A Milestone Review on How Macrophages Affect Tumor Growth

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In this 75th Anniversary issue commentary, we discuss a seminal review published in 2006 by Claire E. Lewis and Jeffrey W. Pollard in Cancer Research, entitled "Distinct Role of Macrophages in Different Tumor Microenvironments" (1). This was a milestone review for the journal that became very highly cited because of its incisive overview of important advances in learning how macrophages exert distinct effects on tumor pathophysiology based on their response to the specific microenvironment of tumors that they rove. This review also helped popularize the nomenclature of tumor-associated macrophages, or TAM, now a widely understood moniker used broadly in the field.

Key Messages from the Review

TAM infiltration was already shown to correlate with poor prognosis of cancer patients. The central message of this seminal review from Drs. Lewis and Pollard was that different TAM populations coexist in tumor, in distinct microenvironments, including areas of invasion, where TAMs promote cancer cell motility, stromal and perivascular areas, where TAMs promote metastasis, and avascular and perinecrotic areas, where hypoxic TAM stimulate angiogenesis. In terms of phenotype and diversity, this review provided a highlight complementing the M1/M2 polarization model proposed by Mantovani and colleagues (2), where exposure to IL4 and IL10 in tumors induced type II polarized TAM (alternatively activated) or M2 macrophage development. Here, distinct TAM populations were described according to their localization in different zones of the tumors and associated functions. An important aspect of the work of Pollard’s team was the use of the MMTV-PyMT–induced mammary tumor model, allowing longitudinal analyses of TAM at different stages of tumor development, a model that is now used very widely in cancer biology, pathology, and pharmacology studies. Distinct contributions of TAM to malignant development discussed in the review are noted below.

Invasion

TAMs present in areas of basement membrane breakdown and invasion during early-stage lesion development produce upregulated levels of proteolytic enzymes (cathepsin B and matrix metalloproteinases) and promote directional movement and invasion of the tumor cells.

Growth

TAM infiltration positively correlates with tumor cell proliferation (i.e., Ki67 levels), and TAMs express a number of soluble factors (i.e., EGF, PDGF, HGF, bFGF) stimulating tumor cell proliferation and survival.

Angiogenesis

Starting in 1991 (3), a large number of studies have described the important role of TAMs in regulating angiogenesis. TAMs release potent proangiogenic cytokines and growth factors (VEGF, TNFα, IL8, and bFGF) and CSF-1/M-CSF (macrophage colony-stimulating factor), resulting in accelerated vascularization. Increased TAM frequency correlates with high vascular grades in many tumor types. In MMTV-PyMT–induced mammary tumors, macrophages are recruited to premalignant lesions immediately before the so-called "angiogenic switch," a critical step in the transition of an early lesion to frank malignancy. Importantly, TAMs accumulate in hypoxic/necrotic areas, where they produce upregulated levels of VEGF and other proangiogenic factors in response to hypoxia. Indeed, a subset of monocytes expressing the angiopoietin receptor Tie-2 is a critical inducer of angiogenesis (4). Targeted deletion of Tie-2–expressing monocytes markedly reduced angiogenesis in human glioma xenografts and prompted substantial tumor regression. Altogether, it is clear that TAMs are important modulators of angiogenesis, being recruited into hypoxic/necrotic areas and are involved both in the formation of new vessels and in their remodeling into a coherent functional network needed for local tumor growth and survival.

Metastasis

Clear associations exist between TAM and sites of tumor cell invasion and blood vessel extravasation. Macrophage deletion greatly reduces formation of lung metastases, arguing that TAMs play major roles in both the release of metastatic cells from the primary tumor as well as the establishment of secondary tumors at distant sites.

Immunosuppression

Unlike healthy tissue macrophages endowed with antigen-presenting capacity, tumor cell lysis ability, and T-cell and natural killer (NK) cell–stimulating functions (IL12 production), TAMs lack these effector functions. Indeed, they are inhibited in the tumor environment through the potent immunosuppressive factors PGE-2 and IL10.

The overall message of the review was that in early preinvasive lesions, tumor cells attract macrophages, where, according to tumor progression and microenvironment sites (tumor cell invasion, perivascular sites, stromal regions, and hypoxic/necrotic areas), TAMs are "educated" to carry out specific functions in
support of tumor cell requirements and activities in those different areas.

**Evolution in Modern Perspectives on TAM**

Since the Lewis and Pollard review appeared, the impact of TAMs on tumor progression and patient outcome has been extensively confirmed in many types of solid tumors. TAMs are now known to release a number of major inflammatory mediators like reactive nitrogen (RNS) or oxygen (ROS) species, TNFα, IL6, IL1β, and IL23. There is also evidence that TAM contributes to DNA damage, oncogenic transformation, survival of transformed cells, and cancer-related inflammation, each of which are causal to carcinogenesis in many settings (5, 6). Specific areas of advance are summarized below.

**TAM origin**

Recent studies demonstrate that tissue-resident macrophages originate from yolk sac-derived progenitors and are renewed from tissue progenitors (7), whereas macrophages involved in pathogen responses appear to arise from circulating bone marrow monocytes. IL34, a recently identified CSF-1R alternative ligand, is predominantly involved in tissue macrophage homeostasis (8). Nevertheless, several recent studies in mice suggest that most TAM subpopulations arise from the circulating Ly6C+ monocytes (9). CSF-1 is the dominant growth factor for TAM development, and CSF-1 genetic deletion results in loss of TAM and delayed tumor initiation, progression, and metastasis. However, genetic gain of function of VEGF-A over a loss of function of CSF-1 in the MMTV-PyMT model also results in a dramatic recruitment of macrophages and a rescue of angiogenesis in favor of an accelerated progression to malignancy (9), suggesting VEGF-mediated CSF-1-independent TAM development. Beyond CSF-1 needed for progenitor development and TAM survival, the chemokine CCL2 is a direct mediator of CCR2+ monocyte recruitment in tumors (9).

**TAM subsets/differentiation state**

Over the past decade, the M1/M2 polarization model has been applied in many systems of cancer study (10). In general, M1-TAMs express high levels of IL12 and IL23 and are efficient producers of effector molecules, such as ROS/RNS (through iNOS) and inflammatory cytokines, participating as inducers and effector cells in Th1 responses. However, as the tumor progresses, macrophages polarize toward an "alternatively activated" or M2 phenotype, differing from M1-TAM in receptor expression, antigen-presenting ability, function (i.e., arginine metabolism), and cytokine production. These M2-TAMs exhibit a protumor, angiogenic, and immunoinhibitory phenotype and are characterized by high IL10 and scavenger receptors expression (i.e., CD163 and CD206).

Importantly, the hypoxic tumor microenvironment, through the production of lactic acid and HIF-1α, induces an M2-type polarization (11) that leads to VEGF production.

Here, it should be noted that the model of segregation for TAMs in extreme forms of polarization as either tumor killing (M1) or tumor promoting (M2), although well established in *vivo*, is widely recognized by experts as limited in *vivo*. For example, transcriptomic analyses have reported mixed profiles of TAMs in *vivo* with different macrophages associated with diverse phenotypes, which argue for a plethora of different populations. However, the definition of true subsets with clear delineating markers has progressed quite slowly except for the identification of the Tie-2–expressing perivascular TAM (see below; ref. 12).

**Tumor angiogenesis**

TAMs drive the "angiogenic switch" associated with malignant transition, as considered in the Lewis and Pollard review, where macrophages act as "bridge cells" to facilitate vascular anastomosis and sprouting. In particular, Tie-2–expressing monocytes/macrophages were confirmed to possess potent proangiogenic properties in a number of human and murine tumors. They are aligned along the abluminal surface of blood vessels through endothelial cell expression of the Tie-2 ligand angiopoietin-2 (ANGPT2), and targeting ANGPT2/Tie-2 releases this macrophage–vessel association and inhibits angiogenesis. CSF-1 upregulates Tie-2 on TAMs. Finally, these perivascular TAMs may mediate relapse after chemotherapy (12, 13).

**Promotion of metastases**

The role of monocytes and/or macrophages as metastasis promoters has been further extended. They can induce epithelial-to-mesenchymal transition and promote tumor cell extravasation to circulation. TAMs also prepare metastatic sites and promote the extravasation, survival, and persistent growth of metastatic cells (12).

Studies have characterized premetastatic niches, influenced by primary tumors, populated by CD11b+ VEGFR1+ myeloid cells. Other studies of lung metastasis showed that tumor cells form microclots with platelets and arrest in the target tissue vessels, leading to CCL2-mediated Ly6C+ macrophages recruitment and their differentiation into CCR2+ VEGFR1+ Ly6C+ F4/80+ metastasis-associated macrophages (MAM) required for metastasis establishment (11). The engagement of the alternative VEGF receptor VEGFR1 (FLT1) on MAMS leads to inflammatory state that promotes breast cancer metastasis (14).

**Immunosuppression**

In the Lewis and Pollard review, the connection between TAMs and immune response was presented mainly as a loss of immunostimulatory function in TAMs. However, since then, it has become clear that TAMs also exert immunosuppressive functions as a dominant feature of the tumor microenvironment. Multiple mechanisms have been reported (15), as summarized below:

(i) TAMs express IL10 and TGFβ and activated STAT3 (16).

(ii) TAMs express arginase-1 as encoded by the ARG-1 gene. The ARG-1 enzyme is an L-arginine catabolic enzyme that suppresses T-cell activity through locoregional depletion of L-arginine. ARG-1 is considered a hallmark of the anti-inflammatory state present in tumors (17).

(iii) The PD-1 ligands PD-L1 and PD-L2, which promote immune escape and favor T-cell exhaustion, are up-regulated in TAMs and blood monocytes from cancer patients (18).

(iv) TAMs expressing the T-cell coregulatory receptor ligand B7-H4 suppress T-cell activation in ovarian cancer and correlate with clinical stage in several cancers.

(v) TAMs also overexpress HLA ligands (HLA-C, E/G) for inhibitory receptors, such as CD94 or ILT2/4, which function to suppress NK cells and various T-cell subsets. TAMs can also express the ILT2/4-inhibitory receptors themselves.
(vi) Tumor microenvironments favor TAM differentiation at the expense of dendritic cells (for review, see ref. 17), a finding that has been extended in multiple studies (18).

(vii) TAMs overexpress indoleamine 2,3-dioxygenase-1 (IDO1), a tryptophan catabolic enzyme widely implicated in T-cell suppression and also angiogenesis in cancer.

Macrophages as therapeutic targets

This important topic has been reviewed recently elsewhere (19). Currently, the most advanced approaches rely on TAM depletion via the inhibition of CSF-1/CSF-1R signaling, based on evidence that this axis is essential for macrophage survival. Recently, a phase I clinical trial using a CSF-1R–blocking mAb (RG7155) in patients with diffuse-type giant cell tumors (DT-GCT), a proliferative disease caused by overexpression of CSF-1 (20), yielded measurable clinical responses. These effects correlated with the depletion of TAM and an increase in CD8+ T-cell infiltration. Clinical trials combining CSF-1R inhibitors and chemotherapy have also been initiated (https://clinicaltrials.gov). However, depletion of nontumor macrophages by these treatments raised safety concerns. Trabectedin, a marine-derived natural product, interferes with transcription and DNA repair but also targets TAMs and induces their depletion through mechanisms as yet obscure (21). Alternative approaches rely on the blockade of monocyte recruitment to tumors by disrupting CCL2–CCR2 signaling, leading to reduced tumor growth and improved survival. However, interruption of anti-CCL2 therapy causes a rapid rebound of monocyte infiltration in tumors, correlating with accelerated metastatic relapse. A more attractive strategy for cancer therapy may be based on functional reprogramming of TAMs to enhance their antitumor properties. In this context, TAMs have been shown to be key effectors of antibody-dependent cellular cytotoxicity/phagocytosis (ADCC/ADCP)–mediated effects of antitumor mAbs (22), acting at the center of anti-CD47 strategies aiming at inducing tumor cell phagocytosis by TAMs (22).

Several recent studies have clearly demonstrated the involvement of TAMs in mediating resistance to therapies (23) and have identified BTK and PI3K-δ as regulators of TAM immunosuppressive pathways (23, 24). Finally, the success of therapy targeting the PD-1 axis may also rely in part on neutralizing PD-L1/2 expressed in T-cell suppression and also angiogenesis in cancer.

Remaining Challenges

Although therapeutic strategies targeting TAMs, through the CSF-1/CSF-1R pathway, are currently being evaluated clinically, further translational opportunities must be developed on the basis of emerging knowledge. In particular, we need to better decipher the molecular mechanisms controlling TAM activity in vivo; the heterogeneity of human TAM subsets, e.g., their origin as CD14+ CD16+ or Tie-2 monocytic; TAM function and phenotypes, e.g., the relationship between the Tie-2+ TAM subset and the M2-immunosuppressive type; and the basis for TAM plasticity during progression in terms of their microenvironment reprogramming potential.

Translational challenges relate in part to the fact that the present knowledge about TAM is based mainly on preclinical studies conducted in mouse models of cancer, mainly those employing transplanted tumors or multifocal spontaneous tumors. In-depth analyses of TAM subsets in human subjects are needed to validate the utility of the knowledge gained in these models. It would also be useful to have further information from spontaneous sporadic tumor models in which TAM populations are tracked longitudinally, so that their origin, functions, and phenotypes can be evaluated as tumors evolve.

One important current question concerns the antitumoral role of TAMs during early immunosurveillance, the studies of which have focused mainly on the reported “don’t eat me” signal enabled by CD47 upregulation on tumor cells as an escape mechanism to TAM-mediated phagocytosis via SIRPα engagement (22). It would be important to further document TAM antitumor functions in spontaneous sporadic tumor models. Such demonstration would strengthen the interest of strategies to restore antitumor capabilities of TAMs, rather than to eradicate them by depletion, perhaps most productively in combination strategies employing antitumor mAbs with ADCC activity (21, 25), or with inhibitors of “don’t eat me” signals, such as elicited by CD47 mAbs (25). In our view, TAM reprogramming strategies offer an exciting avenue for future investigations, for example, through CD40/TLR agonists, which we speculate may possibly engage TAMs to restore antitumor potential.

Another limitation of the current knowledge is the respective role of IL34, an alternate ligand of the important macrophage receptor CSF-1R, versus soluble and membrane-bound forms of the primary ligand CSF-1. Furthermore, a better understanding of the relative impact of the CSF-1/IL34 pathway versus the GM-CSF/IL3 and VEGF-R1/Tie-2 pathways is also important in the development, differentiation, and survival of different TAM subsets (12). The field also needs a deeper understanding of the connections and overlap between the so-called monocytic myeloid-derived suppressive cells, which contribute to cancer, and the TAM populations, which have been characterized, both of which are being regulated by angiogenic factors (26, 27). Here, comparative transcriptomic analyses may offer a first step, particularly in distinguishing the complex myeloid cell phenotypes and functions in cancer, which remain unclear.

The relationships between TAM and cancer-associated inflammation are yet another area requiring attention. TAMs are generally thought to promote tumor initiation and progression, but to understand their roles, further work is needed to molecularly define the subtypes or “flavors” of inflammation to which their functions clearly contribute. It is growing increasingly clear that multiple forms of inflammation exist, for example, as programmed by IDO1 or other metabolic constraints (28). Thus, beyond the M1/M2-polarized extreme phenotypes, other functional phenotypes certainly exist, depending on tumor stage, microenvironment, and therapeutic response. Resolving TAM heterogeneity to define more specific parameters will likely be important in directing more personalized treatment of cancer patients.

Disclosure of Potential Conflicts of Interest

G.C. Prendergast has ownership interest (including patents) in and is a consultant/advisory board member for New Link Genetics Corporation. No potential conflicts of interest were disclosed by the other authors.
Grant Support
C. Caux and colleagues are very grateful for grant support from the French National Institute of Cancer (INCa), in particular the SIBRC project (UVRC, grant no. INCa_4664), and from the French National Agency for Research (ANR), in particular the LABEX DEWECAN (ANR-10-LABX-0061) of Université de Lyon (ANR-11-IDEX-0007). R.N. Ramos is supported by the São Paulo Research Foundation (FAPESP#2012/13429-2).

Received September 26, 2016; accepted September 26, 2016; published online November 15, 2016.

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Cancer Res 2016;76:6439-6442.

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