

EDITORIAL

Mast cells and angiogenesis

In health and disease there are two main mechanisms by which blood vessels are formed, namely vasculogenesis and angiogenesis.

Vasculogenesis is the formation of networked microvasculature by incorporation of cells (angioblasts). This happens mainly during embryogenesis; the origin of the angioblasts is in the mesoderm [1].

Angiogenesis is the growth of new blood vessels from pre-existing microcirculation [2]. This is the predominant mechanism of blood vessel formation in later stages of embryonic development and in the adult, for example in the female reproductive organs [3,4] and in the retina [5]. It also happens in pathological conditions in direct response to tissue demands such as wound healing, inflammation, tumour growth and others [4,6–8].

A third mechanism has been recently described in which blood channels are formed by tumour cells of uveal melanoma in the absence of endothelial cells [9]. This mechanism has been termed 'vasculogenic mimicry'.

New vessel formation is a multistep, highly orchestrated process not only involving vessel sprouting but also endothelial cell migration, proliferation, tube formation and survival [1,2]. Various growth factors and proteins, elements of the extracellular matrix, components of the coagulation/fibrinolytic system, and platelets, interact with the endothelial cells and pericytes of blood vessels to regulate new vessel formation [1,2]. Mesoderm-inducing factors of the fibroblast growth factor family (FGFs) are crucial in causing mesoderm to form angioblasts and haematopoietic cells in vasculogenesis [2]. In angiogenesis the following are among the most important factors: vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), basic fibroblast growth factor (bFGF), tumour growth factor-beta (TGF- β), epidermal growth factor (EGF), platelet derived growth factor (PDGF), tumour necrosis factor-alpha (TNF- α) and platelet-derived endothelial cell growth factor (PD-ECGF) [1,2].

VPF/VEGF is the essential growth factor for endothelial cells which is sufficient for the formation of new blood vessels if over expressed *in vivo* [10]. VEGF acts as a true growth factor. It induces an increase in the number of endothelial cells [1], and is their survival factor [7]. It can also act as a lumenizing factor by virtue of its ability to increase vascular permeability [1]. Specific VEGF receptors are VEGF-R1 (Flt-1), VEGF-R2 (Flk-1), TIE-1 and

TIE-2 [2]. VEGF and its high affinity receptor tyrosine kinase Flk-1 represent a paracrine signalling system crucial for the differentiation of endothelial cells and the development of the vascular system [2]. In contrast, bFGF, a member of the heparin binding growth factor family, does not induce new vessel formation. However, it is a potent mitogenic factor and it has been shown to be one of the most potent angiogenic factors *in vitro* and *in vivo* [11]. The role of other factors such as PDGF, PD-ECGF, TNF- α , TGF- β , TGF- α and EGF seem to be complementary [1,12].

The producing cells of the main angiogenic growth factors are variable according to the process in which they are involved. In embryonic development where the endoderm is adjacent to the mesoderm, VEGF can be produced by the endoderm reaching the mesoderm by the paracrine way [1]. In some tissues like the retina in which the endoderm does not exist, VEGF is produced by astrocytes and Müller cells, that are the glial cells of the retina, and is secreted to the adjacent endothelial cells [5].

In pathological situations the producer cells vary according to the organ system affected and the aetiopathogenesis of the disease. In these cases, the angiogenic factor can be secreted by cells other than those that secrete in normal conditions [8].

In the vast majority of the pathological processes the main mechanism of new vessel formation is angiogenesis. In most of these disorders but not in all, the angiogenesis is accompanied by and possibly requires inflammation [2].

In inflammation, infiltrating inflammatory cells and some resident cells are the producers of the angiogenic factors. All inflammatory cell types have been demonstrated to produce VEGF. For example, human neutrophils [13,14], CD4⁺ and CD8⁺ peripheral blood lymphocytes [15] and T-lymphocytes synthesize and secrete VEGF [16]. Moreover, plasma cells have been found to express mRNA for VPF/VEGF [17]. Peritoneal macrophages of cirrhotic patients express mRNA for VEGF and secrete, upon activation, the protein [18]. Very recently also human peripheral eosinophils were found to express VEGF and especially when stimulated by granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-5 [19]. Fibroblasts, as resident cells, are also a demonstrated rich source of VEGF [20,21].

Mast cells that are widely distributed in the connective tissues, where they are frequently located in close

proximity to blood vessels, can be considered both as resident cells and as infiltrating cells that participate in a number of inflammatory reactions [22]. Mast cells have been linked for almost two decades to neovascularization. Actually, several lines of evidence, many of them derived from histological studies, have implicated mast cells in the regulation of pathological or physiological examples of angiogenesis, including that associated with haemangiomas [23], neoplasms [24,25], rheumatoid arthritis [26], nasal polyps [27], wound healing [28] and ovulation [29]. However, the exact contribution of mast cells to the neovascularization process as a potential source of pro-angiogenic factors still remains largely unknown and only recently some research has focused on the production and secretion of angiogenic factors from mast cells.

For example, mast cells from human tissues with chronic inflammation [30,31] and rat/mouse tissues with anaphylaxis were shown to possess bFGF in their cytoplasmic granules that can be released through degranulation [31].

Boesiger et al. [32] showed that murine or human cord blood derived mast cells release VPF/VEGF upon stimulation through Fc ϵ RI or c-Kit or after challenge with phorbol myristate acetate, or calcium ionophore. Such mast cells can rapidly release VPF/VEGF apparently from a pre-formed pool, and can then sustain release by secreting newly synthesized protein.

Also, the human mast cell leukaemic cell line HMC-1 can constitutively express and secrete three isoforms of VPF/VEGF and stimulation of these cells for 24 h results in enhanced secretion of this factor [33].

Mast cells can contribute to various aspects of angiogenesis not only through the production of bFGF and VEGF but also through other pre-formed mediators or cytokines. For example, heparin that is contained in large amounts in mast cell secretory granules, is important in angiogenesis. In fact, it stimulates endothelial cell chemotaxis [34] and proliferation [35] and, by binding bFGF, it renders it biologically active and protected from proteolysis. Histamine and more recently tryptase have been shown to be angiogenic factors [36,37]. In addition, TGF- β , TNF- α , IL-8 and other cytokines produced by mast cells might contribute to the angiogenic process [22,38]. Also the production of collagen type VIII by human mast cells *in vivo* may influence angiogenesis since this collagen is believed to facilitate the assembly of endothelial cords and tubes and its synthesis precedes that of pro-collagen type I [39].

Interestingly, angiogenic factors are not only produced by mast cells but they have also been shown to stimulate mast cell migration at sites of angiogenesis [40].

The paper by Ribatti et al. [41] that is published in this issue is an important contribution since it demonstrates a direct role of mast cells in angiogenesis in an *in vivo*

model. The authors show that in the chick embryo chorioallantoic membrane assay, degranulated mast cells and their secretory granules induce an angiogenic response. The addition of anti-FGF-2 (bFGF) or anti-VEGF antibodies significantly reduces the angiogenic response, indicating that these two factors are primarily responsible for the mast cell vasoproliferative activity.

In allergic inflammation, the recognized key initiator and effector cells are the mast cells. Also in allergy, as a consequence of tissue damage, coordinated sequelae of re-epithelization, angiogenesis and remodelling are evident. Mast cells, that have the capability of surviving for extended periods of time and being activated and reactivated on a multiple basis, have been proposed to influence these processes as well [42]. Recently, it has been recognized that in the asthmatic lung, fibrosis can develop as a major previously overlooked pathological process [43]. The contribution of mast cells to lung fibrosis and especially to angiogenesis will have to be evaluated in depth for a better understanding of the pathogenesis of asthma.

Angiogenesis has been proposed as a target for anti-cancer therapy [44] and for treatment of pathological neovascularization such as in hypoxic retina [45]. We believe that angiogenesis might be one of the targets also for anti-inflammatory therapy.

Indeed, various anti-inflammatory drugs have also been found to be anti-angiogenic. For example, glucocorticosteroids that are the most potent anti-inflammatory drugs employed, have anti-angiogenic properties [46]. Notably glucocorticosteroid anti-angiogenic activity is not only due to their recognized ability to decrease inflammatory cell numbers and infiltration into the tissues, but also to their direct inhibition of the expression of the VEGF gene [47]. This latter mechanism can be due to an interaction between the glucocorticosteroid receptor with transcription factors, such as AP-1 and NF- κ B [48], whose response elements are located in the VEGF gene promoter [49]. Also non-steroidal anti-inflammatory drugs such as aspirin, indomethacin and ibuprofen were found to be anti-angiogenic through a direct effect on COX1 and COX2 of endothelial cells [50].

Other therapeutic possibilities would include the use of neutralizing antibodies for the main angiogenic factors such as VEGF and bFGF, as suggested by the report of Ribatti et al. [41]. In addition, anti-receptor antibodies and anti-sense therapies can be considered as well.

In conclusion, our current state of knowledge indicates that the cascade of angiogenic events depends on complex processes that include cell-cell interactions, various intracellular signalling pathways, and the appropriate extracellular micro-environment. Therefore, the important observation that mast cells produce and release angiogenic

factors is not enough to define the role of these cells in neovascularization. It remains to be determined under what circumstances mast cells represent a critical source of angiogenic factors *in vivo*, and, in such instances, what signals regulate their production and secretion in order to build new therapeutic interventions associated with mast cell presence and activation.

References

- 1 Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 1995; 11:778–91.
- 2 Risau W. Mechanisms of angiogenesis. *Nature* 1997; 386:671–4.
- 3 Augustin HG, Braun K, Telemeniakis I et al. Ovarian angiogenesis; phenotypic characterization of endothelial cells in a physiological model of blood-vessel growth and regression. *Am J Pathol* 1995; 147:339–51.
- 4 Dvorak HF. VPF/VEGF and the angiogenic response. *Semin Perinatol* 2000; 24:75–8.
- 5 Stone J, Itin A, Alon T et al. Development of retinal vasculature is mediated by hypoxia-induced expression of vascular endothelial growth factor (VEGF) by neuroglia. *J Neurosci* 1995; 15:4738–47.
- 6 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. *Nat Med* 1995; 1:27–31.
- 7 Alon T, Hemo I, Itin A et al. Vascular endothelial growth factor acts as a survival factor for newly-formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1995; 1:1024–8.
- 8 Pe'er J, Shweiki D, Itin A et al. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. *Lab Invest* 1995; 72:638–45.
- 9 Maniotis AJ, Folberg R, Hess A et al. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. *Am J Pathol* 1999; 155:739–52.
- 10 Flamme I, von Reutern M, Drexler H et al. Overexpression of vascular endothelial growth factor in the avian embryo induces hypervascularization and increased vascular permeability without alterations of embryonic pattern formation. *Dev Biol* 1995; 171:399–414.
- 11 Reed JA, Albino AP, McNutt NS. Human cutaneous mast cells express basic fibroblast growth factor. *Lab Invest* 1995; 72:215–22.
- 12 Riley BB, Savage MP, Simandl BK et al. Retroviral expression of FGF-2 (bFGF) affects patterning in chick limb bud. *Development* 1993; 118:95–104.
- 13 Gaudry M, Bregerie O, Andrieu V et al. Intracellular pool of vascular endothelial growth factor in human neutrophils. *Blood* 1997; 15:4153–61.
- 14 Scapini P, Calzetti F, Cassatella MA. On the detection of neutrophil-derived vascular endothelial growth factor (VEGF). *J Immunol Methods* 1999; 17:121–9.
- 15 Iijima K, Yoshikawa N, Nakamura H. Activation-induced expression of vascular permeability factor by human peripheral T cells: a non-radioisotopic semiquantitative reverse transcription-polymerase chain reaction assay. *J Immunol Methods* 1996; 196:199–209.
- 16 Freeman MR, Schneck FX, Gagnon ML et al. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. *Cancer Res* 1995; 15:4140–5.
- 17 Ito A, Hirota S, Mizuno H et al. Expression of vascular permeability factor (VPF/VEGF) messenger RNA by plasma cells: possible involvement in the development of edema in chronic inflammation. *Pathol Int* 1995; 45:715–20.
- 18 Perez-Ruiz M, Ros J, Morales-Ruiz M et al. Vascular endothelial growth factor production in peritoneal macrophages of cirrhotic patients: regulation by cytokines and bacterial lipopolysaccharide. *Hepatology* 1999; 29:1057–63.
- 19 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.
- 20 Pinney E, Liu K, Sheeman B et al. Human three-dimensional fibroblast cultures express angiogenic activity. *J Cell Physiol* 2000; 183:74–82.
- 21 Cho CS, Cho ML, Min SY et al. CD40 engagement on synovial fibroblast up-regulates production of vascular endothelial growth factor. *J Immunol* 2000; 164:5055–61.
- 22 Church M, Levi-Schaffer F. The human mast cell. *J Allergy Clin Immunol* 1997; 99:155–60.
- 23 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.
- 24 Imada A, Shijubo N, Kojima H et al. Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. *Eur Respir J* 2000; 15:1087–93.
- 25 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* 2000; 436:243–8.
- 26 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.
- 27 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.
- 28 Trabucchi E, Radaelli E, Marazzi M et al. The role of mast cells in wound healing. *Int J Tissue React* 1988; 10:367–72.
- 29 Kirshna A, Beesley K, Terranova PF. Histamine, mast cells and ovarian function. *J Endocrinol* 1989; 120:363–71.
- 30 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* 1998; 115:47–54.
- 31 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* 1998; 46:1119–28.

- 32 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 Fcε receptor I expression. *J Exp Med* 1998; 188:1135–45.
- 33 Grutzkau A, Kruger-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.
- 34 Azizkhan RG, Azizkhan JC, Zetter BR et al. Mast cell heparin stimulates migration of capillary endothelial cells in vitro. *J Exp Med* 1980; 152:931–44.
- 35 Folkman J. Regulation of angiogenesis: a new function of heparin. *Biochem Pharmacol* 1985; 34:905–9.
- 36 Sozbo J, Jakobsson A, Norrby R. Mast cell histamine is angiogenic through receptors for histamine 1 and histamine 2. *Int J Exp Pathol* 1994; 75:43–50.
- 37 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.
- 38 Norrby K. Mast cells and de novo angiogenesis: angiogenic capability of individual mast-cell mediators such as histamine, TNF, IL-8 and bFGF. *Inflamm Res* 1997; 46(Suppl 1):57–8.
- 39 Ruger B, Dunbar PR, Hasan Q et al. Human mast cells produce type VIII collagen in vivo. *Int J Exp Pathol* 1994; 75:397–404.
- 40 Gruber BL, Marchese MJ, Kew R. Angiogenic factors stimulate mast-cell migration. *Blood* 1995; 86:2488–93.
- 41 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.
- 42 Levi-Schaffer F, Weg VB. Mast cells, eosinophils and fibrosis. *Clin Exp Allergy* 1997; 27(Suppl. 1):64–70.
- 43 Fish JE, Peters SP. Airway remodeling and persistent airway destruction in asthma. *J Allergy Clin Immunol* 1999; 104: 509–16.
- 44 Folkman J. Fighting cancer by attacking its blood supply. *Sci Am* 1996; 275:150–4.
- 45 Aiello LP, Pierce EA, Foley ED et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc Natl Acad Sci USA* 1995; 92: 10457–61.
- 46 Foschi D, Castoldi L, Corsi F et al. Inhibition of inflammatory angiogenesis in rats by loco-regional administration of hydrocortisone and protamine. *Agents Actions* 1994; 42:40–3.
- 47 Nauck M, Karakiulakis G, Perruchoud AP et al. Corticosteroids inhibit the expression of the vascular endothelial growth factor gene in human vascular smooth muscle cells. *Eur J Pharmacol* 1998; 341:309–15.
- 48 Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* 1994; 94:1202–13.
- 49 Tischer E, Mitchell R, Hartman T et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternate exon splicing. *J Biol Chem* 1991; 266: 11947–54.
- 50 Jones MK, Wang H, Peskar BM et al. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med* 1999; 5:1418–23.

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