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Dual Role of the Leukocyte Integrin αMβ2 in Angiogenesis

Dmitry A. Soloviev,* Stanley L. Hazen,† Dorota Szpak,* Kamila M. Bledzka,* Christie M. Ballantyne,‡ Edward F. Plow,* and Elzbieta Pluskota* 

Polymorphonuclear neutrophils (PMNs) and macrophages are crucial contributors to neovascularization, serving as a source of chemokines, growth factors, and proteases. αMβ2(CD11b/CD18) and αβ2(CD11a/CD18) are expressed prominently and have been implicated in various responses of these cell types. Thus, we investigated the role of these β2 integrins in angiogenesis. Angiogenesis was analyzed in wild-type (WT), αM-/- knockout (αM-/-), and αβ2-deficient (αβ2-/-) mice using B16F10 melanoma, RM1 prostate cancer, and Matrigel implants. In all models, vascular area was decreased by 50–70% in αM-/- mice, resulting in stunted tumor growth as compared with WT mice. In contrast, αβ2 deficiency did not impair angiogenesis and tumor growth. The neovessels in αM-/- mice were leaky and immature because they lacked smooth muscle cell and pericytes. Defective angiogenesis in the αM-/- mice was associated with attenuated PMN and macrophage recruitment into tumors. In contrast to WT or the αβ2-/- leukocytes, the αM-/- myeloid cells showed impaired plasmin (Plm)-dependent extracellular matrix invasion, resulting from 50–75% decrease in plasminogen (Plg) binding and pericellular Plm activity. Surface plasmon resonance verified direct interaction of the αM-/- domain, the major ligand binding site in the β2 integrins, with Plg. However, the αβ2-domain failed to bind Plg. In addition, endothelial cells failed to form tubes in the presence of conditioned medium collected from TNF-α-stimulated PMNs derived from the αM-/- mice because of severely impaired degradation and secretion of VEGF. Thus, αMβ2 plays a dual role in angiogenesis, supporting not only Plm-dependent recruitment of myeloid cells to angiogenic niches, but also secretion of VEGF by these cells. 

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#one marrow–derived myeloid cells, particularly polymorphonuclear neutrophils (PMNs) and macrophages, are key regulators of tumor progression and metastasis.

One of the major tumor promoting functions of these cells is their facilitation of angiogenesis (reviewed in Refs. 1, 2). PMNs and macrophages contribute to angiogenesis via a variety of well-established mechanisms. One example is their capacity to produce and secrete a variety of proangiogenic factors such as vascular endothelial growth factor A (VEGF-A), fibroblast growth factor (FGF), IL-8, IL-10, CXCL1/GRO, and COX-2 (3, 4). In addition, PMNs and macrophages are a rich source of numerous proteases, including neutrophil elastase, cathepsin G, and several metalloproteinases, which are crucial for extracellular matrix (ECM) degradation and remodeling and regulate the bioavailability of various proangiogenic stimuli (5), all requisite events in angiogenesis (reviewed in Refs. 4, 6–8). In addition, PMNs and macrophages secrete urokinase-type plasminogen activator (uPA), which converts plasminogen (Plg) to plasmin (Plm). There are diverse Plg receptors on leukocyte surface (reviewed in Ref. 9), and bound Plm facilitates leukocyte migration/invasion by directly degrading ECM, and encourages leukocyte recruitment in a variety of in vivo models of inflammation (10–12).

αMβ2 (CD11b/CD18) and αβ2 (CD11a/CD18), the two most broadly studied members of the β2 integrin subfamily, are particularly enriched in PMNs and macrophages, where they regulate diverse cell functions, including migration, adhesion, the respiratory burst, and cytokine production (13). In addition, we have previously demonstrated that αMβ2 enhances uPA-dependent Plg activation on the PMN surface (14, 15), which has the potential to influence their recruitment to inflammatory or angiogenic sites in vivo. Based on these observations, we hypothesized that αMβ2 and αβ2, as key regulators of leukocyte functions, might be implicated in angiogenesis. In this study, we used αM-/- and αβ2-/- mice and three distinct in vivo angiogenesis models to show that αMβ2, but not αβ2, is a critical contributor to angiogenesis. This function of αMβ2 is mediated by two distinct mechanisms: 1) support of Plm-dependent PMN and macrophage recruitment to angiogenic niches; and 2) enhancement of leukocyte production and secretion of the primary angiogenic growth factor, VEGF-A.

Materials and Methods

Materials

Mouse VEGF165 and keratinocyte-derived cytokine (KC) were from Biosource International (Camarillo, CA); heparin was from Sigma-Aldrich (St. Louis, MO); biotin-conjugated anti-mouse CD31 mAb was from BD Pharmingen (San Jose, CA); rabbit anti-smooth muscle actin (SMA; Abcam, Cambridge, MA); rabbit anti-neuronal/glial antigen 2 (NG2; Millipore, Temecula, CA); rabbit anti-mouse laminin (Serotec, Oxford,
UK), goat anti-Fibrin II (Accurate Chemical, Westbury, NY), purified, or FITC-labeled rat anti-Ly6G, clone 1A8, specific for mouse PMNs were from BD Pharmingen (San Jose, CA); anti-mouse macrophage–monocyte mAb (MOMA-2) was from Chemicon (Temecula, CA); and rat LEAF TM purified anti-mouse αM integrin (clone M1/70) was from BioLegend (San Diego, CA). Glu-Plg was isolated from normal human plasma by affinity chromatography on lysine-seraphose followed by gel filtration. Growth factor-reduced Matrigel matrix was from BD Biosciences (San Diego, CA). Murine recombinant TNF-α was from R&D Systems. Cycloheximide and pentoxifylline were from Sigma-Aldrich.

**Mice**

The αM<sup>−/−</sup> mice were generated as described previously (16), and αM<sup>+/−</sup> mice were purchased from the Jackson Laboratory. Both strains were backcrossed for 12 generations into a C57BL/J6 background. The study was conducted under protocols approved by the IACUC of the Cleveland Clinic.

**Angiogenesis models in vivo**

Eight- to twelve-week-old mice were injected s.c. with 10<sup>6</sup> murine B16F10 melanoma or RPMI prostate cancer cells. Tumors were collected 8–14 d after injection and were weighed, photographed, and processed for immunohistochemical staining. Endothelial cells (ECs) were identified using a biotinylated mouse CD31 mAb, SMAs with anti-SMA Ab, pericytes with anti-NG2 Ab, fibrin with anti-Fibrin II Ab, basement membrane with anti-laminin Ab, PMNs with rat anti-Ly6G (clone 1A8), and monocytes–macrophages with MOMA-2 mAbs. Stained sections were analyzed using fluorescent imaging microscopy (Leica, Wetzlar, Germany) and ImagePro Plus Capture and Analysis software (Media Cybernetics). CD31, fibronectin, and Ly6G- or MOMA2-positive area was quantified in 5–10 independent fields. The average area per field was determined from duplicate measurements of each of the fields analyzed. Matrigel plug assay was performed as described (17). Mice were injected with 500 µl growth factor-reduced Matrigel mixed at 4˚C with heparin (26 U/ml) alone or with KC (500 ng/ml) or VEGF 165 (100 ng/ml; R&D Systems). Matrigel plugs were harvested 8 d after injection and snap-frozen, and 8-µm sections were processed for immunohistochemical analyses as described above. In αM<sup>−/−</sup> integrin blocking experiments, WT mice were injected intravenously with rat LEAF anti-mouse αM integrin (clone M1/70; BioLegend) or isotype-matched normal rat IgG<sub>2a</sub> (3.5 mg/kg), 4 h before and then 2, 4, and 6 d after Matrigel-KC implantation. The Matrigel plugs were collected 8 d after injection, sectioned, and stained with anti-CD31 to examine vascular formation.

**Bone marrow transplantation**

Two-month-old recipient mice were lethally X-irradiated with a total dose of 9 Gy and reconstituted with i.v. injection of 10<sup>7</sup> bone marrow (BM) cells isolated from the femurs of donor mice. Mice were used 6–8 wk after BM transplantation (BMT). Engraftment efficiency was examined 6 wk after BMT in chimeric αM<sup>−/−</sup>→WT and WT→αM<sup>−/−</sup> mice using WT and αM<sup>−/−</sup> mice, which did not undergo BMT as controls did. Single-cell suspensions from spleens and thymuses from these mice were prepared, and percentages of individual leukocyte subsets were measured by flow cytometry using FITC-labeled Abs to cell-specific markers (Ly-6G for PMN, F4/80 for macrophages, CD19 for B lymphocytes, CD3 for T lymphocytes) and FITC-labeled isotype-matched Abs as controls.

**Plg binding to αM and αI<sub>2</sub> domains**

Glutathione S-transferase (GST)-fused αM<sub>4</sub> and αI<sub>2</sub> I<sub>10</sub> domains were purified with glutathione chromatography. Real-time protein–protein interactions were analyzed using a Biacore 3000 instrument (Biacore AB). Plg (90 µg/ml) was added to PMNs 60 min before the addition of TNF-α (20 ng/ml) for 2 h at 37˚C. In parallel, lactoferrin concentrations for each cell type (10). PMNs constitute 92% and macrophages constitute 90% of all cells in the 6- and 72-h peritoneal lavages, respectively. PMNs and macrophages harvested from lavages are referred to as “peritoneal PMN” or “peritoneal macrophages” in the manuscript to distinguish them from blood cells.

**Matrix invasion assays ex vivo**

Pretested cell culture inserts with porous (8-µm pore size) polyester membrane (Costar, Corning, NY) were coated with 50 µl/insert Matrigel (10× diluted) overnight at 4˚C. Next, matrices were rehydrated with 600 µl DMEM F-12 for 1 h. Peritoneal PMNs or macrophages were suspended in serum-free DMEM F-12 medium (Life Technologies, Carlsbad, CA), and added to matrix-coated inserts (1 × 10<sup>6</sup>/insert), which were placed in a 24-well plate containing serum-free DMEM F-12 supplemented with or without 100 ng/ml KC. Plg (90 µg/ml) was added to appropriate inserts, and cells were incubated for 18 h at 37˚C. Assays were stopped by removing the inserts and washing the sides with a cotton swab to remove nonmigrated cells. The migrated cells were quantified using the Cyquant Cell Proliferation Kit (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions.

**Plg activation on the leukocyte surface**

Peripheral blood PMNs or lymphocyte–monocyte mixtures were incubated with KC (100 ng/ml) for 1 h at 37˚C in the presence of 25 nM single-chain uPA (sc-uPA) in 10 mM Tris-Cl buffer (pH 7.4) containing 0.14 mM NaCl, 0.1% BSA, 1 mM CaCl<sub>2</sub>, and 1 mM MgCl<sub>2</sub>. The cells were washed three times, and 50 µl of the cell suspension (1 × 10<sup>6</sup> cells/well) were added to each well of the microtiter plates. Peritoneal macrophages, which had not been incubated with KC or sc-uPA, were also added to the plates. Next, 100 µl of a Glu-Plg (1 µM) and Ptn-specific fluorescent substrate H-D-Val-Leu-Lys-7-amido-4-methylcoumarin (2 mM) mixture was added, and Ptn formation was monitored over 45 min at 37˚C at αM<sub>4</sub> = 370 nm and αM<sub>4</sub> = 470 nm using a fluorescence plate reader (SpectraMax GeminiXS; Molecular Devices).

**Regulation of VEGF by PMNs**

Peripheral blood WT, αM<sup>−/−</sup> or αI<sub>2</sub>−/− PMNs were incubated in 24-well tissue culture plates (Costar; 3 × 10<sup>5</sup> cells/well) in 250 µl DMEM F-12 medium in the absence or presence of TNF-α (20 ng/ml) for 2 h at 37˚C. The inhibitors of protein synthesis (cycloheximide-10 µg/ml) or PMN degradation (pentoxifylline, 3,7-dimethyl-1-[5-oxoethyl]-xantine, 300 µM) were added to PMNs 60 min before the addition of TNF-α. Supernatants were collected and centrifuged at 1500 rpm in a Beckman GS-6 centrifuge for 10 min, and VEGF was measured using mouse VEGF Quantikine ELISA Kit (R&D Systems). In parallel, lactoferrin concentrations were measured in PMN supernatants using mouse Lactoferrin LTR/LEL Elisa Kit (Cusabio).

**Quantitative real-time PCR**

Total RNA was isolated from peripheral blood mouse PMNs, either resting or stimulated with TNF-α (20 ng/ml) for 2 h at 37˚C. The inhibitors of protein synthesis (cycloheximide-10 µg/ml) or PMN degradation (pentoxifylline, 3,7-dimethyl-1-[5-oxoethyl]-xantine, 300 µM) were added to PMNs 60 min before the addition of TNF-α. Supernatants were collected and centrifuged at 1500 rpm in a Beckman GS-6 centrifuge for 10 min, and VEGF was measured using mouse VEGF Quantikine ELISA Kit (R&D Systems). In parallel, lactoferrin concentrations were measured in PMN supernatants using mouse Lactoferrin LTR/LEL Elisa Kit (Cusabio).

**PMN and macrophage isolation**

Mouse PMNs for use in mouse aortic endothelial cell (MAEC) tube formation and Plg activation assays (see below) were isolated from blood drawn from hearts of anesthetized animals into sterile acid-citrate-dextrose (1:7 volume 145 mM sodium citrate, pH 4.6, and 2% dextrose). Blood was transferred to 1.25% dextran T500 solution (1:9) to sediment erythrocytes for 30 min at room temperature (14, 18). Leukocyte-rich supernatants were washed with PBS once, and PMNs were isolated by magnetic positive selection using mouse anti-Ly6G MicroBead Isolation Kit (Miltenyi Biotech, Auburn, CA) according to the manufacturer’s instructions. Eluted cells were 98% granulocytes, of which more than 96% were neutrophils and 1–2% were eosinophils. Contaminating lymphocytes and monocytes were less than 2% as determined by Wright staining. PMN viability was >98% as determined by trypan blue staining. The PMN yield was usually >0.5 × 10<sup>5</sup> per mouse, and blood pooled from 10–15 mice was used. For Plg activation assays, lymphocytes and monocytes were collected from buffy coats and washed twice with the HBSS buffer. Leukocytes were obtained from blood pooled from 7–10 mice.

For matrix invasion and Plg binding assays, macrophages and PMNs were isolated from peritoneal lavages. PMNs at 6 h and macrophages at 72 h after i.p. thioglycollate injection, when their recruitment was at the highest levels for each cell type (10). PMNs constitute 92% and macrophages constitute 90% of all cells in the 6- and 72-h peritoneal lavages, respectively. PMNs and macrophages harvested from lavages are referred to as “peritoneal PMN” or “peritoneal macrophages” in the manuscript to distinguish them from blood cells.

**Intracellular free calcium analysis**

Mouse PMNs for use in tube formation were isolated from blood drawn from hearts of anesthetized animals into sterile acid-citrate-dextrose (1:7 volume 145 mM sodium citrate, pH 4.6, and 2% dextrose). Blood was
PCR were performed in triplicate. Results were calculated as expression of the target gene relative to expression of the reference gene (GAPDH).

**MAEC tube formation assay**

Twenty-four-well tissue culture plates were coated with 250 μL Growth Factor-Reduced Matrigel (BD Biosciences, San Diego, CA) and incubated at 37°C for 30 min. When the Matrigel solidified, 1.25 × 10^5 of WT MAECs were added to each well in 250 μL of chosen PMN-conditioned DMEMF-12 medium obtained as described in Regulation of VEGF by PMN and supplemented with 10% FBS and 90 μg/ml heparin. Inhibitors of VEGF, neutralizing LEAF rat anti-mouse VEGF mAb (BioLegend), isotype matched rat IgG2a (100 μg/ml; BioLegend), and recombinant mouse soluble VEGF receptor-1 (sFLT-1; 100 ng/ml; R&D Systems) were preincubated for 60 min with conditioned media of WT TNFα–stimulated PMNs before its addition to MAECs. Live time-lapse photography was performed for 12 h, using 5-min intervals on a Leica DMIRB Inverted Microscope equipped with a Roper Scientific CoolSNAP HQ Cooled CCD camera, a temperature controller, and a CO2 incubation chamber. Snapshots were taken using MetaMorph Software. Tube formation was analyzed and quantified using Imaged software version 1.34.

**Statistical analysis**

Data are expressed as mean ± SEM. To determine significance, a one-way ANOVA test was performed to compare angiogenic responses between the three mouse genotypes, and a two-tailed Student t test was performed for comparisons between WT and the α5β1−/− mice using the Sigma-Plot software program (SPSS); p < 0.05 was considered to be statistically significant.

**Results**

**Integrin α5β2 is critical in angiogenesis in vivo**

Leukocytes, particularly PMNs and macrophages, are important supporters of angiogenesis as a source of proteases and angiogenic factors (reviewed in Refs. 1, 2). The β2 integrins are crucial in the regulation of a variety of leukocyte responses, including adhesion, migration, and cytokine production (reviewed in Ref. 13). Accordingly, we examined the role of two prominent members of the β2 integrin subfamily, α5β1 and α5β2, in angiogenesis. These two integrins share the same β2 subunit, and their α-subunits are 94% identical (19). Angiogenesis was analyzed in the α5β1−/−, α5β2−/−, and control WT mice using two tumor models, murine B16F10 melanoma, and RM1 prostate cancer. These tumors are highly vascularized, and their growth is heavily dependent on an angiogenic response (20, 21). Staining of tumor sections for endothelial cell markers such as CD31 and NG2 revealed reduced pericytes interacting with blood vessels. We double-stained melanoma and prostate tumor sections with Abs to CD31 and to SMA (Fig. 2A, bottom panel). In prostate tumors grown in WT and α5β1−/− mice 30–37% of total CD31+ blood vessels stained for SMA, whereas only 15% of blood vessels formed in α5β2−/− mice expressed this marker (p < 0.02, n = 8; Fig. 2B). Cotaining for CD31 and NG2 revealed reduced pericytes interacting with blood vessels in prostate tumors in α5β2−/− mice as compared with WT and α5β1−/− mice (25 ± 8 μm versus 50–55 ± 10 μm; p < 0.05, n = 6; 4 mice per group; Fig. 2D, top panel, and 2E). Immunostaining for laminin showed a 50% reduction in basement membrane thickness of blood vessels in α5β2−/− mice (2.7 ± 0.3 μm) compared with WT and α5β1−/− mice (5.8–6 ± 1.3 μm; p < 0.03, n = 60; 4 mice per group; Fig. 2D, lower panel, and 2F). With decreased maturation and laminin deposition, we considered that vasculature in α5β2−/− mice might be leaky. Plasma leakage measured as an area that is positive for plasma-derived fibrin was enhanced by 2.5-fold in tumors grown in α5β2−/− mice compared with WT and α5β1−/− mice (13.8 ± 1.8% versus 5.2 ± 1.1% and 3.8 ± 0.7%, respectively; p < 0.03, n = 20; 4 mice per group; Fig. 2G, 2H). The vasculature in melanoma tumors grown in α5β2−/− mice also showed reduced maturity and leakiness (data not shown). In addition, the permeability of preexisting blood vessels in dorsal skin of α5β2−/− and WT mice was examined using Evans blue dye injected i.v. Baseline permeability upon injection of control PBS and VEGF-A–induced vascular permeability were similar in α5β2−/− and WT mice, indicating that preexisting vasculature in α5β2−/− mice was normal (Supplemental Fig. 1).

**Impaired PMN and macrophage recruitment to angiogenic sites in α5β2−/− mice**

The CD11b+/Gr-1+ myeloid cells consisting primarily of PMNs are crucial enhancers of angiogenesis, and they contribute to angiogenic switch in many tumors (4, 5, 22). Tumor growth and angiogenesis were impaired in the α5β2−/− (CD11b−/−) mice; therefore, we considered the possibility that this integrin regulates recruitment of these cells to growing tumors. First, we examined infiltration of CD11b+Gr-1− cells in prostate and melanoma tumors in WT and α5β1−/− mice by double staining with anti-CD11b (green) and anti-Ly6G (Gr-1) mAbs (red). As shown in Fig. 3A, 70–80% of CD11b+ cells were also positive for Gr-1, and numerous CD11b+/Gr-1+ cells were detected in prostate tumors in with Matrigel alone or with Matrigel supplemented with VEGF or KC (keratinocyte-derived factor) to stimulate angiogenesis. CD31 staining of Matrigel plugs containing VEGF or KC revealed well-formed vasculature in the implants from WT and α5β1−/− mice, whereas the Matrigel plugs from α5β2−/− mice showed no distinct vascular formations, although a few CD31-positive ECs were discerned within the plugs (Fig. 1E). Regardless of the angiogenic factor used, blood vessel area in the Matrigel implants in the α5β2−/− mice was reduced by ~75% compared with the implants from the α5β1−/− and WT animals (p < 0.01, n = 8 per group; Fig. 1F). In control Matrigel plugs without proangiogenic cytokines, no blood vessels were detected in any of the three mouse genotypes tested (data not shown).

**Neoangiogenesis in α5β2−/− mice is immature**

The presence of smooth muscle cells and pericytes within vasculature is a key indicator of its maturity because they stabilize blood vessels. We double-stained melanoma and prostate tumor sections with Abs to CD31 and to SMA (Fig. 2A, top panel) or to NG2 chondroitin sulfate proteoglycan, a marker of pericytes (Fig. 2A bottom panel). In prostate tumors grown in WT and α5β1−/− mice 30–37% of total CD31+ blood vessels stained for SMA, whereas only 15% of blood vessels formed in α5β2−/− mice expressed this marker (p < 0.02, n = 8; Fig. 2B). Cotaining for CD31 and NG2 revealed reduced pericytes interacting with blood vessels in prostate tumors in α5β2−/− mice as compared with WT and α5β1−/− mice (25 ± 8 μm versus 50–55 ± 10 μm; p < 0.05, n = 6; 4 mice per group; Fig. 2D, top panel, and 2E). Immunostaining for laminin showed a 50% reduction in basement membrane thickness of blood vessels in α5β2−/− mice (2.7 ± 0.3 μm) compared with WT and α5β1−/− mice (5.8–6 ± 1.3 μm; p < 0.03, n = 60; 4 mice per group; Fig. 2D, lower panel, and 2F). With decreased maturation and laminin deposition, we considered that vasculature in α5β2−/− mice might be leaky. Plasma leakage measured as an area that is positive for plasma-derived fibrin was enhanced by 2.5-fold in tumors grown in α5β2−/− mice compared with WT and α5β1−/− mice (13.8 ± 1.8% versus 5.2 ± 1.1% and 3.8 ± 0.7%, respectively; p < 0.03, n = 20; 4 mice per group; Fig. 2G, 2H). The vasculature in melanoma tumors grown in α5β2−/− mice also showed reduced maturity and leakiness (data not shown). In addition, the permeability of preexisting blood vessels in dorsal skin of α5β2−/− and WT mice was examined using Evans blue dye injected i.v. Baseline permeability upon injection of control PBS and VEGF-A–induced vascular permeability were similar in α5β2−/− and WT mice, indicating that preexisting vasculature in α5β2−/− mice was normal (Supplemental Fig. 1).
WT and $\alpha_M^{-/-}$ mice, with no differences in their recruitment observed in the two mouse strains. Similar results were obtained with melanoma tumors (data not shown). An assessment of CD11b$^+$/Gr-1$^+$ cell recruitment to tumors in $\alpha_M^{-/-}$ (CD11b$^{-/-}$) mice was not feasible because of the absence of the $\alpha_M$ integrin subunit on these cells; therefore, we stained tumor sections only with PMN-specific anti-Ly6G (Gr-1) mAb. Indeed, PMN infiltration into both melanoma and prostate tumors was significantly reduced in the $\alpha_M^{-/-}$ mice compared with WT and $\alpha_L^{-/-}$ littermates (Fig. 3B). Quantification of Ly6G-positive areas in tumor sections verified these observations: $900 \pm 640 \mu m^2$ in WT and $1120 \pm 750 \mu m^2$ in $\alpha_M^{-/-}$ mice in melanomas ($p < 0.01, n = 8$) and $1200 \pm 265 \mu m^2$ in $\alpha_M^{-/-}$ versus $620 \pm 840 \mu m^2$ in WT and $7135 \pm 1780 \mu m^2$ in $\alpha_L^{-/-}$ mice in prostate tumors ($p < 0.01, n = 8$ per group; Fig. 3C). Next, tumor sections were stained with MOMA-2. Macrophage infiltration into both tumors was decreased by 50–60% in the $\alpha_M^{-/-}$ mice as compared with WT and $\alpha_L^{-/-}$ littermates (Fig. 3D, 3E). In melanomas grown in the $\alpha_M^{-/-}$ mice, macrophage-positive area was $1820 \pm 160 \mu m^2$ compared with $5610 \pm 985 \mu m^2$ in WT and $5240 \pm 450 \mu m^2$ in $\alpha_L^{-/-}$ mice, whereas in prostate tumors it was $3600 \pm 750 \mu m^2$ in $\alpha_M^{-/-}$ mice versus $7500 \pm 1100 \mu m^2$ in WT and $7280 \pm 1300 \mu m^2$ in $\alpha_L^{-/-}$ littermates ($p < 0.05, n = 8$ per group). In addition, staining of Matrigel implant sections with anti-PMN and MOMA-2 Abs revealed robust VEGF- and KC-dependent leukocyte infiltration into the centers of the implants in WT and $\alpha_L^{-/-}$ mice, whereas it was inhibited in the $\alpha_M^{-/-}$ mice (data not shown).

**BMT and suppression with blocking Abs confirm the importance of $\alpha_M\beta_2$ in angiogenesis**

Next, we sought to confirm that reduced tumor growth and angiogenesis in the $\alpha_M^{-/-}$ mice are due to impaired functions of bone marrow-derived cells, including leukocytes, and are not caused by defective vascular cells. Therefore, we performed BMT experiments and examined growth and angiogenesis in RM1 tumors. Transplantation of $\alpha_M^{-/-}$ BM into WT hosts ($\alpha_M^{-/-} \rightarrow$WT) resulted in reduced RM1 tumor growth and angiogenesis (tumor weight: $p = 0.0169$; vascular area: $p = 0.028$ for $\alpha_M^{-/-} \rightarrow$WT versus WT$\rightarrow$WT; $n = 5$; Fig. 4A, 4B). Alternatively, transplantation of WT
BM into the \( \alpha_M^{−/−} \) hosts (WT→\( \alpha_M^{−/−} \)) restored growth and angiogenesis of prostate tumors to these of control WT mice receiving WT bone marrow (RM1 weight: \( p = 0.0183 \)); vascular area: \( p = 0.012 \) for WT→\( \alpha_M^{−/−} \) versus \( \alpha_M^{−/−} \rightarrow WT; n = 5 \); Fig. 4A, 4B). These results suggest that blunted tumor growth and angiogenesis in the \( \alpha_M^{−/−} \) mice is a consequence of altered bone marrow-derived cell functions. In addition, image analysis of RM1 tumor sections stained with PMN-specific anti-Ly6G and anti-monocyte–macrophage MOMA-2 Abs revealed impaired recruitment of these cells to tumors grown in WT recipients receiving \( \alpha_M^{−/−} \) BM as compared with control WT→WT mice, indicating a crucial role of \( \alpha_M \beta_3 \) in this process (\( p = 0.0184 \) for PMNs [Ly6G] and \( p = 0.0167 \) for MOMA-2; \( n = 5 \); Fig. 4C, 4D). In contrast, transplantation of WT BM to \( \alpha_M^{−/−} \) recipients restored not only angiogenesis, but also PMN and macrophage migration into tumors (PMNs: \( p = 0.021 \); MOMA-2: \( p = 0.0454 \) for WT→\( \alpha_M^{−/−} \) versus \( \alpha_M^{−/−} \rightarrow WT; n = 5 \); Fig. 4C, 4D). To exclude the possibility that \( \alpha_M \) deficiency might impair recovery of the immune system upon BMT, we assessed engraftment efficiency 6 wk after BMT in chimeric \( \alpha_M^{−/−} \rightarrow WT \) and WT→\( \alpha_M^{−/−} \) mice using WT and \( \alpha_M^{−/−} \) mice not undergoing BMT as controls. We have collected spleens and thymuses from these mice, prepared single-cell suspensions, and measured percentages of PMN, macrophage, B cells, and T cells by flow cytometry using FITC-labeled Abs to cell specific markers (Ly-6G, F4/80, CD19, CD3, respectively) and FITC-labeled isotype-matched Abs as controls. We found that the content of individual leukocyte subsets was similar in respective organs in both chimeric mouse lines as well as in control mice (no BMT), indicating that \( \alpha_M \) deficiency did not affect BMT engraftment efficiency (Supplemental Table I). In addition, flow cytometry of circulating total leukocytes with anti-mouse \( \alpha_M \) mAb confirmed its absence in the \( \alpha_M^{−/−} \rightarrow WT \) chimeras and its presence in the WT→\( \alpha_M^{−/−} \) mice (data not shown).

To confirm the importance of the \( \alpha_M \) integrin in angiogenesis, WT mice were injected with a rat anti-\( \alpha_M \) blocking Ab (clone M1/70) that has been shown to inhibit neointima formation in rabbits specifically (23). These neutralizing Abs bind to the ligand-binding site within the \( \alpha_M \) subunit and inhibit its interactions with ligands thereby mimicking the gene ablation. Matrigel implants were harvested after 8 d, and the Ab was injected before Matrigel injection and then every 2 d. Vasculature was analyzed in Matrigel sections stained with anti-CD31 mAb (Fig. 4E). The anti-\( \alpha_M \) Ab reduced KC-induced angiogenesis in Matrigel implants by ~60–80% as compared with noninjected mice, whereas isotype-matched normal rat IgG3s had no effect indicating specificity (\( p < 0.05 \) mice injected with anti-\( \alpha_M \) versus not injected; \( n = 4\) mice per group; Fig. 4E, 4F). These experiments verify the importance of \( \alpha_M \beta_3 \) on myeloid cells in tumor-induced angiogenesis via regulation of leukocyte recruitment to sites of neovascularization.
$\alpha_M\beta_2$ facilitates leukocyte recruitment to angiogenic sites via interaction with Plg and enhancement of Plg activation

As Plm-dependent ECM proteolysis greatly facilitates leukocyte invasion (10–12), we hypothesized that the inability of $\alpha_M^{-/-}$ leukocytes to invade tumors could be caused by impaired Plg activation on the leukocyte surface. Because $\alpha_M\beta_2$ and $\alpha_L\beta_2$ are the most abundant $\beta_2$-integrins on PMNs and macrophages, we used a modified Boyden chamber system to elucidate the role of

**FIGURE 3.** Reduced leukocyte infiltration of tumors in the $\alpha_M^{-/-}$ mice. (A) Prostate tumor sections from WT and $\alpha_M^{-/-}$ mice were double stained with FITC-labeled rat anti-CD11b (M1/70) mAb (green) and rat anti-Ly6G (Gr-1; clone 1A8-red). Numerous CD11b$^+$Gr-1$^+$ cells are present on merged images (yellow-orange) in both mouse strains. Representative images of the melanoma and prostate tumor sections stained with PMN-specific anti-Ly6G (B) and the monocyte–macrophage-specific MOMA-2 (D) Abs. Image analysis shows reduced Ly6G-positive (C) and MOMA-2-positive (E) area in melanoma and prostate tumors in the $\alpha_M^{-/-}$ mice. Data are means ± SEM ($n = 8$ mice per group) and are representative of three independent experiments. Scale bars, 50 μm. *$p < 0.05.$

**FIGURE 4.** Defective hematopoietic cells contribute to reduced tumor growth and angiogenesis in the $\alpha_M^{-/-}$ mice. (A) Average weight and (B) CD31-positive vascular area in RM1 prostate tumors in mice undergoing BMT with WT or $\alpha_M^{-/-}$ donor marrow. Data represent mean ± SEM ($n = 5$ mice per group). (C) Ly6G-positive and monocyte–macrophage-positive (D) area in RM1 tumors in mice undergoing BMT with WT or $\alpha_M^{-/-}$ donor marrow. Data represent mean ± SEM ($n = 5$ mice per group). (E and F) Intravenous administration of blocking mAb (M1/70) to $\alpha_M$ inhibits KC-dependent angiogenesis in Matrigel plug model in WT mice. M1/70 and normal rat IgG3_a (3.5 mg/kg) were injected before Matrigel injection and on days 2, 4, and 6. Matrigel implants were harvested on day 8, sectioned, and stained with anti-CD31 mAb. (E) Representative images of Matrigel sections stained with anti-CD31 (brown). Scale bars, 50 μm. (F) Quantification of the CD31-positive area in Matrigel implants. Data are means ± SEM ($n = 4$) and are representative of two independent experiments. *$p < 0.05.$
αMβ2 and αLβ2 in mouse peritoneal PMN and macrophage migration through a Matrigel matrix barrier (ECM extracted from Engelbreth-Holm-Swarm mouse sarcoma) in response to KC (Fig. 5A, 5B). In the absence of KC, there was minimal migration of leukocytes into the lower chambers in the presence or absence of added Plg. Although KC-induced migration of PMNs and macrophages was significantly impeded by Matrigel, this barrier effect was overcome by addition of Plg to the αL−/− leukocytes (Fig. 5A, 5B). In contrast, addition of Plg to the αM−/− cells failed to improve their migration through the Matrigel barrier, suggesting that the capability of these cells to bind and activate Plg was limited (p = 0.014 αM−/− versus WT PMNs and p = 0.005 αM−/− versus WT macrophages; n = 3; Fig. 5A, 5B). Indeed, flow cytometry showed a 50-60% reduction in binding.

**FIGURE 5.** αMβ2 directly interacts with Plg, enhances its activation, and facilitates Plm-dependent leukocyte recruitment. KC-directed transmigration of peritoneal PMNs (A) and macrophages (B) through Matrigel-coated inserts is Plg-dependent and is impaired in αM−/− leukocytes. The data are means ± SEM of triplicate samples and are representative of two independent experiments including three mice per group. (C) Reduced binding of soluble Alexa488-labeled Plg to peritoneal αM−/− PMNs and macrophages. The cells were incubated with increasing concentrations of Plg as indicated for 30 min at 37°C. After two washings, Plg binding to cell surface was analyzed using a FACSCalibur flow cytometer and CellQuest software. The cells incubated without Plg were set as negative controls. The data are means ± SEM of triplicate samples (n = 3 mice per group) and are representative of two independent experiments. (D) Plg was immobilized on the CM5 sensor chip surfaces (500 RU). Sensorgrams obtained for a concentration series of GST-αM-I (left panel) and GST-αL-I domain (right panel). (E and F) Reduced Plg activation on the surface of peripheral blood αM−/− PMNs and peritoneal macrophages. The results are means ± SEM of triplicate samples (n = 5 mice per group) and are representative of two independent experiments. *p < 0.05.
of Alexa488-conjugated soluble Plg to \( \alpha_M^{-/-} \) peritoneal PMNs and macrophages as compared with WT and \( \alpha_L^{-/-} \) leukocytes (Fig. 5C).

To determine whether Plg interacts directly with the \( \alpha_M \) and \( \alpha_L \) domain, the major site of ligand binding in \( \beta_2 \) integrins (13), we performed SPR experiments. GST-tagged \( \alpha_M \)-domain interacted with Plg immobilized on biosensor chips in a concentration-dependent manner, whereas the GST-tagged \( \alpha_L \)-domain did not bind Plg (Fig. 5D). GST alone also did not interact with Plg. From the progress curves of the Plg-\( \alpha_M \)-domain interaction, we estimated a \( K_d = 1.76 \pm 0.9 \times 10^{-7} \). This value was derived by fitting the kinetic data to a 1:1 global Langmuir model, and the stoichiometry observed at ligand saturation was 1:1.

Next, we compared Plg activation on the surface of WT, \( \alpha_L^{-/-} \), and \( \alpha_M^{-/-} \) leukocytes using a fluorogenic plasmin-specific peptidomimetic substrate. Peripheral blood PMNs and lymphocytes were stimulated with KC and pretreated with sc-uPA to activate the integrin and enable sc-uPA binding to leukocyte surface, respectively. No plasmin activity was detected in the absence of leukocytes. The \( \alpha_M^{-/-} \) PMNs showed a 50% reduction in Plm generation as compared with WT and \( \alpha_L^{-/-} \) PMNs (\( p < 0.03, \ n = 5 \); Fig. 5E). In contrast, Plg activation was similar on WT, \( \alpha_M^{-/-} \), and \( \alpha_L^{-/-} \) lymphocytes (Fig. 5E). As with PMNs, peritoneal \( \alpha_M^{-/-} \) deficient macrophages also exhibited severely reduced (by 75%) Plg activation compared with the \( \alpha_L^{-/-} \) and WT macrophages (\( p < 0.05, \ n = 5 \); Fig. 5F). In control experiments, we examined \( \alpha_L \) expression on \( \alpha_M^{-/-} \) deficient PMN, \( \alpha_M \) levels on \( \alpha_L^{-/-} \) null, and control WT PMNs (both peritoneal and circulating) by flow cytometry. Neither \( \alpha_L \) nor \( \alpha_M \) deficiency altered expression of its counterpart \( \beta_2 \)-integrin on PMNs (data not shown), confirming that Plg recognition and activation is \( \alpha_M \beta_2 \)-specific. In addition, \( \alpha_M \beta_2 \) functions as a Plg receptor, and this function is critical in Plm-dependent leukocyte recruitment to angiogenic sites.

\( \alpha_M \beta_2 \) regulates secretion of VEGF-A by PMNs

Our data demonstrate a critical role of \( \alpha_M \beta_2 \) in leukocyte recruitment to angiogenic sites. However, we considered a possibility that \( \alpha_M \beta_2 \) might also regulate other proangiogenic leukocyte functions such as production and secretion of angiogenic stimulators. The CD11b\(^+/\)Gr-1\(^+\) cells, which primarily constitute PMNs, are of particular interest because they secrete high levels of MMP-9 and VEGF, leading to “angiogenic switch” in many tumors and to a failure of anti-VEGF therapies (5, 22). Therefore, to investigate whether \( \alpha_M \beta_2 \) supports angiogenesis via regulation of VEGF secretion by PMNs, we harvested from WT or \( \alpha_M^{-/-} \) peripheral blood PMNs (Table I). In control experiments, we examined \( \alpha_L \) expression on \( \alpha_M^{-/-} \)-deficient PMN, \( \alpha_M \) levels on \( \alpha_L^{-/-} \)-null, and control WT PMNs (both peritoneal and circulating) by flow cytometry. Neither \( \alpha_L \) nor \( \alpha_M \) deficiency altered expression of its counterpart \( \beta_2 \)-integrin on PMNs (data not shown), confirming that Plg recognition and activation is \( \alpha_M \beta_2 \)-specific. In addition, \( \alpha_M \beta_2 \) functions as a Plg receptor, and this function is critical in Plm-dependent leukocyte recruitment to angiogenic sites.

FIGURE 6. \( \alpha_M \beta_2 \) supports angiogenesis via regulation of VEGF secretion by PMNs. (A) Peripheral blood WT, \( \alpha_M^{-/-} \), or \( \alpha_L^{-/-} \) PMNs (3 \( \times \) 10\(^6\) cells/well) were incubated in 24-well TC plates in the absence or presence of TNF-\( \alpha \) (20 ng/ml) for 2 h at 37°C. Cycloheximide (10 \( \mu \)g/ml) or pentoxifylline (300 \( \mu \)M) were added 60 min before addition of TNF-\( \alpha \). VEGF concentration was measured in supernatants using mouse VEGF Quantikine ELISA Kit. Data are means \( \pm \) SEM of triplicate samples and are representative of three independent experiments. (B) Bright field microscopy of tube formation by WT MAECs in the presence of conditioned media collected from WT, \( \alpha_M^{-/-} \), or \( \alpha_L^{-/-} \) peripheral blood PMNs stimulated with TNF-\( \alpha \) (upper panels). Inhibitors of VEGF: neutralizing anti-VEGF mAb, isotype matched rat IgG2a (100 \( \mu \)g/ml) or pentoxifylline (300 \( \mu \)M) were added 60 min before addition of TNF-\( \alpha \). VEGF concentration was measured in supernatants using mouse VEGF Quantikine ELISA Kit. Data are means \( \pm \) SEM of triplicate samples and are representative of three independent experiments.
Table I. Comparison of VEGF-A mRNA content in WT, αM<sup>−/−</sup>, and α<sub>L</sub><sup>−/−</sup> peripheral blood PMNs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WT</th>
<th>αM&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>αL&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.22</td>
<td>0.19</td>
<td>0.2</td>
</tr>
<tr>
<td>TNF-α (20 ng/ml)</td>
<td>0.24</td>
<td>0.20</td>
<td>0.24</td>
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Values are expressed relative to GAPDH mRNA levels. PMNs were incubated in the presence or absence of TNF-α (20 ng/ml) for 2 hr at 37°C. Total RNA was isolated using Trizol reagent, and RT-PCR has been performed as described in Materials and Methods.

To corroborate this conclusion, we measured the concentration of lactoferrin, a marker of PMN-specific granules, in PMN supernatants. The relative changes of lactoferrin and VEGF in the PMN-conditioned media were highly similar. Importantly, the αM<sup>−/−</sup> PMNs, but not the αL<sup>−/−</sup> PMNs, showed severely impaired release of lactoferrin into the supernatants of TNF-α-stimulated αM<sup>−/−</sup> PMNs, which were almost as low as in supernatants of resting PMNs from each mouse strain (Supplemental Fig. 2). We also sought to examine VEGF production and secretion in CD11<sup>L</sup><sup>+</sup> progenitor cells isolated from bone marrow of WT, αM<sup>−/−</sup>, and αL<sup>−/−</sup> mice. However, we failed to detect mRNA for VEGF in these immature cells.

Next, we analyzed the capacity of WT MAECs to form tubes in the presence of the conditioned media derived from WT, αM<sup>−/−</sup>, or αL<sup>−/−</sup> TNF-α-stimulated PMNs. MAECs in the presence of media collected from WT or αL<sup>−/−</sup> PMNs formed well-organized tubelike networks. In contrast, tubes formed by MAECs in the presence of the αM<sup>−/−</sup> PMN-conditioned media were incomplete (p < 0.05, n = 20; Fig. 6B, 6C). In control samples, TNF-α alone did not support tube formation by ECs. To confirm that tube formation by MAECs in the presence of WT or αL<sup>−/−</sup> PMN supernatants is VEGF-dependent, we added neutralizing rat anti-mouse VEGF mAb (clone 2G11-2A6/5), its isotype control rat IgG<sub>2a</sub>, or sFLT-1. The effectiveness of these VEGF inhibitors has been previously established (26, 27). These inhibitors of VEGF almost completely inhibited (by 75–80%) tube formation by MAEC (p < 0.05, n = 20). In contrast, the isotype control Ab did not have any effect (Fig. 6B, bottom panel, and 6C). Although other proangiogenic factors are likely to be present in PMN supernatants, VEGF appears to be the key stimulator of this process in our experimental system. Finally, supplementation of αM<sup>−/−</sup> PMN-conditioned medium with recombinant mouse VEGF to the same concentration as in medium from WT PMNs (520 pg/ml) enhanced the numbers of closed tubes by 2.5-fold and almost completely closed tubes by 2.5-fold and almost completely.

Discussion

The goal of this study was to examine involvement of the two major leukocyte β<sub>2</sub> integrins in angiogenesis: αMβ<sub>2</sub> and αLβ<sub>2</sub>. Using the αM<sup>−/−</sup> and αL<sup>−/−</sup>-deficient mice, we demonstrate that αMβ<sub>2</sub> promotes angiogenesis in model melanoma and prostate tumors, as well as in Matrigel implants, whereas the αLβ<sub>2</sub> integrin does not. Blood vessel formation and tumor growth were impaired in αM<sup>−/−</sup> mice as compared with the αL<sup>−/−</sup> or WT mice. Impaired angiogenesis in αM<sup>−/−</sup> mice was due to dramatic reduction in recruitment of PMNs and macrophages into the tumors and Matrigel implants. Furthermore, we showed that Plg binding and activation on the surface of αM<sup>−/−</sup> PMNs and macrophages and their Plg-dependent invasion through Matrigel were significantly attenuated as compared with the αL<sup>−/−</sup> and WT cells. These data were consistent with the SPR sensorgrams showing that recombinant αM<sup>−/−</sup>-domain directly interacts with Plg, but the αL<sup>−/−</sup>-domain does not. These findings are in agreement with prior studies showing that αMβ<sub>2</sub> recognizes urokinase (uPA) and Plg enhancing their reciprocal activation on PMN surface (14, 15, 28). To our knowledge, this is the first report implicating αMβ<sub>2</sub> in angiogenesis and demonstrating its intimate interplay with Plg in vivo. Although, the β<sub>2</sub>-deficient mice showed slowed angiogenesis in healing wounds (29), none of the individual β<sub>2</sub> integrin family members was shown to contribute to this process. The implication of αMβ<sub>2</sub> in angiogenesis and Plg binding and activation is highly specific as αLβ<sub>2</sub> did not show any impairment in these responses. This distinction may be explained, at least in part, by the relatively low sequence identity between the ligand binding αMβ<sub>2</sub>- and αLβ<sub>2</sub>-domains resulting in the αMβ<sub>2</sub> promiscuity for many structurally unrelated ligands, whereas αLβ<sub>2</sub> shows a limited ligand repertoire with little overlap in ligand recognition with αMβ<sub>2</sub> (19). Regarding the ligand repertoire, the two β<sub>2</sub> integrins αMβ<sub>2</sub> and αLβ<sub>2</sub> are more similar to αMβ<sub>2</sub> than to αLβ<sub>2</sub>, and it would be interesting to examine their roles in angiogenesis. The critical role of αMβ<sub>2</sub>-dependent Plg activation in PMN and macrophage recruitment to angiogenic niches is in accord with previous studies demonstrating the importance of cell-bound plasmin in leukocyte recruitment in a variety of in vivo models of inflammation (10–12) and with a crucial role of the Plg system in angiogenesis (30–36). Pericellular proteolysis is critical for initiation of angiogenesis as evidenced by suppression of neovascularization in mice deficient in various proteases (31, 37–39) and by administration of a variety of protease inhibitors (40, 41). Among the proteases implicated in angiogenesis, in addition to plasmin, are its activators and metalloproteinases (reviewed in Ref. 4, 8). Plasmin is one of pro-MMP-9 activators (42) and PMN-derived MMP-9 is responsible for angiogenic switch in some tumors (5). Consistent with the finding of decreased Plm activity and the capacity of αMβ<sub>2</sub> to bind and activate MMP-9 (43), we observed reduced MMP-9 activity in tumor extracts from the αM<sup>−/−</sup> mice (data not shown).

As a key source of proteases and proangiogenic factors, PMNs and macrophages are essential for angiogenesis. Angiogenesis is severely blunted in neutropenic mice (5, 29, 44, 45) or in mice in which macrophages have been eliminated (2). Robust PMN and macrophage recruitment is observed into ischemic tissues including tumors (46), and in many tumors recruitment of these cells correlates with poor host survival (reviewed in Ref. 2). With such evidence, the virtual absence of PMNs and macrophages in the angiogenic tissue of the αM<sup>−/−</sup> mice provides a mechanism to account for profound reduction in neovascularization and tumor growth in these animals. In addition to defective recruitment, the αM<sup>−/−</sup> PMNs also exhibited severely attenuated secretion of VEGF-A because of impaired degranulation, and supernatants collected from these cells did not support EC tube formation in vitro. As enhanced VEGF is the hallmark and a key contributor to CD11b<sup>+</sup>/Gr-1<sup>−</sup>-dependent (mostly PMNs) resistance of many tumors to VEGF targeting anticancer therapies, we have focused our investigations on the regulation of this pivotal cytokine. We cannot exclude that other proangiogenic factors, particularly those stored in PMN granules, might also be regulated by αMβ<sub>2</sub> via its influence on degranulation. This important issue is open for further investigation. Even if αM<sup>−/−</sup> PMNs had been able to migrate, they would likely have failed to promote angiogenesis because of an inability to supply the requisite components of the major proangiogenic stimulus, VEGF. The neovascularization in growing tumors, but not preexisting blood vessels, in αM<sup>−/−</sup> mice showed immature and leaky phenotype. This might be caused by insufficient infiltration of CD11b<sup>+</sup>/Ly6G<sup>+</sup>
PMNs known to support vascular maturation via elevated levels of proangiogenic factors VEGF and MMP-9 (22).

Taken together, our studies demonstrate that integrin αβ₂ promotes angiogenesis in vivo via a dual mechanism: first, as a Plg receptor, αβ₂ supports Plg-dependent recruitment of myeloid cells to angiogenic niches; second, αβ₂ enhances VEGF-A secretion by PMN degranulation. Based on our findings, selective antagonists of αβ₂ can be considered as a new target to inhibit tumor angiogenesis.

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Disclosures

The authors have no financial conflicts of interest.

References


2. Qian, B.-Z., and J. W. Pollard. 2010. Macrophage diversity enhances tumor angiogenesis via a dual mechanism: first, as a Plg receptor, αβ₂ supports Plg-dependent recruitment of myeloid cells to angiogenic niches; second, αβ₂ enhances VEGF-A secretion by PMN degranulation. Based on our findings, selective antagonists of αβ₂ can be considered as a new target to inhibit tumor angiogenesis.


