Review Article
Macrophages, Neutrophils, and Cancer: A Double Edged Sword

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1. Introduction

The tumor microenvironment plays important roles in cancer development and behaviour [1–4]. The tumor microenvironment consists of various host components such as stromal cells, growing blood vessels, and inflammatory infiltrate that cause a local chronic inflammation. Infiltrating leukocytes in the tumor microenvironment may exert a dual role on tumor development and progression. Tumor cells can be directly eliminated by the immune cells that had developed an anti-tumoral immune response but can also recruit and instruct immune cells to favour tumor growth and progression. The recruited immune cell consists of many players, including macrophages and neutrophils.

Tumor-associated macrophages (TAM) are components of the inflammatory infiltrate of several tumors and produce many mediators (e.g., chemokines) responsible for the activation and maintenance of the chronic inflammatory process [5]. In human peripheral blood neutrophils are the most abundant leukocyte subset and have a primary role in the defence against infective pathogens. They have long been considered to have a negligible role in cancer-related inflammation because of their short life span and their terminal differentiation. However, it has been recently recognized, on the basis of evidence in animal models, that tumor-associated neutrophils (TAN) can also be polarized in different phenotypes in response to microenvironmental signals. In fact, they can interact with distinct cell populations and produce a wide number of cytokines and effector molecules. Therefore, macrophages and neutrophils are both integrated in the regulation of the innate and adaptive immune responses in various inflammatory situations, including cancer. These findings have triggered efforts to target tumor-associated macrophages and neutrophils. In particular, “reeducation” to activate their antitumor potential or elimination of tumor promoting cells is a new strategy undergoing preclinical and clinical evaluation.
functional and phenotypical similarities with TAM and TAN, this relationship needs further investigations and is beyond the scope of this review.

TAM and TAN exert their protumoral functions participating in the extracellular matrix (ECM) remodelling and enhancing cancer cell invasion and metastasis, angiogenesis, cancer cell proliferation, and lymphangiogenesis, while inhibiting the antitumoral immune surveillance [8, 13–17]. Hypoxia is a key determinant of the localization and phenotype of TAM [18]. On the other end, TAM and TAN exert antitumoral activities by direct killing of tumor cells and releasing a wide range of mediators (e.g., cytokines, chemokines, and growth factors) which are able to recruit and activate cells of both the innate and adaptive immune systems [4, 19–21]. In this review we will explore the immunobiology of TAM and TAN; particular attention will be given to the recruitment and polarization of these tumor-associated cells, to the roles they can play in tumor growth and progression, and to their significance in clinical settings.

2. Tumor-Associated Neutrophils

There is now evidence that the “small eaters” (microphages), neutrophils, in addition to their known antimicrobial functions, play an important role in tumor progression, beyond the “big eaters” macrophages. TANs have been proposed as key mediator of malignant transformation, tumor progression, and angiogenesis and in the modulation of the antitumor immunity [8].

That neutrophil infiltration within tumors which may be associated with poor clinical outcome has been suggested by several epidemiological evidences. This negative correlation between TAM infiltration and clinical outcome has been reported in metastatic and localized clear cell carcinoma [22, 23], in head and neck squamous cell carcinoma [24], in bronchoalveolar carcinoma [25], in hepatocellular carcinoma [26], and in esophageal squamous cell carcinoma [27]. In human gliomas [28] and in aggressive types of pancreatic tumors [29], infiltration of neutrophils correlates with tumor grade. However, a favourable prognosis associated with a high neutrophil count has also been reported in some tumors (e.g., gastric cancer) [30].

How neutrophils exert their role in the promotion and progression of cancer has been only partially elucidated. ROS-dependent and -independent mechanisms [31], including the production of granule proteins, cytokines, and angiogenic factors, have been reported as factors that link neutrophils and carcinogenesis. For instance, epithelial tumor cells adjacent to neutrophils can uptake the neutrophil elastase and hydrolyse the insulin receptor substrate-1 (IRS-1), causing the enhancement of PDGFR signalling and tumor cell proliferation [32]. In addition, in patients with bronchoalveolar carcinoma, the hepatocyte growth factor (HGF) is released by neutrophils upon stimulation with tumor derived cytokines (TNF-α, GM-CSF) and HGF promotes tumor cell migration [25]. In breast cancer, neutrophils release oncostatin M (OSM) when stimulated by the GM-CSF released by cancer cells. In turn, OSM induces VEGF in breast cancer cells and promotes their detachment and invasive behaviour [33]. Moreover a new mechanism by which TAN affect tumor growth has been very recently reported. In an experimental tumor model Mishalian and coworkers showed that TAN from established tumors produce CCL17, recruiting regulatory T cells (Tregs) into the tumor. Recruitment of Tregs was inhibited by anti-CCL17 monoclonal antibodies treatment and neutrophil depletion using anti-Ly6G monoclonal antibodies reduced the migration of Tregs into the tumors, highlighting a clear link between TAN and regulatory T cells (Tregs) in tumor immunosurveillance [34].

Neutrophil depletion in two murine models of transplantable tumors (melanoma and fibrosarcoma) revert the increased tumor growth, angiogenesis and metastasis observed in IFN-β deficient mice [35]. Neutrophil depletion decreased tumor growth also in normal C57Bl/6 mice transplanted with the T cell lymphoma RMA [36, 37].

TANs have been reported as key mediators in angiogenesis. In a genetic mouse model of pancreatic cancer, neutrophil-derived MMP9 was responsible of VEGF release from the ECM and neutrophil depletion inhibited VEGF-dependent angiogenic switch [38]. An increase in neutrophil infiltration into tumors is significantly correlated with glioma grade and in glioblastoma with acquired resistance to anti-VEGF therapy. In this model the effect was mediated by neutrophil-mediated overexpression of S100A4 in tumor cells, since downregulation of S100A4 inhibited tumor progression independently of the infiltration of neutrophils into the tumor [39]. It has been recently proposed that neutrophils exert their protumoral activities, as progression and dissemination, through neutrophil extracellular traps (NETs), especially in the context of systemic infections that might occur in surgery oncology patients [40]. This study reports that circulating tumor cells become trapped within NETs in vitro under static and dynamic conditions. In a murine model of infection in tumor-bearing mice, microvascular NET deposition and consequent trapping of circulating lung carcinoma cells have been demonstrated. NET trapping was associated with increased formation of hepatic metastases. NET inhibition with DNase or a neutrophil elastase inhibitor abrogated these effects. Neutrophil elastase profoundly influences cancer growth and development directly inducing tumor cell proliferation [32]. Interestingly it can also modify the tumor suppressor role of some ECM components, as has been reported for EMILIN1 that is cleaved by neutrophil elastase in inactive fragments. The consequence of this proteolytic process was the impairment of its antiproliferative role [41].

As has been described for TAM, also neutrophils can display surprising plasticity [9]. In models as lung cancer and mesothelioma, infiltration of TANs, their tumor cytotoxicity, and their immunostimulatory profile were increased by TGF-β blockage. Interestingly, in these experimental animal models, CD8+ T-cell activation increased after TAN depletion, whereas after TGF-β blockage, depletion of TANs decreased CD8+ T-cell activation [6].

Therefore, as already established for macrophage polarization, it has been proposed that TAN can be polarized to N1 and N2 phenotypes. Thus TANs infiltrating the tumor are driven by TGF-β to acquire a N2 protumoral phenotype. In
contrast, TGF-β inhibition induces an antitumoral N1 phenotype [6]. TGF-β signaling in myeloid cells has been reported to be required for tumor metastasis [42]. This study identifies myeloid-specific TGF-β signaling as a critical mediator in tumor metastasis, distinct from the tumor-suppressive effect of TGF-β signaling on epithelial cells, fibroblasts, and T cells, suggesting a cell-type-specific cancer-targeting strategy.

Plasticity of TAN has been recently described, using two models of murine tumor cancer cell lines, the Lewis Lung carcinoma and the AB12 mesothelioma [43]. TAN from early tumors were more cytotoxic toward tumor cells and produce higher levels of TNF-α, NO, and H₂O₂, while in established tumors these functions were downregulated and TAN acquire a more protumorigenic phenotype, showing how the evolution of tumor microenvironment influence TAN phenotype.

Moreover, it is now clear that cancer is associated with the expansion of the myeloid compartment and with the appearance of a heterogeneous population of immunosuppressive cells functioning as myeloid-derived suppressor cells (MDSCs) [44]. MDSCs and TANs present with shared phenotypic (e.g., cell markers and morphology) and functional properties (e.g., recruitment, angiogenesis, and immunosuppressive activity) [45]. It is not yet completely elucidated whether TAN activity is at least in part due to MDSCs. In this regard, Fridlender and colleagues recently conducted transcriptomic analysis of TANs, naïve bone marrow neutrophils (NN), and granulocytic MDSCs (G-MDSC). Their data showed that TANs are a neutrophil population that markedly differs in their genetic profile from both NN and G-MDSC. They concluded that TAN are not “tissue-based G-MDSC” that undergo phenotypic modulation because of the tumor. In parallel, the NN and G-MDSC are more closely related to each other than TANs [46].

Finally, it can be remembered here that, as will be described in the following section regarding macrophage, cell polarization is a phenomenon that displays notable differences between mice and humans. Therefore, the existence and properties of N1-N2 in cancer-related inflammation in humans need to be carefully investigated.

3. Polarized Activation of Macrophages

Monocytes/macrophages undergo reprogramming when exposed to signals derived from microbes, damaged tissues, or activated lymphocytes. Functional reprogramming activates a spectrum of distinct functional phenotypes, in principle described as two different polarization states [47, 48]. IFNγ alone or in concert with microbial stimuli (e.g., LPS) or cytokines (e.g., TNF and GM-CSF) induces classically activated M1 macrophages. In contrast IL-4 and IL-13 induce the alternative M2 form of macrophage activation [49]. Moreover, IL-33 and IL-21 are associated with Th2 and M2 polarization [50, 51].

Classical M1 cells play a central role as inducer and effector cells in polarized Th1 responses; they are also effectors of resistance against intracellular parasites and tumors. They are high producers of IL-12 and IL-23 and low producers of IL-10. Moreover they are efficient producers of effector molecules (e.g., reactive oxygen and nitrogen intermediates) and inflammatory cytokines (e.g., IL-1β, TNF, and IL-6) [48, 52]. They can also be potent antitumor effectors, controlled by CD4+ Treg cells that prevent the recruitment and/or differentiation of Ly-6C<sup>hi</sup> monocytes favoring tumor progression [21].

In contrast, M2 macrophages generally produce low levels of IL-12 and IL-23 and high levels of IL-10 and release variable amounts of inflammatory cytokines. M2 cells are poor antigen presenting cells, suppress Th1 responses, actively scavenge debris, contribute to the dampening of inflammation, and promote wound healing, angiogenesis, tissue remodelling, and tumor progression [47]. M2 macrophages contribute to Th2 response and to elimination of parasites [53]. The IL-1 system, central in all inflammatory processes, appears differentially regulated in the two polarized populations of macrophages; in fact M2 cells express and produce lower amounts of IL-1β and higher IL-1α and decoy type II receptor in comparison with M1 cells [54].

M1 and M2 macrophages have a distinct ability to produce chemokines: M1 macrophages release Th1 lymphocyte attracting chemokines, such as CXCL9 and CXCL10, whereas M2 macrophages release CCL17, CCL22, and CCL24, chemokines that are involved in the recruitment of Tregs, Th2, eosinophils, and basophils [13, 55].

Metabolism of M1- and M2-polarized macrophages can also differ, as regards iron, folate, and glucose [56, 57]. Recently metabolism has been reported to be important in shaping the functional phenotype of macrophages in response to distinct polarizing stimuli in the tissue microenvironment, under normal as well as pathological setting. Indeed, recent data suggest that macrophage functional polarization is regulated by their distinct metabolic features [58].

It is now clear that M1 and M2 macrophages are an oversimplification: in the presence of various stimuli, such as antibody immune complexes together with LPS or IL-1, glucocorticoids, transforming growth factor-β (TGF-β), and IL-10, macrophages can acquire M2-like functional phenotypes with properties shared with IL-4- or IL-13-activated macrophages. In vivo modulation of M2 polarization has also been demonstrated (e.g., during helminthic and *Listeria* infection, in the placenta and embryo development, in obesity and cancer) [59]. Thus, M1 and M2 polarized macrophages are extremes in a continuum in a wide range of functional states [48]. Interestingly exposure of M2 macrophages to some immunological mediator molecules, as, for instance, IFN-γ, can induce “reeducation” into M1 cells and abrogate their immunosuppressive abilities [60].

4. Mechanisms of Recruitment

Stromal and tumor cells produce a wide spectrum of chemokines and growth factors that recruit circulating monocytes to tumor sites and cause their differentiation into macrophages. For instance, CCL5/RANTES, CCL12/SDF-1, and CXC3L1/fractalkine were found in neoplastic tissues and contribute to macrophage recruitment and tumor promotion [61–65]. In addition to chemokines and growth...
factors monocyte recruitment and macrophage differentiation are induced by noncanonical chemotactic peptides, such as vascular endothelial growth factor (VEGF), transforming growth factor beta (TGFB), basic fibroblast growth factor (bFGF), macrophage colony stimulating factor (M-CSF/CSF-1), and uronosine plasminogen activator (uPA), by the antimicrobial peptide β-defensin-3, by the lectin Reg3β [64, 66–72], and by tumor products, as oxysterols which recruit tumor-supporting neutrophils [37]. CXC chemokines (CXCL8, CXCL1, CXCL2, CXCL3, and CXCL5), known for their role in neutrophil recruitment in both physiological and pathological conditions and involved in cancer progression, are also produced in tumor-associated inflammation. This favors tumor angiogenesis and metastasis [8, 73, 74]. In a preclinical experimental model of tumor transplant CXCL17, the latest identified member of the CXC family, enhanced tumor growth increasing the recruitment of immature myeloid derived CD11b+Gr1+F4/80+ cells in the tumor [75]. In animal studies, tumor-derived CXCL5 recruited intratumoral infiltrative neutrophils and these promoted tumor growth and metastasis. Immunohistochemical analysis of human ICC samples showed that overexpression of CXCL5 correlated strongly with intratumoral neutrophil infiltration, shorter overall survival, and high tumor recurrence [76]. In addition, in murine models of inflammation-induced skin tumors and intestinal colitis-associated or spontaneous cancer, myeloid cells infiltrated tumors, and CXC2 deficiency or neutrophil depletion suppressed inflammation-related tumorigenesis and the onset of spontaneous tumors [77]. In a mouse model of colitis-associated cancer (CAC) infiltrated neutrophils have been shown to produce large amounts of interleukin-1 (IL-1β), that in CAC milieu promotes tumorigenesis by inducing IL-6 production by intestine-resident monocellular phagocytes. It was also noted that commensal flora-derived lipopolysaccharide (LPS) was identified to trigger IL-1β expression in neutrophils [78]. Interestingly, epithelial barrier deterioration caused by colorectal-cancer initiating genetic lesions (i.e., loss of the adenomatous polyposis coli tumor suppressor) resulted in adenoma invasion by microbial products. These products are responsible for the production of IL-23 in myeloid cells and the development of a pro-tumoral IL-17 response [79]. In a mouse model of lung adenocarcinoma driven by conditional activation of K-ras and of p53 inactivation, Cortez-Retamozo et al. reported that spleen was the site of localization of TAM and TAN precursors, from where they physically relocated to the tumor stroma [80]. Removal of the spleen caused reduced accumulation of TAM and TAN in the tumor and affected tumor growth, showing that the spleen is a reservoir for TAM and TAN precursors which play a central role in the control of tumor evolution [80]. Moreover overproduction of angiotensin II was also present in this model and it amplified self-renewing hematopoietic stem cells and macrophage progenitors [81]. In a K-ras mutant mouse model of lung cancer, depletion of neutrophils or inhibition of CXCR2 receptor or genetic deficiency of neutrophil elastase significantly inhibited lung cancer development [82].

In humans, CXC chemokines, notably CXCL8/IL-8, are produced by liver epithelial cells from hepatocellular carcinoma (HCC). These chemokines in turn promote neutrophil migration in peritumoral stroma [26]. Recently, Zhou and colleagues analyzed by immunohistochemistry samples from 919 HCC patients and found correlation of CXCL5 overexpression with neutrophil infiltration, shorter overall survival, and tumor recurrence [83]. Very recently the same group analyzed CXCL5 expression in tumor samples from patients with intrahepatic cholangiocarcinoma and reported that in multivariate analysis CXCL5 overexpression alone, or combined with the presence of intratumoral neutrophils, was an independent prognostic indicator for ICC patients and thus a potential therapeutic target [76]. In addition, neutrophil chemotaxis was induced by IL-8 present in the medium conditioned by head and neck squamous cells carcinoma (HNSCC) and macrophage migration inhibitory factor (MIF) produced by HNSCC induced neutrophil recruitment in a CXCR2-dependent manner [24, 84]. CCL4-STAT3 signaling induced in prostate cancer by androgens contributes to tumor promotion highlighting a connection between chemokines and hormones in prostate cancer promotion [85].

5. Functions of Tumor-Associated Macrophages

Plasticity is a well-known characteristic of macrophages; that is, they are able to integrate distinct signals from the microenvironment and to acquire distinct phenotypes [48]. From a simplistic point of view, as already mentioned in a previous paragraph, macrophages may result in two opposite polarization states. M1 macrophages are induced by the exposure to Th1 cytokines (e.g., IFN-γ) alone or in combination with microbial components (e.g., LPS); M1 participate in Th1 response and are mediators of resistance against microbes and tumor cells. Typically M1 polarized macrophages are efficient producers of effector molecules (e.g., reactive oxygen and nitrogen intermediates) and inflammatory cytokines (e.g., IL-1β, TNF, IL-6), produce high levels of IL-12, and display a low expression of IL-10 [48]. In agreement with their functions in Th1 responses, M1 macrophages produce Th1 attracting chemokines, such as CXCL9/Mig and CXCL10/IP-10. The cytokines IL-4 and IL-13 inhibit this classical activation and induce the alternative M2 form of macrophage polarization [49]. Unlike M1, M2 macrophages produce high amounts of IL-10 and low amounts of IL-12 and display a poor antigen presenting capacity [53]. Being involved in Treg cell, Th2, eosinophil, and basophil recruitment, M2 macrophages produce chemokines that recruit these cell populations, such as CCL17/TARC, CCL22/MDC, and CCL24/Eotaxin-2 [19, 55, 86]. Moreover, they suppress Th1 adaptive immunity, participate in the resolution of inflammation and in the protection against parasites, and promote wound healing, angiogenesis, and tissue remodelling [47]. A strong evidence of macrophage plasticity consists in their potential to be “reprogrammed” by some immunological stimuli, such as IFN-γ or IFN-α, from immunosuppressive M2 macrophages into immunostimulatory cells [60, 87]. Interestingly it has been reported that IFN-γ driven intratumoral microenvironment exhibits superior prognostic effect compared with
an IFN-α driven microenvironment in patients with colon carcinoma, giving a successful proof-of-principle approach that complex cytokine interaction networks can be found and dissected in human tissues [88]. It can be remembered here that a Th1-dominated tumor micromilieu is strongly associated with a positive prognosis in CRC [89–91]. Tumor-produced molecules contribute to TAM polarization; thus modulation of the microenvironment can contribute to macrophage “reeducation.” For instance, Wang et al. recently reported that Nodal, an embryonic morphogen member of the TGF-β superfamily, not detectable in healthy adult tissues, but that can reemerge in a number of human cancers and contribute to tumorigenesis, invasion, and metastasis, promotes to the in vitro generation of M2-like macrophages and downregulates the expression of IL-12. Its inhibition reversed TAMs to classically activated macrophage phenotype [92]. Drugs can also influence the TAM phenotype: using the HER2/neu transgenic (Tg-neu) mouse model lovastatin (Lov) was shown to significantly reduce the number of new oncogenic lesions in these mice, reducing the number of immunosuppressive and proangiogenic M2-like tumor-associated macrophages (TAM) and enhancing tumor infiltration by effector T cells [93]. Interestingly, microarray analysis identified a Lov-elicited genetic program in Tg-neu tumors that included downregulation of placental growth factor, which triggers aberrant angiogenesis and M2-like TAM polarization. Therapeutically “reeducated” macrophages to treat glioblastoma multiforme (GBM) have been described in a mouse model of proneural GBM. Treatment of mice with an inhibitor of the CSF-1 receptor (CSF-1R) to target TAMs significantly increased survival and regressed established tumors. [94]. Surprisingly, TAMs were not depleted in treated mice. Instead, glioma-secreted factors, including granulocyte-macrophage CSF (GM-CSF) and interferon-γ (IFN-γ), facilitated survival of TAM, whose expression of alternatively activated M2 markers decreased, consistent with impaired tumor-promoting functions. Moreover, multiple proneural GBM human xenografts responded similarly to CSF-1R inhibition, underscoring the therapeutic relevance of these findings [94]. Macrophages infiltrating the tumor may display dual functions, though often they participate to local inflammation, thus favoring tumor formation and progression. In facts several studies reported that TAMs have a M2-like phenotype [95]. The M2-like phenotype of TAM can be induced by the tumor cells. Katara et al. reported that vacuolar ATPase (V-ATPase) produced by tumor cells can promote tumor survival and growth. In particular cancer tissues and cells overexpress the a2 isoform of V-ATPase (a2V) and exposure of macrophages to the cleaved N-terminal domain of a2V induces expression and secretion of distinct TAM associated molecules, as elevated expression of mannose receptor-1, Arginase-1, interleukin-10, TGFβ, MMP9, and VEGF [96]. During tumor growth and progression macrophages can perform many functions, including extracellular matrix remodelling, promotion of tumor cell invasion and metastasis, angiogenesis, lymphangiogenesis, and immune suppression [19] (Table 1).

TAMs produce a number of proteolytic molecules, such as plasmin, urokinase-type plasminogen activator, cathepsin B, and matrix metalloproteases (MMP) which may directly remodel the ECM [98, 99, 105]. For instance, MMP participate to tumor progression through their capacity to degrade the basement membrane, activate growth factors, and enhance angiogenesis [103, 114, 115]. Moreover, the expression of nonproteolytic molecules by TAM can also have a role in the invasiveness of cancer cells. For instance, when macrophages were cultured in vitro in the presence of conditioned media from mammary epithelial cells containing FGF receptor 1-induced soluble factors, they increased the expression of CXCR2-binding chemokines. In turn, these CXCR2-binding chemokines induced migration of primary and tumor mammary epithelial cells [116]. Expression of scavenger-receptor A in hematopoietic cells, as well as on macrophages, has been reported to be necessary for metastasis, in a mouse model of subcutaneously transplanted pancreatic cancer cells [100]. Moreover, expression of MMP-9 in glioma stem-like cells was induced by macrophages-derived TGF-β1, and MMP9 increased the invasiveness of tumor cells [101]. Finally, macrophages increased the expression of the antimicrobial peptide hCAP18/LL37 in the presence of tumor-derived versican V1 and the increased availability of hCAP18/LL37 contributed to ovarian tumor cell proliferation and invasion [102].

As mentioned above, macrophages have been described to be associated with the metastatic potential of several tumors [117, 118]. For instance, transfer of thioglycollate-elicited peritoneal macrophages in mice increased by up to 100-fold the number of artificially induced metastatic lung nodules induced by the intravenous injection of melanoma or Lewis lung carcinoma tumor cells [119]. In a mouse model of breast cancer, IL-4-treated macrophages upregulated the expression of cysteine protease cathepsin B, which promoted lung metastasis [120]. Moreover, macrophages exposed to M2 polarizing cytokines or tumor cells conditioned media express a truncated fibronectin isoform, namely, migration-stimulating factor (MSF), which exerts a potent chemotactic effect on tumor cells [121]. The metastatic potential of TAM is further supported by depletion studies demonstrating a reduced incidence of metastasis [122, 123]. Moreover, some TAM phenotypes can differ in primary tumor or metastasis. CX3CR1 expression can have a role in metastasis, as shown in an experimental colon cancer tumor model, where CX3CR1 deficiency significantly inhibited liver metastasis [124]. TAM density and their phenotype were evaluated in 246 samples from primary tumor and brain metastasis from renal cell cancer (RCC) patients. While the density of CD68(+) TAMs was similar in primary RCC and brain metastases, TAMs were more frequently CCR2-positive in brain metastases than in primary RCC (P < 0.001). Since CCL7 expression in cancer cells of brain metastases was more frequent compared with primary tumours, it was concluded that monocyte recruitment by CCR2 contributes to brain metastasis of RCC [125].

TAMs are associated with tumor angiogenesis and lymphangiogenesis: in fact factors that are directly or indirectly involved in new vessel formation and sprouting are expressed by TAM. These include TGF-β, VEGF-A, VEGF-C, PDGF, MMP-9, thymidine phosphorylase (TP), and chemokines...
Table 1: Effector molecules involved in tumor promotion by TAM.

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<tr>
<th>Molecules</th>
<th>Effect</th>
<th>Selected reference</th>
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<tr>
<td>Cathepsins, MMP-2, MMP-9</td>
<td>ECM Remodeling, Tumor Invasion, Metastasis</td>
<td>Gocheva et al., 2010 [98]</td>
</tr>
<tr>
<td>SR-A</td>
<td>Angiogenesis and Lymphangiogenesis</td>
<td>Huang et al., 2002 [103] Granata et al., 2010 [104]</td>
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<td>TGF-β</td>
<td>Immunosuppression</td>
<td>Wang et al., 2011 [105] Hagemann et al., 2006 [109]</td>
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<td>MMP9</td>
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<td>Kuang et al., 2009 [110]</td>
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<td>VEGFs</td>
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<td>Chen et al., 2013 [111]</td>
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<td>PDGF</td>
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<td>Chen et al., 2012 [112]</td>
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<td>CXCL8</td>
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<td>Chang et al., 2001 [113]</td>
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<td>IDO</td>
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<td>Zhao, et al., 2012 [146]</td>
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<td>IL-10</td>
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<td>Sica et al., 2000 [108]</td>
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<td>B7-H1 (PD-L1)</td>
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<td>Hagemann et al., 2006 [109]</td>
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<td>B7-H3</td>
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<td>Arginine Depletion</td>
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(e.g., CXCL8/IL-8) [104, 106, 107, 126–128]. For instance, TAMs induce the release of heparin-bound growth factors, such as VEGF-A, that have a crucial role in the angiogenesis switch, releasing MMP-9 [129]. On-site education of VEGF-recruited monocytes improves their performance as angiogenic cells [130]. The recruited monocytes derive from the abundant pool of circulating Ly6Chi monocytes and upon arrival in the VEGF-rich environment they undergo multiple phenotypic and functional changes, endowing them with enhanced proangiogenic capabilities and, importantly, with a markedly increased capacity to remodel existing blood vessels.

In the tumor microenvironment, a proangiogenic program is triggered in macrophages by the increasing expression levels of HIF-1 and HIF-2 induced by low-oxygen tension. In this environment macrophages increase their expression levels of VEGF, bFGF, CXCL8/IL-8, and glycolytic enzymes [131]. Moreover, during local hypoxia the levels of adenosine markedly increase; this causes the release by human macrophages of angiogenic and lymphangiogenic factors [104]. Casazza and coworkers recently reported that TAMs’ localization into hypoxic tumor areas is controlled by a Sema3A/Neuropilin-1 signaling axis. Confining TAMs inside normoxic regions by blunting the Sema3A/Neuropilin-1 pathway restores antitumor immunity and abates angiogenesis, overall inhibiting tumor growth and metastasis. Thus cancer cell-derived Sema3A, not VEGF, is responsible for TAM entry into hypoxic niches, through NrP1 signaling, where TAM escape antitumor immunity and promote vascularization [132]. Modulating TAM localization and thus their phenotype can be a new approach to guide TAM activities against cancer. Moreover Laoui et al. reported that hypoxia is not a major driver of the TAM subset differentiation found in tumor infiltrate, namely, CD11bLo F4/80Lo Ly6CLo/MHC-IIlo or MHC-IIhi TAM, both of which derived from tumor-infiltrating Ly6CLo monocytes, but rather specifically fine-tune the phenotype of M2-like MHC-IIlo TAM, that as a consequence contain higher mRNA levels for hypoxia-regulated genes than their MHCIIhi counterparts [18].

The angiogenic potential of TAM has been further proved by depletion studies demonstrating a reduced blood vessel density in tumor environment [133]. TAM express also immunosuppressive potential, through a wide range of molecules, such as TGF-β, iNOS, arginase-1, IDO, and IL-10, known for their immunosuppressive role [97, 108, 109, 134]. In murine models of breast cancer, T-cell suppression is dependent, at least in part, on TAM metabolic activities via arginase-1 or iNOS expression [113, 135–137]. Moreover, TAM have been shown to express the immunosuppressive molecule B7-H1 in hepatocellular carcinoma, B7-H4 in ovarian and lung cancer, and B7-H3 in lung cancer [110–112, 138]. In addition, TAM have the capacity to induce the expression of these molecules on cancer cell surface, thus providing a novel mechanism by which cancer cells escape the immune surveillance [111].

The immunosuppressive molecules of the B7 family can be expressed also by tumor cells. For instance, B7-H3 was highly expressed in non-small-cell lung cancer and its expression, evaluated by immunohistochemistry, significantly correlated with the patients’ survival time; higher levels of B7-H3 expression were associated with a shorter survival time. Interestingly, in vitro soluble B7-H3 was capable of inducing
Macrophages with an anti-inflammatory/reparatory (alternative/M2) phenotype; these M2 macrophages express high levels of macrophage mannose receptor (MMR) and IL-10 and low levels of HLA-DR and IL-1β [139]. The interaction between tumor-infiltrating hematopoietic cells and epithelial cancer cells can result in their fusion and the expression by tumor cells of hematopoietic markers, including CD45 and CXCR4. In this case tumor infiltrating leukocytes promote tumor growth equipping tumor cells with molecules that enhance cancer cells dissemination and escape from immune mechanism destruction [140]. This observation may have implications on the bone marrow contribution to the cancer stem cell population. It was hypothesized that the superior migratory potential gained by the cancer cells due to the fusion helps in its dissemination to various secondary organs upon activation of the CXCR4/CXCL12 axis. This finding has repercussions on CXCR4-based therapeutics and opens new avenues in discovering novel molecular targets against fusion and metastasis.

Due to their overall protumoral effects, targeting TAM is a useful and promising tool for new antitumoral therapies. Accordingly, several therapeutic strategies were proposed to inhibit their recruitment, interfere with their survival, or reprogram them into a M1 antitumoral phenotype [141–145].

6. Prognostic Significance

Prognostic significance of TAM in humans has been recently critically evaluated [146]. Though there is epidemiological evidence that in many human cancers, such as breast, cervix, bladder, and gastric cancer, high numbers of TAM are significantly associated with poor patient prognosis, [14, 147]; other studies have reported that the prognostic significance of TAM can be controversial [146]. For instance, in high-grade osteosarcoma patients CD68+ TAM associated with reduced metastasis and improved survival [148], whereas in patients with classical Hodgkin's lymphoma and with large B-cell lymphoma no such correlation was found [149, 150]. However a recent study in patients with diffuse large B-cell lymphomas treated with Rituximab-CHOP reported that an increase of CD163(+) cells predicts poor prognosis, while an increase in CD68(+) cells was related to improved overall survival [151]. No correlation was also found for CD68+ cells in epithelial and stromal tumor compartments and cancer progression in patients with endometrial carcinomas [152].

In gastric cancer, number of TAM has been positively correlated with tumor cell apoptosis and the presence of CD8-positive cells [153]. Moreover, the number of TAM was found to be independent predictor of patient better survival [153]. The prognostic significance of TAM in patient with colorectal cancer (CRC) is controversial and could depend on distinct phenotypes acquired on distinct localization within the tumor [154]. In CRC it has been recently reported that an IFN-γ driven tumor microenvironment exerts a superior effect on patients’ survival and outcome compared with microenvironments driven by IFN-α or without an IFN-associated immune reaction. IFN-γ was found expressed mainly in the desmoplastic stroma and only rarely in the tumor cells, while most of the IFN-α-positive cells were found to be of tumor epithelial origin. These results suggest that complex cytokine interaction networks can have greater relevance for the determination of the prognosis of patients than the mere presence of tumor infiltrating leukocytes [88]. Moreover the positive role of macrophages in CRC has been found to be related to factors secreted by CRC cells that induced TAMs of a mixed M1/M2 phenotype, which in turn could contribute to a “good” inflammatory response [155]. This suggests that reeducation of macrophages might allow for important therapeutic advances in the treatment of human cancer.

These controversies can also be the consequence of the consistent heterogeneity of the studies performed (i.e., experimental procedures and techniques used to identify tissue macrophages) could lead to these controversies [146].

The relationship between TAN infiltration and prognosis in human cancer has been recently reviewed [22]. In many cases neutrophil infiltration was associated with a worse clinical outcome, as in patients with metastatic and localized clear cell carcinomas, bronchioalveolar carcinoma, hepatocellular carcinoma, colorectal carcinoma, head and neck squamous cell carcinoma, and resectable esophageal squamous cell carcinoma [23–27, 156]. Correlation between neutrophils infiltration with the tumor grade in human gliomas and with more aggressive types of pancreatic tumors has been reported [28, 29]. In contrast, TAM have been associated with better prognosis in gastric carcinoma [30]. In analogy to TAM, also the prognostic significance of TAN largely depends on the type of tumor and on the methodology used to detect and evaluate the neutrophils within the tumors (e.g., hematoxylin-eosin stain versus immunohistochemistry) [30, 134, 157].

7. Therapeutic Targeting

Preclinical cancer models in the mouse indicate that it may be possible to “reeducate” tumor-promoting TAMs to reject neoplastic cells [14, 158]. In this regard, Beatty and coworkers investigated in both humans and mice whether the systemic CD40 activation with an agonist CD40 monoclonal antibody can circumvent tumor-induced immune suppression and favour antitumor immunity. In human studies they conducted a phase II clinical trial of the fully human CD40 agonist antibody CP-870,893 in combination with gemcitabine chemotherapy, in twenty-one patients with advanced pancreatic cancer and observed tumor regression in some patients. Investigating the mechanisms responsible for the observed partial clinical effect in a murine model of pancreatic cancer, they found a modified macrophage phenotype with upregulated MHC class II and CD86 expression [141]. In relation to these data it is interesting that the Th1 cytokine IFNγ promoted the antitumor effector function of CD40 ligand-activated macrophages [159].

Recruitment is a key determinant sustaining macrophage infiltration at sites of inflammation. To interfere with infiltration sustained by the CCL2/CCR2 axis, antibodies against CCL2 or its cognate receptor CCR2 have been tested in experimental models. In some tumors as prostate cancer [160] and breast metastasis [161] anti-CCL2 therapy reduced
tumor growth. Combination of anti-CCL2 with chemotherapy improved survival in preclinical settings [144]. On these bases, anti-CCL2 antibodies are being evaluated in humans in prostate and ovarian cancer [162]. Targeting CSF-1 receptor can be another way to modulate recruitment. To this aims, CSF-1 receptor (CSFR1) kinase inhibitors have been generated and have been shown to display antiangiogenic and antimitastatic activity in acute myeloid leukemia and melanoma models [163]. Anti-CSF-1 antibodies and anti-sense oligonucleotides suppressed macrophage infiltration and xenograft mammary tumor growth in mice [164, 165]. As described above, TAMS have a role in promoting invasion of breast carcinoma cells via a CSF-1/EGF paracrine loop [166]. Thus, new therapeutic strategies to inhibit macrophage and tumor cell migration and invasion can include blockade of EGF receptor or interference with CSFR1 signalling. TAMS may also influence the tumor’s response to chemotherapy. In a genetic mouse model of breast cancer, chemotherapy with paclitaxel caused upregulation of the macrophage chemotactic factors CSFI, CCL8, and IL-34 and increased in CSFR1 expression in TAM. Inhibitors of CSFR1 that block macrophage recruitment combined with chemotherapy enhanced therapeutic activity, inhibited metastases, and increased T cells in the tumors and mRNA for various cytotoxic effector molecules such as granzyme A and B and perforin-1. If CD8+ cytotoxic effector T cells (CTL) were depleted, the positive effects of the block of macrophage infiltration disappeared, suggesting that macrophages depletion enhances chemotherapeutic response in a CD8+ CTL-dependent manner [167].

Yondelis (Trabectedin) is an EMEA-approved natural product derived from the marine organism Ecteinascidaria turbinata, with potent antitumor activity; it is specifically cytotoxic for human and murine macrophages and TAMS and inhibits of the production of CCL2 and IL-6 both by TAMS and tumor cells [168]. Evidence now indicates that targeting TAM represents a major determinant of the antitumor action of this agent [143].

A therapeutic strategy alternative to the reduction of macrophage recruitment can be the inhibition of TAM effector functions. The bisphosphonate zoledronic acid is a prototypical MMP inhibitor. In cervical cancer this compound suppressed MMP-9 expression by infiltrating macrophages and inhibited metalloprotease activity, reducing angiogenesis and cervical carcinogenesis [169]. The halogenated bisphosphonate derivative clodronate is a macrophage toxin, which depletes selected macrophage populations. In particular, clodronate encapsulated in liposomes has been shown to efficiently deplete TAMs in tumor models, as murine teratocarcinoma and human rhabdomyosarcoma xenografts. TAM depletion significantly inhibited tumor growth [133].

Recently, Daurkin and coworkers suggested that enhanced 15-Lipoxygenase-2 (15-LOX2) activity in renal cell carcinoma (RCC) microenvironment could be implicated in monocytes recruitment through the CCL2-CCR2 axis. In turn, TAMs could exert immunosuppressive actions on T cells by inducing FOXP3 and CTLA4 in LOX-independent fashion. Therapeutic approaches directed toward the manipulation of 15-LOX2-mediated arachidonic metabolism could represent a novel strategy to counteract cancer-related inflammation and subvert the immunosuppression in patients with RCC [170]. Liver X receptor (LXR) ligands, also named oxysterols, are released by cancer cells and inhibit CCR7 expression on maturing DCs, therefore dampening DC migration to draining lymph nodes and antitumor immune responses [171]. At the experimental level it has been demonstrated that interfering with the oxysterol-CXCR2 axis delays tumor growth and prolongs the overall survival of tumor-bearing mice. Moreover some freshly isolated human tumor cells release oxysterols able to bind both LXR and CXCR2 [36] and higher numbers of intratumor neutrophils severely affect overall survival of kidney cancer patients [23]. Results of Racosta et al. identify an unanticipated protumor function of the oxysterol-CXCR2 axis and suggest that manipulating LXR ligands and their interaction with CXCR2 and immune cells could provide additional targets for the development of new antitumor therapies.

Monoclonal antibodies and molecular inhibitors targeting the VEGF and EGF pathways are already approved for treatment of various carcinomas alone or in combination, although none were specifically targeted on TAMs function, and their clinical efficacy has been variable [172].

TAMS can also be potential immunotherapeutic target [141, 167, 173, 174]. Cavnar and coworkers investigated their role in gastrointestinal stromal tumor (GIST) and demonstrated that tumor cell oncogene activity determined TAM phenotype and function. In mice, established GIST tumors contained M1-like TAMSs, which were antitumoral, and proven by depletion studies. Imatinib therapy in mouse GIST polarized TAMSs to become M2-like through the activation of cC/EBPα. Human TAMS were also M1-like at baseline and became M2-like after imatinib therapy. In patients whose tumors developed resistance to imatinib, TAMS reverted to M1-like and had a remarkably similar gene expression profile as M1-like TAMSs from untreated patients. These findings reveal the central importance of tumor cell oncogene activity in TAM polarization [175].

Progress in understanding the biology of TAM paves the way to development of different and alternative strategies to target these cells or modulate their function, although the careful definition of the immunobiology of the context in different tumors may be required for successful therapeutic exploitation.

8. Concluding Remarks

Resolution of inflammation, tissue repair, and remodelling involve the function of cells of the mononuclear phagocytes system. Tumor progression is modified by a wide variety of host myeloid cell types, including macrophages and neutrophils. Under the influence of multiple microenvironmental signals, macrophages as well as neutrophils polarize toward distinct phenotypes with protumoral or antitumoral activities. Moreover, elucidating the relationship between MDSC, TAM, and TAN needs further investigations. Early studies indicated a possible cytotoxic activity of neutrophils toward tumor cells, and more recent reports have shown that
neutrophils promote tumor progression via matrix degrada-
tion, tumor cell proliferation, increased metastasis, and
enhanced angiogenesis, as it has been described in many
studies for TAM. A better knowledge of the mechanisms that
myeloid cells use to affect tumor growth and progression can
be clinically relevant to develop new therapeutic strategies in
cancer.

Conflict of Interests
The author declares that there is no conflict of interests
regarding the publication of this paper.

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