Fibronectin promotes VEGF-induced CD34$^+$ cell differentiation into endothelial cells

Errol S. Wijelath, PhD,[a] Salman Rahman, PhD,[b] Jacqueline Murray, PhD,[a] Yatin Patel, PhD,[b] Geoffrey Savidge, MD,[b] and **Michael Sobel, MD,[a]** *Seattle, Wash; and London, United Kingdom*

Background: **Adult endothelial progenitor cells (EPC) may be a useful source for engineering the endothelialization of vascular grafts. However, the optimal factors that promote differentiation of EPCs into endothelium remain to be elucidated. The goal of this current report was to determine which extracellular matrix (ECM) protein might modulate or enhance the effects of EPCs on differentiation into mature endothelium.**

Methods: **Human EPCs (CD34$^+$ cells) were cultured in ECM-coated six-well plates in MCDB-131 medium containing vascular endothelial growth factor (VEGF), insulin-like growth factor−1, and basic fibroblast growth factor. After 21 days, differentiated endothelial colonies were confirmed by immunofluorescence for von Willebrand factor (vWF) and vascular-endothelial (VE)-cadherin and mRNA expression of the endothelial markers Flk-1, vWF, and VE-cadherin. Cell migration toward the VEGF–matrix protein combinations was also measured.**

Results: **As judged by positive staining for endothelial markers vWF and VE-cadherin, the combination of VEGF with fibronectin (FN) produced significantly more endothelial colonies ($P < .05$) than did collagens I or IV or vitronectin. Defined fragments of FN did not enhance VEGF-mediated effects. Fibrinogen produced intermediate stimulation of differentiation. FN also enhanced VEGF-mediated CD34$^+$ cell migration. Blockade of α5β1, but not αvβ3 or αvβ5, inhibited both VEGF-mediated CD34$^+$ cell differentiation and migration.**

Conclusions: **VEGF and FN together significantly promote the migration and differentiation of CD34$^+$ cells. This synergism is specific to FN and the α5β1 integrin. Combinations of VEGF and FN may be useful in promoting differentiation of circulating endothelial progenitors into endothelial cells for tissue engineering. (J Vasc Surg 2004;39: 655-60.)**

Clinical Relevance: **Treatment of injured or diseased tissues with adult stem cells is a promising approach. In particular, bone marrow derived circulating endothelial progenitors (CEP's) have been shown to differentiate into endothelial cells *in vitro* and promote tissue revascularization of ischemic limbs and myocardium *in vivo*. Because of the relative ease of obtaining CEP's and as well as its high proliferative rate, CEP's may have clinical potential for endothelialization of prosthetic vascular grafts and revascularization of injured myocardium. However, there is a need to better understand the molecular pathways involved in the proliferation and differentiation of CEP's to take full advantage of its clinical potential.**

Until recently, differentiation of angioblasts (endothelial progenitors) into endothelial cells (vasculogenesis) was believed to be restricted to early embryogenesis, whereas angiogenesis, the sprouting of capillaries from preexisting blood vessels, occurs both in the developing embryo and in postnatal life.[1,2] However, recent studies have shown the presence of circulating endothelial progenitor cells (EPC) in adults that can differentiate into endothelial cells, suggesting that vasculogenesis can occur in the adult.[3,4] Evidence suggests that, at least in animal models, EPCs play a role in new blood vessel formation during wound healing.[5] Studies have also shown that circulating EPCs can home in to sites of ischemia.[6] Vascular endothelial growth factor (VEGF) is known to be essential for the in vitro differentiation of purified EPCs into mature endothelial cells.[4] This observation is consistent with in vivo studies demonstrating the importance of VEGF in vasculogenesis.[7,8] However, the role that extracellular matrix (ECM) proteins play in the differentiation of EPCs into mature endothelial cells is still unclear.

The interactions between ECM and cells are mediated by the integrin family of cell surface receptors.[9] Binding of integrins to ECM proteins initiates signals that contribute to important cellular processes such as cell proliferation, migration, differentiation, and survival. The formation of new blood vessels requires signals from both angiogenic growth factors and ECM. Recent studies have shown that there is an important synergism between integrins and growth factor receptors,[10] although the exact mechanisms by which these receptors and ligands cooperate is not known. In endothelial cells, we recently showed that fibronectin (FN) contains binding sites for VEGF and that these binding sites were critical for promoting the association of the integrin α5β1 with vascular endothelial growth factor receptor (VEGFR)2 and for promoting the biological responses to VEGF.[11]

From the [a]Department of Surgery, Division of Vascular Surgery, University of Washington School of Medicine and VA Puget Sound Health Care System, Seattle, Wash and [b]Coagulation Research Laboratory, GKT Medical School, St. Thomas Hospital, London, United Kingdom.

Dr Sobel was supported by grants from the National Institutes of Health (HL-39903) and the Veterans Administration Research Service.

Competition of interest: none.

Reprint requests: E. S. Wijelath, PhD, VA Puget Sound Health Care System, 151, 1660 S. Columbian Way, Seattle, WA 98108. (e-mail: errolw@washington.edu).

0741-5214/$30.00

doi:10.1016/j.jvs.2003.10.042

In the present study, we have investigated the role of FN in regulating VEGF-induced $CD34^+$ cell differentiation into endothelial cells. We hypothesize that optimum differentiation of $CD34^+$ cells into mature endothelial cells is dependent on the ECM. We show that FN promotes VEGF-mediated $CD34^+$ cell migration and differentiation. Using integrin-blocking antibodies, we also show that the $\alpha5\beta1$ integrin is involved in regulating $CD34^+$ cell differentiation and migration.

MATERIAL AND METHODS

$CD34^+$ differentiation assay. Differentiation of $CD34^+$ cells was carried out as described elsewhere,[4] with minor modifications. Purified human $CD34^+$ cells (obtained from BioWhittaker) were cultured in six-well plates. The plates were coated overnight with ECM proteins (10 μg/mL; Chemicon) in phosphate-buffered saline. This optimal concentration of ECM coating was based on preliminary experiments. After washing plates with phosphate-buffered saline, the plates were incubated for a further 3 hours in serum-free MCDB-131 medium containing VEGF (50 ng/mL; R&D systems). Medium was then discarded, and $CD34^+$ cells were seeded at 3×10^5 cells in endothelial growth medium consisting of MCDB-131 medium supplemented with VEGF (50 ng/mL), basic fibroblast growth factor (10 ng/mL; R&D systems), insulin-like growth factor-1 (5 ng/mL; R&D systems), the ECM protein to be tested (10 μg/mL), heparin (10 μg/mL), hydrocortisone (1 μg/mL), and 15% fetal bovine serum (stripped of FN and vitronectin by gelatin and antibody affinity columns). One half of the medium was replaced every 3 days. After 3 weeks, endothelial cell colonies were identified by their characteristic morphology and by immunostaining for von Willebrand factor (vWF, Dako) and vascular-endothelial (VE) cadherin (Santa Cruz).

$CD34^+$ migration. Migration assays were performed with some modifications as described elsewhere,[12] with 6.5-mm, 5-μm–pore size Transwells (Costar). ECM proteins (50 μg/mL) in assay medium (600 μL) were placed into 24-well plates together with VEGF (50 ng/mL) and incubated for 30 minutes at 37°C. $CD34^+$ cells were washed twice in MCDB-131/0.25% bovine serum albumin (assay medium) and were resuspended in assay medium to a final concentration of 1×10^6 cells/mL. One hundred microliters of cell suspension was then added to each Transwell and placed on the 24-well plate, where it was incubated for 4 hours. Cells migrating to the underside of the membrane were fixed in 2% paraformaldehyde and stained with crystal violet. Migrated cells were quantified by eluting dye with 10% acetic acid and reading absorbance at 562 nm. For studies with integrin-blocking antibodies, $CD34^+$ cell suspensions in assay medium were incubated with indicated antibodies for 30 minutes at 37°C before adding cells to Transwells. Antibodies were also added to the bottom well.

Reverse transcription polymerase chain reaction analysis. RNA was isolated using the RNeasy kit (Qiagen). RNA were reverse-transcribed and amplified for CD45, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), VEGFR-2, vWF, and VE-cadherin using the Superscript one-step RT-PCR kit (Life Technologies). Briefly, samples were reverse-transcribed for 30 minutes at 45°C and predenatured for 2 minutes at 94°C; this was followed by 35 cycles of denaturing (15 seconds at 94°C), annealing (15 seconds at 55°C), and extension (20 seconds at 72°C) and by 1 cycle of final extension for 5 minutes at 72°C in a final volume of 25 μL. Polymerase chain reaction (PCR) products were loaded and run on ethidium bromide 2% agarose gels. The sizes of PCR products for Flk-1, vWF, VE-cadherin, CD45, and GAPDH are 351, 431, 593, 485, and 576 bp, respectively. The primer sequences used for reverse transcription (RT) PCR were as follows. vWF forward: 5′-tggatgagcttttgcagacc-3′, reverse: 5′-gtgggagccgtcgtggtact-3′; VE-cadherin forward: 5′-ggatgaccaagtacagc-3′, reverse: 5′-acacactttgggctggtagg-3′; Flk-1 forward: 5′-gtgattgccatgttcttctg-3′, reverse: 5′-gaggatcttgagttcagaca-3′; GAPDH forward: 5′-atcaccatcttccaggagcg-3′, reverse: 5′-gcctgcttcaccaccttctt-3′; CD45 forward[13]: 5′-ttcaacttatacccttcgtgtc-3′, reverse: 5′-cctgctttactttgtccacttc-3′.

Statistical analysis. Results are expressed as means ± SD. Statistical significance between control and experimental groups was determined by one-way ANOVA. A value of $P < .05$ was considered significant. Densitometry analysis was performed by NIH Image 1.3 software.

RESULTS

FN promotes VEGF-induced $CD34^+$ differentiation into endothelial cells. Figure 1 shows the number of endothelial colonies derived from $CD34^+$ cell differentiation in response to different ECM. Endothelial colonies were observed in all the VEGF–ECM protein combinations tested. However, when $CD34^+$ cells were incubated on VEGF-FN–coated plates, there was more than a five-fold increase in endothelial colonies, indicating that FN was the most effective ECM protein. VEGF-fibrinogen–coated plates also stimulated endothelial colony formation above that observed for collagen and vitronectin, but not as effectively as did FN. Cell adhesions to the different ECM protein were comparable (data not shown). We also tested whether any FN fragments could support $CD34^+$ cell differentiation. As shown in Fig 1, when used to coat plates, a mixture of VEGF and FN 120-kDa cell-binding fragment was not as effective as the intact FN molecule in differentiating $CD34^+$ cells into endothelial cells. Adding both the FN N-terminal 70-kDa and C-terminal 40-kDa peptides to the VEGF–FN 120-kDa mixture failed to restore endothelial cell differentiation to the levels observed with intact FN.

$CD34^+$ colonies express endothelial markers. To determine whether the endothelial colonies at day 21 expressed mRNA for endothelial markers, we performed RT-PCR analysis of RNA extracted from $CD34^+$ at day 0 and after differentiation at day 21. At day 0, $CD34^+$ cells expressed mRNA for CD45 and VEGFR-2 receptors, and by day 21, mRNA for endothelial markers vWF and VE-cadherin was also expressed (Fig 2A). Using densitometry scanning, quantitative analysis of RT-PCR data for day 0 and day 21 endothelial markers is shown in Fig 2B. These

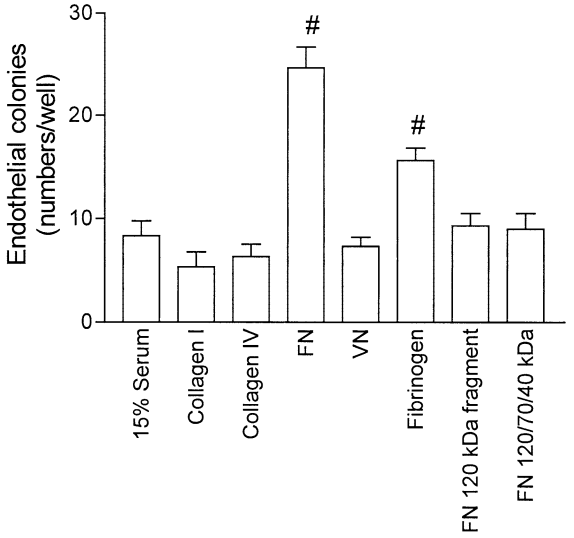

Fig 1. Comparison of ECM proteins on VEGF-induced $CD34^+$ differentiation. 3×10^5 $CD34^+$ cells were cultured for 21 days with endothelial growth medium on ECM-VEGF–coated plates. The number of endothelial colonies formed was identified by staining for vWF and VE-cadherin. Results are expressed as mean ± SD (n = 3). $^{\#}P < .05$ compared with control (15% serum).

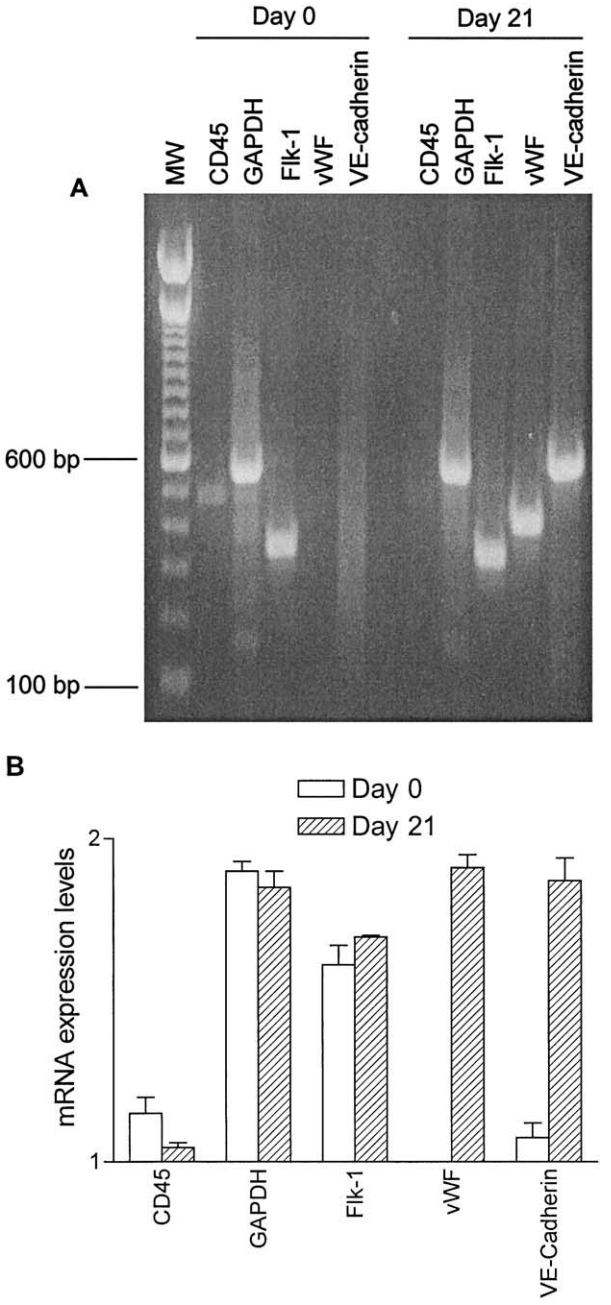

Fig 2. RT-PCR analysis for endothelial markers from VEGF-FN–differentiated $CD34^+$ cells. **A,** RNA was extracted from freshly isolated $CD34^+$ cells (day 0) and VEGF-FN differentiated $CD34^+$ cells (day 21) and was analyzed for endothelial-specific markers by RT-PCR. Data shown are representative of two independent experiments. **B,** Densitometry analysis of endothelial markers. Data are expressed as ratio of mRNA levels to gel background. Data are means ± SEM (n = 2).

data further confirm the differentiation of $CD34^+$ hematopoietic progenitor cells into endothelial cells.

FN promotes VEGF-induced $CD34^+$ migration. We next compared the effect of FN, vitronectin, and FN fragments on VEGF-mediated $CD34^+$ cell migration. VEGF alone mildly stimulated $CD34^+$ cell migration (Fig 3). When VEGF was incubated with FN, migration of $CD34^+$ cell was increased by nearly three-fold. Vitronectin (VN) or FN fragments alone or in combination with VEGF did not promote cell migration more then did VEGF alone.

The effect of FN on VEGF-induced $CD34^+$ cell differentiation and migration is mediated by the $\alpha5\beta1$ integrin. To investigate the role of specific integrins on VEGF-FN–induced $CD34^+$ cell differentiation, $CD34^+$ cells were cultured on FN-coated plates in the presence of VEGF and integrin-blocking antibodies, and endothelial colony formation was measured after 21 days. Antibodies to $\alpha5\beta1$ inhibited endothelial colony formation by 35%, whereas antibodies to $\alpha v\beta3$ and $\alpha v\beta5$ had no effect (Fig 4A). In migration assays, antibodies to $\alpha5\beta1$ inhibited VEGF-induced $CD34^+$ cell migration over a FN substrate, whereas antibodies to $\alpha v\beta3$ and $\alpha v\beta5$ had no effect (Fig 4B).

DISCUSSION

The goal of this study was to determine which ECM modulated VEGF-mediated $CD34^+$ cell migration and differentiation into mature endothelial cells. We showed that FN uniquely promoted VEGF-induced $CD34^+$ cell migration and differentiation. The synergistic effect of FN was most likely mediated through the $\alpha5\beta1$ integrin. These observations suggest that both FN and VEGF are critical for promoting differentiation of EPCs into mature endothelial cells and therefore may contribute to the formation

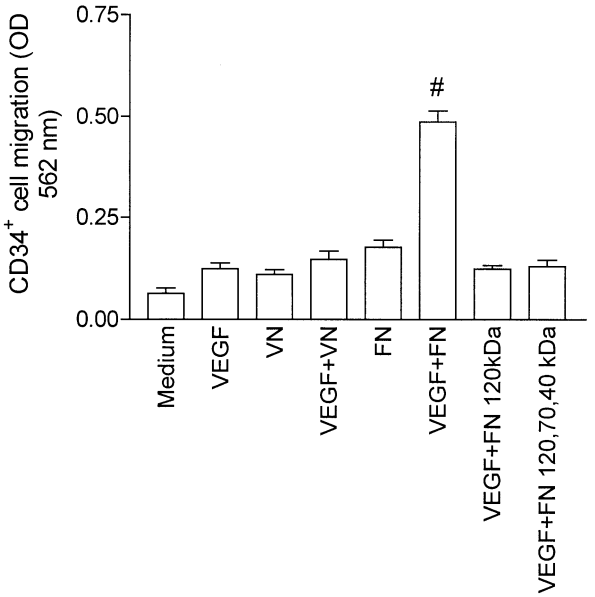

Fig 3. Effect of ECM proteins on VEGF-induced $CD34^+$ cell migration. Migration assays were carried out as described in the Methods section. The migration rates of $CD34^+$ cells to VEGF and different combinations of ECM proteins were measured after 6 hours. Results are expressed as mean ± SD (n = 3). $^{\#}P < .05$ compared with VEGF or FN alone. Negative control was medium alone, and positive controls were VEGF or FN.

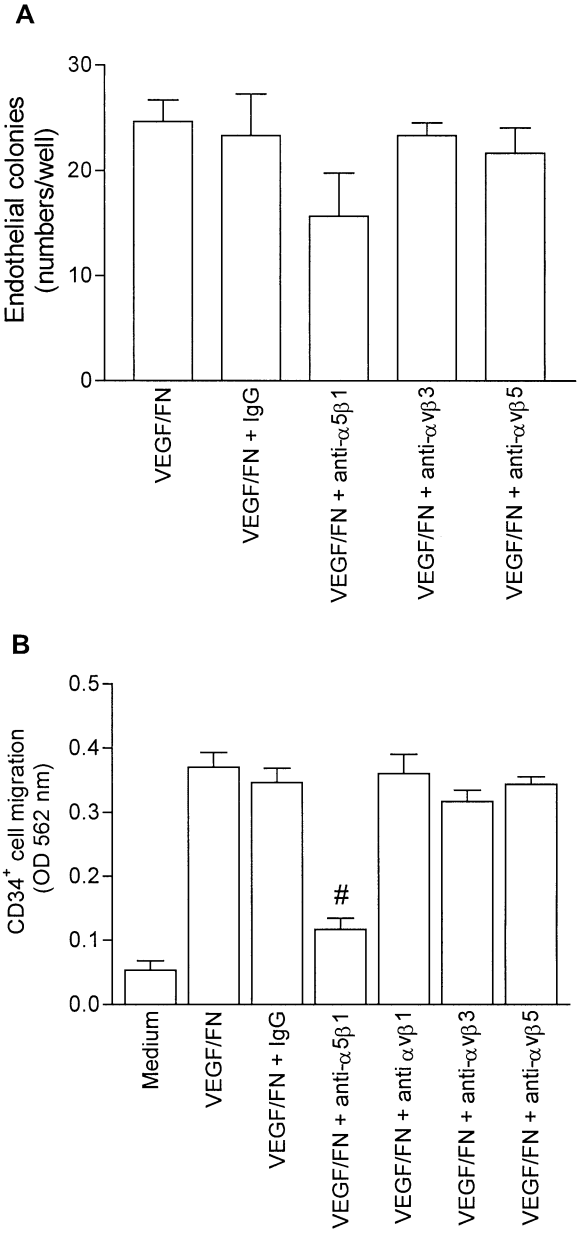

Fig 4. Inhibition of VEGF-FN–induced $CD34^+$ cell differentiation and migration by antibodies to α5β1. **A,** $CD34^+$ cells were cultured on FN-VEGF–coated plates in endothelial growth medium containing blocking antibodies for α5β1, for αvβ3, or for αvβ5 (5 μg/mL). Fresh antibodies were added every 3 days. Endothelial colonies were determined after 21 days by staining for vWF and VE-cadherin. Results are expressed as mean ± SD (n = 3). **B,** Blocking antibodies to α5β1, αvβ3, or αvβ5 (20 μg/mL) were assessed for inhibition of VEGF-FN–induced $CD34^+$ cell migration. Results are expressed as mean ± SD (n = 3). $^{\#}P < .05$ compared with VEGF-FN.

of new endothelium. Indeed, several recent studies using animal models have shown that EPCs can contribute to new blood vessel formation.[3,5,14,15] As we recently showed, platelets are an important source of preformed VEGF-FN complexes.[11] It is therefore likely that release of VEGF-FN complexes after activation of platelets at sites of injury could stimulate neoendothelialization and blood vessel growth, either from preexisting vessels (angiogenesis) or by promoting differentiation of EPCs into endothelial cells (vasculogenesis).

The $CD34^+/VEGFR\text{-}2^+$ cell was recently shown to generate both hematopoietic and endothelial cells and thus may represent the hemangioblast.[13] There is also evidence to suggest that EPCs not only express CD34 and VEGFR-2 but also a newly discovered hematopoietic stem cell marker, AC133.[16] Several studies have shown that AC133 progenitor cells can be induced to differentiate into endothelial cells and form blood vessels.[17-19] Recently, AC133 progenitors were shown to differentiate more efficiently into endothelial cells when plated on FN-coated plates.[20]

Our finding that both FN and VEGF are important for $CD34^+$ cell differentiation into endothelial cells is consistent with findings from gene deletion studies. Mutant mice homozygous for null mutations of FN or α5β1 die in embryonic development because of a defective vascular system.[21-23] Similarly, inactivation of one single VEGF allele caused early embryonic death because of a defective vascular system, whereas homozygous loss of VEGFR-1 or VEGFR-2 also resulted in early embryonic lethality, because of impaired development of blood vessels.[7,8,24] These studies show the pivotal importance of FN, VEGF, and their receptors in blood vessel development.

FN provides attachment sites for the hematopoietic stem cell through its interaction with α5β1 and α4β1 and has been shown to mediate stem cell proliferation.[25-27] Collaboration between α5β1 and c-kit was shown to promote sustained activation of focal adhesion kinase and extracellular-regulated kinase (Erk), which enhanced erythroid progenitor cell survival and proliferation.[28] We recently showed that when endothelial cells bind FN and VEGF, there is collaboration between α5β1 and VEGFR-2, resulting in prolonged Erk activation and enhanced endothelial cell migration.[11] Although not statistically significant, α5β1 antibodies inhibited $CD34^+$ cell differentiation by 35% There are two possibilities as to why antibodies to α5β1 only partially inhibited $CD34^+$ cell differentiation by 35%. First, because the differentiation assay is over 21 days, it is possible that insufficient α5β1 blocking antibodies were used. Second, it is also possible that the α4β1 integrin may be involved in $CD34^+$ cell differentiation. Binding of FN to α5β1 and α4β1 can have opposing effects on hematopoietic stem cells. For example, ligation of FN to α5β1 promoted cell proliferation and survival, whereas to α4β1, it induced cell death.[28] A recent study showed that adhesion and migration of activated $CD34^+$ cells on a substrate of FN was mediated through the α5β1 integrin, whereas adhesion and migration of quiescent $CD34^+$ cells was mediated by α4β1.[29] In our study, we observed significant inhibition of VEGF-induced $CD34^+$ cell migration using antibodies to α5β1.

Not collagen, nor VN, nor FN fragments significantly enhanced VEGF biologic responses. This supports our theory that the synergism between FN and VEGF arises because they form a complex of ligands that coordinately bind their cognate receptors. Collagen and VN are ineffective because they do not bind VEGF.[11] Mixtures of FN fragments including both the integrin and VEGF-binding domains were not effective either. One explanation for this is that the integrin and VEGF-binding domains must be physically connected for FN to "chaperone" and support the sustained binding of VEGF to VEGFR-2, while engaging the α5β1 integrin at the same time.

Among the common ECM proteins, our studies have shown that a combination of FN and VEGF is superior in promoting differentiation of EPCs into endothelial cells and that fibrinogen has an intermediate effect. Fibrinogen was recently shown to directly interact with VEGF.[30] This suggests that like FN, interaction between fibrinogen and VEGF may induce specific integrin signaling that enhances VEGF biological activity. Future studies investigating integrin–FN interactions in EPCs and studies to define the specific molecular interactions between VEGF and ECM proteins will be needed to fully understand the specificity and mechanisms of these interactions.

REFERENCES

1. Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol 1995;11:73-91.
2. Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992;267:10931-4.
3. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-7.
4. Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, et al. Evidence for circulating bone marrow-derived endothelial cells. Blood 1998;92:362-7.
5. Crosby JR, Kaminski WE, Schatteman G, Martin PJ, Raines EW, Seifert RA, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res 2000;87:728-30.
6. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434-8.
7. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435-9.
8. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 1996;380:439-42.
9. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11-25.
10. Eliceiri BP. Integrin and growth factor receptor crosstalk. Circ Res 2001;89:1104-10.
11. Wijelath ES, Murray J, Rahman S, Patel Y, Ishida A, Strand K, et al. Novel vascular endothelial growth factor binding domains of fibronectin enhance vascular endothelial growth factor biological activity. Circ Res 2002;91:25-31.
12. Leavesley DI, Schwartz MA, Rosenfeld M, Cheresh DA. Integrin beta 1- and beta 3-mediated endothelial cell migration is triggered through distinct signaling mechanisms. J Cell Biol 1993;121:163-70.
13. Pelosi E, Valtieri M, Coppola S, Botta R, Gabbianelli M, Lulli V, et al. Identification of the hemangioblast in postnatal life. Blood 2002;100:3203-8.
14. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci USA 2000;97:3422-7.
15. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, et al. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 2000;105:1527-36.
16. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997;90:5002-12.
17. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000;95:952-8.
18. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 2000;95:3106-12.
19. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. Br J Haematol 2001;115:186-94.
20. Kanayasu-Toyoda T, Yamaguchi T, Oshizawa T, Hayakawa T. CD31 (PECAM-1)-bright cells derived from AC133-positive cells in human peripheral blood as endothelial-precursor cells. J Cell Physiol 2003;195:119-29.
21. Yang JT, Rayburn H, Hynes RO. Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development 1993;119:1093-105.
22. Taverna D, Hynes RO. Reduced blood vessel formation and tumor growth in alpha5-integrin-negative teratocarcinomas and embryoid bodies. Cancer Res 2001;61:5255-61.
23. George EL, Baldwin HS, Hynes RO. Fibronectins are essential for heart and blood vessel morphogenesis but are dispensable for initial specification of precursor cells. Blood 1997;90:3073-81.
24. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995;376:66-70.

25. Williams DA, Rios M, Stephens C, Patel VP. Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. Nature 1991; 352:438-41.
26. van der Loo JC, Xiao X, McMillin D, Hashino K, Kato I, Williams DA. VLA-5 is expressed by mouse and human long-term repopulating hematopoietic cells and mediates adhesion to extracellular matrix protein fibronectin. J Clin Invest 1998;102:1051-61.
27. Jiang Y, Prosper F, Verfaillie CM. Opposing effects of engagement of integrins and stimulation of cytokine receptors on cell cycle progression of normal human hematopoietic progenitors. Blood 2000;95:846-54.
28. Kapur R, Cooper R, Zhang L, Williams DA. Cross-talk between alpha(4)beta(1)/alpha(5)beta(1) and c-Kit results in opposing effect on growth and survival of hematopoietic cells via the activation of focal adhesion kinase, mitogen-activated protein kinase, and Akt signaling pathways. Blood 2001;97:1975-81.
29. Huygen S, Giet O, Artisien V, Di Stefano I, Beguin Y, Gothot A. Adhesion of synchronized human hematopoietic progenitor cells to fibronectin and vascular cell adhesion molecule-1 fluctuates reversibly during cell cycle transit in ex vivo culture. Blood 2002;100:2744-52.
30. Sahni A, Francis CW. Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. Blood 2000;96:3772-8.

Submitted Jun 10, 2003; accepted Jul 17, 2003.