

Review

The role of macrophages in tumor development

Gerben J. van der Bij^{a,b}, Steven J. Oosterling^{a,b}, Sybren Meijer^a, Robert H.J. Beelen^b and Marjolein van Egmond^{a,b,*}

^a Department of Surgical Oncology, VU University Medical Center, Amsterdam, The Netherlands

^b Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands

Abstract. Macrophages constitute a large proportion of the immune cell infiltrate, which is present in many tumors. Activation state of macrophages is greatly influenced by their environment, leading to different macrophage subsets with diverse functions. Although previously regarded as potent immune cells that are capable of destroying tumor cells, recent literature focuses on the ability of macrophages to promote tumor development due to secretion of mediators, like growth and angiogenic factors. It is now becoming increasingly clear that a complicated synergistic relationship exists between macrophages and malignant cells whereby tumor cells can affect macrophage phenotype, and vice versa. As such, macrophages and their contribution in cancer development are currently subject of debate.

Keywords: Cancer, macrophages, metastases, tumor associated macrophage, cytotoxicity

Abbreviations:

CSF-1: Colony stimulating factor-1;
ECM: Extracellular matrix;
EGF: Epidermal growth factor;
GM-CSF: Granulocyte macrophage-colony stimulating factor;
GS: Glucocorticosteroids;
HIF: Hypoxia inducible factor;
IFN: Interferon;
IL: Interleukin;
KC: Kupffer cell;
LPS: Lipopolysaccharide;
MCP: Monocyte chemotactic protein;
MHC: Major histocompatibility complex;
MMPs: Matrix metalloproteinases;
NO: Nitric oxide;
PDGF: Platelet derived growth factor;
TAM: Tumor associated macrophages;

TGF: Transforming growth factor;
TNF: Tumor necrosis factor;
VEGF: Vascular endothelial growth factor.

1. Introduction

Solid tumors not only consist of malignant cells, but also contain other components, including fibroblasts, endothelial cells and immune cells as well as extracellular matrix (ECM). Until recently, cancer research mainly focused on the occurrence of changes/mutations within malignant cells, neglecting the influence of other tumor elements. Over the years however, the importance of tumor stroma and infiltrating immune cells for disease onset and progression has been acknowledged [7,13,47,71]. Stromal cells, like fibroblasts and endothelial cells, as well as infiltrating immune cells, are capable of secreting various cytokines, growth factors, chemokines and matrix metalloproteinases (MMPs), which are involved in processes such as proliferation, angiogenesis and metastases development. A large fraction of the non-malignant cells in tumors are macrophages, which have a compli-

* Corresponding author: Marjolein van Egmond, PhD, Dept. of Molecular Cell Biology and Immunology, vd Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. Tel.: +31 20 4448079; Fax: +31 20 4448081; E-mail: m.vanegmond@vumc.nl.

cated dual role in tumor development. On the one hand macrophages might control cancer progression by their capacity to kill tumor cells, but on the other hand they may promote tumor development by secretion of growth factors, cytokines and MMPs. This review therefore addresses the influence of macrophages in tumor development.

2. Normal macrophage function

2.1. Origin

Macrophages are widely distributed throughout the body and constitute a prominent part of the innate immune system. They originate from a common myeloid progenitor in the bone marrow. Proliferation and differentiation into monocytes is dependent on the presence of lineage determining cytokines such as colony stimulating factor 1 (CSF-1, also known as macrophage colony-stimulating factor), and granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as on interactions with stroma in haematopoietic organs [24,45]. In the circulation, monocytes can enter all tissue compartments in the body where they further adapt to the local microenvironment and differentiate into resident tissue macrophages [24,45]. Fully differentiated macrophages have lost the ability to proliferate [24,72]. Additionally, under inflammatory conditions, monocytes are recruited to sites of infection by chemoattractants/chemokines where they differentiate into macrophages.

2.2. Macrophage functions

Macrophages are among the first cells to enter inflamed tissues, and have the ability to kill microorganisms through production of nitric oxide (NO) and reactive oxygen intermediates (ROI). As such, they form a first line of defense against pathogens. Macrophages are furthermore potent producers of a plethora of different (anti-) inflammatory mediators and chemokines by which macrophages can recruit and activate other immune cells. By presenting antigen on their cell surface to T helper cells, which subsequently trigger antibody production by B cells or activate cytotoxic T cells, they also participate in initiating adaptive immune responses. Additionally, macrophages play a significant role in tissue homeostasis by scavenging apoptotic cells and cell debris [25]. During wound

healing, macrophages are essential in controlling infection, rearranging ECM components and in initiating angiogenesis [15,72].

In general, macrophages can be identified by expression of (a combination of) different markers on their cell surface, such as CD11b, CD14, CD68, and CD163. However, it is becoming increasingly clear that, depending on the local stimulus, macrophages are activated in various ways, which leads to different subsets. Although the nomenclature is confusing, some consensus has now been reached, which classifies macrophages subtype based on their activation and function [24,49] (Fig. 1). First, classically activated macrophages – also called M1 macrophages – develop as a result of stimulation with interferon- γ (IFN- γ), either alone or in combination with exposure to microbial products or tumor necrosis factor α (TNF- α). Activation of M1 macrophages leads to enhanced production of toxic oxygen species, including nitric oxide (NO) and reactive oxygen intermediates, which can facilitate killing of pathogens and tumor cells. In addition, classically activated/M1 macrophages can efficiently present antigen, and are able to induce Th1 adaptive immune responses [24,49].

Second, alternative activation of macrophages is induced after stimulation with interleukin (IL) 4 and/or IL-13. Alternatively activated, or M2a macrophages, have increased mannose receptor expression, whereas production of pro-inflammatory cytokines decreased. Additionally, increased arginase metabolism accounts for low NO production, subsequently leading to diminished cytotoxic and increased stroma-forming capacities of macrophages [24,50]. Additionally, alternatively activated/M2a macrophages are poor antigen presenting cells, even though major histocompatibility complex (MHC)-class II molecules are upregulated [24,53]. Functions associated with alternatively activated/M2a macrophages include phagocytosis of debris, wound healing and induction of Th2 responses. Furthermore, T-cell deactivation by alternatively activated/M2a macrophages has been reported [65].

Third, type II activated macrophages, also referred to as M2b macrophages, have been identified, which differentiate from immature macrophages after ligation of Fc γ receptors and a signal through any of the Toll-like receptors, CD40 or CD44 [75]. These macrophages are characterized by high IL-10 and absence of IL-12 production [74]. Additionally, type II activation of macrophages inhibits the potential to exert cytotoxicity and promotes Th2 cytokine and antibody production [2]. Mice that were challenged with

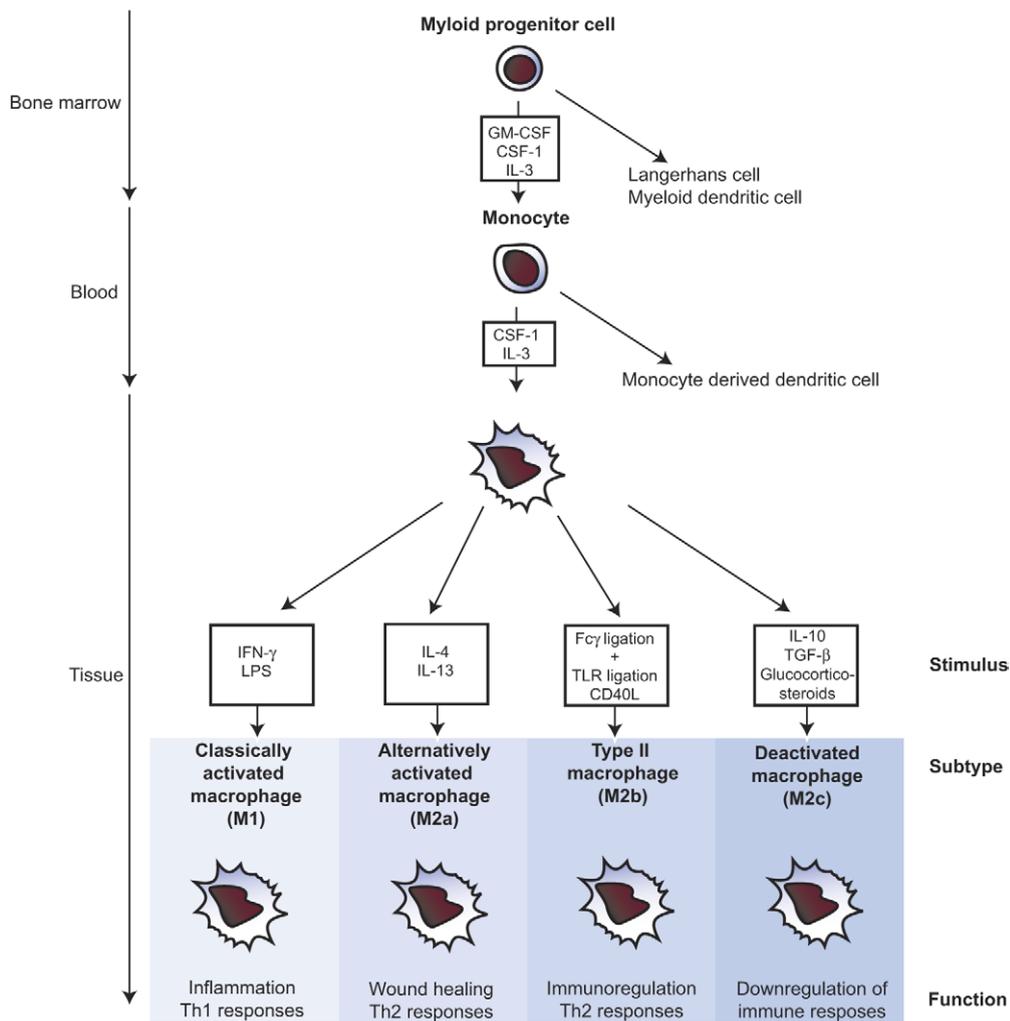


Fig. 1. Origin and activation of macrophages. Myeloid progenitor cells undergo differentiation into monocytes/macrophages after stimulation with GM-CSF, CSF-1 and IL-3. The presence of diverse environmental stimuli leads to differentiation into different macrophage subsets with dissimilar functional characteristics.

lipopolysaccharide (LPS) after infusion of type II activated macrophages, were able to survive a six times lethal dose of systemically administered LPS, compared to control mice, indicating the potential of these macrophages to dampen inflammatory reactions [23].

Fourth, deactivation of macrophages is achieved following stimulation with IL-10, transforming growth factor (TGF)- β , glucocorticosteroids (GS) or after phagocytosis of apoptotic cells [59]. MHC-class I and II expression is downregulated in these so-called M2c macrophages and cytotoxicity is impaired [45]. Cytokine production skews towards an anti-inflammatory profile and as such, M2c macrophages participate in downregulating inflammation [24].

3. Macrophage recruitment into tumors

In many clinical studies correlations have been found between the extent of macrophage infiltration in tumors, the occurrence of angiogenesis and disease progression [8]. In breast, prostate, cervical and ovarian carcinomas a link between poor prognosis and macrophage infiltration has been reported, whereas an opposite correlation is found in colorectal cancers [55]. Data concerning stomach and lung cancers are contradictory [8]. Overall, macrophage infiltration tends to associate with increased angiogenesis and poor prognosis, but differences between macrophage infiltration and clinical outcome are not yet fully understood. As the type of stimulus profoundly influences

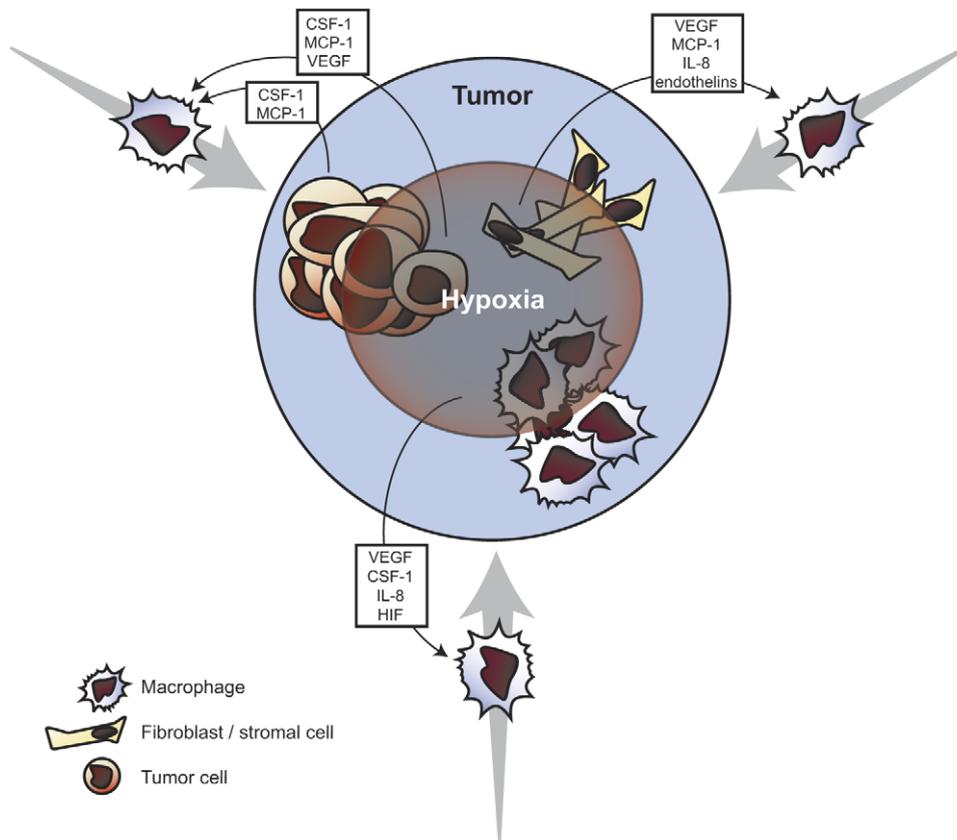


Fig. 2. Recruitment of macrophages by tumors. Tumor cells, macrophages and fibroblasts produce various chemoattractants for macrophages. Hypoxia, which is present in most tumors, attracts monocytes/macrophages and vast quantities of macrophages generally accumulate in hypoxic areas. Under hypoxic conditions secretion of many pro-angiogenic mediators is increased. Additionally, levels of various transcription factors, including HIF, are upregulated.

macrophage functions, heterogeneity between different tumor types, and thus tumor milieu, may represent one explanation [8,37]. For instance, presence of IL-4 or IL-13 in the tumor may stimulate the development of alternatively activated macrophages, whereas presence of IFN- γ might direct differentiation into effector M1 macrophages. Alternatively, the location of infiltrated macrophages may represent a factor for their prognostic value. For instance, in endometrial cancer, presence of macrophages in contact with tumor cells – which may favor macrophage cytotoxicity and hereby halt tumor development – associates with favorable prognosis. In contrast, presence of macrophages in necrotic or stromal tumor compartments, which results in lack of close contact with tumor cells, was correlated with worse prognosis [58].

Many tumors are able to produce factors such as monocyte chemoattractant protein 1 (MCP-1) and CSF-1, which can recruit monocytes from the blood into tumors [26,46,51] (Fig. 2). It has therefore been sug-

gested that it may be an advantageous event for tumors to attract macrophages. It has even been hypothesized that recruited macrophages are educated by tumors and as such may benefit developing tumors by facilitating tumor cell proliferation, angiogenesis and metastases formation [60]. However, factors such as MCP-1 and CSF-1 can also have autocrine effects on cancer cells, hereby directly facilitating tumor cell proliferation and/or invasion [34,36,80]. In addition, many of the chemoattractants produced by tumor cells have paracrine effects on other cells like fibroblasts and endothelial cells, which in turn can assist tumor growth [64].

3.1. MCP-1

MCP-1 (also referred to as CC chemokine ligand 2) is commonly present in tumors, and can be produced either by tumor cells themselves or by infiltrating

macrophages, fibroblasts and endothelial cells [63]. In many studies correlations have been found between MCP-1 expression in tumors and the presence of macrophages [38,63,70,77]. In an animal model low-level MCP-1 secretion by tumors correlated with modest monocyte infiltration resulting in enhanced tumor formation [57]. High-level secretion was, however, associated with massive infiltration of macrophages and subsequent destruction of the tumor mass within a few days. Blocking MCP-1 activity was furthermore shown to increase tumor formation and accelerate tumor growth [57]. In human tumors, it has been reported that MCP-1 expression correlates with the presence of potent pro-angiogenic factors like vascular endothelial growth factor (VEGF), IL-8 and thymidine phosphorylase [63,77]. As such it has been proposed that macrophages, recruited and activated by MCP-1, stimulate angiogenesis by production of angiogenic factors. However, besides having effects on monocytes/macrophages, MCP-1 is a chemotactic factor for endothelial cells as well. By recruiting endothelial cells directly, MCP-1 can therefore induce angiogenesis in a leukocyte independent manner [64].

3.2. CSF-1

CSF-1 is a potent hematopoietic factor produced by a variety of cells including lymphocytes, monocytes, fibroblasts, endothelial cells, myoblasts and osteoblasts. It is a key regulator of cellular proliferation, differentiation, and survival of blood monocytes, tissue macrophages and their progenitor cells. In various malignancies, including breast and ovarian cancer, high levels of CSF-1 in tumors correlate with the presence of high numbers of macrophages, increased tumor growth and metastases formation, as well as poor prognosis [35,44,66]. Interestingly, localization of CSF-1 present in the tumor appears to affect tumor development. Whereas CSF-1 presence around tumor cells was associated with poor outcome, stromal expression of CSF-1 was linked to prolonged survival [11]. Additionally, the type of CSF-1 expressed in tumors might be of importance in disease outcome. Cell-surface bound CSF-1 has been reported to facilitate tumor cell killing by macrophages and thus may restrict cancer development, whereas secretion of soluble CSF-1 by malignant cells increases tumor growth [31,32].

In addition to influencing macrophage recruitment, CSF-1 may act as a growth factor for tumor cells directly. In many tumor cells mutations in the CSF-1

receptor (CSF-1R) are found, resulting in constitutive activation [61]. It was shown that CSF-1R activation on mammary epithelium resulted in hyperproliferation and profound progressive disruption of junctional integrity [80]. In a similar way, excess secretion of CSF-1 may activate CSF-1R and stimulate tumor cell proliferation. Thus, CSF-1 production by tumor cells may promote malignancy and growth in an autocrine manner, as well as having effects through the recruitment of macrophages.

3.3. Hypoxia

Hypoxic and anoxic regions, with oxygen tensions of less than 10 mmHg, are present in most human cancers [19,79]. These oxygen deprived regions commonly result from insufficient blood supply as a consequence of disorganized vessel structure, which is frequently associated with tumors [10]. Hypoxia triggers cells to produce angiogenic factors. Under hypoxic conditions, tumors show increased expression of VEGF, IL-8 and endothelins, as well as increased expression of the transcription factor hypoxia inducible factor (HIF) [39,41,43]. These molecules can directly induce angiogenesis by stimulating endothelial cell recruitment and proliferation, but also can exert their effects by stimulating macrophage infiltration, as macrophages were shown to preferentially cluster in hypoxic regions [42,43,54,82].

4. Tumor promoting functions of macrophages

The influence of macrophages on tumor development was elegantly shown when mice – susceptible for the development of mammary carcinoma – were crossed with mice that were CSF-1 deficient, and thus lack macrophage recruitment [44]. Although the incidence and growth rate of primary tumors was not altered, development of metastases was delayed and less extensive, supporting a role for macrophages involvement in tumor progression. Additionally, blocking of CSF-1 with antisense oligonucleotides suppressed growth of human colon and embryonic tumor cells that had been xenografted in immunocompromised mice. Direct effects of antisense oligonucleotides on tumor cells were ruled out, and as less macrophages were present in tumors of antisense treated animals, these data indirectly pointed to macrophage mediated effects [1]. When needles, containing gradients of either CSF-1 or epidermal growth factor (EGF) were

inserted in primary mammary tumors, migration of both tumor cells and macrophages into microneedles was stimulated. Blockade of either EGF or CSF-1 stimulated signaling decreased migration of both cell types. Since only tumor cells expressed EGF receptor, whereas CSF-1 receptor expression was only found on macrophages, this observation indicates the presence of a synergistic interaction between tumor cells and macrophages, which are also referred to as tumor associated macrophages (TAM) [81]. TAM are considered as alternatively activated macrophages, as they possess many of the characteristics that apply to this type of activation.

TAM can contribute to tumor cell proliferation by producing a wide array of growth factors, including platelet derived growth factor (PDGF), fibroblast growth factors, hepatocyte growth factor, EGF receptor family ligands and TGF- β [48]. Furthermore, tumors require new blood vessel formation in order to grow beyond a certain size [5,29]. Macrophages are a rich source of both pro- and anti-angiogenic mediators and, as such, play an important role in angiogenesis. Pro-angiogenic mediators include VEGF, PDGF and TNF α , which stimulate endothelial cell recruitment as well as their proliferation [16,73]. Presumably, the tumor microenvironment triggers the production of pro-angiogenic factors by macrophages [16]. It has been suggested that hypoxia, which is present in most tumors, acts as a trigger for macrophage recruitment and expression of increased levels of HIF by macrophages. The latter in turn stimulates the production of VEGF [42,43]. In addition, TAM can produce mediators that indirectly contribute to angiogenesis by stimulating fibroblasts, endothelial cells and newly recruited macrophages to produce pro-angiogenic factors.

In order for tumor cells to become metastatic, remodeling of the stroma that surrounds tumor cells is required. Proteolytic enzymes, like MMP-2 and 9, are capable of cleaving ECM components and basement membranes, thereby creating the necessary routes for invading tumor cells. Levels of MMP-2 and 9 have been found to correlate with depth of tumor invasion as well as histological grade and MMP-9 production by macrophages is found in several types of invasive tumors [17,30,56]. Additionally, these proteolytic enzymes can facilitate angiogenesis by stroma rearrangement [5,6]. Macrophages might contribute to this process as MMP-9 expression by bone marrow derived monocytes was recently shown to contribute to skin carcinogenesis in a mouse model [14].

Finally, TAM can produce immunosuppressive mediators such as IL-10, prostaglandin E2 and TGF- β , which may downregulate the cytotoxic potential of macrophages towards tumor cells. Macrophages isolated from tumors showed a decreased capability to lyse target cells in comparison with more distantly located macrophages. This functional impairment was correlated with a decreased inducible nitric oxide synthase mRNA and protein levels resulting in lower NO production [18]. Furthermore, immunosuppressive mediators produced by TAM may hamper effective immune responses and promote tumor development by suppressing T cell activation and inducing Th2 responses [48].

5. Tumor inhibiting functions of macrophages

Most macrophages cannot efficiently induce tumor cell killing without activation, but have been shown to kill malignant cells in the presence of stimuli such as IFN- γ or TNF- α [37,67]. Macrophages can recognize tumor cells by altered membrane composition such as increased phosphatidylserine or altered carbohydrate structures on the surface [21,78]. Tumor cell lysis occurs through cell-cell contact or via release of soluble lytic factors, such as TNF- α and NO [9]. TNF- α can induce tumor cell directly, but also upregulates release of IL-1 by macrophages, which has both cytotoxic and growth inhibitory effects on tumor cells. Additionally, macrophages are able to induce antibody-dependent cellular cytotoxicity, as antibodies bound to tumor cells can be recognized by the Fc receptors, which are expressed on macrophages. Depending on the activation state of macrophages and the isotype of the antibody, time needed for this type of killing can vary from a few hours to 2 days [22,33]. Furthermore, in several *in vivo* studies macrophages have been identified as effector cells in tumor rejection [12,76]. GM-CSF potently upregulates macrophage cytotoxicity, survival and proliferation *in vitro* and *in vivo* [68,69]. Additional animal studies have shown that GM-CSF-transduced tumor cells have decreased potential to grow out, which is presumably due to the presence of a large macrophage infiltrate [40]. Additionally to cellular cytotoxicity, macrophages can inhibit tumor growth by presenting antigen and thereby inducing adaptive immune responses against tumors. In animal models, effective macrophage-induced anti-tumor immune responses can be generated by vaccination with GM-CSF transfected melanoma cells

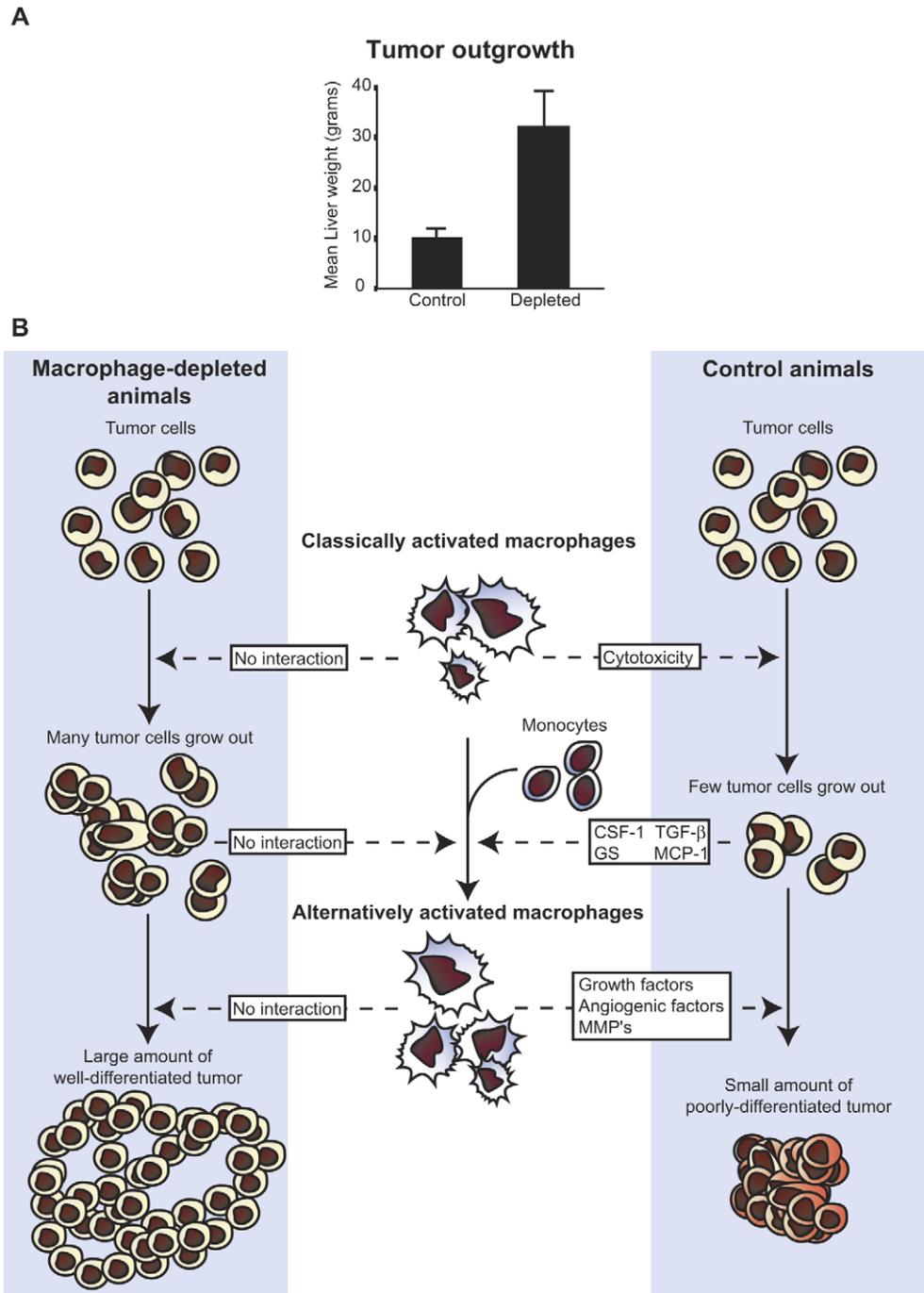


Fig. 3. (A) Tumor outgrowth in the liver increased dramatically when Kupffer cells (liver macrophages) had been depleted prior to inoculation of tumor cells in the portal vein. Total liver weight of Kupffer cell-depleted and control rats is shown. (Adapted from Heuff et al., 2003.) (B) Schematic model of the proposed role of macrophages in tumor outgrowth. Left: depleted animals. Due to the absence of macrophages, the majority of injected tumor cells grow out, which results in a large tumor load. However, presence of growth and angiogenic factors is reduced, leading to well differentiated tumor. Right: control animals. After injection of tumor cells, a large fraction of the cells is killed by macrophages, resulting in outgrowth of few tumor cells. However, the tumors that do develop promote the generation of alternatively activated macrophages, either by recruitment of new monocytes or by conversion of already present macrophages. These in turn can stimulate tumor growth and angiogenesis, which leads to poorly differentiated tumors.

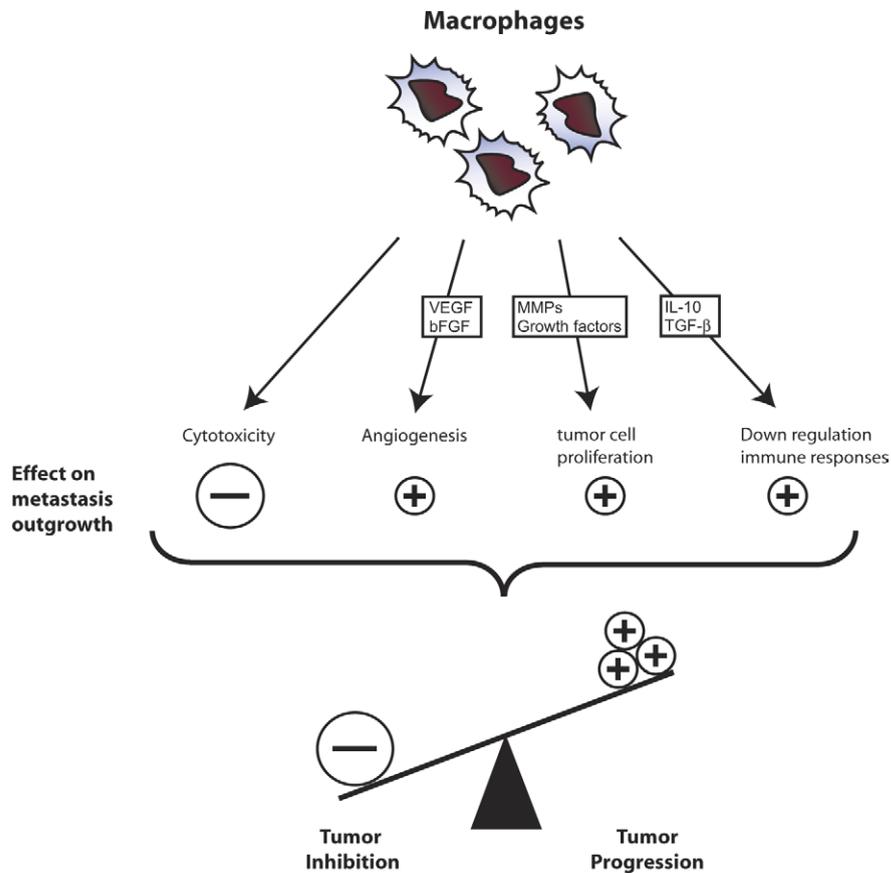


Fig. 4. Net effect of macrophages on tumor outgrowth. Macrophages contribute to tumor growth and differentiation into a malignant phenotype by production of growth factors and MMPs, induction of angiogenesis and downregulation of immune responses. However, depletion of macrophages led to poorer survival compared to control animals, illustrating the importance of macrophage mediated tumor cell killing. Thus, the anti-tumor properties of macrophages are prevalent over the tumor promoting characteristics, resulting in an overall beneficial effect of macrophages on survival.

[20] or adoptive transfer of *ex vivo* targeted peritoneal macrophages [3].

Macrophages were furthermore reported to exert great protective capacity against metastases outgrowth in the liver [4,27,52,62]. Kupffer cells (KC), which represent the main macrophage population in the liver, can kill tumor cells without activation [28] and *in vivo* studies showed that tumor cells are arrested and eradicated by KC [4]. Pre-treatment with GM-CSF furthermore increased the number of rat KC *in vivo*, and led to decreased tumor outgrowth [68]. Importantly, after depletion of KC metastases outgrowth in the liver increased drastically compared to outgrowth in livers of normal rats [4,27,62] (Fig. 3A). Similar results were found when macrophages were depleted from the peritoneal cavity (unpublished data). Remarkably, tumors grown in the presence of macrophages showed hallmark features of malignancy, such as poor differenti-

ation, absence of basement membranes, a desmoplastic stroma reaction and increased angiogenesis. In contrast, tumors grown in the absence of macrophages had a tubulo-papillary structure, well-defined basement membranes and a smaller stromal compartment, which indicates better differentiation of these tumors (Fig. 3B). Nonetheless, rats of which macrophages had been depleted prior to tumor inoculation – either in the liver or peritoneal cavity – showed poorer survival, which is most likely due to the absence of tumor cell killing by macrophages (unpublished data) (Fig. 4).

6. Concluding remarks

In conclusion, macrophages can exert both negative and positive effects on tumor growth, which is greatly affected by the microenvironment. The influ-

ence of TAM not only depends on the activation state of macrophages themselves, but also on the intrinsic characteristics of tumor cells. Because macrophages are abundantly present in most tumors, and as they have great impact on tumor development, these cells may represent a potential target for tumor therapy. Further knowledge on the complicated synergistic relationship between tumor cells and macrophages might therefore open up new prospects for development of new anti-cancer treatments. On the one hand it could be worthwhile to develop strategies that reduce recruitment of alternatively activated macrophages, which can promote tumor growth. On the other hand, stimulation of anti-tumor properties of macrophages may lead to additional therapeutic approaches as well.

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