

The Tumor Microenvironment Innately Modulates Cancer Progression

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Abstract

Cancer development and progression occurs in concert with alterations in the surrounding stroma. Cancer cells can functionally sculpt their microenvironment through the secretion of various cytokines, chemokines, and other factors. This results in a reprogramming of the surrounding cells, enabling them to play a determinative role in tumor survival and progression. Immune cells are important constituents of the tumor stroma and critically take part in this process. Growing evidence suggests that the innate immune cells (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid-derived suppressor cells, and natural killer cells) as well as adaptive immune cells (T cells and B cells) contribute to tumor progression when present in the tumor microenvironment (TME). Cross-talk between cancer cells and the proximal immune cells ultimately results in an environment that fosters tumor growth and metastasis. Understanding the nature of this dialog will allow for improved therapeutics that simultaneously target multiple components of the TME, increasing the likelihood of favorable patient outcomes.

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Introduction

The tumor microenvironment (TME) is complex and continuously evolving. In addition to stromal cells, fibroblasts, and endothelial cells, the TME comprises innate and adaptive immune cells. Previous studies have focused predominantly on adaptive immune cells in the context of cancer. T lymphocytes, in particular, have been a target of interest for their potent cytotoxic capabilities, so much so that their differentiation status became a model for other cell types and was coined the "Th1/Th2 paradigm" (1). This dichotomy posits that T cells orchestrate pathogen-dependent immune responses by differential production of cytokines: Th1 cells govern a proinflammatory phenotype and Th2 cells orchestrate an immunosuppressive phenotype. Current TME-targeted treatments have focused predominantly on T cells; prime examples include checkpoint blockade and chimeric antigen receptor (CAR) T-cell therapies. With an expansion of the literature regarding the TME, it is now evident that the innate immune response not only indirectly influences the TME by controlling T-cell fate, but also critically sculpts the TME. These innate immune cell types include macrophages, dendritic cells (DC), neutrophils, myeloid-derived suppressor cells (MDSC), natural killer cells (NK), and innate lymphoid cells (ILC). Mechanistically, cytokines within the TME manipulate immune functions that culminate in muted immune responses that guide tumor progression. It is essential to develop a comprehensive understanding of the innate immune cells and extend this knowl-

edge to current therapies that target dysfunctional cells in the TME. In this review, we summarize the current knowledge on the ability of the TME to co-opt innate immune cells for cancer promotion and clinical strategies targeting these innate immune responses in the context of cancer.

Macrophages

Of all of the innate immune cells, monocyte-derived macrophages (M ϕ) best reflect the Th1/Th2 paradigm. Simplistically, M ϕ s can be polarized into inflammatory M1 (classically activated) or immune-suppressive M2 M ϕ s (alternatively activated) (2). M ϕ s modulate immune responses through pathogen phagocytosis and antigen presentation, and also function in wound healing and tissue repair, thus necessitating them for immune homeostasis (3). M ϕ s are tissue-specific and ubiquitous; they contribute to all stages of wound healing, tissue formation, coagulation, inflammation, and tissue reorganization (4). M ϕ s first appear in the yolk sac on embryonic day 7, and from there they disseminate to peripheral tissues to establish tissue-resident M ϕ s, although a majority of adult tissue M ϕ populations (including the spleen, lung, and skin), originate in the fetal liver, indicating that the M ϕ s established by the yolk sac are replaced by those that originate in the fetal liver. Specifically, hematopoietic stem cells colonizing the fetal liver give rise to all hematopoietic lineages, including monocytes (5). In the context of cancer, one form of M ϕ recruitment includes recruitment from the bone marrow as monocytes by chemokines (CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CXCL1, CXCL2, CXCL4, CX3CL1), leading to M ϕ differentiation in response to cytokines (CSF-1) that are secreted by many different cell types, including tumor cells, osteoblasts, and uterine epithelial cells (4, 6).

The TME potentiates immune suppressive M2 M ϕ s through the secretion of cytokines such as IL4 (Fig. 1A and B). Cumulatively, this enables tumor growth and progression as M ϕ s can make up to 50% of tumor mass (7). High M ϕ infiltration of most tumor types including breast cancer, gastric cancer, lung cancer, hepatoma, and other malignancies correlated with a negative prognosis, further establishing their role in cancer progression (8–10). Also,

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Cancer Res 2019;79:4557–66

doi: 10.1158/0008-5472.CAN-18-3962

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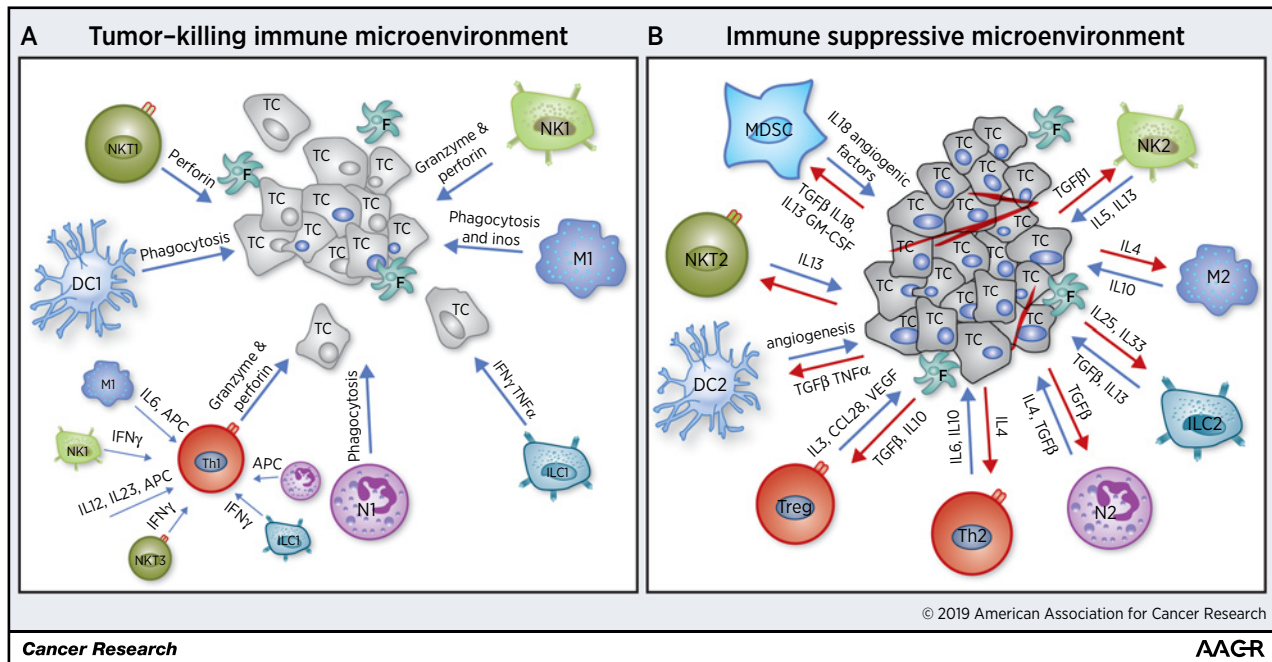


Figure 1. Cross-talk in the tumor microenvironment. **A**, The impact of inflammatory or tumor-suppressive immune cells on tumor cells in the TME. The bold arrows show the impact that immune cells ideally have on tumor cells (TC). The interactions between NKTs, DCs, T cells, neutrophils, ILCs, M ϕ s, and NK cells and tumor cells are depicted. Fibroblasts are denoted with the letter "F." **B**, The cross-talk between immune cells in the TME that have been polarized to an immune-suppressive type and the cytokines secreted by the TCs that contribute to this Th2-like polarization.

an aspect of their normal tissue remodeling abilities includes regulation of epithelial cell movement. This function of M ϕ s is co-opted by tumor cells within the TME; M ϕ s release factors (e.g., EGF) that promote the movement and invasion of cancer cells (11, 12).

While the M1/M2 classification is a simplified understanding of M ϕ phenotype and function, in reality M ϕ s are plastic in nature and exist in a continuum of functional states (7). M2 M ϕ s can further be classified into M2a, M2b, M2c, and M2d subsets (Table 1). These subsets are defined on the basis of their different inducers namely: IFN γ , and LPS for M1; IL4, IL10, IL13 for M2a; TLR agonists for M2b; IL10, TNF α , and glucocorticoids for M2c; and TLR and adenosine A2A receptor for M2d (13). Furthermore, these differential M ϕ subtypes have different functional roles as outlined in Table 1. Therefore, it is unsurprising that M ϕ s that exhibit properties of both M1 and M2 exist in distinct proportions in the TME, depending on the tumor type, although the M2 phenotype is typically favored. This poses a conundrum because M ϕ -mediated killing of cancer cells is virtually nonexistent in the TME of tumors with high proportions of M2 M ϕ s. The wound-healing phenotype of M2 M ϕ s established by the TME enables tumor growth, proliferation, angiogenesis, and epithelial-mesenchymal transition (EMT; ref. 14). There are many aspects of the TME, including cytokines and hypoxia, which orchestrate M ϕ polarization and function (Table 1; ref. 15). IL4, commonly present in the TME, initiates STAT6 signaling in M ϕ s, launching a transcriptional program that directs alternative polarization of M ϕ s. In a recent publication, Hanna and colleagues have identified that tumor cells engage in a dialog with M ϕ s via secreted Hedgehog

ligands (16). This kindles a feed-forward loop that sustains alternatively polarized M ϕ s within the TME. Interfering with this cross-talk reprogrammed the TME to be immune reactive and diminished the occurrence of metastasis. Given their role in inducing a premetastatic niche ("a favorable microenvironment for survival and outgrowth of tumor cells induced at distal sites by tumors"; ref. 17), aiding in extravasation of circulating cancer cells, and promoting metastasis (18), M ϕ s present as prime candidates for therapeutic intervention.

Dendritic cells

DCs bridge the gap between the adaptive and innate immune systems. They initiate pathogen-specific T-cell responses and are therefore important for bolstering protective immunity. It is important to note that B cells and M ϕ s also perform antigen presentation, albeit with lower activity than that of DCs. To effectively stimulate the adaptive immune response, DCs must recognize, capture, and present antigens, upregulate costimulatory molecules, produce inflammatory cytokines, and then travel to secondary lymphoid organs for antigen presentation to T cells. The inability of DCs to perform these functions greatly hampers the immune response to pathogens, viruses, and tumors. DCs are functionally classified into different subtypes such as classical DCs (cDC), plasmacytoid DCs (pDC), and monocyte-derived inflammatory DCs (moDC). cDCs can be further divided into cDC1 and cDC2. cDC1s develop under the control of the transcription factors IRF8, ID2, and BATF3, and cDC2s develop under the control of transcription factors IRF4, ID2, ZEB, and Notch2/KLF4 (19). These subsets are also functionally distinct: cDC1s are capable of cross-presentation and thus are able to present both

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Table 1. Innate immune cells in the tumor microenvironment

Cell type	Normal functions	Stimulatory cytokines in the TME	Cytokine/chemokine secretion	Human markers	Mouse markers	Effect	Source(s)
MACROPHAGES							
M1	Activate Th1 responses, phagocytosis, type 4 hypersensitivity	IFN γ	IL12, IL23, IL1 β , IL6, IL12, IL23, CCL10, CCL11, CCL2-5, CCL8, CCL9	CD64, IDO, SOCS1, CXCL10, CD80, CD86, CD68, MHC-II, IL1R, SOCS3	CXCL9, CXCL10, CXCL11, NOS2	Antitumor	(2, 13, 94)
M2a	Activate Th2 responses, wound healing, allergy	IL4, IL10, IL13, CSF1, CCL2, CCL3, CCL14	IL4, L-arginine, PGE2, IL10, TGF β , IL1ra, CCL17, CCL22, CCL24	MRC1, TGM2, CD23, CCL22, CD163, IL-1R II	Mrc1, Tgm2, Fizz1, Ym1/2, Arg1, MHC-II, IL1ra	Protumor	(2, 13, 94)
M2b	Th2 activation, immunoregulation	TLR agonists, Immunocomplex	IL1, IL6, IL10, TNF α , CCL1	CD86, MHC-II	CD86, MHC-II	Protumor	(13, 94)
M2c	Tissue repair, immunoregulation, matrix remodeling	IL10, TNF α , Glucocorticoids	TGF β , IL10, CCR2	CD163, Mrc2	CD163, Mrc1	Protumor	(13, 94)
M2d	Angiogenesis, clearance of apoptotic tissues	TLR, adenosine A2A receptor	TNF α , TGF β , VEGF-A, IL10, IL12, CCL5, CXCL10, CXCL16	VEGF	VEGF	Protumor	(13, 94)
DCs							
Immature DCs	Recognize antigens, migrate to secondary lymphoid organs, phagocytosis, minimal APC, induce T-cell energy and promote Th2 and T-reg responses	N/A	N/A	CD11c, HLA-DR, FLT3L	Cd11c, MHCII, FLT3L, CD45	Depends on tumor type	(20, 95)
cDC1	APC to CD8 T cells, cross presentation, secretion of IL12		IL12, TNF α , IFN γ	CD11c, CD141, XCR1, HLA-DR, Nect2, CLEC9A, CD80, CD86, CD40, CCR7, FLT3, TLR3, CD103, CADM1, CD26, BTLA, CD226, CD13, CD33, CXCR3, CXCR4, CLEC9A	CD11c, CD8 α (lymphoid), MHCII, Clec9A, CD103 (Non-Lymphoid), DEC205, XCR1, CD80, CD86, CADM1, CD26, CD24	Depends on tumor type	(19, 96-98)
cDC2	APC to CD4 T cells		TGF β , IL6, IL8, IL1, IL12, IL23, IL10, TNF α	CD11c, HLA-DR, CD1c, CD11b, CD80, CD86, FLT3, CLEC7A, CLEC6A, Dectin 1&2, CD40, CADM1, CD172a, CD2, SIRPA, Fc ϵ R1, DCIR, CD62L, MHCII, ILT1	CD11c, CD11b, MHCII, CD4 \pm , Sirpa+, CD80, CD86, CD172a, CD26	Depends on tumor type	(19, 96-98)
pDCs	Abundant secretory activity (IFN type 1), respond to viral infections		Type 1 IFN, TNF, IL6	CD11c, HLA-DR, CD304, CD303, CD123, FLT3, B220, PDCA1, Fc ϵ R1, ILT3, ILT7, DR6, CD300A, BTLA, CD62L, CD45RA	CD11c, B220, CD45, Siglec H, CD317, Gr-1, Ly6C	Depends on tumor type	(20, 96-98)
MoDCs	Produce high levels of the pro-inflammatory cytokines TNF, IL6, and IL12		TNF α , IL1, IL12, IL23	CD11c, CD14, Factor XIIIa, HLA-DR, CD62L, CXCR3, CD209, CD1c, CD80, CD86, CD64, MAR-1	CD11c, MHC-II, CD11b, F4/80, Ly6C, CD206, CD115, CD107b, Fc ϵ R1, CD80, CD86	Antitumor	(19, 95, 97, 99, 100)
Tolerogenic DCs	Diminished APC, stimulate Th2 responses and Tregs to induce tolerance	PGE2, TGF β , VEGF, IL10, TNF α	TGF β	C1QA, C3AR1, CD163, CD300LF, CFH, CSGALNACT1, Fc γ R11A, Fc γ R11B, P2RY14, ZBT16	SLAM, PDL1, PDL2, DEC205, IDO	Protumor	(99-101)

(Continued on the following page)

Table 1. Innate immune cells in the tumor microenvironment (Cont'd)

Cell type	Normal functions	Stimulatory cytokines in the TME	Cytokine/chemokine secretion	Human markers	Mouse markers	Effect	Source(s)
NEUTROPHILS							
N1	Phagocytosis; release of NETs, inflammatory cytokines, toxin and ROS; respiratory burst, promotion of tumor cell apoptosis	N/A	TNF α , IL1, IFNs, MMP-8, Defensins, Along with toxic substances and reactive oxygen species.	TNF α , I-CAM1, FAS, ROS	TNF α , I-CAM1, FAS, ROS	Antitumor	(23, 30, 33)
N2	Support angiogenesis, cancer cell migration and invasion, immune surveillance, and metastasis as well as secrete chemokines, cytokines and ROS/RNS	TGF β , Angiotensin II	Oncostatin-M, MMP-9, CXCL1, CXCL8, CCL-3, Neutrophil elastase (NE), CXCL6, Collagenase IV, Heparanase, TGF β , PGE2	Arginase, CCL2, CCL5	Arginase, CCL2, CCL5	Protumor	(23, 30, 33)
MDSCs							
M-MDSCs	Suppress innate and adaptive immune responses	CSF-1, CCL2, CCL7, Hif1 α , CXCL1	NO, CCL3, CCL4, CCL5, Arg1, PGE2, IL4	CD11b ⁺ , HLADR ^{lo/-} , CD14 ⁺	Cd11b ⁺ , Ly6C ^{hi} , CD49d ⁺	Protumor	(41-43)
PMN-MDSCs	Suppress innate and adaptive immune responses		ROS, Arg1, PGE2, IL4	CD11b ⁺ , HLADR ⁻ , CD15 ⁺ , CD14 ⁻	Cd11b ⁺ , Gr-1 ^{hi} , Ly6G ⁺ , Ly6C ^{lo}	Protumor	(41-43)
eMDSCs	Suppress innate and adaptive immune responses		N/A	CD33 ⁺ , Lin ⁻ , CD13 ⁻ , CD14 ⁻ CD3 ⁻ , CD6 ⁻	Not well characterized	Protumor	(42)
NK CELLS							
CD56 ^{hi} CD16 ^{lo} \pm NKs	Produce inflammatory cytokines	TGF β , PGE2, IDO, IL10	IFN γ , TNF α	CD16 \pm , CD56, NKG2A, CCR7, CXCR, CXCR3	NKp46, NK1.1, CD122	Depends on tumor type	(48, 51)
CD56 ^{lo} CD16 ^{hi} NKs	Promote antibody-dependent cellular cytotoxicity, high perforin production, enhanced killing	TGF β , PGE2, IDO, IL10	IL22, IL10	CD16 ^{hi} , perforin ^{hi}	Not well characterized	Depends on tumor type	(48, 51)
ILCs							
ILC1 NK Cells	Cytotoxicity, macrophage activation, chronic inflammation, CD8 T-cell activation	N/A	IFN γ , TNF α	CD56, NKp46, NKp44, IL/12RB2, DNAM1	CD56, NKp46, NKp44, IL/12RB2, CD161, TIGIT, CTLA-4, CD96, NKG2A	Antitumor	(57, 62)
ILC1 Non-NK	Macrophage activation, chronic inflammation	N/A	IFN γ , TNF α	ICOS, IL1R, IL/12RB2, CCR6	ICOS, IL1R IL/12RB2	Antitumor	(57, 62)
ILC2	Stimulate T-cell responses through Th2-related cytokines, promotes skin inflammation	IL33, IL25	IL5, IL13	CD117, CD127, ICOS, CD294, IL1R, ST2, IL17RB, CD161, NKp30, PD1, CRTH2	CD127, ICOS, CD294, IL1R, ST2, IL17RB, Sca1, PD1, CRTH2	Protumor and antitumor	(57, 62)
ILC3	Chronic inflammation, intestinal homeostasis, lymphoid development bacterial immunity,	IL23, IL1 β	IL22, IL17, GM-CSF	CD127, CD117, CD25, IL1R, ICOS, IL23R, MHCII, CCR6, NKp44, NKp30, NKp46, CD161	CD127, CD117, CD25, IL1R, ICOS, IL23R, Sca1, MHCII, NKp46, CD161	Protumor	(57, 62)

Abbreviation: N/A, not applicable.

endogenous and exogenous antigens, whereas cDC2s only present exogenous antigens and do not typically perform cross-presentation. cDCs and pDCs are present and active during steady-state conditions, while mDCs tend to only arise during inflammation. DCs specialize in different functions dependent on their stage of maturation and differentiation (Table 1). DCs can localize and acclimate to different tissues such as skin, lung, intestine, and liver and efficiently respond to environmental stimuli (20).

Analogous to M ϕ s, DCs are plastic in nature and can be stratified into specific subtypes. In the context of cancer, DCs are broadly referred to as tumor-infiltrating dendritic cells (TIDC), which will be the predominant focus of this section. TIDCs can be immunogenic or tolerogenic dependent upon environmental signals. Examples of DCs that contribute to immune suppression include CD5^{hi} cDC2s that stimulate Th2, Th17, and T regulatory responses (19). It is important to note that each of the subtypes

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referred to in Table 1 can make up TIDCs that often adopt an immune-suppressive phenotype due to the suppressive nature of the TME.

Tumors classically reprogram their microenvironment to support their survival. In the context of DCs, they do so by secreting cytokines to upregulate transcriptional and metabolic pathways that promote a tolerogenic phenotype, such as those that involve IDO, Arg1, iNOS, and STAT3 (21). These pathways trigger alterations in DC metabolism, metabolite production, energetic shifts, and/or alterations of chromatin accessibility (22). These modifications impact every aspect of DC functionality, including their abilities to secrete inflammatory cytokines and to prime effector T cells. Generally, DCs patrolling the TME encounter immune-suppressive factors such as VEGF, IL10, TGF β , prostaglandin E2 (PGE2), and other cytokines (seen in Fig. 1B) that inhibit DC maturation into immunogenic cells and promote their development into a tolerogenic phenotype, not only stunting their Th1-priming capacities, but also affording them the ability to promote Th2 and T regulatory responses (20). Once removed from the TME, these DCs regain their ability to effectively process antigen and prime T cells (23), demonstrating that stimulating DC inflammatory functions in the TME may be an effective therapeutic strategy.

Further complexity regarding DC plasticity arises when considering different tumor types. DCs have been reported to be tumor-promoting in some TMEs, and tumor-suppressive in others. For example, TIDCs correlate with a positive prognosis in endometrial carcinoma, but not in breast cancer (24, 25). This could be indicative of a tumor stage-dependent phenomenon, that is, DCs are tumor suppressive in early stages and become tumor promoting as the tumor progresses. Furthermore, infiltrating TIDC percentages differ among tumor types, suggesting that TMEs vary in their capacities to potently polarize TIDCs to tolerogenic DCs (26). Adding to this complexity, there are discrepancies among DC phenotypes between subtypes of the same tumor type. For example, transcriptomics of triple-negative breast cancers reveals upregulated IFN pathways for all DC subtypes, whereas this is not the case in luminal breast cancer (27). As such, the DC composition and functionality is tremendously influenced by the tumor type or the tumor subtype and its unique TME.

Neutrophils

Neutrophils account for up to 70% of circulating leukocytes and are the first line of defense against pathogens (28). These cells are typically short-lived, persisting up to five days in circulation (29). Upon tissue damage or infection, epithelial cells secrete neutrophil homing chemokines, compelling them to extravasate from circulation and enter the damaged tissue where they secrete inflammatory cytokines, release neutrophil extracellular traps (NET), and phagocytose invading microorganisms (30). NETs are composed of a chromatin backbone as a vehicle for antimicrobial peptides and toxins and are released as a further method of attack, although to the detriment of the neutrophil (31, 32). In the context of cancer, tumor-associated neutrophils (TAN) also follow the Th1/Th2 paradigm and exhibit an N1 (tumor-suppressive) or N2 (tumor-promoting) phenotype (Table 1). The phenotype of neutrophils in the TME depends on the tumor type and the stage of disease progression. Neutrophils are inflammatory during early tumor stages, but as the tumor progresses, they adopt an immunosuppressive phenotype (33). Neutrophils modulate inflammation via production of reactive intermediates

(ROS/RNS). They also reconfigure the extracellular matrix through secretion of neutrophil elastase (NE) and matrix metalloproteinases (MMP8/9) in the TME and promote angiogenesis (Oncostatin-M), tumor progression (PGE2), and invasion (through the release of ROS/RNS, NE, MMP-9). NETs are comprised of MMPs, cathepsin G, and NE (34, 35). These proteases degrade proinflammatory cytokines and reposition the TME to enhance tumor progression and aid in metastasis (36).

The plasticity of circulating neutrophils is an important feature in patients with cancer. These neutrophils, called high-density neutrophils (HDN) or low-density neutrophils (LDN), correspond to N1 and N2 phenotypes, respectively. In many cancer types, LDNs, which exhibit a more immature phenotype, predominate in the circulation and may contribute to cancer progression and metastasis (29). A detailed understanding of neutrophils and signals that pivot neutrophils to become immune suppressive holds much promise toward reprogramming the TME. This is important given that they are present in the tumor in large numbers. The unique mechanism of NET-osis (NET formation) may prove to be a promising therapeutic target. While preclinical models demonstrate effectiveness of NET targeting, evidence on the clinical front is awaited.

Myeloid-derived suppressor cells

Another cell type that can be found in the TME includes myeloid-derived suppressor cells (MDSC). Some argue that MDSCs are a subtype of neutrophils (33), as there are several overlapping markers between MDSCs and TANs that make distinguishing between these cell types challenging. It is still debated whether MDSCs represent a separate lineage of cells or are polarized immature neutrophils (37). Despite this quandary, MDSCs are defined as, "a heterogeneous population of cells of myeloid origin that comprise myeloid progenitor cells and immature macrophages, immature granulocytes, and immature dendritic cells" (38). Accordingly, MDSCs and TANs clearly differentiate into distinct cell types even though they both stem from myeloid progenitor cells. Other than being hypodense, MDSCs are divergent from neutrophils in several ways, including reduced expression of CD16 and CD62L, and increased expression of Arg1, CD66B, and CD11b (39, 40). MDSCs can be further categorized into subsets: monocytic MDSCs (M-MDSC), which are distinguished by a CD11b^{hi}, LY6C^{hi}, and LY6G^{lo} phenotype, polymorphonuclear MDSCs (PMN-MDSC), which display a CD11b^{hi}, LY6C^{lo}, and LY6G^{hi} phenotype, and early-stage MDSCs (eMDSC) that are CD13⁻ and CD14⁻, and CD33⁺ in humans (41, 42). It is noteworthy that both M-MDSCs and PMN-MDSCs present within the TME have an enhanced suppressive phenotype when compared with MDSCs present within peripheral lymphoid organs, due to increased expression of suppressive molecules by MDSCs in the TME (43).

MDSCs present in the TME contribute to immunosuppression, including T-cell suppression and innate immune regulation, through various mechanisms (Table 1; ref. 43). Furthermore, MDSCs sculpt the primary TME and also initiate formation of the premetastatic niche. In particular, MDSCs enhance tumor cell stemness, increase angiogenesis, and advance the metastatic process by promoting EMT through IL6 secretion (44, 45). MDSCs also are influenced by the TME (Fig. 1B) that further perpetuates their inherent immunosuppressive functions. For example, HIF-1 α , a key player in the hypoxic tumor microenvironment, aids in MDSC differentiation to tumor-promoting TAMs (46). Also,

factors in the TME can alter the metabolism of MDSCs toward fatty acid oxidation, prompting an upregulation of Arg1 and NOS2 production (47). The critical role of MDSCs in tumorigenesis, growth, the establishment of the premetastatic niche, and metastatic outgrowth warrants the need to effectively target them by depletion or blockade. Although their critical role in the survival and advancement of tumors is well known, there are currently no FDA-approved drugs or therapies that directly target MDSCs.

Natural killer cells and natural killer T cells

NK cells are circulatory, innate lymphoid cells recognized for their cytotoxic effector functions. Classically, there are two subsets of NKs defined by their expression of CD16 and CD56 levels: namely, CD56^{hi} CD16[±] and CD56^{lo} CD16^{hi} (48). CD56^{hi} CD16[±] NKs secrete inflammatory cytokines, whereas CD56^{lo} CD16^{hi} NKs specialize in cytotoxic functions and cell-mediated killing. Within the cancer framework, these cells are extremely efficient in eliminating malignant cells and limiting tumor metastases (49). Their significance in tumor surveillance is illustrated by a correlation between low NK-cell activity and increased cancer risk (50). NKs employ death receptor-mediated apoptosis and perforin/granzyme-mediated cytotoxicity to target tumor cells and limit primary tumor growth (51). While NKs characteristically destroy circulating tumor cells, they are much less efficient at cell killing within the TME. Tumors deploy many mechanisms to evade destruction by NKs, including coating themselves in collagen to engage inhibitory NK receptors and utilizing platelets as a shield to avoid NK detection (52). Within the TME, both NK subsets exhibit reduced inflammatory cytokine production and reduced or no cytotoxicity and both subsets will be referred to collectively as tumor-infiltrating natural killer cells (TINK). Many cytokines commonly present in the TME diminish NK effector functions (Table 1). These cytokines can stunt the cytotoxicity of TINKs (Fig. 1B), which not only display diminished cytotoxicity, but also contribute to arresting the proliferation and expansion of T cells, enhancing their immune-suppressive properties (these cells are often referred to as NKregs as well). Future efforts for developing therapeutic approaches could consider augmentation of cytotoxic NKs and/or targeting of TINKs. It is tempting to speculate that administration of NKs may enable a cancer-preventative approach, or at the very least, a metastasis-preventative approach as NKs are extremely efficient at targeting circulating cancer cells.

Also prevalent in the TME are natural killer T cells (NKT), which are CD1d restricted, innate-like T lymphocytes that, like T cells, possess a T-cell receptor, and like NKs, respond quickly to antigenic exposure (53). Also, like T cells, overstimulation of NKTs can render them anergic. There are two major types of NKTs, type I NKTs (NKT1) and type II (NKT2) cells, which are characterized by their distinct T-cell repertoires. While NKT1s express the Va14Ja18 invariant TCR alpha chain, the T-cell repertoire of NKT2s is less defined (54). Both types can be dissected into further subsets that reflect the T-cell subsets that play inflammatory or immune-suppressive roles in the context of the TME. Specifically, NKT1s can be divided into Th1-like, Th2-like, Th17-like, Treg-like, and T follicular helper (TFH)-like NKTs; and NKT2s can be divided into Th1-like and Th2-like NKTs. Furthermore, NKTs are reported to switch back and forth between inflammatory and immune-suppressive subsets in response to their environment. In particular, NKT1s are typically antitumor, whereas NKT2s are predominantly protumor. NKT1s have been reported to prevent metastatic

breast cancer (55) in mouse models. However, NKT2s have been reported to support MDSCs in a B-cell lymphoma mouse model (54, 56). As such, targeting NKT2s and supplementation with NKT1s may provide an exciting therapeutic approach.

Innate lymphoid cells

Another crucial component of the TME is the ILCs that have characteristics similar to those of NK cells. ILCs share a common lymphoid progenitor with B and T cells, but lack B- and T-cell receptors and are thus classified as innate immune cells (57). ILCs contribute to T-cell polarization through antigen presentation and cytokine secretion (58). There are three types of ILCs (ILC1, ILC2, and ILC3) classified on the basis of their production of Th1, Th2, and Th17-based cytokines and distinct transcription factors. (59). ILC1s tend to exhibit antitumor functions through cytokine production (mainly IFN γ). Furthermore, ILC1s can be divided into NK ILC1s and non-NK ILC1s based on their expression or lack thereof of the NK-specific transcription factor, Eomesodermin. Importantly, NK ILCs can be distinguished from conventional NKs by differences in transcriptional regulation, phenotype, and localization as described by Seillet and colleagues (60). While ILC2s can functionally either promote or antagonize tumor growth depending on the tumor type (Fig. 1), ILC3s are classically protumorigenic. ILC polarization is determined by the composition of each specific TME (Table 1). As such, ILCs are differentially associated with different tumor types, likely because different tumor types have distinct TME compositions; for example, ILC2s are typically found in the TME of breast and gastric cancer, ILC3s are implicated in colon cancer (61, 62), and ILC1s prevent melanoma growth through the production of inflammatory cytokines (63, 64). ILC3s may differentiate into ILC1s upon IL12 stimulation, and ILC1s may differentiate into ILC3s upon stimulation by retinoic acid and IL23 (62). The conversion of ILC1 to ILC3 stunts their ability to aggressively target the tumor. This plasticity offers an attractive opportunity for therapeutically reprogramming ILC3s to ILC1s.

Immune cells and other components of the microenvironment

While the importance of direct interactions between tumor cells and immune cells is clear, it is also noteworthy to mention that immune cell interactions with other components in the TME can impact tumor fate. For example, it has been reported that the extracellular matrix can play both supportive and inhibitory roles to the adaptive immune response by providing migratory pathways that allow T cells to invade the tissue or by directly inhibiting T-cell proliferation, respectively (65). Also, lymphatic vessels can regulate the immune microenvironment. Lymphatic vessels have been linked to providing nutrients to tumors through increased angiogenesis. They may also serve as migratory highways for immune cells (66), and lymphatic endothelial cells have also been reported to directly regulate DC activation (67). Immune cells also interact with stromal cells, including cancer-associated fibroblasts (CAF). CAFs exhibit wound-healing properties and have been implicated as contributors to tumor proliferation, invasion, and metastasis. CAFs may secrete immune-suppressive cytokines that polarize M ϕ s to the M2 phenotype and contribute to CD8⁺ T-cell exhaustion and deletion (68).

These observations indicate a complex series of interactions between immune cell types and nontumor cells within the TME that clearly impact tumor progression, invasion, and metastasis. Therefore, not only should therapy designs consider tumor-

immune cell cross-talk and tumor–stromal cross-talk, but also stromal–immune cell cross-talk as it contributes significantly to tumor development.

Current and future therapeutics

The tumor masterfully controls its surrounding environment to promote its establishment, growth, survival, and spread. One of the chief ways it does this is through reprogramming innate immune cells to foster tumor growth and survival, leaving the patient with a weakened defense and often a worse prognosis. This is a potential Achilles heel of the tumor; as such, reprogramming the innate immune system is a potentially important approach to improve patient outcomes.

Macrophage therapies

Previous clinical trials targeting Mφs in the TME have been unsuccessful. Many prior trials involved the activation and injection of Mφs into patients with cancer using various activation methods such as IFN γ , mifamurtide, and LPS, but none of these methods were therapeutically efficacious (69–71). There have been some promising clinical trials utilizing anti-M-CSFR antibodies. One such example includes the administration of RG7155, an anti-M-CSFR antibody, to diffuse-type giant cell tumor (Dt-GCT) patients. This strategy led to decreased TAM infiltration and overall positive patient responses (72). It is noteworthy that anti-M-CSFR antibodies have yet to be successful in glioblastoma models, and there is still work to be done on this front. Ongoing clinical trials that target Mφ receptor, CSF-1R, and the CCL2–CCR2 signaling axis ablate tumor-infiltrating Mφs and show promise in advanced solid tumors (73). Moreover, the efficacy of CSF-1R inhibition is vastly improved when combined with receptor tyrosine kinase inhibitors. In addition to targeting CSF-1R and the CCL2–CCR2 signaling axis, there are ongoing clinical trials targeting CXCL12/CXCR4, CD40, and angiopoietin1/2 (74). Treatment with IFN α has yielded favorable outcomes in patients with melanoma. IFN α promotes an inflammatory environment, stimulates Mφs toward an M1 type, and has been demonstrated to reduce tumor growth and diminish metastasis (75).

DC therapies

Targeting DC activation via DC vaccination is another therapeutic option. An important consideration in using DC vaccinations as cancer treatment is the method of priming DCs with tumor antigen. Options including priming with whole tumor cells, tumor cell lysate, apoptotic bodies, exosomes, or DNA or RNA need to be considered when designing an effective DC vaccine (76–78). Thus far, whole-cell vaccines seem to be the most promising. Several DC vaccination trials are currently ongoing (clinicaltrials.gov). One trial (NCT01204684) involves enrichment of DCs from patients with glioma, pulsation with tumor lysate, and autologous intradermal injection. In their phase I clinical trial, Hus and colleagues primed DCs from patients with B-cell chronic lymphocytic lymphoma with tumor lysates and autologously vaccinated patients with these primed DCs (79). This strategy resulted in an increase in cytotoxic T-cell response. An example of a successful DC-based therapy for prostate cancer is Provenge. The regimen for Provenge therapy involves harvesting monocytes from prostate cancer patients, differentiating and activating them *in vivo* with PAP antigen, and introducing them

back into the patient. This therapy has achieved significant success marked by diminished tumor burden in patients with prostate cancer. A new DC vaccine targeting glioblastoma is DCVax-L that includes autologous DCs loaded with glioblastoma tumor lysate. This vaccine has been tested in a phase III clinical trial for glioblastoma, and overall patient survival was shown to increase by 6 months (80).

Despite success with DC vaccinations, there are challenges associated with them, including high cost, the absence of universal vaccine, the need for massive amounts of DCs, and issues with polarizing conventional DCs *in vitro*. Previous attempts at DC vaccinations focused on moDCs that are rare and do not functionally resemble cross-presenting DCs *in vivo* (81). It is now recognized that cDCs comprise the DC subtype that is most likely to come into contact with cancer cells in the TME and mount the ensuing immune response. While cDCs are challenging to isolate, a cDC vaccine for melanoma has been reported to elicit a cytotoxic T-cell response making them functionally more relevant (82). Further work is required to standardize methods to effectively isolate cDCs for antigen loading and DC vaccination. A new focus for DC therapy involves directly targeting them *in vivo*. *In vivo* delivery of antibodies to cDC1 receptors conjugated to tumor antigens results in better DC activity and a higher rate of primed T cells. This is expected to reduce treatment costs due to the universality of the therapy and improve therapeutic effectiveness because DCs *in vivo* are already at the tumor site (in contrast to direct tumor injections that are not always possible or effective depending on tumor type). Combining this approach with immune checkpoint inhibitor blockade therapy will allow for rapid, effective T-cell priming without T-cell exhaustion.

Neutrophil therapies

There are ongoing efforts to target neutrophils in the TME. Preclinical models have yielded optimistic success in reducing neutrophil number by squelching G-M-CSF from the TME. Reparixin is a noncompetitive allosteric inhibitor of CXCR1 and CXCR2 (83) and targets neutrophil maturation to inhibit the immunosuppressive impact of tumor-induced N2 neutrophils. Reparixin is currently in one phase I and two phase II clinical trials for metastatic breast cancer. Targeting neutrophil polarization is another enticing therapeutic option through TGF β inhibitors (84). While there are currently many clinical trials that use TGF β inhibitors, off-target effects, and cytotoxicity have been reported (85).

MDSC therapies

There are currently several ongoing clinical trials that target MDSCs in different cancer types including leukemia, melanoma, glioblastoma, and breast cancer (86). These trials utilize different mechanisms of indirectly impacting MDSC function, including targeting Arg1, iNOS, and STAT3 activities, metabolism through CD36, and trafficking through CXCR2 (86). MDSC depletion is another tested avenue for cancer therapeutics. There has been some success in triggering MDSC apoptosis with gemcitabine and 5-fluorouracil, correlating with diminished tumor growth. Docetaxel, doxorubicin, paclitaxel, and tyrosine kinase inhibitors have also been demonstrated to reduce the numbers and effectiveness of MDSCs in the TME (86). There also are therapies targeting MDSCs in combination with immune checkpoint inhibitors. A phase I/II clinical trial in patients with renal cell

carcinoma using atezolizumab and a histone deacetylase inhibitor shows promise (NCT03024437). Also, a phase II clinical trial in patients with melanoma combines ipilimumab and ATRA, which blocks retinoic acid signal transduction, leading to the differentiation of MDSCs into Mφs and DCs (NCT02403778). ATRA alone also leads to a reduction of MDSC frequencies in small-cell lung cancer (87, 88). While these trials show moderate yet encouraging success, off-target effects of these drugs may contribute to diminished therapeutic efficacy.

NK-cell therapies

Multiple enduring clinical trials aim to stimulate the immune system with NK-cell therapy. For example, there is a phase I trial targeting advanced biliary tract cancer via allogeneic NK injection (NCT03358849). Yang and colleagues pioneered allogeneic NK-cell therapy by activating allogeneic NKs with IL2, followed by administration to patients with advanced lymphoma (89). The results revealed diminished T-reg and MDSC populations and increased expression of NKG2D on cytotoxic T cells (90). NK-cell therapy in combination with chemotherapy for small-cell lung cancer (NCT03410368) is also an effective strategy (91). Also, the use of CAR-NK cells, genetically engineered cells that directly target tumor-specific antigens in an HLA-unrestricted manner, has shown favorable outcomes in preclinical studies for B-cell malignancies, ovarian, breast, prostate, and colon cancers (92). All of these approaches have exhibited varying degrees of positive outcomes, but they also are limited by toxicity and detrimental side effects, high cost, and low efficacy (51, 93). In contrast, there have been few successful clinical trials for ILC therapy in cancer.

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Conclusion

Each of the therapeutic approaches discussed in this review has focused on targeting one aspect of the immune system. While some of these treatments yield positive outcomes, a more definitive and likely more effective approach involves altering multiple facets of the TME through a strong inflammatory response by promoting the inflammatory innate immune cells. There are multiple strategies that target immune-suppressive cells, but unfortunately many of these responses are important for self-tolerance mechanisms and aid in protection against autoimmunity. Targeting immune-suppressive cells cannot focus on a global depletion of all innate cells in the TME as this could cause dire effects in the host. The solution must be an intricate combination that involves selective inhibition or depletion of robust tumor-suppressive cytokines and cell types in addition to bolstering the inflammatory phenotype of immune cells.

Disclosure of Potential Conflicts of Interest

L.A. Shevde is a consultant/advisory board member for NIH and CDMRP. No potential conflicts of interest were disclosed by the other author.

Acknowledgments

We acknowledge funding from the Department of Defense (W81XWH-14-1-0516 and W81XWH-18-1-0036), NCI R01CA169202, and The Breast Cancer Research Foundation of Alabama (BCRFA; to L.A. Shevde). We would also like to acknowledge Will Jackson who contributed to figure development and conceptualization of this work, Dr. Rajeev Samant, Dr. Ann Hanna, Brandon Metge, and Sarah Bailey for editorial suggestions, and the UAB O'Neal Comprehensive Cancer Center (P30CA013148).

Received December 27, 2018; revised February 28, 2019; accepted May 21, 2019; published first July 26, 2019.

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