Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Innate effector cells in angiogenesis and lymphangiogenesis

Gilda Varricchi[1,2,3], Stefania Loffredo[1,2,3], Maria Rosaria Galdiero[1,2,3], Giancarlo Marone[4,5], Leonardo Cristinziano[1,2], Francescopaolo Granata[1,2,3] and Gianni Marone[1,2,3,6]

Angiogenesis and lymphangiogenesis are distinct and complex processes requiring a finely tuned balance between stimulatory and inhibitory signals. During adulthood, angiogenesis and lymphangiogenesis are activated at sites of tumor growth, tissue injury and remodeling, and chronic inflammation. Vascular endothelial growth factors (VEGFs), angiopoietin (ANGPTs) and a multitude of additional signaling molecules play distinct roles in the modulation of angiogenesis/lymphangiogenesis. VEGFs and ANGPTs activate specific tyrosine kinase receptor (e.g., VEGFR1, VEGFR-2, VEGFR-3 and TIE2 respectively), expressed on blood endothelial cells (angiogenesis) and lymphatic endothelial cells (lymphangiogenesis). Although tumor cells produce VEGFs and other proangiogenic mediators, tissue resident (e.g., macrophages, mast cells) and circulating immune cells (e.g., basophils, neutrophils, monocytes, eosinophils) are an important source of angiogenic/lymphangiogenic mediators in inflammation and in tumor microenvironment and at site of chronic inflammation. Certain immune cells can also release anti-angiogenic factors. Mast cells, basophils, neutrophils and presumably other immune cells are not only a source of angiogenic/lymphangiogenic molecules, but also their target. Cells of the immune system need consideration as major players and possible targets for therapeutic manipulation of angiogenesis/lymphangiogenesis in chronic inflammatory disorders and tumors.

Addresses
[1] Department of Traslational Medicine, University of Naples Federico II, Naples, Italy
[2] Center for Basic and Clinical Immunology Research (CISI), Naples, Italy
[3] World Allergy Organization (WAO), Center of Excellence, Naples, Italy
[4] Department of Public Health, Section of Hygiene, University of Naples Federico II, Naples, Italy
[5] Monaldi Hospital Pharmacy, Naples, Italy
[6] Institute of Experimental Endocrinology and Oncology "Gaetano Salvatore", National Research Council, Naples, Italy

Corresponding authors: Varricchi, Gilda (gildanet@gmail.com), Marone, Gianni (marone@unina.it)

Current Opinion in Immunology 2018, **53**:152–160

This review comes from a themed issue on **Lymphangiogenesis: heterogeneity and function**

Edited by **Silvano Sozzani** and **Marco Presta**

For a complete overview see the Issue and the Editorial

Available online 17th May 2018

https://doi.org/10.1016/j.coi.2018.05.002

Introduction

Angiogenesis and lymphangiogenesis are distinct and complex processes requiring a finely tuned balance between stimulatory and inhibitory signals [1••,2•,3]. Analogous to blood vessels, the formation of new lymphatic vessels occurs vigorously during embryogenesis, but is restricted in adults [1••]. During adulthood, angiogenesis/lymphangiogenesis is limited to sites of chronic inflammation [4], tissue injury or remodeling [5], and cancer [6••].

Angiogenesis is initiated by activation of vascular endothelial growth factor receptor 2 (VEGFR2, a tyrosine kinase (TK) receptor expressed on blood endothelial cells (BECs), by soluble vascular endothelial growth factor-A (VEGF-A)). Although cancer cells are an important source of VEGF-A and other pro-angiogenic mediators [7–9], immune cells in tumor microenvironment (TME) increase VEGF-A availability during the angiogenic switch [10•]. Angiogenesis requires the participation of additional signaling molecules, including angiopoietin 2 (ANGPT2) and delta ligand-like4 (DLL4), which respectively, destabilizes endothelial cells (EC) junctions and controls the tip-cell phenotype [11].

VEGFs and their endothelial TK receptors are central regulators of vasculogenesis, angiogenesis, and

lymphangiogenesis [12]. VEGF-A signaling through VEGFR2 is the major angiogenic pathway, and blockage of VEGF-A/VEGFR2 signaling is the major antiangiogenic strategy [13]. VEGF-C and VEGF-D, through the engagement of VEGFR3 on lymphatic endothelial cells (LECs) induce lymphangiogenesis in tumors and stimulate the formation of metastasis [14,15].

The vascular endothelial growth factor family and their receptors

The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF) [10•]. VEGFs bind with different specificity to three mainly endothelial transmembrane receptors, VEGFR-1, VEGFR-2, and VEFGR-3 [16••,17•,18]. All VEGFRs have a series of immunoglobulin-like domains in the extracellular part and an intracellular split TK domain [12]. Neuropilins 1 (NRP1) and 2 (NRP2) function as co-receptors for specific VEGFs [3]. VEGF-A signaling through VEGFR2 activates angiogenesis by inducing the survival, proliferation, sprouting and migration of BECs, increases endothelial permeability [19••,20••] and induces inflammation [16••,21•,22].

There are several splicing isoforms of VEGF-A (121, 165, 189, and 206) which differ in their binding to matrix and to co-receptors; for example, VEGF-A_{121} is acidic and freely diffusible whereas VEGF-A_{165}, VEGF-A_{189}, and VEGF-A_{206} are basic and bind to heparin and heparin proteoglycans on cellular surfaces and extracellular matrices [23]. VEGF-A binding to VEGFR2 causes receptor dimerization and autophosphorylation at several TK residues [24]. Activation of the receptor leads to its internalization, degradation in the lysosomes or through the proteasome pathway. These are mechanisms for VEGFR2 signal downregulation and the rapid turnover of VEGF-A/VEGFR2 is important for the tight control of VEGFR signaling [17•].

VEGF-A is primarily known for its essential role in physiologic and pathologic angiogenesis [25] and also retains lymphangiogenic properties [26]. The angiogenic activity of VEGF-A is mediated by interaction with VEGFR2, whereas the lymphangiogenic activity is promoted by binding to VEGFR2/VEGFR3 heterodimer receptor [12]. VEGF-A stimulates lymphangiogenesis also indirectly by recruiting immune cells (e.g., macrophages, mast cells) that produce VEGF-C and VEGF-D [21•,27•]

PlGF is expressed in the placenta, heart and lungs and has four isoforms (PlGF1–4) [28,29]. VEGF-B is highly expressed in heart, skeletal muscle and brown fat in adults and has two major isoforms in humans: VEGF-B_{167} binds to heparin proteoglycans, whereas VEGF-B_{186} does not bind heparin and is more soluble [30]. PlGF and VEGF-B bind with high affinity to VEGFR1 whose TK activity is weak and downstream signaling poorly understood [17•]. VEGFR1 is expressed on ECs, some immune cells and pericytes, and its TK activity is required for cell migration towards VEGFs or PlGF [16••,22,31]. VEGF-B and PlGF have been shown to be angiogenic in certain pathophysiological settings [32]. VEGF-B can modulate coronary vessel growth and cardiac hypertrophy and lipid metabolism [29,33].

The VEGF-C/VEGFR3 signaling pathway is the main pathway implicated in lymphangiogenesis [18]. VEGF-C is produced as a precursor protein, which is activated by intracellular convertase [34]. The secreted disulfide-linked VEGF-C subunit only binds VEGFR3, but the factor is further proteolysed in the extracellular environment by proteases to generate homodimeric proteins with high affinity for both VEGFR2 and VEGFR3 [35]. VEGF-C is crucial for the survival, proliferation, and migration of LECs [36]. VEGF-D also binds VEGFR3 to promote lymphangiogenesis. Maturation of VEGF-D is similar to that of VEGF-C and occurs by the cleavage of N- and C- terminal regions. VEGF-D overexpression correlates with lymphatic vessels growth and lymphatic metastasis [37]. VEGFR3, expressed on LECs, is activated by both VEGF-C and VEGF-D [14]. The role of VEGFR2 in lymphangiogenesis is still not completely clarified. VEGFR2 is expressed in LEC at low levels, but VEGF-A overexpression in skin and in models of wound healing and skin tumors cause lymphatic vessels hyperplasia [38,39].

Angiopoietins (ANGPTs) play an important role in modulating angiogenesis and lymphangiogenesis. The ANGPT/Tie system consists of two cell-surface TK receptors (TIE1 and TIE2) and two ligands ANGPT1 and ANGPT2. TIE2 is primarily expressed on ECs and binds both ANGPTs, whereas TIE1 is an orphan receptor that can modulate TIE2 activity. ANGPT1 and ANGPT2 bind TIE2 on ECs, but elicit different responses. ANGPT1 is expressed by perivascular cells, such as pericytes and sustains EC survival of blood endothelial cells. By contrast, ANGPT2, secreted by ECs, acts autocrinally and paracrinally as TIE2 ligand to promote angiogenesis and lymphangiogenesis [40•].

Several chemokines, produced by immune and non-immune cells, play an important role in the modulation of angiogenesis and anti-angiogenesis [41•]. These molecules are also involved in the recruitment of tumor infiltrating immune cells [42•].

Inflammatory angiogenesis and lymphangiogenesis

The association between angiogenesis and tumor growth attracted paramount interest during the last decades for the obvious implications of the nature of initiation of tumors and the possibility to inhibit cancer growth by

blocking angiogenesis [13]. By contrast, inflammation-associated angiogenesis and lymphangiogenesis was a rather neglected field until recently. The interest in inflammatory angiogenesis/lymphangiogenesis increased exponentially during last years for a number of reasons. First, chronic low grade inflammation is an essential hallmark of cancer [43••]. Second, angiogenesis and lymphangiogenesis are components of several inflammatory disorders [10•,44]. Third, there is compelling evidence that several immune cells can be involved, directly and indirectly, in the modulation of angiogenesis and lymphangiogenesis [4,8,10•,16••,19••,21•,27•,41•,44]. The latter observation led to the recognition that the interactions between immune cells and the vascular system are involved in a multitude of human inflammatory diseases in addition to cancer [45•]. Moreover, accumulating evidence suggests that inflammation-associated angiogenesis/lymphangiogenesis is not merely an endpoint phenotype of inflammation, but rather a dynamic reaction that can alter the process of inflammatory tissue repair and resolution [4]. Finally, there is evidence that circulating progenitor cells can be incorporated into the growing lymphatic vessels and trans-differentiate into LECs [4,46].

Human innate effector cells modulate angiogenesis and lymphangiogenesis

In 1971, Judah Folkman, the father of angiogenic research suggested that tumor growth is angiogenesis-dependent and inhibition of angiogenesis could be of therapeutic value [47]. He also suggested that immune cells, in particular macrophages and mast cells, could be a source of angiogenic factors modulating tumor growth. There is now compelling evidence that several cells of innate and adaptive immune system can play major roles in the complex processes of inflammatory and tumor angiogenesis/lymphangiogenesis [2•,45•].

Monocytes

Transcriptomic, epigenetic, and functional studies have delineated three mononuclear cell lineages within myeloid network: monocytes, macrophages, and dendritic cells [48]. In the past it was assumed that monocytes merely constitute a transient precursor for tissue macrophages. Classical dendritic cells and macrophages originate independently of monocyte input. Conversely, monocytes have emerged as a highly plastic and dynamic cellular system. There are currently three main human monocyte subpopulations defined as $CD14^+$ $CD16^+$ (classical monocytes), $CD14^+$ $CD16^+$ (intermediate), and $CD14^-$ $CD16^+$ (non-classical monocytes) [49].

Several stimuli (e.g., M-CSF, ATP, cysteinyl-leukotriene D_4) can stimulate VEGF production from human monocytes [50–52]. These cells are also a target of angiogenic mediators. Human monocytes express VEGFR1 whose activation by VEGF-A induce migration [22]. VEGF-A-induced chemotaxis of $CD16^+$ monocytes is reduced compared to $CD16^-$ monocytes due to a lower VEGFR1 expression [53].

De Palma and collaborators have identified a unique subset of human monocytes which express the TIE2 receptor [54•]. TIE2-expressing monocytes are angiogenic and lymphangiogenic and have been associated with different human tumors [55,56]. Moreover, ANGPT2 stimulates TIE2-expressing monocytes to suppress T cell activation [57] and it has been shown that targeting the ANGT2/TIE2 axis inhibits tumor growth [58].

Macrophages

Macrophages play a pivotal role in chronic inflammation and tumor growth [59]. These cells are the predominant immune cells in inflamed tissues as well as in various solid tumors where they are referred to as tumor-associated macrophages (TAMs) [60]. Macrophages can promote tumor progression either directly, by stimulating the proliferation of cancer cells, or indirectly, by producing angiogenic and lymphangiogenic factors [61]. These programs are induced by TAMs in TME by signals resulting from metabolic conditions (e.g., hypoxia), low pH or from mediators (e.g., cytokines, LPS, adenosine) in TME [10•,61].

Macrophages purified from human lung parenchyma (HLMs) activated by secreted phospholipases A_2 ($sPLA_2$) [27•] and lipopolysaccaride (LPS) expressed VEGF-A, VEGF-B, VEGF-C, and VEGF-D at both RNA and protein levels [62•]. Adenosine induced a synergistic increase of VEGF-A released from HLMs through the engagement of A_{2a} and A_3 receptors [27•]. These results demonstrate that human tissue resident macrophages can be a source of both proangiogenic and lymphangiogenic factors. Interestingly, HLMs express the cannabinoid receptors types 1 and 2 (CB1 and CB2 receptors) and the activation of these receptors by specific agonists inhibits LPS-induced production of VEGF-A, VEGF-C, ANGPT1 and ANGPT2. The latter findings suggest that activation of cannabinoid receptors on tissue resident macrophages may be a novel strategy to modulate vascular remodeling in cancer and chronic inflammation.

There is increasing evidence of the heterogeneity of the origin of monocytes and monocyte-derived macrophages in rodents and in humans [63•]. Interestingly, human monocyte-derived macrophages produce VEGF-A and VEGF-C, but the release is not modified by the CB1 and CB2 agonists [62•]. Macrophage-derived proteases including matrix metalloproteinases (MMPs) (e.g., MMP2, MMP9, MMP12) and serine or cysteine proteinases, such as cathepsins and plasminogen activator, facilitate the infiltrative growth of tumor cells in the TME [64•,65].

Mast cells

Mast cells are tissue-resident immune cells, and their involvement in tumor angiogenesis has long been demonstrated [8,44]. Tissue mast cells and peripheral blood basophils are the only cells expressing the high-affinity receptors (FcεRI) for IgE and synthesizing a plethora of pro-inflammatory and immuno-regulatory factors. Although these cells share some similarities, they play different roles in several aspects of innate and adaptive immunity [66•].

Several immunologic and non-immunologic stimuli (e.g., anti-IgE, PGE_2, substance P, corticotropin-releasing hormone, adenosine) induce the production of VEGF-A from human mast cells (HMCs) [67–69]. We have demonstrated that human lung mast cells (HLMCs) constitutively express several isoforms of VEGF-A (121, 165, 189, and 206) and that the IgE-mediated activation of these cells can induce the release of VEGF-A [21•]. In addition to VEGF-A, HMCs express the two canonical isoforms of VEGF-B (167 and 186). Importantly, supernatants of immunologically activated HLMCs induced an angiogenic response in the chick embryo-chorioallantoic membrane (CAM). Interestingly, HLMCs expressed lymphangiogenic factors, such as VEGF-C and VEGF-D and adenosine increased VEGF-A, and VEGF-C production. Both VEGFR1 and VEGFR2 are expressed on HMCs and VEGF-A, VEGF-B, VEGF-C and VEGF-D induce mast cell chemotaxis *in vitro* [21•] and *in vivo* [7] through the activation of both VEGFR1 and VEGFR2. Collectively, these results indicate that HMCs are a major source of both angiogenic and lymphangiogenic factors. Moreover, VEGFs produced at sites of inflammation and tumors can amplify mast cell infiltration by interacting with VEGFRs. Mast cell-derived proteases, such as tryptase, MMP2 and MMP9 chymase, can modulate angiogenesis [66•]. Mast cells can reduce the efficacy of anti-angiogenic therapy by releasing granzyme B [70•]. It remains to be investigated whether human mast cells from different anatomic sites and subpopulations of these cells also express anti-angiogenic molecules when exposed to immunological stimuli.

Basophils

Basophils purified from peripheral blood of healthy donors constitutively express mRNA for three isoforms of VEGF-A (121, 165 and 189) and two isoforms of VEGF-B (167 and 186) [16••]. In addition, basophils infiltrating human nasal polyps contained VEGF-A stored in secretory granules. IgE-mediated activation of basophils induced the rapid release of VEGF-A. Human basophils also express on their surface VEGFR2 and the co-receptors NRP1 and NRP2. VEGF-A induces basophil chemotaxis through the engagement of VEGFR2. These results can have relevance in inflammatory disorders [10•,44] and in certain tumors [71] in which basophils can infiltrate the site of chronic inflammation and TME. In contrast to mast cells, basophils do not express/release lymphangiogenic factors. An interesting cross-talk between HMCs and basophils is mediated by the ANGPT1/TIE2 system. ANGPT1 is present in cytoplasmic vesicles of human basophils and their activation rapidly release ANGPT1 that induce HMC chemotaxis through the activation of TIE2 on their surface [72]. Although basophils can play a role in tumor growth [8,66•] and have been detected in draining lymph nodes of cancer patients [71], the production of lymphangiogenic factors by these cells should be further evaluated.

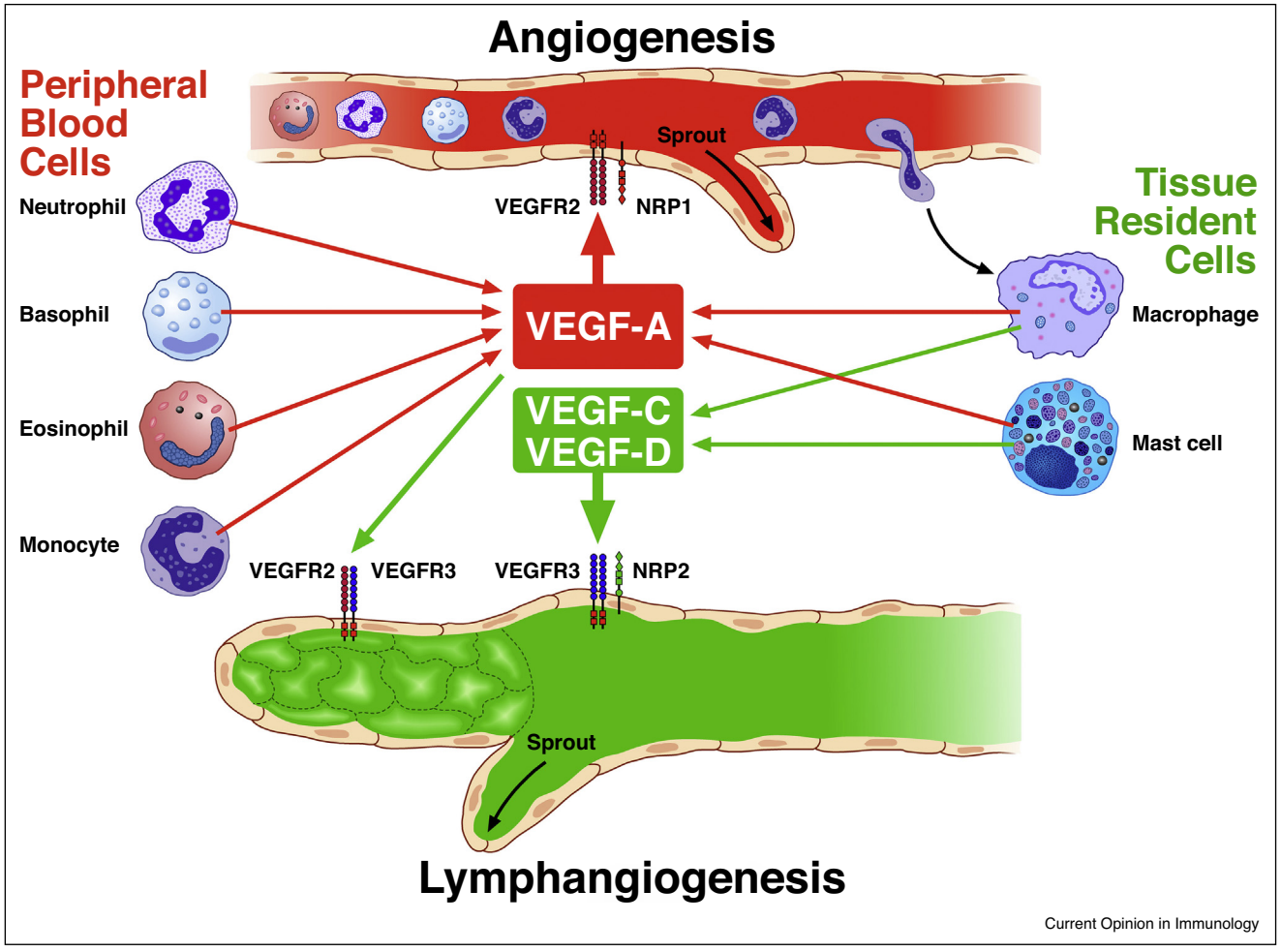
Neutrophils

Neutrophils (PMNs) are abundant granulocytes in human blood and account for a substantial proportion of immune cells in TME in experimental and human tumors [61,73]. Because of their terminally differentiated phenotype and short-half life, the role of neutrophils in tumor development has been erroneously considered marginal. Recent evidence indicates that tumor-associated neutrophils (TAN) can exert anti-tumoral as well as pro-tumoral functions and PMNs display unsuspected plasticity [74•]. Human PMNs release VEGF-A in response to various stimuli (e.g., fMLF, LPS) [75]. We found that several $sPLA_2$ induce the release of pro-angiogenic molecules such as VEGF-A, CXCL8/IL-8 and ANGPT1 [19••]. Interestingly, $sPLA_2$ induced the secretion of the anti-angiogenic VEGF-A isoform, namely VEGF-A_{165b}. While the release of VEGF-A, CXCL8/IL-8, and ANGPT1 was mediated by $sPLA_2$ enzymatic activity and/or binding to a cell membrane receptor(s), the release of VEGF-A_{165b} required the interaction with β integrins. These results suggest that the engagement of different receptors on human PMNs results in the release of different isoforms of VEGF-A exerting opposite effects (e.g., proangiogenic vs antiangiogenic). The translational relevance of these findings is supported by the observation that sPLAs are increased in human cancer [76] that are infiltrated by PMNs [73]. There is no evidence that human the activation of PMNs induces the production of lymphangiogenic factors. Neutrophil-derived MMP9 prompts the angiogenic switch in TME [77].

Eosinophils

Eosinophils represent a minor granulocytic cell infiltrate in experimental and human tumors and have been erroneously neglected for decades. Immunologists and Oncologists are now appreciating the role of these cells in experimental and human tumors [78••,79]. Eosinophils are present in TME of several human solid and hematologic tumors [78••] and their infiltration depends on a combination of cytokines/chemokines produced by tumors and by tumor-infiltrating immune cells. Human eosinophils produce several angiogenic factors such as VEGF-A [80,81•], fibroblast growth factors (FGF-2) [82,83], CXCL8/IL-8 [84], and osteopontin [85]. Human eosinophils also produce MMP9 [86–88]. Eosinophils

Figure 1



Several cells of innate and adaptive immune system play a role in the complex process of inflammatory and tumor angiogenesis and lymphangiogenesis. Activation of human peripheral blood neutrophils, basophils, eosinophils and monocytes induce the production of VEGF-A, which is the main proangiogenic factor. This molecule signals through the engagement of VEGFR2 on blood endothelial cells (BECs). VEGF-A also has pro-lymphangiogenic properties by binding to VEGFR2/VEGFR3 heterodimer receptor on lymphatic endothelial cells (LECs). By contrast, tissue resident macrophages and mast cells produce both proangiogenic (VEGF-A) and lymphangiogenic factors (VEGF-C and VEGF-D). The lymphangiogenic activity of VEGF-C and VEGF-D is mediated by binding to VEGFR3 on LECs. Neutropilins (NRP1 and NRP2) function as co-receptors and amplify the tyrosine kinase activity of VEGFRs.

have been detected in metastatic lymph nodes of cancer patients, but the production of lymphangiogenic factors by these cells should be further addressed.

Concluding remarks and perspectives

Compelling evidence indicates that cells of innate and adaptive immune system can participate in the complex processes of inflammatory angiogenesis and lymphangiogenesis by releasing a wide spectrum of factors which varies enormously among the various cells. Neutrophils, basophils, monocytes and eosinophils isolated from peripheral blood of healthy donors are a major source of several isoforms of proangiogenic molecules. By contrast, macrophages and mast cells isolated from human tissues produce both angiogenic and lymphangiogenic factors (Figure 1). A growing list of immunologic and non-immunologic stimuli present at sites of inflammation and in TME activate *in vitro* cells of innate immunity to produce angiogenic/lymphangiogenic molecules. However, a note of caution should be mentioned because studies *in vitro* are performed in physiological conditions which are different from the hostile environment at sites of inflammation and tumor growth (e.g., low pH, high concentration of adenosine and oxygen radicals). All the above factors can profoundly influence the metabolic activities of immune cells.

Mast cells, basophils, neutrophils, monocytes and presumably other immune cells are not only a source of angiogenic/lymphangiogenic molecules, but also a target

[16••,21•,22]. In fact, HMCs and basophils express specific receptors (VEGFR1, VEGFR2 and TIE2) and co-receptors (NRP1 and NRP2) for angiogenic and lymphangiogenic factors. These mediators can also exert pro-inflammatory effects (e.g., chemotaxis) by engaging receptors and co-receptors present on HMCs, basophils and presumably other immune cells. Thus, it is likely that angiogenic/lymphangiogenic mediators produced by immune cells at site of inflammation or in TME can contribute to chronic inflammation and cancer growth by recruiting further immune cells.

Human basophils, mast cells, macrophages, and PMNs are among the few cells that express both isoforms of VEGF-B [16••,19••,21•,27•]. Although VEGF-B can modulate coronary vessels growth [33,89•] and lipid metabolism [90], it remains the most mysterious member of the VEGF family [91]. We have found that VEGF-B can play a role in inflammation by inducing immune cells chemotaxis [16••,21•]. After being neglected for years, the role of VEGF-B in inflammation and immunity deserves further investigations.

Angiogenesis and lymphangiogenesis are controlled by a delicate balance between a plethora of stimulating and inhibitory factors which are incompletely understood. We have found that the engagement of different receptors on human neutrophils can produce distinct isoforms of VEGF-A exerting pro-angiogenic and anti-angiogenic activity [19••]. It will be important to verify whether the activation of subpopulations of PMNs (e.g., N1 vs N2) leads to the preferential production of pro-angiogenic or anti-angiogenic molecules. Moreover, additional studies are urgently needed to identify the release of anti-angiogenic factors from other human immune cells activated by different stimuli.

TAM phenotypes are highly plastic, and recent reports suggest that the canonical model distinguishing polarized anti-tumor M1 and alternatively polarized pro-tumor M2 incompletely accounts for the phenotypic diversity *in vivo* [92]. In addition, there is evidence, at least in rodents, of subpopulations of N1 and N2 neutrophils [74•]. Recent advances in single-cell genomics technologies are beginning to allow us the identification of a wide spectrum of TAM and T cell phenotypes [92]. The characterization of different phenotypes of cells of the adaptive and innate immune system will reveal subpopulations of immune cells producing distinct patterns of pro-angiogenic and anti-angiogenic/lymphangiogenic molecules.

Recent studies have shown that tumor regression or refractoriness after immune checkpoint blockade (ICB) with monoclonal antibodies targeting cytotoxic T lymphocytes associated antigen 4 (CTLA-4) or programmed cell-death protein 1 (PD-1) were associated with altered serum levels of ANGPT2 [93•]. Moreover, ICB activates $CD4^+$ T lymphocytes which favor tumor blood vessel normalization [94]. Thus, tumor response to immunotherapy may involve immune-mediated anti-angiogenic mechanisms. Cells of the immune system and their mediators need consideration for future therapeutic manipulation of angiogenesis/lymphangiogenesis in chronic inflammatory disorders and tumors.

Conflict of interest statement

Nothing declared.

Acknowledgements

We regret that, because of space limitations, we could not always adequately quote the work of our colleagues who have greatly contributed to the field reviewed here. This work was supported in part by grants from Regione Campania CISI-Lab Project, CRèME Project, and TIMING Project. The authors thank Dr. Gjada Criscuolo for critical reading of the manuscript and Fabrizio Fiorbianco for the elaboration of the figure.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Zheng W, Aspelund A, Alitalo K: **Lymphangiogenic factors, mechanisms, and applications**. *J Clin Invest* 2014, **124**:878-887.
This comprehensive review summarizes the recent progress on lymphangiogenic factors and mechanism and their applications in disease treatment.

2. • Marone G, Granata F: **Angiogenesis, lymphangiogenesis and clinical implications. Preface**. *Chem Immunol Allergy* 2014, **99**:XI-XII.
See annotation to Ref. [1••].

3. Zachary I: **Neuropilins: role in signalling, angiogenesis and disease**. *Chem Immunol Allergy* 2014, **99**:37-70.

4. Kim H, Kataru RP, Koh GY: **Inflammation-associated lymphangiogenesis: a double-edged sword?** *J Clin Invest* 2014, **124**:936-942.

5. Lee SJ, Park C, Lee JY, Kim S, Kwon PJ, Kim W, Jeon YH, Lee E, Yoon YS: **Generation of pure lymphatic endothelial cells from human pluripotent stem cells and their therapeutic effects on wound repair**. *Sci Rep* 2015, **5**:11019.

6. •• Karaman S, Detmar M: **Mechanisms of lymphatic metastasis**. *J Clin Invest* 2014, **124**:922-928.
This elegant review illustrates the complex role of lymphatic vessel and lymphangiogenic factors in tumor growth and immune modulation in tumor microenvironment.

7. Melillo RM, Guarino V, Avilla E, Galdiero MR, Prevete N, Rossi FW, Basolo F, Ugolini C, de Paulis A, Santoro M: **Mast cells play a protumorigenic role in human thyroid cancer**. *Oncogene* 2010, **29**:6203.

8. Varricchi G, Galdiero MR, Loffredo S, Marone G, Iannone R, Granata F: **Are mast cells MASTers in cancer?** *Front Immunol* 2017, **8**:424.

9. Prevete N, Liotti F, Visciano C, Marone G, Melillo RM, de Paulis A: **The formyl peptide receptor 1 exerts a tumor suppressor function in human gastric cancer by inhibiting angiogenesis**. *Oncogene* 2015, **34**:3826-3838.

10. • Varricchi G, Granata F, Loffredo S, Genovese A, Marone G: **Angiogenesis and lymphangiogenesis in inflammatory skin disorders**. *J Am Acad Dermatol* 2015, **73**:144-153.
In this review, the authors discuss the role of cells of innate and adaptive immunity in the modulation of angiogenesis and lymphangiogenesis in skin disorders.

11. Potente M, Gerhardt H, Carmeliet P: **Basic and therapeutic aspects of angiogenesis**. *Cell* 2011, **146**:873-887.

12. Simons M, Gordon E, Claesson-Welsh L: **Mechanisms and regulation of endothelial VEGF receptor signalling**. *Nat Rev Mol Cell Biol* 2016, **17**:611-625.

13. Welti J, Loges S, Dimmeler S, Carmeliet P: **Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer**. *J Clin Invest* 2013, **123**:3190-3200.

14. Karpanen T, Egeblad M, Karkkainen MJ, Kubo H, Yla-Herttuala S, Jaattela M, Alitalo K: **Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth**. *Cancer Res* 2001, **61**:1786-1790.

15. Achen MG, Williams RA, Baldwin ME, Lai P, Roufail S, Alitalo K, Stacker SA: **The angiogenic and lymphangiogenic factor vascular endothelial growth factor-D exhibits a paracrine mode of action in cancer**. *Growth Factors* 2002, **20**:99-107.

16. •• de Paulis A, Prevete N, Fiorentino I, Rossi FW, Staibano S, Montuori N, Ragno P, Longobardi A, Liccardo B, Genovese A *et al.*: **Expression and functions of the vascular endothelial growth factors and their receptors in human basophils**. *J Immunol* 2006, **177**:7322-7331.
This is the first demonstration that human basophils could play a role in angiogenesis and inflammation through the expression of several forms of VEGF and their receptors.

17. • Shibuya M, Claesson-Welsh L: **Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis**. *Exp Cell Res* 2006, **312**:549-560.
This review illustrates the signal transduction by VEGFR in regulation of blood endothelial cells and lymphatic endothelial cells.

18. Heinolainen K, Karaman S, D'Amico G, Tammela T, Sormunen R, Eklund L, Alitalo K, Zarkada G: **VEGFR3 modulates vascular permeability by controlling VEGF/VEGFR2 signaling**. *Circ Res* 2017, **120**:1414-1425.

19. •• Loffredo S, Borriello F, Iannone R, Ferrara AL, Galdiero MR, Gigantino V, Esposito P, Varricchi G, Lambeau G, Cassatella MA, Granata F, Marone G: **Group V secreted phospholipase A2 induces the release of proangiogenic and antiangiogenic factors by human neutrophils**. *Front Immunol* 2017, **8**:443.
This is the first demonstration that human neutrophils activated by different stimuli can release pro-angiogenic and anti-angiogenic factors.

20. •• Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF: **Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid**. *Science* 1983, **219**:983-985.
This is the first and elegant identification and characterization of vascular permeability factor (VPF), now defined as VEGF.

21. • Detoraki A, Staiano RI, Granata F, Giannattasio G, Prevete N, de Paulis A, Ribatti D, Genovese A, Triggiani M, Marone G: **Vascular endothelial growth factors synthesized by human lung mast cells exert angiogenic effects**. *J Allergy Clin Immunol* 2009, **123**:1142-1149 1149 e1–5.
This report demonstrates that human mast cells are both a source and a target of angiogenic and lymphangiogenic factors and therefore may play a role in inflammatory and neoplastic angiogenesis and lymphangiogenesis.

22. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D: **Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1**. *Blood* 1996, **87**:3336-3343.

23. Houck KA, Leung DW, Rowland AM, Winer J, Ferrara N: **Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms**. *J Biol Chem* 1992, **267**:26031-26037.

24. Dougher M, Terman BI: **Autophosphorylation of KDR in the kinase domain is required for maximal VEGF-stimulated kinase activity and receptor internalization**. *Oncogene* 1999, **18**:1619-1627.

25. Carmeliet P: **Angiogenesis in life, disease and medicine**. *Nature* 2005, **438**:932-936.

26. Wuest TR, Carr DJ: **VEGF-A expression by HSV-1-infected cells drives corneal lymphangiogenesis**. *J Exp Med* 2010, **207**:101-115.

27. • Granata F, Frattini A, Loffredo S, Staiano RI, Petraroli A, Ribatti D, Oslund R, Gelb MH, Lambeau G, Marone G, Triggiani M: **Production of vascular endothelial growth factors from human lung macrophages induced by group IIA and group X secreted phospholipases A2**. *J Immunol* 2010, **184**:5232-5241.
This is the first demonstration that secretes phospholipases A2 can induce the production of both angiogenic and lymphangiogenic factors from human macrophages suggesting that secreted phospholipases may play a role in inflammatory and neoplastic angiogenesis and lymphangiogenesis.

28. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG: **Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor**. *Proc Natl Acad Sci U S A* 1991, **88**:9267-9271.

29. Bry M, Kivela R, Leppanen VM, Alitalo K: **Vascular endothelial growth factor-B in physiology and disease**. *Physiol Rev* 2014, **94**:779-794.

30. Maglione D, Guerriero V, Viglietto G, Ferraro MG, Aprelikova O, Alitalo K, Del Vecchio S, Lei KJ, Chou JY, Persico MG: **Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14**. *Oncogene* 1993, **8**:925-931.

31. Clauss M, Weich H, Breier G, Knies U, Rockl W, Waltenberger J, Risau W: **The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis**. *J Biol Chem* 1996, **271**:17629-17634.

32. Fischer C, Mazzone M, Jonckx B, Carmeliet P: **FLT1 and its ligands VEGFB and PlGF: drug targets for anti-angiogenic therapy?** *Nat Rev Cancer* 2008, **8**:942-956.

33. Karpanen T, Bry M, Ollila HM, Seppanen-Laakso T, Liimatta E, Leskinen H, Kivela R, Helkamaa T, Merentie M, Jeltsch M *et al.*: **Overexpression of vascular endothelial growth factor-B in mouse heart alters cardiac lipid metabolism and induces myocardial hypertrophy**. *Circ Res* 2008, **103**:1018-1026.

34. Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N, Alitalo K: **A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases**. *EMBO J* 1996, **15**:1751.

35. Alitalo K, Tammela T, Petrova TV: **Lymphangiogenesis in development and human disease**. *Nature* 2005, **438**:946-953.

36. Tammela T, Saaristo A, Lohela M, Morisada T, Tornberg J, Norrmen C, Oike Y, Pajusola K, Thurston G, Suda T, Yla-Herttuala S, Alitalo K: **Angiopoietin-1 promotes lymphatic sprouting and hyperplasia**. *Blood* 2005, **105**:4642-4648.

37. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, Prevo R, Jackson DG, Nishikawa S, Kubo H, Achen MG: **VEGF-D promotes the metastatic spread of tumor cells via the lymphatics**. *Nat Med* 2001, **7**:186-191.

38. Wirzenius M, Tammela T, Uutela M, He Y, Odorisio T, Zambruno G, Nagy JA, Dvorak HF, Yla-Herttuala S, Shibuya M, Alitalo K: **Distinct vascular endothelial growth factor signals for lymphatic vessel enlargement and sprouting**. *J Exp Med* 2007, **204**:1431-1440.

39. Hirakawa S, Kodama S, Kunstfeld R, Kajiya K, Brown LF, Detmar M: **VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis**. *J Exp Med* 2005, **201**:1089-1099.

40. • Eklund L, Saharinen P: **Angiopoietin signaling in the vasculature**. *Exp Cell Res* 2013, **319**:1271-1280.
In this review, the authors illustrate the current understanding and the signaling functions of the angiopoietin–Tie pathway and its potential future development of targeted vascular therapies.

41. • Bosisio D, Salvi V, Gagliostro V, Sozzani S: **Angiogenic and antiangiogenic chemokines**. *Chem Immunol Allergy* 2014, **99**:89-104.
Elegant review highlighting that chemokines can exert both pro-genic and angiogenic roles either by acting directly on endothelial cells or by recruiting that, in turn, secreted angiogenic mediators.

42. Schulz O, Hammerschmidt SI, Moschovakis GL, Forster R:
• **Chemokines and chemokine receptors in lymphoid tissue dynamics**. *Annu Rev Immunol* 2016, **34**:203-242.
In this review, the authors focus on the rule of the chemokines and chemokine receptors in the migration dynamics of immune cells in infectious and inflammatory processes.

43. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next**
•• **generation**. *Cell* 2011, **144**:646-674.
In this seminal review, the authors illustrate the fundamental hallmarks of cancer that provide a framework for understanding the remarkable diversity of neoplastic diseases.

44. Marone G, Varricchi G, Loffredo S, Granata F: **Mast cells and basophils in inflammatory and tumor angiogenesis and lymphangiogenesis**. *Eur J Pharmacol* 2016, **778**:146-151.

45. De Palma M, Biziato D, Petrova TV: **Microenvironmental**
• **regulation of tumour angiogenesis**. *Nat Rev Cancer* 2017, **17**:457-474.
This review illustrates the complex regulation of angiogenesis by the tumor microenvironment, highlighting potential targets of anti-angiogenic cancer therapies.

46. Kerjaschki D, Huttary N, Raab I, Regele H, Bojarski-Nagy K, Bartel G, Krober SM, Greinix H, Rosenmaier A, Karlhofer F, Wick N, Mazal PR: **Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants**. *Nat Med* 2006, **12**:230-234.

47. Folkman J: **Tumor angiogenesis: therapeutic implications**. *N Engl J Med* 1971, **285**:1182-1186.

48. Sander J, Schmidt SV, Cirovic B, McGovern N, Papantonopoulou O, Hardt AL, Aschenbrenner AC, Kreer C, Quast T, Xu AM *et al.*: **Cellular differentiation of human monocytes is regulated by time-dependent interleukin-4 signaling and the transcriptional regulator NCOR2**. *Immunity* 2017, **47** 1051–1066 e12.

49. Ginhoux F, Jung S: **Monocytes and macrophages: developmental pathways and tissue homeostasis**. *Nat Rev Immunol* 2014, **14**:392-404.

50. Eubank TD, Galloway M, Montague CM, Waldman WJ, Marsh CB: **M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes**. *J Immunol* 2003, **171**:2637-2643.

51. Hill LM, Gavala ML, Lenertz LY, Bertics PJ: **Extracellular ATP may contribute to tissue repair by rapidly stimulating purinergic receptor X7-dependent vascular endothelial growth factor release from primary human monocytes**. *J Immunol* 2010, **185**:3028-3034.

52. Poulin S, Thompson C, Thivierge M, Veronneau S, McMahon S, Dubois CM, Stankova J, Rola-Pleszczynski M: **Cysteinyl-leukotrienes induce vascular endothelial growth factor production in human monocytes and bronchial smooth muscle cells**. *Clin Exp Allergy* 2011, **41**:204-217.

53. Czepluch FS, Olieslagers S, van Hulten R, Voo SA, Waltenberger J: **VEGF-A-induced chemotaxis of CD16+ monocytes is decreased secondary to lower VEGFR-1 expression**. *Atherosclerosis* 2011, **215**:331-338.

54. De Palma M, Venneri MA, Galli R, Sergi Sergi L, Politi LS,
• Sampaolesi M, Naldini L: **Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors**. *Cancer Cell* 2005, **8**:211-226.
This is the first identification of a hematopoietic lineage of proangiogenic Tie2 monocytes required for tumor vessel formation.

55. Matsubara T, Kanto T, Kuroda S, Yoshio S, Higashitani K, Kakita N, Miyazaki M, Sakakibara M, Hiramatsu N, Kasahara A *et al.*: **TIE2-expressing monocytes as a diagnostic marker for hepatocellular carcinoma correlates with angiogenesis**. *Hepatology* 2013, **57**:1416-1425.

56. Turrini R, Pabois A, Xenarios I, Coukos G, Delaloye JF, Doucey MA: **TIE-2 expressing monocytes in human cancers**. *Oncoimmunology* 2017, **6**:e1303585.

57. Coffelt SB, Chen YY, Muthana M, Welford AF, Tal AO, Scholz A, Plate KH, Reiss Y, Murdoch C, De Palma M, Lewis CE: **Angiopoietin 2 stimulates TIE2-expressing monocytes to suppress T cell activation and to promote regulatory T cell expansion**. *J Immunol* 2011, **186**:4183-4190.

58. Mazzieri R, Pucci F, Moi D, Zonari E, Ranghetti A, Berti A, Politi LS, Gentner B, Brown JL, Naldini L, De Palma M: **Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells**. *Cancer Cell* 2011, **19**:512-526.

59. Bonavita E, Galdiero MR, Jaillon S, Mantovani A: **Phagocytes as corrupted policemen in cancer-related inflammation**. *Adv Cancer Res* 2015, **128**:141-171.

60. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M: **Macrophage plasticity and polarization in tissue repair and remodelling**. *J Pathol* 2013, **229**:176-185.

61. Galdiero MR, Bonavita E, Barajon I, Garlanda C, Mantovani A, Jaillon S: **Tumor associated macrophages and neutrophils in cancer**. *Immunobiology* 2013, **218**:1402-1410.

62. Staiano RI, Loffredo S, Borriello F, Iannotti FA, Piscitelli F,
• Orlando P, Secondo A, Granata F, Lepore MT, Fiorelli A *et al.*: **Human lung-resident macrophages express CB1 and CB2 receptors whose activation inhibits the release of angiogenic and lymphangiogenic factors**. *J Leukoc Biol* 2016, **99**:531-540.
This is the first demonstration that human lung macrophages express the cannabinoid receptors and that their activation selectively inhibits the release of angiogenic and lymphangiogenic factors.

63. Gordon S, Pluddemann A: **Tissue macrophages: heterogeneity**
• **and functions**. *BMC Biol* 2017, **15**:53.
This review describes macrophage phenotypic heterogeneity in different tissue environments, drawing particular attention to organ-specific functions.

64. Kessenbrock K, Plaks V, Werb Z: **Matrix metalloproteinases:**
• **regulators of the tumor microenvironment**. *Cell* 2010, **141**:52-67.
This is a comprehensive review describing the role of matrix metalloproteinases as modulators of the tumor microenvironment.

65. Olson OC, Joyce JA: **Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response**. *Nat Rev Cancer* 2015, **15**:712-729.

66. Varricchi G, Raap U, Rivellese F, Marone G, Gibbs B: **Human mast**
• **cells and basophils—how are they similar, how are they different?** *Immunol Rev* 2018 http://dx.doi.org/10.1111/imr.12627.
This comprehensive review illustrates the similarities and differences of human mast cells and basophils and their distinct role in inflammatory disorders and in cancer.

67. Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, Dvorak HF, Galli SJ: **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**:1135-1145.

68. Abdel-Majid RM, Marshall JS: **Prostaglandin E2 induces degranulation-independent production of vascular endothelial growth factor by human mast cells**. *J Immunol* 2004, **172**:1227-1236.

69. Theoharides TC, Zhang B, Kempuraj D, Tagen M, Vasiadi M, Angelidou A, Alysandratos KD, Kalogeromitros D, Asadi S, Stavrianeas N *et al.*: **IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin**. *Proc Natl Acad Sci U S A* 2010, **107**:4448-4453.

70. Wroblewski M, Bauer R, Cubas Cordova M, Udonta F, Ben-
• Batalla I, Legler K, Hauser C, Egberts J, Janning M, Velthaus J *et al.*: **Mast cells decrease efficacy of anti-angiogenic therapy by secreting matrix-degrading granzyme B**. *Nat Commun* 2017, **8**:269.
This report demonstrates that granzyme B, released by activated mast cells, can decrease the efficacy of anti-angiogenic therapy.

71. De Monte L, Wormann S, Brunetto E, Heltai S, Magliacane G, Reni M, Paganoni AM, Recalde H, Mondino A, Falconi M *et al.*: **Basophil recruitment into tumor-draining lymph nodes correlates with Th2 inflammation and reduced survival in pancreatic cancer patients**. *Cancer Res* 2016, **76**:1792-1803.

72. Prevete N, Rossi FW, Rivellese F, Lamacchia D, Pelosi C, Lobasso A, Necchi V, Solcia E, Fiocca R, Ceppa P *et al.*: ***Helicobacter pylori* HP (2-20) induces eosinophil activation and accumulation in superficial gastric mucosa and stimulates VEGF-alpha and TGF-beta release by interacting with formyl-peptide receptors**. *Int J Immunopathol Pharmacol* 2013, **26**:647-662.

73. Galdiero MR, Bianchi P, Grizzi F, Di Caro G, Basso G, Ponzetta A, Bonavita E, Barbagallo M, Tartari S, Polentarutti N *et al.*: **Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer**. *Int J Cancer* 2016, **139**:446-456.

74. • Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM: **Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN**. *Cancer Cell* 2009, **16**:183-194.
This is the first identification of neutrophils subpopulations (N1 vs N2) and their different role in tumor growth.

75. Tecchio C, Micheletti A, Cassatella MA: **Neutrophil-derived cytokines: facts beyond expression**. *Front Immunol* 2014, **5**:508.

76. Triggiani M, Giannattasio G, Calabrese C, Loffredo S, Granata F, Fiorello A, Santini M, Gelb MH, Marone G: **Lung mast cells are a source of secreted phospholipases A2**. *J Allergy Clin Immunol* 2009, **124**:558-565 565 e1–3.

77. Nozawa H, Chiu C, Hanahan D: **Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis**. *Proc Natl Acad Sci U S A* 2006, **103**:12493-12498.

78. •• Varricchi G, Galdiero MR, Loffredo S, Lucarini V, Marone G, Mattei F, Marone G, Schiavoni G: **Eosinophils: the unsung heroes in cancer?** *OncoImmunology* 2017 http://dx.doi.org/10.1080/2162402X.2017.1393134.
This comprehensive review illustrates the complex roles of eosinophils and their mediators in tumor initiation and progression.

79. Lucarini V, Ziccheddu G, Macchia I, La Sorsa V, Peschiaroli F, Buccione C, Sistigu A, Sanchez M, Andreone S, D'Urso MT *et al.*: **IL-33 restricts tumor growth and inhibits pulmonary metastasis in melanoma-bearing mice through eosinophils**. *Oncoimmunology* 2017, **6**:e1317420.

80. 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**:70-77.

81. • Nissim Ben Efraim AH, Levi-Schaffer F: **Roles of eosinophils in the modulation of angiogenesis**. *Chem Immunol Allergy* 2014, **99**:138-154.
This elegant review describes the current understanding of the role of human eosinophils in the modulation of angiogenesis.

82. 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.

83. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hammerling GJ: **Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells**. *Nat Immunol* 2015, **16**:609-617.

84. Yousefi S, Hemmann S, Weber M, Holzer C, Hartung K, Blaser K, Simon HU: **IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma**. *J Immunol* 1995, **154**:5481-5490.

85. Puxeddu I, Berkman N, Ribatti D, Bader R, Haitchi HM, Davies DE, Howarth PH, Levi-Schaffer F: **Osteopontin is expressed and functional in human eosinophils**. *Allergy* 2010, **65**:168-174.

86. Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA: **Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF-alpha**. *J Immunol* 2001, **167**:4008-4016.

87. Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM: **Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9**. *Am J Respir Cell Mol Biol* 1997, **17**:519-528.

88. Schwingshackl A, Duszyk M, Brown N, Moqbel R: **Human eosinophils release matrix metalloproteinase-9 on stimulation with TNF-alpha**. *J Allergy Clin Immunol* 1999, **104**:983-989.

89. • Bry M, Kivela R, Holopainen T, Anisimov A, Tammela T, Soronen J, Silvola J, Saraste A, Jeltsch M, Korpisalo P *et al.*: **Vascular endothelial growth factor-B acts as a coronary growth factor in transgenic rats without inducing angiogenesis, vascular leak, or inflammation**. *Circulation* 2010, **122**:1725-1733.
This review summarizes what is currently known about the main functions of VEGF-B in physiology and disease.

90. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, van Meeteren LA, Samen E, Lu L, Vanwildemeersch M *et al.*: **Vascular endothelial growth factor B controls endothelial fatty acid uptake**. *Nature* 2010, **464**:917-921.

91. Li X, Kumar A, Zhang F, Lee C, Tang Z: **Complicated life, complicated VEGF-B**. *Trends Mol Med* 2012, **18**:119-127.

92. Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, Bacac M, Ries CH, Ailles L, Jewett MAS, Moch H *et al.*: **An immune atlas of clear cell renal cell carcinoma**. *Cell* 2017, **169** 736–749 e18.

93. • Wu X, Giobbie-Hurder A, Liao X, Connelly C, Connolly EM, Li J, Manos MP, Lawrence D, McDermott D, Severgnini M *et al.*: **Angiopoietin-2 as a biomarker and target for immune checkpoint therapy**. *Cancer Immunol Res* 2017, **5**:17-28.
This report suggests that angiopoietin-2 could be a predictive and prognostic biomarker in metastatic melanoma patients receiving immune checkpoint therapy.

94. Tian L, Goldstein A, Wang H, Ching Lo H, Sun Kim I, Welte T, Sheng K, Dobrolecki LE, Zhang X, Putluri N *et al.*: **Mutual regulation of tumour vessel normalization and immunostimulatory reprogramming**. *Nature* 2017, **544**:250-254.