

Mast cells and eosinophils: A novel link between inflammation and angiogenesis in allergic diseases

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Mast cells and eosinophils are the key cells in the early and late stages of allergic inflammation. There is increasing evidence that angiogenesis plays an important role both in the development of inflammation and in the pathophysiology of tissue remodeling during allergic disorders. In this review we provide recent data showing a link between allergy and angiogenesis and some possible mechanisms through which vascular endothelial growth factor and the immune system can interact. We discuss the multifaceted roles of mast cells and eosinophils in tissue remodeling and angiogenesis during allergic diseases and whether these cells can be both source and target cells for proangiogenic mediators. (*J Allergy Clin Immunol* 2005;116:531-6.)

Key words: Mast cells, eosinophils, allergy, angiogenesis, tissue remodeling, asthma, vascular endothelial growth factor

MAST CELLS AND EOSINOPHILS IN ALLERGIC INFLAMMATION AND TISSUE REMODELING

Allergic inflammation

Allergy is a complex disease characterized by a specific pattern of inflammation that is driven by IgE-dependent triggering of tissue resident mast cells and characterized by the influx and persistence of the eosinophils in the relevant tissue.^{1,2}

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Abbreviations used

FGF:	Fibroblast growth factor
HSPG:	Heparan sulfate proteoglycan
MMP:	Metalloproteinase
NGF:	Nerve growth factor
PG:	Prostaglandin
RTK:	Receptor tyrosine kinase
TIMPs:	Tissue inhibitors of metalloproteinases
VEGF:	Vascular endothelial growth factor
VEGFR:	High-affinity receptor tyrosine kinases for VEGF

Activated mast cells release mediators that contribute to eosinophil recruitment, activation, and survival. For example, rat peritoneal mast cell–derived TNF- α enhances eosinophil survival through autocrine production of GM-CSF, and mast cell–derived tryptase induces IL-6 and IL-8 release in eosinophils through the mitogen-activated protein kinase and activating protein 1 pathways. On the other hand, eosinophil-derived mediators can influence mast cell survival and activation. Among them, major basic protein was found to induce histamine and prostaglandin (PG) D₂ release from human lung- and cord blood–derived mast cells through an IgE-independent mechanism. Eosinophils synthesize, store, and release important mast cell survival and activating factors, such as stem cell factor and nerve growth factor (NGF). All this evidence indicates that mast cells and eosinophils, through their specific preformed mediators, can prolong and even intensify allergic inflammation, with possible development of tissue damage and consequent remodeling.

Tissue remodeling

One of the initial features of tissue remodeling in allergic disorders is the fibrotic response, in which fibroblasts are the main direct targets of the inflammatory mediators.

Mast cells and eosinophils can directly affect fibroblasts.^{1,2} The human mast cell line HMC-1 induces human lung, skin, and intestinal fibroblast proliferation

by histamine and tryptase and enables contraction of a collagen matrix and their differentiation into myofibroblasts.¹ Eosinophil granule basic proteins modulate tissue remodeling by acting on the extracellular matrix components and on some fibroblast properties. For example, eosinophil cationic protein inhibits proteoglycan degradation and increases intracellular accumulation of glycosaminoglycans in human lung fibroblasts. Major basic protein acts synergistically with IL-1 and TGF- β to increase IL-6 production, whereas eosinophil-derived neurotoxin stimulates fibroblast proliferation. In the *in vivo* system of human atopic dermatitis, eosinophils infiltrate the skin after allergen-induced IgE-dependent activation and parallel myofibroblast formation and deposition of tenascin and procollagen I. Some of the mast cell- and eosinophil-derived cytokines, such as TGF- β and fibroblast growth factor (FGF) 2, are well-known, potent profibrogenic mediators. Others mediators have been only recently discovered to display selective profibrogenic properties. For example, NGF enhances human lung, skin, and conjunctival fibroblast migration, differentiation into myofibroblasts, and contraction of collagen gels but not their proliferation and collagen synthesis. Mast cells and eosinophils also contain preformed matrix metalloproteinases (MMPs), such as MMP-9, and tissue inhibitors of MMPs (TIMPs; ie, TIMP-1 and TIMP-2), indicating that they can also modulate extracellular matrix formation. During tissue remodeling, fibroblasts are not only a target for inflammatory cells but also a source of important survival and activating mediators for mast cells and eosinophils. Therefore they contribute to the perpetuation and amplification of the inflammatory process.

ANGIOGENESIS AND INFLAMMATION

Angiogenesis

Angiogenesis is the growth of new blood vessels from preexisting ones. This is the predominant mechanism of blood vessel formation in the later stages of embryonic development and in postnatal life in wound repair and cyclically in the female reproductive system. Under physiologic conditions, angiogenesis depends on the balance of positive and negative angiogenic mediators within the vascular microenvironment and requires the functional activities of a number of molecules, including angiogenic factors, extracellular matrix proteins, adhesion receptors, and proteolytic enzymes.³ Angiogenesis is also associated with pathologic conditions in direct response to tissue demands, such as chronic inflammation, fibrosis, and tumor growth.⁴

New vessel formation is a multistep and highly orchestrated process not only involving vessel sprouting but also involving endothelial cell migration, proliferation, tube formation, and survival.³

Angiogenic mediators

In normal tissues vascular quiescence is maintained by the dominant influence of endogenous angiogenesis

inhibitors over angiogenic stimuli. On the other hand, in pathologic situations angiogenesis might be excessive because of the overproduction of angiogenic factors, downregulation of angiogenesis inhibitors, or both.

Numerous inducers of angiogenesis have been identified, including members of the FGF family, vascular permeability factor/vascular endothelial growth factor (VEGF), angiogenin, TGF- α and TGF- β , platelet-derived growth factor, TNF- α , hepatocyte growth factor, GM-CSF, interleukins, chemokines, and angiopoietin 1 and 2. Among them, VEGF is the most potent direct-acting regulator of angiogenesis, and its expression is often excessive in chronic inflammation, fibrosis, and cancer.

VEGF and its receptors

Over the years, at least 6 VEGF isoforms of variable amino acid numbers have been produced through alternative splicing: VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₃, VEGF₁₈₉, and VEGF₂₀₆. VEGF₁₂₁, VEGF₁₆₅, and VEGF₁₈₉ are the major forms secreted by most cell types. The various VEGF isoforms differ primarily in their bioavailability, which is conferred by heparin and heparan sulfate binding domains encoded by exons 6 and 7. After secretion, VEGF₁₂₁ might diffuse relatively free in tissues, whereas approximately half of the secreted VEGF₁₆₅ binds to cell-surface heparan sulfate proteoglycans (HSPGs). VEGF₁₈₉ and VEGF₂₀₆ remain almost completely sequestered by HSPGs in the cell surface and in the extracellular matrix, making HSPGs a reservoir of VEGF that can be mobilized through proteolysis.⁴

The effects of VEGF on endothelial cells are mediated mostly by signals generated by binding to receptor tyrosine kinases (RTKs). Several high-affinity RTKs for VEGF (VEGFRs) have been identified, including VEGFR-1, VEGFR-2, and VEGFR-3. Neuropilin 1 and 2 constitute another class of high-affinity non-RTKs of VEGF receptors and can bind certain isoforms of VEGF. Both VEGFR-1 and VEGFR-2 have 7 extracellular immunoglobulin-like domains, a single-transmembrane region, and a consensus tyrosine kinase sequence that is interrupted by a kinase-insert domain. VEGFR-1 binds VEGF, VEGF-B, and placental growth factor with high affinity. VEGFR-1, like VEGFR-2, is expressed mostly on endothelial cells, as well as on other structural and hematopoietic stem cells. An alternatively spliced soluble form of VEGFR-1 has been shown to be a natural inhibitor of VEGF activity in human subjects and mice. VEGFR-2 binds VEGF, VEGF-C, and VEGF-D. VEGFR-2 is the primary receptor transmitting VEGF signals in endothelial cells. Sequestration of VEGF results in downregulation of VEGFR-2 and in apoptotic death of some capillary endothelial cells *in vivo*. Moreover, VEGFR-2 might be associated with integrin-dependent migration of endothelial cells because it forms a complex with integrin α V β 3 on binding VEGF.

Biologic functions and regulation of VEGF

VEGF induces proliferation, migration, and tube formation of endothelial cells. It promotes secretion of

interstitial collagenase (MMP-1) and von Willerbrand factor and the expression of chemokines, as well as leukocyte adhesion molecules, such as intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin.⁵ VEGF is also a potent survival factor for endothelial cells, and it induces in endothelial cells the expression of antiapoptotic proteins, such as survivin. VEGF also causes vasodilatation through the induction of the endothelial nitric oxide synthase and the subsequent increase in nitric oxide production. Therefore VEGF acts principally on endothelial cells, even though it can influence other cell types, including hematopoietic stem cells, monocytes, osteoblasts, and neurons.

Interestingly, VEGF modulates immune cell functions in different ways. For example, it inhibits dendritic cell maturation and increases the production of B cells and immature myeloid cells. It can also inhibit the development of T cells from early hematopoietic progenitor cells. In addition, VEGF stimulates monocyte chemotaxis and contributes to hematopoietic stem cell survival and recruitment of bone marrow-derived endothelial cells in angiogenesis.

Many growth factors and cytokines can regulate VEGF expression. TGF- β was shown to induce VEGF gene expression and secretion in fibroblasts and epithelial cells. IL-5 and GM-CSF have a similar effect on eosinophils (see later).⁶ CD40–CD40 ligand signaling, which has multiple functions in inflammation, increases the expression of VEGF by rheumatoid synovial fibroblasts and endothelial cells. VEGF expression is also regulated by a variety of environmental factors. For example, blood and tissue hypoxia are potent stimuli for VEGF production in endothelial cells, mainly through hypoxia inducible factor transcriptional complex.

Concerning negative regulation of angiogenesis and VEGF, at least 15 molecules are currently known to be endogenous inhibitors, including endostatin, thrombospondin 1, IFN- α , angiostatin, and TIMPs.⁷ Some of them, such as endostatin, can downregulate the migration and proliferation of microvascular endothelial cells in the tumor bed, helping in the suppression of pathologic angiogenesis.

Angiogenesis and chronic inflammation

There is considerable evidence to suggest that angiogenesis and chronic inflammation are codependent.⁸ In chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis, the proliferating tissue contains an abundance of inflammatory cells and angiogenic blood vessels. When the environment in the tissue becomes hypoxic or inflammatory, many resident cells, such as fibroblasts, keratinocytes, smooth muscle cells, and/or infiltrating cells, such as monocytes-macrophages, neutrophils, and lymphocytes, synthesize and secrete VEGF, promoting neovascularization in different tissues. Angiogenesis contributes to the perpetuation of chronic inflammation by promoting the migration of inflammatory cells to the site of inflammation. As the inflammatory disease evolves, the microvasculature undergoes progressive

changes in structure and function. Blood vessels enlarge or proliferate to supply nutrients and oxygen to the proliferating inflamed tissue.⁸

Many inflammatory mediators, such as PGE₁, PGE₂, TNF- α , IL-1, IL-6, IL-8, nitric oxide, and platelet-activating factor, have been shown to induce expression of VEGF, angiogenesis, or both.

MAST CELLS AND EOSINOPHILS IN ANGIOGENESIS DURING ALLERGIC DISEASES

Role of angiogenesis in allergic diseases: The asthma example

Angiogenesis is an important event both in the development of allergic inflammation and in the pathophysiology of tissue remodeling in atopic diseases.^{9–18} In the 1960s, a study by Dunnill¹⁹ demonstrated for the first time that asthmatic subjects who die in acute attacks have an enlarged capillary bed in the airway wall. Later on, increased vascularity in the airways has been recognized not only in patients with severe asthma but also in those with mild disease.^{9–11} Recent studies in the airways of asthmatic patients have revealed that the ratio between the level of VEGF and endostatin, proangiogenic and antiangiogenic mediators, respectively, is increased in the sputum of asthmatic subjects in comparison with that of control subjects.¹⁵ Therefore it seems that an imbalance in favor of proangiogenic factors leads to the abnormal growth of new blood vessels in asthma. The submucosa of the airways of asthmatic subjects also exhibits higher VEGF, FGF-2, and angiogenin immunoreactivity than did those of control subjects,¹³ and the expression of VEGF and its receptors VEGFR-1 and VEGFR-2 inversely correlates with airway function.¹⁴ An increase in FGF-2 levels in the bronchoalveolar lavage fluid of atopic asthmatic subjects in comparison with that of healthy subjects has also been reported.¹² Interestingly, a further increase of FGF-2 in the bronchoalveolar lavage fluid after acute allergen exposure suggests that the upregulation of angiogenic mediators might be the result of mast cell degranulation after IgE-dependent activation.

Role of VEGF in asthma

The direct contribution of VEGF in allergic response in asthma has been recently proposed by Lee et al.¹⁷ They generated lung-targeted VEGF₁₆₅ transgenic mice and evaluated the role of VEGF in antigen-induced T_H2-mediated inflammation. Overexpression of VEGF induced leukocyte infiltration in the lung, overproduction of IL-13, and increases in mucus production, collagen deposition, and smooth muscle hyperplasia. This was in addition to the predictably marked angiogenesis, edema, and vascular remodeling. Interestingly, these mice had an increase in the number of activated dendritic cells, and they had an exaggerated immune response after respiratory allergen challenge. These results prove that VEGF has direct effects on the immune system, and it amplifies a T_H2-mediated response in an animal model of experimental asthma.

On the other hand, it has been demonstrated that T_H2 cytokines modulate the synthesis and release of VEGF by different inflammatory and structural cells of the airways. For example, in the airway smooth muscle cells, IL-4, IL-5, and IL-13 enhanced VEGF production, whereas T_H1 cytokines, such as INF- γ , inhibited their spontaneous and IL-4-, IL-5-, or IL-13-induced VEGF release. These results, together with those of Lee et al,¹⁷ introduce a new concept of how VEGF and the immune system can interact in the allergic airway disorders: T_H2 cytokines, such as IL-13, induce structural cells to produce VEGF, which, in turn, enhances allergen-induced inflammation and consequent remodeling.

MAST CELLS AND EOSINOPHILS: EFFECTOR CELLS IN ANGIOGENESIS

For almost 2 decades, mast cells, anatomically located in proximity to blood vessels, have been associated with angiogenesis during hemangioma, rheumatoid arthritis, nasal polyps, wound healing, and ovulation.¹⁸ Recent studies have highlighted the contribution of mast cells in angiogenesis during allergic inflammation. For example, after IgE-dependent activation, mast cells released several proangiogenic mediators stored in their granules, such as VEGF and FGF-2, promoting angiogenesis even in the early phase of allergic inflammation. Histamine stimulates new vessel formation by acting through H_1 and H_2 receptors. In an *in vivo* system of induced subcutaneous granuloma, histamine increased angiogenesis by upregulating the level of VEGF. This effect was delayed when the subcutaneous granuloma was induced in mice lacking histamine (L-histidine decarboxylase knockout mice). Heparin and, more recently, tryptase have been shown to be proangiogenic factors by acting directly on endothelial cells and by degrading extracellular matrix components with consequent release of VEGF or FGF-2 from their matrix-bound state. In particular, mast cell-derived heparin has been shown to stimulate capillary endothelial migration and proliferation *in vitro*. In *in vivo* experiments the subcutaneous injection of commercial standard heparin (mean, 15 kd) and high-molecular-weight heparin (mean, 22 kd) stimulates angiogenesis, whereas low-molecular-weight heparin (mean, 2.5 kd and 5.0 kd) inhibits it. These different effects of heparins, which were unrelated to anticoagulant activity, charge density, and sulfate content, suggest that the molecular size and tertiary structure might have an important influence on events controlling angiogenesis.

Eosinophils, like mast cells, have the potential to induce neovascularization.²⁰ They synthesize and store in their granules several proangiogenic mediators, such as VEGF, FGF-2, TNF- α , GM-CSF, NGF, and IL-8, and are positively stained for VEGF and FGF-2 in the airways of asthmatic patients.¹³ In addition to this, we have recently provided evidence for the direct contribution of eosinophils to angiogenesis.²⁰ Eosinophils were shown to promote endothelial cell proliferation *in vitro* and to induce

new vessel formation in the aorta rings and in the chick embryo chorioallantoic membrane models. Interestingly, neutralization of VEGF in eosinophils reduced their angiogenic effects in the chorioallantoic membrane by 55%, suggesting an important but not unique role played by this factor in the induction of the angiogenic response. We obtained similar results for the mast cell-induced angiogenesis in the same *in vivo* system,²¹ in which neutralization of VEGF reduced the angiogenic response by 30%, but a combination of anti-VEGF and anti-FGF-2 antibodies caused a reduction of 70%.

Some experimental evidence indicates that mediators present in allergic inflammation promote the release of proangiogenic mediators by mast cells and eosinophils. Eosinophils can release VEGF after stimulation with GM-CSF and IL-5.⁶ Moreover, IgE-mediated mouse and human mast cell activation is an effective inducer of VEGF production. Interestingly, PGE₂, found to be increased in allergic asthma, induced human mast cells to release VEGF through activation of the prostaglandin E2 receptor in the absence of degranulation. Because both mast cells and eosinophils are rich sources of several extracellular matrix-degrading enzymes, we believe that they promote angiogenesis by acting on matrix degradation. HMC-1 cells constitutively express MMP-9 mRNA and secrete the active form of MMP-9 after phorbol myristate acetate activation. Eosinophils were previously found to produce active MMP-9, and more recently, we have shown that they express heparanase, an extracellular matrix-degrading enzyme that is preformed and inhibited by the eosinophil cationic proteins.²² In addition, IL-8, a proangiogenic chemokine preformed in and newly synthesized by eosinophils, enhances the mRNA levels, as well as active MMP-2 and MMP-9 expression, in endothelial cells.

MAST CELLS AND EOSINOPHILS: TARGETS FOR VEGF

Many inflammatory and structural cells, such as macrophages, neutrophils, epithelial cells, fibroblasts, and smooth muscle cells, are important sources of VEGF in the inflamed tissue.⁸ Different conditions can induce these cells to release VEGF. Several cytokines and growth factors involved in allergic inflammation and in remodeling are responsible for increasing the basal level of VEGF in fibroblasts, smooth muscle cells, and keratinocytes. For example, bradykinin, IL-1 β , IL-5, IL-13, and TGF- β are potent inducers of VEGF in airway smooth muscle cells, and TGF- β , together with IL-4 and IL-13, enhances the synthesis of VEGF in bronchial fibroblasts.²³ VEGF is a potent chemoattractant for leukocytes in experimental asthma¹⁷ and induces migration of mononuclear cells across an endothelial cell monolayer *in vitro*. Recent evidence indicates that eosinophil infiltration could be reduced by administration of anti-VEGF receptor antibodies in a murine model of toluene diisocyanate-induced asthma.²⁴ A possible explanation for this downregulatory effect comes from recent *in vitro* studies. Feistritzer et al²⁵ have detected VEGFR-1 and

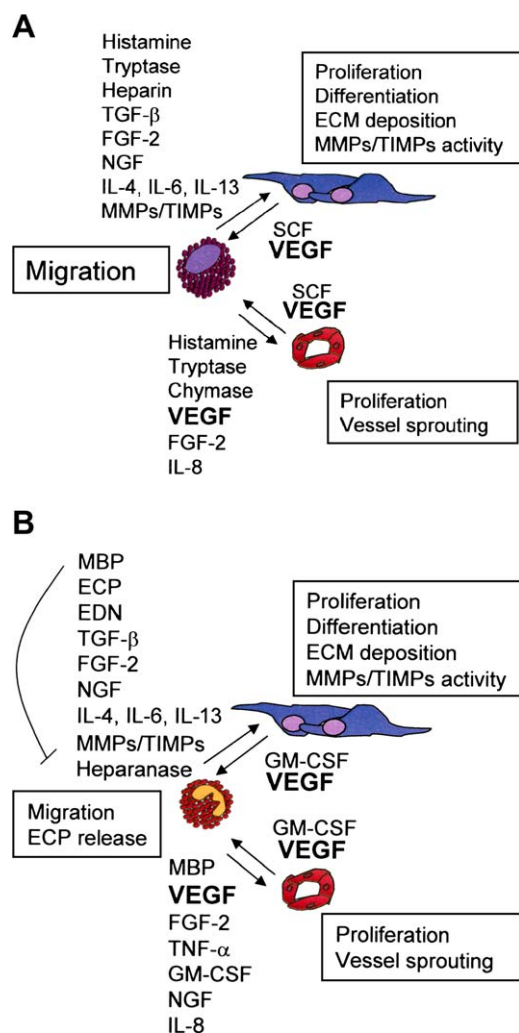


FIG 1. Interactions of mast cells (**A**) and eosinophils (**B**) with fibroblasts and endothelial cells. SCF, Stem cell factor; MBP, major basic protein; EDN, Eosinophil-derived neurotoxin; ECP, eosinophil cationic protein.

VEGFR-2 on human peripheral blood eosinophils and demonstrated that VEGF induces eosinophil migration and eosinophil cationic protein release, mainly through VEGFR-1. Together with eosinophils, mast cells can also migrate *in vivo* and *in vitro*²⁶ in response to VEGF, suggesting their recruitment to sites of neovascularization during physiologic or pathologic angiogenesis.

These results indicate that a positive feedback loop can take place in allergic inflammation, with T_H2 mediators inducing VEGF release by eosinophils and mast cells and consequent angiogenesis and VEGF enhancing activation of mast cells and eosinophils (Fig 1).

THERAPEUTIC APPROCHES IN ANGIOGENESIS

Recognition of the VEGF pathway as a key regulator of angiogenesis has led to the development of various

therapeutic strategies designed either to stimulate or to inhibit VEGF production. For example, angiogenic growth factors, such as members of the VEGF family, are used to treat patients with ischemia heart or limb diseases. On the other hand, there is increasing evidence to suggest that inhibiting VEGF is a valid strategy to suppress tumor-associated angiogenesis and therefore to treat patients with cancer.⁴ However, it is important to acknowledge that VEGF also can inhibit the antitumor immune response, thereby decreasing the host's ability to eradicate tumor cells.

The complex network between allergic inflammation and angiogenesis and the limited benefit of the therapeutically classical approaches of airway remodeling in asthma have led us to consider the use of angiogenic inhibitors to control inflammation, angiogenesis, and remodeling in allergic disorders. The results of the study by Lee et al¹⁷ in an animal model of allergic asthma indicate that VEGF production is a critical event in T_H2 inflammation and T_H2 cytokine production and augments antigen sensitization by acting on pulmonary dendritic cells. Interestingly, specific VEGF neutralization reduces not only angiogenesis but also some features of inflammation, such as infiltrating inflammatory cells, IL-13 production, and airway hyperresponsiveness. Similar results have been obtained in toluene diisocyanate-induced asthma. In this animal model administration of VEGF receptor inhibitors²⁴ blocked the VEGF-induced plasma leakage and the migration of inflammatory cells through the endothelial basement membrane. Therefore on the basis of the strong evidence obtained in these 2 different animal models of asthma, therapeutic agents that antagonize the effect of VEGF and prevent its production might be useful for prevention and/or treatment of asthma and other allergic diseases.

CONCLUSION

Changes in the vasculature still represent an important gap for a more complete understanding of the pathophysiology of allergic inflammation. We have discussed new insights that demonstrate the active contribution of mast cells and eosinophils in the development and amplification of angiogenesis.

Angiogenesis, through VEGF and other angiogenic mediators, might be partially responsible for the perpetuation of the allergic inflammation and of its consequences. In view of these recent findings, new clinical strategies aiming to downregulate VEGF might be of benefit in the treatment of allergic diseases.

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