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Monocytes in the Tumor Microenvironment

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Abstract

Immunotherapy has revolutionized cancer treatment over the past decade. Nonetheless, prolonged survival is limited to relatively few patients. Cancers enforce a multifaceted immune-suppressive network whose nature is progressively shaped by systemic and local cues during tumor development. Monocytes bridge innate and adaptive immune responses and can affect the tumor microenvironment through various mechanisms that induce immune tolerance, angiogenesis, and increased dissemination of tumor cells. Yet monocytes can also give rise to antitumor effectors and activate antigen-presenting cells. This yin-yang activity relies on the plasticity of monocytes in response to environmental stimuli. In this review, we summarize current knowledge of the ontogeny, heterogeneity, and functions of monocytes and monocyte-derived cells in cancer, pinpointing the main pathways that are important for modeling the immunosuppressive tumor microenvironment.

1. INTRODUCTION

The clinical results obtained with immune checkpoint inhibitors (ICIs) (1) and chimeric antigen receptor T cells (2) have changed the landscape of tumor treatment, highlighting immunotherapy as the latest breakthrough. There is a general consensus pinpointing T cells as the major players in the antitumor response. Indeed, the evaluation of T lymphocyte density (i.e., cell quantity), spatial localization (i.e., invasive margin, tumor core, or tertiary lymphoid structures), cell types (i.e., T helper cells and cytotoxic, memory, and exhausted T cells), and functional immune orientation stimuli (i.e., adhesion receptors, chemokines, cytokines) within the tumor could predict survival in colorectal cancer more accurately than the classical staging systems (3). Indeed, by combining immunohistochemical analysis and gene profiling data for tumor-infiltrating T cells, a new parameter, called an Immunoscore, has been developed to estimate the adaptive immune composition of the tumor microenvironment (TME) and validated as a predictive biomarker in several malignancies (4). Based on their Immunoscore, tumors may be segregated into four major groups: hot, altered-excluded, altered-immunosuppressed, and cold. Immune deserts (cold tumors) are characterized by the absence of T cell infiltration due to a lack of the cells and/or mechanisms required for T cell priming or activation (i.e., low tumor mutational burden and poor antigen presentation). A tumor with an immunosuppressed TME is instead defined by an intermediate infiltration of exhausted T cells [i.e., T cell immunoglobulin and mucin domain-containing protein 3 (TIM3)–expressing cells] and a high density of soluble inhibitory mediators [e.g., interleukin (IL)-10] and immune-suppressive cells [e.g., regulatory T cells (Tregs)]. T cell exclusion inside the tumor core, induced by the presence of aberrant vasculature and fibrotic nets, is the main feature of an altered-excluded immune tumor. Finally, immune-replete tumors (hot tumors) are characterized by high infiltration of programmed cell death 1 (PD-1)- or cytotoxic T lymphocyte–associated antigen 4 (CTLA4)-expressing cytotoxic T lymphocytes (CTLs) and tumor cells expressing costimulatory or drug-targetable molecules [e.g., programmed death-ligand 1 (PD-L1)] that are able to maintain T cell fitness (4, 5). Interestingly, hot tumors are also characterized by the presence of local inflammation, and, moreover, they respond to immunotherapy (5). As expected, T cell composition inside the TME partially depends on the mutational landscape of the tumor (6). In fact, oncogene activation in cancer cells favors the secretion of soluble factors (i.e., growth factors, cytokines, and chemokines) that alter the TME to favor T cell migration and localization inside the cancer core; the aberrant expression of neoantigens, potential immunotherapy targets, can fuel antitumor responses by activating CTLs (7). However, oncogenes can also activate immune regulatory circuits that have a negative impact on adaptive immunity. For instance, in LSL-Kras$^{G12D/+}$; LSL-Trp53$^{R172H/+}$; Pdx1-Cre mice (the KPC mouse model), the simultaneous expression of mutant KRAS^{G12D} and mutant p53^{R172H} in pancreatic epithelial cells promotes tumor growth and increases the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), which favors the accumulation of immunosuppressive myeloid cell subsets (8).

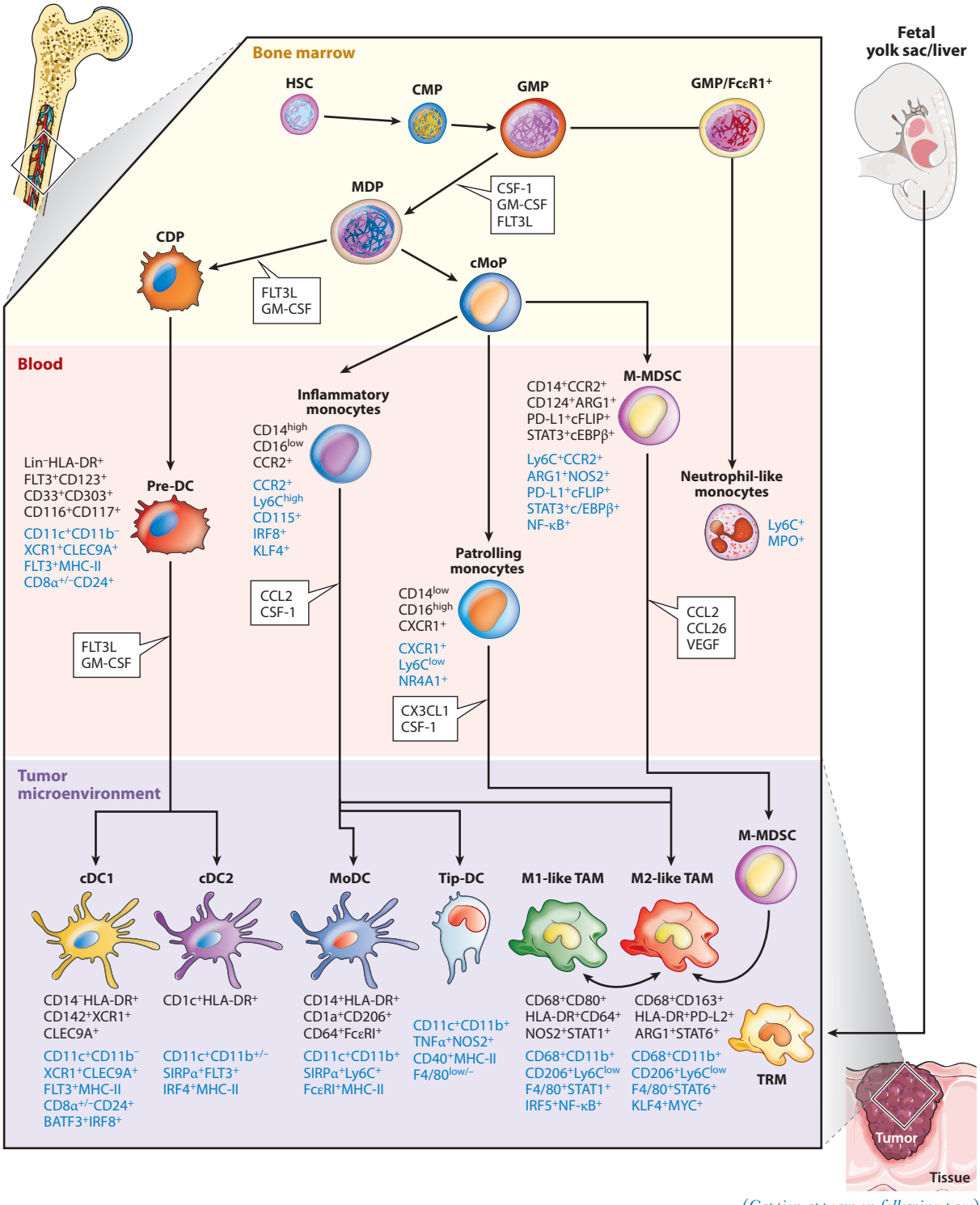
Although the correlation between cancer genotype and infiltration of adaptive immune cells is quite solid in preclinical models, a deeper understanding of cancer–immune cell cross talk is still lacking. Despite the success of cancer immunotherapy, the majority of cancer patients currently do not benefit from immune-based treatments. A great deal of evidence suggests that myeloid cells have a direct role in promoting, supporting, and maintaining tumor growth, either through direct interplay with cancer cells, manipulation of T cell composition and activity (e.g., inhibition of T cell migration or activation), or alteration of the stromal architecture (e.g., matrix protein remodeling) (9, 10). In particular, monocytes serve as a principal source of long-lived TME-infiltrating cells such as macrophages and dendritic cells (DCs), and during cancer progression, they contribute to cancer immune evasion by differentiating into immune regulatory cells. Interpreting

the heterogeneity of monocytes and their roles at different stages of cancer progression is essential for improving our knowledge of TME composition and evolution. In this review, we discuss our current understanding of the tumor-dependent mechanisms that shape myeloid cell polarization, differentiation, and function by altering precise molecular pathways.

2. TUMOR-INDUCED ALTERNATIVE MYELOPOIESIS

As occurs during systemic bacterial infections, myeloid cell turnover is dramatically boosted during cancer progression. The recruitment of myeloid cells to the primary tumor site is supported by the continuous production and mobilization of cells from the bone marrow (BM), causing a state of emergency in which monocytes can be generated from alternative pathways or precursors (11). Given the dynamics of recruitment and differentiation into different myeloid lineages and the involvement of the BM niche, the myeloid circuits affected by tumors are not just local and confined to the TME but spread systemically. The accumulation of cells in the circulation, in addition to the TME, is a sign of the corrupted promyelopoietic process induced by tumors (12) (**Figure 1**).

The classical view of hematopoiesis implies that hematopoietic stem cells (HSCs) in the BM give rise to heterogeneous multipotent progenitors (MPPs), which in turn differentiate in a colony-stimulating factor (CSF)-1-dependent manner (13) into common myeloid progenitors (CMPs). Granulocyte and monocyte precursors (GMPs), originating from the CMPs, differentiate into monocytes through a series of steps, going from monocyte and dendritic cell precursors (MDPs) to unipotent common monocyte progenitors (cMoPs) (14, 15). Differentiated monocytes circulating in the blood can be divided into two main subpopulations in mice, $Ly6C^{hi}CX3CR1^{low}$ (classical) and $Ly6C^{low}CX3CR1^{hi}$ (nonclassical) cells, and into three subsets in humans, $CD14^{hi}CD16^{low/-}$ (classical or inflammatory), $CD14^{low}CD16^{hi}$ (nonclassical or patrolling), and $CD14^{hi/mid}CD16^{+}$ (intermediate). However, recent single-cell and family tracing methodologies, including single-cell RNA sequencing (RNA-seq), mass cytometry, and cellular barcoding, raise questions about the classical branching nature of the hematopoietic tree and suggest that lineage commitment is already present in oligopotent progenitors (16). Specifically, this redefined model envisions a developmental shift in the progenitor cell architecture from the fetus (where many stem and progenitor cell types are multipotent) to the adult (where the stem-cell compartment is multipotent, but the progenitors are unipotent) and provides a revised framework for understanding hematopoiesis in health and disease. Seminal work defined the role of hematopoietic cytokines and growth factors in shaping hematopoiesis (17). Under steady-state conditions, myeloid lineage commitment and differentiation are orchestrated by both lineage-specific cytokines, mainly CSFs (such as CSF-1, CSF-2, and CSF-3) and transcription factors (TFs). Focusing on monopoiesis, CSF-1 receptor (CSF-1R), which binds the ligands CSF-1 and IL-34, regulates the development of monocytes in the BM and, therefore, is considered the master regulator of monocyte differentiation (18). In mice deficient for CSF-1R and CSF-1, the number of monocytes in the blood is profoundly reduced (18). In patients with solid cancers, hematopoiesis is generally perturbed, with a bias toward myeloid differentiation at the expense of erythroid and lymphoid precursor polarization (19). Besides the function of the lineage-commitment cytokines mentioned before, other tumor-secreted, proinflammatory cytokines [e.g., IL-1β, IL-6, interferons (IFNs), and tumor necrosis factor-α (TNF-α)] and Toll-like receptors (TLRs) can influence the rate and route of HSC differentiation (20). This can lead to the accumulation of cells with immunosuppressive activity, primarily myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory DCs (21). In the MMTV-PyMT mouse model of spontaneous breast cancer, the release of CSF-2 by tumor cells was shown to induce the expansion of



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Tumor-induced emergency myelopoiesis and its impact on the bone marrow, peripheral blood, and tumor microenvironment. Tumors produce and secrete a variety of soluble factors that affect steady-state myelopoiesis. Text next to or below the cell types shows human phenotypes (*black text*) and/or mouse phenotypes (*blue text*). Abbreviations: ARG, arginase; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; cDC, conventional dendritic cell; CDP, common dendritic cell progenitor; c/EBP, CCAAT/enhancer-binding protein; cFLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; cMoP, common monocyte progenitor; CMP, common myeloid progenitor; CSF, colony-stimulating factor; CX3CL, C-X3-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; FLT3L, Flt3 ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMP, granulocyte and monocyte progenitor; HLA-DR, human leukocyte antigen DR; HSC, hematopoietic stem cell; IRF, interferon response factor; KLF, Krüppel-like factor; MDP, monocyte and dendritic cell progenitor; MHC-II, major histocompatibility complex II; M-MDSC, monocytic-myeloid derived suppressor cell; MoDC, monocyte-derived dendritic cell; NF-κB, nuclear factor κB; NOS, nitric oxide synthase; PD-L, programmed cell death ligand; pre-DC, dendritic cell precursor; STAT, signal transducer and activator of transcription; TAM, tumor-associated macrophage; Tip-DC, TNF-α/inducible (i)NOS-producing dendritic cell; TNF, tumor necrosis factor; TRM, tissue-resident macrophage; VEGF, vascular endothelial cell growth factor; XCR, X-C motif chemokine receptor. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

HSCs to replenish the short-lived MDSCs (22). Similarly, in Lewis lung carcinoma, the increased signaling of insulin-like growth factor type 1 receptor (IGF-1R) in HSCs leads to a skewed differentiation of $Lin^{-}Sca1^{+}CD117^{+}$ (LSK) progenitors toward myeloid cells with a suppressive phenotype (23). Even though this is true for some tumors, the majority of solid tumors do not secrete hematopoietic cytokines, yet they still present with altered hematopoiesis. In this scenario, the altered hematopoiesis can be supported by TNF-α released by immune cells, mainly $CD4^{+}$ T cells, in several mouse models of tumorigenesis. Depletion of $CD4^{+}$ and $CD8^{+}$ T cells was shown to normalize the number of LSKs and CMPs, resulting in a significant reduction in MDSCs and associated immunosuppression. Alternatively, the loss of TNF-α in TNF receptor (TNFR)1/2-deficient mice or the neutralization of TNF-α by blocking antibodies normalized hematopoiesis and reduced the number of peripheral granulocytic (G)- and monocytic (M)-MDSCs (24, 25). Moreover, TNF-α not only increases the number of MDSCs by regulating HSCs but also directly enhances MDSC survival through cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP)-mediated inhibition of caspase-8 (26), suggesting that targeting TNF-α could restore cancer immune surveillance and enhance the efficacy of cancer immunotherapy (27).

In addition to being affected by hematopoietic cytokines and growth factors, HSCs can also be modulated by trained immunity, at least during inflammation and sepsis. Through epigenetic and metabolic switches, administration of β-glucan to mice induces an expansion of myeloid progenitors, mainly LSKs and MPPs, which then differentiate into myeloid cells that secrete proinflammatory mediators such as IL-1β, CSF-2, TNF-α, and IL-6, providing a robust and quick (e.g., trained) response to subsequent insults (28). CSF-2 was also shown to be a stimulus for trained immunity, resulting in increased TNF-α production upon subsequent lipopolysaccharide stimulation through a mechanism dependent on the MAPKs ERK1 and ERK2 (29). Like in inflammation and sepsis, a similar program may also occur in cancer. In this context, the function of MDSCs is determined by their epigenetic program, including DNA methylation, histone modifications, and modulatory noncoding RNAs (30). A similar process occurs when monocytes infiltrate tumors and differentiate into TAMs that promote tumor growth and suppress antitumor immune responses (31). Epigenetic reprogramming is a central feature of TAM differentiation; long-term histone modifications, such as changes in H3K4me3 and H3K9me3, underlie and induce a protumorigenic profile in these cells (31). Rewiring the epigenetic and functional program of MDSCs, TAMs, or both by inducing trained immunity may be a compelling option for cancer immunotherapy (28). While the exact impact of trained immunity on HSC precursors has not been fully clarified in the context of cancer, and in human cancer in particular, new data suggest that targeting

trained immunity, in particular myeloid-biased progenitors in the BM, may facilitate the induction of durable, reliable, and precise responses without severe immune-related adverse effects.

As mentioned earlier, the commitment toward monocyte differentiation is determined by major lineage-determining TFs, namely PU.1, CCAAT/enhancer-binding protein (c/EBP)β and c/EBPα. Together with IFN regulatory factor (IRF)8 and Krüppel-like factor (KLF) 4, PU.1 binds to chromatin to coordinate the activation of genomic regions necessary for monocyte origin and differentiation. Indeed, mice lacking PU.1 are not vital and the transfer of stem cells carrying the genetic ablation of PU.1 favors a skewed myelopoiesis with a profound contraction in monocytes and DCs and an expansion of lymphocytes (32). Moreover, together with IRF8, PU.1 binds to promoters and enhancers supporting the monocyte genetic program. IRF8-deficient mice accumulate monocyte-committed progenitors and monoblasts that are unable to differentiate into mature monocytes, suggesting that IRF8 is dispensable for monocyte lineage commitment but regulates later maturation stages (33). Additionally, IRF8 affects the expression of monocyte genes through the direct activation of the TF KLF4, induces the activation of enhancers to sustain the expression of monocyte-related genes, and interacts with c/EBPα to inhibit the granulocyte differentiation program (11).

Proteins of the c/EBP family can have opposing impacts on the development of myeloid cells (11). Indeed, while c/EBPα inhibits the development of nonmyeloid cells by blocking the function of their specific TFs, c/EBPβ must contribute directly to monopoiesis since mice lacking c/EBPβ have a drastic reduction in the number of circulating monocytes (34). Of note, c/EBPβ controls altered myelopoiesis in cancer, contributing to the accumulation of MDSCs and the maintenance of an immunosuppressive TME (35). Fate-mapping analysis and in-depth RNA interrogation at the single-cell level have revealed what seems to be an endless series of monocyte progenitors, hinting that the activation of the monocyte gene-specific lineage is sustained by TFs acting in a combinatorial way and is functionally controlled by autoregulatory loops (34). Whether the TFs themselves are regulated by environmental or intrinsic signals is still a matter of debate. Transcriptomic data identified an RNA-binding protein named DZIP3 as an E3 ubiquitin ligase that might regulate monocyte development by controlling TF access to lineage-specific promoter and/or enhancer regions in progenitor cells (36). Recently, single-cell RNA-seq revealed zinc finger E-box-binding homeobox 2 (Zeb2) and GATA2 as TFs implicated in monocyte differentiation, with Zeb2 deletion leading to the depletion of Ly6C^{hi} monocytes in the BM and GATA2 mutations being associated with monocyte deficiencies in humans (37, 38).

During emergency hematopoiesis, like in cancer or inflammation, there is an accumulation of monocytes that have escaped the canonical MDP-cMoP-monocyte developmental pathway and acquired similarities to neutrophils, such as the neutrophil-like Ly6C^{hi} monocytes originating from GMPs (39). Interestingly, GMP-derived neutrophil-like Ly6C^{hi} monocytes have a high level of expression of growth factor independent 1 (Gfi1), whose normal role is to sustain granulopoiesis (39). Yet, whether Gfi1 plays a role in the functional programming of these monocytes, such as by regulating the expression of intracellular granule content, remains to be determined. Intense investigation is ongoing to clarify whether other noncanonical monocytes also play a role in cancer. This is the case for segregated nucleus-containing atypical Ly6C^{low} monocytes (SatMs), a monocyte subset that appears during lung fibrosis (40). Fate-mapping studies and extensive proteomic analysis revealed that SatM cells derive from GMP FcεR1^{+} progenitors, are characterized by a highly active c/EBPβ-associated gene program, and are also rich in granules containing myeloperoxidase and neutrophil elastase, similar to neutrophils (40). At present, the lack of surface markers to unequivocally distinguish SatM (Ly6C^{low}Ceacam1^{hi}Msr1^{hi}) cells from nonclassical Ly6C^{low} monocytes and neutrophil-like Ly6C^{hi} monocytes from classical Ly6C^{hi} monocytes

prevents these populations from being assessed in primary and metastatic tumors, as well as the complete tracking of their ontogeny in cancer in both humans and mice.

Epigenetic modifications represent another layer for hematopoiesis regulation, including but not restricted to both distribution and access of TFs to chromatin. Even though our knowledge of how and when epigenetic modifications occur during both steady-state and cancer-driven emergency myelopoiesis is just being defined, some paradigms are emerging. Mice with reduced DNA (cytosine-5)-methyltransferase (DNMT)1 activity have only myelo-erythroid cells and lack lymphoid progeny (41). Moreover, CMPs differentiate into megakaryocyte-erythroid lineages when histone deacetylase 1 (HDAC1) expression is sustained by GATA1, whereas when HDAC1 expression is downregulated by c/EBP TFs, committed CMPs give rise to myeloid cells, in particular granulocytes (42). Among several epigenetic modifications, DNA methylation is one of the most studied in the context of hematopoiesis, being involved in regulating HSC self-renewal, facilitating the commitment to lymphoid or myeloid progeny, and establishing the identities of differentiated cell types (11). DNMT1 is indispensable for protecting HSCs from the premature activation of differentiation programs (41), and DNMT3A and DNMT3B are required to silence the expression of TFs related to self-renewal and multipotency, such as RUNT-related transcription factor 1 (RUNX1) and GATA3 (43). In addition, analysis of the epigenome of HSCs has revealed that genes encoding TFs that are important for hematopoietic cell differentiation, such as c/EBPα, PU.1, and paired-box protein (PAX)5, have low levels of DNA methylation and are enriched in both activating H3K4me3 and repressive H3K27me3 modifications (44). Myelo-monocytic cells are evolutionarily more ancient than the lymphoid compartment, and the DNA methylation patterns of HSCs resemble those seen in myeloid cells rather than lymphoid cells (41), suggesting an intrinsic myeloid bias of the HSC methylome (45).

3. THE TUMOR MICROENVIRONMENT SHAPES MONOCYTE DIFFERENTIATION IN SITU

Monocytes are circulating cells able to migrate inside tissues in response to damage signals (46). Similarly, monocytes can be recruited to the TME, where they locally differentiate (47, 48) (**Figure 1**). However, the cues that drive the monocyte fate decision between remaining a monocyte, further differentiating, or undergoing programmed cell death are still not completely known. Recent data from RNA-seq on myeloid cell composition in the TME of human breast cancers highlight a monocyte-activation gene signature that supports the existence of a trajectory from blood monocytes to intratumoral monocytes and, more interestingly, to different myeloid cell subsets (49). In general, TME-infiltrating, monocyte-derived cells can be grouped into three main subsets: TAMs, tumor-associated DCs (TADCs), and MDSCs.

3.1. Tumor-Associated Macrophages

TAMs are the most prominent myeloid cell subset inside the TME (50). TAMs have an intrinsically heterogeneous nature, and their presence is generally associated with poor prognosis in different tumors (51), even though in some cancers, such as endometrial cancer, a clear correlation between TAM infiltration and improved cancer-patient survival has also been described (52). Recently the heterogeneity of breast (53), lung (47), liver (54), and renal (55) carcinoma patients' immune ecosystems was deeply mapped by single-cell sequencing able to identify up to 17 different TAM clusters, each characterized by a specific genetic profile. In liver cancer, for example, TAM-like cells highly express a marker gene, *SLC40A1*, that encodes ferroportin, an iron exporter that is able to regulate the release of proinflammatory cytokines including IL-6,

IL-23, and IL-1β via TLR-mediated signaling. Moreover, this peculiar TAM-associated gene signature is significantly associated with a survival disadvantage for patients, highlighting the role of iron metabolism in shaping innate immunity into a protumor component (54). In breast cancer, TAM infiltration correlates with tumor aggressiveness, as evidenced by a greater macrophage presence in the more aggressive luminal B tumors compared to the luminal A subtype. Notably, in luminal B tumors, estrogen receptor–positive cancer regions are massively infiltrated by immunosuppressive PD-L1/CD38-expressing TAMs, which are preferentially located in the inner space of the tumor while monocytes are present in the juxta-tumoral tissue (53). These spatial and genetic features of TAMs could partially explain the limited success of ICI-based therapy in estrogen receptor–positive breast-cancer patients. Interestingly, TAM molecular signatures from KEP or NeuT transgenic mice, whose phenotypes resemble human invasive lobular carcinoma (ILC) and HER2$^+$ tumors, respectively, are unique, suggesting that the cancer subtype dictates the TAM phenotype (56). Furthermore, the KEP-derived TAM signature is consistently correlated with poor overall survival in ILC but not in triple-negative breast-cancer patients, indicating that translation of mouse TAM signatures to patients is cancer-subtype dependent (56).

TAMs can be broadly defined on the basis of their origin as either tissue-resident macrophages (TRMs), which develop from fetal–yolk sac or fetal-liver progenitors (14, 57), or monocyte-derived macrophages (58), although the precise kinetics of monocyte to TAM differentiation is still not completely understood. In a seminal work, classical monocytes were found to upregulate markers of TAM terminal differentiation (e.g., F4/80) in primary mouse breast tumors within 5 days of their arrival, following the activation of the transcriptional regulator of Notch signaling RBPJ (58). Unlike other hematopoietic-derived cells, TRMs derive from two precursors during embryonic development (59). In an early phase of embryogenesis, macrophages originate from the yolk sac, directly from erythro-myeloid progenitors (EMPs) and independently of the transcriptional control of c-Myb. These macrophages seed some tissues and constitute microglia, for example. Alternatively, during the late phase of embryonic development, fetal monocytes generated from c-Myb$^+$ EMPs in the fetal liver can give rise to the majority of TRMs (59). The long-standing question regarding the kinetics and extent of the circulating monocyte contribution to TRM replacement during homeostasis, inflammation, and disease was recently addressed by the generation of a series of *Ms4a3*TdT reporter and *Ms4a3*^{Cre} and *Ms4a3*^{CreERT2} fate-mapping mouse models, which were developed to trace monocytes (60). As mentioned before, monocytes are hypothesized to arise from the following hierarchical sequence: CMP to GMP, then MDP, cMoP, and finally monocyte (12). However, this model was recently challenged by Yanez et al. (39), who proposed that MDPs arise directly from CMPs independently of GMPs and that GMPs and MDPs give rise to distinct monocytes through monocyte-committed progenitors and cMoPs, respectively. These two pathways may be mobilized in response to different microbial components to which the immune system is exposed (39). Fate-mapping studies using the *Ms4a3*^{Cre} model also showed that MDPs do not arise from GMPs, and in turn, they do not generate cMoPs. In particular, *Ms4a3* was found to be a marker for BM-resident GMPs and cMoPs and may be suitable for labeling GMPs and their progeny, including monocytes. While TRMs in the brain, i.e., microglia, do not undergo monocyte-dependent replacement after birth, those in other tissues exhibit either fast (dermis) or slow (kidney) replacement under steady-state conditions (60). Moreover, the monocyte contribution to inflammation caused by different stimuli is variable and associated with the death of tissue macrophages, which are monocytes that are able to seed the tissue and fill the empty niches left after macrophage dismissal (60). Within the tissues, subsets of macrophages coexist and respond differently to the same homeostatic and damage signals, and these fate-tracing models will be extremely useful in the future to study how cancer modifies the balance between different subsets.

Recent insights suggest that resident macrophages exhibit different transcriptional features in the TME compared to their monocyte-derived counterparts. Indeed, TRMs have increased expression of genes involved in tissue remodeling and wound healing, while monocyte-derived TAMs are enriched in genes associated with immunosuppression and antigen presentation (61), suggesting a distinct contribution to tumor progression. RNA-seq analysis of TAMs purified from endometrial and breast cancers and compared with TRMs from healthy tissues validated the presence of TME cues that are able to modulate TAM features independently from their precursor cells (62). Moreover, monocyte-derived TAMs are transcriptionally distinct from circulating monocytes, and TAMs located in collagen-rich stroma differ from TAMs placed in perivascular cancer lesions, suggesting that the differentiation trajectory of TME-infiltrating monocytes depends on their spatiotemporal infiltration within the tumor tissue (63). While some TRMs maintain expression of tissue-associated markers (62), the heterogeneous phenotype of TAMs makes their enumeration inside TMEs quite challenging, and new strategies need to be developed to define their functional state. Indeed, a TAM's functional state is strictly controlled by epigenetic and molecular pathways (31), and this plasticity is oversimplified into the classical (M1) or alternative (M2) macrophage polarization model based on in vitro studies with macrophages undergoing either IFNγ or IL-4 stimulation, respectively (64). Even if the classification of M1 and M2 macrophages cannot take into consideration the broad spectrum of macrophage activation that occurs in vivo, defining only the extremes of macrophage plasticity, it is interesting that this dichotomy may still underlie a correlation between TME-infiltrating TAMs and patient outcome. M2-polarized macrophages are involved in tissue remodeling, repair, and angiogenesis processes, mostly via the induction of arginase (ARG)1- and IL-10-associated networks; in line with these functions, the frequency of M2-like TAMs in the TME generally correlates with poor prognosis in a pan-cancer analysis (65), and thus M2 macrophages are considered protumorigenic elements. Conversely, M1-polarized macrophages have been traditionally classified as antitumor effectors since they express high levels of inflammatory mediators, such as TNF-α, nitric oxide (NO) [via inducible NO synthase (iNOS; also called NOS2)], and reactive oxygen species (ROS), which are able to directly kill tumor cells and stimulate an antitumor T cell response (66).

3.2. Tumor-Associated Dendritic Cells

TADCs represent a minor population in the TME but their immune control function is significant since they are specialized in antigen processing and presentation to naive T cells (67, 68). Indeed, high densities of TADCs are associated with increased overall survival for patients with breast, lung, or head and neck cancer (67), as well as with an increased antitumor T cell response (69). Moreover, DCs contribute to the generation of tertiary lymphoid structures (TLSs) inside the TME. TLSs are ectopic lymphoid organs in nonlymphoid tissues, such as stroma and the invasive margin and/or core of different tumors, that are formed upon long-lasting exposure to inflammatory signals. They are composed of a T cell–rich zone containing mature DCs juxtaposed with B cell follicles that contain a germinal center and are surrounded by plasma cells. TLSs might represent privileged sites for local presentation of neighboring tumor antigens to T cells by DCs, which activate effector-memory T helper cells, effector-memory CTLs, memory B cells, and antibody-producing plasma cells, as elegantly reviewed by Fridman and colleagues (70). Interestingly, TLS presence is associated with a favorable prognosis for most solid malignancies (71) and correlates with the immunotherapy response in melanoma (72) and renal-cell carcinoma patients (73).

The two main conventional DC subsets (cDCs) located within tumors, defined as cDC1s and cDC2s, originate from lineage-restricted precursors characterized by the activation of the BAFT/IRF8- and IRF4-dependent pathways, respectively (67). Interestingly, these circulating

precursors can also be detected inside TMEs (67). cDC1s are not only involved in promoting T cell activation; they cross-present tumor antigens, are the primary producers of IL-12, and also promote $CD8^+$ T cell proliferation and effector functions, which correlate with higher rates of responsiveness to chemotherapy (67). Conversely, cDC2s preferentially control the activation of $CD4^+$ T cells though major histocompatibility complex (MHC)-II-mediated pathways (68). Similar to TAMs, the classification of TADCs is probably simplified, and these cell subsets show a great deal of heterogeneity. Single-cell analysis of innate immune cells in lung adenocarcinoma demonstrated the presence of two distinct clusters of TADCs, one expressing high levels of *CD1c*, *CXCR1*, and *IRF4* and resembling cDC2s (the $CD1c^+$ DC cluster), while the other was characterized by *CD207*, *CLEC9A*, and *XCR1* expression typical of cDC1s (the $CD141^+$ DC cluster) (47). Interestingly, the $CD141^+$ DC cluster but not the $CD1c^+$ DC cluster was significantly reduced in tumors compared to normal tissue, suggesting that tumor escape might be preferentially linked to a contraction in cDC1-like cells. Indeed, the tumor-infiltrating $CD141^+$ DC subset expresses lymphotoxin beta transcripts that can help preserve TLS architecture and favor lymphocyte recruitment (47).

The TADC pool inside the TME can also be maintained by C-C motif chemokine receptor $(CCR)2^+$-monocyte differentiation after their infiltration into the tumor mass. Indeed, monocyte adoptive transfer restores TADC-mediated antitumor responses in CD11c-DTR tumor-bearing mice in which DCs are genetically depleted (74). Normally, monocyte-to-TADC differentiation in the TME is characterized by the acquisition of specific markers (i.e., CD11c, CD103, CD80, CD86), morphological changes, and antigen-presenting cell (APC)-associated functions (75). Monocyte-derived TADCs are usually immunogenic, due to the upregulation of positive costimulatory molecules and IL-12 secretion (76), even though in some contexts they can help dampen protumor immune responses by abrogating T cell functions (77). Since monocyte conversion in either protumoral or antitumoral TADCs may diverge in the TME of different cancer types, one challenge for the field is the identification and characterization of molecular switches that underlie different TADC functional states, which goes beyond standard phenotypical characterization. In line with this goal, Sharma and colleagues (78) demonstrated that $Ly6C^+CD103^+$ monocyte-derived (Mo)DCs in the TME are distinct from $CD103^+$ cDCs and derive from both cMoPs and circulating M-MDSCs. This cell conversion is induced by TME-associated local inflammation and depends on the activation of p53, which controls upregulation of Batf3 and acquisition of the MoDC phenotype. Furthermore, $Ly6C^+CD103^+$ MoDC polarization is affected by $PTEN^+$ Tregs, suggesting that the TME immune composition can steer monocyte cell plasticity (78). We also demonstrated that monocytes can differentiate into potent APCs in the TME, acquiring distinctive features of DCs called TNF-α/iNOS-producing (Tip)-DCs that are specialized in innate immunity against pathogens. Tumor-infiltrating Tip-DC generation requires CD40/CD40L signaling and offers a source of potent antitumor elements, not only APC activity but also TNF-α- and NO-dependent tumor killing, which promote the antitumor activity of transferred T lymphocytes bearing a T cell receptor with high affinity for tumor antigens (79). These two examples of monocyte differentiation in TMEs suggest that monocyte-derived DCs, in addition to cDCs, participate in the induction of effective antitumor immunity, and the development of therapeutic strategies based on monocyte reprogramming might be exploited for cancer immunotherapy.

3.3. Myeloid-Derived Suppressor Cells

MDSCs are a heterogeneous myeloid cell population characterized by protumoral functions such as the ability to abrogate adaptive antitumor immune responses and favor the metastatic

process (9). MDSCs have been abundantly observed in both tumor-bearing mice and cancer patients to accumulate within primary tumor and metastatic lesions as well as in lymphoid tissues, BM, and peripheral blood (80). Interestingly, circulating MDSC frequency is correlated with the clinical outcome of patients treated with immunotherapy (81) as well as with shorter overall survival and the development of metastatic disease in pancreatic cancer patients (82). Currently, MDSC characterization is based on surface markers that define three main cell subsets: monocytic-MDSCs (M-MDSCs) that are identified as $CD11b^{+}CD14^{+}CD15^{-}HLA\text{-}DR^{low/-}CD124^{+}$ cells in cancer patients and $CD11b^{+}Ly6C^{+}Ly6G^{-}$ cells in tumor-bearing mice; granulocytic-MDSCs (G-MDSCs) characterized as $CD11b^{+}CD14^{-}CD15^{+}HLA\text{-}DR^{low/-}CD124^{+}$ cells and $CD11b^{+}Ly6C^{-}Ly6G^{+}$ cells in humans and mice, respectively; and early immature–MDSCs (eMDSCs) described as $Lin^{-}CD11b^{+}CD34^{+}CD33^{+}CD117^{+}HLA\text{-}DR^{low/-}$ in human cells and $CD11b^{+}Gr1^{+}CCR2^{+}Sca1^{+}CD31^{+}$ in mouse cells (83). Since MDSCs share some morphological and phenotypical features with their nonsuppressive counterparts, it is essential to confirm their identity with functional in vitro testing (83). Indeed, M-MDSCs isolated from the blood of pancreatic cancer patients are clearly distinguishable from normal monocytes by their cytological features (e.g., the presence of granules), molecular signatures, and immunosuppressive properties but not by surface markers (82). M-MDSCs are generally more immunosuppressive compared to G-MDSCs on a per cell basis both in tumor-bearing mice (84) and in cancer patients (82), mainly due to contact-dependent but nonantigen-specific immune tolerance (26). Interestingly, tumor-isolated M-MDSCs display a more potent suppressive activity than spleen-derived MDSCs, suggesting that MDSC-mediated immunosuppression is dynamically enforced within the TME more than in the periphery. The net result of these enhanced suppressive features is the generation of a TME that prevents and inhibits T cell–mediated cancer elimination (9). In addition, the tissue distribution of the M- and G-MDSC subsets is quite different: G-MDSCs are preferentially located in the spleen, circulation, and BM, while M-MDSCs are enriched within the majority of tumors (80); furthermore, M-MDSCs and G-MDSCs activate different pathways for regulating cell-intrinsic death programs (26). In fact, c-FLIP and MCL-1 are essential for the survival of M-MDSCs and G-MDSCs, respectively (26). Preventing the expression of c-FLIP altered the immunosuppressive TME by reducing the number and activity of M-MDSCs (27). M-MDSCs exhibit a higher cell plasticity compared to G-MDSCs; in the TME they act as precursors of TAMs as well as transdifferentiate into G-MDSCs to maintain their level in the blood (85, 86). Thus, it appears that the TME not only affects MDSC function but also modifies M-MDSC fate by promoting their differentiation into more long-lived cells, such as TAMs. A critical role for miR-142-3p in this process was recently suggested, since its downregulation was required for the generation of immunosuppressive TAMs (87). Mechanistically, miR-142-3p downregulates gp130 by binding to the mRNA 3′ untranslated region and represses C/EBPβ LAP* by noncanonically binding to its 5′ mRNA, impairing macrophage differentiation both in vivo and in vitro. Indeed, tumor-bearing mice constitutively expressing miR-142-3p in the BM show an increase in survival following immunotherapy with antigen-specific T lymphocytes (87). M-MDSC plasticity and function are strictly controlled by several signaling pathways (reviewed in 9, 80); specifically, c/EBPβ, nuclear factor κB (NF-κB), and signal transducer and activator of transcription (STAT)3 have been reported to be involved in MDSC generation, differentiation, and function. The key role of c/EBPβ in controlling MDSC generation was demonstrated using myeloid-restricted, c/EBPβ-deficient mice engrafted with different tumor models, in which MDSC accumulation was completely abrogated (35). NF-κB also regulates MDSC differentiation and function. Indeed, MDSC immunosuppressive activity was recently linked to NF-κB p50 protein translocation into the nucleus: By limiting the generation of p50:p50 homodimers through the abrogation of NF-κB p50 nuclear translocation, MDSCs lose their immunosuppressive properties (88). In agreement

with these data, we demonstrated that c-FLIP promotes the nuclear translocation of NF-κB p50 protein, inducing immunosuppressive properties in monocytes independently of the antiapoptotic properties of the protein (27). STAT3 protects MDSC survival by upregulating c-Myc, survivin, and Cyclin D1 as well as by blocking cell differentiation through IRF8 (89). STAT3 also controls several immunosuppressive circuits; in particular, STAT3 induces ARG1 by binding its specific promoter (90) and also activates the production of proinflammatory soluble mediators (cytokines and growth factors), such as S100A8/A9, which prevents MDSC conversion to DCs (91).

4. HOW THE TUMOR MICROENVIRONMENT SHAPES MONOCYTE DIFFERENTIATION

Myeloid cell complexity depends on intrinsic local factors of the TME such as low levels of nutrients, pH, and oxygen as well as cancer-released soluble factors and vesicles that are able to activate stress-dependent molecular pathways in monocytes, shaping their phenotype and pro- or antitumor function (**Figure 2**). Three main monocyte-shaping cues dominate the TME: environmental stress, metabolic stress, and cell cross talk–dependent signals.

4.1. Environmental Stress Signals

In general, chronic stress and alterations to cellular homeostasis can steer tumor progression by fueling inflammation, which is among the hallmarks of cancer (92). Stress signal pathways are highly intertwined with each other and can shift the balance of protumor and antitumor elements inside the TME.

One of the most prominent features of the TME is hypoxia, i.e., a nonphysiological level of oxygen tension (0.1–3% O_2), the combined result of anarchic vasculogenesis and intense metabolic activity inside the tumor. Tumor-infiltrating monocytes and monocyte-derived cells adapt themselves to the local conditions, stabilizing hypoxia-inducible factor 1α (HIF-1α) by activating oxygen-sensing rheostats including mTOR (mammalian target of rapamycin) and the unfolded protein response (UPR) (93). HIF-1α transactivates a broad spectrum of genes that control T cell activation, cell metabolism, cell recruitment, and cell differentiation (94). Indeed, tumor-infiltrating MDSCs lacking HIF-1α are defective in generating TAMs but instead undergo conversion to TADCs (95). HIF-1α increases ARG1 and iNOS expression in tumor MDSCs cultured in vitro under hypoxic conditions; this can be halted by genetic deletion of HIF-1α, and abrogation of the HIF-1α-mediated polarization of myeloid cells increased the efficacy of gp100-specific T cell adoptive transfer combined with vaccination in controlling the outgrowth of melanoma cells (95). Interestingly, hypoxic conditions fuel PD-L1 expression in both MDSCs and TAMs within the TME. This process is mediated by direct binding of HIF-1α to the PD-L1 promoter (96). Moreover, hypoxia orchestrates the angiogenic activities of MHC-II^{low} M2-like TAMs, enabling the different spatial distribution of TAMs inside the TME, with increased infiltration of either M2-polarized or M1-like TAMs in hypoxic versus normoxic tumor regions, respectively (66). Contributing to the hypoxia-dependent alteration of the TME cell composition, the secretion of soluble factors such as vascular endothelial cell growth factor (VEGF) and C-C motif chemokine ligand (CCL)26 favors the recruitment of MDSCs and the migration of other VEGF receptor (VEGFR)–positive cells such as Treg lymphocytes, exacerbating the TME immune impairment (97). In response to hypoxia, TAMs and MDSCs release proteases [e.g., cathepsin and matrix metalloproteinase 9 (MMP9)], which sustain angiogenesis by freeing heparin-bound growth factors, such as VEGF-A, and inducing extracellular matrix (ECM) remodeling (51). Although hypoxia

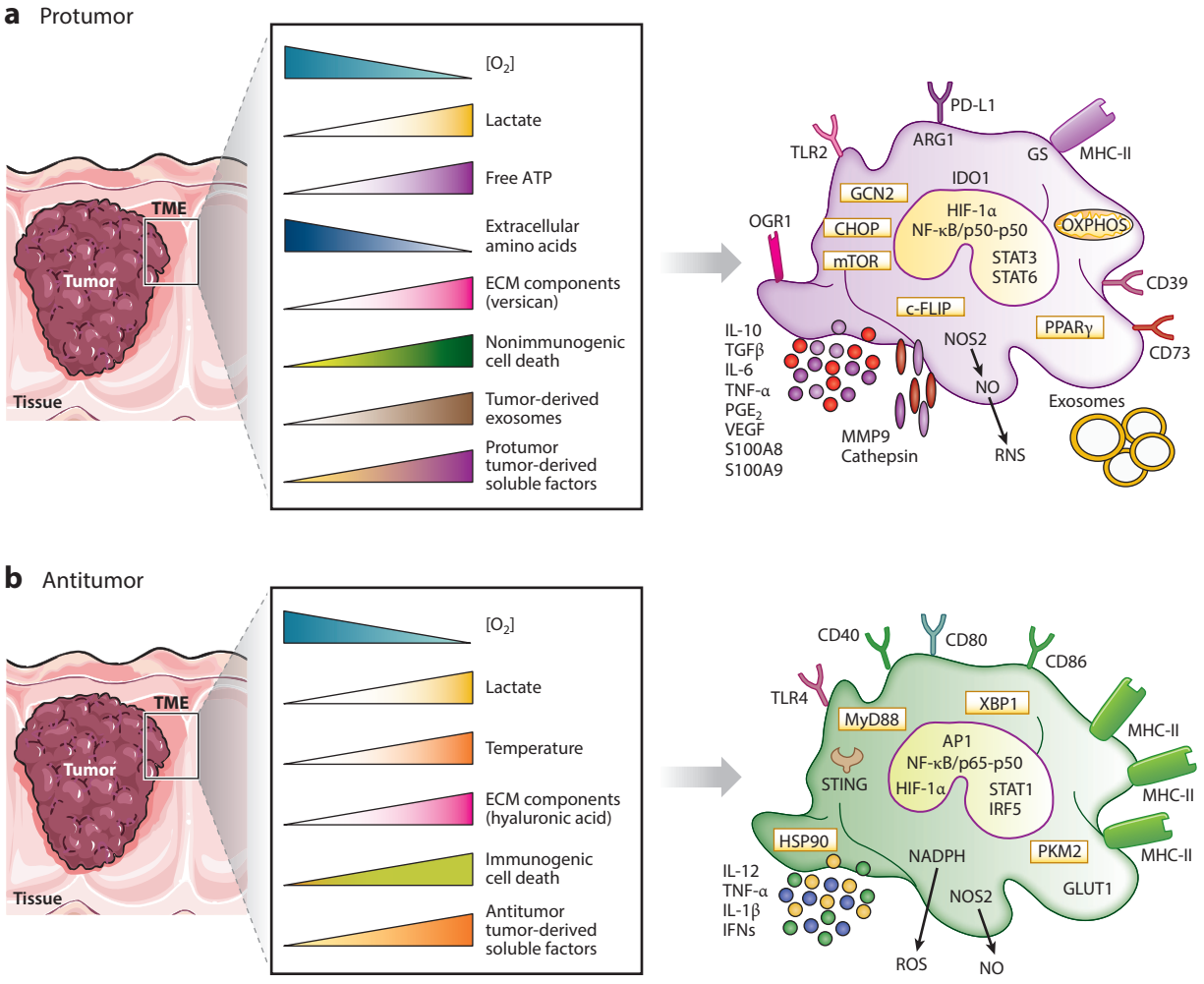


Figure 2

(*a*) Tumor-derived factors and tumor-supported conditions driving the differentiation of myeloid cells toward protumor phenotypes and functions. (*b*) Tumor-derived factors and tumor-supported conditions driving the differentiation of myeloid cells toward antitumor phenotypes and functions. Abbreviations: AP1, activator protein 1; ARG1, arginase 1; CD, cluster of differentiation; CHOP, CCAAT-enhancer-binding protein homologous protein; c-FLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; ECM, extracellular matrix; GCN2, general control nonderepressible 2; GLUT1, glucose transporter 1; GS, glutamine synthetase; HIF-1α, hypoxia-inducible factor 1α; HSP90, heat shock protein 90; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; IRF, IFN regulatory factor; MHC-II, major histocompatibility complex II; MMP, matrix metallopeptidase; mTOR, mammalian target of rapamycin; MyD88, myeloid differentiation primary response 88; NF-κB/p65-p50, nuclear factor κB protein 65 and 50; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NO, nitric oxide; NOS2, nitric oxide synthase 2; OGR1, proton-sensing ovarian cancer G protein–coupled receptor; OXPHOS, oxidative phosphorylation; PD-L1, programmed death-ligand 1; PGE_2, prostaglandin E2; PKM2, pyruvate kinase isozyme M2; PPARγ, peroxisome proliferator activated receptor γ; RNS, reactive nitrogen species; ROS, reactive oxygen species; S100, S100 calcium binding protein; STAT, signal transducer and activator of transcription; STING, stimulator of IFN genes; TGFβ, transforming growth factor β; TLR, Toll-like receptor; TME, tumor microenvironment; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial cell growth factor; XBP1, X-box binding protein 1. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

has been shown to promote the migration of $CD8^+$ T cells into the TME, it also favors the induction of T lymphocyte exhaustion through the expression of inhibitory receptors, such as PD-1 and CD223 [best known as lymphocyte activating gene 3 (LAG3)] (98). Since hypoxic conditions in the TME not only drive the migration and conversion of monocytes but also reprogram monocyte-derived cells into protumor elements, targeting hypoxic stress is considered a potential strategy to shield or awaken antitumor immune responses.

Besides secreting immune-suppressive cytokines and chemokines, tumor cells produce large amounts of lactic acid, which causes an acidification of the TME with profound consequences for several immune cells and local inflammation. In fact, T cell proliferation and functions are inhibited at acidic pH, as demonstrated by an increase in the apoptosis rate and a reduction in IL-2, IFNγ, and perforin/granzyme (99). Local low pH enhances the expression of P-selectin glycoprotein ligand 1 (PSGL-1) on tumor vascular endothelia, which facilitates monocyte migration via P- or L-selectin binding, whereas acidic pH does not alter the expression of E-selectin, which preferentially mediates T cell extravasation (100). Most importantly, low environmental pH influences monocyte-derived cell function inside the TME. For instance, monocyte-derived cells express proton-sensing ovarian cancer G protein–coupled receptor (OGR1), a pH-sensing receptor that reacts to extracellular protons and activates phospholipase C, triggering the secretion of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (101). In an autocrine loop, macrophage-derived TNF-α, synthesized in response to low pH, activates the NF-κB pathway, which upregulates iNOS at both the mRNA and protein levels (64). Monocyte-derived cells can thus participate in the early phases of tumor progression by sustaining precancerous inflammation, while in later stages the low pH in the TME might be an important modulator of monocyte-derived cell functions that enable tumor escape.

Environmental stress can rapidly disrupt correct protein folding and activate cell death in resident TME cells, generating an increased local level of cellular debris that needs to be eliminated by innate immune cells. These cellular components are able to alter the function of phagocytes since they can deliver damage-associated molecular patterns (DAMPs) (102). DAMP-mediated signals can promote TADC activation but also M2-like TAM polarization, as in the case of high-mobility group protein B1 (HMGB1) signaling. This molecule has pleiotropic and context-specific effects on TME-infiltrating immune cells; it fuels DC maturation and antitumor immune responses via TLR4 in response to chemotherapy-induced immunogenic cell death (102). However, it can also sustain IL-10 production after binding receptor for advanced glycation end products (RAGE) in TAMs (103). Moreover, tumor cells undergoing genotoxic stress can activate TME-infiltrating cells that express stimulator of IFN genes (STING), also known as transmembrane protein 173 (TMEM173). The STING pathway is essential for detecting cytoplasmic DNA. In fact, interactions between STING and cyclic GMP-AMP synthase (cGAS) promote the activation of the TBK1-STAT6-IRF3 signaling cascade, which leads to type I IFN release. Phagocytosis of tumor cells and DNA by TADCs after gaining access to the cytosol likely triggers STING pathways that promote IFN-dependent priming of tumor-specific $CD8^+$ T cells (104). Also, some components of the ECM, such as versican, tenascin, and hyaluronic acid (HA), are recognized as DAMPs by TLRs and promote changes in the recruitment, function, survival, and activation states of monocyte-derived cells within the TME (105). HA-rich stroma recruits TAMs, likely through the creation of an ECM scaffold that enhances chemokine retention. TAM recruitment and the resulting sustained tumor angiogenesis can be abrogated by disrupting the HA synthase 2 gene in stromal fibroblasts (106). Versican binding to TLR2 triggers a tolerogenic phenotype in DCs through a doubly synergistic process: Induction of IL-6 and IL-10 synthesis and expression of their cognate receptors sustain the activation of STAT3 signaling in an autocrine manner while TLR2 blockade improves the antitumor functions of DCs and the efficacy

of immunotherapy (107). Tenascin also plays a role in the TME by inducing the phagocytosis of CD47-deficient glioblastoma cancer cells and the release of proinflammatory cytokines (e.g., TNF-α) after triggering TLR4 in macrophages (108). Notably, apoptotic cell debris can be taken up by macrophages using the Mer tyrosine protein kinase (MERTK) receptor, which activates an immunosuppressive signaling program based on IL-10, ARG1, and transforming growth factor β (TGFβ) (109). In agreement with these data, loss of MERTK increases iNOS expression in macrophages and T cell infiltration into the TME (110).

Environmental pathophysiological conditions can activate endoplasmic reticulum (ER) stress in tumor-infiltrating myeloid cells, since under these circumstances there might be an impairment of the protein folding machinery or unrestrained protein synthesis and loading in the ER lumen. In response to these alterations, an adaptive homeostatic response based on the UPR is activated to restore ER proteases or induce apoptosis. The UPR fuels proinflammatory cascades via NF-κB and JNK-AP1 activation, resulting in the production of protumor cytokines such as IL-6, IL-23, and TNF-α (102). Moreover, APCs experiencing ER stress alter their antigen processing machinery, resulting in defective presentation of immune-dominant peptides from tumor antigens and, hence, decreased activation of antigen-specific T cells (102). Indeed, the conditional deletion in DCs of X-box binding protein 1 (XBP1) compromises tumor progression by enhancing T cell activation in the TME; XBP1 is the main target of inositol-requiring enzyme-1α, a major sensor of the UPR signaling cascade (111). Notably, TME-infiltrating MDSCs express high levels of the ER stress–induced proapoptotic TF CHOP (CCAAT-enhancer-binding protein homologous protein), which is essential for their survival and immunosuppressive functions. Another mechanism of immunosuppression that has gained attention as a potential therapeutic target is the purinergic signaling axis, whereby the production of the purine nucleoside adenosine (ADO) in the TME can effectively suppress T- and natural killer (NK)–cell function (112). While the concentration of ADO in normal tissue is in the nanomolar range, it can swell to micromolar concentrations in solid tumors and is particularly enriched in the hypoxic tumor core (113). The production of ADO is mediated by the cell-surface ectoenzymes CD73, CD39, and CD38, and, once generated, ADO signals through the adenosine receptors (A1R, A2AR, A2BR, A3R), members of the G protein–coupled receptor family. MDSCs and TAMs in the TME can contribute to immunosuppression both by upregulating the ectoenzymes CD73 and CD39 and by overexpressing A2BR/A2AR and A2BR/A2AR/A3R, respectively (114). Thus, macrophages stimulated with ADO secrete increased amounts of IL-10 and less IL-12, TNF-α, and chemotactic factors, dampening T cells' killing activity. Using an elegant approach in which A2AR was specifically eliminated in myeloid cells (by Lys-Cre-dependent deletion), it was shown that A2AR limited antitumor immune responses, in part through the modulation of myeloid cells and the subsequent enhancement of antitumor T cell responses (115). Similarly, A2BR blockade has been shown to enhance antitumor immune responses, partly through a reduction in MDSC differentiation (116) and enhancement of the capacity of DCs to evoke antitumor T cell responses (117). A seminal study from the Sitkovsky group (112) demonstrated that the genetic deletion of A2AR can enhance the responses mediated by activated anti-melanoma T cells in the TME. In addition, the A2AR antagonist ZM241,385 was found to enhance the antitumor effect of $CD8^+$ T cells in studies of lung metastasis that originated from a sarcoma model. More recently, CD73 and CD38 expression on tumor cells was shown to confer resistance to anti-PD-1 treatment, since activation of T cells (i.e., the production of IFNγ) and PD-1 blockade upregulate the expression of A2AR, making these cells more susceptible to ADO-mediated suppression (118). Hence, the inhibition of CD73, A2AR, or CD38 in combination with anti-PD-1 was shown to elicit antitumor $CD8^+$ T cell responses mediated by enhanced IFNγ and granzyme B release (118).

4.2. Metabolic Stress Signals

It is well established that cancer cells undergo a metabolic shift toward relying on aerobic glycolysis as the primary energy source to maintain their rapid proliferation. This process is called the Warburg effect, and it is also utilized by tumor-infiltrating macrophages (119). Since metabolic stress is not as well studied in other myeloid cells within the TME, we mainly focus here on TAMs. It may seem counterintuitive that activated cells like macrophages use glycolysis as their main energy source, as oxidative phosphorylation (OXPHOS) produces 36 molecules of ATP compared to glycolysis, which generates a mere 2 ATP per molecule of glucose. However, glycolysis can be switched on faster than OXPHOS since it does not require mitochondrial biogenesis, and it provides essential biosynthetic intermediates to be used in other cellular processes. Glycolysis is an essential metabolic activity for M1-polarized macrophages since it can affect several functions that are generally controlled by the activation of the TF HIF-1α, such as phagocytosis, ROS production, and secretion of proinflammatory cytokines (120). Indeed, HIF-1α regulates the expression of genes encoding for glycolytic enzymes and transporters such as GLUT1 (121), which facilitates glucose uptake in M1-polarized macrophages. In contrast, M2 macrophages rely on OXPHOS over glycolysis as their main source of ATP (122). Evidence for this distinction was obtained by using extracellular flux analysis to study the metabolic features of classical and alternative macrophages (122). Moreover, IL-10 suppresses glycolysis in in vitro–differentiated macrophages, highlighting how a Warburg-like effect is predominantly associated with M1-polarized macrophages. Conversely, M2 macrophages display an increased rate of oxidative metabolism as well as amplified mitochondrial metabolism (123). In alternatively activated macrophages, glucose metabolism is normally regulated by mTOR through an AKT-dependent pathway; however, this process is modulated by several environmental signals, such as IL-4 and M-CSF, that enforce the use of glycolysis via the mTOR/IRF4 pathway. This increased glycolysis during M2 activation also reduces glucose availability for M1-polarized macrophages (124). This aspect of M2 macrophage metabolism suggests that they have a more flexible metabolism since they can supply OXPHOS even in the absence of glycolysis by using glutamine (122). Several glycolytic enzymes influence the functional properties of monocyte-derived cells. Among them, pyruvate kinase M2 (PKM2) acts in M1-polarized macrophages as a nuclear coactivator of HIF-1α, promoting the expression of proinflammatory cytokines such as IL-1β via direct binding to the hypoxia response element site of their promoters (125). Notably, PKM2 has also been shown to regulate PD-L1 expression in both macrophages and other immune cells; indeed, the PKM2/HIF-1α complex specifically binds the promoter of the PD-L1 gene (126).

Other metabolic pathways, such as lipid and amino acid metabolism, are adapted by tumor-infiltrating, monocyte-derived cells in response to TME nutrients. Fatty acid synthesis and fatty acid beta oxidation are strictly controlled and take place in different cellular compartments, i.e., the cytosol and mitochondria, respectively. Fatty acid synthesis is closely linked to the proinflammatory functions of myeloid immune cells and monocyte conversion into macrophages under M-CSF-driven differentiation (127). In agreement with these observations, a newly identified protein named FAMIN is actively involved in de novo lipogenesis but also regulates the inflammasome and the release of inflammation-associated cytokines in macrophages (128). Notably, alterations to the triglyceride biosynthetic pathway in DCs significantly impair their ability to activate antitumor responses by priming T cells (111). Lipolysis in the TME produces free fatty acids that are taken up by CD36-expressing monocyte-derived cells that mostly resemble M2-polarized macrophages. M2 macrophages rely on fatty-acid uptake and oxidation to stimulate the activation of STAT6- and peroxisome proliferator-activated receptor γ (PPARγ)-dependent programs (129).

The extracellular amino acid reservoir is directly related to the activation state of immune cells inside the TME. The first evidence that amino acid metabolism can regulate macrophage function comes from data on the ability of macrophages to block tumor growth through the consumption of arginine (130). The consumption of amino acids strongly affects the immune cell composition of the TME, and it represents a key process required for monocyte-derived cells to abolish adaptive immune responses (described in Section 4.3). Glutamine metabolism has a profound impact on both tumor growth and the cellular composition of the TME. M2-like macrophages express high levels of glutamine synthase (GS), the glutamine-synthesizing enzyme that controls the activation of several metabolic mediators, including the key sensor mTOR (131). Pharmacological inhibition of GS skews M2-polarized macrophages toward the M1-like phenotype, which is characterized by reduced intracellular glutamine and increased succinate, enhanced glucose flux through glycolysis, and HIF-1α activation. As a result of these metabolic changes and HIF-1α accumulation, GS-inhibited macrophages have an increased capacity to induce T cell recruitment, a reduced ability to suppress T cells, and an impaired ability to foster endothelial-cell branching and cancer-cell motility. Thus, blocking glutamine metabolism would not only reduce tumor growth but also restore an effective antitumor response (132), supporting the use of glutamine-pathway inhibitors to treat cancer.

4.3. Cell Cross Talk–Dependent Signals

Interactions among neighboring cells in the TME are indispensable for tumor growth and development. Besides direct cell-cell contact, intracellular communication involves complex systems using secreted factors. Several factors released by cancer cells can regulate the generation, differentiation, and function of tumor-infiltrating monocyte-derived cells. Among them, prostaglandin E2 (PGE_2), which is secreted by various cancer types, plays multiple roles in the recruitment and conversion of monocytes. Indeed, it inhibits cancer-cell secretion of CCL5 and other proinflammatory components and limits the expression of CCR5 in TAMs, but at the same time, it prompts the release of MDSC chemoattractants such as C-X-C motif chemokine ligand (CXCL)12 (133). The genetic ablation of cyclooxygenase 1 (COX1), an enzyme critical for the production of PGE_2, significantly increases trafficking of DCs and their expression of IL-12 and costimulatory molecules in preclinical models of melanoma tumors. In accordance, COX1 inhibition synergizes with anti-PD-1 blockade in controlling tumor growth, suggesting that COX inhibitors could be useful adjuvants for immune-based therapies in cancer patients (134). Other tumor-derived soluble factors such as cytokines also regulate myeloid cells' identity and function. Well-characterized examples are IL-4, IL-10, and TGFβ (5). In the last few years, attempts to understand the role of tumor-derived exosomes (TEXs) in modulating the TME have been of great interest. Exosomes are nanometer-sized membranous vesicles packed with proteins, lipids, DNA, mRNA, and microRNAs (135). Interestingly, exosomes are generally present at higher concentrations in the blood of cancer patients compared to healthy controls, and their cargo can vary according to the patient's disease, suggesting that cancer cells might use exosome-mediated communication to modify not only local immune responses but also distal immune sites (136). In line with this hypothesis, recent data clearly highlight the ability of TEXs to edit and program premetastatic and metastatic niches (137). Normally, TEXs activate NF-κB-dependent pathways in macrophages, leading to the release of IL-6 and TNF-α and thus promoting the proliferation of cancer cells (135). Moreover, TEXs favor the expression of PD-L1 in MDSCs (138). However, TEXs may contribute to efficient DC-mediated priming of antitumor adaptive responses (135). Macrophage-derived exosomes can also affect tumor-cell biology. In fact, macrophage-derived exosomes can promote

tumor invasion by transferring Wnt5a to cancer cells, leading to the activation of the β-catenin-independent Wnt signaling pathway; M2 TAM-derived exosomes containing miR-21 can also mediate resistance to cisplatin chemotherapy through the activation of the phosphoinositide 3-kinase (PI3K)/AKT pathway, which promotes prosurvival functions in gastric cancer cells (139). These data suggest the existence of a bidirectional exosome-mediated engagement between cancer cells and tumor-infiltrating myeloid cells in the TME. The use of standardized protocols for TEX isolation and characterization is essential for studying exosome biogenesis and the mechanisms of exosomal cargo delivery. Taking current limitations into account, TEXs may provide a new source of biological markers for predicting patient prognosis or response to therapy as well as important targets for developing therapeutic strategies based on exosome-mediated communication between TME elements.

5. HOW MONOCYTE-DERIVED CELLS SHAPE THE TUMOR MICROENVIRONMENT

Tumor-infiltrating monocyte-derived cells support tumor growth through both immunological and nonimmunological mechanisms.

5.1. Promotion of Immune Dysfunction

MDSCs and TAMs synergize to establish local and peripheral tolerance, which hides tumors from the effector arm of the immune system. To this end, myeloid cells use an array of cytokines, metabolites, and surface receptors that directly affect the function of innate and adaptive immunity by both directly modulating the fitness of NK and effector T cells and indirectly skewing the polarization status of other immune subsets toward a suppressive phenotype, i.e., pDCs, Tregs, and regulatory B lymphocytes (86). TAMs and MDSCs deplete the TME of arginine, tryptophan, and cysteine, essential amino acids for T cells, through the cooperative catabolism of iNOS, ARG1, and indoleamine 2,3-dioxygenase (IDO) enzymes.

Arginine deprivation has multiple negative effects on T cell function. It rewires T cell metabolism by switching it from OXPHOS to glycolysis, reducing T cell fitness, central memory–like functions, survival, and antitumor efficacy (140). The transcription regulators BAZ1B, PSIP1, and TSN are responsible for arginine sensing in the TME, modifying metabolism and regulating prosurvival actions in T lymphocytes. Accordingly, arginine deprivation affects the expression of the TFs E2F1, cdk2, and cyclin D3, which in turn prevent cell cycle progression at the G0/G1 phase, resulting in proliferative arrest of T cells (9). Moreover, arginine starvation results in reduced TCR signaling due to the downregulation of the CD3ζ chain, which limits IFNγ release and T cell function (141). ARG1 and iNOS are the two main enzymes involved in arginine catabolism. ARG1, which is mainly produced by M2-polarized macrophages and MDSCs, metabolizes arginine to urea and ornithine and is considered a marker of immunosuppression in myeloid cells (141). Its expression is regulated by cytokines (i.e., IL-4, IL-6, IL-10, and IL-13), prostaglandins, tumor metabolic products such as lactic acid, and hypoxia (141). Pharmacological blockade of ARG1 or genetic deletion in myeloid cells results in improved immunotherapy efficacy (79). Interestingly, T cells are also endowed with cell-autonomous arginine-dependent regulatory pathways that depend on the activity of mitochondrial ARG2, which intrinsically regulates T cell function, proliferation, differentiation, and antitumor activity in vivo (142).

iNOS competes with ARG1 for arginine as a substrate to generate NO and citrulline. However, iNOS's immunosuppressive and protumor contribution is more context dependent. Indeed, iNOS is considered a classic hallmark of antitumor M1-polarized macrophages, and

its upregulation has a beneficial effect on host immunity and tumor restriction in both TAMs (64) and tumor-infiltrating Tip-DCs (79). However, iNOS is also expressed by MDSCs and is responsible (sometimes together with ARG1) for MDSC-mediated suppression of T cell proliferation and activity (35, 84). NO negatively affects IL-2R signaling and promotes T cell apoptosis through p53 accumulation and Fas (CD95/APO-1) signaling (80). The genetic deletion of iNOS in MDSCs completely restores T cell proliferation (79). Similar to arginine, tryptophan shortage has multiple negative consequences for T cell proliferation and function.

Tryptophan deprivation activates the amino acid sensor general control nonderepressible 2 (GCN2) through its binding to uncharged tRNAs, which results in the phosphorylation of polypeptide chain initiation factor 2 subunit α (eIF2α). This posttranslational modification decreases the turnover of eIF2α and dramatically affects protein synthesis by interrupting mRNA translation and downstream cellular functions, such as proliferation and activation (143). Tryptophan starvation also results in downregulation of the CD3ζ chain and impairment of TCR downstream signaling in T cells (144). Notably, GCN2 was recently reported to drive and sustain the immunosuppressive functions of MDSCs in the TME (145). IDO is the main enzyme expressed by tolerogenic DCs and MDSCs responsible for tryptophan catabolism into kynurenines. The latter molecules have immune regulatory functions as well: By interacting with the aryl hydrocarbon receptor, they trigger CD4-expressing lymphocytes and DCs to differentiate toward Tregs and pDCs, respectively (144).

Cysteine is another limiting amino acid for protein biosynthesis and T cell function. This molecule can be obtained by converting methionine with cystathionine γ-lyase or imported in its oxidized form (cystine) from extracellular space via the SLC7A11 cystine/glutamate antiporter. T cells, which do not express any of these proteins, mostly rely on amino acids provided by macrophages and DCs that reduce cystine to cysteine and release it through the alanine-serine-cysteine (ASC) transporter. In contrast, MDSCs do not express ASC and store cystine and cysteine for their own use as a source of glutathione, an antioxidative molecule that can detoxify cells from the dangerous consequences of the free radicals they produce (146).

Macrophages and MDSCs are the main source of ROS within the TME. ROS include hydrogen peroxide, singlet oxygen, superoxide anions, and hydroxyl radicals that are generated during electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) to oxygen in a reaction catalyzed by the NADPH oxidase (NOX) protein family. ROS generation has a direct negative impact on IFNγ secretion and the proliferation of antigenic-specific T cells by reducing the CD3ζ chain and an indirect role in the evolution of the immune-suppressive tumor milieu by inhibiting M-MDSC differentiation toward macrophages and DCs, supporting the recruitment of other MDSCs, and increasing VEGFR expression and STAT3 signaling in an autocrine manner (147).

NOX2 genetic deficiency disrupts MDSC-dependent suppression of T cell function and enhances MDSC differentiation toward macrophages and DCs (147). More importantly, NO generated by iNOS quickly reacts with ROS to produce more dangerous and reactive nitrogen species (RNS), which have additional negative effects on T cell recruitment and function. Indeed, both ROS and RNS avidly react with macromolecules (i.e., lipids, nucleic acids), modify the tridimensional structure of proteins (by preferentially nitrating tyrosine residues), and consequently impair the signaling pathway activity and biological processes of innate and adaptive immunity. For example, RNS can affect both the trafficking and function of T lymphocytes by modifying chemokines (e.g., CCL2 and CCL5), altering antigen presentation and peptide loading onto MHC-I molecules, or affecting the TCR structure to prevent signaling and promote dissociation of the CD3ζ chain (148). Notably, TME preconditioning with an NO donor named [3-(aminocarbonyl)furoxan-4-yl]methyl salicylate (AT38), which is able to limit endogenous NO

production by iNOS and ARG1 expression in myeloid cells, efficiently restores T cell infiltration within the tumor and improves the efficacy of T cell adoptive therapy (149). RNS can also negatively affect the FcR-mediated functions of NK cells such as antibody-dependent cellular cytotoxicity and cytokine secretion, thus impairing the efficacy of antibody-based immunotherapy in pancreatic cancer (150).

ARG1 and iNOS targeting represents a valid strategy to neutralize MDSC-triggered immune suppression; however, the current literature suggests that myeloid cells can also use receptor-ligand interactions to induce T cell apoptosis. Indeed, PD-L1 expression by MDSCs and TAMs was reported in solid tumors with different histology, and the efficacy of anti-PD-1 therapy (alone or in combination with CTLA4) in melanoma and ovarian cancer patients was directly correlated with the expression of its ligand on TAMs and monocytes rather than on tumor cells (151). B7H4 is another immune-checkpoint molecule expressed on monocytes and TAMs in ovarian and liver cancers (152, 153) that plays a negative role in T cell function. Its expression is induced by the cytokines IFNγ, IL-6, and IL-10, and, after interacting with an unknown receptor on T cells, B7H4 impairs IL-2 production and induces cell exhaustion. Ectopic expression of B7H4 turns normal macrophages into suppressive cells (152), whereas its pharmacological inhibition restrains the growth of subcutaneous tumors and synergizes with anti-PD-1 to enhance antitumor immune responses (153).

Macrophages also express a variety of lectins, which can have a potential role in tuning T cell responses. Mannose receptor (CD206), for example, has been shown to inhibit CD45 phosphatase activity in $CD8^+$ T cells, resulting in impaired cytotoxic activity (154). Both CTLs and myeloid cells use receptor-ligand interactions that can directly target tumor cells to induce apoptosis, such as the interaction between Fas ligand and TNF-related apoptosis-inducing ligand (TRAIL); however, tumors can hijack these proapoptotic pathways to establish a microenvironment that assures its outgrowth and invasion. TRAIL-resistant tumor cells respond to TRAIL-receptor triggering by activating a TRAIL-dependent secretome in a FADD- and caspase-8-dependent manner, which drives monocyte polarization to MDSCs and M2-like macrophages (25). Moreover, myeloid cells can trigger Fas-mediated apoptosis of T lymphocytes, contributing to the establishment of an immune-suppressive microenvironment (155).

Tumor-infiltrating, monocyte-derived cells display membrane B7-1 and B7-2 proteins, which can have either protumor or antitumor activity by triggering CTLA4 or CD28 receptors exposed on T cell membranes, respectively (156). Tumor and stromal cells synergize to establish an immunosuppressive milieu by releasing an array of cytokines and chemokines that orchestrate the infiltration and polarization of immune-suppressive leukocyte subsets. This is the case for TGFβ, whose production by monocyte-derived macrophages and MDSCs is a major source of the cytokine in the TME. TGFβ has multiple negative effects on tumor immunity: excluding T cell infiltration in the tumor bed (157), sustaining Treg generation, and impairing effector T cell and NK function (158). MDSCs and TAMs also shift the IL-10/IL-12 balance toward increased IL-10; more specifically, MDSCs skew the polarization of TAMs and DCs toward protumor elements via the release of IL-10. TAM-derived IL-10 has negative effects on T cell immunity, as indicated by the experimental observation that IL-10 pharmacological blockade can interrupt this switch by inducing IL-12-producing $CD103^+$ DCs that are able to support T cell antitumor functions in breast cancer models (159).

5.2. Promotion of Tumor Growth by Other Mechanisms

Recent findings confirm the hypothesis that TAMs and MDSCs can directly support tumor growth through mechanisms that extend beyond the regulation of the antitumor immune

response. Indeed, myeloid cells play a pivotal role in sustaining the metabolic needs of tumor cells, assisting tumor evolution during all phases of progression, from the initial oncogenic cascade to its spread to distal anatomical sites (160). The increased demand for nutrients and oxygen sensed by HIF-1α triggers the release of inflammatory cytokines in glioblastoma cells, including stromal cell-derived factor-α (SDF1α), which supports the infiltration of inflammatory monocytes. The recruited cells in turn increase VEGF availability via MMP9-mediated matrix remodeling (93). A self-perpetuating positive loop also sustains tumor angiogenesis: TNF-α and VEGF released in the TME supports the infiltration of both intermediate and classical monocytes, which in turn increase VEGF availability via MMP9 activity (161). MDSCs can also contribute to angiogenesis by activating a Bv8-dependent pathway that bypasses VEGF restriction (9, 80). Refractoriness to antibody-mediated antiangiogenic therapy in colorectal cancer is linked to SDF1α secretion in the TME, supporting the hypotheses that many compensatory mechanisms sustain tumor angiogenesis and that myeloid cells play a pivotal role in these processes (162). Thus, a therapeutic approach with multiple targets is necessary to restrict the supply of nutrients to cancer (163).

MDSCs and TAMs are able to shape the tumor ECM to support tumor proliferation and promote cell invasion by releasing an array of digestive enzymes, including cathepsins and MMPs (164). However, TAMs also support tumor metastases by directly guiding tumor cells across the ECM toward the basal membrane and endothelium, opening a gate for tumor-cell entry into the blood. This is mediated by the establishment of a paracrine signaling loop between CSF-1 and epidermal growth factor (EGF) between the two cells, which can be interrupted by inhibiting either CSF-1R or EGFR downstream signaling (165). Accordingly, EGF and CSF-1 expression in TAMs and tumor cells, respectively, are independent markers of poor prognosis in breast cancer (56). Tumor-infiltrating, monocyte-derived cells also support the metastatic process by inducing the epithelial-to-mesenchymal transition (EMT). By activating a β-catenin-mediated pathway, macrophage-derived TGFβ inhibits expression of the epithelial marker E-cadherin and increases the expression of mesenchymal markers, triggering an invasive phenotype in breast cancer cells (166). These findings are clinically relevant since macrophage infiltration and TGFβ levels are positively correlated with mesenchymal markers and tumor grade in non–small cell lung cancer patients (166).

ECM composition plays an important role in defining the fate of tumor cells: Secreted protein acidic and rich in cysteine (SPARC)-rich ECM is associated with poor clinical outcomes and preferential EMT in breast cancer. SPARC orchestrates the infiltration and immune-suppressive function of MDSCs (88), and targeting of MDSCs with aminobisphosphonates [zoledronic acid (ZA)] is able to reverse the EMT (167). Indeed, ZA treatment affects both MDSC-dependent immune-suppressive and protumor functions by directly impairing STAT3, ARG1, and TGFβ expression, resulting in increased T cell proliferation and higher E-cadherin expression on tumor cells.

TAMs and MDSCs revert senescence in tumor cells and support cancer stem cells (CSCs) via many mechanisms. TAM-derived IL-6 activates STAT3-induced expansion of $CD44^+$ hepatocellular carcinoma (HCC)-derived cells, the formation of spheroids in vitro, and tumor establishment in vivo. Importantly, levels of IL-6 are clinically correlated with cancer progression and markers of CSCs in HCC (168). However, TAMs also sustain CSCs through juxtacrine signaling mediated by activating CD90 and Ephrin-4 receptors on breast CSCs. This interaction activates signaling via the Src and NF-κB pathways and ends in the secretion of an array of cytokines (i.e., IL-6, IL-8, and GM-CSF) that sustain the EMT state of CSCs (169). MDSCs revert cancer senescence in PTEN-null prostate cancer in a paracrine manner by releasing IL1-RA, and strategies that target MDSC expansion or genetically delete IL1-RA critically affect this process (170).

Monocytes can either contribute to or limit metastatic spreading depending on their origin and polarization status. Inflammatory CCR2^{+} monocytes are recruited to primary breast tumors following CCL2 secretion by tumor and stromal cells; at the premetastatic site, they support tumor extravasation in a VEGF-dependent manner (171). CCL2 and macrophage infiltration are correlated with worse clinical outcomes and higher metastatic probability in breast cancer patients. The pharmacological inhibition of CCL2-CCR2 signaling (by targeting either CCL2 or CCR2) decreases inflammatory monocyte recruitment to primary tumors, thus reducing metastatic spreading and increasing mouse survival (171). Patrolling monocytes control tumor seeding in the lungs, fostering cancer immune surveillance in many mouse tumor models, including breast cancer. Indeed, their removal by Nr4a1 genetic deletion resulted in higher metastatic spreading (172). Even if the underlying mechanism is not completely defined, it involves efficient patrolling by monocytes to remove tumor material engulfed in the lung vasculature in a C-X3-C chemokine receptor (CX3CR)1-dependent manner and promotes the recruitment and activation of NK cells (172).

6. CONCLUDING REMARKS

Accumulating evidence indicates that tumors influence monocyte fate inside the TME, favoring the accumulation of protumoral cells with angiogenic, trophic, and immunosuppressive properties. However, this tumor imprinting is reversible since monocyte-derived cells maintain plasticity, which allows them to be reprogrammed into antitumor elements. Rewiring tumor-infiltrating, monocyte-derived cells has been shown to induce antitumor effects in preclinical models (173, 174). Indeed, strategies to directly manipulate the transcriptional programs in monocyte-derived cells have been developed, including methods to modulate key signaling pathways associated with immune suppression, i.e., the STAT3, NF-κB, and PI3Kγ pathways. Furthermore, combined actions might improve the final pharmacological activity: For example, PI3Kγ targeting inhibits not only the growth of tumor vasculature but also local immunosuppression by reducing immunosuppressive TAMs or stimulating TADCs to produce cytokines that induce effective T cell responses (175). The CD40:CD40 ligand axis may represent another interesting target for turning immunosuppressive cells into effective APCs. Indeed, agonistic CD40 antibody-based therapies abrogate MDSC:Treg cross talk, convert MDSCs into functional T cell–priming cells, and prompt TAMs to exert tumoricidal activity (176). Therefore, a new era of cancer immunotherapy based on targeting tumor-infiltrating myeloid cells to enhance current therapeutic protocols is just around the corner. More caution is warranted when neutralizing immune factors that are not unique to tumors and their TMEs (e.g., iNOS) since they are essential for maintaining body homeostasis. Blocking these factors will require the targeting of specific cell subsets that are induced by cancer programming. Thus, to develop future therapies, it will be essential to expand our knowledge of the mechanisms regulating monocyte differentiation inside the TME. In particular, we need to decipher the complex repertoires of tumor-infiltrating myeloid cells by applying novel technologies (e.g., single-cell RNA-seq approaches) to precisely map the steps that monocytes take as they differentiate into either tumor-promoting or tumor-suppressing cells.

DISCLOSURE STATEMENT

V.B. is a Strategic Advisor for IO Biotech ApS, Xios Therapeutics, Codiak BioSciences, Inc., and EMD Serono; is a consultant for Ganymed Pharmaceuticals AG and Incyte Corporation; and holds patents related to NO-releasing furoxan-derived compounds and myeloid cell targeting or generating approaches for c-FLIP modulation. S.U. received a research grant from IO Biotech ApS and holds a patent related to c-FLIP.

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Errata

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