Monocytes and Macrophages in Cancer: Development and Functions

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Abstract Monocytes and tumor-associated macrophages are part of the myeloid family, a group of hematopoietic derived cells. Monocytes are direct precursors of hematopoietic stem cell-derived macrophages. After their recruitment into the tumor tissue, they can differentiate into tumor-associated macrophages, a very heterogeneous cell population in terms of phenotype and pro-tumor function, supporting tumor initiation, local progression and distant metastasis. Therefore, targeting monocytes and macrophages is a promising immunotherapeutic approach. This review will focus on the development of monocytes as macrophage precursors, the functions of tumor-associated macrophages and the possibility of interfering with tumor development and progression by targeting these myeloid cells.

Keywords Tumor-associated macrophages · Monocytes · Myeloid cells · Immunotherapy

Introduction

The mononuclear phagocyte system (MPS) represents a body-wide, specialized system of different phagocytic cell types derived from bone marrow and yolk sac progenitors [1, 2]. Monocytes (Mo) and macrophages (MΦ) are essential components of the MPS and critically involved in

central processes of the MPS such as innate immune functions, support of adaptive immunity and tissue homeostasis [1, 3].

Ilya Mechnikov, a bacteriologist, first described macrophages more than a century ago. They colonize every organ in the body and, based on the location, some specialized macrophages have received specific names, e.g., Kupffer cells in the liver, microglia in the neuronal tissue and osteoclasts in the bone. They have specific functions under tissue homeostasis including phagocytosis of apoptotic cells, growth factor production and angiogenesis induction [4, 5]. In response to inflammation, they are needed for pathogen clearance, followed by a phase of tissue repair and wound healing that promotes restoration of homeostasis [5]. Monocytes are precursors of resident macrophages. Differentiation occurs upon entering the tissue and is under the control of local environmental cues [6, 7]. As a second origin, it has recently been reported that yolk sac-derived cells can constitute a different line of macrophages in adult mice. Their development occurs independently of monocytes derived from adult hematopoietic stem cells (HSC) and the transcription factor *Myb* [2].

The tumor tissue comprises a very large and diverse set of myeloid cell lineages. This review will focus on monocytes and tumor-associated macrophages (TAMs), their differentiation, function and strategies to target them for immunotherapy.

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Steady-State Development of Monocytes

Monocytes are found in the blood and spleen, but their largest reservoir under homeostatic conditions is the bone marrow, the primary site of monocyte generation [3, 8].

Still, monocytes are also produced by extra-medullary hematopoiesis in the spleen and increase under inflammatory conditions such as atherosclerosis and cancer as recently shown [9, 10]. In the bone marrow, monocytes are generated from HSC in a sequential process of differentiation and commitment steps [3, 11], collectively called monopoiesis (Fig. 1). Monopoiesis is tightly regulated by microenvironmental cues, modulating gene expression in developing cells and leading to the often irreversible, phenotypic and functional changes associated with hematopoietic differentiation. Although the key role of cytokines, such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3), within this developmental niche has been intensively studied [12–14], the impact of other niche components, like immobilized factors on stromal cells or extracellular matrix (ECM) components, on monopoiesis remains still unclear. Early-phase monopoiesis proceeds via distinct proliferative progenitor stages, such as common myeloid progenitors (CMP) and granulocyte/macrophage

progenitors (GMP) [15], to the macrophage dendritic cell progenitor (MDP) [12, 16], which serves as a common precursor for monocytes, macrophages and dendritic cells (DCs). During late-phase monopoiesis, the MDP finally gives rise to monocytes (Fig. 1) [12, 16]. These bone marrow-derived monocytes have lost their proliferative potential and can be sub-divided by expression of chemokine receptors and additional cell surface markers into “classical” $\text{Ly6C}^{\text{high}}$ and “non-classical” Ly6C^{low} monocytes [11, 17]. However, the two subsets can also be distinguished by functions such as the patrolling behavior found in Ly6C^{low} but not $\text{Ly6C}^{\text{high}}$ monocytes [18]. As these phenotypic and functional differences are found in both human and murine monocyte subsets, evolutionary conservation of monocyte heterogeneity has been suggested [19–22]. The developmental relationship between the monocyte subsets is still not completely understood [11]. Previous studies have demonstrated that $\text{Ly6C}^{\text{high}}$ monocytes shuttle between the blood and bone marrow and eventually lose expression of Ly6C , suggesting a linear conversion model of $\text{Ly6C}^{\text{high}}$ into Ly6C^{low} monocytes within the

Fig. 1 Origins of monocytes and macrophages in steady-state and disease. Different subsets of monocytes (Mo) are generated in the bone marrow from hematopoietic stem cells (HSC) via common myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP) and monocyte/macrophage dendritic cell progenitors (MDP). Currently, the origin of Ly6C^{low} monocytes is unclear (dotted lines). The monocyte subsets are mobilized from the bone marrow into the blood stream and form a local reservoir in the spleen (re-mobilized in response to injury or inflammation). In the steady-state, blood monocytes are recruited to different tissues and give rise to populations of monocyte-derived macrophages (Mo-MΦ) and DCs (Mo-DCs) involved in tissue development and

maintenance of homeostasis. Importantly, these populations are distinct from yolk sac-derived macrophages (YS-MΦ), renewing locally and independently from monocytes, and DCs derived from common DC progenitors (not displayed). Under inflammatory conditions, monocyte mobilization and recruitment to tissues is greatly enhanced, leading to generation of populations of inflammatory macrophages and monocyte-derived DCs supporting innate and adaptive immune responses. In presence of tumors, monocytes give rise to populations of immunosuppressive tumor-associated macrophages (TAMs) and monocytic MDSCs (M-MDSCs) at the tumor site promoting tumor progression and immune evasion

bone marrow (Fig. 1) [23]. Alternatively, a divergent model is possible in which the monocyte subsets arise independently from each other from a common progenitor, however supporting evidence for this model is limited [11]. Novel transgenic mouse models with subset-specific defects, such as the recently described transcription factor Nr4a1-deficient mouse which lacks most of the Ly6C^{low} monocytes [24], as well as novel subset-specific molecular targets, e.g. the recently identified microRNA *miR-146a* [25], will be of great value for answering these and further questions.

Development of Monocytes in Cancer

In cancer, many characteristics of myeloid cells, such as migratory and functional properties, are altered, but whether the principal developmental processes in the bone marrow are also affected is currently under investigation. Recently, Pittet and colleagues revealed alterations in myeloid development in a model of lung adenocarcinoma driven by activation of *K-Ras* and inactivation of *p53* [10]. In this model, early hematopoietic progenitors (such as the GMP) relocated to the spleen during tumor progression and locally produced monocytes and granulocytes that contributed to tumor growth. Thus, the bone marrow partially outsources the production of myeloid cells to extra-medullary sites in cancer (and potentially also in other inflammatory diseases). The findings of Pittet and colleagues are reminiscent of myeloid-derived suppressor cells (MDSCs) [26–28]. MDSCs, comprising monocytic and granulocytic subpopulations, are

potent immune suppressors in various pathological conditions (like cancer and other inflammatory diseases) and promising targets for upcoming therapies (as reviewed by Dr. Umansky and colleagues in this issue and [29]). Previous reviews have discussed developmental aspects of MDSCs and suggested the generation of monocytic MDSCs (M-MDSCs) in bone marrow without a monocytic intermediate stage from a separate developmental branch in monopoiesis [27, 28]. Usually identified as CD11b⁺ Ly6C⁺ Ly6G[−] cells in mice, M-MDSCs yet share a common phenotype and morphology with normal Ly6C^{high} monocytes (Table 1) [26, 27]. Furthermore, the limited marker panel used for M-MDSC identification and the use of antibodies recognizing less specific markers like Gr-1 (clone: RB6-8C5), a shared epitope of Ly6C and Ly6G [30, 31], make distinguishing M-MDSC from normal monocytes challenging. Thus, M-MDSCs are currently defined by their suppressive activity, which is absent in normal monocytes [27]. This definition may result in an image that reflects monocytes and their macrophage progeny, which acquired immuno-suppressive properties in response to tumor-derived factors, rather than a separate developmental branch from defined monocyte/macrophage progenitors (such as the GMP or MDP) (Fig. 1). Although tumors produce factors involved in several aspects of monocyte/macrophage biology (such as GM-CSF) [27], the current evidence supporting a concept of M-MDSC development without a monocytic intermediate is limited and would benefit from comprehensive fate-mapping approaches. The identification of additional markers or progenitors specific for M-MDSCs (compared to normal monocytes) will certainly help elucidating this issue.

Table 1 Phenotypic identification of murine monocytes and macrophages. This table summarizes currently used phenotype markers for identification of monocytes, macrophages and M-MDSCs in mice

Population	Subset	Phenotype	Ref.
HSC	Long-term repopulating HSC	c-Kit ⁺ Sca-1 ⁺ CD34 [−] CD135 [−] CD48 [−] CD150 ⁺	[112]
CMP	–	c-Kit ⁺ Sca-1 [−] CD34 [−] IL-7Rα [−] FcγR ^{low}	[15]
GMP	–	c-Kit ⁺ Sca-1 [−] CD34 ⁺ IL-7Rα [−] FcγR ^{high}	[15]
MDP	–	c-Kit ⁺ Sca-1 [−] CD11b [−] CD115 ⁺ CD135 ⁺ CX3CR1 ⁺ Ly6C [−] Ly6G [−]	[11, 12, 16, 51]
Mo	Ly6C ^{high} Mo	c-Kit [−] CD11b ⁺ CD115 ⁺ CD135 [−] CX3CR1 ^{low} Ly6C ^{high} Ly6G [−] CCR2 ⁺ F4/80 ^{low}	[11, 17, 19]
	Ly6C ^{low} Mo	c-Kit [−] CD11b ⁺ CD115 ⁺ CD135 [−] CX3CR1 ^{high} Ly6C ^{low} Ly6G [−] CCR2 [−] F4/80 ^{low}	
MΦ ^a	Yolk sac-derived MΦ	CD11b ⁺ F4/80 ⁺⁺	[2, 113–115]
	Monocyte-derived MΦ	CD11b ⁺⁺ F4/80 ⁺	
Mo-DC ^a	DC-SIGN ⁺ Mo-DC	CD11b ⁺ CD115 [−] CD209a/DC-SIGN ⁺ CD11c ^{high} Ly6C [−] Ly6G [−] F4/80 ^{low}	[11, 48]
	CX3CR1 ⁺ Ip Mo-DC	CD11b ⁺ CD14 ⁺ CX3CR1 ^{int/high} CD103 [−] CD11c ^{high/int}	[11, 52]
	Tip-DC	CD11b ⁺ CD11c ^{int} Tnf-α ⁺ iNOS ⁺ CX3CR1 ⁺ Ly6C ⁺ Ly6G [−] CCR2 ⁺	[11, 116–118]
MDSC	Monocytic MDSC	CD11b ⁺ CD115 ⁺ Ly6C ^{high} Ly6G [−] CCR2 ⁺ F4/80 ^{low}	[29, 119]

^a MΦ and Mo-DC populations at different anatomical sites and under different conditions may exhibit strong phenotypic heterogeneity. For MΦ, a combination of high F4/80-expression and further tissue-specific markers is commonly used for identification [113–115], whereas relative levels of F4/80 and CD11b may discriminate between monocyte-derived macrophages and yolk sac-derived macrophages (such as Kupffer cells in liver, Langerhans' cells in skin or microglia in brain) [2]. For Mo-DC, markers for discrimination from other DC-subsets have been identified, such as DC-SIGN for DCs in inflamed lymph nodes [48], CX3CR1 for intestinal lamina propria DCs [52] and TNF-α/ROS-production for Tip-DCs [116, 117]

Mobilization and Recruitment of Monocytes to Target Sites

Monocytes are mobilized from the bone marrow and spleen in response to chemotactic signals and recruited to target tissues guiding their further differentiation [32]. These migration processes have been recently reviewed in detail [32]. Due to distinct expression of chemokine receptors (such as CCR2 and CX₃CR1), the monocyte subsets have different migratory properties [19]. A major role in mobilization and recruitment of Ly6C^{high} monocytes, under both homeostatic and pathological conditions, has been attributed to the chemokine receptor CCR2 (CD192), bound by the chemokines CCL2 and CCL7 [32–34]. CCL2 (monocyte chemoattractant protein-1 or MCP-1) is expressed by many nucleated cell types in response to pro-inflammatory or microbial stimuli and the role of the CCL2-CCR2-axis in recruitment of Ly6C^{high} monocytes to sites of inflammation has been demonstrated for various pathological conditions (such as infection, atherosclerosis and diabetes) [32–36].

In cancer, CCL2 was identified as the first tumor-derived factor (TDF) that induced chemotaxis in monocytes [37]. Produced by tumor cells and the associated stromal cells, CCL2 is currently acknowledged as the major tumor-derived factor responsible for recruiting circulating monocytes from the blood into a variety of mouse and human tumors [28, 38]. Interestingly, recent work from Qian and colleagues demonstrated that metastatic tumor cells and target-site stromal cells also produce CCL2 [39]. Using a mammary tumor model, they observed that a sub-population of CCR2-expressing inflammatory monocytes was recruited to the target-site and promoted extravasation, establishment and persistent growth of the tumor metastasis [39].

Tumor cells and the associated stromal network also produce additional molecules that are traditionally involved in monocyte recruitment to, and differentiation in, inflammatory sites. These TDFs include a number of chemokines, including CCL3, CCL4, CCL5 and CXCL12, and various growth factors, including M-CSF, transforming growth factor- α (TGF- α), fibroblast growth factor (FGF), Angiopoietin-2 (Ang-2, critical for Tie2-expressing monocytes) and vascular endothelial growth factor (VEGF) [26, 28, 40, 41].

Development and Functions of Macrophages

Following tissue recruitment, monocytes are polarized by the local microenvironment and differentiate into resident macrophages under the control of multiple tissue-specific factors [6, 7]. This differentiation process is accompanied by vast phenotypic, functional and morphological changes, establishing a spectrum of diverse macrophage populations throughout the body [6, 7]. Notably, monocyte-derived

macrophages are phenotypically and functionally distinct from yolk sac-derived macrophages, which represent a major fraction of F4/80⁺ macrophages in some tissues [2]. As recently demonstrated by Geissmann and colleagues, these yolk sac-derived macrophages develop independently of the transcription factor *Myb*, renew locally and persist in tissues in adult mice independently from HSC in contrast to monocyte-derived macrophages [2] (Fig. 1). In the future, delineation between the functions of monocyte-derived versus yolk sac-derived macrophages, under steady-state and disease conditions, may be of great value for the field, as it may help to understand the diversity of macrophages and hold therapeutic potential.

Tissue macrophages display enormous functional and phenotypical plasticity in response to changing microenvironmental stimuli [42, 43]. Under steady-state conditions, macrophages support either homeostatic processes or trophic processes for tissue development/remodeling in developing or healing tissues [4, 5]. The mechanisms involved in this support include phagocytosis, growth factor production, angiogenesis and degradation of ECM components by secreted MMPs (matrix metallo-proteinases) [4, 5]. In response to immunogenic signals (such as Toll-like receptor (TLR) signals or inflammatory cytokines), the functional properties of macrophages are polarized towards processes needed for immunity and pathogen defense. These include pathogen phagocytosis, release of cytotoxic ROS/RNS (reactive oxygen/nitrogen species), production of pro-inflammatory cytokines and major histocompatibility complex (MHC) class II-mediated antigen presentation [5]. Usually, these immuno-stimulatory processes are followed by a phase of tissue remodeling and restoration of homeostatic conditions. Interestingly, macrophage polarization requires some transcriptional regulators that are also involved in monopoiesis. This was recently shown in Nr4a1-deficient mouse models yielding pro-inflammatory macrophages during atherosclerosis [44].

According to their polarization status, tissue macrophages were originally categorized on a linear scale from pro-inflammatory M1-macrophages (classically activated) to anti-inflammatory M2-macrophages (alternatively activated) as the two extreme ends of the scale [45–47]. Although still commonly used, and useful for conceptual understanding, the applicability of the M1/M2-classification is limited due to its oversimplification of the functional diversity of macrophages [6, 45]. More recently, alternative classification systems have been suggested that are based on macrophage function and relative contribution to tissue development, tissue remodeling and immune responses, thus reflecting the functional plasticity of macrophages in response to different stimuli [6].

Although established as precursors of macrophages, monocytes can develop into cells with more robust antigen-presentation capacity compared to macrophages [1, 3].

Recently, these cells were termed monocyte-derived DCs (Mo-DCs) to discriminate them from classical and plasmacytoid DCs which are derived from a separate progenitor [48–51]. These Mo-DCs were characterized in particular under inflammatory conditions and include Tip-DCs (TNF- α - and iNOS-producing DCs), DC-SIGN⁺ DCs and CX3CR1⁺ lamina propria DCs of the small intestine [23, 48, 52, 53] (Fig. 1). The biology of DCs and their relationship to monocytes have been discussed elsewhere in detail [3, 11, 54, 55].

Tumor-Associated Macrophages

Tumor-derived factors attract circulating monocytes into the tumor tissue where they differentiate into macrophages (Fig. 1). In cancer, it is becoming clear that TAMs, like other members of the myeloid family, are incredible heterogeneous and depending on the tissue and type of tumor, the stage of tumor progression and location within the tumor tissue, different subpopulations of TAMs may differ considerably in terms of function and phenotype [27, 56].

A recent study, using multiple mouse tumor models, demonstrated that multiple subpopulations of TAMs (and/or potentially monocyte-derived transition stages) could be consistently identified based on their expression of surface markers such as Ly6C and MHC [56]. These sub-populations localized to different regions of the tumor microenvironment (such as hypoxic and normoxic regions) and had distinct functions. In addition, the relative frequencies of these subsets changed as the tumor microenvironment changed during disease progression [27, 56].

These results highlight a potential reason for the dynamic (and often seemingly contradictory) nature of the TAM function in cancer. Historically, a high TAM density has been associated with poor clinical outcome [57]. Although the majority of animal and human data support the idea that TAMs promote tumor growth, there are exceptions that seem to depend on the tumor location and the stage of tumor development. For example, TAM infiltration has been shown to correlate with poor prognosis in breast, cervical, and bladder cancer but there is conflicting evidence in cancer of the prostate, lung and brain [57]. Furthermore, more recent research has suggested that not the number, but the phenotype of TAMs is more informative for patient prognosis. In this regard, increased density of M2-like TAMs is a marker of bad prognosis, whereas increased density of M1-like TAMs is a marker of better prognosis [57–61]. Thus, different macrophage subsets or polarization states are critical for cancer patient prognosis.

Functions of Tumor-Associated Macrophages

TAMs can influence nearly every stage of tumor development and progression. They have a wide range of anti-tumor

functions, including production of cytotoxic factors, phagocytosis of tumor cells, especially metastatic cells, and, recently described, participation in cancer immuno-editing [62, 63]. In that regard, new therapies, such as targeting the CD47-signal regulatory protein- α (SIRP α) signaling pathway, are being investigated that aim to activate and expand these functions. TAMs also have a wide range of pro-tumor functions. In the pre-tumor stage, high production of inflammatory mediators can contribute to neoplastic transformation. In more established tumors, TAMs produce factors that contribute to tumor growth, angiogenesis, immune suppression, and tissue remodeling. In addition, TAMs can promote metastatic spread of cancer cells [26, 47, 64, 65]. In this review, we will focus on new insights in the pro-tumor functions of TAMs and describe how understanding these functions can benefit anti-tumor therapy.

Inflammation-Induced Genetic Alterations and Instability

It is becoming increasingly clear that chronic unresolved inflammation is the underlying cause of many types of cancer [63, 64, 66]. Mantovani and colleagues have called this “smoldering” inflammation the seventh addition to Hanahan and Weinberg’s “Hallmarks of Cancer” [67, 68]. In sites of chronic unresolved inflammation, macrophages initially triggered by a pathogen or tissue stress, recruit monocytes that develop into additional inflammatory macrophages, producing cytokines and chemokines propagating and amplifying the inflammatory cascade [66]. Activated macrophages, located in the sub-epithelial spaces, can contribute to genetic mutations in the adjacent epithelial cells through the production of DNA-damaging reactive nitrogen and oxygen species [69–71]. Yet, if DNA repair fails and these mutations accumulate and stabilize, neoplasia can develop and lead to the production of a new wave of TDFs, including M-CSF and CCL2, which will influence the recruitment of more monocytes/macrophages.

Production of Trophic Growth Factors and Promotion of Angiogenesis

During the next stage of tumor development, recruited monocytes differentiate into macrophage subpopulations, unlike those found in the acute inflammatory environment, which support tissue remodeling and thereby promote tumor growth. This change in polarization state, exemplified by the M1/M2 macrophage classification system, plays a key role in the pro-tumoral versus anti-tumoral functions of TAMs and, as such, is of high importance for tumor therapy [63].

TAMs are well-documented producers of trophic and activating factors that directly promote the proliferation and survival of tumor and stromal cells [66]. This includes, among many others, endothelial growth factor (EGF), FGF,

VEGF, platelet-derived growth factor (PDGF), and transforming growth factor- β (TGF- β) [28, 66, 72]. In addition, TAMs secrete proteolytic enzymes that degrade the ECM and facilitate the diffusion of growth factors in the tumor microenvironment [40, 66].

One of the most important roles for macrophages in tumor growth seems to be in promoting angiogenesis. Tumor development begins with an “avascular phase” characterized by a limited number of cells and acquisition of nutrients by simple diffusion. As the tumor becomes larger, the metabolic demands increase and a more developed vascular infrastructure is required. This transition to this “vascular phase”, termed the “angiogenic switch”, is stimulated by macrophages in the tumor [63, 73, 74]. Of note, it might be a very standard physiological reaction of macrophages to respond to tissue hypoxia and initiate, or support, angiogenesis and not at all specific to the tumor environment. Although most TAM populations have been shown to play a role in vascular growth, the Tie2-expressing monocyte/macrophages (TEMs) seem to be a critical promoter of tumor angiogenesis [28, 75–77]. TEMs are a small subset of tumor-associated myeloid cells characterized by the expression of the Ang-2 receptor Tie2 [41, 77, 78]. They derive from circulating Tie2-expressing monocytes, which are recruited into the hypoxic areas of solid tumors by hypoxia-induced, endothelial-derived chemotactic factors, such as Ang-2 and CXCL12 (the CXCR4 ligand) [76–78].

Promotion of Metastasis by Monocytes and Tumor-Associated-Macrophages

TAMs play a role in multiple stages of tumor metastasis, from promoting tumor cell escape from the primary site to facilitating tumor cell arrival and establishment at distant sites. The association between monocytes/macrophages and tumor metastasis has been established by extensive clinical correlations as well as genetic experiments where certain myeloid cell types have been eliminated [40, 63, 79, 80]. Recent results, particularly from new advances in multi-photon imaging, have begun to shed light on the specific mechanisms of monocyte/macrophage-mediated metastasis.

Migration and Invasion of Cancer Cells

After detachment from the primary tumor, tumor cells begin the process of migration/invasion that brings them into contact with blood or lymph vessels. Wyckoff and colleagues elegantly demonstrated that interactions between macrophages and tumor cells facilitate their simultaneous migration through the primary tumor [81]. They concluded that tumor-derived factors, like M-CSF, stimulate macrophage migration and production of EGF, which then activates tumor cell migration. In addition, inhibition of either

M-CSF or EGF signaling blocked migration of both cell types [81].

Macrophages also produce proteases that facilitate the escape of tumor cells from the ECM at the tumor border through a process called invasion [63]. At specific stages of tumor development, especially at the transition to metastatic disease, macrophages can be found at locations of basement-membrane breakdown suggesting that tumors could exploit the normal matrix remodeling capacities of macrophages [80].

Although monocytes/macrophages play an important role in the migration and invasion of solid tumors, their role in hematopoietic tumors is not as well understood. Recently, Lee and colleagues utilized *in vitro* assays to examine the role of monocytes/macrophages in the progression of human B-cell precursor acute lymphoblastic leukemia (BCP-ALL) [82]. They observed that BCP-ALL “conditioned” monocytes as well as BCP-ALL patient-derived monocytes were able to promote migration and invasive capacity of BCP-ALL cells in a CXCL10-dependent manner [82].

Intravasation of Cancer Cells

TAMs also enhance the ability of tumor cells to enter the blood vessels, a process called intravasation. Intravasation efficiency correlates with migration/invasion capability and density of macrophages at the tumor-vascular interface [63, 80]. Intravital multi-photon microscopy showed that tumor cell intravasation occurs through clusters of macrophages located on the abluminal side of the vessels [83]. Recently, Zervantonakis and colleagues described a three-dimensional micro-fluidic model to test tumor cell intravasation and endothelial barrier permeability [84]. They reported that macrophage production of tumor necrosis factor- α (TNF- α) resulted in endothelial barrier impairment and a higher intravasation rate.

Extravasation of Cancer Cells and Establishment of Local Metastasis

The tumor microenvironment and TAMs both play crucial roles in the initiation of metastasis at the primary tumor site. However, the functions of monocytes/macrophages at the metastatic site have remained largely unknown [27]. Recently it was shown that unique monocyte/macrophage sub-populations are recruited to extravasating pulmonary metastatic cells in an animal model of breast cancer metastasis [85]. These populations were required for extravasation as well as establishment and proliferation of the metastasis.

Qian and colleagues extended these earlier findings by demonstrating that the Gr1⁺ CCR2⁺ inflammatory monocyte sub-population is preferentially recruited to pulmonary metastases but not to primary mammary tumors in mice [39]. An *ex vivo* whole-lung imaging system showed that CCL2 produced

by both metastatic tumor cells and target-site stromal cells was critical for this recruitment. In addition, the sub-population of CCR2-expressing monocytes enhanced the extravasation of the tumor cells through the production of VEGF-A [39].

Even after successful extravasation into potential metastatic sites, the vast majority of tumor cells are killed in the unfriendly stromal microenvironment. Chen and colleagues used a mouse metastatic breast cancer model to investigate tumor cell infiltration and survival in the leukocyte-rich microenvironment of the lungs [86]. They found that vascular cell adhesion molecule-1 (VCAM-1 or CD106) is aberrantly expressed on lung metastatic breast cancer cells. VCAM-1, generally expressed by vascular endothelial cells, binds to the $\alpha 4\beta 1$ integrin (also called Very Late Antigen-4 or VLA-4), a hetero-dimer composed of CD49d ($\alpha 4$) and CD29 ($\beta 1$). VCAM-1 expression facilitates the tethering of metastasis-associated macrophages to the tumor cells in an $\alpha 4$ -integrin-dependent manner. These interactions result in VCAM-1 clustering and activation of downstream signals that lead to protection from pro-apoptotic cytokines, such as TRAIL, in the stromal microenvironment, thus supporting cancer cell survival and establishment of local metastasis [86].

Therapeutic Targeting of Monocytes and Macrophages in Cancer

Having discussed that monocytes and TAMs have multiple pro-tumor functions as well as an anti-tumor repertoire, we

want to highlight some findings on how to exploit this for anti-tumor therapy (Fig. 2). It is important to note that some of the following reports may describe targeting of monocytes, TAMs and/or MDSCs equally due to a lack of specificity. A more comprehensive view on MDSC-specific studies has recently been published [27].

Cytotoxic Compounds for Monocyte/Macrophage Depletion

Therapies based on using cytotoxic compounds for monocyte/macrophage depletion are loosely based on their general characteristics including location, proliferation, and phagocytic ability. Their ability to target proliferating cells has made these compounds attractive general chemotherapeutic agents. Only recently has their ability to specifically deplete monocytes and macrophages been investigated. Trabectedin (or Yondelis), a tetrahydroisoquinoline alkaloid produced by the marine tunicate *Ecteinascidia turbinata*, was discovered in a large anti-cancer screen of plant and marine material by the National Cancer Institute performed in the 1950s and 1960s. It is a DNA minor groove binder that blocks cell cycle and interferes with inducible gene transcription in a selective manner. Trabectedin was recently registered in Europe for the treatment of soft tissue sarcoma and ovarian cancer and is currently in a number of clinical trials for other types of cancer [87]. In addition to its anti-neoplastic activity, Trabectedin was shown to selectively deplete circulating monocytes in a small group of tumor patients, while other cells (including neutrophils and

Fig. 2 Targeting monocytes and macrophages in cancer. Monocytes (Mo) and tumor-associated macrophages (TAMs) are promising targets for a variety of diseases. This figure illustrates some recently developed strategies to target these cell types for immunotherapy of cancer. Several approaches intend to reduce the local pool of TAMs, thus preventing pro-tumoral functions in situ. Such strategies include the blockade of monocyte/macrophage development, the interference with

monocyte recruitment to the tumor as well as the direct deletion of TAMs following phagocytic uptake of cytotoxic agents or antigen-specific targeting. In contrast, other therapeutic strategies aim at functional modification of TAMs, thus activating anti-tumoral functions, rather than eliminating TAMs. Such approaches include the cytokine- and antibody-based therapeutic reprogramming of TAMs as well as licensing phagocytosis of targeted tumor cells by TAMs

lymphocytes) were significantly less sensitive to the drug [88]. Furthermore, sub-cytotoxic doses of Trabectedin inhibited both *in vitro* and *ex vivo* differentiation of monocytes to macrophages. Bisphosphonates are another class of anti-neoplastic compounds being tested for their ability to deplete macrophages *in vivo*. They are the primary treatment of bone metastases, secondary to several tumor types and have direct effects on different cancer cell lines [89]. To selectively target macrophages, the drugs are encapsulated into liposomes, which are then specifically endocytosed and degraded by macrophages. For example, clodronate-encapsulated liposomes have been used for *in vivo* depletion of macrophages and have shown subsequent reduction of tumor growth in a variety of tumor models [63, 90].

Antigen-Specific Targeting of Tumor-Associated Macrophages

Antigen-specific tumor-targeting strategies have been widely investigated and developed for clinical use. They have been used to target, and generally kill, certain tumor cells as well as blocking the factors that promote recruitment or drive tumor progression [91]. Unfortunately, the tremendous heterogeneity of macrophage populations and redundancy of cell-surface markers has made it difficult to use adaptive immune-based techniques, such as cytotoxic T cells (CTLs) or monoclonal antibodies, to specifically target TAMs. However, recent comprehensive gene and protein expression analyses have identified potential TAM-specific profiles that could be used for antigen-specific therapies [56, 92]. For example, DNA vaccines against MHC class I-restricted epitopes derived from legumain were developed to generate TAM-specific CTLs. Legumain is a member of the asparaginyl endopeptidase family and is a stress protein highly over-expressed by TAMs in the tumor microenvironment. These vaccines induced a robust CD8⁺ T cell response against TAMs and protected mice from lethal tumor cell challenges by suppression of angiogenesis, tumor growth, and metastasis [28, 92]. In addition, Movahedi and colleagues generated single-domain antibodies (sdAb) that are specific for the macrophage mannose receptor (MMR or CD206), which they found to be highly expressed on certain TAM subpopulations [56]. The MMR-specific sdAb were able to bind TAMs isolated from multiple tumor types *ex vivo* as well as *in vivo* following intravenous injection of sdAb [93]. These pre-clinical results suggest that antigen-specific targeting of TAMs is possible, although it remains to be seen if this will translate to effective therapy.

Targeting Monocyte Recruitment

High densities of M2-like TAMs in tumors are often associated with poor clinical outcomes and TAM depletion

strategies are successful at improving clinical outcomes. Therefore, blocking migration of monocytes into the tumor tissue could be an effective, and potentially less harmful, therapeutic strategy. As previously mentioned, a number of tumor-derived factors and their receptors, including CCL2, M-CSF and CXCL12 play important roles in recruiting monocytes into tumor tissue.

Using a CCL2-specific monoclonal antibody, Qian and colleagues inhibited the tumor recruitment of monocytes, decreased metastases and increased survival in a mammary tumor transgenic mouse system [39]. In addition, Leuschner and colleagues targeted monocyte recruitment to tumors using siRNA-mediated silencing of the chemokine receptor CCR2 in EL4 lymphoma and CT26 colon carcinoma models in mice, which resulted in decreased TAM accumulation and tumor growth [94].

M-CSF is critical for recruitment and differentiation of monocytes, and thus an important target for therapy. Many complementary strategies have been used to demonstrate that blocking this pathway significantly reduces monocyte infiltration, tumor growth and metastasis in a variety of tumor models. This includes targeting both M-CSF and M-CSFR with antisense-RNA, siRNA, monoclonal antibodies and chemical signaling inhibitors [27, 63, 95–97].

The CXCL12-CXCR4 pathway has been shown to participate in recruiting monocytes to tumor tissues. For example, interrupting this pathway with a CXCR4 antagonist is sufficient to significantly reduce recruitment of monocytes and inhibit tumor growth in multiple tumor models [27, 98, 99]. Chemokines promote monocyte recruitment into tissue, in part, by promoting changes in integrin affinity and avidity that increases attachment to endothelial cells bordering tumor tissue. In a subcutaneous Lewis lung carcinoma model, CXCL12 was shown to activate the $\alpha 4 \beta 1$ integrin on monocytes, increase integrin clustering and monocyte attachment to VCAM-1-expressing endothelial cell monolayers *in vitro* [98]. The importance of the $\alpha 4 \beta 1$ integrin in monocyte recruitment to tumor tissue was confirmed by using an $\alpha 4$ -specific blocking antibody and an $\alpha 4$ -specific small-molecule inhibitor to decrease monocyte infiltration and tumor growth [98]. These advancements in understanding the mechanisms behind CXCL12-CXCR4-mediated recruitment will provide additional targets for therapy.

Therapeutic Reprogramming of Tumor-Associated Macrophages

The numerous uniquely polarized sub-populations of TAMs found in different stages (and locations) of tumor development are potential targets for developing therapies that interfere with (or “reprogram”) macrophage differentiation. Manipulation of macrophage polarization is an especially broad topic that has been more comprehensively reviewed

[27, 28]. In this review, we will highlight some of the more recently described and further developed therapeutic ideas and give examples of two distinct techniques, monoclonal antibodies to surface-expressed proteins and recombinant cytokines, used to reprogram macrophages.

Beatty and colleagues used a human clinical trial and a mouse model to test if CD40 activation could reverse immune suppression and promote the anti-tumor T cell responses in pancreatic ductal adenocarcinoma (PDA) [100]. They tested a humanized CD40 agonist antibody (in combination with gemcitabine chemotherapy) in a small group of patients with advanced PDA and observed tumor regressions in some patients. Unexpectedly, tumor regression did not correlate with lymphocyte infiltration in primary lesions. In fact, further experiments in the mouse model found that tumor regression required macrophages but not T cells (or gemcitabine) [100]. They observed that treatment with the CD40 agonist antibody resulted in reprogramming of TAMs, including up-regulation of MHC class II and the costimulatory molecule CD86, accumulation in tumor tissue and ability to lyse tumor cells *ex vivo*. These results demonstrated a novel mechanism for CD40-mediated tumor elimination involving TAMs [100].

IL-12 is an important cytokine that is involved in lymphocyte and macrophage polarization and differentiation. Intravenous treatment of tumor-bearing mice with recombinant IL-12 has been shown to induce tumor regression and TAM conversion from a pro- to anti-tumoral phenotype [27, 101]. The following two examples are interesting because they describe IL-12-mediated TAM reprogramming using a unique cytokine delivery method. Kerkar and colleagues generated IL-12-secreting, tumor-specific CD8⁺ T cells in the B16 mouse model of established melanoma. By adoptively transferring these tumor-derived antigen-specific T cells, they were able to deliver IL-12 directly into the tumor tissue. They found that anti-tumor activity depended on the ability of the myeloid-derived cells, but not lymphocytes or NK cells, to respond to IL-12. In addition, this T cell-delivered IL-12 resulted in significant reprogramming of multiple myeloid-derived cell populations, including MDSCs, macrophages, and DCs within the tumor [102]. Chmielewski and colleagues took a different approach by engineering cytotoxic T cells with a chimeric antigen receptor (CAR) with specificity for a tumor-associated antigen. These cells release an inducible IL-12 (iIL12) upon CAR engagement in the tumor tissue [103]. Adoptive transfer of these CAR-iIL12 T cells resulted in an accumulation of activated macrophages within the tumor tissue that was critical to the anti-tumor response [103].

TNF- α is also an important cytokine involved in lymphocyte and macrophage polarization. Like the previous examples, this report is particularly interesting because of the delivery method. In a recent study, Johansson and colleagues engineered TNF- α with a tumor vasculature-targeting peptide to accurately deliver specific doses of the cytokine into mice

carrying pancreatic neuro-endocrine tumors [104]. They demonstrated that intravenous injections of low-dose TNF- α enhanced anti-tumor immunotherapy not by destroying the tumor vasculature, like intra-tumor interferon- γ (IFN- γ), but instead by increasing vascular stability and vessel perfusion. This vascular remodeling was mediated, in part, by TAMs, reprogrammed towards a more inflammatory anti-tumor profile [104].

Licensing Phagocytosis

CD47, a cell surface protein in the immunoglobulin superfamily, is expressed in the majority of normal tissues. It serves as an anti-phagocytic signal by binding to SIRP α , a protein expressed on macrophages and dendritic cells [105]. A number of recent reports have described how tumor cells use this pathway to avoid phagocytosis. Tumor cells were shown to constitutively up-regulate CD47 expression in multiple tumor entities and animal models. Furthermore, over-expression of CD47 was a poor prognostic factor and correlated with increased pathogenicity [106–110]. Treatment of solid or hematopoietic tumors with either CD47-specific blocking antibodies or SIRP α -fusion proteins resulted in an increase in macrophage-dependent phagocytosis of tumor cells and a corresponding decrease in tumor size and number of metastases [107–111]. Importantly, there did not seem to be a significant increase in the phagocytosis of normal cells *in vitro* or *in vivo*.

Concluding Remarks

Targeting the myeloid immune cell compartment in tumors is a promising approach for immunotherapy. Three cell types may be of special interest here: monocytes, TAMs and MDSCs. Currently, two main strategies are investigated: reduction of the pool size and functional modification of these myeloid cells (Fig. 2). The number of tumor infiltrating monocytes and macrophages can be reduced by controlling the development, recruitment and survival of these cells. Since their half-life is relatively short, interfering with generation and differentiation may have great potential. Several tools are available to modify the cellular functions. The overall goal is to activate the anti-tumoral potential of these myeloid cells. This can be achieved either by agonistic antibodies targeting surface structures such as CD40 or by reprogramming the cytokine profile of the cells. Another interesting approach is to license the phagocytosis potential by disrupting the CD47-SIRP α axis. Multiple manipulation strategies have been mentioned in this review and many more have been tested, but unfortunately, could not be discussed in-depth here.

In summary, targeting myeloid cells for immunotherapy is very encouraging, but whether a monotherapy will be

sufficient or a combination with adoptive immunotherapy or other strategies will be more effective remains to be determined.

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