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Mechanism for laser-induced neovascularization in rat choroid: Accumulation of integrin α chain-positive cells and their ligands

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Purpose: Inhibitors binding to integrins α5 and αv are antiangiogenic in models of choroidal neovascularization (CNV). However, a comprehensive understanding of the accumulation of integrin α isoform-positive cells, their ligands, and associations is limited. The purpose of the present study was to examine the localization of integrin α chain-positive cells and their extracellular matrix (ECM) ligands in the RPE/choroid after laser injury.
Methods: CNV, observed with fluorescein isothiocyanate (FITC)-labeled isolectin, was produced in Brown Norway rats with a 532 nm green laser. Localization of α5 and αv integrins and their ligands was performed with immunohistochemistry in consecutive cryosections. To test the binding specificity between the integrin α chains and ECM ligands, an in vitro cell adhesion assay was performed using retinal endothelial cells and specific antibodies.
Results: Angiogenesis was observed on day 7 after laser injury in choroidal flat mounts and cryosections. The number of integrin α5- and αv-positive cells markedly increased at day 3 and then gradually decreased, but was still elevated on day 14. One day after laser treatment, α integrin ligands fibronectin (FN) and vitronectin (VN) were markedly increased, and localized closely to integrins in the laser-injured regions. FN decreased on day 7, but was still retained until 14 days. In contrast, VN disappeared. Cell adhesion assays showed specific association of integrin α5 to FN, and integrin αv to VN.
Conclusions: Laser-induced choroidal injury increased FN and VN, followed by accumulation of integrin α5- and αv-positive cells. The interaction between integrin α chain-positive cells and their specific ligands FN and VN may be important steps leading to CNV.

Choroidal neovascularization (CNV) is a major cause of severe central vision loss in patients with exudative, age-related macular degeneration (AMD) [1]. In patients with exudative AMD, choroidal blood vessels grow through Bruch's membrane into the subretinal space. This is followed by leakage and accumulation of serum or blood beneath the RPE, leading to retinal damage and rapid loss of vision [2].

Human ocular neovascularization may be caused or facilitated by altered expression of integrins [3,4]. Integrins are heterodimeric, cell surface receptors found in nearly all metazoan cell types. These receptors are composed of non-covalently linked α- and β-subunits [5,6]. In mammals, 18 α-subunits and eight β-subunits have been identified [7]. The subunit composition of the heterodimer determines binding to specific ligands in the extracellular matrix (ECM). For example, α5β1 and αvβ3 integrins bind to their ligands fibronectin (FN) and vitronectin (VN), respectively, which are strongly expressed around developing vasculature [5,8,9]. Therefore, α5β1 and αvβ3 integrins have been investigated the most as potential regulators of angiogenesis [10]. Integrin αvβ3 was the first integrin associated with angiogenesis [11], and deletion of the α5 gene is embryonically lethal with vascular and cardiac defects [12]. These integrin-mediated interactions are also required for pathological processes such as angiogenesis [13], tumor survival, and metastasis [14].

Laser irradiation encircling the optic nerve is widely used for inducing CNV in rodent [15] and primate [16] models of AMD. During experimental choroidal neovascularization, the binding of inhibitors to integrins α5 and αv is antiangiogenic. For example, systemic administration of α5β1 integrin antagonist causes CNV suppression and regression [17]. An αvβ3 specific antagonist also inhibits laser-induced CNV in mice [18]. However, the expression of integrin α5 and αv subunits with time is limited in these models, and the localization of integrin ligands such as FN and VN has not been reported. Thus, the purposes of the present study in the laser-induced CNV model were as follows: 1) to identify integrin α chain-positive cells and their ligands FN and VN and 2) to measure the binding of integrins to their ligand ECMs.

METHODS

Animals: Twenty adult male Brown Norway rats (8–9 weeks-old) were obtained from Charles River Laboratories (Yokohama, Japan) and maintained under a 12 h:12 h light-dark

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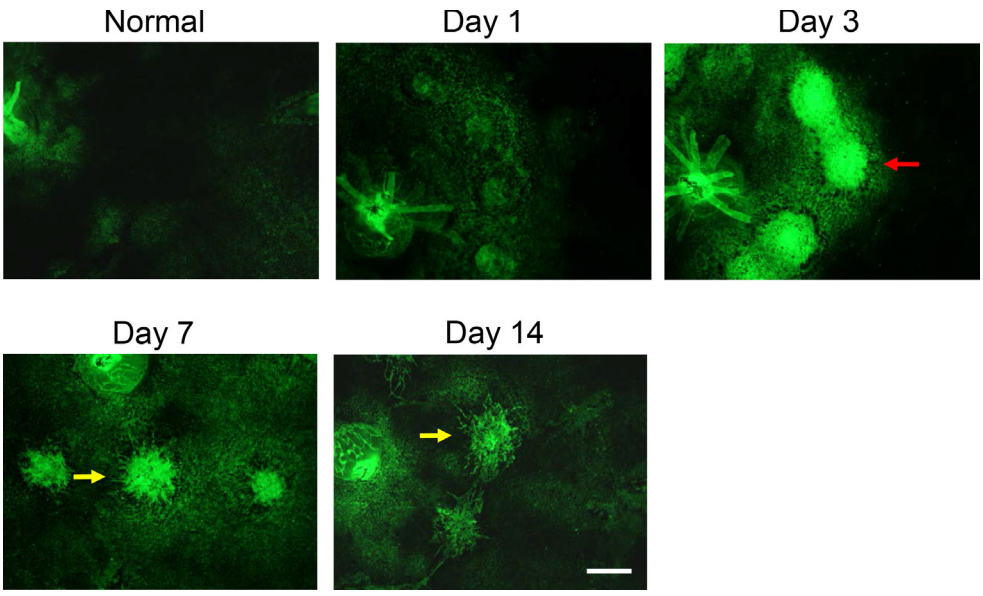


Figure 1. Representative flatmount images of macrophages and microglial and endothelial cells labeled with green fluorescent antibody for isolectin in normal and laser-treated rat RPE-choroid preparations. The red arrow indicates macrophages and microglial and endothelial cells; the yellow arrows indicate new tubular structures. Scale bar = 200 µm.

cycle. Senju follows the international IACUC animal research laws, policies and guidelines, and all experimental animals were handled in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and the NIH Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80–23).

Laser-induced choroidal neovascularization model: Rats were anesthetized with a 1 ml/kg bodyweight intraperitoneal injection of a mixture containing ketamine hydrochloride (50 mg/ml; Daiichi Sankyo, Tokyo, Japan) and xylazine hydrochloride (10 mg/ml; Bayer, Tokyo, Japan). Pupils were dilated with topical tropicamide/phenylephrine (Midrin-P, Santen Pharmaceutical, Osaka, Japan). Visualization of the fundus was aided by placing a cover glass on the eye over hydroxyethylcellulose. A slit-lamp biomicroscope (NIDEK, Aichi, Japan) was used to encircle the optic nerve on the retina with six to seven laser spots (100 µm each, 532 nm, 150 mW, 0.1 s). Breakage of Bruch's membrane was confirmed by bubble formation and used as an end point for treatment. Animals with severe hemorrhages in the retina were excluded from the study. Two rat eyes in the experimental group were choroidal flatmounted and subjected to immunohistochemistry as described below.

Choroidal flatmounts: After euthanasia on day 1, 3, 7, or 14 following laser photocoagulation, the eyes were enucleated and prefixed with 4% paraformaldehyde (Nacalai tesque, Kyoto, Japan) for 30 min. Retina-RPE-choroid complexes were microsurgically isolated from the prefixed eyes and further fixed with 4% paraformaldehyde for 1 h. The retina was removed from RPE-choroid complexes. The RPE-choroid complexes were then washed with PBS (1X; 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4:Nacalai tesque) and incubated for 30 min with PBS blocking buffer containing 0.5% Triton X-100 (GE Healthcare UK, Buckinghamshire, UK) and 1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO). To visualize neovascularization, RPE-choroid complexes were incubated overnight at 4 °C with 0.01% fluorescein isothiocyanate (FITC)-conjugated isolectin B4 derived from *Griffonia (Bandeiraea) simplicifolia* agglutinin (Vector Laboratories, Peterborough, UK) diluted with PBS containing 0.5% Triton X-100. After the RPE-choroid complexes were washed with PBS and sealed with VECTASHIELD (Vector Laboratories), CNV was observed with a fluorescence microscope (IX71; Olympus, Tokyo, Japan). Note that FITC-labeled isolectin binds to microglial cells and macrophages as well as endothelial vascular cells [19].

Immunohistochemistry for integrins and extracellular matrix: Enucleated eyes in embedding medium (Tissue-Tek OCT Compound; Sakura Finetek, Torrance, CA) were flash-frozen with liquid nitrogen. Serial sections (10 µm) were cut on a cryostat at −20 °C and collected with adhesive film (Cryofilm type 2C(9); SECTION-LAB, Hiroshima, Japan). The sections were fixed with 75% ethanol at −20 °C for 30 min, washed three times with Tris-Buffer Saline (TBS; 2 mM Tris, 50 mM NaCl, pH7.4; BioRad Laboratories, Hercules, CA) for 5 min each and dried for 30 min at room temperature. Dried sections were incubated in PBS blocking buffer containing 0.3% Triton X-100 and 10% goat serum (Dako, Glostrup,

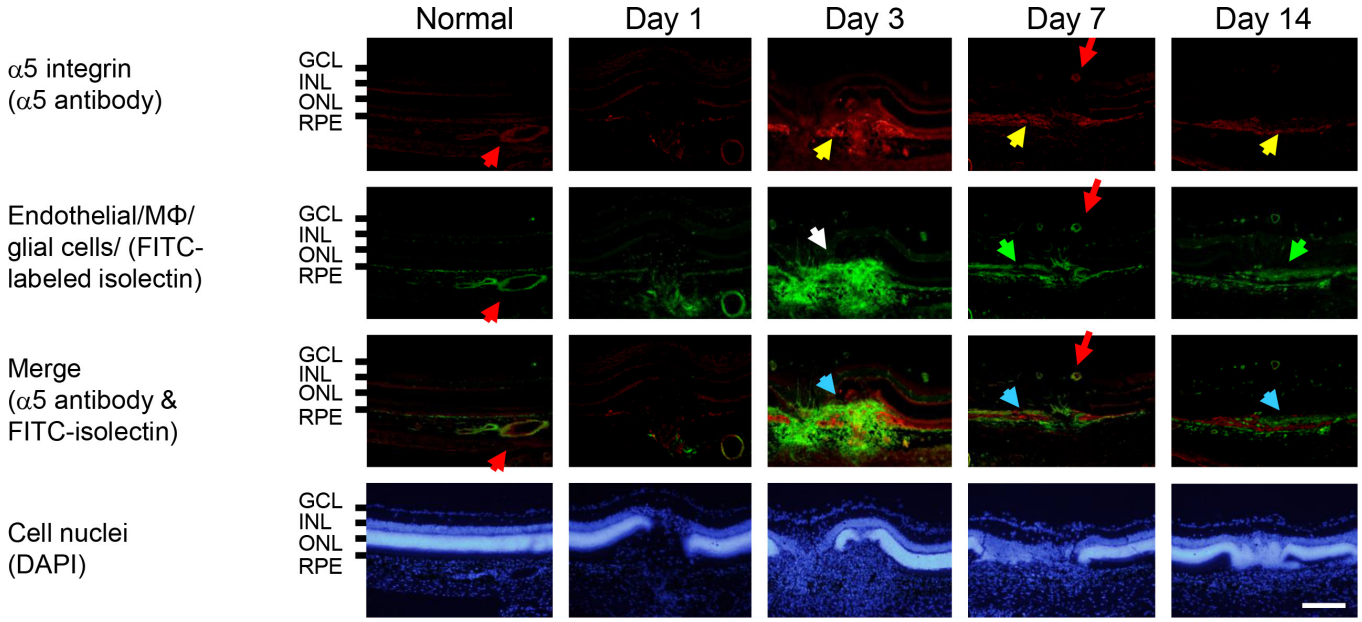


Figure 2. Fluorescence microscopy with red fluorescent antibody for integrin α5 in normal and laser-treated rat RPE-choroid. The red arrows show integrin α5 expression near normal blood vessels. The yellow arrows show positive red antibody binding for integrin α5 between the subretinal and suprachoroidal spaces with ruptured Bruch's membranes after laser treatment. The white arrow indicates isolectin binding in green macrophages and microglial and endothelial cells and near new tubular structures (green arrows). The blue arrows show colocalization of red integrin α5 with green macrophages and microglial and endothelial cells. Blue 4',6-diamidino-2-phenylindole (DAPI) nuclear stain highlights the disruption and wound healing in the inner and outer nuclear layers. Scale bar = 100 µm.

Denmark) for 30 min and incubated overnight at 4 °C with primary antibody diluted with Can Get Signal Solution B (NKB-601, TOYOBO, Osaka, Japan) containing 10% goat serum. Dilution of the primary antibodies was as follows: rabbit anti-integrin α5 (1:50; H-104, Santa Cruz, Dallas, TX), rabbit anti-integrin αv (1:50; Q-20-R, Santa Cruz), anti-isolectin B4 (1:25; Vector Laboratories, Burlingame, CA), rabbit anti-vitronectin (1:50; Santa Cruz), rabbit anti-laminin (1:50; Santa Cruz), and mouse anti-fibronectin (1:50; Santa Cruz). After washing with TBS for 5 min three times, the sections were incubated for 1 h with Alexa Fluor 546 goat anti-rabbit immunoglobulin G (IgG; Life Technologies, Carlsbad, CA) or Alexa Fluor 488 goat anti-rabbit IgG (Life Technologies) diluted to 1:250 with Can Get Signal Solution B containing 10% goat serum. Sections were washed three times with TBS containing 0.3% Triton-X 100 and sealed with Fluoromount (Diagnostic BioSystems, Pleasanton, CA). As a negative control, normal rabbit IgG diluted to 1:3,750 (Dako) was used in place of the primary antibody. Cellstain (DOJINDO, Kumamoto, Japan) diluted to 1:3,000 was used for nuclear staining.

Cell adhesion assay: To confirm the selectivity and specificity of the association between integrins and ECM ligands, a cell adhesion assay was performed using primary human retinal endothelial cells (HRECs; Cell Systems, Kirkland, WA). Briefly, 75 µl vitronectin (5 µg/ml; BD, Franklin Lakes, NJ) or fibronectin (10 µg/ml, Sigma-Aldrich; concentrations showing best adhesion to HRECs) was incubated for 1 h at 37 °C in 96-well microtiter plates. After excess ECM was removed from the wells, the uncoated area in the wells was blocked with 1% BSA. HRECs suspended at 10^5 cells per ml in CSC medium (Cell Systems) containing 0.1% BSA were incubated for 30 min with antibodies for α5β1 (clone JBS5, EMD Millipore, Billerica, MA) or αvβ3 (LM609, Millipore). Ten-microliter cell suspensions were inoculated into the ECM-coated wells. After incubation for 10 min at 37 °C, the cells were fixed with 10% neutral formalin (Nacalai tesque) and stained with 0.1% crystal violet solution (Sigma-Aldrich). The statistical test and variance is stated in figure legend. The stained areas were quantified with Image-Pro (Media Cybernetics, Rockville, MD).

RESULTS

Neovascularization after laser treatment: Immunohistochemistry with FITC-labeled isolectin as a marker for laser-induced neovascularization in the RPE-choroid showed blood vessels in normal, non-laser treated rats (Figure 1, Normal), but only a few macrophages and microglial and endothelial

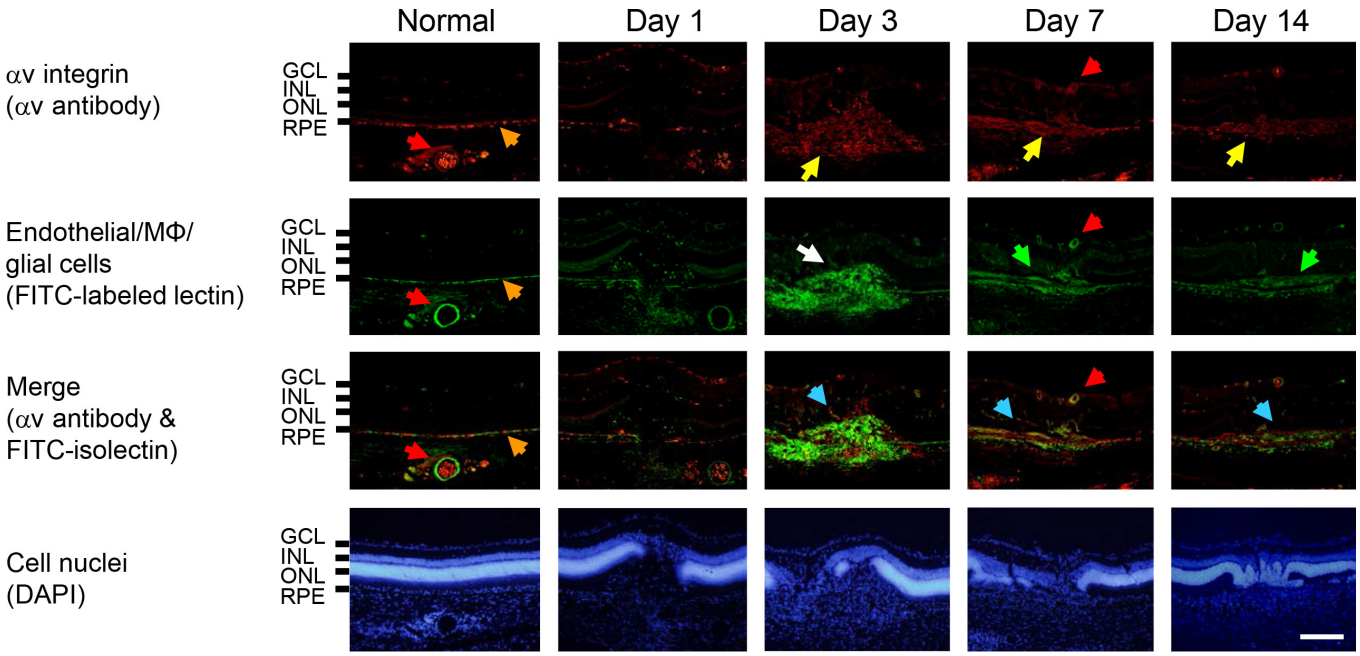


Figure 3. Fluorescence microscopy with red fluorescent antibody for integrin αv in rat RPE-choroid. Non-treated retinas show positive antibody binding for integrin αv around the normal vessels (red arrows) and in the RPE (orange arrows). The yellow arrows show positive red antibody binding for integrin αv between the subretinal and suprachoroidal spaces after laser treatment. The white arrow indicates isolectin binding in green macrophages and microglial and endothelial cells and near the new tubular structures (green arrows). The blue arrows show colocalization of red integrin αv with green macrophages and microglial and endothelial cells. Scale bar = 100 µm.

cells stained 1 day after laser injury (day 1). Staining on day 3 indicated early accumulation of mainly macrophages and microglial cells with some endothelial cells (red arrow). On days 7 and 14, macrophages and microglial cells decreased, and tubular structures were observed (Figure 1, yellow arrows), indicating active retinal neovascularization in this rat model.

Colocalization of integrin α5 and αv subunits with tubule formation: Fluorescence microscopy with red fluorescent labeling for the α5 subunit of integrin showed weak reactivity near blood vessels throughout the RPE-choroid of normal rats (Figure 2, red arrows). On day 1 after laser treatment, binding of the antibody for integrin α5 slightly increased; and on day 3, integrin α5 markedly increased between the subretinal and suprachoroidal spaces with ruptured Bruch's membranes (row 1, yellow arrows). Integrin α5 remained visible for 14 days. Macrophages and microglial and endothelial cells also accumulated around the laser spots on day 3 (row 2, white arrow), and angiogenic tubules (green arrows) were observed on days 7 and 14. Integrin α5 colocalized with macrophages and microglial and endothelial cells (row 3, blue arrows) on day 3 and endothelial cells on days 7 and 14. Another subunit of integrin, αv, was also observed in normal blood vessels and the RPE (Figure 3, red and orange arrows). The appearance and accumulation of integrin αv after laser treatment (Figure 3) were similar to those described for integrin α5. Negative controls using IgG in place of primary antibodies did not show significant binding (data not shown). Blue nuclear staining (row 4, Figure 2 and Figure 3) showed overall retinal disruption on day 1 followed by progressive healing over 14 days, suggesting increased α5 and αv integrin subunit-positive cells during retinal neovascularization.

Extracellular matrix components precede accumulation of integrin-positive cells: We found that FN and VN weakly localized around blood vessel in normal rats (Figure 4, red arrows) but markedly increased between the subretinal space and sclera with ruptured Bruch's membranes on day 1 after laser treatment (Figure 4, orange arrows). FN and VN antibody binding on day 3 was somewhat decreased but remained (yellow and blue arrows). On day 7 after laser injury, FN further decreased, but closely localized with tube-like structures (Figure 4, green arrows). In contrast, VN almost disappeared. Day 14 was similar in appearance to day 7. Binding of antibody for laminin, another extracellular matrix protein that binds to integrin [6], did not change after laser treatment (data not shown). These data suggested that specific binding ligands, such as FN and VN, may precede and possibly

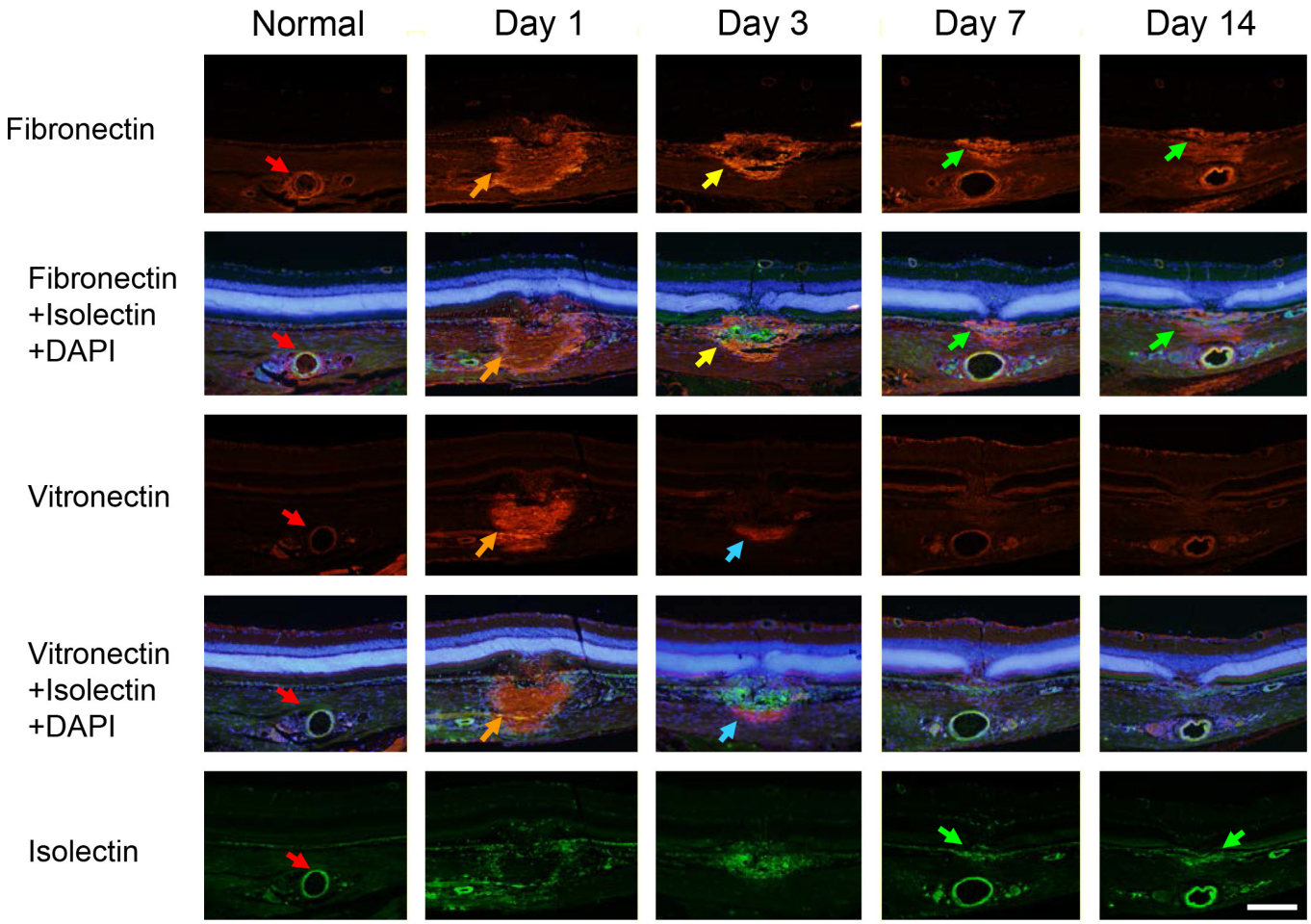
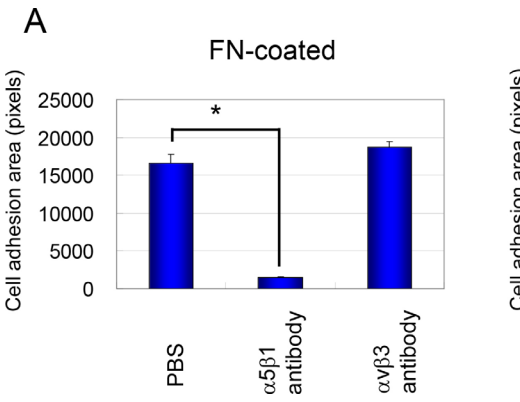


Figure 4. Fluorescence microscopy in normal and laser-treated rat RPE-choroid, triple labeled red for fibronectin (FN, row 2) and vitronectin (VN; row 4), green for isolectin, and blue for nuclear staining. The red arrows indicate normal blood vessels. The orange arrows indicate the antibody binding for FN and VN between the subretinal space and sclera after laser treatment. The yellow and blue arrows indicate the moderate decrease in FN and disappearance in VN, respectively; the green arrows show FN localization around forming tubules. Scale bar = 100 µm.



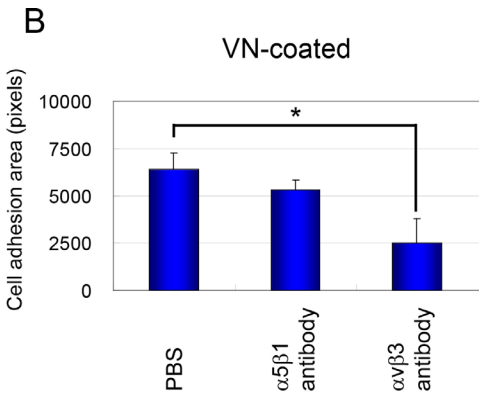


Figure 5. Cell adhesion assay of primary human retinal endothelial cells. Adherence of human retinal endothelial cells to (**A**) fibronectin (FN) and (**B**) vitronectin (VN)-coated plates were conducted in the absence (PBS) and presence of antibodies to specific integrin subunit α5β1 and αvβ3. Data are means ± standard deviation (SD; n = 3). *p<0.01 relative to PBS (Dunnett's test).

influence the accumulation of integrin α5- and αv-positive cells during retinal neovascularization.

Fibronectin and vitronectin matrix proteins bind to specific integrin subunits: An in vitro cell adherence-inhibition assay using HRECs showed that the antibody for integrin α5β1 subunit significantly ($p<0.01$) inhibits adhesion of HRECs onto FN-coated plates (Figure 5A). An integrin αvβ3 antibody had no effect. Conversely, the integrin αvβ3 antibody significantly inhibited adhesion of cells onto VN-coated plates, while the integrin α5β1 antibody had no effect (Figure 5B). These data indicated that integrin α5 and αv bind specifically to FN and VN, respectively.

DISCUSSION

The major finding of the present studies was the sequence of molecular events that occur during the onset and development of neovascularization in the laser-induced CNV model in rats (Table 1). The first major event observed 1 day after laser treatment was the accumulation of FN and VN between the subretinal space and sclera around the injured areas (Figure 4). mRNA specific for FN is transiently increased by the terminal complement proteins C5b-8 and C5b-9 in glomerular mesangial cells [20]. VN also increases in complement-stimulated human RPE cells [21]. The end product of complement activation, membrane attack complex (MAC), increases as early as 1 h after laser treatment [22]. Thus, complement activation could be a mechanism for the early expression of FN and VN in our laser model.

On day 3, binding of antibodies for integrins α5 and αv and for FITC-labeled isolectin (for macrophages and microglial, perivascular, and endothelial cells) increased markedly (Table 1, Figure 1, Figure 2, Figure 3, and Figure 4). Increased chemokine, (C-C motif) ligand 2 (CCL2), causes migration and accumulation of microglia and macrophages for uptake of cell debris [22,23]. mRNA for CCL2 peaks at 12 h within laser-injury spots [22]. In addition, mRNA for vascular endothelial growth factor (VEGF) increases 2 days after laser treatment [22], and VEGF is secreted from macrophages and microglial and RPE cells [24]. Specific adherence of retinal endothelial cells, where integrins α5 and αv were expressed (confirmed with western blotting, data not shown), to FN and VN through integrin α5 and αv was also observed (Figure 5). These data suggest that under the influence of cytokines, such as CCL2 and VEGF, integrin α5- and αv-positive macrophages and microglial and endothelial cells migrated between the subretinal and suprachoroidal spaces with ruptured Bruch's membranes. This is also where FN and VN, the specific ligands for integrins α5 and αv, accumulated. These data suggest that the increased FN and VN in laser injury may be important for the accumulation of integrin α5- and αv-positive cells. This was followed by uptake of cell debris by the macrophages and microglia and neovascularization by the endothelial cells.

On day 7, FN was retained, tube-like structures formed, and VN disappeared (Table 1). These data suggest that VN was important for the onset of neovascularization, while FN was needed for onset and further vessel development during neovascularization. Interestingly, laminin, another extracellular matrix protein found in the RPE basement membrane [25], did not increase after laser injury. These specific changes in ECM localization suggest that the association between integrin subunits and their specific ligands may be important for VEGF-induced proliferation and differentiation of endothelial progenitor cells for subsequent CNV.

Antagonists targeting single integrin subunits inhibit CNV [17,18,26,27]. However, our current data suggested that αv and α5 integrins are important for neovascularization. Blocking of multiple integrins thus may be useful for the therapy for AMD. Only limited data are available concerning the efficacy of using antagonists to block multiple integrin subunits [28]. Antagonism of multiple integrin subunits may also cause stronger side effects; further experiments testing multiple integrin antagonists are needed.

Table 1. Summary of changes in integrins and their ligand ECMs during neovascularization.

Post laser treatment, days	Staining intensity*			
	1	3	7	14
Fibronectin	+++	++	+	+
Vitronectin	+++	++	-/+	-/+
α5 Integrin	-/+	+++	++	+
αv Integrin	-/+	+++	++	+
Neovascularization	-	-/+	+	+

* (-)=none, (-/+)=minimal, (+)=mild, (++)=moderate, (+++)=strong.

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REFERENCES

1. Yeoh J, Sims J, Guymer RH. A review of drug options in age-related macular degeneration therapy and potential new agents. Expert Opin Pharmacother 2006; 7:2355-68. [PMID: 17109611].
2. Nowak JZ. Age-related macular degeneration (AMD): pathogenesis and therapy. Pharmacol Rep 2006; 58:353-63. [PMID: 16845209].
3. Friedlander M, Theesfeld CL, Sugita M, Fruttiger M, Thomas MA, Chang S, Cheresh DA. Involvement of integrins alpha v beta 3 and alpha v beta 5 in ocular neovascular diseases. Proc Natl Acad Sci USA 1996; 93:9764-9. [PMID: 8790405].
4. Cui J, Maberley D, Samad A, Ma P, Ning A, Matsubara JA, Baciu P. Expression of integrins on human choroidal neovascular membranes. J Ocul Biol Dis Infor 2009; 2:12-9. [PMID: 20072642].
5. Takada Y, Ye X, Simon S. The integrins. Genome Biol 2007; 8:215-[PMID: 17543136].
6. Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res 2010; 339:269-80. [PMID: 19693543].
7. Millard M, Odde S, Neamati N. Integrin targeted therapeutics. Theranostics 2011; 1:154-88. [PMID: 21547158].
8. Ffrench-Constant C, Hynes RO. Alternative splicing of fibronectin is temporally and spatially regulated in the chicken embryo. Development 1989; 106:375-88. [PMID: 2591321].
9. Jang YC, Tsou R, Gibran NS, Isik FF. Vitronectin deficiency is associated with increased wound fibrinolysis and decreased microvascular angiogenesis in mice. Surgery 2000; 127:696-704. [PMID: 10840366].
10. Hynes RO. Cell-matrix adhesion in vascular development. J Thromb Haemost 2007; 5:32-40. [PMID: 17635706].
11. Brooks PCCR, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994; 264:569-71. [PMID: 7512751].
12. Francis SEGK, Hodivala-Dilke K, Bader BL, Stark M, Davidson D, Hynes RO. Central roles of alpha5beta1 integrin and fibronectin in vascular development in mouse embryos and embryoid bodies. Arterioscler Thromb Vasc Biol 2002; 22:927-33. [PMID: 12067900].
13. Rüegg C, Alghisi GC. Vascular integrins: therapeutic and imaging targets of tumor angiogenesis. Recent Results Cancer Res 2010; 180:83-101. [PMID: 20033379].
14. Nemeth JA, Nakada MT, Trikha M, Lang Z, Gordon MS, Jayson GC, Corringham R, Prabhakar U, Davis HM, Beckman RA. Alpha-v integrins as therapeutic targets in oncology. Cancer Invest 2007; 25:632-46. [PMID: 18027153].
15. Semkova I, Peters S, Welsandt G, Janicki H, Jordan J, Schraermeyer U. Investigation of laser-induced choroidal neovascularization in the rat. Invest Ophthalmol Vis Sci 2003; 44:5349-54. [PMID: 14638737].
16. Miller H, Miller B, Ishibashi T, Ryan SJ. Pathogenesis of laser-induced choroidal subretinal neovascularization. Invest Ophthalmol Vis Sci 1990; 31:899-908. [PMID: 1692312].
17. Umeda N, Kachi S, Akiyama H, Zahn G, Vossmeyer D, Stragies R, Campochiaro PA. Suppression and regression of choroidal neovascularization by systemic administration of an alpha5beta1 integrin antagonist. Mol Pharmacol 2006; 69:1820-8. [PMID: 16527907].
18. Honda S, Nagai T, Negi A. Anti-angiogenic effects of non-peptide integrin alphavbeta3 specific antagonist on laser-induced choroidal neovascularization in mice. Graefes Arch Clin Exp Ophthalmol 2009; 247:515-22. [PMID: 19048271].
19. Bejarano-Escobar R, Blasco M, Duran AC, Martin-Partido G, Francisco-Morcillo J. Chronotopographical distribution patterns of cell death and of lectin-positive macrophages/microglial cells during the visual system ontogeny of the small-spotted catshark Scyliorhinus canicula. J Anat 2013; 223:171-84. [PMID: 23758763].
20. Wagner C, Braunger M, Beer M, Rother K, Hansch GM. Induction of matrix protein synthesis in human glomerular mesangial cells by the terminal complement complex. Exp Nephrol 1994; 2:51-6. [PMID: 8081997].
21. Wasmuth S, Lueck K, Baehler H, Lommatzsch A, Pauleikhoff D. Increased vitronectin production by complement-stimulated human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 2009; 50:5304-9. [PMID: 19407013].
22. Liu J, Jha P, Lyzogubov VV, Tytarenko RG, Bora NS, Bora PS. Relationship between complement membrane attack complex, chemokine (C-C motif) ligand 2 (CCL2) and vascular endothelial growth factor in mouse model of laser-induced choroidal neovascularization. J Biol Chem 2011; 286:20991-1001. [PMID: 21515678].
23. Liu J, Copland DA, Horie S, Wu WK, Chen M, Xu Y, Paul Morgan B, Mack M, Xu H, Nicholson LB, Dick AD. Myeloid cells expressing VEGF and arginase-1 following uptake of damaged retinal pigment epithelium suggests potential mechanism that drives the onset of choroidal angiogenesis in mice. PLoS ONE 2013; 8:e72935-[PMID: 23977372].
24. Yang XM, Wang YS, Zhang J, Li Y, Xu JF, Zhu J, Zhao W, Chu DK, Wiedemann P. Role of PI3K/Akt and MEK/ERK in mediating hypoxia-induced expression of HIF-1alpha and VEGF in laser-induced rat choroidal neovascularization. Invest Ophthalmol Vis Sci 2009; 50:1873-9. [PMID: 19098317].

25. Booij JC, Baas DC, Beisekeeva J, Gorgels TG, Bergen AA. The dynamic nature of Bruch's membrane. Prog Retin Eye Res 2010; 29:1-18. [PMID: 19747980].

26. Fu Y, Ponce ML, Thill M, Yuan P, Wang NS, Csaky KG. Angiogenesis inhibition and choroidal neovascularization suppression by sustained delivery of an integrin antagonist, EMD478761. Invest Ophthalmol Vis Sci 2007; 48:5184-90. [PMID: 17962472].

27. Yasukawa T, Hoffmann S, Eichler W, Friedrichs U, Wang YS, Wiedemann P. Inhibition of experimental choroidal neovascularization in rats by an alpha(v)-integrin antagonist. Curr Eye Res 2004; 28:359-66. [PMID: 15287373].

28. Kim H, Csaky KG. Nanoparticle-integrin antagonist C16Y peptide treatment of choroidal neovascularization in rats. J Control Release 2010; 142:286-93. [PMID: 19895863].

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