Angiogenesis Review Series *J. Cell. Mol. Med. Vol 13, No 9A, 2009 pp. 2822-2833*

Guest Editor: N. Moldovan

Immune cells and angiogenesis

Domenico Ribatti [a, *], Enrico Crivellato [b]

[a] *Department of Human Anatomy and Histology, University of Bari Medical School, Bari, Italy*
[b] *Department of Medical and Morphological Research, Anatomy Section, University of Udine Medical School, Udine, Italy*

Received: March 5, 2009; Accepted: May 6, 2009

Abstract

Both innate and adaptive immune cells are involved in the mechanisms of endothelial cell proliferation, migration and activation, through the production and release of a large spectrum of pro-angiogenic mediators. These may create the specific microenvironment that favours an increased rate of tissue vascularization. In this review, we will focus on the immune cell component of the angiogenic process in inflammation and tumour growth. As angiogenesis is the result of a net balance between the activities exerted by positive and negative regulators, we will also provide information on some antiangiogenic properties of immune cells that may be utilized for a potential pharmacological use as antiangiogenic agents in inflammation as well as in cancer.

Keywords: angiogenesis • antiangiogenesis • chronic inflammation • immune cells • tumour growth

Introduction

Immune cells can be divided into innate (myeloid) and adaptive (lymphoid) cells. Innate immune cells, including macrophages, polymorphonuclear granulocytes (neutrophils, basophils and eosinophils) mast cells, dendritic cells (DC), natural killer (NK) cells and platelets represent the first line of defence against pathogens and foreign agents. The innate immune cells can directly eliminate pathogenic agents *in situ*. DCs, on the other hand, take up foreign antigens and migrate to lymphoid organs where they present their antigens to adpative immune cells. Adaptive immune cells are lymphocytes (T and B cells), which undergo clonal expansion and elaborate an adaptive response targeted to the foreign agent.

Whereas the cells of the innate immune system are found in the blood stream and in most organs of the body, lymphocytes are localized to specialized organs and tissues. There is tight interplay between innate immune cells and the vascular system. Endothelial cells mediate immune cell recruitment to extravascular tissues by expressing a repertoire of leucocyte adhesion molecules. On the other hand, innate immune cells synthesize a number of soluble factors that influence endothelial cell behaviour. Recruitment of an inflammatory infiltrate supports angiogenesis and tissue remodelling.

The importance of angiogenesis in physiological and pathological conditions

Angiogenesis, *i.e.* the formation of new vessels from pre-existing ones such as capillaries and post-capillary venules, plays a pivotal role during embryonal development [1] and later, in adult life, in

*Correspondence to: Prof. Domenico RIBATTI,
Department of Human Anatomy and Histology,
University of Bari Medical School,
Piazza G. Cesare, 11,
Policlinico, 70124 Bari, Italy.
Tel.: +39 080 5478240
Fax: +39 080 5478310
E-mail: ribatti@anatomia.uniba.it

doi:10.1111/j.1582-4934.2009.00810.x

Table 1 Major angiogenic and anti-angiogenic factors that regulate angiogenesis

Angiogenic factors
Vascular endothelial growth factor (VEGF)
Fibroblast growth factor-2 (FGF-2)
Placental growth factor (PlGF)
Platelet derived growth factor (PDGF)
Transforming growth factors (TGF-α and -β)
Hepatocyte growth factor (HGF)
Platelet activating factor (PAF)
Tumour necrosis factor α (TNF-α)
Insulin-like growth factor (IGF)
Angiogenin
Angiopoietin-1
Granulocyte colony stimulating factor (G-CSF)
Granulocyte-macrophage colony stimulating factor (GM-CSF)
Erythropoietin
Interleukin-6
Interleukin-8
Anti-angiogenic factors
Thrombospondin-1
Angiostatin
Endostatin
Interferon-α and -γ
Interleukin-12
Angiopoietin-2
Tissue inhibitors of metalloproteinases

several physiological (*e.g.* corpus luteum formation) and pathological conditions, such as tumour and chronic inflammation, where angiogenesis itself may contribute to the progression of disease. In 1971, Folkman published in the 'New England Journal of Medicine' a hypothesis that tumour growth is angiogenesis-dependent and that inhibition of angiogenesis could be therapeutic [2]. This paper also introduced the term anti-angiogenesis to mean the prevention of new vessel sprout from being recruited by a tumour. The hypothesis predicted that tumours would be enable to grow beyond a microscopic size of 1 to 2 mm^3 without continuous recruitment of new capillary blood vessels. This concept is now widely accepted because of supporting data from experimental studies and clinical observations carried out over the intervening years [3].

The process of angiogenesis begins with local degradation of the basement membrane surrounding the capillaries, which is followed by invasion of the surrounding stroma by the underlying endothelial cells, in the direction of the angiogenic stimulus. Endothelial cells migration is accompanied by the proliferation of endothelial cells and their organization into three-dimensional structures that join with other similar structures to form a network of new blood vessels [1]. Angiogenic factors are potent growth factors that promote proliferation and differentiation of endothelial cells. The major angiogenic and anti-angiogenic factors are listed in Table 1.

Under physiological conditions, angiogenesis is dependent on the balance of positive and negative angiogenic modulators within the vascular microenvironment [4] and requires the functional activities of a number of molecules, including angiogenic factors, extracellular matrix proteins, adhesion receptors and proteolytic enzymes. As a consequence, angiogenic endothelial cells have a distinct gene expression pattern that is characterized by a switch of the cell proteolytic balance towards an invasive phenotype as well as by the expression of specific adhesion molecules. In normal tissues, vascular quiescence is maintained by the dominant influence of endogenous angiogenesis inhibitors over angiogenic stimuli [5].

Pathological angiogenesis is linked to a switch in the balance between positive and negative regulators, and mainly depends on the release by inflammatory or neoplastic cells of specific growth factors for endothelial cells, that stimulate the growth of the blood vessels of the host or the down-regulation of natural angiogenesis inhibitors [6].

The contribution of immune cells to angiogenesis in inflammation and tumour growth

There is increasing evidence to support the view that angiogenesis and inflammation are mutually dependent [7]. During inflammatory reactions, immune cells synthesize and secrete pro-angiogenic factors that promote neovascularization. On the other hand, the newly formed vascular supply contributes to the perpetuation of inflammation by promoting the migration of inflammatory cells to the site of inflammation [7]. The extracellular matrix and basement membrane are a source for endogenous angiogenesis inhibitors. On the other hand, many extracellular matrix molecules promote angiogenesis by stabilizing blood vessels and sequestering angiogenic molecules [8].

It is well established that tumour cells are able to secrete pro-angiogenic factors as well as mediators for inflammatory cells [6]. They produce indeed angiogenic cytokines, which are exported from tumour cells or mobilized from the extracellular matrix. As a consequence, tumour cells are surrounded by an infiltrate of inflammatory cells. These cells communicate *via* a complex network of intercellular signalling pathways, mediated by surface adhesion molecules, cytokines and their receptors [9]. Immune cells cooperate and synergise with stromal cells as well as malignant cells in stimulating endothelial cell proliferation and blood vessel formation. These synergies may represent important mechanisms for tumour

development and metastasis by providing efficient vascular supply and easy pathway to escape. Indeed, the most aggressive human cancers are associated with a dramatic host response composed of various immune cells, especially macrophages and mast cells [7].

Neutrophils

Evidence for the possible role of polymorphonuclear granulocytes in inflammation-mediated angiogenesis and tissue remodelling was initially provided by the finding that CXC receptor-2 (CXCR-2)-deficient mice, which lack neutrophil infiltration in thioglycollate-induced peritonitis [10], showed delayed angiogenesis and impaired cutaneous wound healing [11].

During the acute inflammatory response, neutrophils extravasate from the blood into the tissue, where they exert their defence functions. Neutrophils are a source of soluble mediators which exert important angiogenic functions. Vascular endothelial growth factor (VEGF), interleukin (IL)-8, tumour necrosis factor-α (TNF-α), hepatocyte growth factor (HGF) and matrix metalloproteinases (MMPs) are the most important activators of angiogenesis produced by these cells [12–14]. In this perspective, microarray analysis has recently revealed about 30 angiogenesis-relevant genes in human polymorphonuclear granulocytes [15]. Thus neutrophil contribution to pathological angiogenesis may be sustained by an autocrine amplification mechanism that allows persistent VEGF release to occur at sites of neutrophil accumulation. Production and release of VEGF from neutrophils has been shown to depend on the granulocyte colony-stimulating factor (G-CSF) [16]. Interestingly, neutrophil-derived VEGF can stimulate neutrophil migration [17]. Human polymorphonuclear granulocytes have demonstrated the ability to directly induce the sprouting of capillary-like structures in *in vitro* angiogenesis assay, mediated by secretion of both preformed VEGF from cell stores and *de novo* synthesized IL-8 [4].

In breast cancer, release by tumour-associated and tumour-infiltrating neutrophils of oncostatin M, a pleiotropic cytokine belonging to the IL-6 family, promotes tumour progression by enhancing angiogenesis and metastases [18]. In addition, neutrophil-derived oncostatin M induces VEGF production from cancer cells and increases breast cancer cell detachment and invasive capacity [18]. Expression of HPV 16 early region genes in basal keratinocytes of transgenic mice elicits a multi-stage pathway to squamous carcinoma. Infiltration by neutrophils and mast cells, and activation of MMP-9 in these cells coincided with the angiogenic switch in premalignant lesions [19]. In the Rip-Tag2 model of pancreatic islet carcinogenesis, MMP-9-expressing neutrophils were predominantly found in the angiogenic islets of dysplasias and tumours, and transient depletion of neutrophils clearly reduced the frequency of the initial angiogenic switch in the dysplasias [20]. The lack of both MMP-9-positive neutrophils and MMP-2-expressing-stromal cells in mice with a double deficiency for MMP-2 and MMP-9 resulted in a lack of tumour vascularization followed by a lack of tumour invasion [21].

Expression of G-CSF or co-expression of G-CSF and granulocyte macrophage-colony stimulating factor (GM-CSF) together induced malignant progression of previously benign factor-negative HaCaT tumour cells. This progression was associated with enhanced and accelerated neutrophil recruitment into the tumour vicinity. The neutrophil recruitment preceded the induction of angiogenesis in the HaCaT heterotransplantation model for human squamous cell carcinoma and in nude mouse heterotransplants of head and neck carcinomas [22, 23].

In some tumours, like melanoma, neutrophils are not a major constituent of the leucocyte infiltrate, but they might have a key role in triggering and sustaining the inflammatory cascade, providing chemotactic molecules for the recruitment of macrophages and other inflammatory and stromal cells. Neutrophils produce and release high levels of MMP-9. By contrast, neutrophils secrete little, if any, MMP-2, which plays an important role in the turnover of various extracellular matrix components [24]. However, neutrophils release a not yet identified soluble factor as well as a specific sulphatase and a heparanase that activate latent MMP-2 secreted by other cells and allow releasing of embedded growth factors from the extracellular matrix [25, 26]. Remodeled matrix facilitates the escape of tumour cells leaving the tumour mass to metastasize at distance, because it offers less resistance. In addition, proteolytic enzymes released by neutrophils can diminish cell-cell interactions and permit the dissociation of tumour cells from the original tumour site [27].

Neutrophils also produce important anti-angiogenic factors. Human neutrophils, for instance, synthesise and secrete small anti-microbial peptides known as alpha-defensins, which exert inhibition of endothelial cell proliferation, migration and adhesion, impaired capillary tube formation *in vitro* and reduced angiogenesis *in vivo* [28]. In addition, neutrophil-derived elastase can generate the anti-angiogenic factor angiostatin [29] a well known inhibitor of IL-8-, macrophage inflammatory protein (MIP)-2- and growth-related oncogen-α (GRO-α)-induced angiogenesis *in vivo* [30]. Remarkably, all-trans retinoic acid, a promising molecule with potential anti-angiogenic use in clinical treatment, has been shown to inhibit VEGF formation in cultured neutrophil-like HL-60 cells [31].

Basophils

Basophils express mRNA for three isoforms of VEGF-A (121, 165 and 189) and two isoforms of VEGF-B (167 and 186) [32]. Peripheral blood and basophils infiltrating sites of chronic inflammation such as nasal polyps contain VEGF-A in their secretory granules. Supernatants of activated basophils induced an angiogenic response *in vivo* in the chick embryo chorioallantoic membrane (CAM) assay. In addition, basophils express VEGF receptor-2 (VEGFR-2) and neuropilin-1 that acts as co-receptor for VEGFR-2 and enhances VEGFR-2-induced responses. Remarkably, VEGF-A also functions as basophil chemoattractant providing a novel

autocrine loop for basophils self-recruitment [32]. Overall, these data suggest that basophils could play a role in angiogenesis and inflammation through the expression of several forms of VEGF and their receptors. Moreover, basophils release histamine, which displays angiogenic activity in several *in vitro* and *in vivo* settings [33].

Eosinophils

Eosinophils are pro-angiogenic through the production of an array of cytokines and growth factors, such as VEGF [34], fibroblast growth factor-2 (FGF-2) [35], TNF-α [36], GM-CSF [37], nerve growth factor (NGF) [38], IL-8 [39], angiogenin [40], and are positively stained for VEGF and FGF-2 in the airways of asthma patients [35]. Eosinophils release VEGF following stimulation with GM-CSF and IL-5 [35], both expressed in the tissue and in the bronchoalveolar lavage fluid of patients with allergic asthma. Eosinophils have the capacity to generate VEGF by *de novo* synthesis and to release it [34]. Feistritzer *et al.* [41] have detected VEGFR-1 and -2 on human peripheral blood eosinophils and they demonstrated that VEGF induces eosinophil migration and eosinophil cationic protein release, mainly through VEGFR-1. Eosinophils promoted endothelial cells proliferation *in vitro* and induce new vessel formation in the aorta ring and in the CAM assays [42]. Interestingly, neutralization of VEGF in eosinophils reduced their angiogenic effects in the CAM by 55% suggesting the important, but not unique role played by this factor in the induction of the angiogenic response. Eosinophils are not the only source of VEGF but they can also be targets for VEGF in allergic inflammation. Eosinophil infiltration could be reduced by administration of an anti-VEGF receptor antibody in a murine model of toluene diisocyanate (TDI)-induced asthma [43]. Because eosinophils are a rich source of preformed MMP-9, it is reasonable to believe that they may promote angiogenesis also by acting on matrix degradation.

Recently, Puxeddu *et al.* [44] have demonstrated that eosinophil-derived major basic protein (MBP) induced endothelial cell proliferation and enhanced the pro-mitogenic effect of VEGF, but did not affect VEGF release. Moreover, MBP promoted capillarogenesis by endothelial cells seeded on Matrigel and angiogenesis *in vivo* in the CAM assay. Finally, the pro-angiogenic effect of MBP was not due to its cationic charge because stimulation in the CAM with the synthetic polycation, poly-L-arginine did not induce any angiogenic effect [44].

Monocytes-macrophages

Cells belonging to the monocyte-macrophage lineage are a major component of the leucocyte infiltration in tumours [45, 46]. The number of tumour-derived chemoattractants ensures macrophage recruitment, including colony-stimulating factor-1 (CSF-1), the CC chemokines CCL2, CCL3, CCL4, CCL5 and CCL8, and VEGF secreted by both tumour and stromal elements [46]. Besides killing tumour cells once activated by interferon-γ (IFN-γ) and IL-12, tumour-associated macrophages produce several pro-angiogenic cytokines as well as extracellular matrix-degrading enzymes [47]. The stimulating effect exerted by tumour-associated macrophages on the growth of the tumour mass is partly related to the angiogenic potential of these cells. In the tumour microenvironment, macrophages are mainly represented by polarized type II (alternatively activated) or M2 elements, which would derive from tumour-associated macrophages upon local exposure to IL-4 and IL-10 [46]. These cells have poor attitude to destroy tumour cells but are better adapted to promoting angiogenesis, repairing and remodelling wounded or damaged tissues, and suppressing adaptive immunity [48]. Tumour-associated macrophages represent a rich source of potent pro-angiogenic cytokines and growth factors, such as VEGF, TNF-α, IL-8 and FGF-2. In addition, these cells express a broad array of angiogenesis-modulating enzymes, including MMP-2, -7, -9, -12, and cycloxygenase-2 (COX-2) [49–51]. In human beings, a significant relationship between the number of tumour-associated macrophages and the density of blood vessels has been established in tumours like breast carcinoma [52], melanoma [53], glioma [54], squamous cell carcinoma of the esophagus [55], bladder carcinoma [56], and prostate carcinoma [57]. In the mouse cornea model, killing of COX-2 positive infiltrating macrophages with clodronate liposomes reduces IL-1-β-induced angiogenesis and partially inhibits VEGF-induced angiogenesis [58]. In one model of subcutaneous melanoma, both angiogenesis and growth rate correlate with tumour infiltration by macrophages that express angiotensin I receptor and VEGF [59]. In addition, Lewis lung carcinoma cells expressing IL-1-β develop neovasculature with macrophage infiltration and enhance tumour growth in wild-type but not in monocyte chemoattractant protein-1 (MCP-1)-deficient mice, suggesting that macrophage involvement might be a prerequisite for IL-1-β-induced neovascularization and tumour progression [58]. In a murine model of mammary carcinoma, deficiency of macrophage-colony stimulating factor (M-CSF), a potent inductor of macrophage recruitment in tumour tissues, does not affect early stages of tumour development but reduces progression to invasive carcinoma and metastasis [60]. This result highlights the possible role of tumour-associated macrophages in contributing to the angiogenic switch that accompanies transition into malignancy. In polyoma middle-T (PyMT)-induced mouse mammary tumours, indeed, focal accumulation of macrophages in premalignant lesions precedes the angiogenic switch and the progression into invasive tumours. Depletion of tumour-associated macrophages reduces to about 50% tumour vascular density, leading to areas of necrosis by loss of blood supply within the tumour mass. Interestingly, macrophages have been shown to accumulate particularly in such necrotic and hypoxic areas in different neoplasia, like human endometrial, breast, prostate and ovarian carcinomas [61, 62]. It is otherwise known, indeed, that up-regulation of the pro-angiogenic programme in tumour-associated macrophages, followed by increased release of VEGF, FGF-2, TNF-α, urokinase and MMPs, is stimulated by hypoxia and acidosis [63]. Moreover, activated macrophages synthesize and

release inducible nitric oxide synthase, which increases blood flow and promotes angiogenesis [64]. Last, the angiogenic factors secreted by macrophages stimulate migration of other accessory cells that potentiate angiogenesis, in particular mast cells [65]. Osteopontin deeply affects the pro-angiogenic potential of human monocytes [66]. Reports suggest that osteopontin may affect angiogenesis by acting directly on endothelial cells and/or indirectly *via* mononuclear phagocyte engagement, enhancing the expression of TNF-α and IL-1-β in mononuclear cells [67, 68].

It should also be mentioned that monocytes and macrophages are primary producers of IL-12. This multifunctional cytokine can cause tumour regression and reduce metastasis in animal models, because of the promotion of anti-tumour immunity and also to the significant inhibition of angiogenesis [69]. The anti-angiogenic activity is mediated by IFN-γ production, which in turn induces the chemokine IFN-γ-inducible protein-10 [70, 71]. There is *in vitro* evidence that IL-12 inhibits VEGF produced by breast cancer cells and regulates stromal cell interactions, leading to decreased MMP-9 and increased tissue inhibitor of metalloproteinase (TIMP)-1 production [72].

Liposomal delivery of the nonaminobisphosphonate clodronate depleted macrophages in the synovial fluid of rheumatoid arthritis patients and depleted macrophages and inhibited tumour angiogenesis in mouse tumour transplantation models [73, 74].

Lymphocytes

Lymphocytes are essential for the airway remodelling. Studies have been performed in mice chronically infected with *Mycoplasma pneumoniae*. Mice lacking B cells expressed a great reduction of angiogenesis when infected with this microorganism [75]. The humoural response, indeed, causes deposition of immune complexes on the airway wall, followed by recruitment of inflammatory cells at sites of infected airways which, in turn, are responsible for local production of remodelling factors. Lymphocytes may cooperate to the generation of an anti-angiogenic microenvironment that is essential for causing regression of the tumour mass. For instance, Th cells and cytotoxic T cells are needed to mediate the anti-angiogenic effect of IL-12 [76].

In mice, NK cells have been found essential for the initiation of pregnancy-associated spiral arterial modification through their production of IFN-γ and VEGF. VEGF provides not only a potent pro-angiogenic stimulus but works as an important stem cell survival factor with ability to recruit cells into the hypoxic environments [77]. Thus, it might act as endothelial tip cell guidance towards hypoxic endometrium not only in the endometrial/decidual environment occupied by the trophoblasts but also in the necrotic milieu that occurs during endometrial destruction in the menstrual cycle. Experimental work suggests that NK cells are required mediators of angiogenesis inhibition by IL-12, and that NK cell cytotoxicity of endothelial cells is a potential mechanism by which IL-12 can suppress neovascularization [78]. IL-12 receptors indeed are present primarily on NK cells and T cells [79]. IL-12-activated lymphocytes influence inhibition of tumour growth and function as an anti-vascular agent, by releasing higher level of IFN-γ and down-modulating VEGF [80].

Dendritic cells

DCs are bone marrow, hematopoietic-derived, professional antigen-presenting cells (APCs), able to induce both primary and secondary T- and B-cell responses as well as immune tolerance [81]. They participate in the regulation of the inflammatory reaction through the release of cytokines and chemokines [82]. DCs express both pro- and anti-angiogenic mediators when exposed to different combinations of cytokines and microbial stimuli and both positive and negative mediators of the angiogenic process can affect the biology of DCs. DCs express both VEGFR-1 and VEGFR-2 [83]. Furthermore, expression of the VEGF co-receptor neuropilin-1 is induced during *in vitro* differentiation of monocytes into DCs [84]. Riboldi *et al.* [85] reported that DCs can be activated to an angiogenesis-promoting phenotype. They demonstrated that alternative activation of DCs by anti-inflammatory molecules, such as calcitriol, prostaglandin E_2 (PGE_2) or IL-10 prompts them to secrete VEGF and inhibit their secretion of IL-12, a potent anti-angiogenic molecule that is secreted by classical activated DCs.

The contribution of progenitor cells and adult cell transdifferentiation

Myeloid-derived suppressor cells (MDSCs) are a heterogenous population, comprising myeloid progenitors, monocytes and neutrophils that express low to undetectable levels of MHC-II and co-stimulatory molecules. Yang *et al.* [86] found that MDSCs obtained from spleens of tumour-bearing mice promoted angiogenesis and tumour growth when co-injected with tumour cells. Reducing the levels of MDSCs by either treatment of mice with gemcitabine or by interfering with the Kit ligand/c-Kit receptor axis impaired tumour growth and angiogenesis [87, 88]. MDSCs isolated from tumours of STAT-3-deficient mice were markedly less potent in inducing endothelial tube formation *in vitro* as compared to STAT-3 wild-type cells, concomitant with markedly reduced exepression levels of several angiogenic factors [89].

Monocytes-macrophages and endothelial cells share phenotypical and functional features, including the expression of common metabolic and surface markers, as well as the ability to transdifferentiate into endothelial cells *in vitro* and *in vivo* [90–93]. Venneri *et al.* [94] have reported the identification in human peripheral blood of a novel subset of Tie-2 expressing monocytes (TEMs) that promote angiogenesis in paracrine manner. Although recruited to tumours in lower numbers than tumour-associated macrophages (TAMs), TEMs

are a more potent source of pro-angiogenic signals, suggesting that they significantly contribute to tumour angiogenesis.

Gottfried *et al.* [95] demonstrated that incubation of tumour-associated DCs with VEGF and oncostatin M led to transdifferentation into endothelial-like cells. These cells showed strong expression of classical endothelial cell markers, such as von Willebrand factor and vascular endothelial cadherin, while leukocytic markers were reduced. Moreover, they were able to vascular-like tubes on Matrigel.

These data indicate that while the concept of immature vascular cells delivered to the site of tumour blood vessels was originally developed on bone marrow derived endothelial precursor cells (EPCs), it is become evident that other classes of vascular cells differentiate from progenitors and adult cells *in situ*.

Mast cells

An increased number of mast cells have been reported in angiogenesis associated with chronic inflammatory diseases, like rheumatoid arthritis and nasal polyps [96, 97].

Mast cells produce a large spectrum of pro-angiogenic factors. Human, rat and mouse mast cells release preformed FGF-2 from their secretory granules [98, 99]. Human cord blood-derived mast cells release VEGF upon stimulation through FcεRI and c-kit. Both FGF-2 and VEGF have also been identified by immunohistochemistry in mature mast cells in human tissues [100, 101]. Human mast cells are a potent source of VEGF in the absence of degranulation through activation of the EP (2) receptor by PGE_2 [102]. Following IgE-dependent activation mast cells released several pro-angiogenic mediators stored in their granules, such as VEGF [103] and FGF-2 [104], that promote angiogenesis even in the early phase of allergic inflammation. Mast cells can also migrate *in vivo* [105] and *in vitro* [65] in response to VEGF. Recently, Detoraki *et al.* [106] have demonstrated that human lung mast cells express VEGF-A, VEGF-B, VEGF-C and VEGF-D at both mRNA and protein level. PGE_2 enhanced the expression of VEGF-A, VEGF-B and VEGF-C, whereas an adenosine analogue (5'-[N-ethylcarboxamido] adenosine [NECA]) increased VEGF-A, VEGF-C and VEGF-D expression. In addition, supernatants of PGE_2- and NECA-activated human lung mast cells induced angiogenic response in the CAM assay that was inhibited by an anti-VEGF-A antibody. Finally, placental growth factor-1 induced mast cell chemotaxis [106].

Granulated mast cells and their granules, but not degranulated mast cells, are able indeed to stimulate an intense angiogenic reaction in the chick embryo CAM assay. This angiogenic activity is partly inhibited by anti-FGF-2 and -VEGF antibodies, suggesting that these cytokines are involved in the angiogenic reaction [107]. Similarly it has been demonstrated, using the rat-mesenteric window angiogenic assay, that intraperitoneal injection of compound 48/80 causes a vigorous angiogenic response [108]. The same treatment in mice also causes angiogenesis [109].

Mast cells store large amounts of preformed active serine proteases, such as tryptase and chymase, in their secretory granules [110]. Tryptase stimulates the proliferation of endothelial cells, promotes vascular tube formation in culture and also degrades connective tissue matrix to provide space for neovascular growth. Tryptase also acts indirectly by activating latent MMPs and plasminogen activator (PA), which in turn degrade the extracellular connective tissue with consequent release of VEGF or FGF-2 from their matrix-bound state [111]. Mast cell-derived chymase degrades extracellular matrix components and therefore matrix-bound VEGF could be potentially released.

Histamine and heparin stimulate proliferation of endothelial cells induce the formation of new blood vessels in the CAM-assay [33, 112]. Histamine stimulates new vessel formation by acting through both H1 and H2 receptors [33]. Heparin may act directly on blood vessels or indirectly by inducing release of FGF-2 from the extracellular storage site. In addition, other cytokines produced by mast cells, such as IL-8 [113], TNF-α [114], TGF-β, NGF [115] and urokinase-type PA have been implicated in normal and tumour-associated angiogenesis [116]. Last, mast cells also contain preformed MMPs, such as MMP-2 and MMP-9, and TIMPs, which enable mast cells to directly modulate extracellular matrix degradation. This, in turn, allows for tissue release of extracellular matrix-bound angiogenic factors.

Mast cells play a role in tumour growth and angiogenesis. Mast cell-deficient W/W^v mice exhibit indeed a decreased rate of tumour angiogenesis [117]. Molecules like heparin could facilitate tumour vascularization not only by a direct pro-angiogenic effect but also through its anti-clotting effect [118]. In addition, mast cell-derived MMPs can degrade the interstitial tumour stroma and hence release matrix-bound angiogenic factors. An increased number of mast cells have indeed been reported in angiogenesis associated with vascular neoplasms, like haemangioma and haemangioblastoma [119], as well as a number of solid and haematopoietic tumours. In general, mast cell density correlates with angiogenesis and poor tumour outcome. Association between mast cells and new vessel formation has been reported in breast cancer [120, 121], colorectal cancer [122] and uterine cervix cancer [123]. Tryptase-positive mast cells increase in number and vascularization increases in a linear fashion from dysplasia to invasive cancer of the uterine cervix [124]. An association of VEGF and mast cells with angiogenesis has been demonstrated in laryngeal carcinoma [125] and in small lung carcinoma, where most intratumoural mast cells express VEGF [126–128]. Mast cell accumulation has also been noted repeatedly around melanomas, especially invasive melanoma [129, 130]. Mast cell accumulation was correlated with increased neovascularization, mast cell expression of VEGF [131] and FGF-2 [132], tumour aggressiveness and poor prognosis. Indeed, a prognostic significance has been attributed to mast cells and microvascular density not only in melanoma [133] but also in squanous cell cancer of the oesophagus [134]. Recently, angiogenesis has been shown to correlate with tryptase-positive mast cell count in human endometrial cancer. Both parameters were found to increase in agreement with tumour progression [135].

Mast cell density, new vessel rate and clinical prognosis have also been found to correlate in haematological tumours. In benign

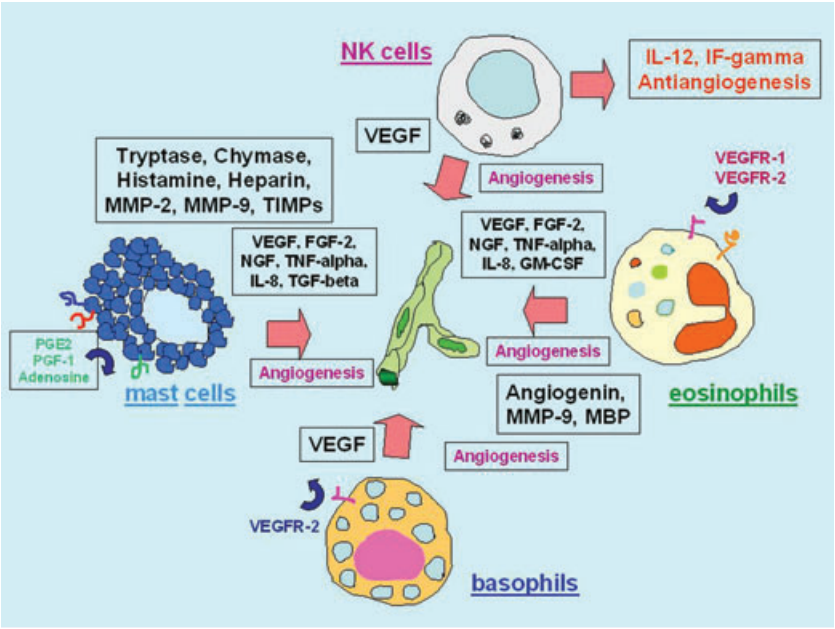

Fig. 1 Interplay between angiogenic and anti-angiogenic molecules secreted by NK cells, mast cells, basophils and eosinophils.

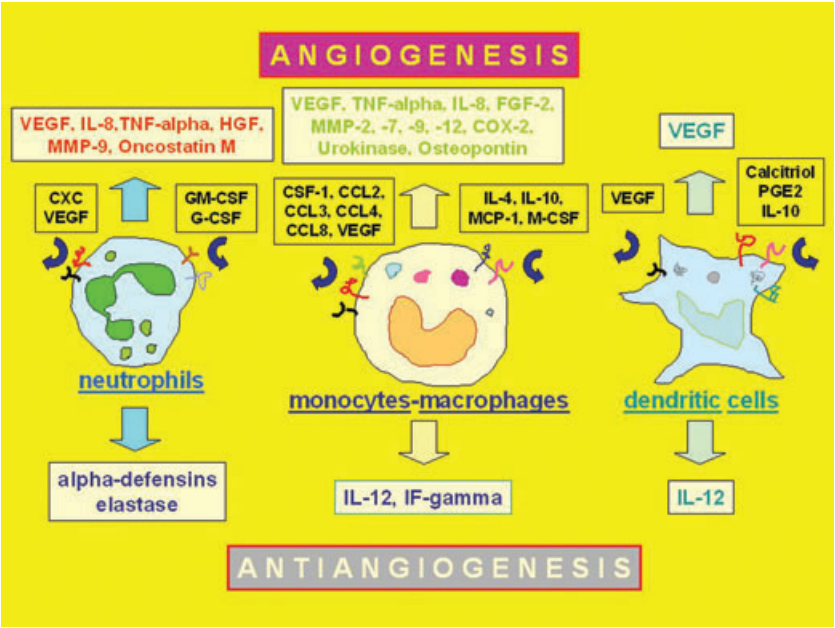

Fig. 2 Interplay between angiogenic and anti-angiogenic molecules secreted by neutrophils, monocytes-macrophages and dendritic cells.

lymphadenopathies and B-cell non-Hodgkin's lymphomas, angiogenesis correlates with total and tryptase-positive mast cell counts, and both increase in step with the increase with malignancy grades [136, 137]. In non-Hodgkin's lymphomas, a correlation has been found between vessel count and the number of mast cells and VEGF-expressing cells [138]. In the bone marrow of patients with inactive and active multiple myeloma as well as those with monoclonal gammopathies of undetermined significance, angiogenesis highly correlates with mast cell counts [139]. A similar pattern of correlation between bone marrow microvessel count, total and tryptase-positive mast cell density and tumour progression has been found in patients with myelodysplastic syndrome [140] and B-cell chronic lymphocytic leukemia [141]. In the early stages of B-cell chronic lymphocytic leukemia, the density of tryptase-positive mast cells in the bone marrow has been shown to predict the outcome of the disease [142].

Platelets

Human platelets carry in their alpha granules a set of angiogenesis stimulators, such as FGF-2, VEGF and thymidine phosphorylase, and inhibitors, such as endostatin, platelet factor-4 and thrombospondin-1 (TSP-1) [143]. These findings may have implications for release of angiogenic molecules at the initiation of wound healing, followed by release of anti-angiogenic molecules at the later stage of wound healing.

These angiogenesis-regulatory molecules are packed into separate and distinct alpha granules [143]. In fact, the treatment of human platelets with a selective proteinase-activated receptor-4 (PAR-4) agonist resulted in release of endostatin-containing granules, but not VEGF-containing granules, whereas a selective PAR-1 agonist liberated VEGF, but not endostatin-containing granules [143]. Moreover, these molecules are sequestered in platelets in higher concentration than in plasma. In fact, VEGF-enriched Matrigel pellets implanted subcutaneously into mice result in an elevation of VEGF levels in platelets, without any changes in its plasma levels [144]. Accumulation of platelets in some tumours and release of angiogenic molecules could further stimulate tumour growth. In fact, it has been recently demonstrated that accumulation of angiogenesis regulators in platelets of animals bearing malignant tumours exceeds significantly their concentration in plasma or serum, as well as their levels in platelets from non-tumour bearing mice [144]. It is likely that novel angiogenesis-regulatory molecules that could be developed into drugs will be discovered in platelets.

Conclusions

This review summarizes the most recent experimental and clinical data providing evidence for the involvement of immune cells in pathological angiogenesis. The cross-talk between the different immune cells and the structural tissue cells establishes a definite microenvironment, which promotes the growth, migration and activation of endothelial cells leading to expansion of the pre-existing vascular supply. This process is the result of a complex balance between pro- and anti-angiogenic stimuli generated locally in the tissue milieu (Figs. 1 and 2). Manipulating this mediators' puzzle by potentiating the local production of anti-angiogenic cytokines would allow to modulate and even inhibit the angiogenic process. Given the detrimental effects pro-angiogenic molecules may exert in inflammation and cancer, it seems of primary importance to understand the contribution of immune cells to pathological angiogenesis.

Acknowledgements

Supported in part by MIUR (PRIN 2007), Rome, and Fondazione Cassa di Risparmio di Puglia, Bari, Italy.

References

1. **Ribatti D.** Genetic and epigenetic mechanisms in the early development of the vascular system. *J Anat.* 2006; 208: 139–52.
2. **Folkman J.** Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285: 1182–6.
3. **Ribatti D.** Judah Folkman, a pioneer in the study of angiogenesis. *Angiogenesis.* 2008; 11: 3–10.
4. **Hanahan D, Folkman J.** Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell.* 1996; 86: 353–64.
5. **Ribatti D, Nico B, Crivellato E, *et al.*** The history of the angiogenic switch concept. *Leukemia.* 2007; 21: 44–52.
6. **Ribatti D, Vacca A.** Overview of angiogenesis during tumor growth. In: Figg WG, Folkman J, editors. Angiogenesis. An integrative approach from science to medicine. New York: Springer; 2008. pp. 161–8.
7. **Mueller MM.** Inflammation and angiogenesis: innate immune cells as modulators of tumor vascularization. In: Marmé D, Fusenig N, editors. Tumor angiogenesis. Basic mechanisms and cancer therapy. Berlin-Heidelberg: Springer-Verlag; 2008. pp. 351–62.
8. **Nyberg P, Salo T, Kalluri R.** Tumor microenvironment and angiogenesis. *Front Bioscience.* 2008; 13: 6537–53.
9. **Ribatti D, Nico B, Vacca A.** Importance of the bone marrow microenvironment in inducing the angiogenic response in multiple myeloma. *Oncogene.* 2006; 25: 4257–66.
10. **Calcano G, Lee J, Kikly K, *et al.*** Neutrophil and B cell expansion in mice that lack murine IL-8 receptor homolog. *Science.* 1994; 265: 682–4.
11. **Devalaraja RM, Nanney LB, Qian Q, *et al.*** Delayed wound healing in CXCR2 knockout mice. *J Invest Dermatol.* 2000; 115: 234–44.
12. **Dubravec DB, Spriggs DR, Mannick JA, *et al.*** Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor alpha. *Proc Natl Acad Sci USA.* 1990; 87: 6758–61.
13. **Bazzoni F, Cassatella MA, Rossi F, *et al.*** Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *J Exp Med.* 1991; 173: 771–4.
14. **Grenier A, Chollet-Martin S, Crestani B, *et al.*** Presence of a mobilizable intracellular pool of hepatocyte growth factor in human polymorphonuclear neutrophils. *Blood.* 2002; 99: 2997–3004.
15. **Schruefer R, Sulyok S, Schymeinsky J, *et al.*** The proangiogenic capacity of polymorphonuclear neutrophils delineated by microarray technique and by measurement of neovascularization in wounded skin of CD18-deficient mice. *J Vasc Res.* 2006; 43: 1–11.
16. **Ohki Y, Heissig B, Sato Y, *et al.*** Granulocyte colony-stimulating factor promotes neovascularization by releasing vascular endothelial growth factor from neutrophils. *FASEB J.* 2005; 19: 2005–7.
17. **Ancelin M, Chollet-Martin S, Herve MA, *et al.*** Vascular endothelial growth factor VEGF189 induces human neutrophil

chemotaxis in extravascular tissue via an autocrine amplification mechanism. *Lab Invest.* 2004; 8: 502–12.

18. **Queen MM, Ryan RE, Holzer RG, *et al.*** Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. *Cancer Res.* 2005; 65: 8896–904.
19. **Coussens LM, Werb Z.** Matrix metalloproteinases and the development of cancer. *Chem Biol.* 1996; 3: 895–904.
20. **Nozawa H, Chiu C, Hanahan D.** Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci USA.* 2006; 103: 12493–8.
21. **Masson V, De La Ballina LR, Munaut C, *et al.*** Contribution of host MMP-2 and MMP-9 to promote tumor vascularization and invasion of malignant keratinocytes. *FASEB J.* 2005; 19: 234–6.
22. **Obermueller E, Vosseler S, Fusenig NE, *et al.*** Cooperative autocrine and paracrine functions of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the progression of skin carcinoma cells. *Cancer Res.* 2004; 64: 7801–12.
23. **Gutschalk CM, Herold-Mende CC, Fusenig NE, *et al.*** Granulocyte colony-stimulating factor and granulocyte-macrophage colony stimulating factor promote malignant growth of cells from head and neck squamous cells carcinomas *in vivo*. *Cancer Res.* 2006; 66: 8026–36.
24. **Muhs BE, Plitas G, Delgado Y, *et al.*** Temporal expression and activation of matrix metalloproteinases-2, -9, and membrane type 1-matrix metalloproteinase following acute hindlimb ischemia. *J Surg Res.* 2003; 111: 8–15.
25. **Schwartz JD, Monea S, Marcus SG, *et al.*** Soluble factor(s) released from neutrophils activates endothelial cell matrix metalloproteinase-2. *J Surg Res.* 1998; 76: 79–85.
26. **Bartlett MR, Underwood PA, Parish CR.** Comparative analysis of the ability of leucocytes, endothelial cells and platelets to degrade the subendothelial basement membrane: evidence for cytokine dependence and detection of a novel sulfatase. *Immunol Cell Biol.* 1995; 73: 113–24.
27. **Shamamian P, Schwartz JD, Pocock BJ, *et al.*** Activation of progelatinase A (MMP-2) by neutrophil elastase, cathepsin G, and proteinase-3: a role for inflammatory cells in tumor invasion and angiogenesis. *J Cell Physiol.* 2001; 189: 197–206.
28. **Chavakis T, Cines DB, Rhee JS, *et al.*** Regulation of neovascularization by human neutrophil peptides (alpha-defensins): a link between inflammation and angiogenesis. *FASEB J.* 2004; 18: 1306–8.
29. **Scapini P, Nesi L, Morini M, *et al.*** Generation of biologically active angiostatin kringle 1–3 by activated human neutrophils. *J Immunol.* 2002; 168: 5798–804.
30. **Benelli R, Morini M, Carrozzino F, *et al.*** Neutrophils as a key cellular target for angiostatin: implications for regulation of angiogenesis and inflammation. *FASEB J.* 2002; 16: 267–9.
31. **Tee MK, Vigne JL, Taylor RN.** All-trans retinoic acid inhibits vascular endothelial growth factor expression in a cell model of neutrophil activation. *Endocrinology.* 2006; 147: 1264–70.
32. **de Paulis A, Prevete N, Rossi FW, *et al.*** Expression and functions of vascular endothelial growth factors and their receptors in human basophils. *J Immunol.* 2006; 177: 7322–31.
33. **Sörbo J, Jakobbson A, Norrby K.** Mast cell histamine is angiogenic through receptors for histamine 1 and histamine 2. *Int J Exp Pathol.* 1994; 75: 43–50.
34. **Horiuchi T, Weller PF.** Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte macrophage colony-stimulating factor and interleukin-5. *Am J Respir Cell Mol Biol.* 1997; 17: 70–7.
35. **Hoshino M, Takahashi M, Aoike N.** Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *Allergy Clin Immunol.* 2001; 107: 295–301.
36. **Wong DT, Weller PF, Galli SJ, *et al.*** Human eosinophils express transforming growth factor alpha. *J Exp Med.* 1990; 172: 673–81.
37. **Kita H, Ohnishi T, Okubo Y, *et al.*** Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J Exp Med.* 1991; 174: 745–8.
38. **Solomon A, Aloe L, Pe'er J, *et al.*** Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J Allergy Clin Immunol.* 1998; 102: 454–60.
39. **Yousefi S, Hemmann S, Weber M, *et al.*** IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. *J Immunol.* 1995; 154: 5481–90.
40. **Hoshino M, Takahashi M, Aoike N.** Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol.* 2001; 107: 295–301.
41. **Feistritzer C, Kaneider NC, Sturn DH, *et al.*** Expression and function of the vascular endothelial growth factor receptor FLT-1 in human eosinophils. *Am J Respir Cell Mol Biol.* 2004; 30: 729–35.
42. **Puxeddu I, Alian A, Piliponsky AM, *et al.*** Human peripheral blood eosinophils induce angiogenesis. *Int J Biochem Cell Biol.* 2005; 37: 628–36.
43. **Lee YC, Kwak YG, Song CH.** Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Immunol.* 2002; 168: 3595–600.
44. **Puxeddu I, Berkman N, Nissim Ben Efraim AH, *et al.*** The role of eosinophil major basic protein in angiogenesis. *Allergy.* 2009; 64: 368–74.
45. **Balkwill F, Mantovani A.** Inflammation and cancer: back to Virchow? *Lancet.* 2001; 357: 539–45.
46. **Mantovani A, Sozzani S, Locati M, *et al.*** Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 2002; 23: 549–55.
47. **Naldini A, Carraro F.** Role of inflammatory mediators in angiogenesis. *Curr Drug Targets Inflamm Allergy.* 2005; 4: 3–8.
48. **Sica A, Schioppa T, Mantovani A, *et al.*** 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.
49. **Sunderkotter C, Goebeler M, Schiltze-Osthoff K, *et al.*** Macrophage-derived angiogenesis factors. *Pharmacol Ther.* 1991; 51: 195–216.
50. **Lewis CE, Leek R, Harris A, *et al.*** Cytokine regulation of angiogenesis in breast cancer: the role of tumor-associated macrophages. *J Leukoc Biol.* 1995; 57: 747–51.
51. **Klimp AH, Hollema H, Kempinga C, *et al.*** Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. *Cancer Res.* 2001; 61: 7305–9.
52. **Leek RD, Lewis CE, Whitehouse R, *et al.*** Association of macrophage infiltration with

angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res.* 1996; 56: 4625–9.

53. **Makitie T, Summanen P, Takkanen A, *et al.*** Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma. *Invest Ophtalmol Vis Sci.* 2001; 42: 1414–21.
54. **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.
55. **Koide N, Nishio A, Sato T, *et al.*** Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. *Am J Gastroenterol.* 2004; 99: 1667–74.
56. **Hanada T, Nakagawa M, Emoto A, *et al.*** Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol.* 2000; 7: 263–9.
57. **Lissbrant IF, Stattin P, Wikstrom P, *et al.*** Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol.* 2000; 17: 445–51.
58. **Nakao S, Kuwano T, Tsutsumi-Miyahara C, *et al.*** Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1 beta-induced neovascularization and tumor growth. *J Clin Invest.* 2005; 115: 2979–91.
59. **Egami K, Murohara T, Shimada T, *et al.*** Role of host angiotensin II type 1 receptor in tumor angiogenesis and growth. *J Clin Invest.* 2003; 112: 67–75.
60. **Lin EY, Nguyen AV, Russel RG, *et al.*** Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med.* 2001; 193: 727–40.
61. **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.
62. **Leek RD, Landers RJ, Harris AL, *et al.*** Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer.* 1999; 79: 991–5.
63. **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.
64. **Jenkins DC, Charles IG, Thomsen LL, *et al.*** Roles of nitric oxide in tumor growth. *Proc Natl Acad Sci USA.* 1995; 92: 4392–6.
65. **Gruber BL, Marchese MJ, Kew R.** Angiogenic factors stimulate mast cell migration. *Blood.* 1995; 86: 2488–93.
66. **Denhardt DT, Noda M, O'Regan AW, *et al.*** Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *J Clin Invest.* 2001; 107: 1055–61.
67. **Leali D, Dell'Era P, Stabile H, *et al.*** Osteopontin (Eta-1) and fibroblast growth factor-2 cross-talk in angiogenesis. *J Immunol.* 2003; 171: 1085–93.
68. **Naldini A, Leali D, Pucci A, *et al.*** Cutting edge: IL-1beta mediates the proangiogenic activity of osteopontin-activated human monocytes. *J Immunol.* 2006; 177: 4267–70.
69. **Colombo MP, Trinchieri G.** Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev.* 2002; 13: 155–68.
70. **Angiolillo AL, Sgadari C, Taub DD, *et al.*** Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med.* 1995; 182: 155–62.
71. **Romagnani P, Annunziato F, Lasagni L, *et al.*** Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest.* 2001; 107: 53–63.
72. **Dias S, Boyd R, Balkwill F.** IL-12 regulates VEGF and MMPs in a murine breast cancer model. *Int J Cancer.* 1998; 78: 361–5.
73. **Barrera P, Blom A, van Lent PL van Bloois L, *et al.*** Synovial macrophage depletion with clodronate-containing liposomes in rheumatoid arthritis. *Arthritis Rheum.* 2000; 43: 1951–9.
74. **Zeisberger SM, Odermatt B, Marty C, *et al.*** Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new high and highly effective antiangiogenic therapy approach. *Br J Cancer.* 2006; 95: 272–81.
75. **Aurora AB, Baluk P, Zhang D, *et al.*** Immune complex-dependent remodeling of the airway vasculature in response to a chronic bacterial infection. *J Immunol.* 2005; 175: 6319–26.
76. **Strasly M, Cavallo F, Guena M, *et al.*** IL-12 inhibition of endothelial cell functions and angiogenesis depends on lymphocyte-endothelial cell cross-talk. *J Immunol.* 2001; 166: 3890–9.
77. **Carmeliet P, Tessier-Lavigne M.** Common mechanisms of nerve and blood vessel wiring. *Nature.* 2005; 436: 193–200.
78. **Yao L, Sgadari C, Furuke K, *et al.*** Contribution of natural killer cells to inhibition of angiogenesis by interleukin-12. *Blood.* 1999; 93: 1612–21.
79. **Trinchieri G.** Interleukin-12 and its role in the generation of TH1 cells. *Immunology Today.* 1993; 14: 335–8.
80. **Cavallo F, Quaglino E, Cifaldi L, *et al.*** Interleukin 12-activated lymphocytes influence tumor genetic programs. *Cancer Res.* 2001; 61: 3518–23.
81. **Banchereau J, Briere F, Caux C, *et al.*** Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000; 18: 767–811.
82. **Bancherau J, Steinman RM.** Dendritic cells and the control of immunity. *Nature.* 1988; 392: 245–52.
83. **Mimura K, Kono K, Takahashi A, *et al.*** Vascular endothelial growth factor inhibits the function of human mature dendritic cells mediated by VEGF receptor-2. *Cancer Immunol Immunother.* 2007; 56: 761–70.
84. **Bourbié-Vaudanie S, Blanchard N, Hivroz C, *et al.*** Dendritic cells can turn CD4+ T lymphocytes into vascular endothelial growth factor-carrying cells by intercellular neuropilin-1 transfer. *J Immunol.* 2006; 177: 1460–9.
85. **Riboldi E, Musso T, Moroni E, *et al.*** Cuting edge: proangiogenic properties of alternatively activated dendritic cells. *J Immunol.* 2005; 175: 2788–92.
86. **Yang L, DeBusk LM, Fukuda K, *et al.*** Expansion of myeloid immune suppressor Gr+ CD11+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell.* 2004; 6: 409–21.
87. **Suzuki E, Kapoor V, Jassar AS, *et al.*** Gemcitabile selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res.* 2005; 11: 6713–21.
88. **Pan PY, Wang GX, Yin B, *et al.*** Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. *Blood.* 2008; 111: 219–28.
89. **Kujawski M, Kortylewski M, Lee H, *et al.*** Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J Clin Invest.* 2008; 18: 3367–77.
90. **Fernandez Pujol B, Lucibello FC, Gehling UM, *et al.*** Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation.* 2000; 65: 287–300.
91. **Schmeisser A, Garlichs CD, Zhang H, *et al.*** Monocytes coexpress endothelial

and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res.* 2001; 49: 671–80.

92. **Iba O, Matsubara H, Nozawa Y, *et al.*** Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. *Circulation.* 2002; 106: 2019–25.
93. **Anghelina M, Krishnan P, Moldovan L, *et al.*** Monocytes and macrophages form branched cell columns in Matrigel: implications for a role in neovascularization. *Stem Cell Dev.* 2004; 13: 665–76.
94. **Venneri MA, De Palma M, Ponzoni M, *et al.*** Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. *Blood.* 2007; 109: 5276–85.
95. **Gottfried E, Kreutz M, Haffner S, *et al.*** Differentiation of human tumour-associated dendritic cells into endothelial-like cells: an alternative pathway of tumour angiogenesis. *Scand J Immunol.* 2007; 65: 329–35.
96. **Yamada T, Sawatsubashi M, Yakushiji H, *et al.*** Localization of vascular endothelial growth factor in synovial membrane mast cells: examination with "multi-labelling subtraction immunostaining". *Virchows Arch.* 1998; 433: 567–70.
97. **Vento SI, Wolff CH, Salven PJ, *et al.*** Vascular permeability factor/vascular endothelial growth factor in nasal polyps. *Acta Otolaryngol Suppl.* 2000; 543: 170–4.
98. **Qu Z, Liebler JM, Powers MR, *et al.*** Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol.* 1995; 147: 564–73.
99. **Qu Z, Huang X, Ahmadi P, *et al.*** Synthesis of basic fibroblast growth factor by murine mast cells. Regulation by transforming growth factor beta, tumor necrosis factor alpha, and stem cell factor. *Int Arch Allergy Immunol.* 1998a; 115: 47–54.
100. **Qu Z, Kayton RJ, Ahmadi P, *et al.*** Ultrastructural immunolocalization of basic fibroblast growth factor in mast cell secretory granules. Morphological evidence for bfgf release through degranulation. *J Histochem Cytochem.* 1998b; 46: 1119–28.
101. **Grützkau A, Krüger-Krasagakes S, Baumeister H, *et al.*** Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: implications for the biological significance of VEGF206. *Mol Biol Cell.* 1998; 9: 875–84.
102. **Abdel-Majid RM, Marshall JS.** Prostaglandin E2 induces degranulation-independent production of vascular endothelial growth factor by human mast cells. *J Immunol.* 2004; 172: 1227–36.
103. **Boesiger J, Tsai M, Maurer M, *et al.*** Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of fc epsilon receptor I expression. *J Exp Med.* 1998; 188: 1135–45.
104. **Kanbe N, Kurosawa M, Nagata H, *et al.*** Production of fibrogenic cytokines by cord blood-derived cultured human mast cells. *J Allergy Clin Immunol.* 2000; 106: S85–S90.
105. **Detmar M, Brown LF, Schon MP, *et al.*** Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. *J Invest Dermatol.* 1998; 111: 1–6.
106. **Detoraki A, Staiano RI, Granata F, *et al.*** Vascular endothelial growth factors syntesized by human lung mast cells exert angiogenic effects. *J Allergy Clin Immunol.* 2009; 123: 1142–9.
107. **Ribatti D, Crivellato E, Candussio L, *et al.*** Mast cells and their secretory granules are angiogenic in the chick embryo chorioallantoic membrane. *Clin Exp Allergy.* 2001; 31: 602–8.
108. **Norrby K, Jakobsson A, Sörbo J.** Mast cell-mediated angiogenesis: a novel experimental model using the rat mesentery. *Virchows Arch B Cell Pathol Mol Pathol.* 1986; 52: 195–206.
109. **Norrby K, Jakobsson A, Sörbo J.** Mast-cell secretion and angiogenesis, a quantitative study in rats and mice. *Virchows Arch B Cell Pathol Mol Pathol.* 1989; 57: 251–6.
110. **Metcalfe DD, Baram D, Mekori YA.** Mast cells. *Physiol Rev.* 1997; 77: 1033–79.
111. **Blair RJ, Meng H, Marchese MJ, *et al.*** Human mast cells stimulate vascular tube formation: tryptase is a novel potent angiogenic factor. *J Clin Invest.* 1997; 99: 2691–700.
112. **Ribatti D, Roncali L, Nico B, *et al.*** Effects of exogenous heparin on the vasculogenesis of the chorioallantoic membrane. *Acta Anat.* 1987; 130: 257–63.
113. **Moller A, Lippert U, Lessmann D, *et al.*** Human mast cells produce IL-8. *J Immunol.* 1993; 151: 3261–6.
114. **Walsh LJ, Trinchieri G, Waldorf HA, *et al.*** Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA.* 1991; 88: 4220–4.
115. **Nilsson G, Forsberg-Nilsson K, Xiang, Z, *et al.*** Human mast cells express functional TrkA and are a source of nerve growth factor. *Eur J Immunol.* 1997; 27: 2295–301.
116. **Aoki M, Pawankar R, Niimi Y, *et al.*** Mast cells in basal cell carcinoma express VEGF, IL-8 and RANTES. *Int Arch Allergy Immunol.* 2003; 130: 216–23.
117. **Starkey JR, Crowle PK, Taubenberger S.** Mast cell-deficient W/Wv mice exhibit a decreased rate of tumor angiogenesis. *Int J Cancer.* 1988; 42: 48–52.
118. **Theoharides TC, Conti P.** Mast cells: the Jekyll and Hyde of tumor growth. *Trends Immunol.* 2004; 25: 235–41.
119. **Glowacki J, Mulliken JB.** Mast cells in hemangiomas and vascular malformations. *Pediatrics.* 1982; 70: 48–51.
120. **Hartveit F.** Mast cells and metachromasia in human breast cancer: their occurrence, significance and consequence: a preliminary report. *J Pathol.* 1981; 134: 7–11.
121. **Bowrey PF, King J, Magarey C, *et al.*** Histamine, mast cells and tumour cell proliferation in breast cancer: does preoperative cimetidine administration have an effect? *Br J Cancer.* 2000; 82: 167–70.
122. **Lachter J, Stein M, Lichting C, *et al.*** Mast cells in colorectal neoplasias and premalignant disorders. *Dis Colon Rectum.* 1995; 38: 290–3.
123. **Graham R, Graham J.** Mast cells and cancer of the cervix. *Surg Gynecol Obstet.* 1966; 123: 3–9.
124. **Benitez-Bribiesca L, Wong A, Utrera D, *et al.*** The role of mast cell tryptase in neoangiogenesis of premalignant and malignant lesions of the uterine cervix. *J Histochem Cytochem.* 2001; 49: 1061–2.
125. **Sawatsubashi M, Yamada T, Fukushima N, *et al.*** Association of vascular endothelial growth factor and mast cells with angiogenesis in laryngeal squamous cell carcinoma. *Virchows Arch B Cell Pathol Mol Pathol.* 2000; 436: 243–8.
126. **Imada A, Shijubo N, Koijma H, *et al.*** Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. *Eur Resp J.* 2000; 15: 1087–93.
127. **Takanami I, Takeuchi K, Naruke M.** Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. *Cancer.* 2000; 88: 2686–92.
128. **Tomita M, Matsuzaki Y, Onitsuka T.** Effect of mast cells on tumor angiogenesis in lung cancer. *Ann Thorac Surg.* 2000; 69: 1686–9.

129. **Reed JA, McNutt NS, Bogdany JK, *et al.*** Expression of the mast cell growth factor interleukin-3 in the melanocytic lesions correlates with an increased number of mast cells in the perilesional stroma: implications for melanoma progression. *J Cutan Pathol.* 1996; 23: 495–505.
130. **Dvorak AM, Mihm MC Jr, Osage JE, *et al.*** Melanoma. An ultrastructural study of the host inflammatory and vascular responses. *J Invest Dermatol.* 1980; 75: 388–93.
131. **Tóth-Jakatics R, Jimi S, Takebayashi S, *et al.*** Cutaneous malignant melanoma: correlation between neovascularization and peritumoral accumulation of mast cells overexpressing vascular endothelial growth factor. *Human Pathol.* 2000; 31: 955–60.
132. **Ribatti D, Vacca A, Ria R, *et al.*** Neovascularization, expression of fibroblast growth factor-2, and mast cells with tryptase activity increase simultaneously with pathological progression in human malignant melanoma. *Eur J Cancer.* 2003; 39: 666–74.
133. **Ribatti D, Ennas MG, Vacca A, *et al.*** Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. *Eur J.Clin Invest.* 2003; 33: 420–5.
134. **Elpek GO, Gelen T, Aksoy NH, *et al.*** The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. *J Clin Pathol.* 2001; 54: 940–4.
135. **Ribatti D, Finato N, Crivellato E, *et al.*** Neovascularization and mast cells with tryptase activity increase simultaneously with pathological progression in human endometrial cancer. *Am J Obstet Gynecol.* 2005; 193: 1961–5.
136. **Ribatti D, Nico B, Vacca A, *et al.*** Do mast cells help to induce angiogenesis in B-cell non-Hodgkin's lymphomas? *Br J Cancer.* 1998; 77: 1900–6.
137. **Ribatti D, Vacca A, Marzullo A, *et al.*** Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. *Int J Cancer.* 2002; 85: 171–5.
138. **Fukushima N, Satoh T, Sano M, *et al.*** Angiogenesis and mast cells in non-Hodgkin's lymphoma: a strong correlation in angioimmunoblastic T-cell lymphoma. *Leuk Lymphoma.* 2001; 42: 709–20.
139. **Ribatti D, Vacca A, Nico B, *et al.*** Bone marrow angiogenesis and mast cell density increase simultaneously with progression of human multiple myeloma. *Br J Cancer.* 1999; 79: 451–5.
140. **Ribatti D, Polimero G, Vacca A, *et al.*** Correlation of bone marrow angiogenesis and mast cells with tryptase activity in myelodysplastic syndromes. *Leukemia.* 2002; 16: 1680–4.
141. **Ribatti D, Molica S, Vacca A, *et al.*** Tryptase-positive mast cells correlate positively with bone marrow angiogenesis in B-cell chronic lymphocytic leukemia. *Leukemia.* 2003; 17: 1428–30.
142. **Molica S, Vacca A, Crivellato E, *et al.*** Tryptase-positive mast cells predict clinical outcome of patients with B-cell chronic lymphocytioc leukemia. *Eur J Haematol.* 2003; 71: 137–9.
143. **Italiano JE Jr, Richardson JL, Paltel-Hett S, *et al.*** Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood.* 2008; 111: 1227–33.
144. **Klement GL, Yip TT, Cassiola F, *et al.*** Platelets actively sequester angiogenesis regulators. *Blood.* 2009; 113: 2835–42.