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Review

Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation

G. Solinas, G. Germano,* A. Mantovani,*,† and P. Allavena*,1*

*Department of Immunology and Inflammation, IRCCS Istituto Clinico Humanitas, Rozzano, Milan, Italy; and †Department of Translational Medicine, University of Milan, Milan, Italy

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ABSTRACT

The microenvironment of solid tumors is characterized by a reactive stroma with an abundance of inflammatory mediators and leukocytes, dysregulated vessels and proteolytic enzymes. TAM, major players in the connection between inflammation and cancer, summarize a number of functions (e.g., promotion of tumor cell proliferation and angiogenesis, incessant matrix turnover, repression of adaptive immunity), which ultimately have an important impact on disease progression. Thus, together with other myeloid-related cells present at the tumor site (Tie2 macrophages and MDSCs), TAM represent an attractive target of novel biological therapies of tumors. *J. Leukoc. Biol.* 86: 1065–1073; 2009.

Introduction

The tumor mass is undoubtedly a multifaceted show, where different cell types, including neoplastic cells, fibroblasts, endothelial, and immune-competent cells, interact with one another continuously. Macrophages represent up to 50% of the tumor mass, and they certainly operate as fundamental actors. Macrophages constitute an extremely heterogeneous population; they originate from blood monocytes, which differentiate into distinct macrophage types, schematically identified as M1 (or classically activated) and M2 (or alternatively activated) [1–7]. It is now generally accepted that TAM have an M2 phenotype and show mostly pro-tumoral functions, promoting tumor cell survival, proliferation, and dissemination [2, 8, 9]. High levels of TAM are often, although not always, correlated with a bad prognosis, and recent studies have also highlighted a link between their abundance and the process of metastasis [10–15]. This pathological evidence has been confirmed also at gene level, where molecular signatures associated with poor prognosis in lymphomas and breast carcinomas include genes characteristic of macrophages (e.g., CD68) [16, 17]. Macrophage infiltration was studied along tumor carcinogenesis in a mouse model of pancreatic cancer induced by the expression of oncogenic $Kras^{G12D}$. Macrophage infiltration began very early during the preinvasive stage of disease and increased progressively [18]. Moreover, gene-modified mice and cell-transfer experiments have confirmed the pro-tumor function of myeloid cells and of their effector molecules. On the other hand, low macrophage infiltration into the tumor mass correlates with the inhibition of tumor growth and metastasis development in different animal models [19–22]. Lin et al. [19] demonstrated that when MMTV-PyMT mice, which spontaneously develop mammary tumors, were crossed with mice lacking monocytes/macrophages (op/op), the tumor growth and spread were reduced significantly. Accordingly, when cocultured with tumor cells, macrophages secrete substances that stimulate tumor cell proliferation. This countersense in which cells of the immunological system work against self hosts is a result of several refined tumor capabilities to mold immature cells and to suppress anticancer cell activity [8, 23, 24].

In contrast to macrophages, the prognostic relevance of a high percentage of TIL is usually associated with a better prognosis (e.g., melanoma, colorectal, and ovarian cancer) [25–27], although in gastric cancer, a high number of TIL seems to correlate with tumor progression [28], and in head and neck squamous cell carcinomas it is still controversial [29].

Within the tumor mass, another myeloid cell population characterized by immune suppressive activity has also been identified. These bone marrow-derived cells were functionally recognized in early studies [30] and more recently, defined as MDSCs able to suppress T cell blastogenesis in tumor-bearing hosts [31–34].

The huge infiltration of white blood cells together with the presence of several cytokines and chemokines and the occur-

Abbreviations: DC=dendritic cell(s), ECM=extracellular matrix, EGF=epidermal growth factor, FGF=fibroblast growth factor, HIF-1=hypoxia-inducible factor 1, MDSC=myeloid-derived suppressor cell, MINCLE=macrophage-inducible C-type lectin receptor(s), MMP=matrix metalloproteinase, PDGF=platelet-derived growth factor, ROI=reactive oxygen intermediate(s), SHIP1=Src homology 2 domain-containing inositol phosphatase 1, TAM=tumor-associated macrophage(s), TEM=Tie2-expressing monocyte(s), TIL=tumor-infiltrating lymphocyte(s), Treg=T regulatory cell, VEGF=vascular endothelial growth factor

1. Correspondence: Istituto Clinico Humanitas IRCCS, Via Manzoni 56, 20089 Rozzano (MI), Italy. E-mail: paola.allavena@humanitas.it

rence of tissue remodeling and angiogenesis are indicators of the strong inflammation present at tumor sites [35–41]. The association between cancer and inflammation dates back to 1863, when Rudolf Virchow noticed the presence of leukocytes in neoplastic tissues [35]. In particular, several studies have now identified two main pathways linking inflammation and cancer: an intrinsic and an extrinsic pathway. The first one includes genetic alterations that lead to inflammation and carcinogenesis, whereas the second one is characterized by microbial/viral infections or autoimmune diseases that trigger chronic inflammation in tissues associated with cancer development. Both pathways activate pivotal transcription factors of inflammatory mediators (e.g., NF-κB, STAT3, and HIF-1) and inflammatory leukocytes [38, 41, 42].

Here, we will review the current knowledge about TAM and other myeloid-derived tumor-infiltrating cells as pivotal players in the tumor microenvironment. A better understanding of their roles could certainly shed new light on the development of efficient anticancer therapies.

MACROPHAGE HETEROGENEITY

Blood monocytes are not fully differentiated cells and are profoundly susceptible to several environmental stimuli. When recruited into peripheral tissues from the circulation, monocytes could differentiate rapidly in distinct, mature macrophages and exert specific immunological functions. M-CSF is the main regulator of the survival, proliferation, and differentiation of mononuclear phagocytes, and many studies have also identified a role in the subsequent polarization phase for this factor [43].

Macrophages can be divided schematically into two main classes in line with the Th1/Th2 dichotomy (**Fig. 1**). M1 macrophages (classically activated cells) originate upon encounter with IFN-γ and microbial stimuli such as LPS and are characterized by IL-12^{high} and IL-23 production and consequent activation of polarized type I T cell response [2–6, 44], cytotoxic activity against phagocytozed microorganisms and neoplastic cells, expression of high levels of ROI, and good capability as APCs. In general, M1 macrophages act as soldiers: they defend the host from viral and microbial infections, fight against tumors, produce high amounts of inflammatory cytokines, and activate the immune response [6, 45]. On the other hand, distinct types of M2 cells differentiate when monocytes are stimulated with IL-4 and IL-13 (M2a), with immune complexes/TLR ligands (M2b), or with IL-10 and glucocorticoids (M2c) [46]. Hallmarks of M2 macrophages are IL-10^{high} IL-12^{low} IL-1ra^{high} IL-1 decoyR^{high} production, CCL17 and CCL22 secretion, high expression of mannose, scavenger and galactose-type receptors, poor antigen-presenting capability and wound-healing promotion. Further, M2 express specific change in some metabolic pathways: arginine metabolism is oriented toward the production of ornitine and polyamine instead of citrulline and NO. M2 cells are workers of the host: they promote scavenging of debris, angiogenesis, remodeling and repair of wounded/damaged tissues. Of note, M2 cells control the inflammatory response by down-regulating M1-mediated functions [6, 7, 12, 14]. In addi-

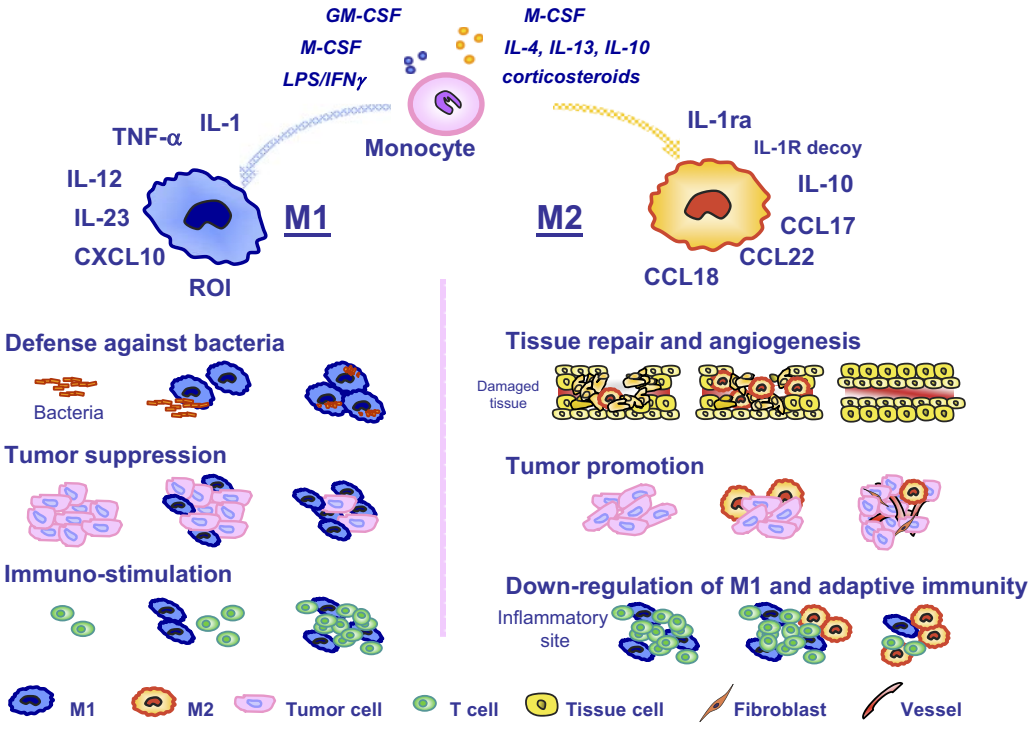

Figure 1. Polarization of macrophage function. Macrophages constitute an extremely heterogeneous population, which could be divided schematically into two main classes: M1 and M2. Blood monocytes differentiating in the presence of LPS/IFN-γ mature into M1-polarized cells (classically activated macrophages). They produce high levels of IL-12, IL-1, IL-23, TNF-α, and CXCL10 and are characterized by cytotoxic activity against microorganisms and neoplastic cells, expression of high levels of ROI, and capability as APCs. On the other hand, when monocytes differentiate in the presence of IL-4, IL-13, IL-10, or corticosteroids, they mature into M2 macrophages (alternatively activated, which secrete IL-10, CCL17, CCL22, CCL18, IL-1ra, and IL-1R decoy. M2 cells are active workers of the host, promoting scavenging of debris, angiogenesis, remodeling, and repair of wounded/damaged tissues. Within the tumor mass, they exert the same functions favoring tumor promotion. In addition, M2 macrophages control the inflammatory response by down-regulating M1-mediated functions and adaptive immunity.

tion, M2 macrophages are competent effector cells against parasitic infections [47].

The loss of equilibrium of M1 and M2 cell number may lead to pathological events: an M1 excess could induce chronic inflammatory diseases, whereas an uncontrolled number of M2 could promote severe immune suppression [12, 14].

As already mentioned, macrophage polarization could also be influenced by M-CSF, a key regulator of the monocyte differentiating process. Martinez at al [48] analyzed the transcriptional profiling of the human monocyte-to-macrophage differentiation and they observed that the polarization to M1 of M-CSF-differentiated macrophages was associated with the most dramatic change in the transcriptome, whereas further stimulation with an M2 stimulus (IL-4) caused a relatively minor alteration in gene expression. This apparently minor effect of IL-4 is a result of the fact that M-CSF-driven differentiation leads to the acquisition of M2 properties per se, including expression of mannose receptor 1 and scavenger receptor SR-A. This finding is in agreement with previous data showing divergent M1–M2 properties of macrophages differentiated in GM-CSF compared with M-CSF [49], which is a homeostatic growth factor circulating at high levels in normal blood. Thus, drifting toward M2 may be a default pathway in macrophage differentiation. Other factors able to influence macrophage polarization toward an M2 profile are IL-10, IL-6, TGF-β, and PGE2 [5, 50].

TAM

TAM originate from blood monocytes recruited at the tumor site [51] by molecules produced by neoplastic and by stromal cells (**Fig. 2**). The chemokine CCL2, earlier described in 1983 as a tumor-derived chemotactic factor, is the main player in this process [52, 53] and experimental and human studies correlate its levels with TAM abundance in many tumors, such as ovarian, breast and pancreatic cancer [23]. TAM themselves produce CCL2, suggesting the action of an amplification loop and anti-CCL2 antibodies combined with other drugs have been considered as an anti-tumor strategy [54]. Other chemokines involved in monocyte recruitment are CCL5, CCL7, CXCL8, and CXCL12, as well as cytokines such as VEGF, PDGF and the growth factor M-CSF [23, 50]. Moreover, monocytes could be attracted by fibronectin, fibrinogen and other factors produced during the cleavage of ECM proteins induced by macrophage- and/or tumor cell-derived proteases [24, 40].

When monocytes (then macrophages) reach the tumor mass, they are surrounded by several signals able to shape the new cells as needed by the tumor. As far as they have been studied, TAM resemble M2-polarized macrophages [2, 8, 9]. This preferential polarization is a result of the absence of M1-orienting signals, such as IFN-γ or bacterial components in the tumor, as well as the expression of M2 polarization factors. In particular, the infiltration of Th2 lymphocytes (driven by Th2-recruiting chemokines such as eotaxins) has been reported in many tumors, and they are a fundamental source of IL-4 and IL-13 cytokines [55–57]. Moreover, neoplastic cells, fibroblasts, and Tregs produce TGF-β and IL-10.

Incoming monocyte differentiation is also influenced by their localization within the tumor mass; for instance, in tumors, there is an established gradient of IL-10. This factor switches monocyte differentiation toward macrophages rather than DC [58, 59], and thus, as observed in breast cancer and in papillary carcinoma of the thyroid, TAM are present throughout the tissues, whereas DC are present only in the periphery [60].

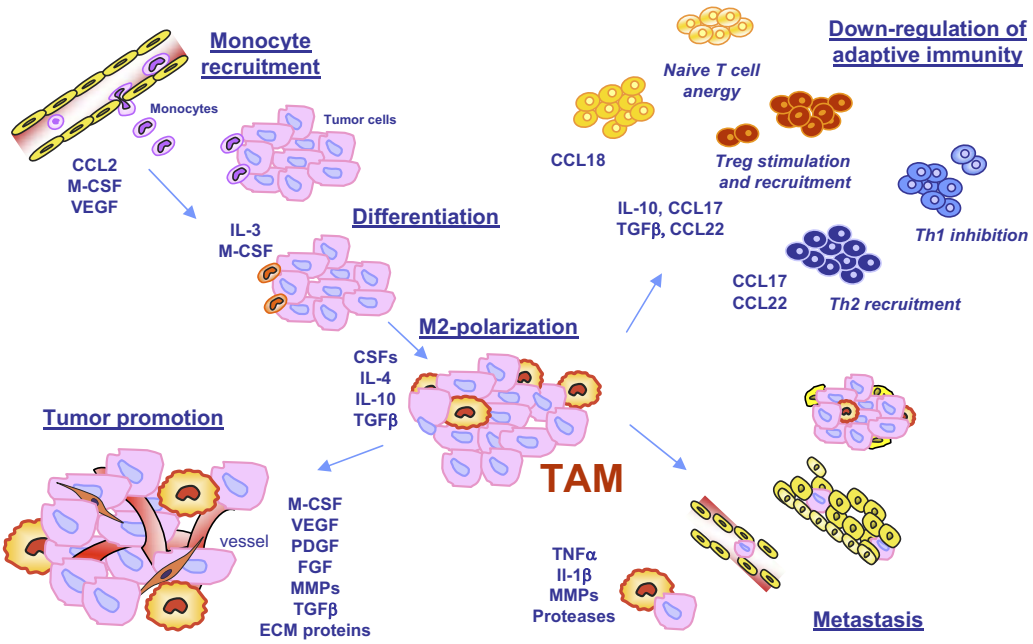

Figure 2. Overview of TAM, which originate from blood monocytes recruited at the tumor site by molecules produced by neoplastic and by stromal cells. Main factors involved in monocyte recruitment are the chemokine CCL2, M-CSF, and VEGF. When monocytes reach the tumor mass, they are surrounded by several microenvironmental signals such as IL-3 and M-CSF, able to induce their differentiation toward mature macrophages (now called TAM) and to shape the "new" cells as needed by the tumor (CSFs, IL-4, IL-10, and TGF-β). Tumor-molded macrophages resemble M2-polarized cells and play a pivotal role in tumor growth and progression. TAM actively work for the tumor: They produce several molecules that sustain malignant cell survival, modify neoplastic ECM proteins, promote the development of a newly formed vessel, and assist tumor cells in their progression. Moreover, TAM affect adaptive immune responses significantly by recruiting and stimulating Tregs and recruiting Th2 lymphocytes, which in turn inhibit Th1 cells, and by inducing anergy of naïve T cells.

The M2 polarization of TAM has also been demonstrated by studying their transcriptional profiling [2]; recent investigations noticed the up-regulation of many M2-associated genes such as CD163, Fc fragment of IgG, C-type lectin domains and heat shock proteins [61–63].

In the tumor milieu, TAM carry on their pro-neoplastic role by influencing fundamental aspects of tumor biology; they produce molecules that affect neoplastic cell growth directly (e.g., EGF), enhance neoangiogenesis, tune inflammatory responses and adaptive immunity and catalyze structural and substantial changes of the ECM compartment [7, 40, 41] (Fig. 2). Another hallmark of TAM is their tendency to accumulate into necrotic regions of tumors, characterized by low oxygen tension [64]. This preferential localization is regulated by tumor hypoxia, which induces the expression of HIF-1-dependent molecules (VEGF, CXCL12, and its receptor CXCR4) that modulate TAM migration in avascular regions [65–67]. HIF-1α also regulates myeloid cell-mediated inflammation in hypoxic tissues [68] and this link between hypoxia and innate immunity was confirmed recently, showing that HIF-1α is also regulated transcriptionally by NF-κB [69].

Biochemical studies have identified the transcription factor NF-κB as a master regulator of cancer-related inflammation in TAM and in neoplastic cells. Constitutive NF-κB activation is indeed observed often in cancer cells and may be promoted by cytokines (e.g., IL-1 and TNF) expressed by TAM or other stromal cells, as well as by environmental cues (e.g., hypoxia and ROI) or by genetic alterations [38, 41, 70]. NF-κB induces several cellular modifications associated with tumorigenesis and more aggressive phenotypes, including self-sufficiency in growth signals, insensitivity to growth inhibition, resistance to apoptotic signals, angiogenesis, migration and tissue invasion [71–73]. In a mouse model of colitis-associated cancer, the myeloid-specific inactivation of the I$\kappa\beta$ kinase inhibited inflammation and tumor progression, thus providing unequivocal genetic evidence for the role of inflammatory cells in carcinogenesis [72, 74].

On the other hand, in established, advanced tumors, where inflammation is typically smoldering [37], TAM usually have defective and delayed NF-κB activation in response to different proinflammatory signals (e.g., expression of cytotoxic mediators such as NO, cytokines, TNF-α, and IL-12) [61, 75, 76]. These observations are in apparent contrast with a pro-tumor function of inflammatory reactions expressed by TAM. This discrepancy may reflect a dynamic change of the tumor microenvironment along tumor progression. In early stages of carcinogenesis, innate responses (inflammatory reactions) are indispensable for the activation of effective surveillance by adaptive immunity [77, 78] but on the other hand, are also likely to promote tumor development. In late stages of neoplasia, the defective NF-κB activation of TAM is insufficient to drive and sustain a potential anti-tumor immune response of the host. Evidence suggests that p50 homodimers (negative regulators of NF-κB) are abundant in TAM and are responsible for its defective activation [79].

As a matter of fact, TAM exert strong immune suppressive activity, not only by producing IL-10 but also by the secretion of chemokines (e.g., CCL17 and CCL22), which preferentially attract T cell subsets devoid of cytotoxic functions such as Treg and Th2 [23, 46]. In normal macrophages, these chemokines are inducible by IL-4, IL-10, and IL-13, thus amplifying an M2-mediated immune-suppressive loop. In addition, TAM secrete CCL18, which recruits naïve T cells by interacting with an unidentified receptor [80, 81]. Attraction of naïve T cells in a microenvironment characterized by M2 cells and immature DC is likely to induce T cell anergy (Fig. 2).

TAM AND ANGIOGENESIS

Angiogenesis is sustained by different mediators produced by neoplastic and by stromal cells. TAM release growth factors such as VEGF, PDGF, TGF-β and members of the FGF family [2, 82, 83], and the proangiogenic role is highlighted by the correlation between their high numbers and high vascular grades in many tumors such as glioma, squamous cell carcinoma of the esophagus, breast, bladder and prostate carcinoma [82, 84–87]. TAM secrete the angiogenic factor thymidine phosphorylase, which in vitro promotes endothelial cell migration [88] and they also produce several angiogenesis-modulating enzymes such as MMP-2, MMP-7, MMP-9, MMP-12, and cyclooxygenase-2 [8, 40, 41]. In estrogen-treated K14-HPV16 transgenic mice (cervical carcinogenesis model), TAM production of MMP-9 is crucial for angiogenesis development [89]. Moreover, by metalloprotease production, TAM and related myeloid cells may also reorganize tumor vasculature after treatment with inhibitors of VEGF signaling [90, 91].

As mentioned above, TAM preferentially localize in tumor hypoxic regions, and hypoxia activates in these cells a specific proangiogenic program. Low oxygen conditions promote HIF-1 and HIF-2 expression with subsequent overexpression of proangiogenic molecules. Of note, TAM express VEGF almost exclusively in avascular and perinecrotic areas of human breast carcinomas [92]. The importance of HIF-1 was underlined by the observation that in hypoxic regions, the ablation of this transcription factor leads to an impaired motility and cytotoxicity of macrophages [68]. Among chemokines, hypoxia tightly regulates the expression of CXCL12, HIF-1-dependent potent chemoattractant for endothelial cells, and its receptor CXCR4 [66]. In addition to CXCL12, TAM release other chemokines involved in angiogenic processes [23] such as CCL2, CXCL8, CXCL1, CXCL13, and CCL5. For instance, levels of CXCL5 and CXCL8 were associated with increased neovascularization and correlated inversely with survival [23, 46, 93, 94].

TAM are also strongly involved in lymphangiogenesis, a process mediated by a number of factors including VEGF-C and VEGF-D via VEGFR3. Lymphatic endothelial growth factors secreted by TAM are related to peritumoral lymphangiogenesis [95, 96]. It has been shown that VEGF-A increases lymphangiogenesis via recruitment of monocytes [97].

Other myeloid cells in the tumor microenvironment play a pivotal role in the angiogenic switch. A distinct subset of monocytes with pro-tumorigenic activity has been identified recently as TEM in mouse models of transplanted and spontaneous tumors [98, 99]. TEM are characterized by the expression of angiopoietin receptor Tie2 and accumulate in hypoxic areas of tumors in response to angiopoietin 2 produced at high levels by hypoxic vascular cells [98–100]. A strong dem-

onstration of TEM involvement in angiogenesis results from the evidence that the selective depletion of these cells in mice during early stages of tumor development leads to a significant reduction of tumor mass and vasculature [98]. TEM are not present in non-neoplastic tissues close to tumors, and their specific tumor-homing ability could be used as a vehicle for anti-tumor gene delivery [101].

TAM, INVASION, AND METASTASIS

The metastasis process unquestionably represents a crucial phase of neoplastic diseases and develops when tumor cells acquire specific capabilities to leave the primary tumor, invade the surrounded matrix, reach through blood or lymphatic vessels' distant sites and there settle down and grow. As a result of its complexity, this process has yet to be analyzed further, but several lines of evidence have already identified a tight link between this process and TAM, which produce inflammatory cytokines likely active on the dissemination stage [14, 40, 41]. The intense cross-talk between macrophages and neoplastic cells guarantees the continuous process of matrix deposition and remodeling, which facilitates tumor growth and invasion of the surrounding tissues. The high tissue remodeling activity of TAM is summarized by Dvorak's definition: "Tumors are never healing wounds" [102].

TAM cooperate on tumor dissemination by promoting invasion characteristics of malignant cells and also by making easier their movement by a direct action on the tumor microenvironment [103]. In particular, one of the main factor involved significantly is TNF-α: cocolture of neoplastic cells with macrophages enhances invasiveness of malignant cells through TNF-dependent MMP induction in macrophages [104, 105].

TAM produce IL-1β, and Giavazzi and colleagues [106] demonstrated the IL-1-induced augmentation of metastasis development in a mouse melanoma model.

In a genetic model of breast cancer growing in monocyte-deficient mice, the tumors developed normally but in the absence of the macrophage-produced EGF, were unable to form pulmonary metastasis [15].

Other evidence comes from the influence of lung macrophages on endothelial cells, which leads to MMP-9 secretion via a VEGFR-1-dependent mechanism [107]. The up-regulation of MMP expression has been demonstrated also in coculture experiments of macrophages with MCF-7 breast cancer cells [108, 109]. Regarding the specific influence on tumor microenvironment, TAM abundantly secrete matrix proteins and several proteases such as serine proteases, MMPs, and cathepsins, which act on cell–cell junctions, modify the ECM composition, and promote basal membrane disruption [110–113]. Moreover, TAM contribute actively to build up the tumor matrix architecture by producing several matrix proteins, including secreted protein acid rich in cysteine, which modulate collagen density, leukocyte and blood vessel infiltration [114].

A better understanding of the metastasis process could certainly be supported by studies performed with innovative techniques such as intravital imaging. By using fluorescently labeled cells, Wyckoff and colleagues [115] showed that in mammary tumors, malignant cell mobilization often occurs next to macrophages, which appear to assist tumor cell intravasation directly on the vessel surface [15, 103].

Pawelek and Chakraborty [116] also reported a macrophage role in the development of metastases; they proposed a challenging theory, where cancer cell fusion with migratory bone marrow-derived cells is the driving force promoting the dissemination process.

FLASH ON MDSCs

MDSCs are raising remarkable interests in the last years, as several studies have reported their presence in different pathologies including tumors and inflammatory diseases [33]. Given their marked heterogeneity, the MDSC world is extremely complex, and this uncertainty also includes their simple definition. Initially, this cell population seemed to consist of granulocytes and monocytes/macrophages [32] but recently, Gallina and colleagues [31] defined these cells as inflammatory monocytes. Regarding the cell-surface phenotype, Bronte et al. [117] characterized bone marrow MDSCs as $CD11b^{+}Gr\text{-}1^{+}CD31^{+}$ cells, but later, the same group and others [31] indicated active MDSCs as CD11b+CD11c+Gr-1+IL-4Ra+ inflammatory monocytes [31]. The studies of chemokine receptors expressed on tumor-infiltrating MDSCs by Umemura et al. [118] demonstrated high RNA levels of CCR2, CCR5, CX3CR1, and CCR7 and protein expression (CX3CR1 and CCR2) by flow cytometry. Of note, CCR2 plays pivotal functions in the turnover of these cells at the tumor site [119].

Among other factors, S-100 proteins, proinflammatory factors present at the tumor site, strongly attract MDSCs, and interestingly, this process is also sustained by an autocrine loop, as MDSCs secrete these proteins themselves [120]. GM-CSF is sufficient to recruit these cells in lymphoid organs, thus inducing a suppression of antigen-specific CD8 T cell proliferation [33]. M-CSF, IL-6 and VEGF are other molecules able to influence MDSC activities significantly and IL-10, in particular, sustains the suppressive phenotype [33, 34].

A great open question about MDSCs concerns their hypothetical, specific polarization. Are these cells M1- or M2-oriented? MDSCs express some, but not all, alternative activation markers as well as some classical activation markers [118]. However, as these cells share properties and gene expression profiles with M2-polarized TAM [34, 61] it is likely that, by nature, they skew toward the M2 orientation.

TAM AND ANTI-CANCER THERAPIES

In this review, we have often underlined how TAM favor neoplastic cells during tumor development and invasion and spread to distant sites. Thus, it is easy to gather that these cells may certainly be considered as an attractive target for novel anti-cancer therapies. If we block macrophages, will we actually disturb tumor progression in human patients? Within a tumor, a heterogeneous microenvironment differentially influences infiltrated macrophages, and this shows clearly the necessity of identifying common TAM targets for the synthesis of new therapeutic molecules. Obviously, the best target would be a pro-

tein expressed or overexpressed only by TAM and neither by resident macrophages of distant, healthy tissues nor by M1 cells, which are important to face pathogens and could take part in anti-cancer actions.

Several "anti-macrophage" approaches are under evaluation currently. Interesting observations come from studies performed with chemokines and chemokine receptors as anti-cancer targets. For instance, in a breast cancer murine model, malignant cells recruit macrophages via the chemokine CCL5, and treatment of murine breast cancers with Met-CCL5 (receptor antagonist) leads to a decreased number of infiltrating macrophages associated with a significantly reduced tumor size [121]. The recently approved (Europe) anti-tumoral drug Trabectedin (Yondelis) also has immunomodulatory properties on mononuclear phagocytes. Our group has demonstrated recently that this drug showed a selective, cytotoxic effect on monocytes and macrophages, including TAM, while sparing the lymphoid subset; moreover, at subcytotoxic concentrations, Trabectedin reduced the production of CCL2 significantly [122]. CCL2 has also been identified as a fundamental regulator of prostate cancer growth and metastases, and its inhibition with anti-CCL2 antibodies, in combination with docetaxel, induces a significant tumor regression in prostate cancer models [123, 124].

TAM proangiogenic functions have also been considered: thalidomide, linomide, pentoxifyline, genistein and other antiangiogenic factors have been demonstrated to inhibit macrophage recruitment and to reduce tumor size [125, 126]. Moreover, recent studies showed that anti-VEGF-A neutralizing antibody therapies reduce the development of new blood and a lymphatic vessel and decrease incidence of lymphatic and pulmonary metastasis in an orthotopic breast tumor model [127]. As already mentioned, VEGF also promotes macrophage recruitment into tumors, and Dineen and colleagues [128] demonstrated recently that specific inhibition of VEGFR2 decreases tumor macrophage infiltration significantly into orthotopic pancreatic tumors.

Other molecules likely efficient are MMP inhibitors (such as the biphosphonate zoledronic acid), which in genetic mouse carcinogenesis models suppressed MMP-9 secretion by TAM, leading to a slower tumor growth [89, 129].

As macrophages infiltrate tumors spontaneously, recent studies used these cells as natural vectors to deliver therapeutic molecules to the neoplastic site. In a preclinical metastatic prostate cancer model, intratumoral injection of macrophages transduced with an IL-12 recombinant adenoviral vector led to the reduction of primary tumor growth and lung metastases with a higher number of tumor-infiltrated CD4+ and CD8+ T cells compared with control animals [130]. Another fascinating approach is targeting stromal–cancer cell interactions with small interfering RNA (reviwed in ref. [131]).

Macrophages have also been used to enhance the immune response or to potentiate chemotherapy specificity. Carta and colleagues [132] engineered a murine macrophage cell line that strongly augmented the production of IFN-γ.

The delicate balance between M1 and M2 cells is a fundamental aspect in anti-cancer treatment also. Several studies have shown that the activation of TLRs (for instance, TLR9) stimulates M1-polarized macrophage responses by inducing the activation of a proinflammatory program [133]. Synthetic CpG oligodendronucleotides demonstrated anti-tumor effects in many preclinical models, although they do not seem to work with large, established tumors [134]. Moreover, when CpG oligodendronucleotides have been added to chemotherapy agents, the response and the survival increased significantly [135]. In general, the restoration of an M1 phenotype in TAM may provide a therapeutic benefit by promoting anti-tumor activities. Rauh and colleagues [136] highlighted the importance of the already mentioned SHIP1, a crucial phosphatase in reverting macrophage M1 versus M2 functions. SHIP1-deficient mice showed a skewed development toward M2 macrophages, and thus, pharmacological modulators of this phosphatase are under investigation currently [137, 138].

Interestingly, a contribution of the immune system to the anti-tumor effects of conventionally used chemotherapy treatments has been suggested. Cells of the innate immunity can be activated by proteins secreted by dying cells—damage-associated molecular patterns [139, 140]. For example, late apoptotic cells express SIN3A-associated protein 130, a ribonucleoprotein that binds to MINCLE. Recent work [140, 141] demonstrated that inhibition of MINCLE interrupts the release of proinflammatory cytokines by macrophages and also blocks neutrophil recruitment.

Also, MDSCs have been considered a target for the development of anti-cancer treatments. The combination of antibodies against myeloid cells with anti-VEGF antibodies leads to a stronger effect in the inhibition of refractory tumor growth than treatment with anti-VEGF alone [142]. In addition, Sinha and colleagues [120] demonstrated a reduced recruitment of MDSCs at the tumor site after anti S100-A8 or S100-A9 protein administrations [143].

CONCLUSION

Several lines of evidence indicate that inflammatory cells and cytokines found in tumors are more likely to contribute to cancer progression rather than to mount an effective host anti-tumor response. TAM are key orchestrators of cancer- related inflammation, and neoplastic cells actively guide monocyte recruitment from blood into tumor tissues to their own advantage. TAM exert several pro-tumoral functions, as they produce a large array of growth factors for tumor cells and for the nascent blood vessels, essential for tumor growth. In addition, together with MDSC, TAM actively participate in the suppression of the adaptive immune response that could potentially attack the transformed cells. In experimental settings, antitumor activity can be achieved by targeting TAM recruitment, survival, polarization, and effector activities. Thus, therapeutic targeting of macrophages in humans may represent a valuable strategy to complement conventional anticancer strategies.

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