Distinct Role of Macrophages in Different Tumor Microenvironments

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Abstract

Macrophages are prominent in the stromal compartment of virtually all types of malignancy. These highly versatile cells respond to the presence of stimuli in different parts of tumors with the release of a distinct repertoire of growth factors, cytokines, chemokines, and enzymes that regulate tumor growth, angiogenesis, invasion, and/or metastasis. The distinct microenvironments where tumor-associated macrophages (TAM) act include areas of invasion where TAMs promote cancer cell motility, stromal and perivascular areas where TAMs promote metastasis, and avascular and perinecrotic areas where hypoxic TAMs stimulate angiogenesis. This review will discuss the evidence for differential regulation of TAMs in these microenvironments and provide an overview of current attempts to target or use TAMs for therapeutic purposes. (Cancer Res 2006; 66(2): 605-12)

Introduction

Compelling evidence has emerged in recent years for macrophages playing an important role in tumor cell invasion into surrounding normal tissues, proliferation and survival, and metastasis to local and distant sites. The onset and maintenance of tumor angiogenesis also seems to be driven, in part, by these cells. Macrophages are derived from CD34\(^{+}\) bone marrow progenitors that continually proliferate and shed their progeny into the bloodstream as promonocytes. They then develop into monocytes and extravasate into tissues where they differentiate into a specific type of “resident” tissue macrophage (1). The phenotype of these fully differentiated, resident macrophages can vary markedly within tissues, from that of microglial cells in the brain, Kupffer cells in the liver, and Langerhans cells in the skin. Despite these regional differences, resident macrophages share a set of common functions, including their ability to guard against microbial infections, to regulate normal cell turnover and tissue remodeling, and to help repair sites of injury (1).

Macrophages also form a major component of the inflammatory infiltrate seen in both primary and secondary tumors (2), where, as will be seen below, they also exhibit a distinct phenotype and are termed tumor-associated macrophages (TAM). Monocytes enter tumors through blood vessels throughout the life span of tumors, from early-stage tumor nodules that are beginning to vascularize to tumors through blood vessels throughout the life span of tumors, termed tumor-associated macrophages (TAM). Monocytes enter the bloodstream as promonocytes. They then develop into monocytes and extravasate into tissues where they differentiate into a specific type of “resident” tissue macrophage (1). The phenotype of these fully differentiated, resident macrophages can vary markedly within tissues, from that of microglial cells in the brain, Kupffer cells in the liver, and Langerhans cells in the skin. Despite these regional differences, resident macrophages share a set of common functions, including their ability to guard against microbial infections, to regulate normal cell turnover and tissue remodeling, and to help repair sites of injury (1).

Macrophages also form a major component of the inflammatory infiltrate seen in both primary and secondary tumors (2), where, as will be seen below, they also exhibit a distinct phenotype and are termed tumor-associated macrophages (TAM). Monocytes enter tumors through blood vessels throughout the life span of tumors, from early-stage tumor nodules that are beginning to vascularize to late-stage tumors that are invasive and metastatic. A number of tumor-derived chemotransports are thought to ensure this ongoing recruitment, including colony-stimulating factor-1 (CSF-1 also known as M-CSF), the CC chemokines, CCL2, CCL3, CCL4, CCL5, and CCL8, and vascular endothelial growth factor (VEGF). The levels of many of these proteins in human tumors correlate positively with the numbers of TAMs present in those tumors (3).

That these cells might help to drive tumor progression has been inferred over a number of years from studies looking at the link between TAM levels and prognosis. Although a few have correlated high TAM numbers with good prognosis, the majority have linked such numbers to reduced patient survival (Table 1). Indeed, high TAM numbers have been shown to be an independent prognostic factor in many forms of cancer (2). These observations accord well with the results of animal studies using macrophage-depleted mice to investigate the role of macrophages in tumor progression in vivo. For example, we used mice homozygous for a null mutation in the CSF-1 gene to deplete macrophages in a mouse model for breast cancer. In this model, mammary tumors are initiated by the mammary epithelial restricted expression of the polyoma virus middle T oncprotein (PyMT). Macrophage depletion resulted in slower progression of preinvasive lesions to malignant lesions and reduced formation of lung metastases (4). Similarly, a recent study using small interfering RNA to inhibit CSF-1 expression by MCF-7 xenografts confirmed these findings, showing that lower numbers of TAMs are accompanied by a marked reduction in tumor growth and angiogenesis (5).

In this review, we will review how the general phenotype of macrophages in tumors differs from that seen in nonmalignant tissues, and then how their migration into distinct tumor sites exposes them to different microenvironmental signals that “educate” them to perform functions that are required by tumor cells in those areas. We will then discuss the possible therapeutic implications of these and the ways in which TAMs could be targeted in new anti-inflammatory therapies.

TAM Phenotypes

TAMs have been studied in some detail with some studies describing their phenotype as relatively immature, characterized by low expression of the differentiation-associated macrophage antigens, carboxypeptidase M and CD51, high constitutive expression of interleukin (IL)-1 and IL-6, and low expression of tumor necrosis factor-α (TNF-α. refs. 6, 7). However, it is not known yet whether the expression of these markers by TAMs varies between different tumors and within specific areas of tumors.

Macrophages derived from healthy or inflamed tissues are capable of lysing tumor cells, presenting tumor-associated antigens to T cells, and expressing immunostimulatory cytokines to stimulate the proliferation and antitumor functions of T cells and natural killer (NK) cells in vitro. In stark contrast, macrophages from experimental or human tumors show greatly reduced levels of these activities, possibly due in part to their exposure to...
such tumor-derived molecules as IL-4, IL-10, transforming growth factor-β1 (TGF-β1), and prostaglandin E2 (PGE2; ref. 8). Moreover, Mantovani et al. (9) have proposed that exposure to IL-4 and IL-10 in tumors may actually induce TAMs to develop into polarized type II (alternatively activated) or M2 macrophages. These cells have poor antigen-presenting capability, produce factors that suppress T-cell proliferation and activity, and are generally better adapted to scavenging debris, promoting angiogenesis, and repairing and remodeling wounded/damaged tissues. This phenotype contrasts markedly with the phenotype of classically activated type I or M1 macrophages that are efficient immune effector cells able to kill microorganisms and tumor cells, present antigen, and produce high levels of T-cell stimulatory cytokines (9).

Roles of TAM in Tumor Progression

**Tumor invasion.** In PyMT-induced mammary tumors, macrophages are present in areas of basement membrane breakdown and invasion during the development of early-stage lesions (Fig. 1A; ref. 4). This finding, together with other results showing the up-regulation of proteolytic enzymes like cathepsin B in macrophages present at the same locations (10), indicates that TAMs could be involved in the invasion of tumor cells into surrounding normal tissue. Indeed, Hagemann et al. (11) have reported that coculturing macrophages with tumor cells enhances their invasive properties in a manner dependent on TNF-α and matrix metalloproteinases (MMP). Subsequently, it was shown in Matrigel invasion assays that TNF-α acted by stimulation of the c-Jun-NH2-kinase and nuclear factor-κB signaling pathways (12). Downstream targets included such proinvasive target genes as *MIF* and extracellular MMP inducer (*EMMPRIN*) in tumor cells (12). These proteins then act in turn to enhance the local release of MMPs by macrophages and possibly other stromal cells such as fibroblasts (12, 13). In other coculture experiments, tumor cell invasion into a collagen matrix was increased by macrophages that synthesized epidermal growth factor (EGF) in response to tumor-derived CSF-1, leading to induction of several genes that increased directional movement and invasion of the tumor cells (14). Further experiments are required to identify the relationship, if any, between EGF and TNF-α signaling in cross-talk between TAMs and tumor cells and to show whether TAMs modulate tumor cell invasion in the same manner *in vivo*.

**Tumor growth.** TAM infiltration positively correlates with tumor cell proliferation as measured by MIB-1 levels in breast carcinomas (15), Ki67 levels in endometrial carcinomas (16), or mitotic index in renal cell carcinoma (17). Indeed, various studies have shown that TAMs express a number of factors that stimulate tumor cell proliferation and survival, including EGF (14, 18), platelet-derived growth factor, TGF-β1, hepatocyte growth factor, and basic fibroblast growth factor (bFGF; Fig. 2; ref. 19). Accordingly, when cocultured with tumor cells, macrophages secrete substances that stimulate tumor cell proliferation (20, 21). Moreover, macrophage depletion studies indicate that the presence of TAMs is essential for the growth of various types of experimental tumors *in vivo* (22). The production of such factors by macrophages in wounds may also help to explain why some tumors (e.g., tumors induced by injections of the Rous sarcoma virus) form more efficiently at sites of wounding or tissue injury. This phenomenon is known to involve factors like TGF-β1 being expressed by inflammatory cells in wounded tissues (23). However, it remains to be seen whether TGF-β1 and/or other molecules specifically expressed by macrophages in these sites are centrally involved in the development of wound-induced tumors.

Other proteins secreted by TAMs have also been implicated in tumor growth, such as MMP-9, which is essential for the growth of human ovarian tumors in nude mice (24). However, these effects may be indirect rather than direct via stimulation of tumor angiogenesis (see below). Also, the role of TAMs in tumor growth may vary by tumor type, because in CSF-1 knockout mice neither the incidence nor the early growth of PyMT-induced benign mammary tumors is adversely affected by macrophage depletion, although growth of more advanced carcinoma stages is impeded (4).3

**Tumor angiogenesis.** It is now widely accepted that the growth and spread of malignant tumors requires angiogenesis, the process by which new blood vessels sprout from the existing vasculature. Considerable evidence indicates that TAMs play an important part in regulating angiogenesis. The possibility that macrophages might be capable of modulating angiogenesis was first proposed by Sunderkotter et al. (25) in 1991. We and others have since elaborated on this to provide a detailed picture of the possible mechanisms used by macrophages to regulate angiogenesis in tumors (Fig. 2; ref. 26).

TAMs release a number of potent proangiogenic cytokines and growth factors, such as VEGF, TNF-α, IL-8, and bFGF. Additionally, they express a broad array of angiogenesis-modulating enzymes, including MMP-2, MMP-7, MMP-9, MMP-12, and cyclooxygenase-2 (COX-2; refs. 25–27). TAM production of MMP-9 has been shown to be crucial for angiogenesis in a mouse model of human cervical carcinogenesis (estrogen-treated K14-HPV16 female transgenic mice; ref. 28). Interestingly, as an important source of MMP-2 and MMP-9 in tumors, TAM may also contribute to the vascular “normalization” that occurs in tumors shortly after their treatment with inhibitors of VEGF signaling (29). The many proangiogenic functions of TAM may help explain reported correlations between increased TAM numbers and high vascular grades of many tumor types, including in breast carcinoma (22, 30), malignant uveal melanoma (31), glioma (32), squamous

| Table 1. High numbers of TAMs correlate with survival in different forms of cancer |
|---------------------------------|---------------------------------|---------------------------------|
| Favorable prognosis             | Reference                        | Poor prognosis                  | Reference                        |
| Stomach (59)                    | Breast**†                        | (15, 37)                        |
| Colorectal (72)                 | Prostate**                        | (35)                            |
| Melanoma (73)                   | Endometrial**                     | (36)                            |
|                                | Bladder**†                        | (34)                            |
|                                | Kidney**                          | (17)                            |
|                                | Esophageal                        | (50)                            |
|                                | Superfiäl?                        | (33)                            |
| Squamous cell carcinoma**       | Malignant uveal melanoma**        | (31)                            |
| Follicular lymphoma             |                                  | (75)                            |

*Correlation with increased tumor angiogenesis.
†Correlation with increased involvement of local lymph nodes. No correlation with survival was found in colon carcinoma (70), high-grade astrocytomas (71), lung carcinoma (74), or cervical carcinoma (76).
cell carcinoma of the esophagus (33), bladder carcinoma (34), and prostate carcinoma (35).

In PyMT-induced mouse mammary tumors, macrophages are recruited to premalignant lesions immediately before the angiogenic switch that presages the transition to malignancy. Depletion of these macrophages results in a ~50% reduction in vascular density even in tumors that progress to advanced stages. This effect is associated with increased necrosis as would be expected by loss of blood supply. TAMs are sufficient to induce angiogenesis in this model because premature recruitment of macrophages into benign hyperplastic lesions by CSF-1 overexpression results in accelerated vascularization of these normally poorly vascularized lesions along with accelerated progression to malignancy.4

Newly formed blood vessels in tumors are often disorganized and prone to collapse, resulting in areas of inadequate perfusion and hypoxia (low oxygen tension). Rapid tumor cell proliferation also outstrips the rate of new blood vessel growth, contributing to hypoxia in some areas. A number of studies have used hypoxic cell markers, like pimonidazole, to highlight the presence of both transient (avascular and nonnecrotic) and chronic (perinecrotic) areas of hypoxia in human and experimental tumors. TAMs accumulate in such hypoxic/necrotic areas in human endometrial, breast, prostate, and ovarian carcinomas (30, 36–39). We have also found TAMs to accumulate in such areas in PyMT-induced mammary tumors (Fig. 1) and human prostate tumor xenografts (3). This pattern of TAM migration is possibly due to a number of mechanisms, including the hypoxic induction of such macrophage chemoattractants as EMAPII, endothelin 2, and VEGF (as reviewed in ref. 3). Furthermore, as macrophages are phagocytes, they may also be attracted into hypoxic, perinecrotic areas along a trail of necrotic debris emanating from such areas. Hypoxia seems to inhibit macrophage migration, thereby immobilizing them in such areas. Increased numbers of TAMs in these sites correlate with increased lymph node involvement and/or poor prognosis in breast cancer (37) and endometrial cancer (38). The increased levels of TAMs at such sites may promote tumor progression in part by stimulating levels of angiogenesis, such as appears to occur in breast carcinoma (37).

TAMs respond to tumor hypoxia by up-regulating the hypoxia-inducible transcription factors HIF-1 and HIF-2. Although both

Figure 1. Location of different TAM subpopulations in tumors. TAMs were visualized by staining fixed sections of spontaneous PyMT-induced murine mammary tumors (A, C, E, and G) or human breast carcinomas (B, D, F, and H) using antibodies that specifically recognize the pan-macrophage markers F4/80 (murine) or CD86 (human). TAMs are seen to gather in areas of tumor cell invasion where they help to degrade the basement membrane and facilitate the migration of tumor cells into the stroma of the surrounding tissue (A and B), and in perivascular areas where they seem to promote metastasis by expressing factors like EGF (C and D). Other subpopulations of TAMs are seen in hypoxic, perinecrotic (E and F), and stromal (G and H) areas of these tumors where they promote angiogenesis and metastasis, respectively. Bar: 50 μm; V, blood vessel; N, necrosis.

factors are up-regulated by macrophages exposed to hypoxia in vitro as well as in hypoxic/necrotic areas of human tumors (40), their relative contribution to the regulation of gene expression by TAMs has yet to be fully elucidated. White et al. (41) used adenoviral infection to overexpress HIF-2 in human macrophages and found it to be the primary inducer of genes encoding angiogenic proteins in these cells. However, the repertoire of genes activated by overexpression of each HIF may differ from those up-regulated in natural settings of hypoxia.

Macrophages also up-regulate VEGF and other proangiogenic factors in response to hypoxia. TAMs express VEGF almost exclusively in avascular and perinecrotic areas of human breast carcinomas (42). Hypoxic induction of this cytokine is largely dependent on HIF-1, at least in murine peritoneal macrophages (43). Macrophages also synthesize elevated levels of MMP-7 when exposed to hypoxia in vitro and in avascular areas of human tumors (44). This multifunctional MMP has many substrates in the extracellular matrix and basement membrane, and is known to stimulate endothelial cell proliferation and migration, which can support tumor angiogenesis (45). A recent cDNA array study has identified up-regulation of messages encoding >30 other proangiogenic genes in primary macrophages exposed to hypoxia, including CXCL8, angiopoietin, COX-2 (the inducible form of nitric oxide synthase), and other factors (41).

We found recently that when macrophages are cocultured in vitro with human tumor spheroids, they infiltrate deep into the central, hypoxic areas of these structures. The release of VEGF by macrophages-infiltrated spheroids was significantly higher than that seen for noninfiltrated spheroids. This increase translated into a significant stimulation of angiogenesis in vivo when spheroids were implanted into microcirculation window chambers on the flanks of nude mice for 3 days (46).

A recent report has identified a subset of monocytes that express the angiopoietin receptor Tie-2 as important inducers of angiogenesis in both spontaneous and orthotopic tumor models (47). Knockout of these Tie-2-expressing cells in vivo markedly reduced angiogenesis in human glioma xenografts and prompted substantial tumor regression. The authors of this study propose that Tie-2-expressing monocytes/macrophages may account for most of the proangiogenic activity of myeloid cells that are

Figure 2. The roles of different subpopulations of TAMs in tumor progression. 1, invasion: TAMs secrete a variety of proteases to breakdown the basement membrane around areas of proliferating tumor cells (e.g., ductal carcinoma in situ in the breast), thereby prompting their escape into the surrounding stroma where they show deregulated growth. 2, angiogenesis: In areas of transient (avascular) and chronic (perinecrotic) tumor hypoxia, macrophages cooperate with tumor cells to induce a vascular supply for the area by up-regulating a number of angiogenic growth factors and enzymes. These diffuse away from the hypoxic area and, together with other proangiogenic stimuli in the tumor microenvironment, stimulate endothelial cells in neighboring, vascularized areas to migrate, proliferate, and differentiate into new vessels. 3, immunosuppression: Macrophages in hypoxic areas secrete factors that suppress the antitumor functions of immune effectors within the tumor. 4, metastasis: A subpopulation of TAMs associated with tumor vessels secrete factors like EGF to guide tumor cells in the stroma toward blood vessels where they then escape into the circulation. In the stromal compartment (both the acellular regions and others where they are in close contact with tumor cells), TAMs secrete growth factors to stimulate tumor cell division and/or undefined factors that promote tumor cell motility.
Macrophages in Different Tumor Microenvironments

present in tumors. In support of this proposal, we have noted that a subset of CD14+ human peripheral blood monocytes also expresses Tie-2.

Hypoxic areas of tumors produced by inadequate vascular perfusion are likely to generate additional adverse conditions in the tumor microenvironment, such as low pH and elevated levels of lactate, which might stimulate proangiogenic gene expression in TAMs (48, 49). Moreover, as macrophages are likely to experience a combination (if not all) of these cellular stresses simultaneously in ischemic areas of tumors, an investigation of their combined effect on macrophage function is clearly warranted.

An interesting recent paper by Ohno et al. (36) confirmed that high numbers of TAMs located to areas of chronic hypoxia (i.e., necrosis) in endometrial carcinomas were associated with reduced relapse-free survival and increased myometrial invasion, as might be expected, but also that the close proximity of TAMs to tumor cell nests in the same tumors correlated with improved survival. Furthermore, TAMs in the stromal compartment of these tumors correlated positively with lymph node metastasis. Together, these observations suggest that TAMs respond to different signals in different areas of a tumor, promoting tumor progression in hypoxic/necrotic areas (although angiogenesis was not examined per se in this study), facilitating metastasis in stromal areas, and reducing tumor cell activity in areas where TAMs make close contact with tumor cells. The identity of the signals present in these three tumor zones regulating these different TAM functions have yet to be determined. However, in model systems, macrophage responses can be influenced through cell-to-cell contact by the type of CSF-1 expressed. For example, in xenograft models of malignant glioma, mice died rapidly if they were engrafted with cells transfected with the secreted form of CSF-1 but survived if the cells expressed the cell surface-bound form of CSF-1. The survival of the mice was associated with macrophage binding to the glioma cells followed by subsequent killing (50, 51).

Together, the data strongly suggest that TAMs are important modulators of angiogenesis, being involved both in the formation of new vessels and in their remodeling into a coherent functional network. They migrate into hypoxic/necrotic areas of the tumor where vascularization is urgently needed for tumor cell survival, and are then activated by local signals, like hypoxia, to synthesize angiogenic regulators. This contributes to the formation of new vessels, facilitating local tumor growth and survival (Fig. 2).

Metastasis. Mounting lines of evidence also implicate TAMs in the regulation of metastasis (Fig. 2). High numbers of TAMs in primary tumors have been correlated with early establishment of metastases in a number of tumor types (30, 34). TAMs seem to play roles in both the release of metastatic cells from the primary tumor as well as the establishment of secondary tumors at distant sites.

As early as 1982, Gorelik et al. (52) showed that inoculation of thioglycollate-elicited peritoneal macrophages reduced the rate of lung clearance of several types of murine tumor cells injected i.v. and increased the formation of metastatic lung nodules by i.v. injected B16 melanoma or Lewis lung carcinoma cells. Furthermore, in PyMT-induced murine mammary tumors as well as in xenografts of rat breast cancer cells, intravital imaging has defined at least two ways that macrophages affect metastasis. First, the motility of tumor cells away from the main body of the tumor almost always occurs in juxtaposition to macrophages (which seem to attract tumor cells). Second, extravasation of tumor cells into blood vessels often occurs at clusters of macrophages found attached to the abluminal side of the vessels. Experiments conducted in vitro and in vivo indicate that each of these cell types exhibit coordinated movement (14, 53). This movement critically requires CSF-1 and EGF signaling in the macrophages and tumor cells respectively, with the ligands being produced by the reciprocal cell type. Inhibition of either pathway blocks cell movement of both cell types. Furthermore, the number of tumor cells entering the bloodstream is dramatically reduced with reduction of macrophage number along the vessels or inhibition of EGF signaling. These observations accord with those of Ohno et al. (36) whose findings show a correspondence between the number of macrophages in the tumor stromal compartment and the metastatic potential of the tumor. The subpopulation of TAM seen in close proximity to some blood vessels in both murine mammary tumors and human breast carcinomas indicate that they may play a similar role in these tissues (Fig. 1, C and D). However, although macrophages isolated from human breast tumors express abundant EGF (15), this has yet to be shown in perivascular TAMs. Thus, it remains to be formally established whether this subpopulation of TAMs is essential for metastasis. Interestingly, TAMs have also been shown to express the lymphatic endothelial growth factor, VEGF-C, suggesting that they may also promote dissemination of tumor cells by stimulating the formation of lymphatic vessels in tumors (54).

In mouse models of PyMT-induced mammary cancer, systemic depletion of macrophages results in reduced formation of lung metastases (4), suggesting that systemic macrophages or TAMs are important in the establishment of metastatic tumors. The ability of tumor cells to colonize and grow in the lungs is dependent on VEGF-induced expression of MMP-9 by alveolar macrophages (and endothelial cells; ref. 55). Also, Oosterling et al. (56) also showed a link between macrophages in metastatic sites and the growth of metastatic tumors. These investigators selectively depleted macrophages in the peritoneal cavity or liver and showed that CC531 colon tumor cells injected into either the peritoneum or liver portal vein formed tumors more slowly than in control mice. Together, these findings suggest that macrophages at metastatic sites support tumor growth, and accords well with clinical studies showing that increased numbers of macrophages in regional lymph node metastases correlates with poor patient survival (57).

Immunosuppression. Unlike macrophages from healthy tissues, which are capable of presenting tumor-associated antigens, lysing tumor cells, and stimulating the antitumor functions of T cells and NK cells, TAMs in the tumor microenvironment lack these activities, leaving the host without the ability to mount an effective antitumor immune response. A number of studies have shown that tumor-derived molecules, like cytokines, growth factors, chemotactic molecules, and proteases, influence TAM functions (8, 25, 58). For example, tumor cells secrete IL-4, IL-6, IL-10, MDF, TGF-β1, and PGE2, which inhibit the cytotoxic activity of TAMs (8, 58). Moreover, TGF-β1, IL-10, and PGE2 may suppress

5 C. Murdoch and C.E. Lewis, unpublished observations.

6 J. Wyckoff, Y. Wang, E.Y. Lin et al., unpublished results.
the expression of MHC class II molecules by macrophages in the tumor microenvironment as well as distant sites like the spleen and peritoneum. This effect may limit the ability of TAMs to present tumor-associated antigens to T cells effectively in these areas (8). Thus, tumors undergo immunoediting to down-regulate macrophage functions that are potentially dangerous to the tumor even in circumstances where recognizable tumor antigens are presented. It should be noted that the tumoricidal activity of TAMs may vary markedly in different tumor microenvironments. For example, in advanced gastric carcinoma, the number of TAMs in close proximity to tumor cells in tumor cell nests correlates positively with tumor cell apoptosis, suggesting that TAMs or TAM effector cells are actively involved in tumor cell destruction. Notably, tumors that have large numbers of “nest TAM” are associated with a significant increase in 10-year disease-free survival (39).

Another important aspect of TAM involvement in antitumor immune mechanisms is the ability of these cells to release immunostimulatory cytokines. For example, macrophage expression of IL-12, a cytokine known to stimulate both the proliferation and cytotoxicity of T cells and NK cells (60), is markedly suppressed in tumors, possibly by exposure to IL-10, PGE₂, and TGF-β (8, 61). By injecting mice with human prostate tumor cells and macrophages engineered to overexpress IL-12, Satoh et al. (62) showed that it was possible to quickly reestablish enhanced antitumor activity of any immune cells that are formed (8, 63).

Hypoxia in the tumor microenvironment is likely to contribute to suppressing the antitumor activity of TAMs as it stimulates the release of the potent immunosuppressive factors PGE₂ and IL-10. These factors impair the development of immune cells by acting on the early stages of their development from primitive pluripotent stem cells (i.e., immunopoiesis), inhibiting the antitumor activity of any immune cells that are formed (8, 63). Additionally, they act on TAMs to reduce their cytotoxicity activity toward tumor cells (8, 63, 64). This may involve reduced oxygen radical formation due to decreased “substrate” availability. Hypoxia also inhibits the ability of macrophages to phagocytose dead or dying cells and present antigens to T cells. One mechanism by which this may be achieved is by reduction in the surface expression of CD80, a costimulatory molecule needed for the full activation of T-cell responses to antigenic peptides. In contrast, hypoxia can also enhance the direct cytotoxicity of macrophages by up-regulating release of TNF-α. As mentioned above, TAMs also up-regulate the expression of MMP-7 protein in hypoxic areas of tumors (44). MMP-7 is known to cleave the Fas ligand from neighboring cells, making tumor cells not only less responsive to chemotherapeutic agents, such as doxorubicin (65), but also lysis by NK and T cells (66).

Conclusion

To summarize, analysis of the expression of protumor molecules by TAMs in human tumors and functional studies in macrophage-depleted murine tumor models have shown TAMs to play an important part in various key steps in tumor progression (Fig. 2). It seems that in early preinvasive lesions, tumor cells release chemokines to attract macrophages (and other inflammatory cells) into stromal areas surrounding the tumor. Macrophages are also found in dense clusters as part of leukocytic infiltrates at areas of focal breakdown of the basement membrane. Tumor cells are thus able to escape the confines of the basement membranes and migrate across into the stroma of surrounding healthy tissues, where they, together with macrophages, stimulate angiogenesis. These newly formed vessels sprout into the early tumor, providing nutrients and oxygen for tumor growth and supplying multiple exit routes for metastasizing cells. The latter may involve a subpopulation of TAMs aligned with the outer surface of tumor-associated blood vessels and cells lying in other regions of the stromal compartment. These vessel-associated TAMs provide chemotactic signals for tumor cells that promote migration and intravasation into the blood and lymphatic vasculature. A separate subpopulation of TAMs also gather at sites distant from the vasculature, where the limited diffusion of oxygen (and possibly nutrients like glucose) activates a quite different TAM phenotype that expresses a broad array of proangiogenic factors. This ensures the revascularization of each hypoxic tumor area and protects tumor cells in these from hypoxia-induced cell death. Growth factors secreted by TAMs in these (and possibly other) areas of tumors then directly promote the proliferation of tumor cells. The consequence of these combined TAM-driven processes is progression of the tumor toward a more highly angiogenic, malignant phenotype.

Thus, it seems that at least four different microenvironments—sites of initial tumor cell invasion, perivascular sites, stromal regions, and hypoxic/necrotic areas—may “educate” macrophages to carry out specific functions in support of tumor cell requirements and activities in those different areas (67). These studies reflect the pitfalls associated with the interpretation of early TAM studies in which these cells were isolated from whole animal or human tumors and characterized as one phenotype in functional in vitro assays.

However, apart from hypoxia, little is known of the micro-environmental signals that regulate the activities of subpopulations of TAMs in these different tumor sites. In vitro studies have shown that CSF-1 synthesized locally by tumors stimulates the expression of EGF by macrophages, causing a two-cell paracrine loop that promotes tumor cell motility (53). It is hoped that recent advances in the use of laser microdissection to “capture” cells from specific areas of tissues sections (68) will now allow the isolation of TAMs from such areas (and more specifically, the RNA or proteins expressed by these cells), so that their phenotype can be more fully characterized using DNA and/or protein microarrays. It is also not known whether different TAM functions in such tumor sites reflect different final differentiation states of these cells, or whether they are entirely plastic and/or reversible, so that TAMs can express one or more phenotypes in a temporal sequence, dependent only on the presence or absence of local signals.

If such studies generate information on macrophage-specific surface receptors uniquely expressed by TAMs in the most “aggressive” areas of tumors (i.e., the hypoxic, stromal and/or perivascular areas, where TAMs drive angiogenesis and metastasis, respectively), then it might prove possible to target cytotoxic therapies to TAMs in these specific areas, leaving other antitumor subpopulations of TAMs and macrophages in other tissues relatively unaffected. In addition, TAMs, through their phagocytic capacity, might engulf liposomes carrying cytotoxic drugs and carry them into the tumor, where, upon their death, these drugs would be released.

Because TAMs migrate into and accumulate in hypoxic areas, it has been postulated that these cells might be used as vectors to
carry genes or drugs activated by hypoxia into these sites. These drugs or gene therapy vectors applied systemically fail to penetrate more than a few hundred micrometers from their points of entry via blood vessels, such that tumor cells in avascular tumor areas go largely untreated in most forms of therapy. Some limited data has been provided recently to suggest that such a macrophage-based system might work. Small breast tumor spheroids have been infiltrated with macrophages infected with adenovirus containing a hypoxia-driven prodrug-activating enzyme (cytochrome P450). When infiltrated, spheroids were then exposed to the corresponding prodrug, cyclophosphamide, the P450 enzyme expressed by hypoxic macrophages within spheroids converted the prodrug into its active, cytoxic metabolite (4-hydroxy-cyclophosphamide). This cell membrane-permeable cytotoxin then diffused from infected macrophages, intercalated into the DNA of surrounding tumor cells, causing cell death during their subsequent mitosis. Because macrophages are terminally differentiated and thus nondividing cells, they are refractory to the cytotoxic effects of the active metabolite. As a result, spheroids infiltrated with P450-engineered macrophages displayed a significant reduction in volume and gross morphologic damage compared with mock-engineered controls (69). These results are encouraging but in vivo studies to test the efficacy of this new approach for targeted gene delivery are now required.

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