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Review

Macrophage Regulation of Tumor Responses to Anticancer Therapies

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Tumor-associated macrophages (TAMs) promote key processes in tumor progression, like angiogenesis, immunosuppression, invasion, and metastasis. Increasing studies have also shown that TAMs can either enhance or antagonize the antitumor efficacy of cytotoxic chemotherapy, cancer-cell targeting antibodies, and immunotherapeutic agents—depending on the type of treatment and tumor model. TAMs also drive reparative mechanisms in tumors after radiotherapy or treatment with vascular-targeting agents. Here, we discuss the biological significance and clinical implications of these findings, with an emphasis on novel approaches that effectively target TAMs to increase the efficacy of such therapies.

Introduction

Macrophages phagocytose microbes and present antigens to T cells, therefore constituting a first line of defense against invading pathogens. They also regulate tissue growth, homeostasis, repair, and remodeling via their expression of numerous cytokines, chemokines, growth factors, proteolytic enzymes, and scavenger receptors (Gordon and Martinez, 2010; Murray and Wynn, 2011). As such, macrophages play a central role in developmental processes, such as tissue morphogenesis and vascular and neuronal patterning, but also in pathophysiological responses, like inflammation and organ healing/regeneration (Mantovani et al., 2013; Nucera et al., 2011; Pollard, 2009).

In selected organs of the adult mouse, the origin of tissue macrophages can be traced back to fetal macrophages that appear before the onset of definitive hematopoiesis (Schulz et al., 2012). In inflamed and remodeling tissues, elevated macrophage turnover is sustained largely from hematopoietic progenitor cells (HPCs), which proliferate and differentiate into promonocytes in the bone marrow (BM) before they are shed into the circulation as monocytes. These then undergo final differentiation into macrophages as they extravasate in the target tissues (Shi and Pamer, 2011). During inflammation and tumor growth, BM-derived HPCs may also accumulate at extramedullary sites, such as the spleen, which can become an important site of monocyte production (Cortez-Retamozo et al., 2012).

Once resident in tissues, macrophages acquire a distinct, tissue-specific phenotype in response to signals present within individual microenvironments. The exact combination of such tissue-specific cues dictates both the differentiation and activation status of these cells. Two extreme forms of the latter are generally referred to as "classical" (or M1) and "alternative" (or M2) activation, which parallel Th1/Th2 programming of adaptive immune cells (Biswas and Mantovani, 2010; Mantovani et al., 2002). During acute inflammation, macrophages are M1-activated by toll-like receptor (TLR) agonists and Th1 cytokines (e.g., interferon [IFN]-γ). This enhances their ability to kill and phagocytose pathogens, upregulate proinflammatory cytokines (e.g., interleukin [IL]-1β, IL-12, and tumor necrosis factor-α [TNF-α]) and reactive molecular species, and present antigens via major histocompatibility complex (MHC) class II molecules (Biswas and Mantovani, 2010; Mantovani et al., 2002). Alternatively, Th2 cytokines, like IL-4 and 13, stimulate monocytes/macrophages to express an M2 activation state. This is characterized by higher production of the anti-inflammatory cytokine, IL-10; lower expression of proinflammatory cytokines; amplification of metabolic pathways that can suppress adaptive immune responses; and the upregulation of cell-surface scavenger receptors, such as mannose receptor (MRC1/CD206) and hemoglobin/aptoglobin scavenger receptor (CD163). As such, M2 macrophage activation may facilitate the resolution of inflammation and promote tissue repair (including angiogenesis) after the acute inflammatory phase (Biswas and Mantovani, 2010; Gordon and Martinez, 2010). In healthy tissues, macrophages often express a mixed M1/M2 phenotype; hence "M1" and "M2" polarization should be regarded as extreme ends of a continuum of activation states, with their exact point on the scale depending on the precise mix of local signals present in a given microenvironment (Biswas and Mantovani, 2010; Lawrence and Natoli, 2011; Sica and Mantovani, 2012).

Tumor-Associated Macrophages

Macrophages are a major cellular component of murine and human tumors, where they are commonly termed tumor-associated macrophages (TAMs). In this article, we specifically review the role of these cells and their monocyte precursors in tumor responses to anticancer therapies. Other tumor-infiltrating myeloid cells not discussed here include neutrophils, eosinophils, and activated dendritic cells (DCs) (de Visser et al., 2006). Tumors also recruit a variety of immature myeloid cells, often referred to as myeloid-derived suppressor cells (MDSCs), which comprise precursors of both the monocyte-DC (mononuclear) and neutrophil (granulocytic) lineages and are commonly identified by their expression of Gr1 (Ly6C/G) and immunosuppressive activity. Mononuclear MDSCs can further mature into TAMs (Coffelt et al., 2010; Gabrilovich et al., 2012). Finally,

there is also evidence for hematopoietic and myeloid progenitor cells homing to tumors and modulating tumor progression (Shaked and Voest, 2009).

Various mouse studies have shown that monocytes are recruited into tumors in large numbers by chemokines secreted by both malignant and stromal cells. These include chemokine (C-C motif) ligand 2 (CCL2, or MCP1), colony-stimulating factor-1 (CSF1), and chemokine (C-X-C motif) ligand 12 (CXCL12, or SDF1) (Murdoch et al., 2008). Upon monocyte differentiation into TAMs, these cells act as a source of local and systemic cues to support the proliferation, survival, and motility of the cancer cells; tumor vascularization (angiogenesis); suppression of antitumor immunity; and intravasation of cancer cells at the primary tumor site and extravasation/growth at distant metastatic sites (Bingle et al., 2006; De Palma et al., 2003; DeNardo et al., 2009; Lewis and Pollard, 2006; Lin et al., 2001; Qian et al., 2011; Qian and Pollard, 2010; Ruffell et al., 2012a; Squadrito and De Palma, 2011; Wyckoff et al., 2004). This impressive array of tumor-promoting functions is consistent with clinical studies showing high macrophage density in many human cancer types to be associated with increased tumor angiogenesis and metastasis, and/or a poor prognosis (Bingle et al., 2002; Clear et al., 2010; Heusinkveld and van der Burg, 2011; Leek et al., 1996). Furthermore, enrichment of a macrophage-related gene signature correlates with reduced survival in some types of human cancer (Engler et al., 2012; Steidl et al., 2010).

A decade ago, it was proposed that TAMs are predominantly polarized in the tumor microenvironment toward an M2-like phenotype and that this underlies their ability to promote the growth and vascularization of tumors (Mantovani et al., 2002). This is also supported by clinical studies showing the predictive value of M2-macrophage associated markers, like CD163 (Heusinkveld and van der Burg, 2011). Flow cytometry and gene expression profiling of mouse and human TAMs has shown that distinct macrophage subpopulations with a variably skewed M2-like phenotype coexist in tumors and that their relative abundance varies with the tumor type (Movahedi et al., 2010; Pucci et al., 2009; Ruffell et al., 2012b). Such complexity likely indicates diverse TAM programming in different microenvironments within individual tumors (Lewis and Pollard, 2006; Qian and Pollard, 2010; Ruffell et al., 2012a; Squadrito and De Palma, 2011). For example, M2-like TAMs reside in both perivascular and hypoxic regions of different mouse and human tumors (Mazzieri et al., 2011; Movahedi et al., 2010; Pucci et al., 2009). A population of vessel-associated TAMs—also referred to as TIE2-expressing monocytes/macrophages (TEMs)—is required for tumor angiogenesis (De Palma et al., 2005) and displays a profoundly M2-skewed phenotype characterized by enhanced expression of scavenger receptors (e.g., MRC1 and CD163) and relatively low levels of MHCII molecules and proinflammatory cytokines (Pucci et al., 2009; Squadrito et al., 2012). Interestingly, vascular endothelial cells (ECs) may induce HPCs to directly differentiate into $TIE2^{+}MRC1^{+}$ macrophages in the perivascular microenvironment, a process that appears to depend on EC-derived CSF1 (He et al., 2012). Also attesting to the complexity of TAM subtypes, recent studies have shown that both the origin and phenotype of TAMs may differ in primary versus metastatic tumors (Qian et al., 2011).

TAMs with a relatively M1-skewed phenotype may be found in incipient or regressing tumors as well as necrotic areas of progressing tumors (Prada et al., 2013; Wang et al., 2011). Gene expression profiling of M1- and M2-like TAMs, however, suggests that such TAM "subtypes" express both canonical M1 and M2 markers, albeit at significantly different levels (Movahedi et al., 2010; Pucci et al., 2009; Squadrito et al., 2012).

Macrophage Involvement in Tumor Responses to Therapy

As will be seen below, TAMs not only enhance tumor growth and progression, but also modulate the efficacy of various forms of anticancer therapy. In some circumstances, they also facilitate tumor regrowth, revascularization, and spread after the treatment.

Chemotherapy

A complex picture has emerged over the past 30 years of the role of TAMs in modulating the antitumor efficacy of chemotherapeutic agents (Figure 1). Early studies showed that the antitumor efficacy of doxorubicin (DOX; an anthracycline formerly known as adriamycin) is reduced when mice bearing immunogenic leukemia or lymphoma transplants were given macrophage toxins (Mantovani et al., 1979; Figure 1A). Furthermore, the in vivo administration of DOX enhanced the tumoricidal activity of macrophages ex vivo. Interestingly, macrophages did not enhance the efficacy of DOX against poorly immunogenic lymphomas, suggesting that tumor immunogenicity may influence the ability of macrophages to modulate the antitumor activity of DOX. In contrast, macrophage depletion failed to limit the antitumor activity of another anthracycline, daunorubicin (formerly daunomycin) (Mantovani et al., 1979), possibly because the latter is per se toxic toward macrophages in vivo (Mantovani, 1977). Together, these early reports suggested that some cytotoxic agents are able to foster the antitumor activities of TAMs, at least in leukemia and/or immunogenic (transplant) tumor models. In this regard, innate immune cells, like macrophages and DCs, are known to mediate "immunogenic cell death" (ICD), a process that encompasses chemotherapy-induced cancer cell death and release of "eat-me" signals (e.g., ATP and high-mobility group B1 [HMGB1]); activation of mononuclear phagocytes and enhancement of their antigen-presenting capacity; and promotion of T cell responses against immunogenic tumors. Of note, only a few chemotherapeutics are known to induce ICD, one of which is DOX (Kroemer et al., 2012).

TAMs can also contribute in other ways to the modulation of tumor responses to chemotherapy. Figure 1 shows that this can vary markedly between different cytotoxic agents and tumor models. For example, the antitumor activity of the taxane docetaxel involves the depletion of immunosuppressive (M2-like) TAMs and the concomitant activation or expansion of antitumoral (M1-like) monocytes/MDSCs in 4T1-Neu mammary tumor implants. Indeed, in vitro T cell assays showed that docetaxel-treated monocytes/MDSCs are able to enhance tumor-specific, cytotoxic T cell responses (Kodumudi et al., 2010). Trabectedin, a DNA-damaging agent approved for soft tissue sarcomas, inhibited the growth of mouse fibrosarcomas primarily by depleting mononuclear phagocytes, including monocytes and TAMs (Germano et al., 2013). Mechanistically, it activates caspase 8 and induces apoptosis specifically in

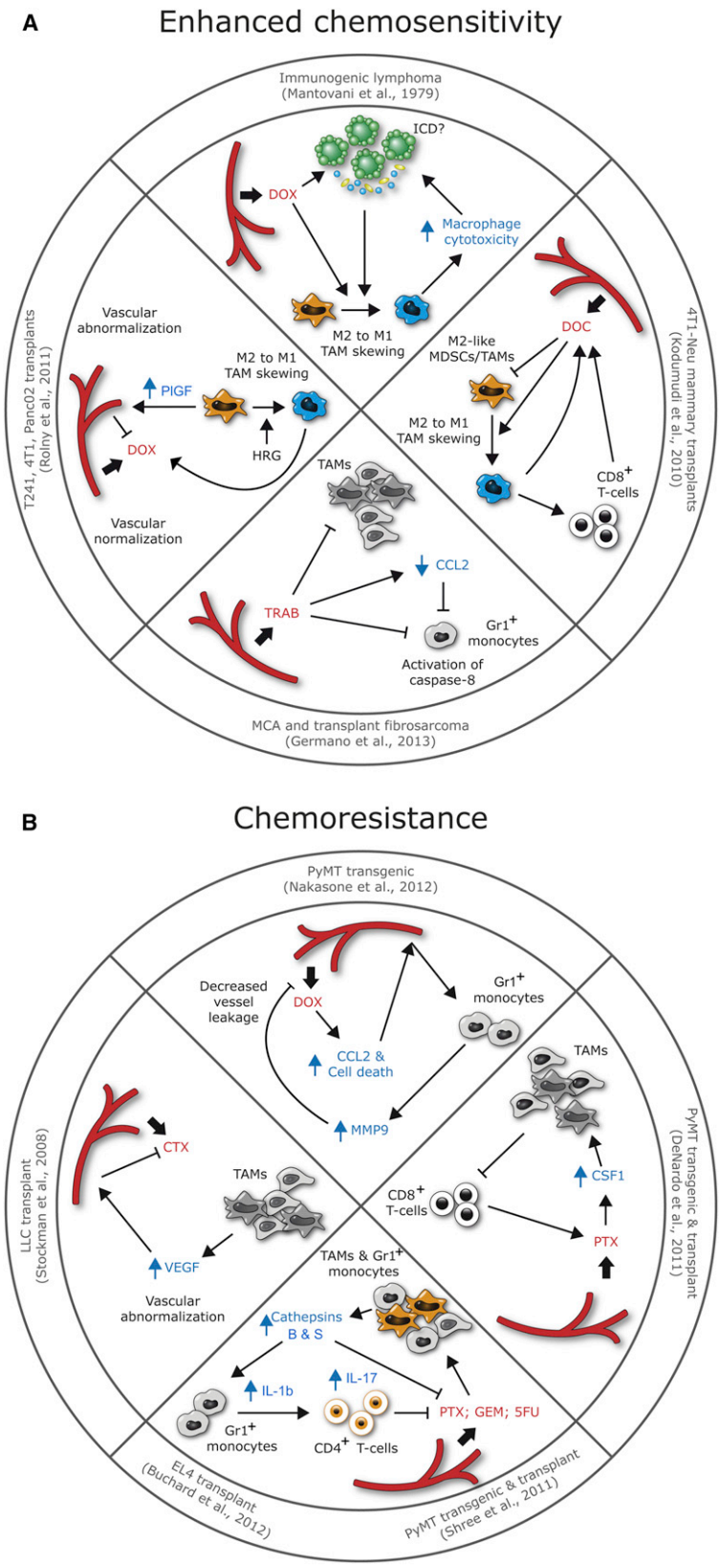

Figure 1. TAMs Enhance or Limit the Efficacy of Chemotherapy Depending on the Cytotoxic Agent Applied and/or Mouse Tumor Model Used

(A) Chemosensitivity is increased when cytotoxic agents, either directly or indirectly, increase the cytotoxicity of TAMs or deplete monocytes, TAMs, or M2-like TAMs. The latter cells can also be reprogrammed by agents like HRG, which in turn enhances chemotherapy delivery. DOX enhances the cytotoxicity of macrophages/TAMs, a process possibly involving ICD (top); DOC promotes the expansion of cytotoxic M1-like MDSCs/TAMs, which enhance antitumor T cell responses (right); TRAB depletes protumoral monocytes/TAMs via caspase-8 activation (bottom); HRG downregulates PlGF in TAMs, reprogramming them toward an M1-like phenotype, and enhances DOX delivery (left).

(B) Chemoresistance is increased when cytotoxic agents, either directly or indirectly, increase protumoral (M2-like) TAM numbers. The latter cells may also limit chemotherapy delivery by affecting vascular leakage. DOX enhances tumor infiltration by MMP9-expressing monocytes via upregulation of CCL2 (top); PTX enhances tumor infiltration by macrophages via upregulation of CSF1 (right); PTX, GEM, and 5FU enhance tumor infiltration by cathepsin-B/S-expressing monocytes/macrophages, which activate chemoprotective T cells through IL-1β and 17 (bottom); VEGF-expressing TAMs augment vascular leakiness and limit CTX delivery (left).

Abbreviations: DOC, docetaxel; TRAB, trabectedin; CTX, cyclophosphamide; GEM, gemcitabine; 5-FU, 5-fluorouracil; IL-1b, interleukin-1β; IL-17, interleukin-17.

monocytes/macrophages via TRAIL-R2, a death receptor not expressed by other leukocytes. Interestingly, trabectedin also depleted circulating monocytes and TAMs in patients with soft-tissue sarcomas. These findings support the notion that the antitumor activity of some cytotoxic agents may depend, at least in part, on their ability to reprogram or deplete protumoral mononuclear phagocytes (Kodumudi et al., 2010; Germano et al., 2013). It remains to be seen whether the mode of action of trabectedin also entails the promotion of adaptive antitumor immune responses, unleashed through the depletion of immunosuppressive TAMs (Figure 1A).

There is also compelling evidence for TAMs limiting the efficacy of chemotherapy (Figure 1B). For example, TAM depletion by anti-CSF1 antibodies enhanced the efficacy of combination chemotherapy (cyclophosphamide, methotrexate, and 5-fluorouracil) in chemoresistant, human breast cancer xenografts grown in immunodeficient mice (Paulus et al., 2006). Similarly, TAM depletion enhanced the efficacy of paclitaxel (PTX, a taxane) in immunocompetent, MMTV-PyMT mouse mammary tumors (DeNardo et al., 2011). At variance with some other cytotoxic drugs (e.g., trabectedin), PTX did not affect tumor growth by depleting TAMs. Rather, it augmented their recruitment to the tumors by upregulating tumor-derived CSF1. Consistent with the known immunosuppressive functions of TAMs, the increased TAM numbers in PTX-treated tumors limited tumor infiltration by $CD8^+$ cytotoxic T cells and possibly reduced their tumoricidal activity. These important findings suggest that TAMs may limit the therapeutic activity of PTX in breast cancer, at least in part, by suppressing specific antitumor immune responses (DeNardo et al., 2011).

TAMs may also release "chemoprotective" factors. Shree et al. (2011) reported increased TAM numbers in PTX-treated MMTV-PyMT tumors and showed that TAM secretion of the lysosomal enzymes, cathepsins B and S, protected cancer cells from PTX-induced cell death and so limited the efficacy of this agent (Shree et al., 2011). Indeed, a pan-cathepsin inhibitor improved the response of MMTV-PyMT tumors to PTX. Interestingly, coculture experiments showed that such macrophage-derived cathepsins protect cancer cells from the direct cytotoxic effects of several chemotherapeutics, including DOX and etoposide (Shree et al., 2011). In this regard, a recent study showed that two broadly used chemotherapeutics, gemcitabine and 5-fluorouracil, induce monocytes/MDSCs to release cathepsin B from lysosomes (Bruchard et al., 2013). This activates the inflammasome and enhances monocyte/MDSC secretion of IL-1β. In turn, IL-1β prompted secretion of IL-17 by $CD4^+$ T cells, which then blunted the anticancer effects of chemotherapy (Figure 1B). These data provide a molecular mechanism linking myeloid cell-derived cathepsins to chemoprotection.

While DOX may stimulate macrophage cytotoxicity toward immunogenic leukemias (Mantovani et al., 1979), its effects on TAMs appear to vary with the tumor type. In the transgenic MMTV-PyMT mammary tumor model, DOX induction of necrotic cell death led to increased tumor infiltration by CCL2 receptor $(CCR2)^+$ monocytes/TAMs, a process that relied on upregulation of CCL2 (Nakasone et al., 2012). Interestingly, the antitumor activity of DOX was enhanced in *Ccr2* knockout hosts, which lack $CCR2^+$ monocytes. While the effect of DOX on the cytotoxic activity of TAMs was not examined in this study, the authors showed that matrix-metalloproteinase (MMP)-9 produced by recruited myeloid cells decreased blood vessel leakiness and limited drug delivery to the tumors, suggesting that, at least in MMTV-PyMT tumors, increased vascular permeability is associated with a better response to DOX (Nakasone et al., 2012). It should be noted that, in other tumor models, downregulating the expression of proangiogenic factors, like vascular endothelial growth factor (VEGF) or placental growth factor (PlGF) by TAMs "normalized" the tumor-associated vasculature, decreased vessel leakiness, and enhanced chemotherapy delivery to tumors (Rolny et al., 2011; Stockmann et al., 2008; Figures 1A and 1B). It remains to be seen whether the different effects of DOX in leukemia versus the above mammary tumor model (Nakasone et al., 2012) reflect differences in tumor immunogenicity or more complex aspects of the tumor microenvironment.

Taken together, the above studies show that different chemotherapeutic agents may induce distinct responses in monocytes/macrophages, which can either enhance or antagonize the activity of the anticancer drug, possibly in a tumor-type dependent fashion. Tumor immunogenicity along with the intrinsic sensitivity of TAMs to the drug and their activation state (M1 versus M2-like) may be important determinants of such TAM-mediated responses. Furthermore, cytotoxic drugs often target multiple cell types in tumors, so tumor-type specific stromal cell signatures (Coussens et al., 2013) could influence the ability of TAMs to respond to and modulate the activity of a given chemotherapeutic. Indeed, cytotoxic drugs could have both direct and indirect effects on TAM behavior. For example, taxanes profoundly alter macrophage gene expression in vitro (Javeed et al., 2009) but also induce tumor damage and cancer cell death, which may trigger a reparative, "wound healing" response in TAMs (Mantovani et al., 2013). Further studies are now warranted to distinguish between the role of TAMs in the chemoprotection described above (DeNardo et al., 2011; Nakasone et al., 2012; Shree et al., 2011) and the reparative responses that occur in tumors after therapy.

Finally, TAMs may enhance tumor chemoresistance by providing survival signals to tumor-initiating/cancer stem cells (CSCs). For example, TAMs were found to release milk fat globule-epidermal growth factor 8 protein (MFG-E8) to help protect lung and colon CSCs from cisplatin. This relied, at least in part, on MFG-E8-induced activation of STAT3, which enhanced CSC chemoresistance (Jinushi et al., 2011). Moreover, TAM depletion has been shown to improve antitumor T cell responses and the efficacy of chemotherapy in a pancreatic cancer model, in part by decreasing the frequency, tumor-initiating capacity, and STAT3 activation of CSCs (Mitchem et al., 2013).

Tumor Irradiation

Tumor irradiation is widely used to treat many cancer types. Early studies correlated high TAM numbers in mouse tumors with poor tumor responses to irradiation (Milas et al., 1987). Recent data suggest that radiation-induced DNA damage and activation of the v-abl Abelson murine leukemia viral oncogene homolog 1 (ABL1) kinase promote *Csf1* gene transcription and upregulation of tumor CSF1, which in turn recruits CSF1R-expressing myeloid cells (including TAMs) that enhance posttherapy tumor regrowth. Indeed, a CSF1R inhibitor improved tumor response to radiotherapy in a prostate cancer model (Xu et al., 2013; Figure 2).

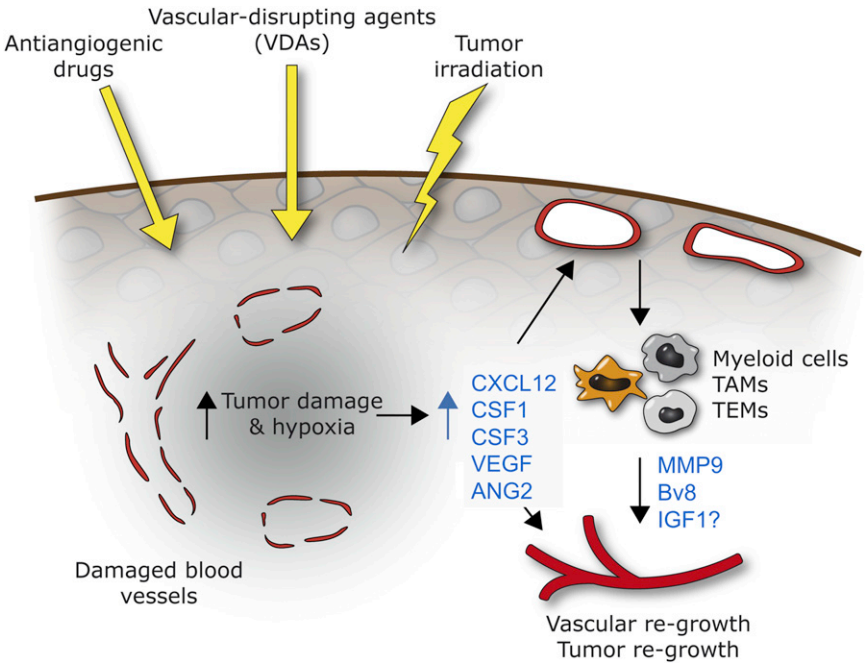

Figure 2. TAMs Promote Tumor Regrowth Following Tumor Irradiation, Antiangiogenic Drugs and VDAs

These anticancer therapies cause tumor necrosis, vascular damage, and hypoxia, which together or separately induce the upregulation of several myeloid cell/monocyte chemoattractants, including CXCL12, CSF1, CSF3, VEGF, and ANG2, in the tumor microenvironment. De novo recruitment of myeloid cells drives tumor regrowth via their effects on the tumor blood vessels (mediated, e.g., by MMP9, Bv8, and IGF1) and, possibly, the cancer cells.

Abbreviations: CSF3, granulocyte-colony stimulating factor; Bv8, prokineticin.

See also Figure 1.

Antibody-mediated depletion of $CD11b^+$ myeloid cells in human head and neck tumors grown in immunodeficient mice also reduced tumor regrowth after therapy (Ahn et al., 2010). In a model of orthotopic human glioblastoma, local irradiation dramatically enhanced tumor infiltration by $CD11b^+$ myeloid cells (Kioi et al., 2010). Interestingly, a high proportion of these cells were $F4/80^+TIE2^+$ TEMs, and their recruitment was dependent on the hypoxic induction of the chemoattractant, CXCL12, in the irradiated tumors (Figure 2). Upregulation of CXCL12 and increased TEM infiltration were also observed in lung and mammary tumors grown subcutaneously following irradiation (Kozin et al., 2010). In the latter study, TEMs congregated mainly around the remaining blood vessels in treated tumors (Kozin et al., 2010), suggesting that they may stimulate tumor recurrence by promoting EC survival and vascular regrowth through their expression of prosurvival factors like insulin growth factor 1 (IGF1) and fibroblast growth factor 2 (FGF2) (De Palma et al., 2005; Pucci et al., 2009). However, the location and, possibly, the function of M2-like TAMs in irradiated tumors may vary with tumor type. In irradiated orthotopic astrocytomas, arginase-1 $(ARG1)^+$ M2-like TAMs were found to accumulate mainly in avascular, hypoxic areas rather than at perivascular sites (Chiang et al., 2012). This suggests that the reparative mechanisms employed by M2-like TAMs in postirradiated tumors may be regulated by distinct microenvironmental signals in different tumor types. It is also conceivable that the functions of M2-like TAMs in irradiated tumors are similar to those of M2-like macrophages driving tissue repair in healing organs, such as following acute renal injury and myocardial infarction (Mantovani et al., 2013).

Vascular-Targeted Therapies

VEGF is a proangiogenic cytokine that also functions as a potent monocyte chemoattractant (Barleon et al., 1996). It is, therefore, possible that the antiangiogenic and antitumor effects of VEGF blockade could result, at least in part, from impaired monocyte/TAM recruitment. However, this seems increasingly unlikely, as it is now established that therapeutic interception of VEGF is counteracted by the compensatory induction of other proangiogenic factors, some of which are involved in monocyte/myeloid cell chemoattraction (Bergers and Hanahan, 2008; Ferrara, 2010).

Tumor hypoxia and necrosis dramatically increase after the selective destruction of tumor blood vessels by high-dose antiangiogenic drugs or vascular-disrupting agents (VDAs) (Bergers and Hanahan, 2008). When tumors are treated with VDAs, like combretastatin-A4-phosphate (CA-4-P), the selective disruption of the tumor-associated vasculature results in vessel collapse, reduced blood flow, induction of tumor hypoxia, and secondary tumor cell death. As in irradiated tumors, VDA-induced hypoxia was associated with elevated levels of CXCL12 and increased TEM infiltration in mammary tumor models (Welford et al., 2011; Figure 2). Blocking this CA-4-P-induced TEM recruitment, either using the CXCR4 antagonist, plerixafor (AMD3100), or by genetic TEM depletion, markedly increased the efficacy of CA-4-P treatment in subcutaneous N202 (Neu^+) mammary carcinomas (Welford et al., 2011).

Blocking the proangiogenic factor angiopoietin-2 (ANG2) also leads to angiogenesis inhibition and increased tumor hypoxia (Daly et al., 2013; Mazzieri et al., 2011). As seen in CA-4-P-treated tumors (Welford et al., 2011), the latter events were associated with an enhanced recruitment of $MRC1^+$ TEMs, which may have limited the efficacy of ANG2 blockade (Mazzieri et al., 2011). Sorafenib, which targets several receptor tyrosine kinases (including VEGF receptor 2 [VEGFR2] and platelet-derived growth factor receptor [PDGFR]) and Raf kinases, was also shown to increase CXCL12 levels and TAM infiltration in hepatocellular carcinoma xenografts. Depletion of TAMs by clodronate-loaded liposomes (clodrolip) augmented the inhibitory effects of sorafenib on tumor angiogenesis, growth, and metastasis in this tumor model (Zhang et al., 2010). Moreover, TAM depletion by clodrolip (Zeisberger et al., 2006) or a CSF1R inhibitor (Priceman et al., 2010) increased the antiangiogenic and antitumor effects of VEGF/VEGFR2 antibodies in subcutaneous tumor models. Together, these data support the rationale for combining antiangiogenic drugs with macrophage

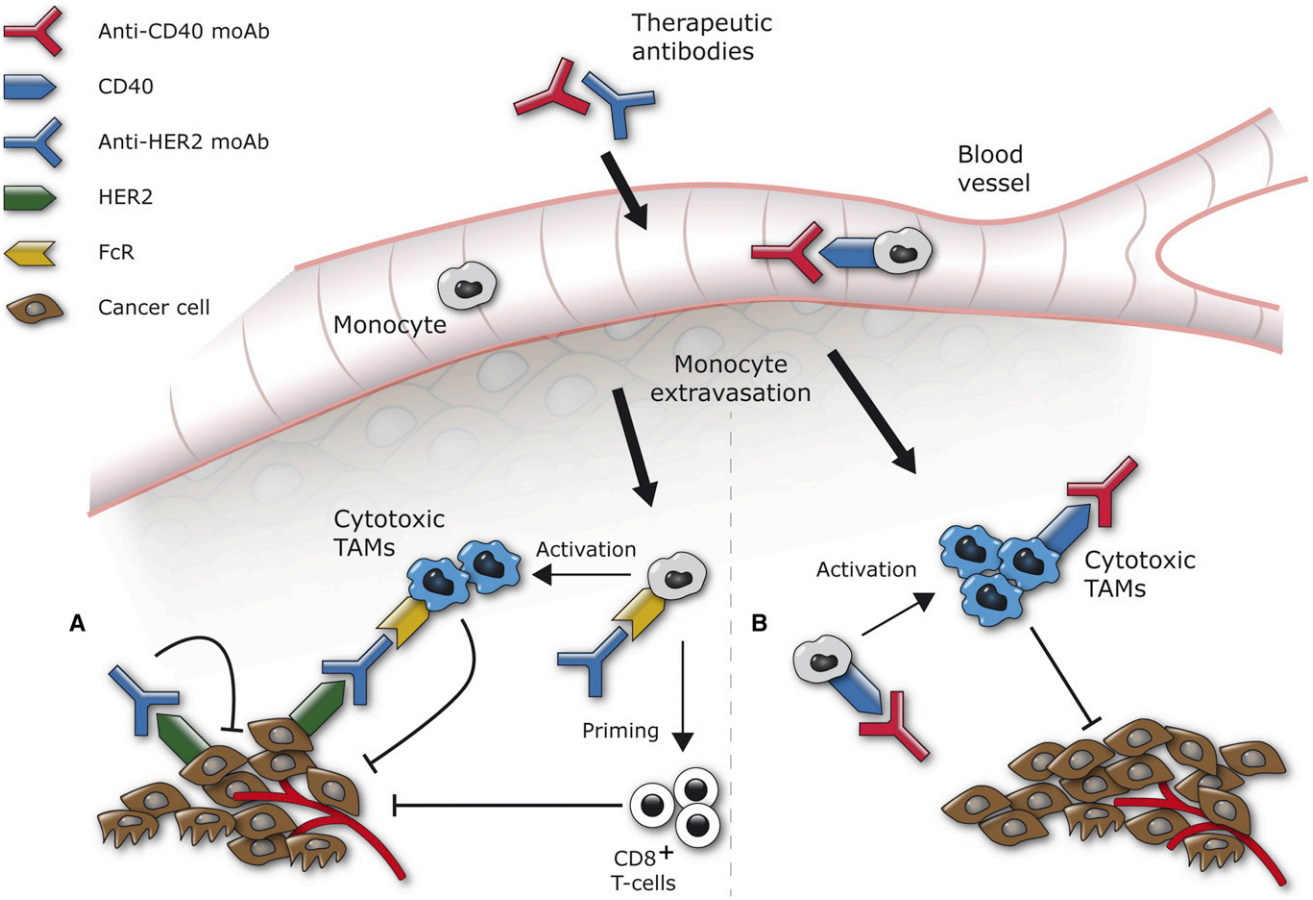

Figure 3. moAbs Activate TAMs to Express a Cytotoxic Phenotype
Binding of therapeutic antibodies to monocytes/macrophages may enhance their tumoricidal activity.
(A) Binding of therapeutic (cancer-cell targeted) moAbs (e.g., anti-HER2) to monocytes/TAMs via Fc-receptors (FcRs) induces FcR-mediated activation of macrophage cytotoxicity/phagocytosis (ADCC/ADCP) and priming of adaptive antitumor immunity (e.g., $CD8^+$ T cells).
(B) Binding of immunotherapeutic moAbs (e.g., anti-CD40) to monocytes/TAMs triggers their activation to a cytotoxic (M1-like) phenotype.

targeting strategies to increase the efficacy of the former, particularly in tumors that are refractory or develop resistance to anti-VEGF therapy.

Targeted Therapies by Monoclonal Antibodies

Although a role for TAMs in modulating the efficacy of oncogene-targeted, small molecule inhibitors has yet to be elucidated, there is now increasing evidence for TAMs contributing to the cytotoxicity of therapeutic monoclonal antibodies (moAbs). TAMs express surface receptors that bind the Fc fragment of antibodies and enable them to engage in Ab-dependent cellular cytotoxicity/phagocytosis (ADCC/ADCP). Trastuzumab, a moAb against the human epidermal growth factor receptor-2 (HER2), not only interrupts HER2 signaling in breast cancer cells, thereby slowing their proliferation rate, but also induces Fcγ receptor (FcγR)-mediated activation of macrophage cytotoxicity (Clynes et al., 2000) and priming of antigen-specific $CD8^+$ T cell responses in MMTV-Neu tumors (Park et al., 2010) (Figure 3A). In one study, TAM depletion limited the efficacy of a moAb directed against tissue factor (CD142)-expressing human breast carcinoma cells inoculated in mice (Grugan et al., 2012). Macrophages also enhance lymphoma elimination in mice in response to rituximab, a moAb against CD20, primarily through FcγR-dependent ADCP (Chao et al., 2010; Minard-Colin et al., 2008). The significance of the aforementioned studies is supported by clinical findings suggesting that certain *FCGR* polymorphisms may bear predictive value for the clinical efficacy of trastuzumab or rituximab therapy in breast cancer and lymphoma, respectively (Mellor et al., 2013). Furthermore, high TAM numbers correlate with a better prognosis in rituximab-treated patients (Taskinen et al., 2007). Engineered recombinant proteins that can enhance the interactions between FcγR-expressing immune cells and moAbs, like the recently described "grababodies" (Cai et al., 2013), may thus have the potential to increase ADCC/ADCP in tumors. It should be noted, however, that engagement of macrophage-FcγRs by serum or therapeutic antibodies (e.g., the anti-EGFR moAb cetuximab) was shown to enhance the immunosuppressive, proangiogenic, and protumoral functions of TAMs both in experimental tumor models and human cancer (Andreu et al., 2010; Pander et al., 2011).

Immunotherapies

As mentioned previously, TAMs can be potent immunosuppressors that limit the cytotoxic activity of $CD8^+$ cytotoxic T cells in progressing tumors (DeNardo et al., 2011). The analysis of human breast cancer tissues showed that a high stromal TAM

density correlates inversely with $CD8^+$ T cell numbers (DeNardo et al., 2011). In a preclinical study, clodrolip-mediated depletion of TAMs enhanced tumor infiltration by HPV16 E7-specific $CD8^+$ T cells in a HPV16 $E6^+/E7^+$ mouse model of cervical cancer (Lepique et al., 2009). TAM-mediated immunosuppression is mediated, at least in part, by induction of T cell apoptosis and nitrosylation of T cell receptors via macrophage products, like ARG1, NOS2, and peroxynitrite (Gabrilovich et al., 2012).

It should be noted that the study by DeNardo et al. (2011) analyzed the leukocyte composition of established tumors (DeNardo et al., 2011), in which immunosuppressive, M2-like TAMs likely predominate over tumoricidal (M1-like) macrophages. It is possible that incipient tumors, which are likely to be more immunogenic than established lesions, contain higher proportions of M1-like TAMs, which could initiate and/or potentiate adaptive immune responses (Prada et al., 2013; Wang et al., 2011). In this regard, macrophages were shown to acutely engulf myeloma cells inoculated subcutaneously in syngenic mice and to activate myeloma-specific $CD4^+$ Th1 cells, which then enhanced the tumoricidal activity of macrophages through IFN-γ secretion (Corthay et al., 2005). In certain immunoprivileged organs, such as the eye, macrophages promote the effector functions of $CD4^+$ T cells, and their depletion enhances rather than inhibits intraocular tumor growth (Dace et al., 2008). Thus, the type of macrophage activation—which may correlate with tumor stage (Prada et al., 2013; Wang et al., 2011)—may dictate the magnitude of antitumoral T cell responses in mouse models of cancer.

Based on the above, strategies to deplete TAMs or block cancer-induced M2-like macrophage programming (see below) may have the potential to enhance T cell-mediated antitumor responses and improve the efficacy of immunotherapies (Coussens et al., 2013; Hagemann et al., 2008; Jaiswal et al., 2010). Intriguingly, increasing data suggest that the efficacy of some forms of immunotherapy may also depend on effective reprogramming of TAMs toward an M1-like phenotype. For example, intravesical instillation of *Mycobacterium bovis* bacillus Calmette-Guérin, which is used for the treatment of superficial bladder cancer, reduces tumor recurrence by stimulating the cytotoxic activity of macrophages. Macrophage-mediated killing of bladder cancer cells relies on both direct effector-target cell contacts and the release of soluble cytotoxic factors, such as TNF-α, IFN-γ, and NO, from the macrophages (Luo and Knudson, 2010). An agonistic antibody to the TNF receptor superfamily member, CD40, was recently reported to bind to circulating monocytes, trigger their recruitment into mouse pancreatic tumors, and activate their tumoricidal functions (Figure 3B). These CD40-activated, cytotoxic (M1-skewed) TAMs were also found to enhance the efficacy of gemcitabine in a small cohort of patients with surgically incurable pancreatic cancer (Beatty et al., 2011). Finally, macrophages and DCs express programmed cell death ligand-1 (PDL1, also known as B7-H1), a major negative regulatory ligand that suppresses T cell activation through its receptor-programmed cell death protein 1. The promising therapeutic activity of anti-PDL1 moAbs in patients with advanced cancer (Brahmer et al., 2012) will no doubt prompt further studies of the possible inhibition of PDL1 expression on TAMs to improve the efficacy of chemo- or antiangiogenic therapies.

Concluding Remarks: Implications for Cancer Treatment

In light of this growing body of evidence for TAMs modulating the effects of various anticancer therapies, attempts are now being made to either target key molecules that regulate their recruitment into tumors or re-educate these cells toward a cytotoxic M1-like phenotype. The efficacy of CSF1R inhibitors in blocking the enhanced uptake of monocytes during PTX treatment in preclinical studies (DeNardo et al., 2011) has prompted clinical trials of their use in combination with either PTX or the antiproliferative agent eribulin (http://www.clinicaltrials.gov). Various preclinical studies have also highlighted ways to reprogram TAMs from an M2 to an M1-like phenotype in tumors. These include the use of histidine-rich glycoprotein (HRG), which induces macrophage downregulation of PlGF, promotes the normalization of blood vessels, and increases delivery and efficacy of chemotherapy in mouse tumor models (Rolny et al., 2011; Figure 1A). Other strategies to reprogram TAMs include blockade of nuclear factor-kB signaling (Hagemann et al., 2008) or their exposure to anti-IL-10R antibodies combined with the TLR9 ligand CpG (Guiducci et al., 2005). The latter resulted in hemorrhagic tumor necrosis, activation of DCs and cytotoxic T cells, and clearance of tumor remnants.

However, there is still much to learn about the mechanisms regulating TAM functions during chemotherapy, as well as other forms of therapy discussed in this review. Importantly, a number of key questions need to be addressed before approaches that combine macrophage targeting (or reprogramming) and conventional cancer therapies can be translated into more effective treatments. Why do preclinical studies employing distinct chemotherapeutic drugs and/or tumor models show different and, at times, contradictory roles for TAMs in modulating tumor responses to such agents? Why do TAMs apparently limit the effects of chemotherapy in some tumor types but not others? Are the distinct TAM subtypes present in individual tumors differentially responsive to chemotherapy? If yes, what are the specific features of the TAM subset(s) that either enhance or promote the antitumor activity of cytotoxic agents? And what are the signals in tumors that regulate these TAM responses? Such information might help selectively target the TAMs that limit chemotherapy while leaving antitumoral or tissue-resident macrophages unaffected. Furthermore, most preclinical studies to date have focused on primary, nonmetastatic tumors. So, are we confident that TAMs in metastatic tumors (Qian et al., 2011) behave in the same way during therapy as those in the primary tumor site?

Mouse tumor models, including genetically engineered mouse models (GEMMs), are being used extensively to study mechanisms underlying tumor (and TAM) responses to anticancer therapies. However, even sophisticated GEMMs of cancer cannot simulate the endless variations in TAM abundance, distribution, and phenotypes between and within different types and subtypes of human cancer (Coussens et al., 2013; De Palma and Hanahan, 2012). Nor do they necessarily model the ability of such tissues to recruit monocytes during therapy. Future work should therefore aim to define the identities and molecular profiles of distinct TAM subtypes in human cancer biopsies before, during, and after therapy. Specific TAM signatures could then be used to stratify patients carrying defined genetic lesions

in order to explore how such signatures correlate with the response of individual patients to chemo-, radio-, or targeted therapies, and/or the emergence of secondary resistance (DeVita and Costa, 2010). If such studies demonstrate the predictive value of specific TAM subtypes for individual tumor responses, then their further characterization in mouse tumor models could help develop more effective cancer therapies. Undeniably, such clinical approaches should consider the biological complexities on a tumor (sub)type and individual patient basis and harness them to design effective personalized therapies.

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