Human monocytes/macrophages in the antitumour response of the host

ANNA SZAFLARSKA, MACIEJ SIEDLAR, MAREK ZEMBALA

Department of Clinical Immunology, Polish-American Institute of Paediatrics, Jagiellonian University Medical College, Cracow, Poland

Abstract
Monocytes/macrophages play a significant role in the host's response to tumours. This includes: cytotoxic/cytostatic activity, presentation of tumour-associated antigens and induction of specific anticancer response of lymphocytes. Circulating blood monocytes respond to a gradient of chemoattractants produced by the tumour, migrate out from the blood to the tumour bed and form a large part of the cellular infiltrate as tumour infiltrating macrophages (TIM). Monocytes and macrophages produce a large array of factors (cytokines, reactive oxygen and nitrogen intermediates, growth factors, prostacyclins, ect.) with opposing biological activities. Consequently, TIM exhibit both tumour growth promoting and inhibitory activities. Furthermore, tumour-derived molecules also modulate TIM activity. In some circumstances monocytes/macrophages are involved in the metastatic process. This review summarizes the current state of knowledge in this area indicating that in fact macrophage-tumour interactions are quite complicated and a delicate balance exists between antitumour response and protumour effect of TIM and the suppression of TIM activity by the tumour. The clinical implications of these findings are also discussed.

***Key words**: monocytes/macrophages, tumour cells, cytokines, cytotoxic mediators, tumour infiltrating macrophages, immunotherapy*

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Introduction

Monocytes/macrophages belonging to the mononuclear phagocyte system are involved in the host response against cancer and function not only as cells presenting tumour-associated antigens to (tumour infiltrating) T lymphocytes but also act as cytotoxic/cytostatic effector cells. In addition, they express surface molecules relevant for cell adhesion and cellular interactions and regulate the functions of other cells in the immune system. Monocytes/macrophages are able to distinguish and kill malignant, but not normal, cells and form the major component of the mononuclear cell infiltrate of many tumours, as tumour infiltrating macrophages (TIM). TIM may both inhibit and promote tumour growth and neoangiogenesis. These opposing activities of TIM are best explained by the "macrophage-tumour balance" hypothesis [1]. The exact role of monocytes/macrophages in human malignancy remains not fully understood.

Function of monocytes in malignant diseases

Production of cytokines

Monocytes and macrophages are capable of producing numerous cytokines, e.g. tumour necrosis factor alpha (TNF), interleukins (IL): IL-1, IL-6, IL-10, IL-12, IL-18, colony-stimulating factors (CSF), chemokines and cytotoxic mediators: reactive oxygen (ROI) and nitrogen intermediates (RNI), which appear to play an important role in the regulation of tumour growth.

Tumour necrosis factor

TNF is produced mainly by mononuclear phagocytes and is cytotoxic for some tumour cells. It is proinflammatory cytokine that mediates and induces tumouricidal activity of monocytes and stimulates its own and other cytokine production by monocytes [2], increases their antigen presenting capacity and upregulates HLA-DR and interferon γ receptor

Correspondence: Anna Szaflarska, Medical College, Jagiellonian University, Department of Clinical Immunology, 265 Wielicka st, 30-663 Cracow, Poland. Phone/fax number: +48 12 658 97 30, e-mail: miszafla@cyf-kr.edu.pl

(IFNγ-R) expression on monocytes [3]. TNF downregulates the monocyte CD86 costimulatory molecule expression and mannose receptor-dependent endocytosis [4]. It also enhances HLA-A,B,C and HLA-DR expression on tumour cells [5], but inhibits IFNγ - induced HLA-DR exprssion on normal differentiated cells (fibroblasts, macrophages) [6]. Tumour cells are able to induce TNF-mRNA expression and TNF production by human monocytes [7]. This capacity is not limited to viable but also metabolically inactive tumour cells and their constituents like membranes or hyaluronian [8, 9]. Several surface molecules (CD44, HLA-DR) [7] and protein kinases: a tyrosine-protein kinases (PTK) and calcium-phospholipid-dependent protein kinases (PKC) are involved in signal transduction for TNF release after stimulation with cancer cells [10]. The antitumour effect of TNF is due to its direct cytocidal activity on tumour cells and selective damage of endothelial cells of the tumour vasculature leading to apoptosis and necrosis of the tumour [11-13]. The inverse correlation between TNF-mRNA expression and microvessels count was found in non-small lung carcinoma [14]. TNF also induces neutrophil-mediated cytostasis of tumour cells that is mediated by high local concentration of hydrogen peroxide [15].

On the other hand, TNF produced by some tumour cells may enhance tumour spread and metastatic formation by induction of transcription of metalloproteinase (MMP)-9 gene in stromal cells of giant cell tumour of bone [16] and proMMP-9 production in monocytes [17] that cause degradation of extracellular matrix compounds [18]. TNF also facilitates the adherence of tumour cells to vascular endothelium [19, 20]. TNF receptor type I (TNFRI) is involved in the regulation of intercellular adhesion molecule-1 (ICAM-1), E-selectin, vascular adhesion molecule-1 (VCAM-1) and CD44 expression on vascular endothelial cells [21, 22] that regulate monocyte migration from the blood vessels to the tumour site. Hence, TNF has several opposite effects on the tumour growth and influences the TIM-tumour balance.

Production of TNF by lipopolisaccharide (LPS)-stimulated monocytes is reduced in colon cancer patients and this reduction is more pronounced in Dukes'C compared to Dukes'A and B tumour stages. This suppression is not mediated by IL-10 and disappears following surgical resection of the tumour [23]. On the other hand, an increased spontaneous or LPS-stimulated production of TNF by peripheral blood mononuclear cells (monocytes are the major cellular source of TNF) was found in gastric cancer patients [24]. Furthermore, serum levels of TNF are increased in all stages of gastric cancer (our unpublished observations). An increased serum levels of TNF are also found in patients with hepatocellular carcinoma and metastatic liver carcinoma [25]. On the other hand, TNF is undetectable in the serum of patients with metastatic breast carcinoma [26] and patients with gastrointestinal cancer-associated cachexia [27]. These and other observations suggest that TNF has no role in cancer-related cachexia in man [24, 28] but may play a significant role in the regulation of the inflammatory host response to the growing tumour [29].

Interleukin-1

IL-1 activates tumouricidal activity of monocytes but also trigger the release of IL-1 and TNF by IFNγ-primed monocytes [30, 31]. IL-1 mimics many of the biological actions of TNF [32] and both these cytokines act as stimulators of IFNγ-R synthesis. IL-1 exerts cytotoxic and cytostatic effects *in vitro* on several tumour cell lines [33, 34]. IL-1 and IFNγ have additive growth inhibitory effect on colon cancer cell line [35].

No significant changes in the production of IL-1 by blood monocytes from patients with untreated colorectal [36], lung [37] or head and neck [38] cancers were observed. However, cytoplasmatic expression of IL-1 (α and β) in monocytes was reduced in patients with lung and colorectal cancers [36]. The presence of IL-1 and IL-6 was detected in the effusions from ovarian cancer [39]. Only a few patients with metastatic breast cancer had detectable IL-1β serum levels [26] but in patients with hepatocellular carcinoma, metastatic liver carcinoma and gastrointestinal cancer serum levels of IL-1 α and β were increased [25, 40]. In contrast, no changes in serum levels of these cytokines were found in endometrial and urinary tract cancer [41, 42], while IL-1 release *in vitro* by unstimulated PBMC of patients with urinary tract cancer was decreased [42]. Hence, no consistent results concerning the production of IL-1 are found in different types of cancer. Moreover, IL-1 is not associated with cancer anorexia-cachexia syndrome [28].

Interleukin-6

IL-6 is a pleiotropic cytokine produced by many different cells, mainly monocytes/macrophages, fibroblasts and endothelial cells. This cytokine acts on activated B cells and induces immunoglobulin production. IL-6 is also involved in growth, differentiation and activation of T cells. It synergises with IL-1 in induction of IL-2 production and IL-2 receptor (CD25) expression on T cells. IL-6 is one of the hepatocyte stimulating factors regulating the biosynthesis of acute phase proteins and an important regulator of hematopoiesis [43].

IL-6 plays an important role in the pathogenesis of plasmocytoma [44, 45]. Its increased serum level positively correlates with severity of disease [46]. Monocytes from patients with head and neck cancers produce an increased amount of IL-6 [38]. Raised serum level of IL-6 is observed in patients with pancreatic [47, 48], gastric [25] and liver carcinomas (hepatocellular and metastatic liver carcinomas) [25,49]. In contrast to these observations [25], we have not found any significant changes in the serum levels of IL-6 in patients with different stages of gastric cancer (unpublished). An increased serum level of IL-6 correlates with tumour progression and poor prognosis in metastatic breast cancer [26]. IL-6 enhances acute phase response and its serum level correlates with poor nutritional status, impaired patient performance status and shorter survival in lung cancer patients [50]. However, other studies revealed no correlation between IL-6 serum levels and the presence of the cancer anorexia-cachexia syndrome [28].

Interleukin-10

IL-10 downregulates antitumour activity of monocytes by suppressing production of IL-1β and TNF and inhibits HLA-DR expression on antigen presenting cells [51]. Although IL-10 inhibits ROI formation in activated monocytes, it has no inhibitory effect on ROI production by activated macrophages [52]. On the other hand, in experimental systems, it inhibits angiogenesis and suppresses growth and metastasis formation by human melanoma cells [53]. An increased serum level of IL-10 in patients with resectable hepatocellular carcinoma [49] and advanced solid tumours [54] appears to be an independent prognostic factor [49, 54]. Monocytes from breast cancer patients show an increased production of IL-10 and decreased IL-12 [55]. Colon and renal carcinoma cell lines stimulate peripheral blood monocytes and lamina propria mononuclear cells to produce increased levels of IL-10 [4, 56]. Tumour-cell-derived TGFβ1 and PGE_2 are the potent IL-10 synthesis stimulators [56]. In this context, it is of interest that increased serum levels of PGE_2 have been found in some types of cancers and in Hodgkin's disease [4, 57, 58]. However, no changes in the serum levels of IL-10 nor its production by PBMC from gastric cancer were found (our unpublished observations).

Interleukin-12

IL-12 has a powerful antitumour activity and is primarily produced by monocytes/macrophages. It skews the immune response in favour of Th_1 cells that preferentially induce cell-mediated immunity. IL-12 acts as a growth factor for activated NK and T cells [59] and stimulates the production of TNF, IFN-γ, GM-CSF and IL-8 by T lymphoctes and/or NK cells [60, 61]. IL-12 enhances its own production by dendritic cells [61]. On the other hand, IL-12 also stimulates the production of IL-10 by T lymphocytes [61]. Antitumour activity of IL-12 (tumour regression or tumour growth inhibition due to administration of IL-12) was demonstrated *in vivo* using 17 different lines of transplantable murine tumours including carcinomas, sarcomas, melanomas and lymphomas [61, 62]. The antitumour effect of IL-12 may be independent of NK cells, since comparable activity was observed in NK-deficient mice [63]. Tumours undergoing IL-12-mediated regression have large numbers of TIM [63, 64, 65]. There are three main mechanisms of antitumour activity of IL-12: induction of CD8 cytotoxic cells, production of IFN-γ by T and NK cells and inhibition of neoangiogenesis [62].

IL-12 induces cytolytic activity of PBMC from patients with lung cancer against lung cancer cells and Daudi lymphoma cells [66]. However, monocytes from patients with lung cancer show decreased production of IL-12 [66]. Also PBMC from patients with colorectal cancer show decreased IL-12 and increased IL-10 production, the latter known to antagonise IL-12 synthesis. The decrease in IL-12 production is most clearly seen in advanced colorectal cancer [67]. However, in some types of cancer, serum or ascitic fluid levels of IL-12 are increased [68, 69]. There is no correlation between LPS-stimulated IL-12 secretion by blood monocytes and survival of patients with head and neck cancer [70]. The effectiveness of IL-12 therapy was demonstrated in several types of experimental tumours [64, 65, 71-75]. Human IL-12 is undergoing phase I clinical trial in metastatic renal cancer and malignant melanoma [76].

Interleukin-18

IL-18 is monocyte-derived pleiotropic cytokine that synergises with IL-12 and induces IFN-γ, IL-1β, NO production by T lymphocytes and promotes Th_1-mediated immune response. IL-18 also enhances IL-13 production by T and NK cells [77]. Antitumour effects of IL-18 may also involve FasL-mediated apoptosis of tumour cells by cytokine enhanced Fas-ligand (Fas-L) expression on NK cells [78] and inhibition of neoangiogenesis in experimental tumours [79]. The decreased production of this cytokine by colon adenocarcinoma cells in comparison to normal epithelial cells of the colon mucosa is associated with immunosuppresion observed in colon cancer [80]. However, our unpublished observations indicate an increased IL-18 serum level in patients with gastric cancer.

Colony stimulating factors

G-CSF is produced primarily by activated monocytes/macrophages and enhances the expression of complement receptor (CR) type 1, 3, FcγRI (CD64) and FcγRIII (CD16) on monocytes and upregulates their tumouricidal capacity [81, 82]. M-CSF is also produced by monocytes/macrophages and stimulates the differentiation of monocytes and macrophages from their progenitor cells. Its high serum levels were found in patients with breast, endometrial and ovarian cancers and correlated with poor prognosis [83, 84]. M-CSF is also produced by tumour cells. Expression of M-CSF and its receptor by breast cancer cells is associated with high macrophage infiltration and poor prognosis [85, 86]. Monocytes from patients undergoing GM-CSF therapy showed a significant increase in MHC class I and II expression, production of TNF and monocyte-mediated cytotoxicity against U937 tumour cells [81]. GM-CSF from transfected human colon cancer cells stimulates monocytes to secrete monocyte chemotactic protein (MCP)-1 and induces expression of the CD11b adhesion molecule [87].

Production of reactive nitrogen intermediates

NO is involved in tumouricidal activity of monocytes/macrophages [88, 89]. Cytotoxicity of NO is mainly due to peroxynitrite ($ONOO^-$) or nitrosothiols (RSNO) production [90, 91]. Peroxynitrite causes the inhibition of mitochondrial respiration and damage of variety of mitochondrial components, nitrosothiols inhibit respiratory complex I, while NO inhibits cytochrome oxidase [91]. Furthermore, NO selectively inhibits IL-12 synthesis by activated monocytes [92, 93] and suppresses T lymphocyte proliferation [94].

Inducible nitric oxide synthase (iNOS) is responsible for biosynthesis of NO by monocytes. Some cytokines (IL-1β, IFN-γ, IL-2, TNF) and LPS induce iNOS-mRNA synthesis but not NO release by monocytes [95-98]. However, some cancer cells may stimulate monocytes for de novo production of NO [95]. Both iNOS-mRNA and iNOS protein were observed in monocytes stimulated with colon carcinoma cell line, but not with human pancreatic cancer cell line [96]. We have recently found that following stimulation with cancer cells, $CD14^+$/$CD16^+$subpopulation of monocytes show an increased expression of iNOS protein and release of NO in comparison to "classical" ($CD14^{++}$) monocytes [97]. Human urothelial carcinoma cell line also fails to induce NO production by monocytes, while tumour cells display iNOS expression and NO production in cocultures with monocytes [98]. The above findings may cast some doubts about the ability of monocytes to produce NO. However, our other observations indicate the expression of iNOS in monocytes stimulated by the supernatants from the culture of cancer cell lines. This phenomen is probably due to microparticles released by tumour cells. The CD29, CD44, CD58, HLA-DR, and MHC class I of monocytes are engaged in tumour cell-induced production of NO [99]. The signal transduction pathways for NO and TNF production seem to be different as at least three protein kinases: PKC, PTK and cAMP-dependent kinase (PKA) are involved in the induction of NO by monocytes stimulated with tumour cells [10].

L-arginine is the substrate molecule for NO synthesis, but some tumours stimulate monocytes for biosynthesis of ornithine, a precursor for polyamine growth factors: putrescine, spermidine and spermine [100]. Polyamines may promote experimental tumour growth not only by increasing proliferation of tumour cells, but also by induction of neoangiogenesis [101]. NO is involved in apoptosis of some cells (it promotes or inhibits apoptosis) and its effect is dose-dependent and cell-type specific [102]. In cholangiocarcinoma cells, NO inhibits apoptosis directly by blocking caspse 9 activation [103]. On the other hand, the presence of iNOS in pancreatic cancer cells positively correlates with their apoptosis [104]. Also mononuclear cells, including macrophages infiltrating colorectal cancer, show both the increased apoptosis and expression of iNOS [105].

Production of reactive oxygen intermediates

In man, there is no evidence for the local production of ROI in the tumour bed. The role of ROI in the antitumour response in human has been indirectly implicated by observation that myeloperoxidase-deficient individuals show an increased incidence of malignancy [106]. Patients with renal cancer show an increased production of ROI by peripheral blood monocytes [107]. *In vitro* stimulation of monocytes with tumour cells, but not with untransformed cells, induces the production of ROI [108]. O_2^-, hydrogen peroxide, $OH^*$and probably hypohalites are involved in the spontaneous cytotoxic activity of monocytes towards tumour cells [108]. On the other hand, the significant inhibition of ROI formation in *in vitro* co-cultures of macrophages and tumour spheroids of colon carcinoma cell lines or supernatants from cultures of tumour cells is observed [52]. TGF-β1, IL-10 and IL-4 are not involved in this tumour-induced suppression of ROI production [52]. It is interesting that ROI may suppress lymphocyte and NK cell function [109]. CD18, CD29 and CD44 adhesion molecules are engaged in the induction of ROI production by monocytes stimulated with cancer cells [110]. Hyaluronan, the major ligand for CD44, which is overexpressed on many cancer cells, triggers ROI generation by monocytes via ligation of CD44 and allows them to distinguish cancer from non-malignant cells. On the other hand, blocking of CD44 on monocytes by free hyaluronan inhibits their response to tumour cells [110].

Production of eicosanoids

Cancer cells may affect monocytes function through alteration of arachidonic acid (AA) metabolism and production of eicosanoids: prostaglandins (PGs), tromboxane, leukotriens (LTs) and hydroxyeicosatetraenoic acid [111]. Serum level of PGE_2 is increased in cancer patients [112]. Stimulation by cancer cells induces monocyte PGE_2 production [4]. Also the level of PGs in tumour tissue is increased [113, 114]. PGs may promote tumour cell proliferation [115]. Blood monocytes and peritoneal macrophages from ovarian cancer patients show enhanced tumouricidal activity following inhibition of cyclooxygenase. However, this has no effect on tumouricidal activity of alveolar macrophages from lung cancer patients [116]. Metabolism of AA occurs preferentially via lipoxygenase pathway [117], which is not altered in circulating monocytes or TIM in cancer patients [116]. Antitumour activity of peritoneal macrophages is correlated with the production of PGE_2 and positively associated with synthesis of LTC_4 and LTD_4 [118].

Chemotactic response of monocytes to the tumour

Migration of blood monocytes to the tumour bed involves a response to a positive gradient of chemoattractants and induces the adherence to vascular endothelium, emigration from the blood vessels and directed movement within the extracellular matrix (ECM). Tumour cells secrete chemotactic factors, such as IL-8 and monocyte chemotactic protein-1, 2, 3 (MCP-1, 2, 3), the members of CC chemokine family: CCL-2, CCL-8, CCL-7. These molecules selectively attract monocytes, but not neutrophils [119]. MCP-1 gene expression within the tumour, predominantly in stromal cells, is correlated with the degree of invasiveness of breast carcinomas [120] and MCP-1 expression by both tumour cells and macrophages is positively associated with macrophages infiltration [121]. MCP-1 regulates the cell surface expression of adhesion molecules, especially β_2 integrins (CD11b/CD18 and CD11c/CD18) on monocytes, thus facilitating their adherence to vascular endothelium. MCP-1 also induces production of IL-1 and IL-6 by monocytes [122]. In ovarian cancer, MCP-1 serum level correlates with the histological grade of the tumour and the age of patients [123]. The production of MCP-1 by human tumours engrafted into mice enables early recruitment of monocytes and tumour growth inhibition [124].

Defective monocyte chemotaxis is observed in patients with head and neck, lung, gastric, breast and genitourinary cancers [125-129] and melanoma [130]. This defect is more apparent in advanced disease and reversed by tumour removal [125, 126, 130]. Chemokine receptor expression is an important factor for monocyte chemotaxis. TIM isolated from ovarian carcinoma and, to lesser extent, blood monocytes display defective mRNA and surface expression of chemokine receptor CCR2 (MCP-1 receptor). Downregulation of CCR2 is largely dependent on the local production of TNF [131]. However, monocyte chemotactic activity and the levels of CC chemokines are higher in non-small-cell lung cancer than in normal lung tissue [132]. Furthermore, blood monocytes from patients with breast cancer display a higher transendothelial migration than those from patients with benign diseases of the breast. It is not concerned with the differences in monocytes phenotype (HLA-DR, CD64, CD11a and CD11b expression) [133].

Cytotoxic activity of monocytes

Human monocytes possess high spontaneous cytotoxic activity against malignant, but not normal, cells [134-136]. ROI, TNF and NO may act synergistically in the cytotoxic damage of neoplastic cells. In most instances, an increased cytotoxic or cytostatic activity of monocytes in patients with different neoplasms, e.g. lung, breast, and gastrointestinal cancers [137], primary and metastatic brain tumours [138], squamous cell carcinoma [139] is observed. However, no changes in cytotoxic activity of monocytes are observed in patients with renal cancer [140] and non-Hodgkin's lymphoma [141]. A variety of agents are capable to induce tumouricidal activity of monocytes, e.g.: LPS, IL-1, IL-2, GM-CSF, laminin, LPS or IFNγ [142-148]. IFNγ prevents the loss of cytotoxic activity, which occurs during monocyte maturation to macrophages [143]. Cytotoxic potential of monocytes is age dependent. Monocytes from aged healthy subjects show decreased *in vitro* cytotoxity against tumour cells which is associated with compromised IL-1, ROI and RNI production [149].

Tumour cells are heterogeneous in their susceptibility to cytocidal activity of monocytes. Human tumours of the same histological origin are affected to different degrees by monocytes [150] or their cytotoxic mediators [151]. The maximal tumouricidal activity usually requires a direct contact between monocytes and target cells [152]. It is suggested that outer membrane phosphatidyloserine [153] or hyaluronan [110] may be involved in the "recognition" of tumour cells by activated monocytes [153].

Phenotypic characteristics of monocytes in malignancy

Changes in monocyte function in malignant diseases are often correlated with changes in the expression of functionally important cell surface molecules.

FcγR1

The receptor for Fc part of IgG (FcγRI) of monocytes is involved in antibody-dependent cellular cytotoxity (ADCC) against tumour cells [154-156]. Its expression is increased in patients with lung, colon [157], kidney [158] and gastric [159] cancers but decreased in patients with squamous cell carcinoma [139]. However, monocytes from metastatic squamous cell carcinoma show an increased expression of FcγRI [160]. In patients with breast cancer the expression of FcγRI on monocytes is unchanged [161].

MHC class II

MHC class II expression is critical for antigen presentation [161]. Furthermore, HLA-DR determinants of monocytes play a role in signal transduction for TNF gene activation [7] and NO production [99]. Monocytes from the *in vitro* co-culture with tumour cells show significant enhancement of HLA-DR expression [162]. Macrophages from the cellular infiltrate surrounding tumour express an abundant quantity of HLA class II determinants, which suggests that they are activated in the tumour bed [163]. There is also an opposite observation that cancer cells may induce a down-regulation of HLA-DR expression on monocytes [4]. In patients with squamous cell and breast carcinoma, HLA-DR expression on monocytes remains unchanged [139, 161].

Subpopulations of monocytes

On the basis of CD14 expression, two main monocyte subpopulations are distinguished. The major population that shows an enhanced expression of CD14 antigen ($CD14^{++}$ monocytes) and the minor one with a weak expression of

CD14 and the presence of CD16 ($CD14^+/CD16^+$ monocytes). In healthy donors $CD14^+/CD16^+$ subpopulation account for 5 +/- 3% of total monocytes. This subpopulation appears to represent more mature monocytes [164], is the main producer of TNF and show low or no production of IL-10 following stimulation with LPS. Therefore, $CD14^+/CD16^+$ subpopulation has been defined as "pro-inflammatory" monocytes [165, 166]. $CD14^+/CD16^+$ monocytes are also defined as the subpopulation containing dendritic cell precursors [167, 168]. The absolute number of $CD14^+/CD16^+$ monocytes is increased in various inflammatory diseases, like bacterial sepsis, viral infections, major trauma [166, 169, 170] and in patients with metastatic gastrointestinal cancers and other solid tumours [171]. We have also observed an elevated absolute number of these cells in the blood of gastric cancer patients (unpublished observations). However, no changes in the expression of CD16 on monocytes are found in patients with kidney cancer [158]. Patients with gastrointestinal carcinoma treated with M-CSF show a significant increase in the percentage of $CD16^+$ monocytes in comparsion to healthy subjects [172]. Our unpublished observations indicate that among all monocytes, $CD14^+/CD16^+$ cells posses the highest antitumour activity as they are the main producers of TNF, IL-12, NO and ROI (O_2^-) and produce low levels of immunosuppressive IL-10.

Adhesion molecules

The CD11b (CR3α chain) is an important receptor for phagocytosis and subsequent activation of respiratory burst in mononuclear phagocytes [173]. The interaction of CD11b with ICAM-1 promotes attachment to endothelium and extravasation of leukocytes [174]. CD11b molecule is lost upon their migration to the tissues [175]. Anti-CD11b monoclonal antibodies inhibit monocytes recruitment to both MCP-1 producing and nonproducing human tumours [176]. CD11b is involved in adhesion of MCP-1-stimulated monocytes to laminin of ECM. [177]. MCP-1 induces the expression of CD11b and CD11c and IL-1 and IL-6 production by monocytes [178]. The expression of CR3 on monocytes from kidney, but not breast, cancer patients is increased [179, 161]. Monocytes also express β1 integrin - very late antigen-4 (VLA-4) and use this molecule in interactions with activated endothelial cells [180] or with tumour cells [181]. The decreased surface expression of VLA-4 on monocytes is observed during tumour growth, which suggests a reduced monocytes ability to bind ECM.

Immunoregulatory activity

The progression of malignant diseases is associated with immune dysfunction. Different monocyte populations may act as suppressor cells. The elevated supressor activity of monocytes in cancer patients is related to tumour burden and the stage of disease. The presence of activated monocytes, which also showed an increased suppressor activity for T cells, is associated with favourable prognosis in some patients with gastric cancer [182]. An increased monocyte-mediated cytostasis of lymphoid cell lines has been observed also in breast and lung cancer patients [183]. Monocytes of some patients with gastrointestinal cancer possess suppressor activity as well as increased cytostatic capacity against L1210 lymphoma cell line [184]. The question arises whether monocyte cytostatic and suppressor activities are interrelated and both indicate an activated state of the cell.

Tumours produce a number of factors (vascular endothelial growth factor, VEGF, M-CSF, IL-6) that block the differentiation of $CD34^+$ stem cells into dendritic cells. However, tumours may promote the altered maturation and early apoptosis of human monocyte-derived dendritic cells. Upregulation of surface markers (CD80, CD86, HLA-DR), nuclear translocation of RelB and allostimulatory activity is associated with the lack of capacity to produce IL-12 and rapid apoptosis of monocytes [185]. Apoptosis of monocytes is also induced during their direct contact with cancer cells *in vitro* [162]. It may be one of the mechanisms by which tumours evade the immune response of the host. The inhibition of the cellular immune response of the host is also due to the gangliosides shedding by the tumour cells and its binding to leukocytes in the tumour microenviornment [186, 187].

Tumour infiltrating macrophages (TIM)

Function of TIM in the tumour growth

Macrophages represent a major component of the mononuclear cell infiltrate of tumours [163, 188, 189] and may consists up to 80% of the total tumour mass [190]. They are located within the tumour mass (intratumourally) or at the periphery of the tumour (peritumourally).

The process of leukocyte migration from the circulation into the tumour involves their interactions with vascular endothelium, i.e. leukocyte rolling and adhesion. This may be reduced in tumour microvessels due to decreased expression of adhesion molecules caused by tumour-derived angiogenic factors [191]. TIM have been demonstrated in the stroma of numerous malignant tumours including colon [192], breast [192, 193], skin [194], lung, ovary or thyroid gland cancers and melanoma [195]. The number of TIM is more increased in advanced (Duke's C) than in early colon cancer and in tumours expressing MHC class II molecules [196]. The composition of the cellular infiltrate depends on the properties of invaded tissue. The extend of TIM infiltration in tumours of the same histological origin varies, but the average number of TIM in particular tumour during its growth is relatively stable. Having divergent functional properties, TIM may modulate tumour growth by affecting cell proliferation, vascularization (angiogenesis), stroma formation, killing and dissolution of neoplastic cells.

The role of TIM in human malignancy is complex. Experimental evidence indicates that the intratumoural as well as peritumoural TIM limit tumour size in early stages of tumour development [174, 176]. In colon cancer TIM accumulate along the invasive edge, are in a direct contact with lymphocytes and express costimulatory molecules: CD80 (B7-1) as well as CD86 (B7-2). In contrast, the expression of CD80 and CD86 is usually inconspicuous in the tumour stroma [197]. These observations are in accordance with clinicopathological observations suggesting that peritumoural lymphocytic infiltration is a favourable prognostic factor in colorectal cancer [198]. The large number of CD16$^+$ macrophages was found in renal cancer, melanoma and colon carcinoma [188]. As these tumours are susceptible to immunotherapy with lymphokine activated killer cells, it may indicate that CD16$^+$ macrophages are involved in antitumour cytotoxic response [188]. In contrast, high TIM content within breast cancer stroma is associated with poor prognosis. This is due to a significant number of suppressor macrophages producing IL-10 that decreases the expression of MHC class II determinants and IL-2R on T cells [192]. However, no significant difference in the spontaneous and LPS-stimulated IL-10 production by alveolar macrophages is observed in patients with metastatic lung cancer [199]. The presence of iNOS was observed in TIM, especially intratumoural, infiltrating gastric [200] and breast cancers [201, 202]. The expression of iNOS in infiltrating cells positively correlates with the metastasis formation in breast cancer [202]. Although TIM from ovarian and colorectal cancers show the presence of TNF-mRNA [203, 204], the production of proinflammatory cytokines like IL-1 and IL-6 by TIM from ovarian cancer, upon stimulation with endotoxin, is decreased in comparsion to monocytes from the same patients [205]. TIM from non-small-cell lung cancer exhibit decreased tumouricidal potential after stimulation with different stimuli in comparsion to peripheral blood monocytes and normal alveolar macrophages [137].

Antitumour activity of TIM is considerably decreased in comparison to blood monocytes [206]. This indicates the suppressive role of tumour microenvironment on TIM *in situ*. In advanced stages of breast cancer, TIM may be ineffective or even promote tumour growth [189]. It is known that activated macrophages are able to produce several growth factors, including: TGF-α and -β, fibroblast growth factor (FGF), IL-1 and endothelial growth factor (EGF). There is a positive association between the degree of TIM infiltration and progression of breast tumours [207]. In breast cancer TIM are involved in stroma formation by transformation into fibroblast-like cells, which produce collagen type I [207]. Tumour may influence the activity of TIM by modulating the binding of TIM to ECM proteins. TIM can secrete proteases, which degrade the surrounding tissue and could facilitate tumour cell expansion and infiltration of the tissues. The activities of two families of proteases: MMPs and urokinases are associated with tumour invasiveness and are important in angiogenesis. MMPs faciliate tumour invasion and metastasis through degradation of ECM compounds like collagens, laminins, proteoglycans and modulation of cell adhesion. MMPs may paradoxically stimulate the creation of biologic active proteins including chemotactic molecules derived from laminin-5 and angiostatin from plasminogen [208, 209, 210]. Colorectal cancer cells are able to stimulate monocytes production of MMP-2 and MMP-9, and this is dependent on metastatic potential of tumour cells. Also soluble products of metastatic colorectal cancer cells induce the expression of MMP-9 in monocytes [211]. The role of tissue inhibitors of MMPs (TIMPs) in cancer is complex. They are produced both by tumour cells and stromal fibroblasts. In experimental tumours TIMPs reduce tumour growth, metastasis and angiogenesis. On the other hand they may promote tumourigenesis and cancer progression through the influence on cell proliferation, apoptosis and MMP activity [212, 213].

Cell adhesion molecules are required for the cellular interactions and development of effective immune response. Contact between macrophages and cancer cells induces changes in the expression of adhesion molecules on both types of interacting cells. TIM from gastrointestinal cancers, especially localised along the invasive edge, show the expression of ICAM-1 (CD54) and lymphocytes from the invasive margin express LFA-1 (receptor of ICAM-1). In diffuse-type gastric cancer, majority of TIM are ICAM-negative [214]. ICAM-1 expression has also been observed on malignant cells including lymphomas [215], melanomas [216] and carcinomas [217-220]. Its expression may be upregulated by macrophage products: IFN-γ, TNF, IL-1α, β, IL-6 and ROI [218, 219, 221, 222] and by interaction of hyaluronan (present on or shed from cancer cells) with CD44 [223]. Expression of ICAM-1 on melanoma cells is associated with their susceptibility to monocyte cytotoxicity [224]. LFA-1 is also present on cancer cells. The enhancement of this molecule on cancer cells and concomitant upregulation of ICAM-1 on monocytes occur after coculture of these cells [162].

The role of TIM in neoangiogenesis

In breast cancer a significant correlation exists between the vascular grade of the tumour, shortened patient survival and number of TIM [190]. Several soluble products of TIM are responsible for neoangiogenesis. These include EGF, VEGF, FGF, platelets derived growth factor (PDGF), GM-CSF, TGF-α and β, IL-1, IL-6 and PGs. PDGF expression in TIM from breast cancer positively correlates with the tumour size and microvessel count [225]. The role of VEGF in the tumour growth is not limited to promotion of angiogenesis as it also stimulates extravasation of plasma fibrinogen that leads to fibrin deposition and increase of ECM within the tumour. This in turn promotes the ingrowth of TIM, fibroblasts and endothelial cells [226]. The release of VEGF is stimulated by hypoxia [227]. Thus, TIM from avascular and necrotic areas of breast cancer display an increased production of VEGF [228]. VEGF has also a crucial role in carcinoma-dependent ascites formation [229]. Not only TIM but also tumour cells are able to produce VEGF, which in turn acts as chemoattractant for macrophages [230]. Angiogenesis is also regulated by chemokines. ECR-CXC chemokines, including CXCL8 (IL-8), CXCL7 (NAP-2), CXCL5 (ENA-78) and CXCL1 (GROα), are a potent angiogenic factors, whereas non-ELR-CXC chemokines such as CXCL10 (IP-10) and CXCL9 (Mig) are angiostatic [231, 232]. Also the member of CC chemokine family, CCL2 (MCP-1), is the angiogenic factor that acts by augmenting both TIM accumulation and angiogenesis [233].

Both tumour cells and monocytes release urokinase plasminogen activator (uPA). It promotes angiogenesis through plasminogen activation and degradation of stroma components and vessel walls (probably the first step of neoangiogenesis) [234]. Some ECM compounds after degradation by plasmin induce angiogenesis [235]. In breast cancer uPA is mainly localized in peripheral parts of the tumour [236]. A specific cell surface uPA receptor (uPAR) has been identified on human monocytes and a variety of cancer cells. In colon adenocarcinoma, uPAR is expressed by tumour cells and by TIM localized at the invasive edge [237], which may facilitate tumour invasion and metastasis [238].

The role of TIM in metastasis formation

There is evidence that TIM are involved in the metastatic process. The association between the presence of large numbers of TIM and lymph node metastases in human breast cancer has been observed [239]. Highly invasive and metastatic tumours can secrete glycoproteins that act as tumour-associated antigens evoking production of antibodies that promote tumour cells invasion and growth. It is due to activation of tumour infiltrating immune cells and proteases secretion followed by ECM degradation and angiogenesis [240].

Tumour invasion and metastasis formation begins from blood vessels basement membranes degradation due to relase of matrix metalloproteinases by tumour cells and tumour cells activated monocytes/macrophages [241]. The next step is the formation of emboli in the microvasculature of different organs. PBMC form aggregates with renal cancer cells through the Siglec7 - the receptor for disialogangliosides, which is expressed by monocytes and NK cells. Metastatic potential of renal cell carcinoma is associated with the expression of gangliosides on tumour cells [242]. Coagulation associated with metastasis formation may also be the result of inappropriate expression of tissue factor in monocytes. Tissue factor (CD142), the main initiator of blood clotting, is produced by activated monocytes [243]. Tissue factor induces thrombin and in turn fibrin formation. Fibrin is known to stimulate the migration of endothelial cells and thus potentiate angiogenesis [244]. Cancer patients have higher monocyte CD142 expression [245] that correlates with tumour progression [246]. uPA produced by macrophages and tumour cells, play also an important role in tissue invasion and metastases formation [247]. High uPA levels are correlated with high vessel density in tumour and higher vascular invasion of tumour cells. uPA and its receptor is localised mainly in the periphery of tumour [236, 237]. uPA content in peripheral parts of the tumour is increased in patients with metastases [236]. It is connected with the proteolytic activity of plasmin on ECM, basement membrane and vessel walls [234, 248]. Cathepsins (lysosomal proteinases) are also involved in metastasis formation. Their expression have been found in TIM from bladder tumours [249] and breast cancers [250].

Some studies suggested the fusion of monocytes with certain haematopoietic tumour cells as an important mechanism of metastases formation [251]. Highly metastatic variant of T cell lymphoma cell line is derived *in vitro* from the spontaneous fusion of the lymphoma cells with the host macrophages [252]. However, the mechanisms of fusion in vivo remain unknown. TIM also participate in the osteolysis associated with bone metastases. They are the major cellular component of the inflammatory infiltratrates in the bones and can release local mediators that stimulate osteoclast activity. Moreover, they can also resorb the bone on their own and differentiate into osteoclast-like cells [253]. Although no data on the role of TIM in the peritoneal dissemination of human malignancy are available, in the murine model milky spots, which are aggregates of macrophages, are considered to be the locus for early peritoneal metastases formation [254, 255].

Monocytes in cancer immunotherapy

In local and systemic adoptive immunotherapy, the autologus effector cells harvested from the blood are activated *in vitro* and reinfused into the host [256-258]. Macrophages can be activated for tumour cell killing by some immunopotentiators [259]. Antitumour activity of monocytes may be enhanced by their incubation in the presence of IFN-γ, LPS, GM-CSF and (OH_2) VitD3 [147, 260-262]. In 1987, Stevenson et al. reported the first clinical trial with adoptive transfer of activated macrophages. They used IFNγ-activated macrophages for intraperitoneal infusions in patients with colorectal cancer [263]. Adoptive transfer of macrophages has undergone phase I clinical trials for patients with metastatic cancer (colon, ovarian, lung, renal, pancreatic cancer and melanoma) infused systemically or intraperitoneally [261, 264-266]. Monocytes differentiated in the presence of IFNγ were used for phase II adoptive therapy of advanced colorectal cancer. Commonly, continuous flow centrifugal leukapheresis and counterflow centrifugal elutriation are used for monocyte isolation. However, the clinical results of adoptive immunotherapy are still controversial [264]. No significant partial or complete responses have been reported, though prolonged disease free intervals were observed [261, 266].

Monocyte cytocidal activity can be enhanced by chemotherapeutic drugs, e.g. cisplatin [268], IFNγ and murapeptides [269]. There is some evidence that activated monocytes are cytotoxic to drug resistant tumour cells [270, 271]. They can also carry cytotoxic drugs and immunomodulators [272, 273]. Immunostimulation with IFN(may reverse monocyte deactivation in patients with chemotherapy-induced neutropenia and the serious infections and cause clinical improvement and increase the level of CD14+ DR+ circulating monocytes [274]. The novel approche is to utilize the ability of macrophages to migrate into hypoxic areas of the tumour and use them for delivering gene therapy [275]. However, currently adoptive therapy with the use of monocytes/macrophages is still at infancy.

Abbreviations

AA – arachidonic acid
CCL – CC chemokine family
CSF – colony stimulating factor
CCR – CC receptors
CR – chemokine receptor
ECM – extracellular matrix
EGF – endothelial growth factor
FasL – Fas-ligand
FcR – the receptor of Fc part of immunoglobulin
FGF – fibroblast growth factor
GM–CSF - ggranulocyte-macrophage colony stimulating factor
ICAM-1 – intercellular adhesion molecule-1
IFN – interferon
IL – interleukin
iNOS – inducible nitric oxide synthase
LFA–3 - leucocyte functional antigen-3
LPS – lipopolysaccharyde
LT – leukotrien
MCP – monocyte chemotactic protein
M-CSF – macrophage colony stimulating factor
MMPs – matrix metalloproteinases
NO – nitric oxide
PAI – inhibitor of plasminogen activation
PBMC – peripheral blood mononuclear cells
PDGF – plated derived growth factor
PG – prostaglandin
PKA – camp-dependent kinase
PKC – calcium-phospholipid-dependent protein kinase
PTK – tyrosine protein kinase
RNI– reactive nitrogen intermediates
ROI – reactive oxygen intermediates
TGF – transforming growth factor
TIM – tumour infiltrating macrophages
TIMP – tissue inhibitor of matrix metalloproteinases
TNF – tumour necrosis factor
TX – tromboxane
uPA – urokinase plasminogen activator
VCAM–1 - vascular adhesion molecule-1
VEGF – vascular endothelial growth factor
VLA–4 - very late antigen-4

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