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Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression

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Abstract

Macrophages are versatile, plastic cells which respond to micro-environmental signals with distinct functional programmes. In the tumour microenvironment, tumour-associated macrophages (TAM) polarise towards a type II phenotype, oriented to the promotion of tissue remodelling and repair. As polarised type II macrophages, TAM are a key component of the inflammatory circuits that promote tumour progression.

Keywords: Tumour-associated macrophages; Polarised phagocyte populations; Tumour progression

1. Introduction

It has long been recognised that leucocytes infiltrate neoplastic tissues [29]. Cells belonging to the monocyte-macrophages lineage are a major component of the leucocyte infiltrate of neoplasms. Tumour-associated macrophages (TAM) originate from circulating blood monocytes. Their recruitment and survival *in situ* is directed by chemokines [29] and by cytokines which interact with tyrosine kinase receptors. TAM have complex dual functions in their interaction with neoplastic cells (the ''macrophage balance'' hypothesis [29], but strong evidence suggests that they are part of inflammatory circuits that promote tumour progression [5,29].

It was in 1863 that Rudolf Virchow noted leucocytes in neoplastic tissues and made a connection between inflammation and cancer. He suggested that the ''lymphoreticular infiltrate'' reflected the origin of cancer at sites of chronic inflammation. Over the past ten years, our understanding of the inflammatory microenvironment of malignant tissues has supported Virchow's hypothesis, and the links between cancer and inflammation are starting to have implications for prevention and treatment. Here, we will review evidence consistent with the view that TAM are a polarised type II macrophage population. Polarised type II (or M2 or alternatively activated macrophages) are oriented to angiogenesis, tissue remodelling and repair. We propose that the neoplastic tissue represents a Darwinian microenvironment which selects for macrophage properties that are favourable, for tumour growth and progression.

2. Macrophage plasticity

Macrophages are versatile, plastic cells, which respond to environmental signals with diverse functional programmes. Classic macrophage activation in response to microbial products and interferon-γ (IFN-γ) has long been recognised. More recently, it was realised that anti-inflammatory molecules, such as glucocorticoid hormones, interleukin-4 (IL-4), IL-13 IL-10, are more than simple inhibitors of macrophage activation, in that they induce a distinct activation programme (alternatively activated macrophages) [15,33]. In analogy with the Th1/Th2 dichotomy in T cell responses, macrophages exposed to IFN-γ and IL-4 have also been referred to as Ml and M2. Here, we will refer to these polarised ends

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of the spectrum of macrophage activation as type I and type II cells, or Ml and M2.

Polarised macrophages differ in terms of receptor expression, cytokine and chemokine production and effector function. Arginine metabolism is characterised by high levels of inducible nitric oxide (NO) synthase (iNOS) in type I macrophages, whereas the arginase pathway predominates in the type II polarised macrophages with the generation of ornithine and polyamines.

Differential cytokine production is a key feature of polarised macrophages. The type I phenotype includes IL-12 and tumour necrosis factor (TNF), while type II macrophages typically produce IL-10 and IL-1 receptor antagonist (IL-1ra) and the type II decoy receptor [31]. Components of the chemokine network are differentially modulated in polarised macrophages. Cytokine mediators that polarise macrophages in a type II direction inhibit the production of various chemokines. Production of IL-8/CCL8, RANTES/CCL5 and MIPs as well as of the IFNγ-inducible chemokines IP-10/CXCL10 and MIG/CXCL9 are inhibited by IL-4 and IL-10. On the contrary, IL-4 selectively induces eotaxin-2/CCL24, CCL18 and MDC/CCL22 in macrophages and dentritic cells (DC) and these effects are inhibited by IFNγ. CCL18 is also upregulated by IL-10 and anti-inflammatory drugs. Therefore, differential production of chemokines which attract Th1 (CXCL9, CXCL10) and Th2 or T regulatory cells (CCL22) integrates type I and type II macrophages in circuits of amplification and regulation of polarised T cell responses.

Different inducers of type II polarisation, elicit distinct functional phenotypes in macrophages. Therefore, polarisation of macrophage function should be viewed as an operationally useful, simplified conceptual framework describing a continuum of diverse functional states. With this general caveat, available information suggests that classically activated type I macrophages (M1) are potent effector cells which kill micro-organisms and tumour cells and produce copious amounts of pro-inflammatory cytokines. In contrast, type II macrophages (M2) tune inflammatory responses and adaptive Th1 immunity, scavenge debris, promote angiogenesis, tissue remodelling and repair.

3. Recruitment, survival and differentiation of TAM

TAM derive from circulating monocytic precursors and *in situ* proliferation is generally not an important mechanism that sustains the mononuclear phagocyte population, at least in human tumours [29]. Several lines of evidence, including correlation between production and infiltration in murine and human tumours, passive immunisation and gene modification, indicate that chemokines play a pivotal role in the recruitment of monocytes in neoplastic tissues [41]. Indeed, tumours have been invaluable for the discovery of several members of the chemokine superfamily. Chemokines are usually classified according to their constitutive (e.g. CXCL12) or inducible production (e.g. CCL2, CXCL8). In tumours, inducible chemokine are generally constitutively expressed [30].

CCL2 is probably the most frequently found CC chemokine in tumours, since its description as a tumour-derived chemotactic factor. Human tumours shown to express CCL2 *in vivo* include sarcomas, gliomas, lung tumours, carcinomas of the breast, cervix and ovary and melanomas. A recent careful analysis of the impact of CCL2 on tumour growth in a non-tumourigenic melanoma system revealed a biphasic effect [36]. Low-level CCL2 secretion, with ''physiological'' accumulation of TAM, promoted tumour formation, while high CCL2 secretion resulted in massive macrophage infiltration into the tumour mass and in its destruction [36]. These results in a model of melanoma progression are consistent with the ''macrophage balance'' hypothesis [29] and emphasise the protumour potential of levels of macrophage infiltration similar to those observed in human malignant lesions.

CCL5 is produced by breast carcinomas and melanomas. In breast carcinomas, CCL5 expression by tumour cells correlates with a more advanced stage of disease, suggesting that CCL5 may be involved in breast cancer progression [4]. A variety of other chemokines have been detected in neoplastic tissues as products of tumour cells or stromal elements. These include CXCL12, CXCL8, CXCL1, CXCL13, CCL17 and CCL22. CXCL1 and related molecules (CXCL2, CXCL3, CXCL8 or IL-8) have been shown to play an important role in melanoma progression [17]. They do so by direct stimulation of neoplastic growth, promotion of inflammation and induction of angiogenesis. In spite of constitutive production of CXCL8 and related chemokines by tumour cells, neutrophils are not a major and obvious constituent of the leucocyte infiltrate. However, these cells, although present in minute numbers, may play a key role in triggering and sustaining the inflammatory cascade.

Vascular endothelial growth factor (VEGF) and macrophage colony stimulating factor (M-CSF) are cytokines commonly produced by tumours, which interact with tyrosine kinase receptors and elicit monocyte migration. There is evidence that M-CSF and VEGF can significantly contribute to macrophage recruitment in tumours [14,25]. These molecules also promote macrophage survival and proliferation, the latter generally limited to murine TAM. Studies in M-CSF-deficient mice (*op*/*op*) have provided strong support to the concept of the protumour function of the mononuclear phagocyte system. It was originally reported that M-CSF-deficiency in *op*/*op* mice diminishes macrophage recruitment, stroma formation and tumour growth in

the Lewis lung carcinoma model [37]. In a mammary carcinoma model, M-CSF-deficiency did not affect early stages of tumour development, but reduced progression to invasive carcinoma and metastasis [25]. Genetic restoration of M-CSF production in epithelial cells restored macrophage infiltration, as well as malignant behaviour.

Chemokines in tumours are more than leucocyte attractants. Transcriptional profiling has shown that CC chemokines activate a restricted and distinct programme in human monocytes [26]. The chemokine activated transcriptional profile includes matrix metalloproteases (see below for discussion) and cytochrome CYP1B1, that are involved in carcinogenesis. Moreover, CXC chemokines with an ELR motif stimulate angiogenesis. Tumour cells express receptors for chemokines [34,45,50] and can respond to these mediators with increased proliferation and survival. Finally, chemokine-driven leucocyte recruitment results in digestion of the extracellular matrix which paves the way for the tumour cell to leave the primary lesions (counter-current invasion [39]). Chemokine receptors can then guide localisation of neoplastic elements at distant anatomical sites [34,45,50]. Interestingly, recent evidence has shown that plasminogen production by sarcoma cancer cells acts as a negative regulator of TAM recruitment [12]. Therefore, TAM recruitment is likely to be the net effect of chemotactic and repulsive inhibitory signals within the neoplastic tissues.

It is likely that sustaining macrophage survival in tumours contributes to the levels of infiltration. CSFs, and M-CSF in particular, are likely to promote the macrophage lifespan as well as, in some murine tumours, the proliferation of TAM [14,25,29]. Recently, placenta-derived growth factor (P1GF), a molecule related to VEGF in terms of its structure and receptor usage, has been reported to promote the survival of TAM [1].

Cytokines present in the tumour microenvironment have the potential to promote and orient the differentiation of recruited mononuclear phagocytes. IL-10, as well as TGFβ, are produced by a variety of tumour cells (including ovarian cancers) and by TAM themselves [47]. IL-10 has been shown to promote the differentiation of monocytes to mature macrophages and to block their differentiation to DC [2]. The effect of IL-10 on monocyte differentiation may be an important determinant of the relative proportion of TAM versus tumour-associated DC (TADC) and of their relative distribution. For instance, in papillary carcinoma of the thyroid, TAM are evenly distributed throughout the tissue, in contrast to DC which are present in the periphery [51]. A gradient of tumour-derived IL-10 may account for differentiation along the DC versus the macrophage pathway in different micro-anatomical localisations in a tumour. To the extent that they have been investigated, differentiated mature TAM have a phenotype and function similar to type II macrophages. TAM from poorly immunogenic malignant tumours have little cytotoxicity for tumour cells and they actually promote tumour cell proliferation, in particular under suboptimal culture conditions [29]. In apparent contrast, a recent report has shown that TAM from various cancers appear to be activated by cancer cells to produce the TNF-related apoptosis-inducing ligand (TRAIL) and to induce the expression of the TRAIL death receptors, DR4 and DR5, tumour cells [18]. However, in this study, pleural effusion cells were studied rather then cells from solid neoplasms. Further work is needed to investigate the expression and significance of TRAIL in TAM.

TAM are poor producers of NO [13] and in ovarian cancers only a minority of macrophages localised at the periphery scored positive for iNOS [22]. Moreover, in contrast to Ml polarised macrophages, TAMs have been shown to be poor producers of reactive oxygen intermediates (ROIs), compared with normal macrophages, consistent with the hypothesis that these cells represent a skewed M2 population [22].

Arginase expression in TAM has not been studied. However, it has recently been proposed that the carbohydrate-binding protein, galectin-1, which is abundantly expressed by ovarian cancers and shows specific anti-infiammatory effects, promote the classic pathway of L-arginine resulting in a strong inhibition of the nitric oxide production by lipopolysaccharide-activated macrophages.

T cells infiltrating various types of human tumours have a type II phenotype, with a predominance of CD8+ (e.g. Kaposi's sarcoma) or CD4+ cells (e.g. cervical carcinoma) in different neoplasms [5]. By producing IL-4, IL-13 and IL-10, tumour infiltrating T cells may reinforce the skewing of monocyte differentiation in tumours towards a type II phenotype.

In addition to being a target for chemokines, TAM are a source of a selected set of these mediators (CCL2, CCL22, CCL18). CCL18 was recently identified as the most abundant chemokine in human ovarian ascites fluid [44]. When the source of CCL18 was investigated, it was tracked to TAM, with no production by the ovarian carcinoma cells. CCL18 is a CC chemokine produced constitutively by immature DC and inducible in macrophages by IL-4, IL-13 and IL-10. Since IL-4 and IL-13 are not expressed in substantial amounts in ovarian cancer, it is likely that IL-10, produced by tumour cells and macrophages themselves, accounts for CCL18 production by TAM. CCL18 is an attractant for naive T cells by interacting with an unidentified receptor. Attraction of naive T cells in a peripheral microenvironment dominated by type II macrophages and immature DC may induce anergy.

Reed-Sternberg cells in Hodgkin's lymphoma have been shown to express CCL22 (MDC) and CCL17

(TARC) [49]. These chemokines recognise CCR4 which is preferentially expressed on polarised Th2 cells and on T regulatory cells (Treg), as well as on monocytes [30]. Interestingly, in the same tumour, stromal cells produce CCL11 (eotaxin), which attracts eosinophils and Th2 cells. Therefore, in this human tumour, neoplastic elements and stroma use complementary tools to recruit cells associated with polarised type II responses. In the same vein of driving into tumours polarised Th2 cells, the oncogenic virus human herpesvirus 8 (HHV8), involved in the pathogenesis of Kaposi's sarcoma and haematological malignancies, encodes three CC chemokines (vMIPI, II and III) which interact as agonists with CCR3, CCR4 and CCR8 and, accordingly, preferentially attract polarised type II T cells [5] and, presumably, Treg cells. Consistently with these *in vitro* observations, Kaposi's sarcoma is infiltrated by CD8+ and, to a lesser extent, CD4+ cells with a predominant type II phenotype. Therefore, HHV8 virus-encoded chemokines represent a strategy to subvert effective antiviral/antitumour immunity by favouring type 2 responses and, possibly, Treg cells.

4. Modulation of adaptive immunity by TAM

It has long been known that TAM have poor antigen presenting capacity and can actually suppress T cell activation and proliferation [5]. The suppressive mediators produced by TAM include prostaglandins, IL-10 and TGFβ [47]. Moreover, they do not produce IL-12 spontaneously and they are refractory to stimulation by IFNγ and lipopolysaccharide (LPS). The IL-10^{high} IL-12^{low} phenotype is characteristic of polarised type II macrophages (see above). Antibody blocking experiments suggest that autocrine IL-10 (in part) accounts for the defective IL-12 production [47].

The mechanisms underlying the IL-10^{high} IL-12^{low} phenotype are complex. IL-10 derived from tumour cells and T cells may favour differentiation along this pathway, as discussed above. In a recent eleganl study, Ibe *et al*. [20] have suggested that during tumour establishment T cells condition TAM to produce IL-10 and that inactivation of T cells results in a switch of TAM towards IFNγ production and elicits tumour rejection.

The molecular basis responsible for the unresponsiveness of TAM to stimulation of IL-12 production has been investigated [47]. TAM display a massive and costitutive nuclear localisation of the nuclear factor-κB(NF-κB) inhibitory p50 homodimer (A. Sica, data not shown), which most likely provides a molecular mechanism for other alterations of TAM functions dependent on NF-κB activation, including defective iNOS and defective NO production expression [13,22].

Immature myeloid cells generated as a consequence of CSF production during tumour growth have been

Table 1
Nomenclature of the chemokines/chemokine receptors cited in the test (adapted from IUIS World Health Organisation [21])

Ligand	Alternative designations		
	Human	Mouse	Receptors
CXC family			
CXCL1	GROα/MGSA-α	GRO/MIP-2/KC?	CXCR2 > CXCR1
CXCL2	GROβ/MGSA-β	GRO/MIP-2/KC?	CXCR2
CXCL3	GROγ/MGSA-γ	GRO/MIP-2/KC?	CXCR2
CXCL8	IL-8	Unknown	CXCR1, CXCR2
CXCL9	Mig	Mig	CXCR3
CXCL10	IP-10	IP-10/CRG-2	CXCR3
CXCL12	SDF-1α/β	SDF-1/PBSF	CXCR4
CXCL13	BCA-1	BLC	CXCR5
CC family			
CCL1	1-309	TCA-3/P500	CCR8
CCL2	MCP1/MCAF/TDCF	JE?	CCR2
CCL3L1	LD78β	Unknown	CCR1,CCR5
CCL5	RANTES	RANTES	CCR1,CCR3,CCR5
(CCL6)	Unknown	ClO/MRP-1	Unknown
CCL8	MCP-2	MCP-2?	CCR3,CCR5
CCL11	Eotaxin	Eotaxin	CCR3
CCL17	TARC	TARC/ABCD-2	CCR4
CCL18	DC-CK1/PARC/AMAC-1	Unknown	Unknown
CCL19	MIP-3 β ELC/exodus-33	MIP-3β/ELC/exodus-3	CCR7
CCL22	MDC/STCP-1	ABCD-1	CCR4
CCL24	Eotaxin-2/MPIF-2	MPIF-2	CCR3

Chemokines are a superfamily of small cytokines, most of which are chemotactic for leucocytes. 47 genes for ligands and 18 for signalling receptors have been identified. Chemokine receptors are G protein-coupled seven transmembrane receptors. IL-8, interleukin-8; IUIS, International Union of Immunological Societies; RANTES, regulated upon activation normal T cell expressed and secreted; BCA-1, B cell-attracting chemokine 1; BLC, B lymphocyte chemoattractant.

shown to be potent suppressors of T cell responses. These immature myeloid suppressors have been shown to respond to undefined tumour attractants and to be present in the neoplastic tissue in head and neck squamous cell carcinoma [6,54]. The relationship if any, of immature myeloid suppressor cells with TAM remains to be defined. Table 1 outlines the novel chemokine nomenclature according to the International Union of Immunological Societies [21].

5. Tissue repair and angiogenesis

Phagocytes play a central role in tissue remodelling and repair during oncogenesis and adult life. This ancestral function of mononuclear phagocytes is expressed by TAM which orchestrate the function of other components of the tumour stroma. TAM produce a host of growth factors which affect tumour cell proliferation, angiogenesis, and the deposition and dissolution of connective tissues. These include epidermal growth factor (EGF), members of the fibroblast growth factor (FGF) family, TGFβ, VEGF chemokines. In lung cancer, TAM may favour tumour progression by contributing to stroma formation and angiogenesis through their release of platelet-derived endothelial cell growth factor (PDGF) in conjunction with TGF-β1 production by cancer cells.

Macrophages can produce enzymes and inhibitors which regulate the digestion of the extracellular matrix, such as MMPs, plasmin, urokinase-type plasminogen activator (uPA) and the uPA receptor. Direct evidence have been presented that MMP9 derived from haematopoietic cells of host origin contributes to skin carcinogenesis [8]. Chemokines have been shown to induce gene expression of various MMPs and, in particular, MMP9 production, along with the uPA receptor [26]. Induction of these molecules is part of a programme of activation which arms monocytes with tools (receptors and enzymes) important for migration in tissues. Therefore, expression of enzymes and receptors which degrade the extracellular matrix could, at least in part,be induced and sustained by chemokines. Evidence suggests that MMP9 has complex effects beyond matrix degradation including promotion of the angiogenesis switch and release of growth factors [8]. Of relevance, distant primary tumours may induce pre-metastatic lung endothelial cells and macrophages to express MMP-9 via VEGFR-l/F1t-1 tyrosine kinase (TK), a mechanism which potentiates specifically pulmonary metastasis formation.

Angiogenesis is a key event in tumour growth and progression. Macrophages can exert a dual influence on blood vessel formation and function. On the one hand,macrophages produce molecules that are pro-angiogenic, on the other hand,they can express anti-angiogenic molecules and damage the integrity of blood vessels. On the anti-angiogenic side, in a murine model, CSF-induced, TAM-derived metalloelastase generates angiostatin. In general, as for interaction with neoplastic cells, the pro-angiogenic functions of TAM prevail. In several studies in human cancers, TAM accumulation has been associated with angiogenesis and with the production of angiogenic factors such as VEGF and PDGF [5].

More recently, in human cervical cancer, VEGF-C production by TAMs was proposed to play a role in peritumoural lymphoangiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis [43]. Additionally, TAM participates in the pro-angiogenic process by producing the angiogenic factor, thymidine phosporylase (TP), which promotes endothelial cell migration *in vitro* and whose levels of expression are associated with tumour neovascularistion [19]. Moreover, TAM accumulate in hypoxic regions of tumours and hypoxia triggers a pro-angiogenic programme in these cells (see above). Therefore, macrophages recruited *in situ* represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumour cells.

Formation of a fibrous capsule and parasite encapsulation are late events associated with polarised Th2 responses. Fibrosis is a prominent feature of certain human tumours (e.g. scirrhous carcinomas). CCL2 and IL-13, present in the tumour microenvironment as products of tumour cells, TAM or T cells, induce TGFβ production and fibrosis. Therefore, it is tempting to speculate that polarised type II macrophages are part of the circuits that regulate the function of fibroblasts in the tumour stroma.

6. Hypoxia and TAM

Uneven vascularisation and hypoxia are characteristics of neoplastic tissues which affect macrophage distribution and function. TAM accumulate preferentially in the poorly vascularised region of tumours which are characterised by low oxygen tension. Macrophage migration is suppressed in hypoxic conditions [16] and TAM are immobilised in avascular [27] and necrotic hypoxic areas of tumours [28]. Evidence suggests that in hypoxic conditions, TAM are stimulated to co-operate with tumour cells and promote angiogenesis. Thus, hypoxia represents a stress factor that, along with other micro-environmental parameters, such as low pH, low glucose levels and high lactate levels, affects the biology of TAM. Expression of the Hypoxia-inducible factor-2α (HIF-2α) was observed in TAM from breast carcinomas [48], and HIF-1 is produced by macrophages in hypoxic condition *in vitro*, and in avascular areas of breast carcinomas [11]. Under low oxygen conditions, TAM

switch their metabolism to anaerobic pathways and this adaptation is achieved by the increased expression of hypoxia-inducible genes, such as VEGF, bFGF and CXCL8, whose transcription is controlled by the transcription factor, HIF-1 and which stimulate angiogenesis. The relevance that the hypoxia-dependent pathways play in macrophage functions was recently demonstrated by Cramer *et al.* [10]. By using conditional knockouts of the hypoxia-responsive transcription factor, HIF-1α, these authors. provided *in vivo* demonstrations that in macrophages this factor is essential to generate adenosine triphosphate (ATP) in low-oxygen conditions and to promote functions such as migration and antibacterial activity. This observation highlights the relevance that the hypoxia-HIF-1α pathway may play in the recruitment and activation of TAM into solid tumours.

We have recently identified a new mechanism by which hypoxia can influence the positioning and function of TAM [42]. It was observed that hypoxia selectively upregulates expression of the echemokine receptor, CXCR4, in various cell types, including mononuclear phagocytes. Hence, following initial recruitment by inflammatory chemokines, TAM may accumulate preferentially at hypoxic sites guided by upregulation of CXCR4.

7. Therapeutic intervention

Having established the protumoural activity of TAM, possible strategies that involve these cells should include reducing the number of host macrophages and/or increasing their tumouricidal activity. Reduction of macrophages has indeed been associated with tumour inhibition [25,37]. We and other groups have observed that TAM are committed to high production of the inhibitory cytokine IL-10 [47], which mediates defective IL-12 production and NF-κB activation in tumour-associated macrophages. This observation suggests that blocking IL-10, as well as other immunosuppressive cytokines present in the tumour microenvironment, may complement therapeutic strategies aimed at activating type I antitumour immune responses [47]. Similarly, paclitaxel and Prolactin were shown to enhance IL-12 production by TAM and contribute to restore a Th1 response in tumour bearers [32,35].

Chemokine and chemokine receptors are a prime target for the develoment of innovative therapeutic strategies in the control of inflammatory disorders. Recent results suggest that chemokine inhibitors could affect tumour growth by reducing macrophage infiltration [40]. Preliminary results in MCP-1/CCL2 gene targeted mice suggest that this chemokine can indeed promote progression in a Her2/neu-driven spontaneous mammary carcinoma model [7]. Thus, available information suggests that chemokines represent a valuable therapeutic target in neoplasia. Adoptive macrophage immunotherapy has been applied in clinical cancer immunotherapy. A likely problem with this approach was the poor recruitment of autologous activated macrophages at the tumour site [3], suggesting the need for further elucidation of the mechanisms of tumours infiltration by TAM. In a study by Joseph and Isaacs [23], Linomide caused significant reduction of the tumour volume, in a murine prostate cancer model, by inhibiting the stimulatory effects of TAM on tumour angiogenesis. Based on this, the effects of Linomide, or other anti-angiogenic drugs, on the expression of anti-angiogenic molecules by TAM (e.g. IL-12, IL-18, IP-10 (CXCL10), MIG (CXCL9)) may be considered valuable targets for anticancer therapy [24]. The adaptation of TAM to hypoxia results in the increased expression of hypoxia-inducible genes, such as VEGF, whose transcription is controlled by the transcription factor HIF-1 [46]. Along with HIF-2α [48], HIF-1 is produced by macrophages in hypoxia *in vitro*, and in avascular areas of breast carcinomas [11]. Inhibition of HIF-1 activity in TAM may represent a novel therapeutic approach to cancer therapy. Due to the accumulation of TAM into the hypoxic regions of tumours, viral vectors, were used to transduce macrophages with therapeutic genes,such as IFNγ, that were activated only in low oxygen conditions [52,53]. Granulocyte macrophage-colony stimulating factor (GM-CSF) gene therapy was recommended as a therapeutic choice for the treatment of cancer, based on its capability to promote chemokine production by TAM and, in turn, to trigger recruitment of myeloid cells into the tumour site [38]. At present, additional studies on TAM are necessary to better identify the molecular basis accounting for their functional phenotype and to explore novel therapeutic strategies.

8. Concluding remarks

The available information suggests that TAM are a prototypic, polarised type II mononuclear phagocyte population (Fig. 1). We are aware that the view of TAM as a skewed type II macrophage population is problably an oversimplification. Yet, the view of TAM as a polarised M2 macrophage is efficacious in summarising the current understanding of the immunobiology of these cells.

As polarised type II macrophages, TAM participate in circuits that regulate tumour growth and progression, adaptive immunity, stroma formation and angiogenesis and are a key component of the inflammatory circuits that, promote tumour progression and metastasis (for review, see [5]). Several lines of evidence including genetic analysis and gene targeting support the general hypothesis of a protumour

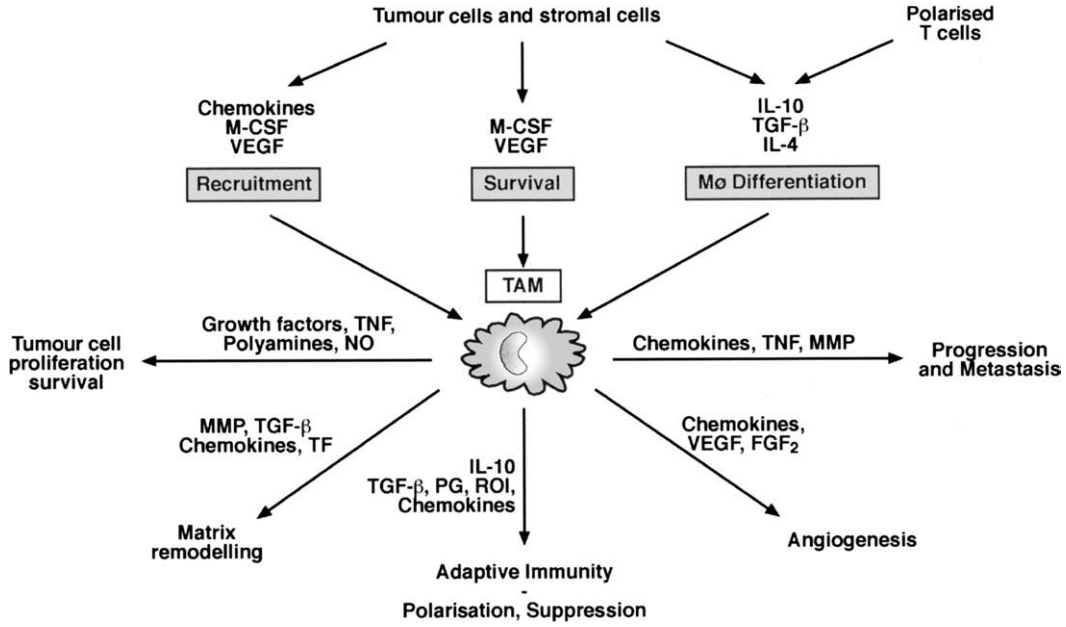

Fig. 1. The role of TAM in tumour growth and progression. TAM, tumour-associated macrophages; M-CSF, macrophage-colony stimulating factor; MMPs, matrix metalloproteinase; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; ROI, reactive oxygen intermediates; NO, nitric oxide TNF, tumour necrosis factor; PG, prostaglandin; IL-10, interleukin-10; FGF_2, fibroblast growth $factor_2$; TF, tissue factor.

role of inflammation, and for macrophages in particular (discussed here, in [9] and [55]). The available information suggesting that inflammatory reactions, and polarised infiltrating macrophages in particular, promote tumour progression, raises the possibility that the molecules and cells involved may represent novel, valuable therapeutic targets.

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