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# Commentary

## Cellular Adhesion Molecules

### *Newly Identified Mediators of Angiogenesis*

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Adhesive interactions between cells or cells and the extracellular matrix (ECM) are thought to play an essential role in a number of diverse physiological and pathological settings. These include processes such as cellular recognition, specification, and signaling, provision of positional cues during vertebrate development, cell proliferation and differentiation during wound healing, blood coagulation, and leukocyte adherence and emigration.[1–9] The molecular determinants responsible for coordinating these diverse functions are the cellular adhesion molecules (CAMs). In addition, cellular adhesion mechanisms have been implicated in the pathogenesis of certain inflammatory disease processes and in malignancy where they participate in tumor invasion and metastasis.[10–14] The CAMs are classified into four major families: the integrins, the immunoglobulin superfamily, cadherins, and selectins.[10–14]

The selectins, the smallest and most recently identified CAM gene family, consists of three members: L-, P-, and E-selectin.[15–17] In comparison with the other CAMs, the selectins are unique in two respects. First, all three selectins are exclusively involved in the binding of leukocytes and some metastatic cells to endothelium. E-selectin, a 115-kd cytokine-inducible protein, was first identified as a cytokine-inducible adhesion protein on human umbilical vein endothelial cells (HUVECs) and found to participate in the rolling and adhesion of neutrophils and monocytes along endothelium at sites of inflammation.[18,19] *In vitro*, E-selectin is maximally expressed on the surface of endothelial cells (ECs) within 3 to 4 hours of activation with either interleukin-1 or tumor necrosis factor (TNF)-$\alpha$. *In vivo*, E-selectin expression has been shown to be restricted to post-capillary venules at sites of inflammation and immune activation.[20] Thus, E-selectin participates very early in the cascade of molecular events that lead to the adhesion of neutrophils and monocytes to the blood vessel wall and enable them to enter sites of inflammation. Second, E-selectin, like other members of the selectin family, functions as a carbohydrate-binding protein in that it recognizes sialylated, fucosylated oligosaccharides and related molecules on neutrophils, monocytes, and certain tumor cells.[15–17,21] This is in contrast to the other members of the CAM families, which function via protein-protein interactions. In addition, a soluble form of E-selectin has been described in several disease states in which it has been associated with several clinical parameters of disease activity. Unlike several other CAMs, no function for E-selectin during organogenesis or tissue remodeling has been reported. Recently, however, three reports have appeared implicating E-selectin and its ligand, the sialyl Lewis$^x$ (sL$^x$), and VCAM-1, a member of the immunoglobulin superfamily of adhesion molecules, in angiogenesis.

Kraling et al[22] analyze the expression of E-selectin in childhood hemangiomas and in two tissues undergoing active neovascularization, the placenta and neonatal foreskin. E-selectin was found to be highly expressed in proliferating microvascular ECs in hemangiomas, third-trimester placenta, and neonatal foreskins. Furthermore, E-selectin expression was observed to decline significantly in involuting phase

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hemangiomas, thus correlating expression levels with the angiogenic phenotype of hemangiomas. These findings document, for the first time *in vivo*, E-selectin expression in blood vessels participating in active neovascularization. These observations are also noteworthy in that E-selectin appears to be up-regulated in the absence of an identifiable inflammatory cytokine-inducing stimulus. Also, the results suggest that E-selectin may be a proximal signal in orchestrating events that lead to the proliferation of ECs.

## *Role of Cellular Adhesion Molecules in Angiogenesis*

The formation of new capillary blood vessels, a process termed angiogenesis, is one of the most pervasive and essential biological processes encountered in mammalian organisms. Angiogenesis is an important event in a variety of physiological settings such as embryonic development, chronic inflammation, and wound repair. It is a process that is tightly regulated in both time and space. Angiogenesis is driven by a cocktail of growth factors and proangiogenic cytokines and is tempered by an equally diverse group of inhibitors of neovascularization. Angiogenesis is also central to the etiology and pathogenesis of a number of pathological processes that include, among others, solid tumors, diseases of the eye, and chronic inflammatory disorders such as rheumatoid arthritis, psoriasis, and periodontitis.[22–30] Several lines of evidence support a role for CAMs in various steps in the cascade of events that lead to angiogenesis.

Expression of several EC integrins in association with components of the ECM are important for the attachment, alignment, and subsequent migration of ECs during angiogenesis.[31,32] ECs utilize the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins to bind to collagens I and IV and laminin.[33,34] Two major receptors for fibronectin, the RGD (Arg-Gly-Asp) peptide-dependent $\alpha_5\beta_1$, as well as $\alpha_4\beta_1$, also bind to the vascular cell adhesion molecule VCAM-1.[33] The former is present on most ECs, whereas others have confirmed that $\alpha_4\beta_1$ is also expressed by some ECs.[33,35] Another fibronectin, laminin, and collagen receptor, $\alpha_3\beta_1$, is present on ECs.[33,34] Integrins containing the $\beta_3$-subunit mediate EC adhesion to fibronectin, vitronectin, fibrinogen, and thrombospondin. The $\alpha_v$-integrin subunit can be associated with several $\beta$-chains ($\beta_1$, $\beta_3$, $\beta_5$, $\beta_6$, and $\beta_8$) and mediates EC adhesion to a variety of ECM components, depending on the $\beta$-subunit pairing.[10,11,33,34] As mentioned above, gpIV, a thrombospondin receptor, is also present on ECs.[10,11] A number of EC integrins, primarily $\beta_1$ and $\beta_3$ have been implicated in angiogenesis.[7–9,33,36,37] Although a number of $\alpha$- and $\beta$-subunits can be expressed on the EC surface, the expression of CAMs on ECs can be altered under certain conditions, such as angiogenesis.[33] For example, integrin $\alpha_2\beta_1$ is expressed during collagen-induced capillary formation,[36,38] and the integrin $\alpha_v\beta_3$ has been reported to be necessary for neovascularization of wounds and promotes EC cord formation *in vitro*.[7–9,39,40] Antibodies to $\alpha_2\beta_1$ and $\alpha_v\beta_3$ inhibited EC proliferation but promoted capillary tube formation on ECM substrates, suggesting that these integrins convert ECs from a proliferative to a differentiated phenotype.[38] Also, antibodies to the $\alpha_6\beta_1$ integrin are able to block capillary formation of an EC line on Matrigel.[41] A direct link between proangiogenic cytokines and CAM during *in vivo* angiogenesis has recently been elucidated in a series of reports from the Cheresh laboratory.[7–9]

Brooks et al[7,8] and Friedlander et al[9] have identified two cytokine-dependent pathways of angiogenesis that are dependent on distinct vascular cell integrins and are distinguished by their dependency on specific $\alpha_v$-integrin subunits and on the intracellular serine/threonine kinase protein kinase C. Using the rabbit cornea and the chick chorioallantoic membrane, they showed that angiogenesis induced by basic fibroblast growth factor (bFGF) or by TNF-$\alpha$ depended on $\alpha_v\beta_3$, whereas angiogenesis initiated by vascular EC growth factor, transforming growth factor-$\alpha$, or phorbol ester depended upon $\alpha_v\beta_5$. They also showed that these pathways could be further distinguished by their sensitivity to calphostin, an inhibitor of protein kinase C that blocked angiogenesis by $\alpha_v\beta_5$ but not $\alpha_v\beta_3$.

Several lines of study suggest that interactions of ECs with other adhesion molecules may also play a role during angiogenesis. Intercellular CAMs present on ECs include VCAM-1, intercellular adhesion molecules ICAM-1 and -2, CD31, E- and P-selectin, CD34 and other carbohydrate ligands for L-selectin, and vascular adhesion protein VAP-1.[10,11,13,42,43] Regarding other EC CAMs, antibodies to E-selectin, as well as to its ligands $sL^x$ and sialyl A, inhibit tube formation of bovine capillary ECs *in vitro*.[44] CD31 is involved in tumor stromal angiogenesis, and its expression on ECs correlates with metastatic potential and prognostic indices in breast cancer.[45] CD31 exhibits an EC expression in melanomas different from that on normal vessels.[39] The L-selectin ligand CD34 shows high EC expression in developing tissues as well as in tumors and healing wounds.[42] The expression of both CD31 and CD34 is increased on

sinusoidal ECs in hepatocellular carcinoma when compared with normal liver.[46] The complexity of CAM-mediated interactions can be demonstrated during tumor progression. A number of EC CAMs, including integrins, ICAM-1 and -2, VCAM-1, CD31, CD34, CD36 (gpIV), CD44, and selectins show differential expression in malignant tumors when compared with their normal counterparts.[12,42,47]

As evidence continues to accumulate, a regulatory network consisting of angiogenic mediators, ECM components, and CAMs is beginning to emerge. FGF bound to ECM substrate has been found to promote EC adhesion, proliferation, and protease production. As bFGF contains RGD sequences, the angiogenic activity associated with this potent growth factor may involve integrins and RGD-containing ECM components.[48] The RGD motif is present in fibronectin, vitronectin, collagen, laminin, and thrombospondin.[10,11,49] FGF treatment of ECs *in vitro* reportedly results in increased expression of the integrin subunits $\alpha_2$, $\alpha_3$, $\alpha_5$, $\alpha_6$, $\beta_1$, $\beta_3$, $\beta_4$, and $\beta_5$ integrins, as well as in enhancing adherence of ECs to collagen, fibronectin, laminin, and vitronectin compared with untreated cells.[50,51] Transforming growth factor-$\beta$ induces $\alpha_2$, $\alpha_5$ and $\beta_1$ expression on these cells, suggesting that various growth factors can selectively alter the integrin profile on ECs.[51] Treatment of ECs with the angiogenic cytokine TNF-$\alpha$ stimulates $\alpha_1\beta_1$ expression and adhesion to laminin.[34,52] Interactions between the proangiogenic mediator bFGF, the angiogenesis inhibitor thrombospondin, and CAMs have also been demonstrated. Thrombospondin inhibits angiogenesis by blocking the effects of bFGF on ECs.[53] In addition, thrombospondin receptors, such as $\beta_3$-integrins and gpIV, have been implicated in neovascularization.[26,34,38] Different signaling mechanisms are likely involved in these interactions. Capillary tube formation induced by bFGF or integrins involves protein kinase activation, G proteins, and calcium influx.[36,40,41,55–57] Thus, it would appear that CAMs, in part regulated by angiogenic factors, are involved in EC adhesion to ECM and other cells during capillary formation. However, there has been no proof until recently showing that CAMs themselves could initiate neovascularization *in vivo*.

## *Soluble Endothelial Adhesion Molecules as Angiogenic Factors*

Koch et al[58] recently examined the role of certain soluble EC CAMs in neovascularization. Soluble (s)VCAM-1 and sE-selectin were examined both *in vitro* for their ability to promote EC migration *in vivo* for their ability to promote and angiogenesis. They reported that both sE-selectin and sVCAM-1 induced in a dose-dependent manner up to a twofold significant increase in the number of migrating HUVECs and dermal microvascular ECs in blind-well chemotaxis chambers. In the rat cornea model of angiogenesis, both soluble CAMs incorporated into Hydron pellets induced an angiogenic response at 10 nmol/L concentration. The angiogenic effect of these soluble CAMs was comparable to that of the potent angiogenic mediator bFGF. Soluble VCAM-1 and sE-selectin did not stimulate HUVECs to produce bFGF, TNF-$\alpha$ or interleukin-8 as determined by enzyme-linked immunosorbent assay, suggesting that these soluble CAMs did not act as indirect angiogenic mediators. Moreover, monoclonal antibodies to the E-selectin ligand $sL^x$ and to the VCAM-1 counter-receptor $\alpha_4\beta_1$ integrin significantly reduced soluble CAM-mediated HUVEC chemotaxis by 27 and 24%, respectively. Both $sL^x$ and the $\alpha_4\beta_1$ integrin have been detected on HUVECs by immunohistochemistry.[60] Although $sL^x$ has been implicated in bovine capillary morphogenesis *in vitro*,[44] the results of Koch et al[58] and Kraling et al[22] suggest a direct link between the sE-selectin- $sL^x$ as well as sVCAM-1-$\alpha_4\beta_1$ adhesion pathways. Thus sE-selectin and sVCAM-1 may act as proangiogenic factors. CAMs shed from the cell surface upon cytokine activation may directly trigger neovascularization by binding to their respective EC ligands and recruit adjacent ECs.[58,61,62]

## *Interactions of Endothelial Cells, Angiogenic Mediators, and Cellular Adhesion Molecules in Disease*

Rheumatoid arthritis is a systemic chronic inflammatory disease that is characterized by the development of a proliferating mass of invasive granulation tissue that invades and degrades cartilage and bone of diarthrodial joints. The rheumatoid synovium contains many different cell types including synovial cells, fibroblasts, lymphocytes, monocytes, and macrophages as well as numerous capillary blood vessels. Normal synovial tissue is poorly vascularized. In contrast, the inflamed synovium contains large numbers of vessels with activated ECs, some of which resemble the morphology of high endothelial venules.[63–67] It has been shown that synovial tissue macrophages produce several factors, which account for the increased vessel formation in the inflamed synovium.[68–70] The intensive angiogenic activity correlates with clinical and inflammatory scores as well as synovial hyperplasia.[63] Several angiogenic factors and CAMs are present in the inflamed synovium.

Among the angiogenic mediators implicated in the persistent vasoproliferative response that characterizes this angiogenesis-dependent disease are TNF-$\alpha$, interleukin-8, vascular endothelial growth factor, acidic FGF, bFGF, epidermal growth factor, and platelet-derived growth factor. Some other angiogenesis-modulating or -inhibiting factors, such as interleukin-1$\beta$ and transforming growth factor-$\beta$, are also present. These factors may control neovascularization within the pannus.[63,66,71,72] EC CAMs, such as ICAM-1, VCAM-1, E-selectin, CD31, $\beta_3$-integrins, and VAP-1 are also present on ECs in the inflamed synovium, and most of them show increased expression in the highly neovascularized synovium.[43,64,70–75] Fibronectin and its CS1 domain have been detected on the luminal surface of synovial ECs.[76] It has been suggested that thrombospondin may modulate EC adhesion to other ECM components and thus angiogenesis in the rheumatoid synovium.[77] Among the angiogenic mediators, TNF-$\alpha$ may also induce the expression of ICAM-1, VCAM-1, E-selectin, and some integrins on synovial and other types of ECs,[10,14,26,33,34,78] resulting in the increased shedding of these CAMs from the EC surface. In this regard, high levels of soluble ICAM-1, VCAM-1, and E-selectin have been found in the synovial fluids of rheumatoid arthritis patients.[79–81] As discussed earlier, soluble VCAM-1 and E-selectin may act as angiogenic mediators. Koch et al[58] have shown that rheumatoid arthritis synovial fluid exhibits chemotactic activity for HUVECs, with a potency comparable to bFGF. Anti-E-selectin and anti-VCAM-1 antibodies compared with isotype-matched control antibodies are able to significantly attenuate the *in vitro* rheumatoid arthritis synovial-fluid-mediated chemotactic activity for HUVECs by 21 and 18%, respectively. In addition, neutralization of these two soluble CAMs also diminished angiogenesis *in vivo*.[58] The effect of cell-cell adhesion on CAM expression has been demonstrated.[82,83] Monocytes layered on cultures of synovial cells induced ICAM-1 and VCAM-1 expression and cytokine production by the latter cells. As shown in neutralization experiments, this interaction was highly TNF-$\alpha$ dependent.[83] In addition, $\alpha_4\beta_1$ integrins on tumor cells can induce VCAM-1 expression on adjacent ECs.[82] These data suggest that not only soluble but also cell surface CAMs may regulate EC adhesion during angiogenesis.

## Summary and Conclusions

In conclusion, cell surface CAMs, through a mechanism not yet well understood, appear capable of mediating interactions between ECs, the ECM, and cytokines leading to the initiation and maintenance of angiogenesis. There is now compelling evidence that angiogenic responses that accompany physiological processes such as wound healing, tissue remodeling, and embryogenesis are dependent in part on CAM-mediated interactions between ECs and components of the ECM. In addition, conditions that result in the overexpression of cell-surface-associated or soluble forms of CAM may contribute to the persistent neovascularization that is associated with diseases that are now recognized as angiogenesis dependent. One could envision that in rheumatoid arthritis or other disorders characterized by a disordered cytokine network, such as in the chronic inflammatory skin disease psoriasis,[84,85] the production of a number of abundantly produced, soluble mediators may directly induce neovascularization by various signals. Some of them, such as TNF-$\alpha$, may act indirectly by inducing CAM expression on and shedding from the EC surface. Increased CAM expression might result in stronger EC adhesion to ECM components. Cell-ECM and cell-cell interactions may further trigger EC CAM expression and cytokine production. Soluble and possibly surface-bound EC CAMs can act as direct mediators of angiogenesis. In addition, as one of the possible ligands for E-selectin is closely related to bFGF, these CAMs may also participate in neovascularization-associated signaling triggered by other mediators. It is possible that E-selectin, which mediates the early leukocyte-EC adhesive interactions may initiate early steps in the angiogenesis cascade while angiogenic mediators secreted by monocyte-derived cells, interstitial macrophages, diseased synovial lining cells, or psoriatic keratinocytes may be important in the later stages of diseases.[13] Thus, the complex interactions between EC, ECM macromolecules, CAMs, and other angiogenic factors may lead to pathological neovascularization. Similarly, the overexpression of CAMs such as E-selectin or their persistent induction by tumor cells would provide an environment highly conducive to tumor progression. No doubt, future studies of CAMs and angiogenesis will focus on determining how cytokines regulate expression of CAMs, identifying the signals resulting from such adhesive interactions that lead to angiogenesis, and analyzing the spectrum of physiological and pathological processes in which CAMs participate in angiogenic responses.

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