Pleural macrophages are involved in local defense mechanisms against environmental pollution, bacteria and cancer. Their main function encompasses phagocytosis of degenerated mesothelial cells. In human pleural effusions macrophages represent more than half of all cells. A model of polarized macrophage activation (M1 and M2) was proposed, which defines a functionally different macrophage populations generated in response to various factors present in the inflamed environment. Tumor associated macrophages are a major component of the inflammatory infiltrate of most cancers. They can promote the proliferation and spread of cancer cells in the early stages of carcinogenesis and during metastasis. Macrophages isolated from malignant pleural effusions as well as tumor associated macrophages exhibit weak cytotoxic activity against tumor cells, increase their proliferative activity and may protect tumor cells from apoptosis. Defining biology of macrophages present in specific environment of the pleural effusion could allow the introduction of innovative diagnostic and therapeutic strategies.

Key words: pleural macrophages, malignant pleural effusion, M1/M2, tumor associated macrophages, TAM.

Macrophages in malignant pleural effusions – alternatively activated tumor associated macrophages

Mariusz Kaczmarek, Jan Sikora

Department of Immunology, Chair of Clinical Immunology, Poznan University of Medical Sciences, Poland

Pleural macrophages

Macrophages are phagocytic cells of myeloid origin that possess the ability of antigen presentation. They can be classified as resident (attached to a particular tissue), or migrating (free) cells. Resident macrophages adapt to the local environment in order to carry out specific functions, e.g. control inflammation or restore damaged tissue [1]. Despite heterogeneity resulting from the settlement of various organs, these cells also show diversity within a single organ. Pulmonary macrophages, according to location, can be divided into alveolar, airway, interstitial, and those present in the vessels of pulmonary circulation. Other mediastinal macrophage populations include pleural macrophages residing in the pleural space and macrophages resident in the local lymph nodes that collect lymph from the lungs. In humans, macrophages constitute over a half of all cells in the pleural fluid (Figure). Pleural macrophages differentiate from peripheral blood monocytes migrating through the lining of the pleural mesothelium. Their increased influx occurs during the parenchymal inflammatory response [2]. Normally, they proliferate locally to maintain a constant number in the pleura. Presumably, similarly to the peritoneum, a constant pool of macrophages resides on the edge of the pleura. These macrophages are believed to accumulate in the pleural fluid during inflammation associated with cancer. Rat pleural macrophages are less frequent in numbers than the alveolar macrophages, have limited capacity of Fcγ receptor-dependent phagocytosis and weaker non-specific esterase activity. They express fewer surface antigens, which conforms them to peritoneal macrophages [3]. Like monocytes, pleural macrophages show an intermediate level of expression of CD16 and increased expression of CD14 antigens. The level of expression of CD11b, CD33 and CD64 is similar to that observed for CD14⁺⁺ monocytes, while expression of CD32, CD54 and HLA-DR is higher [4]. Due to the high density of HLA-DR and ICAM-1 molecules, pleural macrophages exhibit enhanced ability to present antigens. They participate in the local defense against environmental pollution, bacteria and neoplastic cells. Inside the pleural space, they are the active helper cells of the immune response. Through cytokine secretion, they participate in generating an inflammatory and fibrotic response. In comparison to monocytes they live longer, show more efficient phagocytosis and perform more diverse functions, and their biological activity depends mainly on the microenvironment in which they are located [5]. Macrophages contribute to maintaining tissue homeostasis, and their main function is phagocytosis of degenerated mesothelial cells. They also phagocytose biotic material penetrating into the pleural space, and granulocytes which previously absorbed particles [2]. In response to deposition of foreign particles in the lungs, the number of pleural macrophages increases, which is related to increased concentrations of chemotactic factors and mitogens potentiating the penetration of alveolar macrophages into the pleural space [6]. After lipopolysaccharide (LPS) stimulation, pleural macrophages secrete

greater amounts of tumor necrosis factor α (TNF- α) and interleukin 10 (IL-10) than monocytes, although the expression of mRNA encoding the IL-10 in non-stimulated cells remains constantly high. By secreting IL-1 β , TNF- α , IL-6, and GRO- β , macrophages support pleural neutrophils influx [7]. Inflammatory cytokines produced by macrophages that reside in the pleura, e.g. TNF- α and IL-1 β , are necessary for the secretion of chemokines of CXC (e.g. GRO- β , IL-8) and CC (e.g. MCP1) families by mesothelial cells [8].

Two models of macrophage activation: M1 vs. M2

A polarized model of macrophages activation differentiates functionally macrophages formed in response to various inflammatory environmental factors. According to the proposed mechanism, macrophages secrete either IL-12 necessary for the synthesis of interferon γ (IFN- γ) and induction of the Th1 response in lymphocytes, or IL-10 enhancing production of IL-4/IL-13 and inducing the Th2 response. Macrophage phenotype M1 is characterized by IL-12^{high} IL-23^{high} IL-10^{low}. Its development is dependent on IFN- γ , IL-12 and IL-18 [9, 10]. Macrophages type M1 have increased expression of HLA-DR, CD80, CD86, TLR2, TLR4 and TLR6 molecules on their surface [11]. They secrete significant amounts of pro-inflammatory cytokines (i.e. IFN- γ , TNF- α , IL-1 α , IL-1 β , IL-6, IL-18) and produce increased amounts of cytotoxic reactive oxygen and nitrogen species (ROS and RNS) [12].

At the same time they demonstrate a reduced ability to absorb apoptotic cells [13]. Inside the M1 population, two subtypes, M1a and M1b, can be distinguished. Unlike the typical M1, M1b macrophages exhibit reduced phagocytic properties and their main marker is the receptor MARCO [14].

Macrophages type M2 are activated by IL-4, IL-10, IL-13, immune complexes, glucocorticoids (GC) or secosteroid hormones (i.e. vitamin D₃). They exhibit a common phenotype, IL-12^{low} IL-23^{low} IL-10^{high}, but differ in the ability to produce pro-inflammatory cytokines. They have immunoregulatory functions and participate in the Th2 response [15]. Macrophages are activated in the direction of M2 during parasitic infections, development of cancer, atherosclerosis, and central nervous system repair. They participate in wound healing, removing dead tissue, fibroblast activation, as well as in growth and tissue remodeling during angiogenesis [10]. Macrophages M2 predominantly express nonopsonic receptors, mainly scavenger receptors (e.g., SR-A, SR-B) and membrane C-type lectin receptors [16]. These cells exhibit increased expression of CD62L, an adhesion molecule involved in the process of colonization of secondary lymphoid organs [17]. They induce signals that inhibit expression of proinflammatory chemokines and secrete post-inflammatory chemokines associated with an alternative type of immune response (e.g. MCP-4, HCC-1, TARC) [15]. Three variants of M2 polarization can be distinguished: M2a (alternative activation) induced by IL-4 or IL-13; M2b (activation of type II; innate activation) resulting from activation of Fc γ receptors in the presence of Toll-like receptor (TLR) stimulants; and M2c (deactivation), which includes a variety of stimuli-induced inactivation of macrophages, e.g. IL-10, transforming growth factor β (TGF- β) or GC. M2a macrophages do not express

inducible nitric oxide synthase (iNOS) but are characterized by high expression of arginase 1 (ARG1), and thereby stimulate cell growth, collagen formation and tissue repair. Macrophages type M2b secrete chemokine I-309, stimulate the CCR8 receptor present on eosinophils, Th2 and T-regulatory lymphocytes (T-reg), and thus enhance Th2 polarization and immunoregulation [10, 12, 18]. However, expression of sphingosine kinase 1 (SPHK1) assimilates them to type M1 [16]. In contrast to M2a, M2b macrophages, despite large amounts of IL-10, also produce TNF- α , IL-1 β and IL-6 [19]. M2c macrophages are distinguished by reduced expression of proinflammatory cytokines and increased cleaning of tissue and cell residues. On their surface, they exhibit increased expression of CD14 antigen, which indicates a return from the activated status to equilibrium [20]. Macrophages from malignant and non-malignant pleural effusions differ from each other. The phenotype of macrophages isolated from malignant effusions moved in the direction of M2 activation profile [21].

Tumor-associated macrophages

Tumor-associated macrophages (TAMs) are a major component of the inflammatory infiltrate in most cancers. In breast cancer, their proportion reaches up to 80% of all leukocytes [22]. The number of macrophages present in infiltrates of esophageal, breast, pancreatic and ovarian cancers as well as melanoma has been correlated with the level of CCL2 [23–25]. A relationship between infiltration of macrophages, expression of colony stimulating factor 1 (CSF-1), and late phase breast cancer or the formation of lung metastases, has also been reported [26]. *In vitro*, TAMs exhibit a reduced ability to lyse tumor cells, impaired function of presenting tumor-associated antigens to T cells, and expression of cytokines inducing antitumor activity of T lymphocytes and natural killer (NK) cells. Tumor microenvironment rich in IL-4, IL-6, IL-10, TGF β 1 and CSF-1 polarizes them toward the M2 phenotype. Interleukin-10 induces features of TAMs related to phenotype M2c, such as phagocytosis of cell residues, participation in angiogenesis and tissue repair and remodeling [27]. Their phenotype depends on the type and stage of cancer development. In areas of chronic inflammation associated with less mature cancer they exhibit a phenotype similar to M1, while in areas of mature and more diverse cancer they exhibit a phenotype similar to M2. They may also exhibit a mixed phenotype of both M1 and M2 [28]. TAMs exhibit defective activation of NF κ B transcription factor associated with its constitutive translocation of the inhibitor to the nucleus [29]. Their numbers in the specific tissue also depend on tumor hypoxia (low tissue oxygen concentration). *In-situ* environment demonstrating insufficient oxygen concentration reduces the mobility of TAMs and causes their immobilization. Tumor hypoxia also enhances the secretion of HIF-1-dependent proteins associated with the angiogenic, invasive and immunosuppressive phenotype [e.g. vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), angiopoietin 1, cyclooxygenase 2 (COX-2)] [30]. TAM accumulation in hypoxic/necrotic areas correlates with increased risk of metastasis and poor prognosis in breast and endometrial cancers [31, 32]. Depending on the type of secret-

ed regulators, TAMs may act anti- or pro-malignantly. Their activation may result from direct contact with a tumor cell, or from mediators secreted into the environment by cells involved in immunological processes, mainly T helper cells. Macrophages activated by IL-2, interferon γ (IFN- γ) and IL-12 can kill cancer cells, yet they can effectively limit the antitumor cytotoxic T lymphocyte (CTL) response by secreting IL-10 [33]. TAMs are able to eliminate cancer cells by macrophage-mediated tumor cytotoxicity (MTC) or antibody-dependent cellular cytotoxicity (ADCC) [34, 35]. They may also inhibit cancer development by secreting TNF- α or nitric oxide (NO), which induce apoptosis in many tumor types *in vitro* [36]. The presence of macrophages in the leucocyte infiltrate may be associated with a successful outcome for stomach or colon cancers and melanoma [37–39]. However, TAMs more often lead to cancer growth and progression and immunosuppression. They can promote proliferation and spread of cancer cells in early stages of carcinogenesis and during formation of metastases [40]. TAMs play a central role in the relationship between inflammation and initiation of various types of neoplasms. Macrophages present in the areas of inflammation can cause extensive damage to the surrounding epithelial tissue, mutations and damage to cellular DNA, and defective activity of p53. Persistent changes resulting from their activities may lead to the transformation of premalignant epithelial cells and initiate tumor formation. The presence of TAMs may be a poor prognostic factor in the course of cancer of the esophagus, lung, breast, kidney, bladder, endometrium, prostate, uveal melanoma as well as follicular lymphomas [41–48]. TAMs secrete factors stimulating tumor cell proliferation and survival (e.g. TGF- β 1, VEGF, PDGFB) [49]. TAMs producing estrogen stimulate breast cancer cell proliferation and enhance its invasiveness [50]. They are involved in tumor invasion to the surrounding healthy tissue and in tumor stroma formation, through releasing mediators affecting the dissolution of the connective tissue, mainly extracellular matrix degrading enzymes (matrix metalloproteinases and serine proteases) and activators of these enzymes [51, 52]. TAMs are involved in the release of metastatic cells from primary tumors as well as in stabilizing the position of metastases in distant places. A so-called “wolf in sheep’s clothing” model was proposed in which to produce diversity, preservation of chromosomal aberrations, epigenetic regulation, and metastasis, tumor cells fuse with macrophages. Such hybrids gain the ability of tropism, like macrophages [53]. TAMs are important modulators of angiogenesis enabling the formation of new blood vessels and converting them into coherent functional networks. They release many angiogenic cytokines (e.g. TNF- α , IL-1 α , IL-1 β , IL-6), chemokines (e.g. CXCL1, CXCL2, CXCL8), growth factors (e.g. TGF- α , VEGF, PDGF, angiopoietins), and enzymes that modulate angiogenesis (e.g. COX-2, iNOS, MMP2, MMP7, MMP9) [54]. The number of TAMs correlates with increased angiogenesis in gliomas, cancer of the esophagus, breast, bladder, prostate, and uveal melanoma [34, 41, 44, 46, 47, 55]. Secreting immunosuppressive factors, such as prostaglandins (PGs), IL-10, and TGF- β , they can indirectly promote tumor growth. These mediators impair the cytotoxic activity of TAMs, and limit their ability to present tumor-as-

ciated antigens of cytotoxic T lymphocytes, as well as the capacity for phagocytosis [56, 57].

Pleural macrophages and tumor

Macrophages derived from pleural effusions are a convenient material to conduct the evaluation of existing relationships between immune cells and tumor cells. Pleural effusion constitutes a microenvironment rich in soluble factors devoid of connective barriers, which makes interactions between cells much easier. During the inflammatory response associated with infiltration of a primary bronchial cancer in pleura, macrophages from exudate and transudate fluids release IL-1 β , TNF- α , and IL-8. Thereby, they affect T cell proliferation, enhance their accumulation and clonal expansion, and stimulate them to release local cytokines [58]. Additionally, a relationship between a decrease in expression of TCR ζ molecules on T cells, and an increase in the number of monocytes/macrophages in malignant effusions has been reported. In this case, cells with reduced expression of the ζ chain were the main subpopulation of T cells undergoing apoptosis [59]. The pivotal role of pleural macrophages is to phagocytose degenerated mesothelial cells. The phagocytosis of apoptotic cells changes classically activated macrophages from histotoxic inflammatory cells into alternatively activated anti-inflammatory cells that are similar to macrophages type M2c [10]. After the uptake of apoptotic cells, TAMs secrete mediators facilitating growth of cancer cells, and thereby their cytotoxicity is reduced. The impairment of natural cytotoxic activity and the ability to kill autologous tumor cells shown by macrophages isolated from malignant pleural effusions have been demonstrated [60]. Carcinogenesis is a multistep process in which cells undergo malignant transformation, autonomously promote their growth, produce their own circulation and resistance to apoptosis, escape from immune surveillance, and finally acquire the capacity for metastasis. IL-4, IL-10, PGE₂ and TGF- β secreted by TAMs affect the increased expression of anti-apoptotic proteins c-FLIP and BclxL [61, 62]. MMP7 digests the FasL molecules from the cell surface, thereby weakening the sensitivity of tumors to chemotherapeutic agents (i.e. doxorubicin) and cytotoxicity of CTLs and NK cells [63]. Soluble phosphatidylserine (sPS) exfoliated by TAM activity induces an

Fig. 1. Pleural effusion macrophages

inflammatory response of falsely activated TAMs. This process enhances tumor cell proliferation and escape of developing tumor cells from immune surveillance [56]. Conditioned media obtained from *in vitro* culture of macrophages isolated from malignant pleural effusions increase the proliferative activity of selected human cancer cell lines. Breast cancer (MDA-MB231), lung (A549), colon (HT29, HCT116, SW620) and hematopoietic (Jurkat, HL60) cell lines manifested an increased percentage of cells in S phase of the cell cycle when cultured in the conditioned media. Assessment of annexin V binding and TUNEL reaction demonstrated that pleural macrophages may protect cancer cells from apoptosis. The antiapoptotic properties of conditioned media correlated with their effect on the expression and activity of proteins regulating apoptosis (reduction in the expression of Fas receptor, expression and activity of caspase-3, and increased expression of Bcl2 and survivin) [64].

Tumor-associated macrophages in diagnosis and therapy

Macrophages are a significant component of the link between chronic inflammation and cancer development. Understanding their biology may largely benefit the knowledge of tissue reconditioning to a normal, pre-pathogenic state. Full determination of the mode of action in the tumor microenvironment requires precise information on specific areas of macrophage activity. Signals responsible for their polarization, identification of genes regulating their activation, determination of signals processed by them within the damaged tissue leading to their polarity, and development of systems for their manipulation *in vivo* are the most crucial elements [65]. Therapeutic strategies using TAMs involve inhibition of their recruitment and survival in locations of tumor development (e.g. trabectedin, brand name Yondelis), inhibition of their effects on angiogenesis and extracellular matrix remodeling (e.g. Linomid, zoledronic acid bisphosphate), and the reversal of immunosuppression and restoration of anticancer cytotoxicity [66–68]. Because of the correlation between the number of macrophages and the development of various types of cancer, TAM evaluation may be important for clinical or diagnostic purposes. Reducing the number of macrophages in glomerulonephritis decreased kidney damage, while in breast cancer it slowed pre-invasive change to full tumor, with simultaneous reduction of the formation of lung metastases [26]. Elimination of macrophages decreased the density of vascularity of advanced cancer by 50% [69]. Macrophage depletion may also be important due to their strong tendency to migrate to places of hypoxia, which may affect formation of relapses. Resulting from different therapy (e.g. radiotherapy, photodynamic therapy, antiangiogenic therapy), hypoxic areas associated with necrosis attract macrophages to these places [70–72]. In such situations, macrophages can switch their phenotype toward promalignant and instead of destroying cancer cells, promote revascularization and reoxygenation, and therefore re-contribute to tumor growth after therapy. Macrophages can be used in cellular immunotherapy, recovering autologous mononuclear cells from patients, activating them *in vitro* and switching back to the patient

[73, 74]. Alternatively activated macrophages can be used to reduce injuries and promote repair processes (e.g. stabilization of atherosclerotic plaques) [10]. There has been an attempt to shift M2 polarization of TAMs into the M1 direction to restore inflammatory antitumor activity [75]. On the other hand, the natural ability of macrophages to migrate toward areas of hypoxia has been used for direct delivery of a cytotoxin during tumor development, to serve as hypoxia-induced vectors for genes encoding cytotoxic proteins [76]. Macrophages were transfected with the IFN- γ gene [77] and a hypoxia-induced gene encoding a pro-drug which activated cytochrome P450 [69].

The symbiotic relationship between cancerous disease and macrophages in the environment of a tumor, where cancer cells can affect the survival of macrophages by producing factors that cause tumor growth and angiogenesis, is highly unfavorable for therapeutic purposes. However, accurate characterization of this relationship may help to reverse this trend and enable the introduction of innovative diagnostic and therapeutic strategies.

References

- Gordon S. The role of the macrophage in immune regulation. *Res Immunol* 1998; 149: 685-688.
- Lehnert BE. Pulmonary and thoracic macrophage subpopulations and clearance of particles from the lung. *Environ Health Perspect* 1992; 97: 17-46.
- Gjomarkaj M, Pace E, Melis M, Spatafora M, Profita M, Vignola AM, Bonsignore G, Toews GB. Phenotypic and functional characterization of normal rat pleural macrophages in comparison with autologous peritoneal and alveolar macrophages. *Am J Respir Cell Mol Biol* 1999; 20: 135-42.
- Frankenberger M, Passlick B, Hofer T, Siebeck M, Maier KL, Ziegler-Heitbrock LH. Immunologic characterization of normal human pleural macrophages. *Am J Respir Cell Mol Biol* 2000; 23: 419-26.
- Wu HM, Jin M, Marsh CB. Toward functional proteomics of alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2005; 288: L585-95.
- Adamson IY, Bowden DH. Chemotactic and mitogenic components of the alveolar macrophage response to particles and neutrophil chemoattractant. *Am J Pathol* 1982; 109: 71-7.
- Murai N, Nagai K, Fujisawa H, Hatanaka K, Kawamura M, Harada Y. Concurrent evolution and resolution in an acute inflammatory model of rat carrageenin-induced pleurisy. *J Leukoc Biol* 2003; 73: 456-63.
- Cailhier JF, Sawatzky DA, Kipari T, et al. Resident pleural macrophages are key orchestrators of neutrophil recruitment in pleural inflammation. *Am J Respir Crit Care Med* 2006; 173: 540-7.
- Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008; 13: 453-61.
- Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* 2003; 3: 23-35.
- Biswas A, Banerjee P, Mukherjee G, Biswas T. Porin of *Shigella dysenteriae* activates mouse peritoneal macrophage through Toll-like receptors 2 and 6 to induce polarized type I response. *Mol Immunol* 2007; 44: 812-20.
- Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* 2003; 73: 209-12.
- Erwig LP, Kluth DC, Walsh GM, Rees AJ. Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines. *J Immunol* 1998; 161: 1983-8.
- Mukhopadhyay S, Chen Y, Sankala M, Peiser L, Pikkarainen T, Kraal G, Tryggvason K, Gordon S. MARCO, an innate activation marker of macrophages, is a class A scavenger receptor for *Neisseria meningitidis*. *Eur J Immunol* 2006; 36: 940-9.

15. 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.
16. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006; 177: 7303-11.
17. Patarroyo M. Adhesion molecules mediating recruitment of monocytes to inflamed tissue. *Immunobiology* 1994; 191: 474-7.
18. Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. *J Immunol* 1998; 160: 5347-54.
19. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; 5: 953-64.
20. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* 2005; 23: 901-44.
21. Kaczmarek M, Nowicka A, Kozłowska M, Zurawski J, Batura-Gabryel H, Sikora J. Evaluation of the phenotype pattern of macrophages isolated from malignant and non-malignant pleural effusions. *Tumour Biol* 2011; 32: 1123-32.
22. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002; 196: 254-65.
23. Nesbit M, Schaidt 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.
24. Negus RP, Stamp GW, Relf MG, et al. The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. *J Clin Invest* 1995; 95: 2391-6.
25. 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.
26. 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.
27. Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur J Cancer* 2006; 42: 717-27.
28. Biswas SK, Sica A, Lewis CE. Plasticity of macrophage function during tumor progression: regulation by distinct molecular mechanisms. *J Immunol* 2008; 180: 2011-7.
29. Chen JJ, Lin YC, Yao PL, et al. Tumor-associated macrophages: the double-edged sword in cancer progression. *J Clin Oncol* 2005; 23: 953-64.
30. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* 2000; 157: 411-21.
31. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996; 56: 4625-29.
32. Ohno S, Ohno Y, Suzuki N, et al. Correlation of histological localization of tumor-associated macrophages with clinicopathological features in endometrial cancer. *Anticancer Res* 2004; 24: 3335-42.
33. Tsung K, Dolan JP, Tsung YL, Norton JA. Macrophages as effector cells in interleukin 12-induced T cell-dependent tumor rejection. *Cancer Res* 2002; 62: 5069-75.
34. Leek RD, Harris AL. Tumor-associated macrophages in breast cancer. *J Mammary Gland Biol Neoplasia* 2002; 7: 177-89.
35. te Velde AA, Figdor CG. Monocyte mediated cytotoxic activity against melanoma. *Melanoma Res* 1992; 1: 303-9.
36. Klimp AH, de Vries EG, Scherphof GL, Daemen T. A potential role of macrophage activation in the treatment of cancer. *Crit Rev Oncol Hematol* 2002; 44: 143-61.
37. Ohno S, Inagawa H, Dhar DK, et al. The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. *Anticancer Res* 2003; 23: 5015-22.
38. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE. Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. *Oncol Rep* 2003; 10: 309-13.
39. Piras F, Colombari R, Minerba L, et al. The predictive value of CD8, CD4, CD68, and human leukocyte antigen-D-related cells in the prognosis of cutaneous malignant melanoma with vertical growth phase. *Cancer* 2005; 104: 1246-54.
40. Coussens LM, Werb Z. Inflammatory cells and cancer: think different! *J Exp Med* 2001; 193: F23-26.
41. Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S. Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. *Am J Gastroenterol* 2004; 99: 1667-74.
42. Takanami I, Takeuchi K, Kodaira S. Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis. *Oncology* 1999; 57: 138-42.
43. Hamada I, Kato M, Yamasaki T, Iwabuchi K, Watanabe T, Yamada T, Itoyama S, Ito H, Okada K. Clinical effects of tumor-associated macrophages and dendritic cells on renal cell carcinoma. *Anticancer Res* 2002; 22: 4281-4.
44. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol* 2000; 7: 263-9.
45. Tsutsui S, Yasuda K, Suzuki K, Tahara K, Higashi H, Era S. Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. *Oncol Rep* 2005; 14: 425-31.
46. Lissbrant IF, Stattin P, Wikstrom P, Damber JE, Egevad L, Bergh A. Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol* 2000; 17: 445-51.
47. Makitie T, Summanen P, Tarkkanen A, Kivela T. Tumor-infiltrating macrophages (CD68+) cells and prognosis in malignant uveal melanoma. *Invest Ophthalmol Vis Sci* 2001; 42: 1414-21.
48. Farinha P, Masoudi H, Skinnider BF, et al. Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). *Blood* 2005; 106: 2169-74.
49. Goswami S, Sahai E, Wyckoff JB, et al. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res* 2005; 65: 5278-83.
50. Mor G, Yue W, Santen RJ, et al. Macrophages, estrogen and the microenvironment of breast cancer. *J Steroid Biochem Mol Biol* 1998; 67: 403-11.
51. Ishikawa S, Egami H, Kurizaki T, Akagi J, Tamori Y, Yoshida N, Tan X, Hayashi N, Ogawa M. Identification of genes related to invasion and metastasis in pancreatic cancer by cDNA representational difference analysis. *J Exp Clin Cancer Res* 2003; 22: 299-306.
52. Hildenbrand R, Dilger I, Horlin A, Stutte HJ. Urokinase and macrophages in tumour angiogenesis. *Br J Cancer* 1995; 72: 818-23.
53. Hume DA. The mononuclear phagocyte system. *Curr Opin Immunol* 2006; 18: 49-53.
54. Ellis LM, Liu W, Ahmad SA, Fan F, Jung YD, Shaheen RM, Reinmuth N. Overview of angiogenesis: Biologic implications for antiangiogenic therapy. *Semin Oncol* 2001; 28: 94-104.
55. Nishie A, Ono M, Shono T, et al. Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. *Clin Cancer Res* 1999; 5: 1107-13.
56. Kim R, Emi M, Tanabe K. Cancer cell immune escape and tumor progression by exploitation of anti-inflammatory and pro-inflammatory responses. *Cancer Biol Ther* 2005; 4: 924-33.
57. Elgert KD, Alleve DG, Mullins DW. Tumor-induced immune dysfunction: the macrophage connection. *J Leukoc Biol* 1998; 64: 275-90.
58. Park JS, Kim YS, Jee YK, Myong NH, Lee KY. Interleukin-8 production in tuberculous pleurisy: role of mesothelial cells stimulated by cytokine network involving tumour necrosis factor-alpha and interleukin-1 beta. *Scand J Immunol* 2003; 57: 463-9.
59. Sikora J, Dworacki G, Giersz R, Zeromski J. The role of monocytes/macrophages in TCR-zeta chain downregulation and apoptosis.

- tosis of T lymphocytes in malignant pleural effusions. *J Biol Regul Homeost Agents* 2004; 18: 26-32.
60. Yanagawa E, Uchida A, Moore M, Micksche M. Autologous tumor killing and natural cytotoxic activity of tumor-associated macrophages in cancer patients. *Cancer Immunol Immunother* 1985; 19: 163-7.
  61. España L, Fernández Y, Rubio N, Torregrosa A, Blanco J, Sierra A. Over-expression of Bcl-xL in human breast cancer cells enhances organ-selective lymph node metastasis. *Breast Cancer Res Treat* 2004; 87: 33-44.
  62. Jung YJ, Kim JY, Park JH. TGF-beta1 inhibits Fas-mediated apoptosis by regulating surface Fas and cFLIPL expression in human leukaemia/lymphoma cells. *Int J Mol Med* 2004; 13: 99-104.
  63. Mitsiades N, Yu WH, Poulaki V, Tsokos M, Stamenkovic I. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. *Cancer Res* 2001; 61: 577-81.
  64. Kaczmarek M, Frydrychowicz M, Nowicka A, et al. Influence of pleural macrophages on proliferative activity and apoptosis regulating proteins of malignant cells. *J Physiol Pharmacol* 2008; 59 Suppl 6: 321-30.
  65. Wilson HM, Walbaum D, Rees AJ. Macrophages and the kidney. *Curr Opin Nephrol Hypertens* 2004; 13: 285-90.
  66. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* 2008; 66: 1-9.
  67. 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.
  68. 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.
  69. Lewis C, Murdoch C. Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol* 2005; 167: 627-35.
  70. Schouwink H, Oppelaar H, Ruevekamp M, van der Valk M, Hart G, Rijken P, Baas P, Stewart FA. Oxygen depletion during and after mTH-PC-mediated photodynamic therapy in RIF1 and H-MESO1 tumors. *Radiat Res* 2003; 159: 190-8.
  71. Staton CA, Brown NJ, Rodgers GR, Corke KP, Tazzyman S, Underwood JC, Lewis CE. Alphastatin, a 24-amino acid fragment of human fibrinogen, is a potent new inhibitor of activated endothelial cells in vitro and in vivo. *Blood* 2004; 103: 601-6.
  72. Vujaskovic Z, Anscher MS, Feng QF, Rabbani ZN, Amin K, Samulski TS, Dewhirst MW, Haroon ZA. Radiation-induced hypoxia may perpetuate late normal tissue injury. *Int J Radiat Oncol Biol Phys* 2001; 50: 851-5.
  73. Andreesen R, Hennemann B, Krause SW. Adoptive immunotherapy of cancer using monocyte-derived macrophages: rationale, current status, and perspectives. *J Leukoc Biol* 1998; 64: 419-26.
  74. Hennemann B, Scheibenbogen C, Schumichen C, Andreesen R. Intrahepatic adoptive immunotherapy with autologous tumor cytotoxic macrophages in patients with cancer. *J Immunother Emphasis Tumor Immunol* 1995; 18: 19-27.
  75. Guiducci C, Vicari AP, Sangaletti G, Trinchieri G, Colombo MP. Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. *Cancer Res* 2005; 65: 3437-46.
  76. Cowen RL, Garside EJ, Fitzpatrick B, Papadopoulou MV, Williams KJ. Gene therapy approaches to enhance bioreductive drug treatment. *Br J Radiol* 2008; 81 Spec 1: S45-56.
  77. 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.

Address for correspondence

Mariusz Kaczmarek
Department of Immunology,
Chair of Clinical Immunology,
Rokietnica 5D
60-806 Poznan

Submitted: 6.10.2011

Accepted: 7.02.2012