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Angiogenesis and Allograft Rejection

Marlies E. J. Reinders and David M. Briscoe

In this review, the authors discuss the intriguing mechanistic and functional interrelationship between the angiogenesis reaction, cell-mediated immune inflammation, and allograft rejection. They discuss evidence that angiogenesis is a component of allograft rejection, and they review data suggesting that monocytes, as well as T cells, can elicit an angiogenesis reaction in the course of recruitment into an allograft. Some angiogenesis factors, including vascular endothelial growth factor (VEGF), are proinflammatory, whereas others have antiinflammatory properties. Angiogenic factors, including VEGF and fibroblast growth factor, are known to be expressed in allografts undergoing acute rejection and are associated with the development of chronic rejection. It is likely that future research will elucidate a function for angiogenesis in allograft rejection, especially chronic rejection.

Angiogenesis and Inflammation

Angiogenesis, the generation of new blood vessels from preexisting ones, is a complex process involving endothelial cell division, degradation of the vascular basement membrane, and surrounding extracellular matrix and endothelial cell migration.¹ It is a characteristic component of many normal physiologic processes and is pathologic in various diseases including cancer, blindness, and chronic inflammation.²⁻⁴ Angiogenesis is regulated by a balance between pro- and antiangiogenic molecules. To this end, it is proposed that pathologic angiogenesis (and certain diseases) either results from an overproduction of proangiogenic factors or a derailment of the function of biologic antiangiogenic factors. Indeed, excessive production of angiogenesis factors occurs in most chronic inflammatory disorders, and in some diseases, inhibition of their function can attenuate disease progression.

There are several observations suggesting that angiogenesis and inflammatory processes are interactive and mechanistically interrelated (Fig. 1). First, many inflammatory mediators can directly or indirectly promote angiogenesis. Angiogenesis, in turn, can contribute to inflammation. Cell-mediated immune inflammatory reactions, involving delayed-type hypersensitivity mechanisms, are characteristically associated with angiogenesis, a process called

leukocyte-induced angiogenesis. Indeed, lymphocytes and monocytes produce potent angiogenesis factors, including vascular endothelial growth factor (VEGF).⁴⁻⁸ Other products of activated macrophages that promote angiogenesis include TNF- α ,^{9,10} TGF- β , and nitric oxide.^{11,12} Interestingly, some of these factors have been found to function in part by stimulating VEGF production, suggesting that VEGF may be a common mediator of leukocyte-induced angiogenesis.^{13,14} Furthermore, the observation that anti-VEGF antibodies inhibit the development of certain inflammatory diseases is suggestive that VEGF-induced angiogenesis is of functional significance in chronic inflammation.^{15,16}

Second, the angiogenesis process itself is proinflammatory. Neovessels at sites of angiogenesis are "sticky," express adhesion molecules, and facilitate the recruitment of leukocytes, in part via enhanced leukocyte-endothelial adhesion events.^{17,18} Furthermore, many angiogenesis factors including VEGF have proinflammatory properties. And it is well known that many inflammatory mediators can induce angiogenesis. VEGF is proinflammatory via its ability to induce endothelial cell adhesion molecule expression, promote the chemotaxis of monocyte/macrophages, and enhance vascular permeability.¹⁷⁻²⁰ Many other angiogenesis factors are proinflammatory via different mechanisms, and some (e.g., TNF- α ,

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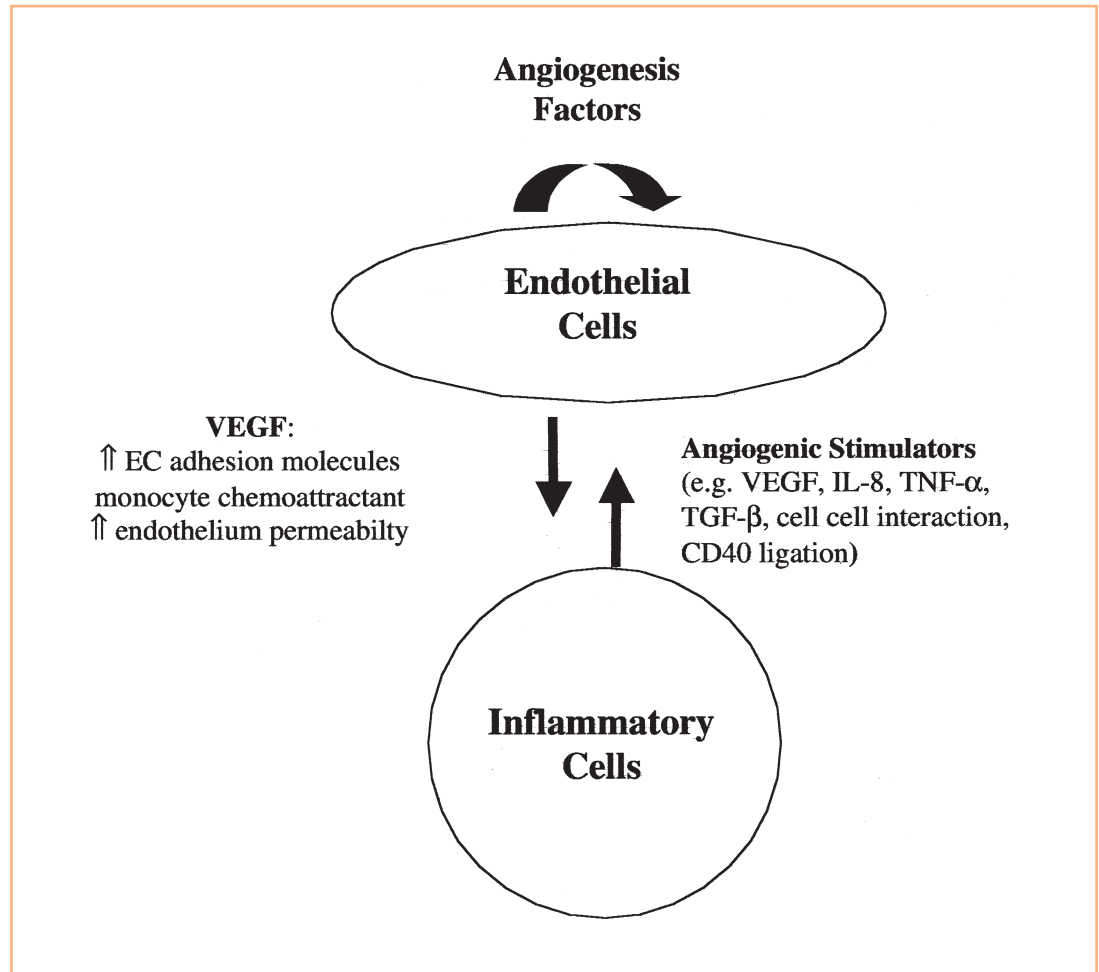


Figure 1. This drawing illustrates the interplay between angiogenesis and inflammation. Angiogenesis stimulates inflammation by producing proinflammatory cytokines including VEGF. VEGF increases endothelial cell (EC) adhesion molecules, is directly monocyte chemoattractant, and increases endothelial cell permeability. Local recruitment of inflammatory cells may also stimulate angiogenesis via the secretion of angiogenesis factors or via cell-cell interactions (e.g., CD40L-CD40 interactions).

soluble adhesion molecules, chemokines) elicit an inflammatory response as their primary function.

On the other hand, inflammation and angiogenesis can also be dissociated and independent. Some angiogenesis factors, such as FGF, may inhibit adhesion molecule expression and have antiinflammatory effects.¹⁷ In addition, some inflammatory mediators can be anti-angiogenic. For example, the competitive binding of chemokines to angiogenesis receptors (and vice versa) results in competition for angiogenesis and inflammatory receptors.²¹ Moreover, potent angiogenesis factors, such as FGF and VEGF, can stimulate angiogenesis in the absence of inflammation.

Taken together, these observations suggest that leukocyte-induced angiogenesis is in part dependent on the relative expression of proinflammatory angiogenesis—as well as antiangiogenesis—factors, at an inflammatory site.

Angiogenesis and Allograft Rejection

In the course of defining the process of leukocyte-induced angiogenesis, Sidky and Auerbach also clearly demonstrated that allogeneic leukocytes stimulate an angiogenic reaction.⁶⁷ In their seminal studies, they demonstrated that direct local infusion of allogeneic spleen cells, but not syngeneic spleen cells, into the skin of nude mice resulted in

marked angiogenesis. The angiogenesis reaction following the infusion of the splenocytes occurred early (within 3-6 days) and was associated with characteristic neovessels showing tortuosity and loop formation. This was very similar to the angiogenesis reaction observed in tumors.⁷ Furthermore, the angiogenesis response was dose dependent, being enhanced in a linear manner with an increase in the number of injected allogeneic spleen cells.⁶

Angiogenesis is also associated with the recruitment of allogeneic cellular infiltrates into skin transplants in the humanized (hu) SCID mouse.²² The SCID mouse is permissive for the transplantation of human skin and for the adoptive transfer of human peripheral blood leukocytes (PBL). Human foreskin engrafts onto SCID mice and becomes vascularized by both mouse and human vessels. The human vasculature within these skin grafts remains stable for many months on the mouse.²³ We and others have shown that adoptive transfer of human PBL into the SCID mouse results in human PBL infiltrates within the human skin transplant.^{22,24} Seven days after transfer, leukocytes are evident in the human skin graft, and after 14 to 21 days, they are associated with a local vasculitis reaction consistent with acute rejection. Most notably, the human infiltrates are selectively recruited into the human skin graft and do not enter the mouse skin, even in mouse skin adjacent to the human skin allograft.

In our analyses of leukocyte-induced angiogenesis in this huSCID model, we found a marked local angiogenesis response at early times in the course of infiltration (by day 7). The angiogenesis reaction could be quantified by both videomicroscopy and immunohistochemistry and was temporally and spatially associated with infiltrates. Furthermore, the angiogenesis response appeared to precede the development of vasculitis and microvascular destruction in association with fulminant rejection. Therefore, local tissue hypoxia was not the likely primary stimulus for initiating the angiogenesis that occurred early in these allografts.²² Rather, the angiogenesis reaction was likely initiated by leukocytes as is typical in leukocyte-induced angiogenesis.⁷

Chronic Rejection

There is also evidence that angiogenesis is associated with chronic rejection and with graft vascular

arteriosclerosis. Graft vascular arteriosclerosis is characteristic of chronic rejection and resembles an accelerated form of atherosclerosis. To this end, it is not surprising that angiogenesis is associated with the graft vascular arteriosclerosis since angiogenesis is characteristically evident within advanced atherosclerotic lesions.^{1,25} Pathologically, graft vascular arteriosclerosis is thought to be initiated by similar immunological mediators as those described for atherosclerosis, involving infiltration of the intima by T lymphocyte and by monocyte/macrophages and the secretion of growth factors and cytokines that result in smooth muscle cell proliferation.⁵

In the course of infiltration of monocyte/macrophages into atherosclerotic lesions, an angiogenesis reaction occurs, and it is evident that this angiogenesis reaction becomes quite prominent. A recent study evaluated the function of angiogenesis in atherosclerosis development and demonstrated that inhibition of angiogenesis could limit the development of atherosclerotic lesion formation.²⁵ This study established for the 1st time that the angiogenesis reaction is of functional significance for lesion development. Furthermore, another study recently demonstrated that VEGF is of functional significance in atherosclerosis lesion development, inasmuch as administration of VEGF was found to accelerate lesion formation and anti-VEGF antibodies inhibited lesion formation in animals prone to develop atherosclerosis.²⁶

In our studies, we also found that angiogenesis is prominent in the intima of large vessels in the classic LEW-into-Fisher rat model of chronic cardiac allograft rejection.²⁷⁻²⁹ This observation is consistent with that published by others and is suggestive that angiogenesis is a component of the graft arteriosclerotic lesion. Tanaka et al. evaluated angiogenesis in graft vascular arteriosclerosis lesions in hypercholesterolemic rabbits.³⁰ A striking finding was that the intima of the transplanted aorta contained prominent microvessels compared with native controls or nontransplanted animals. The increased capillary density was associated with T cell and monocyte infiltrates within the parenchyma of the cardiac transplants.³⁰ These observations suggest that factors produced by these cellular infiltrates are of importance in mediating the intimal angiogenesis reaction. Indeed, as discussed above, angiogenesis

ANGIOGENESIS:

The generation of new blood vessels from preexisting ones.

sis is known to be associated with monocyte activation,⁵ and leukocyte-induced angiogenesis may be dependent on the CD4⁺ lymphocyte.⁶ The close proximity of T cells with monocytes and endothelial cells in the lesion is also consistent with a role for cell-cell interactions in angiogenesis. We suggest that this may result in VEGF-induced angiogenesis events inasmuch as we have found that interactions between CD40L (expressed on activated T cells) and CD40 (expressed on monocytes and endothelial cells) are potent for the induction of VEGF.³¹

Angiogenesis Factors

Vascular Endothelial Growth Factor

VEGF is produced by many cell types in the immune system including endothelial cells, macrophages, and activated T cells.^{3,5,32,33} Hypoxia is reported to be the major stimulus for VEGF expression.³⁴⁻³⁶ Other factors known to induce VEGF expression include some cytokines, cell surface interactions (CD40 ligation), oncogenes, prostaglandins, modulators of protein kinase C, nitric oxide, and stimulators of adenylate cyclase.^{3,31} Several members of the VEGF family have been identified, including VEGF-A (also referred to simply as VEGF), VEGF-B, VEGF-C, and VEGF-D.³⁷⁻⁴⁰ Each VEGF gene has been found to have angiogenesis-inducing properties, but each may be selective for pathophysiologic angiogenesis (VEGF-A), lymphangiogenesis (VEGF-C and -D), or developmental angiogenesis. VEGF binds to 2 major tyrosine kinase receptors, FLT-1 (VEGFR-1) and KDR (VEGFR-2), as well as a 3rd receptor called Neupilin Receptor-1 (NRP1). Binding of VEGF to any of its receptors results in endothelial cell proliferation and cell migration and inhibits apoptosis by inducing expression of the antiapoptotic proteins Bcl-2 and A1 in human endothelial cells.⁴¹

The expression of VEGF has been described in allografts undergoing rejection. One study reported enhanced VEGF expression in rejecting cardiac allografts. VEGF immunoreactivity was confined to areas of fibrin deposition and was spatially associated with infiltrates.⁴² In another study, increased expression of VEGF was reported in human renal allografts with evidence of chronic rejection. VEGF expression was most striking in the interstitium and

was colocalized with CD68⁺ monocyte/macrophages. It has been postulated that VEGF expression by macrophages plays an important role in the development of interstitial fibrosis and myofibroblast stimulation in chronic rejection.⁴³ Its ability to induce the expression of connective tissue growth factor, an important stimulator of fibrosis, is consistent with this possibility.^{44,45}

Fibroblast Growth Factor

Acidic fibroblast growth factor (aFGF or FGF-1) and basic fibroblast growth factor (bFGF or FGF-2) are the best characterized members of the fibroblast growth factor family. The FGFs are synthesized by a wide variety of cells and are recognized by a family of cell surface receptors that have intrinsic tyrosine kinase activity after ligand-induced activation. They have different functions in angiogenesis, wound repair, development, and hematopoiesis.⁴⁶ FGF is expressed in many adult tissues in response to injury. Studies have demonstrated that myocytes and small myocardial vessels overexpress FGF and FGF receptors after transplantation.^{47,48} FGFs costimulate T-cell proliferation and cytokine production and thus may potentiate immune responses to donor antigens. FGF also mediates anti-major histocompatibility complex antibody-induced endothelial cell activation and proliferation.⁴⁷

In a prospective study in human cardiac allografts, aFGF mRNA expression varied between patients. And, in the same patient, expression varied over time was found to correlate with development of graft arteriosclerosis.⁴⁹ In addition, bFGF mRNA and protein was found to be increased in cardiac allografts with chronic rejection, and bFGF-positive cells appear to be associated with activated macrophages but may also be temporally and spatially associated with other cell types including endothelial cells.⁵⁰ Furthermore, aFGF may serve as a growth factor associated with neointima development and angiogenesis during chronic rejection of human renal allografts.⁵¹

Angiogenesis Inhibitors

Therapeutic strategies that interfere with angiogenesis have been shown to reduce inflammation.⁵² An increasing number of angiogenesis inhibitors

have been described and represent a diverse array of agents, ranging from fungal products (fumagillin and its analogue TNP-470)⁴⁵ to breakdown products of collagen type 18, and plasminogen (angiotatin and endostatin).^{53,54} Other agents include monoclonal antibodies directed to critical angiogenic factors, such as VEGF, or to cell surface molecules important in endothelial cell migration and capillary tube formation (the $\alpha 5\beta 3$ integrins). Whereas most agents directly inhibit endothelial cell proliferation (e.g., TNP-470, thalidomide), others interrupt steps critical in new vessel formation such as breakdown of basement membrane proteins (e.g., matrix metalloproteinase inhibitors) and endothelial cell migration (e.g., anti- $\alpha 5\beta 3$ antibody).⁵⁵ The mechanism of action of some agents such as endostatin and angiotatin is still under investigation.^{54,56}

Conclusion

In conclusion, there is significant evidence that angiogenesis is a component of acute and chronic rejection. Multiple angiogenesis inhibitors are currently available and in clinical trials for the treatment of chronic inflammatory diseases and cancer. As of the writing of this review, only 1 study has indicated that angiogenesis may be of functional importance in chronic rejection. We anticipate that this will be an area of intense investigation in the future.

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