inflammatory conditions, including in cancer, constant release of inflammatory mediators and myelopoietic factors such as colony stimulating factors (CSFs) not only exacerbate this myelopoietic response but also interfere with the differentiation of the newly generated cells. As a result, poorly differentiated myeloid cells with reduced effector functions (e.g. limited capacity for antigen presentation) accumulate in secondary lymphoid organs, the blood, and the tumor tissue itself (38). This phenomenon can be observed in a large variety of tumor types (reviewed in (39)). As tumor-induced immature myeloid cells may be derived from various sub-lineages (e.g. granulocytic or monocytic), it is not surprising that they display a large phenotypic heterogeneity. Nonetheless, the general conclusion is that these cells are able to inhibit tumor-specific adaptive immune responses, via expression of arginase, immune checkpoints, and release of reactive oxygen species and nitric oxide. Therefore, these cells are commonly denoted as “myeloid-derived suppressor cells” (MDSCs). It should be noted however that this is a functional definition applied to a complex cell population, implying that MDSC subtypes regulate tumor immunity in various ways, and to various extents (40). Also, it is a matter of debate to what extent MDSC subtypes represent specific cell populations or developmental stages, or overlap with classic leukocyte populations, such as mature neutrophils (41).

Next to immature myeloid cells, tumor-associated myelopoiesis can also affect the composition and function of tumor-associated macrophages (TAMs) (42). TAMs represent an important constituent of the tumor microenvironment in several cancer types, for example in breast cancer, and can exert tumor-promoting or inhibitory functions, regulating tissue remodeling, angiogenesis, and tumor immunity (43). The precise phenotypic and functional make-up of TAMs is considered to depend on microenvironmental cues including cytokines and chemokines (CSF-1, CCL2, TGF- β), hypoxia, nutrients and metabolites, etc. (43). However, at least in some cases, TAMs originate from circulating bone marrow-derived monocytes infiltrating the tumor tissue, replacing the pool of tissue-resident macrophage types (44).

Another aspect is signaling provided by one tumor site to another (indolent) tumor site which has been experimentally addressed by McAllister et al. (45). They showed that systemic

instigation of indolent tumors requires the secretion of osteopontin by the primary “instigating” tumor which stimulated the recruitment of bone marrow-derived cells (BMCs). BMCs will then be incorporated into indolent tumors to stimulate their growth. McAllister et al called this “systemic endocrine instigation” where the primary tumor itself acts as a sort of endocrine organ.

In comparison to tumor-induced alterations in the myeloid arm of the hematopoietic system, very little is known if and how growing tumors may affect the generation and maturation of lymphoid immune cells. There are reports that the growth of several transplantable and transgenic tumor models in mice impairs the generation and maturation of NK cells (46, 47) and of B lymphocytes (46, 48) by release of a soluble factor. Although several candidate cytokines and growth factors, including VEGF, TGF- β and TNF- α have been probed (46), the nature of this tumor cell-derived factor that drives these processes has remained elusive. In the case of one study, tumor growth even reduced the frequency of common lymphoid progenitor cells (46), suggesting that the *de novo* generation of T cells may be reduced as well. Together, these changes likely limit the host’s capacity to mount cytotoxic immune responses, facilitating tumor growth.

3.3 The microbiotic TOE – a multi-faceted regulator of inflammation and immunity

The microbiota in adults is primarily composed of *Bacteroidetes* and *Firmicutes*, and of *Actinobacteria*, *Proteobacteria* and *Fusobacteria* to a lesser extent (49). The distribution of these different microbiota is variable, depending on the anatomical site and the respective individuals. The most extensively explored site is the gut microbiota. Microbiome exposure occurs early in life and may even occur in utero via the placenta (50). However, this is still a matter of debate (51).

Studies which compared germ-free (GF) mice raised in specific pathogen-free conditions to normal mice demonstrated alteration in physiological parameters such as fluid balance, cardiovascular function, nervous system functions and immunity (52). In particular, GF mice showed greater susceptibility to infection partially due to an altered mucosal layer and to alterations of proximal/local lymphoid structures and immune effector functions (53). Furthermore, systemic innate and adaptive immunity are modulated by the microbiota, for instance by induction of Th17 cells and regulatory T cells.

Several studies reported a role of the gut microbiome in tumorigenesis (54, 55). This is true for intestinal tumors where dysbiosis favors a tumor-promoting inflammatory state (56). Microbiota such as *Bacteroides fragilis* and *Helicobacter pylori* may produce inflammatory toxins, impair anti-tumor responses by the immune system, increase ROS, or enforce signaling pathways involved in tumor development (57, 58). However, not only local but also distant tumors seem to be concerned. For instance, hepatocellular carcinoma (HCC) may be promoted by microbiota indirectly by favoring steatohepatitis and cirrhosis and perhaps directly by disturbing the immune cell balance. Indeed, depletion of commensal gut bacteria

in mice by feeding them an antibiotic cocktail comprising vancomycin, neomycin and primaxin reduced primary HCC growth and, for lymphoma, hepatic metastasis (59). This liver anti-tumor effect was due to enhanced NKT cells expressing CXCR6. Mechanistically, NKT cell accumulation may be the result of the conversion of primary bile acids into secondary bile acids by the gut commensal bacteria. Other solid tumors may be impacted as well. For example, in the case of breast carcinoma, gut microbiota have been suggested to control tumor growth and progression by various mechanisms including estrogen metabolism, effects on adiposity and obesity (further discussed below in section 3.5), and immunity (reviewed in (60)). Distant metastatic lesions in colon carcinoma have been reported to harbor bacteria such as *F. nucleatum* and a causal relationship has been suggested (61). Besides, tumor immune-suppressive activity has also been described (62). The exact mechanisms how the microbiome contributes to tumor development are not well understood but seem to involve multiple feed-forward and feed-back loops involving alteration of the immune response, impairment of cell signaling, DNA damage etc. (63). Soluble mediators have been proposed not only to act locally in the gut but to enter the general circulation and to exert their effects at distant sites. These include short-chain fatty acids (SCFA) such as butyrate or tryptophan containing food components (TRP) processed into indole-containing catabolites (64). Mechanisms include reprogramming of macrophages and microglial cells, Tregs and cytotoxic T cells or direct effects by acting in a GPCR-dependent manner and suppressing HDACs. In the context of cancer therapy, the gut microbiome has been shown to have a major impact on the therapeutic outcome. For example, the anti-cancer response to cyclophosphamide in preclinical models has been shown to be improved by the microbiota and was due to increased intestinal permeability of bacteria which triggered maturation of T helper cells (65). On the other hand, chemotherapy can affect the intestinal microbiome and cause dysbiosis (66), which in turn may affect the therapeutic response to cancer treatment. Allogenic transplantation in several malignancies may lead to graft-versus-host-disease (GVHD), which often occurs in places with high bacterial flora. It has been shown that the intestinal bacteria *Blautia* may play a beneficial role in alleviating GVHD in patients (67). Finally, intratumoral bacteria have been evidenced and a role in immunosuppression and chemoresistance has been suggested (68, 69). However, the role of the tumor-associated microbiota will not be discussed here since this does belong to the local tumor environment and not to the TOE.

3.4 The neurogenic TOE in tumor development and progression

Tumor neurogenesis may act locally but also distantly via two mechanisms. Locally, neurogenesis may favor tumor development, and a local control of neurite formation has been evidenced through EphrinB-containing exosome release from tumors (70). Additionally, neuron-derived glutamate has recently been shown to facilitate breast-to-brain metastasis (71). However, there are also distant neurogenic effects from the TOE on tumor growth. The first is signaling from the nervous system via the sympathetic/parasympathetic system, which is thought to influence tumor growth, at least in the case of prostate cancer (72). A

second is the recruitment of neural progenitor cells from the central nervous system (CNS) through the blood stream which accumulate at the tumor site (73). It has been shown that neural progenitors expressing doublecortin (DCX) infiltrate prostate tumors and metastatic lesions. These cells are derived from the subventricular zone and egress from the CNS after disruption of the blood brain barrier. DCX⁺ cells appear to affect various stages of prostate tumor development including initiation and later tumor expansion. The density of DCX⁺ neural progenitors strongly correlates with aggressiveness and recurrence of prostate adenocarcinoma, whereas blockade of accumulation of DCX⁺ cells through genetic depletion strongly impairs tumor development. Thus, progenitors derived from the CNS seem to play a decisive role in prostate tumor development. Consequently, the nervous system may not only provide local control but also long-range effects by recruiting cells from the CNS into extra-nervous system tumors (73).

The tumor by itself may also impact on the TOE. Indeed, complex systemic effects affecting both the neurogenic and the metabolic TOE have been reported (74). Using experimental models of mammary carcinoma in mice, it has been shown that factors derived from primary tumors may affect both sleep and metabolism. The inflammatory cytokine IL-6 was increased but this did not explain the sleep and metabolic effects. In tumor bearing animals, increased activity of heme oxygenase (HO) neurons was observed, and blocking HO activity with almorexant improved sleep and glucose metabolism. Furthermore, the serum leptin (an inhibitor of HO neurons) concentration was reduced in tumor-bearing mice. Thus, the neurogenic and metabolic TOE may interact in a complex manner when challenged by the tumor.

3.5 The metabolic TOE – type II diabetes and obesity and their impact on cancer development

Two major metabolic conditions that may impact on cancer development are represented by diabetes and overweight or obesity. Diabetic patients have been reported to have an approximately 19-27% higher risk for cancer when compared to non-diabetic counterparts (75). Among those, women were at higher risk than men. This appears to be particularly true for diabetes type II, whereas the results for type I diabetes are contradictory with one study showing an increased risk (75) while in another did not (76). Meta-analyses have shown that type II diabetic patients have an increased risk to develop cancer, for example in the breast, by up to 27% (77). The major driving factor is supposed to be hyperinsulinemia and hyperglycemia which may affect directly genetically unstable epithelial tissue and its microenvironment to boost tumor development and spread (78). Additional growth factor loops may be stimulated (FGF-dependent, IGF-1 dependent) to further enhance tumorigenesis. The increased risk for breast cancer is associated with much higher odds to develop triple-negative breast cancer (TNBC) than ER⁺/Her2⁺ breast cancer in the diabetes type II group when compared to the non-diabetic group. In a retrospective study of 4340

patients, an increased risk of 38 % was reported (79). Furthermore, patients treated with the antidiabetic drug metformin had also elevated odds to develop TNBC.

Type II diabetes (like obesity) frequently results in dyslipidemia, including increased serum cholesterol levels, which may further promote tumor growth and progression. For example, mouse models have shown that high levels of cholesterol or its metabolite 27-hydroxcholesterol accelerate not only the growth of several breast cancer models, but also breast-to-lung metastasis (80, 81). Notably, cholesterol not only affected cancer cells directly (80), but had also a significant negative impact on tumor immunity, inducing granulocytes and $\gamma\delta$ -T cells, which were at least partially responsible for its metastasis-promoting effects (82).

Overweight is defined by an increased body-mass index (BMI) of 25-30kg/m², whereas a BMI of 30kg/m² or above defines obesity (83). The frequency of these conditions is rising due to the high calorie food regimens in Weird (Western Industrialized Rich and Developed) countries, and they are causally linked to various pathologies including diabetes, cardiovascular disease and cancer (84, 85). This represents a significant societal burden for industrialized countries. Obesity, the more severe condition, is characterized by an increased accumulation of cutaneous and visceral fat tissue with a deregulated influx of inflammatory cells (86). However, little is understood about the precise impact of obesity on immune responses during cancer progression. Only recently, some new light has been shed on the mechanism of obesity-dependent immune regulation. Curiously, a paradoxical effect of obesity on the immune response and the response to immunotherapy has been reported. Obesity has a negative impact on tumor development and spread with increased immune aging and dysfunction. For instance, Wang et al (87) observed obesity-associated T cell defects across multiple species. PD-1⁺ T cells and exhausted T cells were significantly increased. In spontaneous obese non-human primates, the memory T cell pool as well as PD-1 were increased but proliferation was significantly impaired. Furthermore, tumor growth was accelerated in diet-induced obese (DIO) mice and tumor immunity was also perturbed in DIO mice with an increase in PD-1⁺ CD8⁺ cells in the tumor and in ectopic sites (liver, spleen, draining lymph nodes). This was corroborated by a transcriptome analysis of T cells which showed an increase in exhaustion and inflammation markers. Furthermore, leptin levels and leptin signaling was increased in DIO mice and leptin further stimulated T cell exhaustion. These results were corroborated by adoptive T cell transfer from DIO B16-F0-bearing mice to immunodeficient DIO Rag2/Il2rg^{-/-} mice where adoptive transfer led to an increase in tumor growth and invasion. Because of elevated PD-1 levels, the response to immunotherapy was also altered. This will be discussed later in this review.

All in all, this indicates that metabolic imbalance within the TOE impact significantly tumor development and spread and, as we will see below (Section 4.5), the response to immunotherapy.

4. TOE and anti-tumor immunotherapy

The 5 different TOE subtypes may influence the response to immunotherapy (Figure 2).

4.1 Immunotherapy and lymphatic vessels

As discussed above (Section 3.1), the lymphatic system is essential for endogenous immune responses, providing transport routes for antigen from the periphery to draining lymph nodes, but also interfering directly with the activation of immune cells via LEC-derived signals acting on DCs as well as T cells. This dual role of lymphatic vessels implies that both the extent of tumor-associated lymphatic vessels as well as the specific phenotype of the LECs can have a positive or a negative influence on endogenous tumor immunity and immunotherapy. Consequently, varying results of lymphatic modulation have been reported in the literature. Lack of dermal lymphatic vessels decreased the efficiency of vaccination against tumor antigens, but surprisingly increased the effect of adoptive transfer of tumor antigen-specific CD8⁺ T cells in the B16 mouse melanoma model (18). On the other hand, forced induction of tumor lymphangiogenesis by overexpression of VEGF-C in the tumor reduced the intratumoral proliferation of adoptively transferred tumor-specific CD8⁺ T cells early after transfer (31), but potentiated the anti-tumor effect of adoptive T cell therapy later on (88). Taken together, these animal studies suggest that, whereas vaccination approaches clearly require a functional lymphatic system to be effective, the effect of tumor-induced lymphangiogenesis or its inhibition on other forms of immunotherapy, including adoptive T cell therapy, is still unclear. In line with this, the response of melanoma patients to a tumor peptide vaccine positively correlated with the serum level of VEGF-C in a phase I clinical trial (NCT00112229) (88). However, although VEGF-C is a potent inducer of lymphatic vessel growth, VEGF-C serum levels alone are not a good indicator of tumor-associated lymphangiogenesis, which needs to be quantified histologically. Additionally, VEGF-C may well have additional effects beyond the lymphatic system that could contribute to the increased vaccination response. Additionally, it is not well understood whether local (i.e. within the TME), regional (in draining lymph nodes) or systemic lymphangiogenesis have differential effects on tumor immunity and the effectiveness of immunotherapy.

Due to the multiple roles of the lymphatic TOE in cancer progression and immunity, therapeutic targeting of tumor-associated lymphatic vessels in cancer patients may be a double-edged sword. With regards to their role in dissemination of metastatic cancer cells, various lymphangiogenesis inhibitors have entered clinical development (13). However, given the essential role of lymphatic vessels in transporting tumor-derived antigens to draining lymph nodes, a more precise modulation of the lymphatic system, e.g. by targeting specific aspects of the tumor-promoting lymphatic functions, such as cancer cell invasion or immune inhibition, might be a superior approach. In this regard, the recent transcriptional

characterization of tumor and tumor-draining lymph node-associated LECs in mouse model (89, 90) may be a step towards the identification of potential targets for future drug development.

4.2 Immunotherapy and the hematopoietic / immunological TOE

Tumor-induced anemia resulting in tissue hypoxia and acidosis is known to create an environment which is unfavorable for endogenous anti-tumor immunity, and therefore is likely to impair immunotherapeutic approaches as well. For example, hypoxia can directly induce PD-L1 expression (91), impair lymphocyte function or skew myeloid cells to immunosuppressive phenotypes (92). Furthermore, anemia has recently been shown to induce immune-inhibitory erythroid progenitor cells in cancer patients (93). Consequently, combined targeting of hypoxia or acidosis and immunotherapy has resulted in improved therapy outcomes in some model systems (94).

Similarly, tumor-induced systemic accumulation of myeloid cells and macrophage skewing towards an immune-inhibitory phenotype are detrimental for tumor immunity. At the same time, they may reduce the effectiveness of immunotherapeutic approaches or mediate resistance towards them (reviewed in (95, 96)). Indeed, various clinical studies have shown that the level of circulating MDSCs of the monocytic lineage negatively correlated with the response to checkpoint inhibitor therapies in melanoma (97-101). Curiously, a positive correlation between granulocytic MDSC and nivolumab response has been reported in non-small cell lung cancer (NSCLC) (102). However, there is no consensus yet how to differentiate stringently between granulocytic MDSC and mature granulocytes (41), and the neutrophil-to-lymphocyte ratio (NLR) correlated with poor prognosis and worse checkpoint inhibitor responsiveness in various cancer types, including NSCLC (103, 104).

MDSCs have also been associated with poor results in other types of cancer immunotherapy. For example, in case of dendritic cell-based cancer antigen vaccination, high levels of circulating MDSCs not only correlated with reduced response rates, but also resulted in difficulties to generate activated autologous dendritic cells in the first place (105-109). With regards to adoptive T cell or CAR T cell therapy, the clinical data is still very limited. However, in a recent phase I/IIa trial involving 15 patients suffering from B cell lymphoma or leukemia, a low frequency of monocytic MDSCs correlated with the response to CD19-targeted CAR T cells (110). Thus, targeting of MDSCs may have synergistic effects when combined with a range of immunotherapeutic approaches (111). However, there is still a lack of supportive clinical evidence in cancer patients for this concept, at least in part due to the fact that specific targeting of MDSCs is challenging (112). In this regard, a recent report indicated that Gemtuzumab ozogamicin, a CD33-directed immunotoxin approved for the treatment of myeloid leukemia, could potentially be useful for MDSC targeting in other cancer types (113).

4.3 Immunotherapy and microbiota

An effect of microbiota on checkpoint blockade inhibition by CTLA4 or PD-1/PD-L1 blockade has been first reported by two groups using mouse models (114, 115). *B. fragilis* was reported to enhance anti-CTLA-4 efficacy via a proposed mechanism involving the activation of Th1 cells with cross-reactivity to bacterial antigens and tumor neoantigens. Oral administration of *Bifidobacterium* increased tumor infiltration and IFN- γ production by CD8⁺ tumor-specific T cells and improved both basal tumor control and anti-PD-L1 efficacy via a proposed mechanism involving increased activation of splenic and intratumoral DCs. These mouse studies established the importance of the microbiome in cancer immune checkpoint therapy and inspired clinical pursuits to assess efficacy. This has been reinforced by clinical studies (116-119). Indeed, specific microbiota signatures have been identified in patients responding to treatment. Patients with the respective signatures exhibited better systemic immunity and a more favorable intratumoral immune-score. On the other hand, a microbial signature rich in *Bacteroidales* correlated with a poor response (119). Fecal microbiota transplants (FMT) of human microbiota into mouse models were able to phenocopy the therapeutic effect and durably reshaped the gut microbiota (120).

Immunotherapy efficacy may be perturbed by radiation therapy, chemotherapy or antibiotics since these treatments alter the gut mucosa significantly. In line with this, studies have reported that antibiotic treatment negatively influences immune checkpoint immunotherapy in renal cell carcinoma (121). Furthermore, the risk of checkpoint inhibitor-induced colitis in melanoma patients may be predicted by the gut microbiome (122, 123). Consequently, FMT may be useful in patients with refractory immune checkpoint inhibitor colitis (124)..

The details of the mechanisms by which the gut microbiota influence immunotherapy are not well understood. Several plausible mechanisms have been suggested, which most likely operate in parallel. Bacteria-associated elements may prime or boost the adaptive immune response (Pathogen-associated patterns..), bacteria-associated homologues to cytokine or growth factors (VEGF bacterial homologue..) may stimulate vascular permeability and angiogenesis, as well as bacterial metabolites that may modify tumor metabolism (125).

With respect to immunotherapy, two different microbiota situations need be distinguished. The systemic microbiota may favor the efficacy of immunotherapy by priming of the immune response. On the other side, intratumoral microbiota may have direct tumor-promoting properties (effect on cell metabolism, etc. (126)) and thus not favor the efficacy of immunotherapy. Furthermore, intratumoral bacteria may create an immunosuppressive tumor environment by targeting ligands and receptors that are involved in the response to immunotherapy. However, other studies have also reported immune-stimulatory effects and thus the situation is less clear (127).

Taken together, the composition of the microbiotic TOE has a dramatic impact on host immunity as well as immunotherapy responsiveness in various cancer types, with certain microbial signatures correlating with a good prognosis and other with a poor prognosis (125).

4.4 Immunotherapy and the neurogenic TOE

Little is known about the systemic and central or peripheral nervous effects of immunotherapy. Cognitive impairment has been documented in cancer patients receiving immunotherapy (128). There may also be a higher risk for neuro-inflammation, since immunotherapy with checkpoint blocking agents significantly boosts the immune system (129). It is not established if immunotherapy impacts directly synaptic function or neurogenesis in the CNS. Furthermore, it is not known whether immunotherapy has an impact on tumor neurogenesis. It would be interesting to see, and this already in experimental models, whether combination treatment associating immunotherapy with e.g. neurogenic blockade of DCX⁺ cells would be further beneficial in halting tumor development.

4.5 Immunotherapy and the metabolic TOE

The effect of the metabolic TOE on immunotherapy is not well understood. It has been observed that roughly 1% of patients treated with anti-tumor immunotherapy via checkpoint inhibition develop diabetes (130). It is not clear how diabetes modifies the response to immunotherapy. However, treatment with the anti-diabetic drug metformin associated with venetoclax reduced the tumor size by stimulating cytotoxic lymphocyte infiltration. Indeed, combination with PD-1 immunotherapy after tumor excision led to long term anti-tumor effects (131).

With regards to overweight and obesity, accumulation of adipose tissue correlates with poor outcome and resistance to conventional cancer therapies (132). On the other hand, more recent clinical data paradoxically link obesity to a better response to immunotherapy (133, 134). The mechanistical aspect of this finding has been recently experimentally addressed (87). While obesity has a negative impact on tumor development and spread with increased immune aging and dysfunction, as already discussed, an improved response to checkpoint inhibitors was observed (87). Indeed, administration of anti-PD1 checkpoint inhibitors in DIO mice clearly inhibited tumor development. It is of note that anti-PD-1 therapy was not active in the B16 or 3LL tumor model in control mice, but had a significant effect in DIO mice. This was associated with an increase in the CD8⁺ to CD4⁺ T cell ratio and a reduction of CD8⁺ PD-1⁺ T cells in the TME. Importantly, metastasis was significantly inhibited in B16-bearing DIO mice. These preclinical observations are of translational importance since obese patients exhibited a better response to immunotherapy.

5. Conclusion

The concept of the TOE encompasses the wider tumor environment and it has been recently recognized that it plays an important role in tumor development and spread. Therapeutic interventions must take the existence of these wider tumor control mechanisms into account since they critically influence the response to therapy. This is particular evident for immunotherapy which is now the focus of many translational studies. On one end, the TOE may weaken or reinforce the effect of immunotherapy. On the other end, immunotherapy

may modify the TOE which may then also modulate the therapeutic response to immunotherapy. In this article, we have discussed 5 different TOEs (the lymphatic, the hematopoietic and immunologic, the microbiotic, the neurogenic and the metabolic TOE) which in our opinion have important implications for cancer development and the response to therapy. The separation of these TOEs is not tight since they may interact in various ways such as via the immune system or via metabolic regulators. For example, metabolism or the microbiome may significantly impact cancer development and the response to immunotherapy via the immune system. The neurogenic TOE has strong connection with the metabolic TOE as the example of HO neurons, which regulate sleep and glucose metabolism in tumor bearing animals, shows (74).

Taken together, the existing literature points to an important regulatory role of TOEs in tumor development and metastasis and in the response to therapy, in particular to therapies interfering with or modulating the immune system. However, the exact place and the importance of the TOE in the sequence of cancer causality needs still to be clarified since cancer is a multi-causal phenomenon where deterministic as well as stochastic explanations can be applied.

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Figure legends

Figure 1: Schematic representation of several aspects of the tumor organismal environment (TOE), one of the six layers of the tumor environment. Lymphatic, hematopoietic and immunological, microbiotic, neurogenic and metabolic TOE and their influence on tumor development and progression are shown. TCTCE: tumor cell to tumor cell environment; TE: tumor environment.

Figure 2: The tumor organismal environment influences cancer immunotherapy. Five aspects of the tumor organismal environment (lymphatic, hematopoietic, microbiotic, neurogenic and metabolic) are depicted, together with their overall influence (promoting (green plus) or inhibiting (red minus)) on the major types of immunotherapy (adoptive cell transfer, tumor vaccination, and checkpoint inhibitors). Further details in the text.

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The Lymphatic TOE

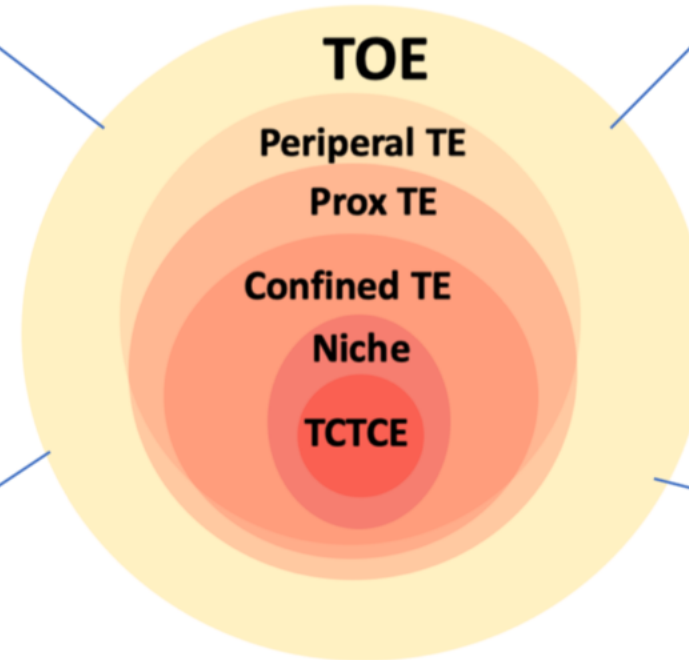
- Connect the tumor to the immune and the vascular system
- Role in distant immune regulation and fluid transport

The hematopoietic and immunological TOE

Myelopoiesis and lymphopoiesis may influence development and spread of solid tumors.

The microbiotic TOE

- The gut microbiome may distantly impact tumor development and metastasis through immune and metabolic regulations
- Complex tumor promoting and inhibitory effects exist



The neurogenic TOE

Effects of both the central and peripheral nervous system on tumor development and metastasis

The metabolic TOE

Systemic glucose and lipid metabolism may impact tumor development and spread such as observed in diabetes type II and obesity

Figure 2

