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More than a scaffold: Stromal modulation of tumor immunity

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ABSTRACT

Current clinical success with anti-cancer immunotherapy provides exciting new treatment opportunities. While encouraging, more needs to be done to induce durable effects in a higher proportion of patients. Increasing anti-tumor effector T cell quantity or quality alone does not necessarily correlate with therapeutic outcome. Instead, the tumor microenvironment is a critical determinant of anti-cancer responsiveness to immunotherapy and can confer profound resistance. Yet, the tumor-promoting environment – due to its enormous plasticity – also delivers the best opportunities for adjuvant therapy aiming at recruiting, priming and sustaining anti-tumor cytotoxicity. While the tumor environment as an entity is increasingly well understood, current interventions are still broad and often systemic. In contrast, tumors grow in a highly compartmentalized environment which includes the vascular/perivascular niche, extracellular matrix components and in some tumors lymph node aggregates; all of these structures harbor and instruct subsets of immune cells. Targeting and re-programming specific compartments may provide better opportunities for adjuvant immunotherapy.

1. Introduction

1.1. Recent advances in anti-cancer immunotherapies

After decades of skepticism, anti-cancer immunotherapy has finally arrived in the clinic. The list of currently available anti-cancer immunotherapies ranges from therapeutic vaccination and adoptive T cell therapy to immunomodulatory antibodies [1]. Dendritic cell (DC)-based vaccines such as the first US Food and Drug Administration (FDA)-approved formulation (sipuleucel-T, Provenge®, Dendreon Corporation) show some benefits in prostate cancer patients [2]. After breakthrough success with ipilimumab (Yervoy®, Bristol-Myers Squibb), an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) monoclonal antibody, particularly in the treatment of malignant melanoma, the field has now fully embraced immune checkpoint inhibition [3–5]. T cell activation mediated by antibodies against programmed cell death ligand-1 and its receptor (PD-L1/PD-1) is currently being evaluated in phase II and phase III clinical trials [6], and the PD-1 antibodies pembrolizumab (KEYTRUDA®, Merck) and nivolumab (Opdivo®, Bristol-Myers Squibb) have most recently been approved by the FDA for non-small cell lung cancer (NSCLC); equally, concurrent anti-CTLA4/anti-PD-1 trials have been conducted with synergistic results [7]. The clinical benefits, including over 30% durable responses, to patients with various cancer types have been impressive. Nevertheless, insights into effector mechanisms conferring long lasting anti-tumor immunity are often lacking. Considerable uncertainties remain as to which patient groups will potentially benefit from immunotherapy [8]. Most important, however, less than half of all patients show objective anti-tumor responses which raises the question of what else is required to improve success rates. Tumor antigenicity and a high rate of somatic mutations as for instance observed in melanoma, NSCLC and bladder cancers seem to correlate with successful immune checkpoint therapy [9], indicating that a high frequency of de novo antigens may improve responsiveness [10]. However, increasing the number of anti-cancer effector T cells does not necessarily lead to tumor rejection [11–13]. Instead, absence of clinical benefits often correlates with impaired homing or functionality of cytotoxic T cells at the tumor site. Thus, one of the major challenges is to identify and eliminate tumor-intrinsic resistance to immunotherapeutic interventions.

1.2. The tumor microenvironment

Tumors create and evolve their own environment which enables and promotes growth. In addition, tumors actively evade immune destruction, which ultimately presents a serious obstacle for immunotherapy

Abbreviations: CAFs, cancer associated fibroblasts; CTLA4, anti-cytotoxic T-lymphocyte-associated protein 4; CSF-1, colony stimulating factor-1; ECM, extracellular matrix; FAP, fibroblast activation protein-α; HEVs, high endothelial venules; IDO, indoleamine-pyrrole 2,3-dioxygenase; LAG-3, lymphocyte activator gene-3; LER, low-expressing region; LFA-1, lymphocyte function antigen-1; LOX, lysyl oxidase; MIF, macrophage migration inhibitor-1; NRP-1, neuropilin-1; NSCLC, non-small cell lung cancer; PD-1, programmed cell death-1; PD-1L, programmed cell death-1 ligand; PGE2, prostaglandin 2; Sema3A, semaphorin 3A; Tim3, T cell immunoglobulin and mucin domain-containing molecule 3; VLA-4, very late antigen-1.

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[14]. The structural framework for tumor cells, the so-called stroma, is crucial for growth promotion, metastatic spread and immune escape. Stoma consists of multiple cell types such as vascular cells, comprised of endothelial cells and supporting pericytes, diverse populations of immune cells, fibroblasts and a non-cellular component, the so-called extracellular matrix (ECM) [15]. In addition, cytokines, chemokines and growth factors form complex signaling networks involving tumor-stromal compartments and other host cells. Tumorigenic factors secreted by cancer cells shape the surrounding stroma and also recruit bone marrow-derived cells to meet the tumor's growing demands. Innate and adaptive immune cells in the tumor environment create an environment of chronic inflammation and thus profoundly contribute to cancer progression and metastasis [16]. Sustained production of growth factors such as vascular endothelial growth factor (VEGF) generates an aberrant and leaky vasculature, inadequate blood flow and a hypoxic environment which directly and/or indirectly affects leukocyte access into tumors [17]. Cancer associated fibroblasts (CAFs) via secretion of cytokines and chemokines may actively contribute to immune suppression and poor prognosis [18–20]. CAFs also produce key components of the tumor-intrinsic ECM which forms an abnormally dense network causing enhanced tumor stiffness. Extensive matrix crosslinking promotes invasive tumor growth and reduces effector T cell migration [21,22]. Collectively, the tumor-intrinsic stromal composition has profound effects on immune cell infiltration, polarization and ultimately effector function. While this is reasonably well understood, stromal function is also highly context dependent and targeting specific compartments of tumor stroma still remains therapeutically under-explored.

2. Immune cell trafficking: an obstacle to immunotherapy

2.1. The blood–tumor barrier

Immune-mediated tumor rejection is intimately linked to effector cell access and function. Even if immune cells are sufficiently activated in tumor-draining lymph nodes, they must be able to migrate to the tumor site, penetrate deep into tumor parenchyma and establish productive contact with antigen-presenting cells to exert their effector function. One of the first hurdles encountered at the tumor site is the tumor neovasculature which is fundamentally different to normal vessels and can restrict lymphocyte infiltration [23,24].

Blood vessels are an integral part of normal tissue immune surveillance and adaptive response to infection. For instance, lymph nodes (LNs) are accessed through high endothelial venules (HEVs), specialized postcapillary venules which support high levels of naïve T cell trafficking. Specifically, HEVs are equipped with peripheral node addressins which bind L-selectin-positive, naïve T cells. Subsequent engagement of CCL21 with $CCR7^+$ T cells, and ICAM-1 with lymphocyte function antigen-1 (LFA-1) selectively facilitates naïve T cell entry into LNs. Secondary lymphoid organs also regulate immune responses by promoting cell–cell and cell–reticular fiber network interactions [25], and by providing a supportive chemokine environment. Following priming, effector T cells change their receptor repertoire and migrate to peripheral organs at sites of inflammation often attracted by chemokines such CXCR9 (Mig) and CXCL10 (IP10) which both engage with CXCR3 expressed by T cells [26]. In the tissue, inflamed blood vessel walls, generally postcapillary venules, support very late antigen-4 $(VLA\text{-}4)^+$ T cell adhesion via intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and transmigration. Overall, this is a multistep process which involves a series of intercellular adhesive interactions, the cytoskeleton and chemokine-G protein-coupled receptor signaling [27].

In contrast to the normal vasculature, angiogenic blood vessels in solid tumors lack hierarchical structures, are highly permeable for plasma molecules, and thus unable to sustain adequate blood flow. Much as vascular leakiness of inflamed normal vessels and transendothelial migration are separate entities [28], tumor vascular leakiness does not facilitate leukocyte extravasation per se. Instead, reduced perfusion in an environment of altered tumor metabolism creates tumor hypoxia, high acidity and increased interstitial fluid pressure [29,30]; this in turn may indirectly restrict lymphocyte access into tumors. Furthermore, lack of adhesion molecules or downstream signaling events involved in leukocyte capture at the vessel wall may contribute to reduced leukocyte transmigration into tumors. Indeed, tumor angiogenesis can induce endothelial "anergy", a state which is mainly described by diminished expression or function of adhesion molecules such as ICAM-1 and VCAM-1 under conditions of high VEGF and basic fibroblast growth factor (bFGF) levels [31–33]. Mechanistically, ICAM-1 expression can be directly regulated by epigenetic modification, but is also indirectly suppressed by sustained MAPK and reduced NFκb signaling in angiogenic endothelial cells [34,35]. Interestingly, nitric oxide (NO) can attenuate cytokine-induced vascular adhesion molecule expression involving NFκb signaling [36]. Failure of adhesion molecules to form functional clusters when NO/caveolin ratios are high in an angiogenic environment can also result in endothelial anergy in vitro [33]. Moreover, in human ovarian cancer, enhanced vascular endothelin signaling prevents tumor infiltration by decreasing ICAM-1 expression and T cell adhesion in a NO-dependent manner [37]. Thus, adhesion molecule expression and T cell trafficking may be controlled by NO levels and subdued vascular inflammation. However, other factors can contribute to reduced ICAM/VCAM levels and leukocyte extravasation. For instance, in human lung and breast carcinomas, Egfl7 (also known as VE-statin) expression correlates with poor immune cell infiltration, and low endothelial adhesion molecule expression specifically in an environment of low IFNγ and IL12 [38]. Angiogenic and immunosuppressive factors in the tumor environment can also regulate anti-tumor immunity by selectively permitting transit of regulatory T cells (Tregs) while excluding effector T cells [39]. Endothelial upregulation of FasL, a pro-apoptotic member of the tumor necrosis factor (TNF) superfamily, in an environment of elevated VEGF, IL10 and prostaglandin E_2 (PGE2) induces killing of activated anti-tumor $CD8^+$ T cells; in contrast, $CD4^+CD25^+$ Tregs are protected by higher expression levels of anti-apoptotic genes. This mechanism may act in concert with other tumor features such as hypoxia-induced upregulation of CCL28 which selectively attracts Tregs [40]. Thus, during tumor angiogenesis the endothelial barrier actively regulates effector T cell trafficking.

2.2. Squeezing through gaps: pericytes and basement membrane

The endothelium is the first port of entry for activated leukocytes, however, migration through the endothelial basement membrane and the pericyte sheath follows suit. Wrapped around endothelial cells, pericytes are strategically placed at the interface between blood and the interstitial space. The extent of pericyte coverage around microvessels varies substantially between different organs and tumor types [41]. Although the role of pericytes in effector T cell trafficking in cancer remains enigmatic, recent work demonstrates that active pericyte–leukocyte interactions facilitate neutrophil transmigration into inflamed tissue [42–44]. For instance, in a model of venous inflammation neutrophils crawl abluminally along the pericyte sheath and exit through gaps between adjacent pericytes. This process is dependent on pericyte-expressed ICAM-1 and corresponding neutrophil ligands, Mac-1 and LFA-1 [42]. Interestingly, pericyte–leukocyte interactions induce pericyte relaxation and opening of pericyte gaps. Simultaneously, the vascular basement membrane develops zones of low matrix expression, so called low-expression regions (LER) which further facilitates the transmigration process [43]. Moreover, arteriolar and capillary pericytes, positive for the proteoglycan NG2 marker, are instrumental in guiding neutrophils and macrophages after extravasation from postcapillary venules by secreting chemoattractants such as macrophage migration-inhibitor factor (MIF) and providing ICAM-mediated anchorage [44]. Pericyte coverage, endothelial alignment and/or differentiation as well as basement membrane structure/