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Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: Potential targets of anti-cancer therapy

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ABSTRACT

Tumour-associated macrophages (TAM) represent the major inflammatory component of the stroma of many tumours, and can affect different aspects of the neoplastic tissue. Many observations indicate that TAM express several M2-associated pro-tumoural functions, including promotion of angiogenesis, matrix remodelling and suppression of adaptive immunity. The pro-tumoural role of TAM in cancer is further supported by clinical studies that found a correlation between the high macrophage content of tumours and poor patient prognosis. Evidence is presented here supporting the view that TAM represent a unique and distinct M2-skewed myeloid population and are a potential target for anti-cancer therapy.

1. Introduction

Accumulation of leukocyte subpopulations is the hallmark of several pathological conditions, including tumours.[1] A prominent component of solid tumours is represented by non-tumoural cells, including stromal cells (fibroblasts and endothelial cells) and leukocytes. Among the latter, macrophages are the major component.[2] Tumour-associated macrophages (TAM) have been studied extensively for their relationship with tumour cells and their multi-faceted functions in the tumour micro-environment. Immunologists have long considered the presence of TAM as evidence of a host response against the growing tumour. Several studies have demonstrated that macrophages have the potential to kill tumour cells in vitro when appropriately stimulated, e.g. following treatment with lipopolysaccharides (LPS) and interferon (IFN)-γ. However, bacterial stimuli and Th1 cytokines inducing M1 type polarisation are usually not present at the tumour site. Here, in contrast, differentiating macrophages are likely to encounter factors that most frequently polarise them toward M2 type macrophages (e.g. interleukin (IL)-10). Over the years it has become increasingly clear that TAM are active players in the process of tumour progression and invasion. In several experimental tumour models, the activation of an inflammatory response (most frequently mediated by macrophages) is essential for full neoplastic transformation and progression.[3] Furthermore, in clinical studies high numbers of intra-tumour macrophages correlate with high vessel density and tumour progression.

The strategic location of TAM suggests that these cells are important regulators of anti-tumour immunity. Characterisation of the phenotype of TAM is therefore essential to the understanding of tumour-derived signals guiding polarisation of innate and adaptive immunity in cancer bearers and to the identification of molecular mechanisms that might be amenable to therapeutic intervention.

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2. Recruitment of myeloid cells at the tumour site

2.1. *Monocyte-macrophages*

Since the first observation by Rudolf Virchow, who noticed the infiltration of leukocytes into malignant tissues and suggested that cancers arise at regions of chronic inflammation, the origin of TAM has been studied in terms of recruitment, survival and proliferation. TAM derive from circulating monocytes and are recruited at the tumour site by a tumour-derived chemotactic factor for monocytes, originally described by this group[4] and later identified as the chemokine CCL2/MCP-1[5,6] (Fig. 1). Evidence supporting a pivotal role of chemokines in the recruitment of monocytes in neoplastic tissues includes: correlation between production and infiltration in murine and human tumours, passive immunisation and gene modification.[7] In addition, the central role of chemokines in shaping the tumour micro-environment is supported by the observation that tumours are generally characterised by the constitutive expression of chemokines belonging to the inducible realm.[8] The molecular mechanisms accounting for the constitutive expression of chemokines by cancer cells have been defined only for CXCL1 and involve NF-κB activation by NF-κB-inducing kinase.[9]

CCL2 is probably the most frequently found CC chemokine in tumours. Most human carcinomas produce CCL2 (Table 1) and its levels of expression correlate with the increased infiltration of macrophages.[2,10,11] Interestingly, CCL2 production has also been detected in TAM, indicating the existence of an amplification loop for their recruitment.[2,12] Other CC chemokines related to CCL2, such as CCL7 and CCL8, are also produced by tumours and shown to recruit monocytes.[13]

Along with the supposed pro-tumoural role of TAM, the local production of chemokines and the extent of TAM infiltration have been studied as prognostic factors. For example, in human breast and oesophagus cancers, CCL2 levels correlated with the extent of macrophage infiltration, lymph-node metastasis and clinical aggressiveness.[14,15] In an experimental model of non-tumourigenic melanoma, low-level of 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.[16] In pancreatic cancer patients, high serum

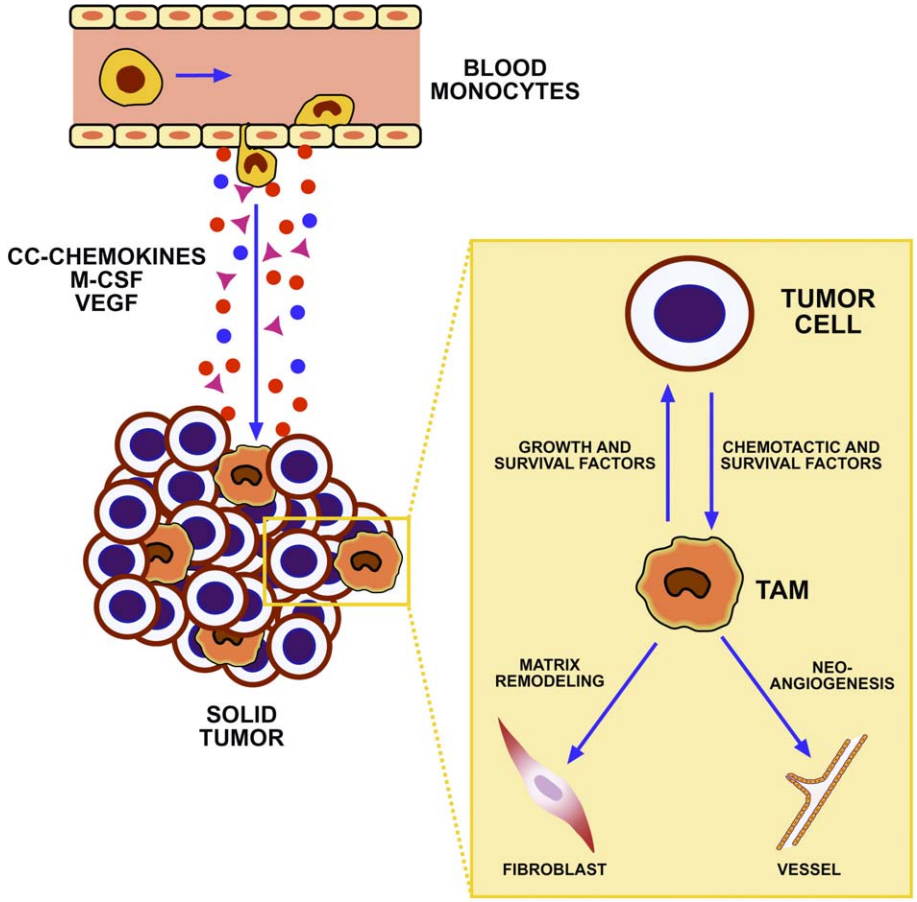

Fig. 1 – Tumour-derived chemotactic factors (CC-chemokines, e.g. CCL2), macrophage colony stimulating factor (M-CSF) and vascular endothelial growth factor (VEGF), actively recruit circulating blood monocytes at the tumour site. In the tumour micro-environment monocytes differentiate into tumour-associated macrophages (TAM), which establish a symbiotic relationship with tumour cells. The above tumour-derived factors positively modulate TAM survival. From their own, TAM secrete growth factors, which promote tumour cell proliferation and survival, regulate matrix deposition and remodelling and activate neo-angiogenesis.

Table 1 – Tumor- and/or stroma-derived chemokines

Ligand	Producing tumor
CXC family	
CXCL1/Groa	Colon carcinoma[88]
CXCL8/IL-8	Melanoma[89], breast[90]
CXCL9/Mig	Hodgkin's disease[91]
CXCL10/IP-10	Hodgkin lymphoma and nasopharygeal carcinoma[92]
CXCL12/SDF-1	Melanoma[93]; prostate, breast, ovary[7]; pancreas[103]
CXCL13/BCA1	Non-Hodgkin B-cell lymphoma[94]
CC family	
CCL1/I-309	Adult T-cell leukemia[95]
CCL2/MCP-1	Pancreas[15]; sarcomas, gliomas, lung, breast, cervix, ovary, melanoma[7,8]
CCL3/MIP-1a	Schwann cell tumors[96]
CCL3LI/LD78b	Glioblastoma[97]
CCL5/RANTES	Breast[12]; melanoma[98]
CCL6	NSLC[99]
CCL7/MCP-3	Osteosarcoma[11]
CCL8/MCP-2	Osteosarcoma[11]
CCL11/eotaxin	T-cell lymphoma[100]
CCL17/TARC	Lymphoma[101]
CCL18/PARC	Ovary[61]
CCL22/MDC	Ovary[59]
CCL28/MEC	Hodgkin's disease[102]

levels of CCL2 were associated with more favourable prognosis and with a lower proliferative index of tumour cells.[17] These biphasic effects of CCL2 are consistent with the 'macrophage balance' hypothesis[104] and emphasise the concept that levels of macrophage infiltration similar to those observed in human malignant lesions express pro-tumour activity.[18]

A variety of other chemokines have been detected in neoplastic tissues as products of either tumour cells or stromal elements (Table 1). These molecules play an important role in tumour progression by direct stimulation of neoplastic growth, promotion of inflammation and induction of angiogenesis. In spite of constitutive production of neutrophil chemotactic proteins by tumour cells, CXCL8 and related chemokines, neutrophils are not a major and obvious constituent of the leukocyte infiltrate. However, these cells, though present in minute numbers, may play a key role in triggering and sustaining the inflammatory cascade.

Macrophages are also recruited by molecules other than chemokines. In particular, tumour-derived cytokines interacting with tyrosine kinase receptors, such as vascular endothelial growth factor (VEGF) and macrophage colony stimulating factor (M-CSF)[19,20] promote macrophage recruitment, as well as macrophage survival and proliferation, the latter generally limited to murine TAM[2,19,20] (Fig. 1). Using genetic approaches, it has been demonstrated that depletion of M-CSF markedly decreases the infiltration of macrophages at the tumour site, and this correlates with a significant delay in tumour progression. By contrast, overexpression of M-CSF by tumour cells dramatically increased macrophage recruitment and this was correlated with accelerated tumour growth.[19,21,22] M-CSF overexpression is common among tumours of the reproductive system, including ovarian, uterine, breast and prostate, and correlates with poor prognosis.[23] Recently, placenta-derived growth factor (PlGF), a molecule related to VEGF in terms of structure and receptor usage, has been reported to promote the survival of TAM.[24]

2.2. *Dendritic cells*

Although usually rare, dendritic cells (DC) have been detected in several tumour types, including lung, prostate, nasopharynx, kidney, thyroid, breast, ovarian carcinoma and melanoma.[25–28] Again, chemokines are involved in the recruitment of DC within the tumour mass.[29,30] A few chemokines are more restricted for DC. CCL20 interacts with CCR6, a receptor expressed by Langerhan's cells but not by monocyte-derived DC. Infiltration of Langerhan's-like DC, positive for the marker Langerin, was noted in breast cancer expressing CCL20.[30] The presence of plasmacytoid DC (P-DC), a distinct DC subset of lymphoid origin, is a more recent finding. P-DC have been shown to accumulate in breast metastatic lymph nodes[31] and in ovarian cancer, in correlation with the expression of CXCL12 to which they respond.[28] Primary melanoma are infiltrated with both myeloid and P-DC.[27,32] DC can also derive from blood monocytes recruited at the tumour site. M-CSF, is expressed abundantly in the tumour cytokine milieu and, together with IL-6, blocks local DC maturation.[33] Indeed, intra-tumour DC have been shown to express an immature phenotype more frequently and therefore to have low immuno-stimulatory properties.

The significance of recruitment of antigen-presenting cells in the tumour, especially whether it is a sign of active immune response, is not clear. DC are well equipped to pick up tumour antigens and cross-present them to T lymphocytes, as documented by several studies.[34–36] However, DC can also potently induce tolerance[37,38] and, as mentioned above, the tumour micro-environment contains immunosuppressive factors. Thus the immunological functions of the newly recruited mononuclear phagocytes, such as antigen presentation and anti-tumour cytotoxicity, are severely inhibited. Nevertheless there are reports of tumour models where chemokines induced DC accumulation and tumour regression, but the exact role of DC has not been investigated.[31] The chemokines involved in these studies included CCL7, CCL16, CCL21 CCL20, CCL19 and CXCL12. Therefore, the role of tumour-associated DC in the establishment of anti-tumour immunity, or in the induction of tolerance, remains to be elucidated.

3. Distinct properties of M1 and M2 macrophages

The ability to express distinct functional programs 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.[39,40] Chronic infections can tightly regulate the immune responses, being able to trigger highly polarised type I or type II inflammation and immunity. Central to the development of type I or type II polarisation is the specificity of the host–pathogen interaction. While intracellular pathogens induce a type I polarised inflammation, with strong neutrophils,

macrophage infiltrate, typical in granulomas, parasites such as helminths trigger strong type II inflammation, characterised by extensive eosinophilia, mastocytosis and tissue remodelling.[3]

Several studies on tumour–host interaction have highlighted the importance of inflammatory responses in the early steps of carcinogenesis, as well as in established progressive tumours and are beginning now to identify the contribution of polarised inflammatory responses in cancer progression.

For a long period, classical or M1 macrophage activation was recognised as the unique activation program in response to microbial products and interferon-γ and has only recently become clear that anti-inflammatory molecules, such as glucocorticoid hormones, IL-4, IL-13 and IL-10, are more than simple inhibitors of macrophage activation, since they induce distinct M2 activation programs.[2,41–44]

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 polarisation.

Classical or M1 macrophage activation in response to microbial products or interferon-γ are characterised by: high capacity to present antigen; high interleukin-12 (IL-12) and IL-23 production[45] and consequent activation of a polarised type I response; and high production of toxic intermediates (nitric oxide (NO), reactive oxygen intermediates (ROI)). Based on this, M1 macrophages are generally considered to be potent effector cells that kill micro-organisms and tumour cells and produce copious amounts of pro-inflammatory cytokines (Fig. 2).

At the opposite extreme, type II macrophages (M2) tune inflammatory responses and adaptive Th1 immunity, scavenge debris, and promote angiogenesis, tissue remodelling and repair. Various signals elicit different M2 activation forms that commonly share selected functional properties (e.g. low IL-12) (Table 2) (reviewed in[39]). Integration with, and promotion of, type II responses prevail for IL-4- or IL-13-stimulated M2a macrophages, whereas suppression and regulation of inflammation and immunity are predominant in IL-10-stimulated M2b cells. Macrophages exposed to immune complexes (IC) and agonists of Toll-like receptors (TLRs) or IL-1R are characterised by an IL-10high and IL-12low phenotype and promote type II responses; they have been called type II

Table 2 – Various forms of M2 polarization

Population	Polarizing signals	Functions
M2a	IL-4 and IL-13	Th2 responses; type II inflammation; allergy; killing and encapsulated parasites
M2b	IC + TLR/IL-1R ligands	Th2 activation; immunoregulation
M2c	IL-10	Immunoregulation; matrix deposition and tissue remodelling

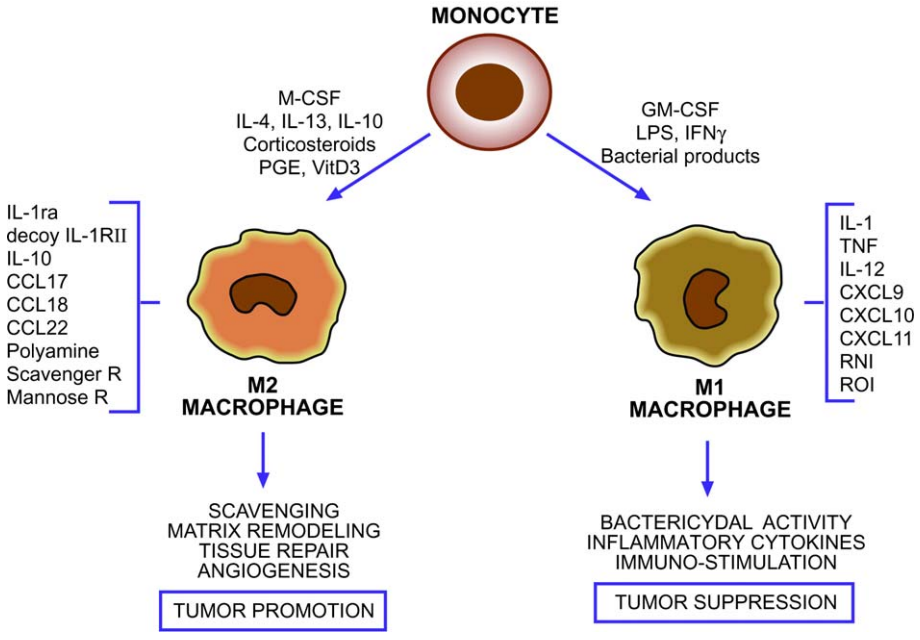

Fig. 2 – Monocytes differentiate into polarised macrophage subsets when exposed to different cytokine milieu. In the presence of granulocyte-macrophage colony stimulating factor (GM–CSF), interferon (IFN)-γ, lipopolysaccharide (LPS) and other microbial products monocytes differentiate into M1 macrophages. In the presence of macrophage colony stimulating factor (M-CSF), interleukin (IL)-4, IL-13, IL-10 and immuno-suppressive agents (corticosteroids, vitamin D3, prostaglandins), monocytes differentiate into M2 macrophages. M1 and M2 subsets differ in terms of phenotype and functions. M1 cells have high microbicidal activity, immuno-stimulatory functions and tumour cytotoxicity. M2 cells have high scavenging ability, promote tissue repair and angiogenesis and favour tumour progression.

activated macrophages.[46] Finally, human monocytes differentiated with granulocyte-macrophage colony stimulating factor (GM–CSF) or M–CSF have M1 and M2 properties, respectively, and have been referred to as Mø1 and Mø2.[45]

A comprehensive picture of the polarised functions of macrophages is represented in Fig. 1. M1 macrophages exposed to the classic activation signals IFN-γ and LPS express opsonic receptors (e.g. FcγRIII (CD16)), whereas M2 macrophages express preferentially non-opsonic receptor (e.g. mannose receptor and scavenger receptors).

Arginine metabolism gives rise to high levels of inducible nitric oxide synthase (iNOS; NOS2) in the M1 population. 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 polarisation programs.[39] The LPS receptor TLR4 and the adapter molecule MyD88 are increased by IFN-γ, while in contrast IL-10 inhibits their expression. By 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 upregulating the signalling IL-1RI, and IL-1R accessory protein.[2] In contrast, 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 IL-1 receptor antagonist (IL-1ra) production and inhibit IL-1.[47] While M1 macrophages express high levels of pro-inflammatory cytokines (IL-1, TNF, IL-6 and IL-23), M2 cells are generally characterised by their low production.

The M1 phenotype is typically IL-12high and IL-10low, whereas M2 macrophages are typically IL-10high and IL-12low. However, macrophages exposed to IC and LPS (M2b) (Fig. 2) are an exception, in that they retain high levels of inflammatory cytokine production with concomitant high IL-10 and low IL-12.[46] In spite of their high production of inflammatory cytokines and toxic molecules, M2b cells protect mice against LPS toxicity,[46,48] promote Th2 differentiation and humoural antibody production.

4. TAM express selected M2 pro-tumoural functions

The cytokine network expressed at the tumour site plays a central role in the orientation and differentiation of recruited mononuclear phagocytes, thus contributing to direct the local immune system away from anti-tumour functions.[2] This idea is supported by both pre-clinical and clinical observations[18,44] that clearly demonstrate an association between macrophage number/density and prognosis in a variety of murine and human malignancies.

The immunosuppressive cytokines IL-10 and transforming growth factor (TGF)-β are produced by both cancer cells (ovary) and TAM.[2] IL-10 promotes the differentiation of monocytes to mature macrophages and blocks their differentiation to DC.[30] Thus, a gradient of tumour-derived IL-10 may account for differentiation along the DC versus the macrophage pathway in different micro-anatomical locations in a tumour. Such a situation was observed in papillary carcinoma of the thyroid, where TAM are evenly distributed throughout the tissue, in contrast to DC, which are present in the periphery.[26] In breast carcinoma, DC with a mature phenotype (DC-LAMP+) were localised in peri-tumoural areas, while immature DC were inside the tumour.[25]

As previously discussed, IL-10 promotes the M2c alternative pathway of macrophage activation and induces TAM to express M2-related functions. Indeed, under many aspects TAM summarise a number of functions expressed by M2 macrophages, involved in tuning inflammatory responses and adaptive immunity, scavenge debris, promote angiogenesis, tissue remodelling and repair. The production of IL-10, TGF-β and prostaglandin E2 (PGE2) by cancer cells and TAM[2] contributes to a general suppression of anti-tumour activities (Fig. 3).

TAM are poor producers of NO[50] and, in situ in ovarian cancer, only a minority of tumours and, in these, a minority of macrophages localised at the periphery scored positive for iNOS.[51] Moreover, in contrast to M1 polarised macrophages, TAMs have been shown to be poor producers of reactive

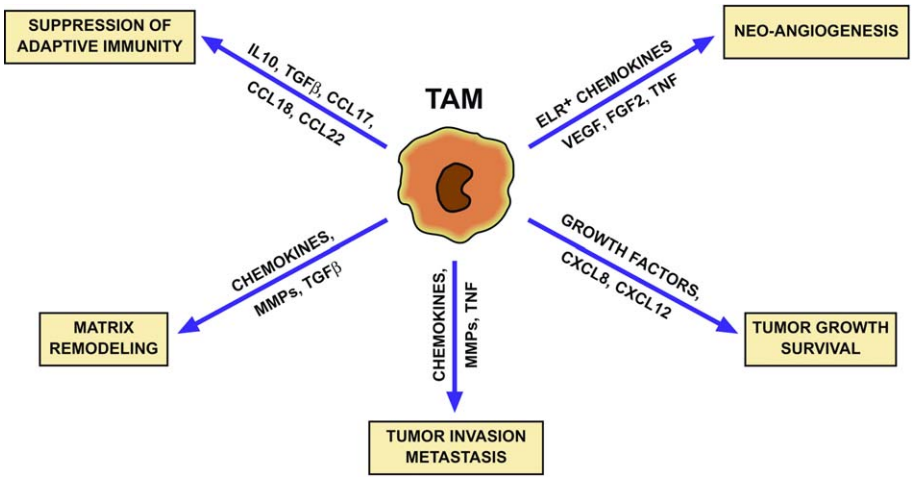

Fig. 3 – Tumour-associated macrophages (TAM) display several pro-tumoural functions. Chemokines have a prominent role as they induce neo-angiogenesis, activate matrix-metalloproteases (MMPs) and stroma remodelling, and direct tumour growth. Selected chemokines and immunosuppressive cytokines inhibit the anti-tumour immune response.

oxygen intermediates (ROIs), consistent with the hypothesis that these cells represent a skewed M2 population.[51]

Moreover, TAM were reported to express low levels of inflammatory cytokines (e.g. IL-12, IL-1β, TNF-α, IL-6).[2] Activation of NF-κB is a necessary event promoting transcription of several pro-inflammatory genes. Our previous studies[49] indicated that TAM display defective NF-κB activation in response to the M1 polarising signal LPS, and we observed similar results in response to the pro-inflammatory cytokine IL-1β (Schioppa and colleagues, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy). Thus, in terms of cytotoxicity and expression of inflammatory cytokines TAM resemble M2 macrophages.

In agreement with the M2 signature, TAM also express high levels of both the scavenger receptor-A (SR-A) (Biswas S, unpublished observation) and the mannose receptor (MR) (P. Allavena, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy). Furthermore, TAM are poor antigen-presenting cells.[2]

Arginase expression in TAM has not been studied. However, it has been proposed recently that the carbohydrate-binding protein galectin-1, which is abundantly expressed by ovarian cancer[52] and shows specific anti-inflammatory effects, tunes the classic pathway of L-arginine, resulting in a strong inhibition of NO production by LPS-activated macrophages.

Angiogenesis is an M2-associated function, which represents a key event in tumour growth and progression. In several studies in human cancer, accumulation of TAM has been associated with angiogenesis and with the production of angiogenic factors such as VEGF and platelet-derived endothelial cell growth factor.[2] More recently, in human cervical cancer, VEGF-C production by TAMs was proposed to play a role in peri-tumoural lympho-angiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis[53] Additionally, TAM participate to the pro-angiogenic process by producing the angiogenic factor thymidine phosphorylase (TP), which promotes endothelial cell migration in vitro and whose levels of expression are associated with tumour neovascularisation.[54] TAM also contribute to tumour progression by producing pro-angiogenic and tumour-inducing chemokines, such as CCL2.[29] Moreover, TAM accumulate in hypoxic regions of tumours and hypoxia triggers a pro-angiogenic program in these cells (see below). Therefore, macrophages recruited in situ represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumour cells. On the anti-angiogenic side, in a murine model, GM–CSF released from a primary tumour upregulated TAM-derived metalloelastase and angiostatin production, thus suppressing tumour growth of metastases.[55]

Finally, TAM express molecules which affect tumour cell proliferation, angiogenesis and dissolution of connective tissues. These include epidermal growth factor (EGF), members of the FGF family, TGF-β, VEGF and chemokines. In lung cancer, TAM may favour tumour progression by contributing to stroma formation and angiogenesis through their release of platelet derived growth factor (PDGF), in conjunction with TGF-β1 production by cancer cells.[2] Macrophages can produce enzymes and inhibitors that regulate the digestion of the extracellular matrix, such as matrix-metalloproteases (MMPs), plasmin, urokinase-type plasminogen activator (uPA) and the uPA receptor. Direct evidence has been presented that MMP-9 derived from haematopoietic cells of host origin contributes to skin carcinogenesis.[56] Chemokines have been shown to induce gene expression of various MMPs and, in particular, MMP-9 production, along with the uPA receptor.[57] Evidence suggests that MMP-9 has complex effects beyond matrix degradation including promotion of the angiogenesis switch and release of growth factors.[56]

5. 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.[2] The suppressive mediators produced by TAM include prostaglandins, IL-10 and TGF-b and indoleamine dioxigenase (IDO) metabolites.[2,44] Moreover, TAM are unable to produce IL-12, even upon stimulation by IFN-γ and LPS.[49] With this cytokine profile, which is characteristic of M2 macrophages, TAM are unable to trigger Th1 polarised immune responses, but rather induce T regulatory cells (Treg) (Fig. 4). Treg cells possess a characteristic anergic phenotype and strongly suppress the activity of effector T cells and other inflammatory cells, such as monocytes. Suppression of T cell mediated anti-tumour activity by Treg cells is associated with increased tumour growth and hence decreased survival.[58] For instance, in patients with advanced ovarian cancer, an increase in the number of functionally active Treg cells present in the ascites was predictive of reduced survival.[59] Immature myeloid suppressor cells present in the neoplastic tissue of some tumours have been shown potently to inhibit T cell responses.[60] The relationship if any, of immature myeloid suppressor cells with TAM remains to be defined.

The complex network of chemokines present at the tumour site can play a role also in the induction of adaptive immunity. Chemokines also regulate the amplification of polarised T cell responses (Fig. 4). Some chemokines may enhance specific host immunity against tumours, but on the other hand other chemokines may contribute to escape from the immune system, by recruiting Th2 effectors and Treg cells.[39] As mentioned above, in addition to being a target for chemokines, TAM are a source of a selected set of these mediators (CCL2, CCL17, CCL18, CCL22). CCL18 was recently identified as the most abundant chemokine in human ovarian ascites fluid. When the source of CCL18 was investigated, it was tracked to TAM, with no production by ovarian carcinoma cells.[61] 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.[62] Attraction of naive T cells in a peripheral microenvironment dominated by M2 macrophages and immature DC is likely to induce T cell anergy.

Work in gene-modified mice has shown that CCL2 can orient specific immunity in a Th2 direction. Although the exact mechanism for this action has not been defined, it may

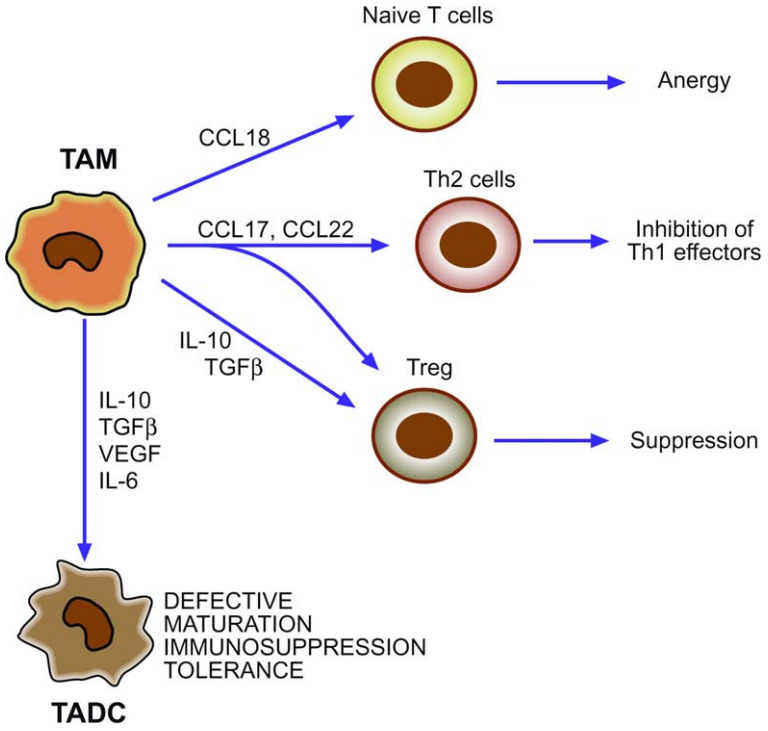

Fig. 4 – Suppressive effects of tumour-associated macrophages (TAM) on adaptive immunity. TAM produce cytokines, negatively modulating the outcome of a potential anti-tumour response. Interleukin (IL)-10, IL-6, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β inhibit the maturation and activation of tumour-associated dendritic cells (TADC). IL-10, TGF-β and selected chemokines act on T helper 2 (Th2)-polarised lymphocytes and T regulatory cells, which are ineffective in anti-tumour immunity and suppress anti-tumour responses.

include stimulation of IL-10 production in macrophages.[63] Overall, TAM-derived chemokines most frequently recruit effector T cell inefficient to mount a protective anti-tumour immunity. TAM also produce chemokine specifically attracting T cells with immuno-suppressive functions.

6. TAM as a therapeutic target

Two major functions of TAM potentially amenable to therapeutic interventions are their recruitment and cytotoxicity. TAM accumulate preferentially in the poorly vascularised region of tumours, which are characterised by low oxygen tension. Such an environment promotes TAM adaptation to hypoxia, which is achieved by the increased expression of hypoxia inducible and pro-angiogenic genes, such as VEGF, bFGF and CXCL8, whose transcription is controlled by the transcription factors HIF-1 and HIF-2.[64] The in vivo relevance of this metabolic adaptation to hypoxia was recently demonstrated by Cramer and colleagues.[65] Ablation of the hypoxia responsive transcription factor HIF-1α resulted in impaired macrophage 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 tumours. In support of this, we have recently described that hypoxia can influence the positioning and function of TAM by selectively upregulating expression of the chemokine receptor CXCR4.[66] Moreover, a recent study has shown that HIF-1 activation may play a role in the induction of the CXCR4 ligand, CXCL12.[67] It appears therefore that targeting HIF-1 activity may disrupt the HIF-1/CXCR4 pathway and affect TAM accumulation.

Due to the localisation of TAM into the hypoxic regions of tumours, viral vectors were used to transduce macrophages with therapeutic genes, such as IFN-γ, that were activated only in low oxygen conditions.[68–70] This work presents promising approaches that use macrophages as vehicles to deliver gene therapy in regions of tumour hypoxia.

Chemokines and chemokine receptors are a prime target for the development of innovative therapeutic strategies in the control of inflammatory disorders. Recent results suggest that chemokine inhibitors could affect tumour growth by reducing macrophage infiltration.[71] 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.[72] Thus, available information suggests that chemokines represent a valuable therapeutic target in neoplasia.

CSF-1 was identified as an important regulator of mammary tumour progression to metastasis, by regulating infiltration and function of TAM. Transgenic expression of CSF-1 in mammary epithelium led to the acceleration of the late stages of carcinoma and increased lung metastasis, suggesting that agents directed at CSF-1/CSF-1R activity could have important therapeutic effects.[19]

Anti-tumour agents with selective cytotoxic activity on monocyte-macrophages would be ideal therapeutic tools for their combined action on tumour cells and TAM. We recently reported that Yondelis (Trabectedin), a natural product

derived from the marine organism *Ecteinascidia turbinata*, with potent anti-tumour activity[73] is specifically cytotoxic to macrophages and TAM, while sparing the lymphocyte sub-set. In addition, Yondelis inhibits the production of CCL2 and IL-6 both by TAM and tumour cells.[74] These anti-inflammatory properties of Yondelis may be an extended mechanism of its anti-tumour activity.

Linomide, an anti-angiogenic agent, caused significant reduction of the tumour volume, in a murine prostate cancer model, by inhibiting the stimulatory effects of TAM on tumour angiogenesis.[75] Based on this, the effects of Linomide, or other anti-angiogenic drugs, on the expression of pro- and anti-angiogenic molecules by TAM may be considered valuable targets for anti-cancer therapy.[76]

The bisphosphonate zoledronic acid is a prototypical MMP inhibitor. In cervical cancer this compound suppressed MMP-9 expression by infiltrating macrophages and inhibited metalloprotease activity, reducing angiogenesis and cervical carcinogenesis.[77]

Defective 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).[2,49] Restoration of NF-κB activity in TAM is therefore a potential strategy to restore M1 inflammation and intra-tumoural cytotoxicity. In agreement, recent evidence indicates that restoration of an M1 phenotype in TAM may provide therapeutic benefit in tumour-bearing mice. In particular, combination of CpG plus an anti-IL-10 receptor antibody switched infiltrating macrophages from M2 to M1 and triggered innate response debulking large tumours within 16 h.[78] It is likely that this treatment may restore NF-κB activation and inflammatory functions by TAM. Moreover, TAM from STA6 –/– tumour-bearing mice display an M1 phenotype, with low level of arginase and high level of NO. As a result, these mice immunologically rejected spontaneous mammary carcinoma.[79] These data suggest that switching the TAM phenotype from M2 to M1 during tumour progression may promote anti-tumour activities. In this regard, Src homology 2 domain-containing inositol 5-phosphatase 1 (SHIP1) was shown to play a critical role in programming macrophage M1 versus M2 functions. Mice deficient for SHIP1 display a skewed development away from M1 macrophages (which have high inducible NOS levels and produce NO), towards M2 macrophages (which have high arginase levels and produce ornithine).[80]

The IFN-γ-inducible enzyme indoleamine 2,3-dioxygenase (IDO) is a well-known suppressor of T cell activation. It catalyses the initial rate-limiting step in tryptophan catabolism, which leads to the biosynthesis of nicotinamide adenine dinucleotide. By depleting tryptophan from the local micro-environment, IDO blocks activation of T lymphocytes.[81] It was reported recently 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), co-operate with cytotoxic agents to elicit regression of established tumours.[82]

Finally, recent reports have identified a myeloid M2-biased cell population in lymphoid organs and peripheral tissues of tumour-bearing hosts, referred to as the myeloid suppressor cells (MSC), which are suggested to contribute to the immunosuppressive phenotype.[83] These cells are phenotypically distinct from TAM and are characterised by the expression of Gr-1 and CD11b markers. MSC use two enzymes involved in arginine metabolism to control T cell response: inducible nitric oxide synthase (NOS2) and arginase (Arg1), which deplete the milieu of arginine, causing peroxinitrite generation, as well as lack of CD3ζ chain expression and T cell apoptosis.[83,84] In prostate cancer, selective antagonists of these two enzymes have been proved beneficial in restoring T cell-mediated cytotoxicity.[85]

7. Conclusion

Though the presence of TAM has long been considered as evidence for a host response against the growing tumour, it has become increasingly clear that TAM are active players in the process of tumour progression and invasion. Molecular and biological studies have been supported by a large number of clinical studies that have found a significant correlation between the high macrophage content of tumours and poor patient prognosis. TAM share many similarities with prototypic polarised M2 mononuclear phagocyte population, in terms of gene expression and functions. In line with known properties of M2 macrophage populations, several studies suggest that TAM promote tumour progression and metastasis by activating circuits that regulate tumour growth, adaptive immunity, stroma formation and angiogenesis (for review, see.[1,2,56]) Analysis of the mechanisms mediating this phenotype involve defective NF-κB activation,[49] an event thought to be responsible for the inability of TAM to mount effective M1 inflammatory responses.[39,49] Thus, the M2-associated pro-tumoural properties of TAM are in apparent contrast with emerging evidence supporting the view that NF-κB activation in the surrounding stroma acts as a tumour promoter at distinct phases of malignant progression.[86,87] This discrepancy appears even more striking when one considers a number of works that have reported diversion of the innate and adaptive immunity toward an M2 and Th2 polarisation state, respectively, in several murine and human malignancies.[2,105] While additional studies are required fully to clarify mechanisms controlling the immune responses in tumour bearers, it is possible that this apparent discrepancy may reflect differential involvement of NF-κB-driven inflammation in early steps of carcinogenesis, as compared with advanced neoplasia, where the established tumour micro-environment would guide TAM functions toward an M2 suppressive and tumour-promoting phenotype. This hypothesis is now accruing new supporting evidence indicating that in vivo functional switching of infiltrating M2 macrophages towards an M1 phenotype provides therapeutic benefit in tumour-bearing mice.[78,79] Identification of mechanisms promoting functional diversion of macrophages towards an M2 direction may disclose new valuable therapeutic targets against tumours.

Conflict of interest statement

None declared.

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REFERENCES

1. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001;**357**:539–45.
2. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002;**23**:549–55.
3. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005;**7**:211–7.
4. Bottazzi B, Polentarutti N, Acero R, et al. Regulation of the macrophage content of neoplasms by chemoattractants. *Science* 1983;**220**:210–2.
5. Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J Exp Med* 1999;**169**:1485–90.
6. Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ. Purification and aminoacid analysis of two human glioma-derived monocyte chemoattractants. *J Exp Med* 1989;**169**:1449–59.
7. Rollins B. *Chemokines and Cancer*. Totowa, NJ: Humana Press; 1999.
8. Mantovani A. The chemokine system: redundancy for robust outputs. *Immunol Today* 1999;**20**:254–7.
9. Yang J, Richmond A. Constitutive IkappaB kinase activity correlates with nuclear factor-kappaB activation in human melanoma cells. *Cancer Res* 2001;**61**:4901–9.
10. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004;**4**:540–50.
11. Conti I, Rollins BJ. CCL2 (monocyte chemoattractant protein-1) and cancer. *Semin Cancer Biol* 2004;**14**:149–54.
12. Ueno T, Toi M, Saji H, et al. Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clin Cancer Res* 2000;**6**:3282–389.
13. Van Damme J, Proost P, Lenaerts JP, Opdenakker G. Structural and functional identification of two human, tumor-derived monocyte chemotactic proteins (MCP-2 and MCP-3) belonging to the chemokine family. *J Exp Med* 1992;**176**:59–65.
14. Azenshtein E, Luboshits G, Shina S, et al. The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. *Cancer Res* 2002;**62**:1093–102.
15. Saji H, Koike M, Yamori T, et al. Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma. *Cancer* 2001;**92**:1085–91.
16. Nesbit M, Schaider H, Miller TH, Herlyn M. Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. *J Immunol* 2001;**166**:6483–90.
17. Monti P, Leone BE, Marchesi F, et al. The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of antimalignant activity. *Cancer Res* 2003;**63**:7451–61.
18. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2000;**196**:254–65.
19. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001;**193**:727–40.
20. Duyndam MC, Hilhorst MC, Schluper HM, et al. Vascular endothelial growth factor-165 overexpression stimulates angiogenesis and induces cyst formation and macrophage infiltration in human ovarian cancer xenografts. *Am J Pathol* 2002;**160**:537–48.
21. Nowicki A, Szenajch J, Ostrowska G, et al. Impaired tumor growth in colony-stimulating factor 1 (CSF-1)-deficient, macrophage-deficient op/op mouse: evidence for a role of CSF-1-dependent macrophages in formation of tumor stroma. *Int J Cancer* 1996;**65**:112–9.
22. Aharinejad S, Abraham D, Paulus P, et al. Colony-stimulating factor-1 antisense treatment suppresses growth of human tumor xenografts in mice. *Cancer Res* 2002;**62**:5317–24.
23. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004;**4**:71–8.
24. Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. *Cancer Res* 2002;**62**:2749–52.
25. Bell D, Chomarat P, Broyles D, et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. *J Exp Med* 1999;**190**:1417–26.
26. Scarpino S, Stoppacciaro A, Ballerini F, et al. Papillary carcinoma of the thyroid: hepatocyte growth factor (HGF) stimulates tumor cells to release chemokines active in recruiting dendritic cells. *Am J Pathol* 2000;**156**:831–7.
27. Vermi W, Bonecchi R, Facchetti F, et al. Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J Pathol* 2003;**200**:255–68.
28. Zou W, Machelon V, Coulomb-L'Hermin A, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 2001;**7**:1339–46.
29. Vicari AP, Caux C. Chemokines in cancer. *Cytokine Growth Factor Rev* 2002;**13**:143–54.
30. Allavena P, Sica A, Vecchi A, Locati M, Sozzani S, Mantovani A. The chemokine receptor switch paradigm and dendritic cell migration: its significance in tumor tissues. *Immunol Rev* 2000;**177**:141–9.
31. Vicari AP, Treilleux I, Lebecque S. Regulation of the trafficking of tumour-infiltrating dendritic cells by chemokines. *Semin Cancer Biol* 2004;**14**:161–9.
32. Salio M, Cella M, Vermi W, et al. Plasmacytoid dendritic cells prime IFN-gamma-secreting melanoma-specific CD8 lymphocytes and are found in primary melanoma lesions. *Eur J Immunol* 2003;**33**:1052–62.
33. Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. *Nat Immunol* 2000;**1**:510–4.
34. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol* 2005;**5**:296–306.
35. Steinman RM, Mellman I. Immunotherapy: bewitched, bothered, and bewildered no more. *Science* 2004;**305**:197–200.
36. Ardavin C, Amigorena S, Reis e Sousa C. Dendritic cells: immunobiology and cancer immunotherapy. *Immunity* 2004;**20**:17–23.
37. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003;**21**:685–711.

38. Rutella S, Lemoli RM. Regulatory T cells and tolerogenic dendritic cells: from basic biology to clinical applications. *Immunol Lett* 2004;**94**:11–26.
39. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;**25**:677–86.
40. Sher A, Pearce E, Kaye P. Shaping the immune response to parasites: role of dendritic cells. *Curr Opin Immunol* 2003;**15**:421–9.
41. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003;**3**:23–35.
42. Anderson CF, Mosser DM. novel phenotype for an activated macrophage: the type 2 activated macrophage. *J Leukoc Biol* 2002;**72**:101–6.
43. Goerdt S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* 1999;**10**:137–42.
44. Mantovani A, Allavena P, Sica A. Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. *Eur J Cancer* 2004;**40**:1660–7.
45. Verreck FA, de Boer T, Langenberg DM, et al. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco) bacteria. *Proc Natl Acad Sci USA* 2004;**101**:4560–5.
46. Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* 2003;**73**:209–12.
47. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991;**77**:1627–52.
48. Mosser DM, Karp CL. Receptor mediated subversion of macrophage cytokine production by intracellular pathogens. *Curr Opin Immunol* 1999;**11**:406–11.
49. Sica A, Saccani A, Bottazzi B, et al. Autocrine production of IL-10 mediates defective IL-12 production and NF-κB activation in tumor-associated macrophages. *J Immunol* 2000;**164**:762–7.
50. Dinapoli MR, Calderon CL, Lopez DM. The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduced expression of the inducible nitric oxide synthase gene. *J Exp Med* 1996;**183**:1323–9.
51. Klimp AH, Hollema H, Kempinga C, van der Zee AG, de Vries EG, Daemen T. Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. *Cancer Res* 2001;**61**:7305–9.
52. Van den Brule F, Califice S, Garnier F, Fernandez PL, Berchuck V, Castronovo V. Galectin-1 accumulation in the ovary carcinoma peritumoral stroma is induced by ovary carcinoma cells and affects both cancer cell proliferation and adhesion to laminin-1 and fibronectin. *Lab Invest* 2003;**83**:377–86.
53. Schoppmann SF, Birner P, Stockl J, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphoangiogenesis. *Am J Pathol* 2002;**161**:947–56.
54. Hotchkiss KA, Ashton AW, Klein RS, Lenzi ML, Zhu GH, Schwartz EL. Mechanisms by which tumor cells and monocytes expressing the angiogenic factor thymidine phosphorylase mediate human endothelial cell migration. *Cancer Res* 2003;**63**:527–33.
55. Dong Z, Yoneda J, Kumar R, Fidler IJ. Angiostatin-mediated suppression of cancer metastases by primary neoplasms engineered to produce granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1998;**188**:755–63.
56. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 2000;**103**:481–90.
57. Locati M, Deuschle U, Massardi ML, et al. Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. *J Immunol* 2002;**168**:3557–62.
58. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005;**6**:345–52.
59. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;**10**:942–9.
60. Bronte V, Serafini P, Mazzoni A, Segal DM, Zanovello P. L-arginine metabolism in myeloid cells controls T-lymphocyte functions. *Trends Immunol* 2003;**24**:302–6.
61. Schutyser E, Struyf S, Proost P, et al. Identification of biologically active chemokine isoforms from ascitic fluid and elevated levels of CCL18/pulmonary and activation-regulated chemokine in ovarian carcinoma. *J Biol Chem* 2002;**277**:24584–93.
62. Adema GJ, Hartgers F, Verstraten R, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 1997;**387**:713–7.
63. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 2000;**404**:407–11.
64. Knowles H, Leek R, Harris AL. Macrophage infiltration and angiogenesis in human malignancy. *Novartis Found Symp* 2004;**256**:189–200.
65. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1α is essential for myeloid cell-mediated inflammation. *Cell* 2003;**112**:645–57.
66. Schioppa T, Uranchimeg B, Saccani A, et al. Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med* 2003;**198**:1391–402.
67. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004;**10**:858–64.
68. Griffiths L, Binley K, Iqball S, et al. The macrophage – a novel system to deliver gene therapy to pathological hypoxia. *Gene Ther* 2000;**7**:255–62.
69. Carta L, Pastorino S, Melillo G, Bosco MC, Massazza S, Varesio L. Engineering of macrophages to produce IFN-gamma in response to hypoxia. *J Immunol* 2001;**166**:5374–80.
70. Burke B, Sumner S, Maitland N, Lewis CE. Macrophages in gene therapy: cellular delivery vehicles and in vivo targets. *J Leukoc Biol* 2002;**72**:417–28.
71. Robinson SC, Scott KA, Wilson JL, Thompson RG, Proudfoot FR, Balkwill FR. A chemokine receptor antagonist inhibits experimental breast tumor growth. *Cancer Res* 2003;**63**:8360–5.
72. Conti I, Rollins BJ. CCL2 (monocyte chemoattractant protein-1) and cancer. *Semin Cancer Biol* 2004;**14**:149–54.
73. Sessa C, De Braud F, Perotti A, et al. Trabectedin for women with ovarian carcinoma after treatment with platinum and taxanes fails. *J Clin Oncol* 2005;**23**:1867–74.
74. Allavena P, Signorelli M, Chieppa M, et al. Anti-inflammatory properties of the novel antitumor agent yondelis (trabectedin): inhibition of macrophage differentiation and cytokine production. *Cancer Res* 2005;**65**:2964–71.
75. Joseph IB, Isaacs JT. Macrophage role in the anti-prostate cancer response to one class of antiangiogenic agents. *J Natl Cancer Inst* 1998;**90**:1648–53.
76. Wahl L, Kleinman HK. Tumor-associated macrophages as targets for cancer therapy. *J Natl Cancer Inst* 1998;**90**:1583–4.

77. Giraudo E, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. *J Clin Invest* 2004;**114**:623–33.
78. Guiducci C, Vicari AP, Sangaletti S, Trinchieri G, Colombo MP. Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. *Cancer Res* 2005;**65**:3437–46.
79. Sinha P, Clements VK, Ostrand-Rosenberg S. Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease. *J Immunol* 2005;**174**:636–45.
80. Rauh MJ, Sly LM, Kalesnikoff J, et al. The role of SHIP1 in macrophage programming and activation. *Biochem Soc Trans* 2004;**32**:785–8.
81. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol* 2003;**24**:242–8.
82. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med* 2005;**11**:312–9.
83. Bronte V, Serafini P, Mazzoni A, Segal DM, Zanovello P. L-arginine metabolism in myeloid cells controls T-lymphocyte functions. *Trends Immunol* 2003;**24**:302–6.
84. Rodriguez PC, Quiceno DG, Zabaleta J, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* 2004;**64**:5839–49.
85. Bronte V, Kasic T, Gri G, et al. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med* 2005;**201**:1257–68.
86. Pikarsky E, Porat RM, Stein I, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* 2004;**431**:461–6.
87. Greten FR, Eckmann L, Greten TF, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004;**118**:285–96.
88. Li A, Varney ML, Singh RK. Constitutive expression of growth regulated oncogene (gro) in human colon carcinoma cells with different metastatic potential and its role in regulating their metastatic phenotype. *Clin Exp Metastasis* 2004;**21**:571–9.
89. Haghnegahdar H, Du J, Wang D, et al. The tumorigenic and angiogenic effects of MGSA/GRO proteins in melanoma. *J Leukoc Biol* 2000;**67**:53–62.
90. Azenshtein E, Meshel T, Shina S, Barak N, Keydar I, Ben-Baruch A. The angiogenic factors CXCL8 and VEGF in breast cancer: regulation by an array of pro-malignancy factors. *Cancer Lett* 2005;**217**:73–86.
91. Teruya-Feldstein J, Tosato G, Jaffe ES. The role of chemokines in Hodgkin's disease. *Leuk Lymphoma* 2000;**38**:363–71.
92. Teichmann M, Meyer B, Beck A, Niedobitek G. Expression of the interferon-inducible chemokine IP-10 (CXCL10), a chemokine with proposed anti-neoplastic functions, in Hodgkin lymphoma and nasopharyngeal carcinoma. *J Pathol* 2005;**20**:68–75.
93. Scala S, Ottaiano A, Ascierto PA, et al. Expression of CXCR4 predicts poor prognosis in patients with malignant melanoma. *Clin Cancer Res* 2005;**11**:1835–41.
94. Smith JR, Braziel RM, Paoletti S, Lipp M, Uguccioni M, Rosenbaum JT. Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. *Blood* 2003;**101**:815–21.
95. Ruckes T, Saul D, Van Snick J, Hermine O, Grassmann R. Autocrine antiapoptotic stimulation of cultured adult T-cell leukemia cells by overexpression of the chemokine I-309. *Blood* 2001;**98**:1150–9.
96. Mori K, Chano T, Yamamoto K, Matsusue Y, Okabe H. Expression of macrophage inflammatory protein-1alpha in Schwann cell tumors. *Neuropathology* 2004;**24**:131–5.
97. Kouno J, Nagai H, Nagahata T, et al. Up-regulation of CC chemokine, CCL3L1, and receptors, CCR3, CCR5 in human glioblastoma that promotes cell growth. *J Neurooncol* 2004;**70**:301–7.
98. Payne AS, Cornelius LA. The role of chemokines in melanoma tumor growth and metastasis. *J Invest Dermatol* 2002;**118**:915–22.
99. Yi F, Jaffe R, Prochownik EV. The CCL6 chemokine is differentially regulated by c-Myc and L-Myc, and promotes tumorigenesis and metastasis. *Cancer Res* 2003;**63**:2923–32.
100. Kleinhans M, Tun-Kyi A, Gilliet M, et al. Functional expression of the eotaxin receptor CCR3 in CD30+ cutaneous T-cell lymphoma. *Blood* 2003;**101**:1487–93.
101. Vermeer MH, Dukers DF, ten Berge RL, et al. Differential expression of thymus and activation regulated chemokine and its receptor CCR4 in nodal and cutaneous anaplastic large-cell lymphomas and Hodgkin's disease. *Mod Pathol* 2002;**15**:838–44.
102. Hanamoto H, Nakayama T, Miyazato H, et al. Expression of CCL28 by Reed-Sternberg cells defines a major subtype of classical Hodgkin's disease with frequent infiltration of eosinophils and/or plasma cells. *Am J Pathol* 2004;**164**:997–1006.
103. Marchesi F, Monti P, Leone BE, et al. Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer Res* 2004;**64**:8420–7.
104. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. *Immunol Today* 1992;**13**:265–70.
105. Clerici M, Shearer GM, Clerici E. Cytokine dysregulation in invasive cervical carcinoma and other human neoplasias: time to consider the TH1/TH2 paradigm. *J Natl Cancer Inst* 1998;**90**:261–3.