Perspectives Series: Cell Adhesion in Vascular Biology

Integrins and Vascular Extracellular Matrix Assembly

Erkki Ruoslahti and Eva Engvall
La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037

Introduction

Integrins, a family of heterodimeric membrane proteins, serve in one of their main roles as adhesion receptors for various extracellular matrix components (1, 2). The integrin–matrix interaction is a two-way street. Most extracellular matrix proteins are ligands for integrins, allowing integrins to mediate cell attachment, and the matrix may, at least to a degree, control the expression and activity of integrins (3). Integrins, in turn, can control the formation of the matrix; this has been most clearly demonstrated for fibronectin (4), but may be true of some other matrix components as well.

The structural and morphological variations in the vasculature throughout the body are matched by an equally large variation in the molecular composition of the vessels. While clear in broad outline, the distribution of integrins and extracellular matrix components in blood vessels is incompletely known in detail.

The various integrins and matrix types are expressed in the different compartments of blood vessels and at different times in development. In principle, the endothelial cells manufacture a basement membrane matrix, the main components of which are laminin, type IV collagen, and heparan sulfate proteoglycan. The smooth muscle fibers in the media are surrounded by another basement membrane with a different set of isoforms of the same basic components. In addition, the media contain sheets of collagenous and elastic tissue, while the adventitia consists of connective tissue rich in collagens. Fig. 1 schematically represents the distribution of some matrix components of recent clinical interest in an arterial wall.

The integrins that have been detected in resting endothelia include α6β1, α5β1, α2β1, and αvβ3 (5), which are receptors for laminins, fibronectin, collagens, and vitronectin. Smooth muscle cells possess a large complement of β1 and αv integrins, of which αvβ3 may be particularly important (6).

Pathological lesions in the vasculature display various changes in integrins and matrix composition. Owing to an improving understanding of the roles of integrins and matrix in these pathologies, drugs modulating integrin activities are beginning to reach the clinic. We review here some of the recent advances in this field.

Integrins and matrix in vascular development

Gene knockout experiments, conducted both by Nature and by experimentalists, have established an important role for the matrix and its receptors in the development of the vasculature.

Fibronectin and type I collagen are necessary for the development and integrity of the vasculature. Mice that lack fibronectin have defects in somitogenesis and vascular development; the embryos die in utero during the 9th and 10th day of gestation (7). Mice that lack type I collagen develop surprisingly far; they die of circulatory collapse just before birth (8).

The absence of the α5β1 integrin, the main fibronectin receptor, is also incompatible with vascular development; the embryos of mice lacking this integrin die around gestational days 10–11. Mice lacking the αv integrin subunit, defining receptors for vitronectin and other RGD-containing proteins, die soon after birth owing to abnormalities in the cerebral blood vessels (7). The reasons for the selective effect of αv on cerebral vasculature are not known, but they may relate to the importance of the αvβ3 and αvβ5 integrins for angiogenesis (see below) or to the preferential expression of two potential ligands of these integrins, vitronectin (9) and merosin (10, 11), in brain vessels. Perhaps brain vessels follow a different developmental path than other blood vessels.

The laminin β2 chain is normally expressed in kidney glomeruli and in smooth muscle basement membranes of blood vessels (in addition to locations such as synapses in the peripheral nervous system and in muscle). In the vascular system, its absence primarily manifests itself in the kidney (12). Similarly, mutations in certain type IV collagen chains cause a kidney disease, Alport's syndrome (13), although some endothelial basement membranes, including those of the eyes, normally contain these collagens (14). Thus, glomerular function appears to be particularly dependent on the correct structure of vascular basement membranes and vascular extracellular matrices.

Integrins and matrix in the maintenance of vascular structure and function

The cell–matrix interactions mediated by integrins are thought to be important in maintaining the polarity and positioning of the cells that make up the vessel wall. In addition, the integrins are likely to be involved in the sensing of and subsequent responses to changes in flow conditions within the vessels. Moreover, certain integrin ligands in the matrix, such as von Willebrand factor, participate in hemostasis and other injury responses (15, 16).

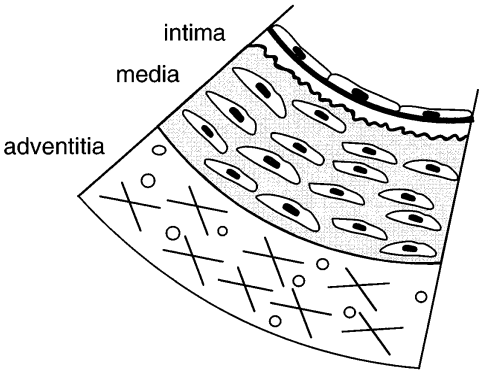
Structural components of the matrix are particularly im-

Address correspondence to Erkki Ruoslahti, La Jolla Cancer Research Center, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037. Phone: 619-646-3125; FAX: 619-646-3198.

Received for publication 7 February 1997.

J. Clin. Invest.

0021-9738/97/03/1149/04 $2.00
Volume 99, Number 6, March 1997, 1149–1152



ECM components	Clinical importance
collagen XVIII	angiogenesis
thrombospondin	angiogenesis
osteopontin	response to injury
fibrillin	Marfan syndrome
elastin	supravalvular aortic stenosis Williams syndrome
collagen III	Ehlers-Danlos type IV

Figure 1. Distribution in blood vessel of some extracellular matrix proteins of clinical interest.

portant in maintaining the integrity of blood vessels. Fibrillin, which is also an integrin ligand, appears to be important for the maintenance of vascular integrity. Mutations in fibrillin that cause its abnormal assembly result in Marfan's syndrome, one hallmark of which is weakness, enlargement, and rupture of large blood vessels (17, 18). Similarly, Ehlers-Danlos type IV patients, who have mutations in type III collagen, often suffer an aortic rupture (19). Interestingly, the absence of elastin, which like fibrillin is concentrated in the elastic laminae of the media, causes aortic constriction rather than widening (20).

Integrins and matrix in vascular pathologies

Disturbances in integrin–matrix interactions underlie or contribute to many vascular pathologies.

Abnormal extracellular matrix accumulation is an important component in the pathophysiology of lesions involving intimal hyperplasia and in the somewhat analogous situation of glomerulosclerosis. Intimal thickening after a lesion, such as that inflicted in angioplasty, occurs in two stages. The first one is characterized by smooth muscle cell proliferation and migration into what will become the neointima (Fig. 2). During the second stage, smooth muscle cells fill the neointima with matrix. The role of integrins in these processes has been probed with antibodies and peptides capable of blocking the binding of integrins to their ligands.

Many integrins recognize the peptide motif RGD (arginine-glycine-aspartic acid) as the key feature in their matrix ligands, and peptides containing this motif, or chemicals that mimic it, inhibit ligand binding (2). These RGD peptides and their mimetics can be designed to be specific for individual integrins. In vitro studies show that RGD peptides and antibodies capable of blocking the ligand binding of the αvβ3 integrin can inhibit the migration of smooth muscle cells. A possible ligand for the αvβ3-mediated migration is osteopontin, an RGD protein the levels of which are greatly increased in intimal hyperplasia (6). Indeed, an antibody that blocks αvβ3, as well as the platelet integrin αIIbβ3, is on the market for the restenosis indication, and drugs based on the RGD motif are also being developed for this purpose (21, 22).

The effectiveness of integrin-blocking agents in preventing restenosis may not be limited to their ability to inhibit cell migration; integrins also control cell proliferation (23) and help assemble matrix, at least fibronectin matrix (4). The αvβ3 integrin is one of the integrins that promotes the assembly of fibronectin matrix (24).

Increased integrin activity, if it plays a role, is not likely to be a major factor in the accumulation of neointimal matrix, because this matrix includes components that are not integrin ligands, such as the proteoglycan versican (25, 26). Instead, increased production of the growth factor TGFβ is the likely

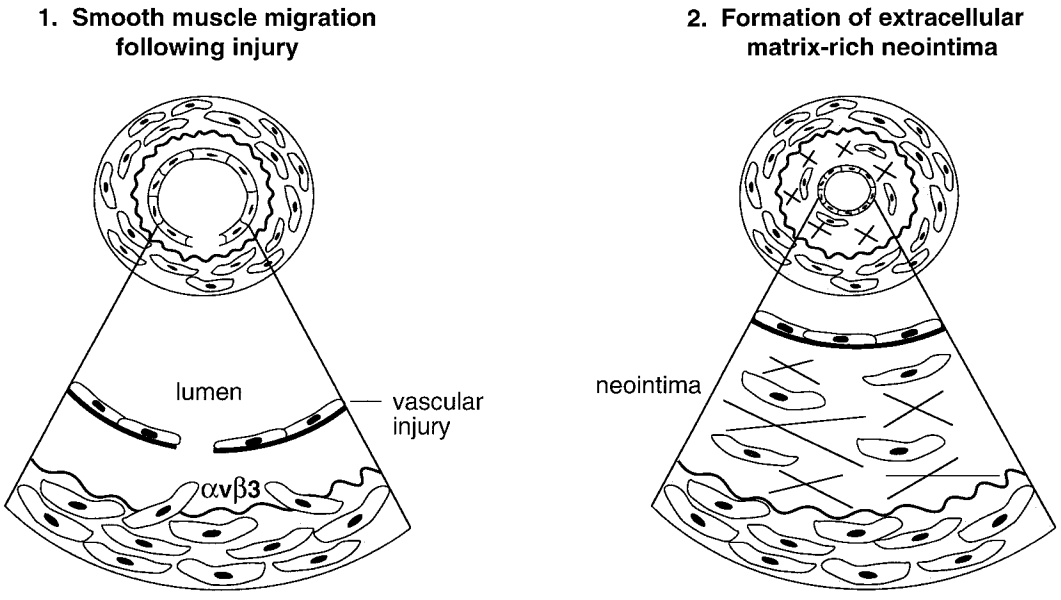


Figure 2. Integrins and extracellular matrix in vascular pathologies that give rise to intimal hyperplasia.

cause of the pathological matrix accumulation in intimal hyperplasia. TGFβ stimulates the synthesis of several matrix components, including proteoglycans. TGFβ activity is increased in intimal hyperplasia and inhibiting this activity with antibodies has been found to reduce the matrix accumulation in both in vitro and in vivo experimental models (26). This facet of intimal hyperplasia seems to resemble many other diseases in which overproduction of TGFβ is an important underlying factor (27). The pathogenetic mechanism is characterized by TGFβ-induced synthesis and accumulation of excessive matrix, accompanied by degeneration of the epithelial component of the tissue; these diseases include glomerulonephritis, liver cirrhosis, pulmonary fibrosis, and others.

Blood vessels in different tissues are specialized and subject to dynamic changes. Lymphocytes recognize these specialized features in tissue-selective homing (28). Specialized tissue recognition may underlie the tendency of certain types of tumors to show preferential patterns of metastasis. Recently, in vivo tissue targeting with phage expressing libraries of random peptides has been used to demonstrate tissue-specific biochemical differences in vascular beds (29). Whether these tissue-specific features include differences in integrins or matrix components in normal vasculature is not known but, as detailed below, highly selective changes in the expression of these proteins do occur in abnormal blood vessels, such as those providing blood supply to tumors.

Integrins and matrix in angiogenesis

Angiogenesis, the formation of new blood vessels, also has important connections to integrins and the matrix. Endothelial cells migrate on extracellular matrix components in vitro and very likely also in vivo. Moreover, the αvβ3 and αvβ5 integrins are upregulated in angiogenic endothelial cells. Thus, the new blood vessels in tumors (30) and in certain inflammatory lesions, such as oxygen-induced retinal neovascularization (31), express these integrins at higher levels than normal resting blood vessels. Furthermore, cultured endothelial cells, which resemble angiogenic cells in that they also express substantial quantities of αvβ3, are dependent on αvβ3 for survival. If αvβ3 is blocked with RGD-containing peptides or antibodies selective for this integrin, endothelial cells grown in vitro and angiogenic endothelial cells in vivo will undergo apoptosis (30, 32). As discussed above, αv knockout mice have problems with their cerebral vasculature (8) and this may also reflect the role of αvβ3 in angiogenesis (and vasculogenesis).

The apparent dependence of angiogenic endothelium on αvβ3 has opened up promising therapeutic possibilities. Thus, αvβ3-directed RGD peptides have been shown to suppress tumor growth and retinal angiogenesis in ocular neovascular diseases (33). Various types of cells, including cultured endothelial cells, require another integrin, the α5β1 fibronectin receptor, for survival under serum-free culture conditions (34; Zhang, Z., and E. Ruoslahti, unpublished observations). However, under other culture conditions, and when engaged in angiogenesis in vivo, endothelial cells seem to be protected against apoptosis by the αvβ3 integrin, because under these conditions apoptosis can be induced with αvβ3 blockers (30, 32). It is unclear how the endothelial cell dependence on αvβ3 is mediated at the molecular level. The ligand that the αvβ3 integrin binds to in mediating angiogenesis is not known but seems unlikely to be vitronectin, the best known ligand for this integrin. Osteopontin is a possible candidate as this RGD protein is expressed at high levels in injured blood vessels (6).

Despite their apparent involvement in adhesive interactions important for the functioning and survival of the angiogenic endothelial cells, a portion of the αv integrins is present on the luminal surface of these cells (5). αv integrins also appear to be active on the luminal surface, because an αv integrin-peptide carried on a phage particle causes the phage to bind to tumor capillaries (Pasqualini, R., E. Koivunen, and E. Ruoslahti, unpublished observations). Clarifying the exact role of the αvβ3 and αvβ5 integrins and their ligands in angiogenesis and tumor targeting is clearly an important task for the future.

A matrix-related marker of angiogenic vasculature is an alternatively spliced variant of fibronectin. Fibronectin is alternatively spliced at three different regions of the molecule. One of these regions contains a type III fibronectin domain known as ED-B. The ED-B domain is preferentially expressed in angiogenic vasculature and, although it has not been proven to be exposed on the luminal side of the endothelium, it has been suggested as a marker suitable for tumor targeting (35). Another link between angiogenesis and vascular matrix is the ability of components of this matrix to inhibit angiogenesis. Such a role has been shown for thrombospondin (36). Most recently, a degradation product of a vascular matrix component, type XVIII collagen, was shown to act as an antiangiogenic agent (37). The physiological significance of this protein fragment, endostatin, may be that its production can serve as a feedback mechanism to suppress angiogenesis in tissue repair.

Clearly the integrins and extracellular matrix play important roles in many facets of vascular biology, and much needs to be learned about those roles. One of the likely payoffs will be new therapies for vascular diseases.

Acknowledgments

We thank Drs. Robert Burgeson, Lynn Sakai, Alexander Ljubimov, and Ulla Wewer for discussions.

The authors are supported by grants CA28896 and Cancer Center Support Grant CA 30199 (E. Ruoslahti) and HD 31636 (E. Engvall).

References

1. Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell.* 69:11–25.

2. Ruoslahti, E. 1996. RGD and other recognition sequences for integrins. *Ann. Rev. Cell Dev. Biol.* 12:697–715.

3. Delcommenne, M., and C.H. Streuli. 1995. Control of integrin expression by extracellular matrix. *J. Biol. Chem.* 270:26794–26801.

4. Mosher, D.F. 1993. Assembly of fibronectin into extracellular matrix. *Curr. Opin. Struc. Biol.* 3:214–222.

5. Conforti, G., C. Dominguez-Jiminez, A. Zanetti, M.A. Gimbrone, O. Cremona, P.C. Marchisio, and E. Dejana. 1992. Human endothelial cells express integrin receptors on the luminal aspect of their membrane. *Blood.* 80: 437–446.

6. Liaw, L., M.P. Skinner, E.W. Raines, R. Ross, D.A. Cheresh, S.M. Schwartz, and C.M. Giachelli. 1995. The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. *J. Clin. Invest.* 95:713–724.

7. Hynes, R.O. 1996. Targeted mutations in cell adhesion genes: What have we learned from them? *Dev. Biol.* 180:402–412.

8. Kratochwil, K., M. Dziadek, J. Lohler, K. Harbers, and R. Jaenisch. 1986. Normal epithelial branching morphogenesis in the absence of collagen I. *Dev. Biol.* 117:596–606.

9. Seiffert, D., K. Crain, N.V. Wagner, and D.J. Loskutoff. 1994. Vitronectin gene expression *in vivo*. *J. Biol. Chem.* 269:19836–19842.

10. Jucker, M., M. Tian, D.D. Norton, C. Sherman, and J.W. Kusiak. 1996. Laminin alpha 2 is a component of brain capillary basement membrane: re-

duced expression in dystrophic dy mice. *Neuroscience.* 71:1153–1161.

11. Engvall, E., and U. Wewer. 1996. Domains of laminin. *J. Cell. Biochem.* 61:493–501.

12. Noakes, P.G., J.H. Miner, M. Guatam, J.M. Cunningham, J.R. Sanes, and J.P. Merlie. 1995. The renal glomerulus of mice lacking s-laminin/laminin beta 2: nephrosis despite molecular compensation by laminin beta 1. *Nat. Genet.* 10:400–406.

13. Tryggvason, K. 1995. Molecular properties and diseases of collagens. *Kidney Int. Suppl.* 49:S24–S28.

14. Ljubimov, A.V., R.E. Burgeson, R.J. Butkowski, J.R. Couchman, R.R. Wu, Y. Ninomiya, Y. Sado, E. Maguen, A.B. Nesburn, and M.C. Kenney. 1996. Extracellular matrix alterations in human corneas with bullous keratopathy. *Invest. Ophthalmol. Vis. Sci.* 37:997–1007.

15. Hynes, R.O., and D. Wagner. 1996. Genetic manipulation of vascular adhesion molecules in the mouse. *J. Clin. Invest.* 98:2193–2195.

16. Ruggeri, Z.M. 1997. von Willebrand factor. *J. Clin. Invest.* 97:559–564.

17. Dietz, H.D., and R.E. Pyeritz. 1995. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. *Hum. Mol. Genet.* 4:799–809.

18. Reinhardt, D.P., R.N. Ono, and L. Sakai. 1997. Calcium stabilizes fibrillin-1 against proteolytic degradation. *J. Biol. Chem.* 272:1231–1236.

19. Steinman, B., P.M. Royce, and A. Superti-Furga. 1993. The Ehlers-Danlos syndrome. *In* Connective Tissue and Its Heritable Disorders: Molecular, Genetic and Medical Aspects. P.M. Royce and B. Steinman, editors. Wiley-Liss, New York. 351–407.

20. Curran, M.E., D.L. Atkinson, A.K. Ewart, C.A. Morris, M.F. Leppert, and M.T. Keating. 1993. The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. *Cell.* 73:159–168.

21. Coller, B. 1997. Platelet GPIIb/IIIa antagonists: the first anti-integrin receptor in therapeutics. *J. Clin. Invest.* In press.

22. Choi, E.T., L. Engel, A.D. Callow, S. Sun, J. Trachtenberg, S. Santoro, and U.S. Ryan. 1994. Inhibition of neointimal hyperplasia by blocking αvβ3 integrin with a small peptide antagonist GpenGRGDSPCA. *J. Vasc. Surg.* 19: 125–134.

23. Assoian, R., and G. Marcantonio. 1996. The relationship between cell adhesion and growth. *J. Clin. Invest.* 98:2436–2439.

24. Wu, C., P.E. Hughes, M.H. Ginsberg, and J.A. McDonald. 1996. Identification of a new biological function for the integrin αvβ3: initiation of fibronectin matrix assembly. *Cell Adhes. Commun.* 4:149–158.

25. Yao, L.Y., C. Moody, E. Schönherr, T.N. Wight, and L.J. Sandell. 1994. Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence analysis, *in situ* hybridization and immunohistochemistry. *Matrix Biol.* 14:213–225.

26. Wolf, Y.G., L.M. Rasmussen, and E. Ruoslahti. 1994. Antibodies against transforming growth factor β-1 suppress intimal hyperplasia in a rat model. *J. Clin. Invest.* 93:1172–1178.

27. Border, W.A., and N.A. Noble. 1994. Transforming growth factor beta in tissue fibrosis. *N. Engl. J. Med.* 331:1286–1292.

28. Butcher, E.C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell.* 67:1033–1036.

29. Pasqualini, R., and E. Ruoslahti. 1996. Organ targeting *in vivo* using phage display peptide libraries. *Nature (Lond.).* 380:364–366.

30. Brooks, P.C., M.P. Montgomery, M. Rosenfeld, R.A. Feisfeld, T. Hu, G. Klier, and D.A. Cheresh. 1994. Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell.* 79:1157–1164.

31. Hammes, H.-P., M. Brownlee, A. Jonczyk, A. Sutter, and K.T. Preissner. 1996. Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits neovascularization. *Nat. Med.* 2:529–533.

32. Brooks, P.C., S. Strömblad, R. Klemke, D. Visscher, F.H. Sarkar, and D.A. Cheresh. 1995. Antiintegrin αvβ3 blocks human breast cancer growth and angiogenesis in human skin. *J. Clin. Invest.* 96:1815–1822.

33. Friedlander, M., C.L. Theesfeld, M. Sugita, M. Fruttiger, M.A. Thomas, S. Chang, and D.A. Cheresh. 1996. Involvement of integrins alphavbeta3 in ocular neovascular diseases. *Proc. Natl. Acad. Sci. USA.* 93:9764–9769.

34. Zhang, Z., K. Vuori, J.C. Reed, and E. Ruoslahti. 1995. The $\alpha_5\beta_1$ integrin supports survival of cells on fibronectin and upregulates Bcl-2 expression. *Proc. Natl. Acad. Sci. USA.* 92:6161–6165.

35. Carnemolla, B., D. Neri, P. Castellani, A. Leprini, G. Neri, A. Pini, G. Winter, and L. Zardi. 1996. Phage antibodies with pan-species recognition of the oncofoetal angiogenesis marker fibronectin ED-B domain. *Int. J. Cancer.* 68:397–405.

36. Bornstein, P. 1995. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J. Cell Biol.* 130:503–506.

37. O'Reilly, M.S., T. Boehm, Y. Shing, N. Fukai, G. Vasios, W.S. Lane, E. Flynn, J.R. Birkhead, B.R. Olsen, and J. Folkman. 1997. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell.* 88:277–285.

"Cell Adhesion In Vascular Biology"

Series Editors, Mark H. Ginsberg, Zaverio M. Ruggeri, and Ajit P. Varki

October 15, 1996	Adhesion and signaling in vascular cell–cell interactions	Guy Zimmerman, Tom McIntyre, and Stephen Prescott
November 1, 1996	Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis	Elisabetta Dejana
November 15, 1996	Genetic manipulation of vascular adhesion molecules in mice	Richard O. Hynes and Denisa D. Wagner
December 1, 1996	The extracellular matrix as a cell cycle control element in atherosclerosis and restenosis	Richard K. Assoian and Eugene E. Marcantonio
December 15, 1996	Effects of fluid dynamic forces on vascular cell adhesion	Konstantinos Konstantopoulos and Larry V. McIntire
January 1, 1997	The biology of PECAM-1	Peter J. Newman
January 15, 1997	Selectin ligands: Will the real ones please stand up?	Ajit Varki
February 1, 1997	Cell adhesion and angiogenesis	Joyce Bischoff
February 15, 1997	von Willebrand Factor	Zaverio Ruggeri
March 1, 1997	Therapeutic inhibition of carbohydrate–protein interactions in vivo	John B. Lowe and Peter A. Ward
March 15, 1997	Integrins and vascular extracellular matrix assembly	Erkki Ruoslahti and Eva Engvall
April 1, 1997	Platelet GPIIb/IIIa antagonists: The first anti-integrin receptor therapeutics	Barry Coller
April 15, 1997	Importance of shear stress in endothelial adhesion molecule expression	Michael Gimbrone
May 1, 1997	Proteoglycans and proteoglycan-binding proteins in vascular biology	Robert Rosenberg
May 15, 1997	New insights into integrin-ligand interaction	Robert Liddington and Joseph Loftus
June 1, 1997	Adhesive interactions of Sickle erythrocytes with endothelium	Robert Hebbel
June 15, 1997	Cell migration in vascular biology	Stephen Schwartz
July 1, 1997	Integrin signaling in vascular biology	Sanford Shattil and Mark Ginsberg
July 15, 1997	Multi-step mechanisms of leukocyte homing	Eugene Butcher
August 1, 1997	Role of PSGL-1 binding to selectins in leukocyte recruitment	Rodger McEver and Richard Cummings