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#### Competing interests statement

The authors declare [competing financial interests](#): see Web version for details.

#### FURTHER INFORMATION

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#### OPINION

# The parallel lives of angiogenesis and immunosuppression: cancer and other tales

Gregory T. Motz and George Coukos

**Abstract** | Emerging evidence indicates that angiogenesis and immunosuppression frequently occur simultaneously in response to diverse stimuli. Here, we describe a fundamental biological programme that involves the activation of both angiogenesis and immunosuppressive responses, often through the same cell types or soluble factors. We suggest that the initiation of these responses is part of a physiological and homeostatic tissue repair programme, which can be co-opted in pathological states, notably by tumours. This view can help to devise new cancer therapies and may have implications for aseptic tissue injury, pathogen-mediated tissue destruction, chronic inflammation and even reproduction.

The vascular system develops through the coordinated actions of both vasculogenesis and angiogenesis. Vasculogenesis gives rise to *de novo* blood vessels, whereas angiogenesis is the sprouting of new vessels from pre-existing ones. Physiological angiogenesis — which occurs during development and wound healing — proceeds through vessel destabilization, endothelial cell migration and proliferation, and sprouting. This is followed by the resolution phase, which is characterized by reduced endothelial cell proliferation and stabilization of the new vessel. Pathological angiogenesis shares many of the same processes, but is characterized by a failure of the resolution phase and the generation of a highly disorganized vascular network. Pathological angiogenesis is a key feature of tumour biology, but is also involved in a broad spectrum of inflammatory diseases, such as rheumatoid arthritis and connective tissue disorders<sup>1</sup>. Although pathological angiogenesis is generally viewed as a process driven by resident endothelial cells and mobilized endothelial progenitor cells, a complex tissue repair programme is responsible for regulating the process of remodelling and vessel formation. It is our view that pathological angiogenesis is integrated with and co-regulated by immunosuppressive processes in a homeostatic tissue repair programme.

There are numerous examples that demonstrate the existence of a biological response characterized by the simultaneous activation of angiogenesis and immunosuppression. This response can be initiated

by diverse physiological stimuli, such as those that occur during aseptic tissue injury resulting from ischaemia–reperfusion injury or wounding, during infection and even during pregnancy. We think that the benefit of such an interconnected and reciprocal tissue repair programme is to ensure tissue homeostasis. Summoning cells that can simultaneously mediate angiogenesis and immunosuppression provides an efficient process that economizes resources at times of homeostatic crisis. This hypothesis is supported by the ever-growing list of haematopoietic cell types that, when appropriately polarized, can promote both immunosuppression and angiogenesis. For example, myeloid-derived suppressor cells (MDSCs)<sup>2</sup>, dendritic cell (DC) subsets<sup>3,4</sup>, natural killer (NK) cells<sup>5</sup>, neutrophils<sup>6</sup>, macrophages, B cells<sup>7,8</sup> and regulatory T (T<sub>Reg</sub>) cells<sup>9</sup>, as well as the angiogenic endothelium itself<sup>10</sup>, have been shown to have this dual capacity. Furthermore, mediators secreted by these cells — such as vascular endothelial growth factor A (VEGFA) and prostaglandin E2 (PGE2) — have well-known functions in both angiogenesis and immunosuppression.

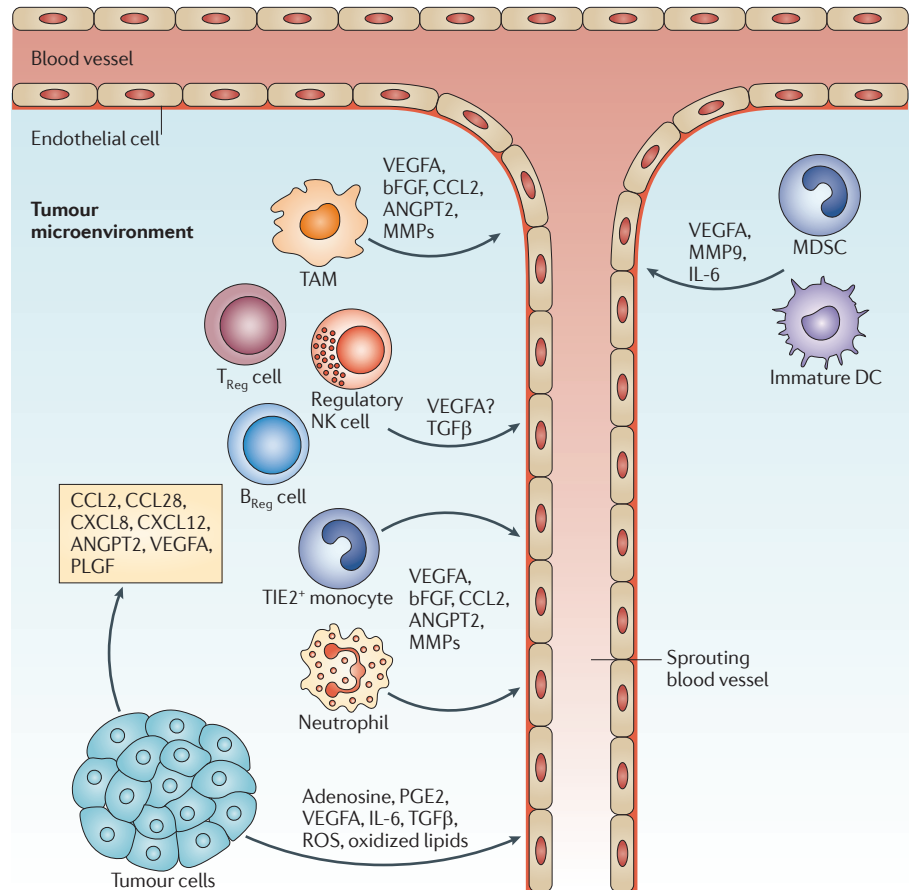
Tumour development, much like tissue repair and wound healing, requires the development of neovasculature and the suppression of excessive inflammation. It is possible that tumour development proceeds by the co-option of the homeostatic tissue repair programme, promoting concurrent angiogenesis and immunosuppression, and that this becomes the overarching biological programme that drives the polarization of the

tumour microenvironment. The dual regulation of angiogenesis and immunosuppression is obviously complex, with often-overlapping and potentially redundant pathways, and this pro-angiogenic and immunosuppressive programme is initiated by the expression of cellular functions and mediators in a context-specific manner. The stimuli responsible for the initiation phase of this programme are most likely to be chemical or physiological in nature (for example, oxidative stress<sup>11</sup> or hypoxia<sup>9</sup>). Under most circumstances, tissues would only encounter these stimuli briefly during a homeostatic crisis (such as that induced by wounding), but genetic mutation and transformation that drives tumour progression chronically activates these pathways. Thus, tumours capitalize on existing tissue repair mechanisms to promote their continued growth and dissemination.

The notion that angiogenesis and immunosuppression work hand-in-hand can be used to study the tumour microenvironment under a new light, in order to derive novel cancer therapies. This paradigm is becoming increasingly relevant to cutting-edge immunotherapy strategies for cancer. For example, we and others have demonstrated that disruption of angiogenesis substantially enhances the efficacy of immune-based cancer therapies such as tumour vaccines and adoptive cell therapy<sup>10,12,13</sup>. Furthermore, this view of the tumour microenvironment could also be used to enhance anti-angiogenesis therapy. For example, combining canonical anti-angiogenesis therapeutics with immunomodulatory drugs that promote pro-inflammatory T helper 1 (T<sub>H</sub>1) cells or deplete pro-angiogenic leukocyte subsets may provide a superior tumour therapy. Ultimately, we envision that future integrative biological treatment of tumours will capitalize on this view to deliver combined therapies that target immune and angiogenesis mechanisms<sup>12,13</sup>. Lessons that have been learnt from cancer biology could then be applied to basic investigation in diverse areas of immunology, including infection, tissue regeneration, autoimmunity, tolerance, transplantation biology and reproduction.

### Cellular players

Various haematopoietic cells that possess both immunosuppressive and pro-angiogenic abilities have been identified in tumours, including both myeloid and lymphocyte populations (FIG. 1). Myeloid cells have received much attention given their prominent roles in the processes of both immunosuppression and angiogenesis,



**Figure 1 | Promotion of angiogenesis by immunosuppressive cells.** Within the tumour microenvironment, multiple cell types with established roles in immunosuppression have been shown to promote angiogenesis through the production of various growth factors. Tumour cells, either in the steady state or in response to hypoxia, secrete soluble factors that recruit immunosuppressive cells to the tumour site. These factors include CC-chemokine ligand 2 (CCL2), CCL28, CXC-chemokine ligand 8 (CXCL8), CXCL12, angiopoietin 2 (ANGPT2), vascular endothelial growth factor A (VEGFA) and placenta growth factor (PLGF). Recruited immunosuppressive cells include tumour-associated macrophages (TAMs), TIE2<sup>+</sup> monocytes, neutrophils, myeloid-derived suppressor cells (MDSCs), immature dendritic cells (DCs), regulatory T (T<sub>Reg</sub>) cells, regulatory B (B<sub>Reg</sub>) cells and regulatory natural killer (NK) cells. The immunosuppressive cells secrete factors — such as VEGFA, basic fibroblast growth factor (bFGF), CCL2 and ANGPT2 — that directly promote endothelial cell proliferation and migration, and/or induce the production of matrix metalloproteinases (MMPs) that act on the extracellular matrix, allowing for the development of new blood vessels. Pro-angiogenic growth factors derived from these cells probably promote angiogenesis in an additive or synergistic manner, together with tumour-derived VEGFA, transforming growth factor-β (TGFβ), adenosine, prostaglandin E2 (PGE2), interleukin-6 (IL-6), reactive oxygen species (ROS) and oxidized lipids.

but lymphocytes also have key roles (see below). It is possible that tumour-derived factors recruit immature myeloid cells and myeloid cell precursors from the periphery, and that in the tumour microenvironment these cells differentiate into cells committed to suppressive and pro-angiogenic functions. However, it may be equally plausible that mature, differentiated myeloid cells are 'edited' by the tumour to acquire a suppressive and pro-angiogenic phenotype, as most of these cell types exhibit at least some degree of plasticity. The notion that the immunosuppressive and pro-angiogenic

phenotype of these myeloid cells may reflect a reversible functional state, rather than terminal and irreversible differentiation, opens the door to designing pharmacological manipulation strategies to reverse this phenotype, and this could have a profound impact on both tumour suppression and angiogenesis.

**Myeloid cells.** Myeloid cells are perhaps the best-studied cell types in terms of their ability to promote immunosuppression and angiogenesis in tumours. Principal among them are MDSCs (reviewed extensively

in REF. 14). MDSC numbers are markedly increased in the spleens of tumour-bearing mice and in the blood of patients with cancer, with reports that up to 40% of splenocytes of tumour-bearing mice are MDSCs<sup>14</sup>. Their functional characterization is ultimately based on their ability to suppress T and NK cell activation<sup>15</sup>, probably through several mechanisms, including the production of nitric oxide (NO), reactive oxygen species (ROS), arginase, interleukin-10 (IL-10) and transforming growth factor- $\beta$  (TGF $\beta$ ). There are also reports that MDSCs may specifically induce the expansion of T<sub>Reg</sub> cell populations<sup>16</sup>.

MDSCs have also been demonstrated to directly promote angiogenesis<sup>2</sup>. Indeed, tumour-bearing mice treated with a neutralizing BV8-specific antibody (which reduces the number of MDSCs) had markedly reduced angiogenesis, strongly

implicating MDSCs in this process<sup>17</sup>. These cells have been shown to secrete the pro-angiogenic factors VEGFA and matrix metalloproteinase 9 (MMP9)<sup>2</sup>. Importantly, the pro-angiogenic function of MDSCs can render tumours refractory to angiogenic blockade by VEGFA-specific antibodies through the secretion of alternative pro-angiogenic factors, such as BV8 (also known as prokineticin 2), which is upregulated by granulocyte colony-stimulating factor<sup>17</sup>.

Several other myeloid cell subsets possess the capacity to promote angiogenesis, including myeloid and plasmacytoid DCs, tumour-associated macrophages (TAMs), monocytes that express the angiopoietin receptor TIE2, mast cells and neutrophils (reviewed in REF. 18) (FIG. 1). For example, myeloid cells such as immature DCs and TAMs can alter their phenotype following their recruitment to the tumour micro-environment in response to tumour-derived chemokines or antimicrobial peptides<sup>19</sup>. These cells acquire a pro-angiogenic profile characterized by the secretion or expression of VEGFA, basic fibroblast growth factor (bFGF; also known as FGF2), CXCL8 and cyclooxygenase 2 (COX2; also known as PTGS2). Moreover, they downregulate their immunostimulatory functions (for example, by downregulating IL-12 expression owing to autocrine IL-10 production)<sup>18,20–22</sup>.

**Lymphocyte populations.** Given the crucial role of lymphocyte populations in immunosuppression and tolerance, it is not surprising that emerging evidence suggests that these cells play key parts in the homeostatic tissue repair programme that is co-opted by tumours. CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T<sub>Reg</sub> cells (which can suppress effector T cell functions) accumulate at tumour sites and are correlated with a poor prognostic outcome<sup>23</sup>. Although the accumulation of T<sub>Reg</sub> cells at tumour sites has been correlated with angiogenesis in endometrial<sup>24</sup> and breast<sup>25</sup> cancer, no direct role for T<sub>Reg</sub> cells in tumour angiogenesis had been demonstrated until we recently uncovered a role for T<sub>Reg</sub> cells in hypoxia-induced angiogenesis in ovarian cancer<sup>9</sup>. Hypoxia is recognized as a major contributor to cancer progression and treatment failures. We found that hypoxic ovarian tumour cells specifically upregulate expression of CC-chemokine ligand 28 (CCL28), and this chemokine preferentially recruits CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T<sub>Reg</sub> cells from peripheral blood through ligation of the cognate

receptor CC-chemokine receptor 10 (CCR10) on T<sub>Reg</sub> cells. Overexpression of CCL28 correlated with shorter survival in patients with ovarian cancer, and resulted in rapid ovarian tumour growth in mice. This was due to increased recruitment of T<sub>Reg</sub> cells to tumour sites, which established an immunosuppressive microenvironment rich in VEGFA and with increased angiogenesis.

Although the pro-angiogenic effects of T<sub>Reg</sub> cells might be indirect, we found that human and mouse CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cells secrete higher amounts of VEGFA in the steady state and under hypoxic conditions than CD4<sup>+</sup>CD25<sup>-</sup> T cells and promote endothelial cell proliferation *in vitro* and *in vivo*. Importantly, depletion of CD25<sup>+</sup> cells or CCR10<sup>+</sup> cells eliminated T<sub>Reg</sub> cells from the tumour microenvironment and substantially suppressed VEGFA expression and angiogenesis at these sites<sup>9</sup>.

These observations are supported by the demonstration that T cells exposed to hypoxia express VEGFA, and T cells within tumours may express VEGFA<sup>26</sup>. Moreover, CD4-deficient mice have an impaired angiogenesis response to hypoxia during ischaemia, suggesting that T cells may also participate in homeostatic tissue repair following ischaemia<sup>27</sup>. However, it remains to be determined whether CD4<sup>+</sup> T cells (particularly T<sub>Reg</sub> cells), make significant contributions to angiogenesis, given that most tumours autonomously produce large amounts of VEGFA. Furthermore, the capacity of T<sub>Reg</sub> cells to contribute to angiogenesis may depend entirely on the context, as T<sub>Reg</sub> cells have been implicated in the prevention of angiogenesis in a mouse model of airway inflammation<sup>28</sup>.

Recently, it has been demonstrated that, during interaction with DCs, activated CD4<sup>+</sup> T cells can acquire mature DC-expressed neuropilin 1 (NRP1; a co-receptor that binds VEGFA) through a process known as trogocytosis<sup>29</sup>. NRP1 expressed in the plasma membrane of DCs is transferred and becomes incorporated into the membrane of recipient T cells, and this was shown to enable T cells to bind DC-secreted VEGFA, potentially converting CD4<sup>+</sup> T cells into VEGFA-shuttling cells<sup>29</sup>. Although activated CD4<sup>+</sup> T cells can capture NRP1 from DCs, CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T<sub>Reg</sub> cells constitutively express NRP1, allowing for the possibility that they could transport additional VEGFA to the tumour site following their recruitment by CCL22 and CCL28 (REFS 9,23,30).

## Glossary

### Adoptive cell therapy

Very simply, the transfusion of lymphocytes into patients for the treatment of cancer. Strategies that have enjoyed success include the rapid *ex vivo* expansion of tumour-infiltrating lymphocytes followed by autologous reinjection. Engineering of patient peripheral blood T cells to express artificial T cell receptors or chimeric antigen receptors that recognize tumour antigens is a recently developed strategy that has proved successful.

### Diapedesis

Leukocyte migration through the endothelium that is mediated by leukocyte-secreted proteases that disrupt the endothelial cell barrier.

### Extravasation

The multistep process of leukocyte infiltration through the endothelium. This process proceeds through the stages of leukocyte rolling, adhesion, diapedesis and finally migration to the surrounding tissues.

### Ischaemia–reperfusion injury

Disruption of proper blood flow, either experimentally or otherwise, followed by restoration of normal blood flow results in significant hypoxia, tissue damage and inflammation followed by angiogenesis and immunosuppression.

### Mural cells

Cells that physically surround the endothelial cells of blood vessels. This population is comprised of vascular smooth muscle cells (associated with veins and arteries) and pericytes (associated with capillaries and developing vessels).

### Pericyte

A mural cell that is thought to have significant roles in supporting the growth and survival of endothelial cells during angiogenesis, particularly in tumours.

### Trogocytosis

Following the formation of the immunological synapse, membrane fragments from the antigen-presenting cell are physically transferred to, and transiently incorporated in, the membrane of the interacting T cell (or B or NK cell). The biological significance remains wholly unknown.

Other lymphocyte subsets with immunosuppressive functions include regulatory B cells<sup>8,31</sup>, type II natural killer T (NKT) cells<sup>32</sup>, NK cells<sup>33</sup> and  $\gamma\delta$  T cells<sup>34</sup>. In addition, B cells<sup>35</sup>,  $\gamma\delta$  T cells<sup>34</sup>, NK cells<sup>5,36</sup> and invariant (type I) NKT cells<sup>37</sup> have been reported to produce VEGFA. The precise role of these cells in tumour angiogenesis is unknown, but some of these lymphocyte subsets can be quite abundant in various tumours, raising the possibility that they make a crucial contribution to tumour development through the acquisition of a dual pro-angiogenic and immunosuppressive phenotype. However, the exact role of these lymphocytes in tumours requires further investigation.

**Stromal cells.** Typically associated with wound healing through the deposition of extracellular matrix, fibroblasts have important roles in both immune modulation and angiogenesis<sup>38</sup>. In the tumour microenvironment, cancer-associated fibroblasts (CAFs) can be activated by TGF $\beta$ , fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF)<sup>38</sup>. In turn, CAFs may secrete angiogenic growth factors such as bFGF and VEGFA<sup>38,39</sup>, while promoting the recruitment and function of immunosuppressive cells (particularly those of the myeloid lineage, such as TAMs and MDSCs) through the secretion of CCL2 and CXCL12 (REFS 18,40). In addition, CAFs may suppress effector T cells through the secretion of TGF $\beta$ <sup>41</sup>.

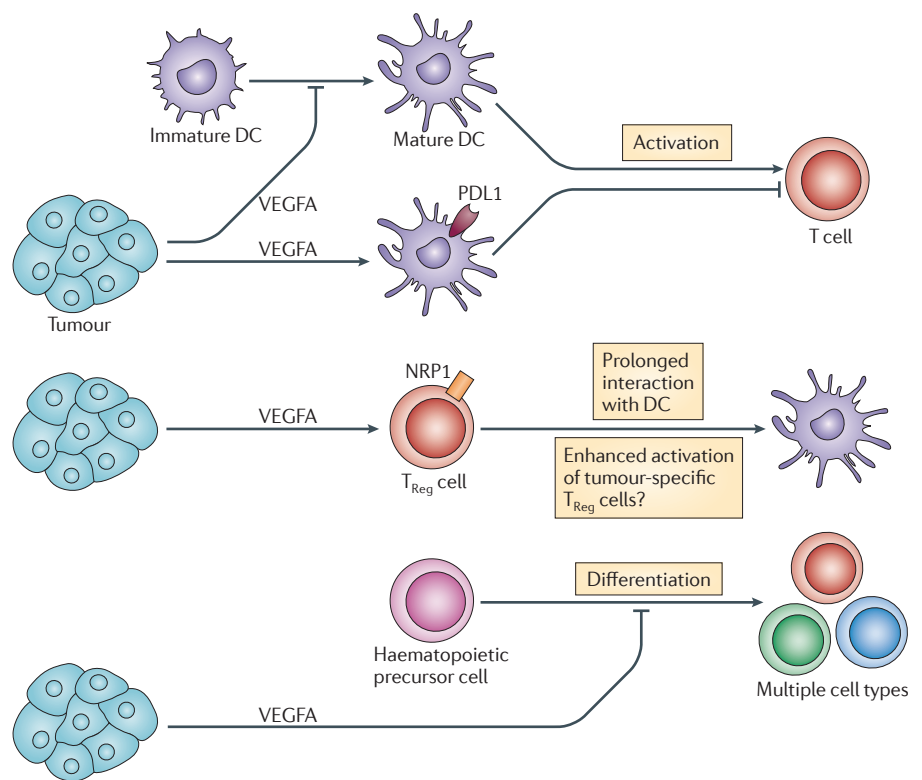
Another adherent stromal cell population is the mesenchymal stem cells (MSCs), which are derived from the bone marrow. Myeloid-derived MSCs secrete VEGFA and promote tumour angiogenesis by differentiating into CAFs or perivascular mural cells, which express  $\alpha$ -smooth muscle actin, TIE2 and other pericyte markers<sup>42</sup>. Importantly, MSCs exert important immunosuppressive functions by blocking the proliferation and function of effector T cells<sup>43,44</sup>. MSCs may be part of a homeostatic programme that responds to tissue injury, and the robust capacity of their immunosuppressive capabilities has been demonstrated from their roles in transplantation tolerance<sup>44,45</sup>. The full extent of the contributions of fibroblasts and myeloid-derived MSCs of the tumour stroma is unknown, but it is becoming apparent that these cells probably have integral roles in the establishment of a tumour-promoting microenvironment, supporting both immunosuppression and angiogenesis.

### Molecular mediators

In addition to its well-established functions as the master regulator of the tumour angiogenic switch<sup>46</sup>, VEGFA regulates a diverse array of immune functions and thus serves as the prototypical molecule that can mediate both angiogenesis and immunosuppression (FIG. 2). VEGFA impairs DC function and maturation, and this effect is thought to be responsible for the defects in antigen presentation and DC maturation observed in patients with cancer<sup>47–49</sup>. Ishida and colleagues demonstrated that tumour-bearing mice have decreased DC numbers and impaired DC function compared with control mice, and that these defects could be reversed by VEGFA blockade<sup>50</sup>. In addition, programmed cell death ligand 1 (PDL1) — a major negative-regulatory ligand that suppresses T cell activation through its receptor programmed cell death protein 1 (PD1) — is highly expressed by tumour-associated myeloid DCs in response to tumour-derived VEGFA<sup>51</sup>.

In addition to its effects on DCs, VEGFA can suppress T cell development and function through the disruption of

haematopoiesis and by increasing the sensitivity of thymocytes to apoptosis<sup>52,53</sup>. Furthermore, VEGFA treatment of mouse splenocytes during T cell stimulation was found to induce IL-10 production by T cells and to suppress interferon- $\gamma$  (IFN $\gamma$ ) production through an undefined mechanism<sup>54</sup>. It has also been shown that overexpression of VEGFA in tumour cells can lead to increased numbers of intratumoural T<sub>Reg</sub> cells<sup>55</sup>, demonstrating its significant role in the establishment of a tolerogenic tumour microenvironment. Lastly, expression of NRP1 — a receptor that interacts with VEGF receptor 1 (VEGFR1) and VEGFR2 — has been detected on CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cells<sup>30</sup>. Neutralizing antibodies specific for NRP1 diminished the interactions of DCs with T<sub>Reg</sub> cells, and ectopic overexpression of NRP1 in T cells enhanced their interactions with DCs<sup>30</sup>. Although not directly tested, VEGFA-expressing DCs could potentially stabilize their interaction with T<sub>Reg</sub> cells via NRP1 (or even VEGFR2 (REF. 56)), leading to enhanced T<sub>Reg</sub> cell activation. This would create a tolerogenic environment and thus promote tumour evasion.



**Figure 2 | The role of VEGFA in immune suppression.** Vascular endothelial growth factor A (VEGFA) has a multitude of suppressive effects on the immune response. For example, VEGFA can inhibit the maturation of dendritic cells (DCs) and disrupt the normal differentiation of haematopoietic precursor cells. VEGFA can also induce the expression of inhibitory molecules such as programmed cell death ligand 1 (PDL1) on DCs, and VEGFA may activate antigen-specific regulatory T (T<sub>Reg</sub>) cells by signalling through neuropilin 1 (NRP1) on T<sub>Reg</sub> cells.

We have chosen to focus on VEGFA owing to the abundance of data regarding its function in the above processes, but numerous mediators that are found in the tumour microenvironment have the capacity to promote both immunosuppression and angiogenesis (see TABLE 1).

Additional factors — such as adenosine<sup>57</sup>, PGE2 (REF. 58) and TGFβ<sup>59</sup> — have key roles in endothelial cell proliferation, survival, migration and vessel formation<sup>11</sup>. Furthermore, many of these mediators have known functions in the suppression of antigen-presenting cell activation,

maturation and antigen presentation, or directly suppress T cell activation while promoting T<sub>Reg</sub> cell functions (TABLE 1).

It is important to note that, although the mediators listed in TABLE 1 possess the capacity for immunosuppression and angiogenesis, their contribution to these processes

Table 1 | **Factors with the capacity to promote both immunosuppression and angiogenesis**

Mediator	Roles in immunosuppression	Roles in angiogenesis	Refs
PGE2	<ul style="list-style-type: none"> <li>Decreases DC maturation, co-stimulatory molecule expression, IL-12 production and CD8<sup>+</sup> T cell cross-priming by tumours</li> <li>Increases tolerogenic DC and T<sub>Reg</sub> cells numbers, arginase 1 expression and the suppressive activity of MDSCs</li> </ul>	<ul style="list-style-type: none"> <li>Induces VEGFA production</li> <li>Activates the RAC and nitric oxide–cGMP pathways</li> <li>Stimulates migration and survival of endothelial cells</li> <li>Directly promotes tube formation and proliferation</li> </ul>	58, 117–120
Adenosine	<ul style="list-style-type: none"> <li>Induces an immunosuppressive DC phenotype</li> <li>Increases IL-10 and IL-6 production by APCs</li> <li>Suppresses T cell effector functions</li> <li>Induces T cell anergy and T<sub>Reg</sub> cell development</li> </ul>	<ul style="list-style-type: none"> <li>Has a mitogenic effect on endothelium</li> <li>Promotes VEGFA, IL-8, bFGF and angiopoietin 1 production by numerous cell types</li> </ul>	57, 121–123
TGFβ	<ul style="list-style-type: none"> <li>Decreases T cell and macrophage functions</li> <li>Drives the proliferation of T<sub>Reg</sub> cells</li> </ul>	<ul style="list-style-type: none"> <li>Stabilizes angiogenic endothelium</li> <li>Can promote the proliferation and migration of endothelial cells</li> </ul>	59,124
IL-6	<ul style="list-style-type: none"> <li>Decreases T<sub>H</sub>1 cell differentiation</li> </ul>	<ul style="list-style-type: none"> <li>Increases VEGFA production</li> </ul>	98
VEGFA	<ul style="list-style-type: none"> <li>Impairs DC maturation</li> <li>Increases PDL1 expression by DCs</li> <li>Blocks T cell activation</li> </ul>	<ul style="list-style-type: none"> <li>Increases the proliferation, migration, activation, recruitment and survival of endothelial cells</li> </ul>	47–49
IDO	<ul style="list-style-type: none"> <li>Inhibits T cell activation through tryptophan depletion</li> </ul>	<ul style="list-style-type: none"> <li>Kynurenine (a tryptophan metabolite produced by IDO) may promote endothelial tube formation and angiogenesis</li> </ul>	125
FASL	<ul style="list-style-type: none"> <li>Induces caspase-mediated cell death of immune cells</li> </ul>	<ul style="list-style-type: none"> <li>FAS ligation with agonist antibody stimulates matrigel neoangiogenesis</li> </ul>	126
CCL2	<ul style="list-style-type: none"> <li>Recruits TAMs and T<sub>Reg</sub> cells</li> </ul>	<ul style="list-style-type: none"> <li>Increases VEGFA production and angiogenesis</li> <li>Promotes the recruitment of endothelial cell precursors</li> </ul>	127
IL-4	<ul style="list-style-type: none"> <li>Promotes alternatively activated macrophages</li> <li>Inhibits T<sub>H</sub>1 cell differentiation</li> </ul>	<ul style="list-style-type: none"> <li>Promotes VEGFA production during hypoxia</li> <li>A low dose promotes angiogenesis</li> </ul>	128–130
M-CSF	<ul style="list-style-type: none"> <li>Promotes the recruitment and/or activation of immunosuppressive myeloid cells</li> </ul>	<ul style="list-style-type: none"> <li>Induces VEGFA production by monocytes</li> </ul>	131
G-CSF	<ul style="list-style-type: none"> <li>Promotes myeloid cell recruitment</li> </ul>	<ul style="list-style-type: none"> <li>Promotes angiogenesis through effects on myeloid cells</li> <li>May increase the numbers of endothelial precursor cells</li> </ul>	132,133
ROS and RNS	<ul style="list-style-type: none"> <li>Inhibit T cell activation</li> </ul>	<ul style="list-style-type: none"> <li>Promote endothelial migration through the production of oxidized lipids</li> </ul>	11, 134,135
Endothelin 1	<ul style="list-style-type: none"> <li>Decreases ICAM1 expression on activated endothelial cells, preventing leukocyte diapedesis</li> </ul>	<ul style="list-style-type: none"> <li>Increases VEGFA and PGE2 production</li> </ul>	10,136
CXCL12	<ul style="list-style-type: none"> <li>Can be involved in the recruitment of TAMs, MDSCs and pDCs that induce IL-10 production by CD8<sup>+</sup> T cells</li> </ul>	<ul style="list-style-type: none"> <li>Induces VEGFA production</li> <li>Promotes the production of MMP9</li> </ul>	137
Angiopoietin 1	<ul style="list-style-type: none"> <li>Has roles in the recruitment of TAMs and MDSCs</li> </ul>	<ul style="list-style-type: none"> <li>Has direct effects on endothelial cells</li> </ul>	17
PDGF	<ul style="list-style-type: none"> <li>Has roles in the recruitment of TAMs and MDSCs</li> </ul>	<ul style="list-style-type: none"> <li>Has direct effects on endothelial cells</li> </ul>	38
PLGF	<ul style="list-style-type: none"> <li>Can impair DC functions</li> <li>Recruits immunosuppressive cells</li> </ul>	<ul style="list-style-type: none"> <li>Has indirect and direct effects on angiogenesis</li> </ul>	138,139
TLR2 ligands	<ul style="list-style-type: none"> <li>Expand T<sub>Reg</sub> cell populations</li> <li>Induce IL-10 production by DCs</li> </ul>	<ul style="list-style-type: none"> <li>Promote angiogenesis</li> <li>MYD88-deficient mice have impaired angiogenesis and wound healing</li> </ul>	11, 140–142
IL-17	<ul style="list-style-type: none"> <li>Can increase IL-6 production and promote tumour growth under certain circumstances</li> </ul>	<ul style="list-style-type: none"> <li>Increases angiogenesis by acting on endothelial cells</li> <li>IL-6–STAT3-mediated activation of tumour cells generates a pro-angiogenic phenotype</li> </ul>	143

APC, antigen-presenting cell; bFGF, basic fibroblast growth factor; CCL2, CC-chemokine ligand 2; cGMP, cyclic GMP; CXCL12, CXC-chemokine ligand 12; DC, dendritic cell; FASL, FAS ligand; G-CSF, granulocyte colony-stimulating factor; ICAM1, intercellular adhesion molecule 1; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; M-CSF, macrophage colony-stimulating factor; MDSC, myeloid-derived suppressor cell; MMP9, matrix metalloproteinase 9; MYD88, myeloid differentiation primary response protein 88; pDC, plasmacytoid DC; PDGF, platelet-derived growth factor; PDL1, programmed cell death ligand 1; PGE2, prostaglandin E2; PLGF, placenta growth factor; PTGER2, PGE2 receptor EP2 subtype; RNS, reactive nitrogen species; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; T<sub>H</sub>1, T helper 1; TLR2, Toll-like receptor 2; T<sub>Reg</sub>, regulatory T; VEGFA, vascular endothelial growth factor A.

may be entirely context dependent. For example, ligands for Toll-like receptor 2 (TLR2) have recently been implicated in the regulation of both physiological and pathological angiogenesis<sup>11</sup>. West and colleagues demonstrated that end products of lipid oxidation that are generated during inflammation are present in high concentrations in both mouse and human tumours, and that these end products are endogenous ligands for TLR2 that promote angiogenesis in a manner dependent on TLR2–MYD88 (myeloid differentiation primary response protein 88) signalling<sup>11</sup>. In addition, the endogenous TLR2 ligand heat shock protein 60 (HSP60) has been shown to enhance the functions of T<sub>Reg</sub> cells in a cell-autonomous manner by increasing IL-10 and TGFβ production by HSP60-treated T<sub>Reg</sub> cells<sup>60</sup>. Furthermore, TLR2 signalling in DCs was found to promote T<sub>Reg</sub> cell differentiation through IL-10 and retinoic acid production following TLR2 stimulation<sup>61</sup>. Injection of mice with TLR2 agonists increased T<sub>Reg</sub> cell numbers and promoted tumour development in a T<sub>Reg</sub> cell-dependent manner<sup>62</sup>.

Despite these observations, TLR2-mediated activation of mast cells was shown to promote antitumour immunity and to inhibit angiogenesis<sup>63</sup>. Moreover, TLR2 ligands have an established role in the activation of DCs to promote antimicrobial immunity, and they possess the capacity to directly enhance the functions of T<sub>H</sub>1 cells<sup>64</sup>. Therefore, it is likely that the context under which stimulation occurs, or how a particular mediator interacts with a particular cellular target<sup>65</sup>, determines how the mediator is integrated into a programme of immunosuppression and angiogenesis.

### Immune regulation by tumour vasculature

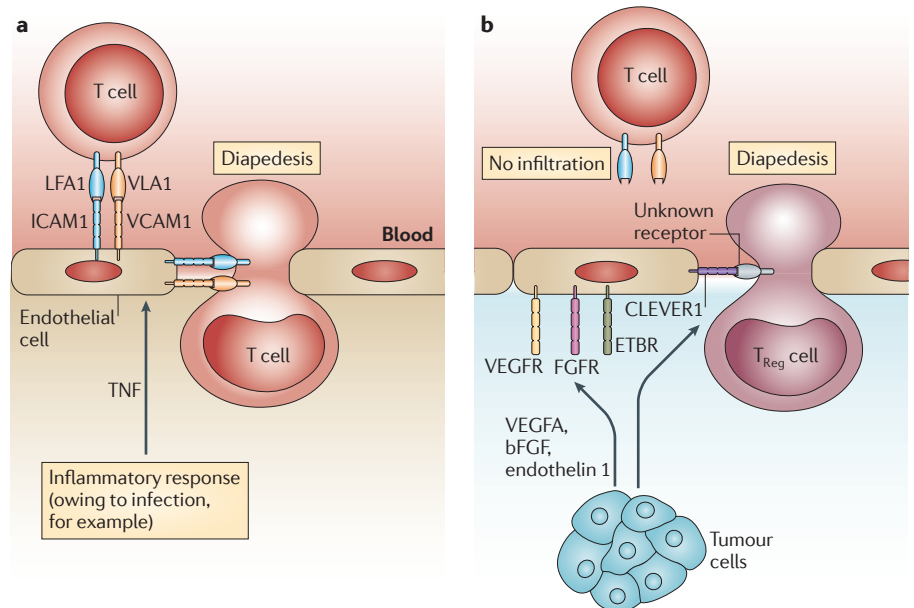
Much of the information presented above is focused on cells and mediators that have the ability to influence endothelial cell activation, resulting in angiogenesis, and to suppress the immune response. However, the angiogenic endothelium can also regulate leukocyte trafficking and can directly suppress and regulate an immune response.

**Adhesion and transmigration of leukocytes.** The endothelium stands as a physical barrier to leukocytes and regulates their extravasation from the circulation into the surrounding tissue. Leukocytes extravasate to tumours by crossing the endothelial cell layer through a multistep process that involves binding to adhesion molecules expressed by endothelial cells followed by diapedesis. The process is mediated by

adhesion molecules such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) (reviewed extensively in REF. 66). Although the tumour vasculature is considered to be 'leaky', a pro-angiogenic tumour microenvironment typically lacks infiltrating immune cells<sup>67,68</sup> and, mechanistically, most of the *in vitro* and *in vivo* data indicate that angiogenic factors reduce the adhesion and migration of leukocytes<sup>69–74</sup> (FIG. 3).

The potent angiogenic growth factors VEGFA and bFGF attenuate the adhesion of T cells to either quiescent or tumour necrosis factor (TNF)-activated endothelial cells<sup>70–72</sup>. Depending on the experimental conditions, these angiogenic factors can suppress the expression of VCAM1 and ICAM1 on endothelial cells or can abrogate the clustering of surface adhesion molecules via caveolin 1 (a process that is important for adhesive interactions with T cells)<sup>72</sup>. We have recently demonstrated an additional endothelial cell-associated mechanism for the regulation of T cell

infiltration to tumours that involves activation of the endothelin B receptor (ETBR)<sup>10</sup>. Endothelial-expressed ETBR was found to be upregulated in ovarian tumours that lacked tumour-infiltrating lymphocytes<sup>10</sup> and, similarly to the absence of tumour-infiltrating lymphocytes<sup>68</sup>, ETBR overexpression was associated with poor survival. Signalling by endothelin 1 through ETBR was found to block T cell adhesion to the endothelium through the suppression of ICAM1 clustering on endothelial cell membranes, an effect mediated by nitric oxide<sup>10</sup>. Importantly, endothelin 1 has been shown to be overexpressed in human ovarian cancer<sup>75</sup>, suggesting that tumours can suppress T cell adhesion to the endothelium in an endothelin 1–ETBR-dependent manner, even in the presence of TNF<sup>10</sup>. This would explain the coexistence of inflammation (TNF is commonly overexpressed in the tumour microenvironment, particularly in ovarian cancer<sup>68</sup>) and a quiescent tumour endothelium phenotype that does not support the homing of T cells<sup>68</sup>.



**Figure 3 | Modulation of immune cell recruitment and diapedesis through the endothelial barrier by the tumour microenvironment. a** | The endothelium acts as a physical barrier to leukocyte traffic to sites of inflammation or tumour growth. In the initial inflammatory response, factors such as tumour necrosis factor (TNF) upregulate the expression of chemotactic factors and adhesion molecules, including intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1). These molecules recruit leukocytes to the site of inflammation and mediate diapedesis through LFA1–ICAM1 and VLA1–VCAM1 interactions. **b** | In the context of a tumour microenvironment (as well as during the resolution phase of infection), growth factors such as endothelin 1, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor A (VEGFA) block the expression of ICAM1 and VCAM1, thus reducing leukocyte trafficking. The tumour endothelium probably also functions as a selective barrier, preventing effector cell infiltration while promoting regulatory T (T<sub>Reg</sub>) cell infiltration through molecules such as common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER1). ETBR, endothelin B receptor; FGFR, FGF receptor; LFA1, lymphocyte function-associated antigen 1; VEGFR, VEGF receptor; VLA1, very late antigen 1.

An open question that remains is whether the endothelium can distinguish between leukocyte subsets, selectively allowing trafficking of only certain immune subsets according to their polarization ( $T_H1$  versus  $T_H2$ ,  $T_H17$  or  $T_{Reg}$  cell), phenotype and/or activation status. One could hypothesize that, under the influence of the local angiogenic milieu, the tumour endothelium could allow immunosuppressive cells to pass, while blocking access to tumour-reactive effector T cells or NK cells. This notion is supported by the observation that  $T_{Reg}$  cells selectively traffic through the tumour endothelium by virtue of enhanced interaction with addressins in human pancreatic cancer<sup>76</sup>. Furthermore, VEGFA and hepatocyte growth factor (HGF) may promote selective migration of  $T_{Reg}$  cells across the endothelium in hepatic cell carcinoma through common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER1; also known as stabilin 1)<sup>77</sup>. Selective

trafficking may also exist for suppressive myeloid populations, and may be mediated by adhesion molecules such as CD31 or CD99 (REF. 78). Thus, it is likely that the tumour endothelium is an active participant in the control of immunosuppression during tumour angiogenesis.

**Suppression of T cell activation by the endothelium.** In addition to regulating the adhesion and extravasation of leukocytes, endothelial cells can express mediators that suppress the actions of effector lymphocytes (FIG. 4). These mediators include PDL1 and PDL2 (REFS 79,80), FAS ligand (FASL; also known as CD95L)<sup>81</sup>, TNF-related apoptosis-inducing ligand (TRAIL)<sup>82</sup> and possibly the endothelial cell marker CD31 (REF. 83). Endothelial cells also express numerous soluble mediators that suppress immune responses, such as IL-6, IL-10, TGF $\beta$  and PGE2 (REFS 84,85).

Very recently, the endothelium within lymphomas was shown to express T cell immunoglobulin domain and mucin domain protein 3 (TIM3; also known as HAVCR2). This protein contributes to immunosuppression through the activation of the STAT3 (signal transducer and activator of transcription 3) pathway for IL-6 production in endothelial cells<sup>86</sup>. TIM3-expressing endothelial cells promoted the onset, growth and dissemination of lymphomas by inhibiting the activation of CD4<sup>+</sup> T cells and  $T_H1$  cell polarization, thus revealing a novel role for the endothelium in immune suppression<sup>86</sup>. In addition, indoleamine 2,3-dioxygenase (IDO) can be expressed by tumour endothelium<sup>87,88</sup>. Endothelial IDO is known to be upregulated by infection and may be required for the regulation of blood pressure through kynurenine<sup>89</sup>, but it can also suppress T cell activation through the depletion of tryptophan<sup>90</sup>.

Endothelial cells can also express several molecules that may be involved in the direct stimulation of T cells — such as ICOS ligand (ICOSL), CD40, CD58, CD80, CD86, CD137 and MHC class I and class II molecules — and many of these molecules are upregulated by angiostatic,  $T_H1$  cell-associated cytokines<sup>91</sup>. Whether the expression and/or function of these immunostimulatory molecules are influenced by angiogenic mechanisms is largely unknown, but it is possible that down-regulation or inhibition of the expression of these molecules on endothelial cells occurs during tumour angiogenesis. Support for this hypothesis comes from the observation that tumour cell supernatants can induce an endothelial suppressor phenotype that is partly dependent on VEGFA<sup>92,93</sup>.

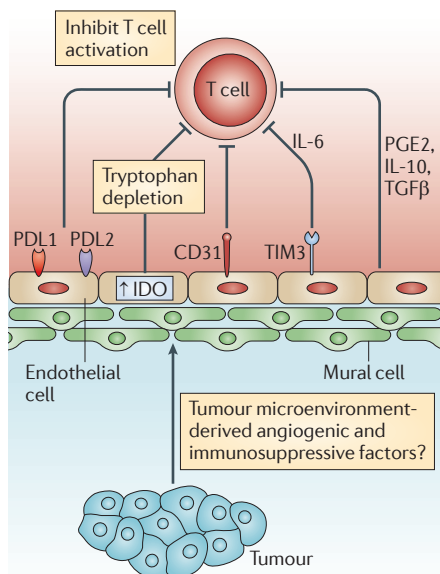
**Additional vascular cells.** Surrounding the endothelial cells, but integral to the vasculature, are mural cells, which are endowed with plasticity and can acquire phenotypes of pericytes or vascular smooth muscle cells<sup>42</sup>. Mural cells have significant roles in angiogenesis, and have also been shown to have an immunosuppressive function<sup>42</sup>. In addition, monocyte populations have been described to participate in the process of blood vessel formation in tissues undergoing post-infarction repair<sup>94</sup>. Similar participation of myeloid cells in rapidly forming vessels could also occur in tumours<sup>2,95,96</sup>, and incorporation of myeloid cells into the developing vasculature has been suggested by several groups<sup>2,95</sup>, but remains controversial.

**Context is key**

Although many of the cells and mediators discussed have the capacity to promote immunosuppression and angiogenesis, it is important to emphasize that their functions in these processes are likely to be entirely context dependent. Therefore, these cells and mediators may require the correct microenvironmental conditions to exert their full potential in this pro-angiogenic-immunosuppressive programme. IL-6 is a particularly relevant example, as it is a pleiotropic cytokine that may have pro-inflammatory or anti-inflammatory (immunosuppressive) effects depending on the context of expression and the cellular target. For example, within the hypoxic tumour microenvironment, IL-6 expression is inducible<sup>97</sup>, and IL-6 could synergize with other factors to induce the expression of VEGFA by multiple cell types<sup>98,99</sup> and/or to promote immunosuppression through the induction of immunosuppressive molecules such as B7H4 (also known as VTCN1)<sup>100</sup>. However, IL-6 can also promote the differentiation of  $T_H17$  cells over  $T_{Reg}$  cells<sup>101</sup>, thereby driving a pro-inflammatory microenvironment, as is seen in rheumatoid arthritis. Thus, it is important to emphasize that the context of mediator expression is crucial to its contribution to functional outcomes.

**Conclusions and implications**

**Implications of this paradigm for cancer immunotherapy.** Solid tumours grow and evolve through a constant crosstalk with the surrounding microenvironment. The evidence discussed here suggests that the tumour stroma and microenvironment activate homeostatic tissue repair mechanisms that include cellular and molecular events traditionally considered to pertain to either angiogenesis or immunosuppressive



**Figure 4 | Direct immune regulation by the endothelium.** In addition to the well-established role for the endothelium in trafficking, the tumour endothelium can express several inhibitory molecules that could limit the antitumour immune response. Programmed cell death ligand 1 (PDL1) and PDL2 can be expressed by endothelial cells, and indoleamine 2,3-dioxygenase (IDO) and T cell immunoglobulin domain and mucin domain protein 3 (TIM3) have been shown to be expressed by the tumour endothelium. The adhesion molecule CD31 is used as a marker of endothelial cells and has been demonstrated to inhibit T cell activation. Furthermore, soluble mediators released from the tumour endothelium — such as prostaglandin E2 (PGE2), interleukin-10 (IL-10) and transforming growth factor- $\beta$  (TGF $\beta$ ) — would further impair immune responses.

mechanisms. The growing understanding of these complex networks has revealed that the same cell populations or soluble factors can simultaneously promote angiogenesis and mediate immunosuppression. The daunting complexity of these overlapping mechanisms could in part explain therapeutic failures, as the field has traditionally targeted only one (but rarely both) of these mechanisms.

40 years of dedicated research following Judah Folkman's anti-angiogenesis hypothesis<sup>102</sup> has yielded only a handful of US Food and Drug Administration (FDA)-approved drugs available to disrupt angiogenesis, all of which interfere with the VEGF pathway (although more than 40 new drugs are in development<sup>103</sup>). The first such drug to be approved, bevacizumab (Avastin; Genentech/Roche), is a neutralizing antibody that specifically inhibits VEGFA, and despite overwhelming preclinical efficacy it does not provide benefit as a monotherapy in patients (except in a few types of tumour, such as glioblastoma multiforme and recurrent ovarian carcinoma). It is possible that blocking VEGFA alone is not sufficient to disable key tissue repair pathways in the tumour stroma, with ensuing therapeutic failure. Indeed, T<sub>Reg</sub> cells recruited by tumour hypoxia and tumour-infiltrating MDSCs have been shown to promote angiogenesis and resistance to VEGFA blockade, respectively<sup>9,17</sup>. Interestingly, bevacizumab has proved to be quite effective when used in combination with traditional chemotherapeutics, extending both progression-free and overall survival (reviewed in REF. 104). Given the emerging off-target effects of chemotherapy drugs on the tumour microenvironment, it is possible that chemotherapy drugs could also help to abrogate some of the tissue repair mechanisms in the tumour microenvironment that account for resistance to bevacizumab. Similarly, cancer immunotherapies that have relied solely on promoting antitumour immune responses without addressing the tumour microenvironment are often met with limited success in the clinic, perhaps owing in part to a lack of immune cell recruitment through the tumour endothelial barrier<sup>105</sup> or to other local immunosuppressive factors.

The notion that the pro-angiogenic and immunosuppressive phenotype is likely to be dynamic and responsive to local conditions has particular relevance for the development of new therapies. Given the degree of cooperation and functional overlap between angiogenesis and immunosuppressive mechanisms, strategies that use anti-angiogenic therapy along with immune modulation

could be more successful at tipping the balance of the tumour microenvironment. However, key questions remain. For example, what are the most important mediators or cells in the establishment of this tissue repair programme in tumours, and how, exactly, do tumours orchestrate this programme? Gene signatures and studies that investigate tissue repair networks could provide hints as to the most relevant interactions<sup>106</sup>.

According to the proposed view that integrates tumour angiogenesis and immunosuppression in the homeostatic tissue repair programme co-opted by tumours, strategies for the elimination of tumours might be more successful if they include complementary approaches to block mediators of both angiogenesis and immunosuppression while simultaneously inducing a strong antitumour immune response. For example, in preclinical models, greater success has been achieved with combinatorial approaches using a tumour lysate vaccine and ETBR blockade<sup>10</sup>, a tumour lysate-pulsed DC vaccine with COX2 inhibitors<sup>107</sup>, or a VEGFA-specific neutralizing antibody and adoptive T cell therapy<sup>12</sup> than with any monotherapy. This strategy is further strengthened by some success with combining IFN $\alpha$  therapy and bevacizumab for the treatment of metastatic renal cell carcinoma<sup>108,109</sup>. In addition, strategies to eliminate the tumour endothelium itself have shown some success<sup>110</sup>. An alternative approach is aimed at eliminating immunosuppressive cells, such as T<sub>Reg</sub> cells, in combination with VEGFA blockade, and this is also currently being investigated<sup>13</sup>. It is important to note that strong T<sub>H</sub>1-type cytokines that are known for their roles in tumour elimination — including IL-12, IFN $\gamma$  and IFN-inducible chemokines such as CXCL9 (also known as MIG) and CXCL10 (also known as IP10) — can exert potent angiostatic effects through direct action on endothelial cells<sup>111–113</sup>. Thus, complete tumour eradication will ultimately require regulation in favour of an immunostimulatory and angiostatic microenvironment. The open question remains as to whether there exists a central regulatory cell type or central mediator that, when blocked, can relieve both the immunosuppression and angiogenesis programmes, thereby promoting an antitumour immune response and leading to the elimination of the tumour.

#### **Implications for other biological processes.**

It is our belief that the fact that so many mediators and cellular players have the capacity to promote both immunosuppression and angiogenesis is a result of an evolutionary pressure to temper excessive inflammatory

responses and avert autoreactivity while promoting the regeneration of damaged, stressed or infected tissues through increased blood supply and tissue rebuilding. As such, many of the processes described above also occur in the context of aseptic tissue injury, ischaemia–reperfusion injury and infection<sup>11</sup>. Perhaps the best example of this hypothesis in action was the demonstration that oxidative stress generates oxidized lipids that act as endogenous ligands for the TLR2–MYD88 pathway — a pathway known for its role during infection. This pathway was shown to control angiogenesis by directly stimulating endothelial cells in different inflammatory contexts, such as in wound healing, ischaemia and tumour angiogenesis<sup>11</sup>. Furthermore, following instances of extreme tissue damage and inflammation (as is the case with sepsis), high levels of VEGFA are expressed<sup>114</sup> and, following the resolution of sepsis, a large expansion occurs in the T<sub>Reg</sub> cell population<sup>115</sup>, indicating that this pro-angiogenic and immunosuppressive programme is also active during the most severe infections.

This paradigm could be extended even to pregnancy, during which immunosuppression and angiogenesis are required for proper development of the fetal–maternal interface. For example, it was demonstrated that NK cells can promote angiogenesis through the secretion of VEGFA at the fetal–maternal interface, while remaining tolerant<sup>5</sup>. The relationship between immunosuppression and angiogenesis may also be present during fetal development, as embryonic macrophages have been shown to express many of the same genes as TIE2<sup>+</sup> monocytes and TAMs<sup>116</sup>. Thus, an understanding of the paradigm presented here should aid new treatment possibilities that perhaps would otherwise be overlooked.

Here, we have presented parallels between cancer and other biological processes that support the hypothesis that angiogenesis and immunosuppression cooperate in the same tissue repair programme. This programme operates normally to ensure homeostasis, but is also co-opted by pathological processes such as cancer. The open challenges are to discover therapeutic approaches to target this tissue repair programme to eliminate cancer, expedite wound healing, promote transplant tolerance and relieve symptoms of autoimmunity.

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#### Competing interests statement

The authors declare no competing financial interests.

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