The inflammatory micro-environment in tumor progression:
The role of tumor-associated macrophages

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Abstract

The link between inflammation and cancer proposed more than a century ago by Rudolf Virchow, who noticed the infiltration of leukocytes in malignant tissues, has recently found a number of genetic and molecular confirmations. Experimental, clinical and epidemiological studies have revealed that chronic inflammation contributes to cancer progression and even predisposes to different types of cancer. Cancer-associated inflammation includes: the presence of leukocyte infiltration; the expression of cytokines such as tumor necrosis factor (TNF) or interleukin (IL)-1; chemokines such as CCL2 and CXCL8; active tissue remodelling and neo-angiogenesis.

Tumor-associated macrophages (TAM) are key regulators of the link between inflammation and cancer. Many observations indicate that, in the tumor micro-environment, TAM have several protumoral functions, including expression of growth factors, matrix proteases, promotion of angiogenesis and suppression of adaptive immunity. In this review we will discuss the role of TAM in the inflammatory micro-environment of solid tumors and will try to identify potential target for future therapeutic approaches.

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1. Origin of TAM

Solid tumors are constituted by variable amounts of neoplastic and stromal cells, the latter comprising fibroblasts, blood/lymphatic vessels and immune-competent cells,
mainly macrophages and lymphocytes. A wide array of biologically active molecules are available in this milieu, either in soluble form or associated to proteins of the extra-cellular matrix. These include, for instance, growth factors for tumor cells and for the newly formed blood vessels, chemoattractants for immune cells recruited into the tumor mass, and a vast number of proteolytic enzymes which actively remodel the surrounding matrix.

Macrophages are usually the most abundant immune population present in the tumor micro-environment [1–3]. Although earlier studies demonstrated that optimally stimulated macrophages can kill in vitro tumor cells, it is now generally accepted that TAM have mostly (though not always) pro-tumoral functions [3–6]. TAM derive from monocyctic precursors circulating in the blood [7]. Previous studies demonstrated the pivotal role of a tumor-derived chemoattractant – later identified as CCL2 – in their recruitment [8,9]. Following this initial observation other chemokines active on TAM (e.g. CCL5 and CXCL1) were detected in the neoplastic tissues as products of tumor or stromal cells [10–13]. The important role of CCL2 in macrophage accumulation at the tumor site is supported by the evidence that levels of tumor-derived CCL2 correlates with the abundance of TAM in several types of adenocarcinoma, including ovarian, breast and pancreas [14–17]. Interestingly, CCL2 production has been detected also in TAM, indicating the existence of an amplification loop [12]. The attraction of blood monocytes within tumors is not only operated by chemokines. Molecules such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor (TGFβ) and macrophage colony stimulating factor (M-CSF) are chemotactic for monocytes/macrophages and also promote macrophage survival and differentiation (primarily M-CSF). In addition, macrophages and tumor cells produce matrix proteases which are able to degrade the extra-cellular matrix (ECM); cleavage of ECM proteins liberate bioactive degradation products, including chemoattractants such as fragments of fibronectin and fibrinogen, in addition to other growth and angiogenic factors [2].

### 2. Plasticity of human macrophages: M1 and M2 polarization

Monocytes recruited from the circulation differentiate into mature macrophages within the tumor micro-environment. The capability to express distinct functional programmes in response to different micro-environmental signals is a biological feature of macrophages, which is typically manifested in pathological conditions such as infections and cancer [18–22]. In response to cytokines and microbial products, mononuclear phagocytes express specialized functional properties. Mirroring the Th1/Th2 nomenclature, many refer to polarized macrophages as M1 and M2 cells. Classically activated M1 macrophages are induced by IFN-γ alone or in concert with microbial stimuli (i.e. LPS) or cytokines (i.e. TNF and GM-CSF). On the other hand, IL-4 and IL-13 induce alternatively activated M2 macrophage. M2 is a generic name covering different forms of macrophage activation other than M1, including cells exposed to IL-4, IL-13, immune complexes, IL-10, glucocorticoids [20,21]. However, this tight distinction between M1 and M2 macrophages does not fully represent the continuum of functional states that macrophages can express and is rather a simplified view of these two extremes of polarization.

M1 and M2 polarized macrophages display a number of distinct features (Table 1). Classical or M1 macrophages are characterized by high capacity to present antigens, high IL-12 and IL-23 production [23] and consequent activation of polarized type I T cell responses. They have cytotoxic ability toward tumor cells as well as toward cells that have ingested intracellular micro-organisms, by releasing high levels of toxic intermediates: nitric oxide (NO), reactive oxygen intermediates (ROI) and TNF [21,22]. When exposed to the classical activation signals IFN-γ and LPS macrophages preferentially express opsonic receptors, e.g. FcγRIII (CD16); moreover they have immune stimulatory functions by producing copious amounts of pro-inflammatory cytokines and eliciting the adaptive immune response. Based on this, M1 macrophages are generally considered as potent effector cells which defend

<table>
<thead>
<tr>
<th>Polarizing stimuli</th>
<th>IFNγ, LPS, GM-CSF, TNF</th>
<th>IL-4 and IL-13 (M2a); IC and LPS or IL-1 (M2b); IL-10, glucocorticoids (M2c)</th>
</tr>
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<tbody>
<tr>
<td>Main functions</td>
<td>TH1 activation, DTH; killing of intra-cellular pathogens; immunostimulation, host defense, tissue destruction</td>
<td>TH1 suppression, TH2 activation (M2a, M2b); killing and encapsulation of parasites (M2a); immunosuppression (especially M2b, M2c); wound healing, tissue remodeling</td>
</tr>
<tr>
<td>Cytokines produced</td>
<td>High IL-12, IL-23, low IL-10; high IL-1, TNF, IL-6; high signaling IL-1RI</td>
<td>High IL-10, low IL-12, TGFβ (M2c); low IL-1, TNF, IL-6 (not for M2b); high decoy IL-1RI. IL-1R-antagonist</td>
</tr>
<tr>
<td>Toxic intermediates</td>
<td>High RNI and ROI</td>
<td>Low RNI and ROI</td>
</tr>
<tr>
<td>Tumor resistance</td>
<td>High</td>
<td>Poor</td>
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IC: immune complex; DTH: delayed type hypersensitivity; RNI: reactive nitrogen intermediates; ROI: reactive oxygen intermediates.
the body against the attack of pathogens and tumor cells.

To the opposite extreme, M2 macrophages have poor antigen presenting capacity, have an IL-12\textsuperscript{low}, IL-10\textsuperscript{high} phenotype, suppress the inflammatory responses and Th1 adaptive immunity, actively scavenge debris, promote wound healing, angiogenesis and tissue remodelling [21,22]. M2 cells have also defensive functions in polarized Th2 reactions, in that they promote killing and encapsulation of parasites [24,25]. Various environmental signals elicit distinct M2 activation forms which commonly share selected functional properties (e.g. low IL-12, high IL-10). Integration and promotion of type II responses prevail for IL-4- or IL-13-stimulated M2a macrophages and for M2b cells exposed to immune complexes (IC) and agonists of Toll-like receptors (TLRs) or IL-1R. Suppression and regulation of inflammation/immunity are predominant in IL-10-stimulated M2c cells [21]. In line with their tissue repair function, M2 cells display high levels of scavenger, mannose and galactose-type receptors [21].

The arginine metabolism gives rise to high levels of inducible nitric oxide synthase (iNOS; NOS2) in the M1 population [19]. In contrast, M2 macrophages express a predominant activation of the arginase pathway and the consequent production of ornithine and polyamines. This metabolic switch occurs preferentially during the activation of the M2a and M2c polarization programmes [20].

The LPS receptor TLR4 and the adapter molecule MyD88 are increased by IFN-γ, while IL-10 inhibits their expression. In analogy, the IL-1 system appears to be differentially regulated by M1 and M2 signals. IFN-γ and LPS foster the IL-1-mediated functions by inhibiting the decoy receptor IL-1RII and by up-regulating the signaling IL-1RI and the IL-1R accessory protein in M1 macrophages [26]. In contrast, in M2 macrophages, IL-4, IL-13 and glucocorticoid hormones attenuate the IL-1 system by inducing expression of the decoy receptor IL-1RII. Moreover, IL-4 and IL-13 induce the IL-1 receptor antagonist (IL-1ra) and inhibit IL-1. While M1 macrophages express high level of pro-inflammatory cytokines (IL-1, TNF, IL-6 and IL-23), M2 cells are generally characterized by their low production and by anti-inflammatory properties. This is reflected in their typical profile: IL-12\textsuperscript{low}, IL-10\textsuperscript{high}. However, macrophages exposed to IC and LPS (M2b) are an exception, in that they retain high levels of inflammatory cytokines with concomitant IL-10\textsuperscript{high} and low IL-12\textsuperscript{low} [20]. In spite of their high production of inflammatory cytokines and toxic molecules, M2b cells protect mice against LPS toxicity [27], promote Th2 differentiation and humoral antibody production.

3. TAM as M2 polarized macrophages

Micro-environmental signals expressed at the tumor micro-environment play a central role in the orientation and differentiation of recruited mononuclear phagocytes, thus contributing to hijack the local immune system away from anti-tumor functions. To the extent that they have been investigated, differentiated mature TAM have phenotype and functions more similar to M2 macrophages [28]. Indeed, under many aspects TAM summarize a number of functions expressed by M2 macrophages: tuning of inflammatory responses and adaptive immunity, tissue remodelling and repair, promotion of angiogenesis. Nevertheless, we have reported that TAM isolated from a murine fibrosarcoma also expressed IFN-inducible chemokines: CXCL9 and CXCL10, via an alternative IRF-3/STAT1 activation pathway [6].

Many are the factors expressed in the tumor microenvironment that have the potential to promote the differentiation and polarization of recruited monocytes into M2 macrophages. These include the growth and differentiation factor M-CSF and PGE2, TGFβ, IL-6 and IL-10.

The immunosuppressive cytokines IL-10 and TGFβ are produced by many types of cancer cells and by TAM themselves [28]. IL-10 promotes the differentiation of monocytes to mature macrophages and blocks their differentiation to DC [29,30]. Thus, a gradient of tumor-derived IL-10 may account for differentiation along the macrophage pathway in different micro-anatomical localizations of a tumor. Such situation was observed in breast cancer and in papillary carcinoma of the thyroid, where TAM were evenly distributed throughout the tissue, in contrast to DC which were present only in the periphery [31,32]. As mentioned in Section 2, IL-10 promotes the M2c alternative pathway of macrophage activation and induce TAM to express M2-related functions.

Activation of NF-κB promotes the transcription of several pro-inflammatory genes. Our previous studies indicated that murine TAM display defective NF-κB activation in response to the M1 polarizing signal LPS [33]. This explain why TAM are poor producers of inflammatory cytokines (e.g. IL-12, IL-1β, TNFα, IL-6) [28]. TAM produce also low levels of NO [34]; in situ, in ovarian cancer, only a minority of macrophages localized at the periphery scored positive for iNOS [35]. Moreover, in contrast to M1 polarized macrophages, TAM have been shown to be poor producers of reactive oxygen intermediates (ROIs) and are generally non cytotoxic toward tumor cells [5,28]. An additional characteristic of TAM as M2-polarized macrophages is their inefficiency to present antigens and trigger adaptive immune responses; low or absent IL-12 production, low levels of MHC molecule expression and high levels of IL-10, concur with their poor APC activity [6].

4. TAM as promoter of tumor progression

Earlier in vitro studies with IFNγ-stimulated macrophages or TAM had indicated that under certain conditions these cells display cytotoxic functions against tumor cells [7,28]. However, it was already clear that in the absence of M1-orienting signals TAM rather promoted tumor cell growth in vitro, as well as in experimental murine models [5,25,28,36,37].
Since then, in many – but not all human tumors – a high frequency of infiltrating TAM has been associated with poor prognosis [3–5,12,37–39]; accordingly, genes associated to macrophage infiltration (e.g. CD68) or differentiation (M-CSF) are part of molecular signatures which herald poor prognosis in lymphoma, breast and liver cancer [40–42]. Of note, SHIP1-deficient mice, which exhibit a spontaneous drift towards M2 polarization, experience increased growth of transplanted tumors [43].

Many macrophage products released in the tumor stroma can directly stimulate the growth of tumor cells and/or promote tumor cell migration and metastasis. These include, for instance, the epidermal growth factor (EGF), cytokines like IL-6 and TNF, as well as chemokines such as CXCL12 [5,6,12,28]. TAM contribute to tumor progression also by producing several factors which enhance neo-angiogenesis and the dissolution and remodeling of the interstitial matrix. Moreover TAM are a source of potent immunosuppressive molecules, such as IL-10 and PGE2, contributing to the tumor immune-evasion (Table 2) [3,5,25,28].

4.1. Angiogenesis

Angiogenesis is an M2-associated function which represents a key event in tumor growth and progression. TAM have been reported to promote angiogenesis with the production of diverse pro-angiogenic factors: TGFβ, VEGF, PDGF, members of the FGF family and angiogenic chemokines [28,44,45]. Indeed it was observed that the density of blood microvessels correlates with the extent of macrophage infiltration in breast cancer and in several other human tumors [44]. In lung cancer, TAM may favour tumor progression by contributing to stroma formation and angiogenesis through the release of PDGF, in conjunction with TGFβ production by cancer cells [28]. In human cervical cancer, VEGF-C production by TAM was proposed to play a role in peritumoral lympho-angiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis [46]. TAM also participate to the pro-angiogenic process by producing the angiogenic factor thymidine phosphorylase, which promotes endothelial cell migration in vitro and whose levels of expression are associated with tumor neo-vascularization [47]. Additionally, TAM have pro-coagulant activity through fibrin deposition, which indirectly enhances blood vessel formation [6].

TAM contribute to angiogenesis also by producing several chemokines. Chemokines have a major impact on the regulation of the angiogenic switch in tumor tissues [12]. The NH2-terminus of several CXC chemokines contains a highly conserved amino acid motif (Glu-Leu-Arg: ELR motif), which immediately preceeds the first cysteine [48–50]. In general, ELR+ chemokines have potent angiogenic activity, while another series of CXC chemokines lacking the ELR motif (non-ELR) are characterized by the ability to block or inhibit angiogenesis. The angiogenic members include CXCL1 through CXCL8, with the exception of CXCL4. These chemokines act through a common receptor, CXCR2. Although some ELR+ chemokines bind also to the receptor CXCR1, it is widely accepted that only CXCR2 mediates the angiogenic activity and, accordingly, endothelial cells express only CXCR2 [51]. Another important ligand-receptor pair is CXCL12 and CXCR4. Even if CXCL12 is a non-ELR chemokine, its activity has been implicated in neo-angiogenesis [52,53] and, as mentioned below, both factors are up-regulated under hypoxic conditions. The importance of ELR+ chemokines in supporting angiogenesis during the neoplastic progression has been established in a variety of tumor cell types [12,49,50]. Both in mouse tumor models and in surgical specimens obtained from tumor patients, expression of CXCL5 and CXCL8 was associated with increased neo-vascularization and inversely correlated with survival. Conversely, depletion of CXCL5 resulted in attenuation of tumor growth and angiogenesis. The finding of the unique use of CXCR2 receptor, despite the redundancy of ELR+ chemokines, provides a good opportunity to target this receptor for therapeutic interventions.

Uneven vascularization and hypoxia are characteristics of neoplastic tissues and have been associated with decreased therapeutic responses, malignant progression, local invasion and distant metastasis [54]. TAM accumulate preferentially in the poorly vascularized regions of tumors which are characterized by low oxygen tension [55]. Hypoxia triggers a pro-angiogenic program in both tumor cells and macrophages. The transcription factor hypoxia-inducible factor-1 (HIF-1) is a major regulator of cell adaptation to hypoxic stress and therefore a potential target of anticancer therapies [54]. HIF-1 mediates the switch from aerobic to anaerobic metabolism thus conferring a glicolytic phenotype to cancer cells and ensuring their energy requirements, thereby allowing their survival in a hostile environment.

In TAM also, adaptation to hypoxia is achieved by the increased expression of HIF-1 and HIF-2 inducible genes, for instance VEGF, betaFGF, CXCL8, as well as glicolytic enzymes [55,56]. The in vivo relevance of this metabolic adaptation to hypoxia by macrophages was demonstrated by Cramer et al. [57]. Ablation of the hypoxia responsive transcription factor HIF-1α resulted in impaired macrophage

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<td>Pro-tumoral functions of TAM</td>
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<tr>
<td>• Production of growth and survival factors for tumor cells e.g. EGF, IL-6, CXCL5</td>
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<tr>
<td>• Production of angiogenic factors e.g. EGF, FGF, VEGF, PDGF, TGFβ, ELR+ chemokines</td>
</tr>
<tr>
<td>• Degradation of extra-cellular matrix and tissue remodelling activity e.g. Expression and release of MMPs, uPA, uPAR, deposition of fibrin and collagen</td>
</tr>
<tr>
<td>• Suppression of adaptive immune responses</td>
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<tr>
<td>Production of immuno-suppressive mediators, e.g. IL-10, PGE2, TGFβ</td>
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<tr>
<td>Low or absent immuno-stimulatory cytokines, e.g. IL-12</td>
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<tr>
<td>Release of chemokines CCL17, CCL18 and CCL22 recruiting T cell subsets devoid of cytotoxic ability (Th2, T naïve and Treg)</td>
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motility and cytotoxicity, in low oxygen conditions. This evidence highlights the relevance that the hypoxia-HIF-1 pathway may play in the recruitment and activation of TAM into solid tumors and may be instrumental for TAM-mediated angiogenesis and tumor metastasis. In support of this, we have described that hypoxia can influence the positioning and function of cancer and stromal cells, including TAM, by selectively up-regulating the expression of the chemokine receptor CXCR4 [58]. Moreover, HIF-1 activation may play a role in the induction of the CXCR4 ligand, CXCL12 [59], a chemokine involved in cancer cell migration [12,60]. Therefore, macrophages recruited in situ represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumor cells. Inhibition of HIF-1α is considered a promising therapeutic approach against cancer and in fact some of its inhibitors (e.g. farnesyl transferase inhibitors, PI3K inhibitors) are now in clinical trials as anti-tumor drugs [54].

4.2. Matrix remodeling

It has long been known that tumor-derived proteases can cleave the extra-cellular matrix proteins and lead to the dissolution of the basement membrane, thus facilitating the process of tumor cell invasion. In the tumor stroma, macrophages can produce enzymes which regulate matrix digestion, such as MMPs, plasmin, urokinase-type plasminogen activator (uPA) and the uPA receptor [61]. The activity of these enzymes has been associated with more aggressive neoplastic behaviour. For example, t-PA and u-PA and their respective receptors, annexin II and u-PAR, were demonstrated to contribute to the invasive behaviour of pancreatic cancer [62]. MMP-2 expression is increased in several tumors and strongly correlates with nodal status and tumor stage [63]. MMPs have complex effects beyond matrix degradation. Digestion of the tumor extracellular matrix releases heparin-bound growth factors and facilitates endothelial cell migration to enhance angiogenesis [64,65]. Direct evidence has been presented that MMP-9 derived from hematopoietic cells of host origin contributes to tumor progression. Human tumor xenografts in MMP9−/− mice, which produce MMP9-defective macrophages, were smaller and contained fewer infiltrating macrophages and vessels, compared with those in wild-type animals [64,65].

Chemokines are potent inducers of proteolytic enzymes and of receptors which degrade the extracellular matrix and favor tumor invasion. In a gene expression analysis of human monocytes, the chemokine CCL5 induced gene expression of various MMPs, especially MMP9, along with the uPA receptor [66].

4.3. TAM as suppressors of anti-tumor immune responses

An additional characteristic of M2 macrophages which is shared by TAM population is the ability to suppress the adaptive immune response through mechanisms including poor antigen-presenting activity and inhibition of T cell proliferation [6,67].

As mentioned above, TAM produce and release several immunosuppressive cytokines, of which IL-10 has been most studied. Moreover they produce low levels of immunestimulatory cytokines such as TNFα, IL-1 and IL-12, mainly due to defective NF-κB activation, at least in TAM of advanced cancer. It has been reported that IL-10, alone or in concert with IL-6 is responsible for the up-regulation of macrophage B7-H4 expression, a molecule implicated in the suppression of tumor-associated antigen-specific T cell immunity [68]. The defective production, in TAM, of the major immunestimulatory cytokine IL-12 may also be due to the activity of IL-10, produced either by TAM and by the tumor cells. At least some of these immunosuppressive activities might be regulated by over-activation of transcription factors such as Stat3. Stat3 inhibition results in enhanced cytotoxicity and antigen-presenting function in activated macrophages and is associated with retardation of tumor growth [69].

Part of the immunosuppressive activity of TAM is exerted indirectly by their release of chemokines that preferentially attract T cell subsets devoid of cytotoxic functions. CCL18 has been identified as the most abundant chemokine in the ascitic fluid of human ovarian carcinoma [70]. When the source of CCL18 was investigated, it was tracked to TAM, with no production by ovarian carcinoma cells. In normal macrophages CCL18 is inducible by Th2 cytokines: IL-4, IL-13 and IL-10, and recruit naive T cells by interacting with an unidentified receptor [71]. Attraction of naive T cells in a peripheral micro-environment dominated by M2 macrophages and immature DC is likely to induce T cell anergy. Two other chemokines, CCL17 and CCL22, are abundantly expressed by TAM [12,28]. These chemokines interact with the CCR4 receptor, expressed mostly by Th2 cells and by Treg [72], two T cell subsets lacking anti-tumor functions.

In addition to TAM, a number of reports have identified a myeloid M2-biased cell population present in tumors and lymphoid organs of tumor-bearing hosts, referred to as myeloid-derived suppressor cells (MDSC), which are suggested to contribute to the immunosuppressive phenotype [73,74]. These cells share properties and gene expression profile with M2 polarized TAM, yet also display distinct features, and are characterized by the expression of the Gr-1 and CD11b markers [75–77]. MDSC use two enzymes involved in the arginine metabolism to control T cell response: inducible nitric oxide synthase (NOS2) and arginase (Arg1), which deplete the milieu of arginine, causing peroxinitrite generation, lack of CD3ζ chain expression by T cells and T cell apoptosis [73,74].

5. Conclusions and therapeutic perspectives

TAM are key orchestrator of the link between smouldering inflammation and cancer progression. In the majority
of experimental and clinical studies the evidence is a pro-
tumoral function of TAM. Thus TAM appear as attractive
candidate of novel therapeutic strategies. Three major aspects
of TAM, potentially amenable of therapeutic interventions are:
(i) inhibition of their recruitment and/or of their survival
at the tumor site; (ii) inhibition of their positive effects on
angiogenesis and tissue remodelling; (iii) reversal of their
immune-suppression and restoration of anti-tumor cytotox-
ity.

Chemokines and chemokine receptors are prime targets
for the development of innovative therapeutic strategies in
the control of inflammatory disorders. Experimental results
have indicated that chemokine inhibitors could affect tumor
growth by reducing macrophage infiltration [78]. In MCP-
1/CCL2 gene targeted mice, this chemokine can indeed
promote progression in a Her2/neu-driven spontaneous mam-
mary carcinoma model [79].

In the tumor milieu, chemokines affect not only leuko-
cytes but also tumor cells. Several types of malignant tumors
have been shown to express selected chemokine receptors
which are involved in their mobilization and invasion [12].
We and other have reported that CXCR4, in addition to
cell movement, increases tumor cell survival and prolifer-
ation [80]. Thus, a complex network of chemokines and
receptors exists in the tumor micro-environment [12]. These
molecules represent a valuable therapeutic target in neoplas-
ia.

M-CSF was identified as an important regulator of
mammary tumor progression to metastasis, by regulating
infiltration and function of TAM. Transgenic expression of
M-CSF in mammary epithelium led to the acceleration of the
late stages of carcinoma and increased lung metastasis, sug-
gesting that agents directed at M-CSF and its receptor could
have important therapeutic effects [81].

Anti-tumor agents with selective cytotoxic activity on
monocyte–macrophages would be ideal therapeutic tools
for their combined action on tumor cells and TAM. We
have reported that Trabectedin, a natural product derived
from the marine organism Ecteinascidia turbinata, with
potent anti-tumor activity [82] is specifically cytotoxic in
vitro to human macrophages and TAM, while sparing the
lymphocyte subset. In addition, at sub-cytotoxic concen-
trations Trabectedin inhibits the production of CCL2 and
IL-6 both by TAM and tumor cells [83]. These anti-
inflammatory properties of Trabectedin may well contribute
to its anti-tumor activity and deserves further investiga-
tion.

Due to the localization of TAM into the hypoxic
regions of tumors, viral vectors were used to transduce
macrophages with therapeutic genes, such as IFNγ, that
were activated only in low oxygen conditions [84–86]. These
works present promising approaches which use macrophages
as vehicles to deliver gene therapy in regions of tumor
hypoxia.

Linomide, an anti-angiogenic agent, caused significant
reduction of the tumor volume, in a murine prostate cancer
model, by inhibiting the stimulatory effects of TAM on tumor
angiogenesis [87]. Based on this, the effects of Linomide,
or other anti-angiogenic drugs, on the expression of pro-
angiogenic molecules by TAM may be considered valuable
targets for anticancer therapy [88].

The biphosphonate zoledronic acid is a prototypical MMP
inhibitor. In an HPV16-induced murine model of cerv-
cical carcinogenesis, this compound suppressed MMP-9
expression by infiltrating macrophages and inhibited metal-
loprotease activity, reducing angiogenesis and tumor growth
[89].

Detective NF-κB activation in TAM correlates with
impaired expression of NF-κB-dependent inflammatory
functions (e.g. expression of cytotoxic mediators, NO)
and cytokines (TNFα, IL-1, IL-12) [28]. Reactivation of
NF-κB function in TAM is therefore a potential strategy
to restore intra-tumor cytotoxicity. Indeed, the combina-
tion of CpG (TLR9 ligand) plus an anti-IL-10 receptor
antibody switched infiltrating macrophages from M2 to
M1 and triggered the innate immune response debulking
large murine tumors within 16 h [90]. TAM from
STA6 /−/− tumor bearing mice display an M1 pheno-
type, with low level of arginase and high level of NO.
As a result, these mice immunologically rejected sponta-
neous mammary carcinoma [91]. Thus, the restoration of
an M1 phenotype in TAM may provide therapeutic ben-
efit by promoting anti-tumor activities. In this regard, the
SHIP1 phosphatase was shown to play a critical role in
programming macrophage M1 versus M2 functions. Mice
deficient for SHIP1 display a skewed development towards
M2 macrophages and produce 10-fold less NO than wild-type
macrophages, because of very high arginase I levels (this
enzyme competes with inducible nitric oxide synthase for
the substrate l-arginine) [43]. Pharmacological modulators
of SHIP1 have been developed and are currently under study
[92].

The IFN-γ-inducible enzyme indoleamine 2,3-
dioxygenase (IDO) is a well-known suppressor of T cell acti-
vation. It catalizes the initial rate-limiting step in tryptophan
catabolism, which leads to the biosynthesis of nicotinamide
adenine dinucleotide. By depleting tryptophan from local
micro-environment, IDO blocks activation of T lymphocytes
[93]. It was reported that the BAR adapter-encoding gene Bin-
1 inhibits IDO expression in cancer cells and macrophages
and that inhibitors of IDO, such as methyl-thiohydantoin-
tryptophan (MTH-trp), cooperate with cytotoxic agents to
elicit regression of established tumors [94].

In conclusion, it seems clear that, at least in the major-
ity of the situations, TAM facilitate tumor progression and
metastatic invasion. An increasing amount of pre-clinical
studies specifically targeting TAM have yielded encouraging
results. Although these results have not yet been success-
fully translated into the clinic, we hope that in the near
future the therapeutic targeting of macrophages will repre-
sent a valuable strategy to complement established anticancer
strategies.
References


Biographies

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