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The Activity of Soluble VCAM-1 in Angiogenesis Stimulated by IL-4 and IL-13

Jun-ichi Fukushi,1* Mayumi Ono,* Wataru Morikawa,* Yukhide Iwamoto,† and Michihiko Kuwano*

IL-13 is a multifunctional lymphokine sharing a number of biological properties with IL-4. We previously observed that IL-4 shows angiogenic activities in vitro as well as in vivo. In this study we examined the effect of IL-13 on angiogenesis in vitro and in vivo and also the underlying mechanisms. Human IL-13 significantly stimulated the formation of tube-like structures in collagen gels by human microvascular endothelial cells and bovine aortic endothelial cells by about 3-fold over the controls in the absence of the cytokines. Administration of murine IL-13 led to neovascularization when implanted in the rat cornea. Coadministration of neutralizing mAb to the IL-4R inhibited both tubular morphogenesis in vitro and activation of STAT6 induced by IL-4 or IL-13. Both IL-4 and IL-13 markedly increased mRNA levels of VCAM-1 in vascular endothelial cells, and the production of the soluble form of VCAM-1 was also stimulated in response to IL-4 or IL-13. Administration of anti-VCAM-1 Ab in vitro blocked tubular morphogenesis induced by IL-4 and IL-13. Angiogenesis induced in vivo in rat cornea by IL-4 and IL-13 was also inhibited by Ab against the rat α4 integrin subunit. These findings suggest that angiogenesis dependent on IL-4 and IL-13 is mainly mediated through a soluble VCAM-1/α4 integrin pathway. The Journal of Immunology, 2000, 165: 2818–2823.

Angiogenesis, the production and organization of newly formed blood vessels, plays a key role in physiological processes, including the development of the embryo, the formation of the corpus luteum, and wound healing, as well as in pathological processes such as the enlargement of solid tumors, ocular diseases, psoriasis, and rheumatoid arthritis (1). A number of factors, including growth factors, cytokines, chemokines, extracellular matrix macromolecules, and adhesion molecules, are involved in angiogenesis. Stimulators include vascular endothelial growth factor (VEGF),2 basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), TNF-α, IL-8, soluble E-selectin, and soluble VCAM-1 (sVCAM-1) (2). Inhibitors include IFN-α, IFN-β, IL-12, TGF-β, platelet factor-4, prolactin fragment, thrombospondins, angiostatin, and endostatin (3).

Angiogenesis-related activities by members of the lymphokine IL-4 superfamily, IL-4 and IL-13, have been reported. Tumors expressing IL-4 have a reduced level of vascular density (4). IL-4 blocked the induction of corneal neovascularization by bFGF in rats (5). Chemotaxis of vascular endothelial cells is stimulated only when small amounts (0.01 ng/ml) of IL-4 are present, but is inhibited when higher doses of IL-4 are present (5). These studies suggest antiangiogenic properties of IL-4 under certain conditions. In contrast, IL-4 induces various activities associated with angiogenesis. IL-4 increases the expression of urokinase-type plasminogen activator (6) and VCAM-1 (7) and induces changes in the morphology of HUVEC (8). IL-4 is a potent mitogen for microvascular endothelial cells (9). We previously reported that angiogenesis is induced in vitro and in vivo in response to IL-4 (10).

Another cytokine derived from T lymphocyte, IL-13, shows about 25% homology to IL-4 and also shares many of the IL-4 activities (11). Their common effects have been explained by using shared receptor subunits consisting of IL-4Ra and IL-13Ra (12). Both IL-4 and IL-13 down-regulate the function of monocytes by inhibiting the secretion of the inflammatory cytokine IL-6, TNF-α, and IL-12 (11) and by inducing the production of IL-1R antagonists (13). Regardless of their predominant anti-inflammatory activities, IL-4 and IL-13 considerably increase the expression of VCAM-1 on the surface of vascular endothelial cells, causing adhesion of T cells (7). In addition, IL-4 and IL-13 induce the synthesis of monocyte chemoattractant protein-1 in endothelial cells (14), and IL-13 is chemotactic for both HUVEC and dermal microvascular endothelial cells (15), suggesting a novel role for IL-13 as a proinflammatory and proangiogenic cytokine. IL-4 and IL-13 thus appear to promote transendothelial migration of leukocytes and act proangiogenically under certain conditions.

In this study, we asked whether IL-13 can induce angiogenesis together with IL-4 through interaction with common receptor molecules on the cell surface of vascular endothelial cells. We examined the angiogenic activity of IL-13 and IL-4 in vitro and in vivo and showed that sVCAM-1 is markedly up-regulated when vascular endothelial cells are treated with IL-4 and IL-13. How IL-4- and IL-13-mediated angiogenesis is controlled is discussed.

Materials and Methods

Materials

Human rIL-4 was a gift from Ono Pharmaceutical (Osaka, Japan). Human rEFG was purchased from PeproTech (London, U.K.). Human rFGF was purchased from Intergen (Purchase, NY). Bovine rFGF, rat rIL-4, human rIL-13, murine rIL-13, and anti-human IL-4 soluble receptor neutralizing Ab were all purchased from R&D Systems (Minneapolis, MN). Anti-human VCAM-1 Ab was purchased from Upstate Biotechnology (Lake Placid, NY), and anti-rat α4 Ab was purchased from Seikagaku (Tokyo, Japan). Anti-STAT6 Ab was purchased from Santa Cruz Biotechnology.
Bovine aortic endothelial cells were isolated and cultured as previously described (16, 17). Human microvascular endothelial cells were isolated from normal omental tissue that was removed during surgery as previously described (18). Because the responsiveness of the endothelial cells to angiogenic factors decreases during serial cultivation, we used cells at passages 5–10 (19).

Quantitative analysis of tube formation in endothelial cells on type I collagen gel

The tube formation assay was made as described previously (16, 20, 21). In brief, bovine aortic endothelial cells or human microvascular endothelial cells were plated onto type I collagen gel in a medium containing 10% FBS. When the cells reached confluency, the medium was replaced with a medium that contained 1% FBS, with or without the various factors, and the cells were incubated for an additional 72 h. On the third day, phase-contrast micrographs (magnification, ×200) were recorded using a still video camera recorder (R5000H, Fuji, Tokyo, Japan). The total length of the tube-like structures for each field was measured using a Cosmozone image analyzer (Nikon, Tokyo, Japan). Eight random fields were measured for each dish, and the total length for each field was calculated.

Corneal pocket assay

The corneal pocket assay was made essentially as previously described (22). In brief, 5 μl of Hydron pellets (IPN Sciences, New Brunswick, NJ) containing 250 ng of rat rIL-4, 250 ng of murine rIL-13, or 100 ng of bovine bFGF were prepared and implanted in the corneas of male Sprague Dawley rats (300–300 g). Abs (2 μg/pellet) were added directly to the cytokine/Hydron solution. After 7 days, the animals were killed, and the corneal vessels were photographed.

Western blot analysis

Human microvascular endothelial cells were grown in medium 199 containing 10% FBS, followed by incubation in medium 199 containing 1% FBS for 24 h. Then, the cells were incubated with anti-IL-4R Ab for 1 h at 37°C and were treated with 50 ng/ml of IL-4 or IL-13 for 15 min. The cells were harvested and then lysed in ice-cold lysis buffer containing 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 10 ng/ml aprotinin, 10 ng/ml leupeptin, and 0.2 mM sodium orthovanadate. After centrifugation, cleared supernatants were collected, electrophoresed by SDS-PAGE on 8% polyacrylamide gel, and blotted onto nitrocellulose filters. The nitrocellulose filters were developed by chemiluminescence according to the enhanced chemiluminescence protocol of Amersham (Piscataway, NJ) (21).

ELISA for VCAM-1

The concentration of soluble VCAM-1 in the conditioned medium and whole-cell lysate of human microvascular endothelial cells was measured using ELISA kits obtained from BioSource (Camarillo, CA) according to the manufacturer’s instructions.

Statistical analysis

Statistical comparisons were performed using Student’s t test.

Results

Tubular morphogenesis of vascular endothelial cells by IL-13 in vitro

We first examined whether IL-13 can increase the formation of tube-like structures by human microvascular endothelial cells. Previous reports showed that human microvascular endothelial cells cultured on the surface of three-dimensional type I collagen gel show a cobblestone-like appearance in the absence of the angiogenic factor, and they form tube-like structures when treated with EGF or IL-4 (10, 20). Human microvascular endothelial cells treated with human IL-13 induced formation of tube-like structures (Fig. 1). To examine the effects of various doses of IL-13 on the formation of tube-like structures by vascular endothelial cells, a quantitative analysis was made to determine the structures’ total length. The total length was 211 ± 45 mm in the absence of treatment and 157 ± 6, 349 ± 69, 457 ± 65, and 462 ± 92 mm after the cells were exposed to 1, 10, 50, and 250 ng/ml of IL-13, respectively. IL-13 at 10–250 ng/ml significantly stimulated the tubulogenesis, but any stimulatory or inhibitory effect was observed at the concentration of 1 ng/ml. The total length was 419 ± 112 mm at 20 ng/ml EGF (Fig. 2A).

We next examined whether IL-13 can induce the formation of these tube-like structures by bovine aortic endothelial cells. We previously reported that bFGF and IL-4 efficiently induce the formation of tube-like structures by bovine aortic endothelial cells (10, 16). In this study we found that 10–100 ng/ml of IL-13 increased tubular morphogenesis by about 3- to 4-fold over the control levels in endothelial cells (Fig. 2B). Thus, IL-13 induced tubular morphogenesis of human and bovine endothelial cells in vitro.

IL-13 induces angiogenesis in rat corneas

We also examined whether IL-13 can induce angiogenesis in vivo. We implanted a pellet of Hydron that had been impregnated with murine IL-13 into the corneas of eight rats. Administration of 250 ng of IL-13 induced an angiogenic response in all eight corneas, while contralateral corneas treated with Hydron alone showed no angiogenic response (Fig. 3). Administration of 250 ng of IL-13 caused a much greater angiogenic response than did administration of 50 ng of IL-13 (data not shown). Consistent with our previous study (10), administration of 250 ng of rat IL-4 or 100 ng of bovine bFGF also induced angiogenesis in the rat cornea. IL-13 thus shows angiogenic activity in vivo as well as in vitro.

IL-13 as well as IL-4 at more than 50 ng could induce angiogenesis in rat corneas. Potent angiogenesis factors, such as VEGF and IL-8, could also induce angiogenesis in rat corneas when 100–500 ng of these factors was administered (data not shown), suggesting that IL-4 or IL-13 could induce angiogenesis at doses comparable to those of VEGF and IL-8.

Anti-IL-4R Ab inhibits STAT6 phosphorylation and tubulogenesis in response to IL-13

The endothelial receptor for IL-13 and IL-4 is a heterodimeric complex of IL-13Rα and IL-4Rα (23). We previously reported that formation of tube-like structures mediated by IL-4 was blocked by coadministration with anti-IL-4R Ab (10). We thus examined whether IL-4Rα is also involved in the IL-13-mediated angiogenesis in vitro and found that pretreatment of human microvascular endothelial cells with anti-IL-4Rα Ab almost completely nullified tubulogenesis induced by IL-13 (Fig. 4). By contrast, EGF-dependent tubular morphogenesis was not inhibited by pretreatment with anti-IL-4Rα Ab.
The interaction of IL-4 and IL-13 with their cognate receptors, IL-4Rα and IL-13Rα, is mediated through activating STAT6 (24). In this study we examined whether STAT6 was activated in vascular endothelial cells treated with IL-4 or IL-13. Human microvascular endothelial cells treated with IL-4 or IL-13 rapidly stimulated STAT6 phosphorylation, as shown by immunoblotting cellular extracts with Ab specific for the tyrosine-phosphorylated form of STAT6 (Fig. 5). Pretreatment of endothelial cells with anti-IL-4Rα Ab almost completely nullified STAT6 phosphorylation induced by IL-13 and IL-4 (Fig. 5).

**IL-4 and IL-13 induce sVCAM-1 expression in vascular endothelial cells**

We previously showed that human microvascular endothelial cells, monocytes, and tumor cells treated with TNF-α increase their production of potent angiogenic factors such as VEGF, bFGF, and IL-8, and also that these angiogenic factors acting in an autocrine/paracrine way result in angiogenesis (25, 26, 27). To learn whether such autocrine/paracrine control by angiogenic factors is involved in angiogenesis induced by IL-4 and IL-13, we determined the expression of various angiogenic mediators, including growth factors, cytokines, proteinases, and adhesion molecules, when treated with IL-4 or IL-13. Administration of IL-4 or IL-13 to vascular endothelial cells did not increase the expression of VEGF, bFGF, IL-8, plasminogen activators, matrix metalloproteinase-2 or -9, ICAM-1, or E-selectin (data not shown). In contrast, and consistent with previous studies (7), vascular endothelial cells treated with IL-4 or IL-13 resulted in increased mRNA expression of VCAM-1 by 13- and 12-fold over controls, respectively (data not shown).

We next examined whether the expression of either membrane-bound VCAM-1 or sVCAM-1 is increased in the presence of IL-4/IL-13 using VCAM-1-specific ELISA. Human microvascular endothelial cells from the omentum constitutively expressed VCAM-1. Vascular endothelial cells treated with IL-4 or IL-13 increased the expression of sVCAM-1 in the conditioned medium by 8-fold over the controls in a time-dependent manner (Fig. 6). We found sVCAM-1 to be present at a level of 7.0 ng/ml in the absence of treatment. The levels of sVCAM-1 were 13.8, 53.6, and

**FIGURE 2.** Quantitative analysis of the formation of tube-like structures by human microvascular endothelial cells (A) and by bovine aortic endothelial cells (B). Cells were plated onto type I collagen gel in medium containing 10% FBS. When the cells became confluent, the medium was replaced with medium containing 1% FBS and the indicated doses of EGF, bFGF, and IL-13. After an additional 72 h of incubation, phase-contrast micrographs were recorded with a still video camera. The total length of the tube-like structures was measured using an image analyzer. Eight random fields were measured, and the total length per field was calculated. At least three tube formation assays were completed. Statistically significant difference (*, p < 0.01; **, p < 0.02) compared with the control is indicated.

**FIGURE 3.** Effect of IL-13 on angiogenesis in vivo. Angiogenic activities of recombinant bFGF, IL-4, and IL-13 were compared following implantation of Hydron pellets containing PBS alone (control), 100 ng of bovine bFGF, 250 ng of rat IL-4, or 250 ng of murine IL-13 into Sprague Dawley rat corneas. Seven days later, vessels in the region of the pellet implant were photographed.

**FIGURE 4.** Effect of anti-IL-4R Ab on formation of the tube-like structures induced by IL-13. Cells were plated onto type I collagen gel in medium containing 199 containing 10% FBS. After the cells became confluent, the medium was replaced with medium containing 199 containing 1% FBS. The cells were incubated with or without 500 ng/ml of anti-IL-4R Ab or nonimmune Ab (IgG) for 1 h and then exposed to 20 ng/ml of EGF and 50 ng/ml of IL-13. Tube formation was quantified after additional incubation for 72 h. At least three tube formation assays were completed. *, Statistically significant difference (p < 0.01) compared with the value obtained in the absence of Ab.
cytokines and either anti-rat 4 integrin mAb or IgG control into Sprague Dawley rats. Pellets also contained anti-rat 4 integrin Ab. Hydron pellets containing 250 ng of IL-13, 250 ng of IL-4, or 100 ng of bFGF were implanted into the corneas of Sprague Dawley rats. Pellets also contained anti-rat 4 integrin Ab or an IgGl isotype control Ab (2 mg/pellet) as indicated. Seven days later, vessels in the region of the pellet implant were photographed.

Discussion

Both IL-4 and IL-13, which are mainly produced from T cells, eosinophils, and mast cells (28, 29), often share their signaling pathways through their heterodimeric receptors, IL-4R and IL-13R (12). We previously reported the angiogenic activity of IL-4 in vitro and in vivo (10). In this study we showed that IL-13 also has angiogenic activity in vitro and in vivo. As an Ab against IL-4Ra blocked the formation of tube-like structures in collagen gel by microvascular endothelial cells in response to IL-4 (10) and IL-13, angiogenesis by IL-4 or IL-13 was mediated through common heterodimeric IL-4Ra/IL-13R. Moreover, both IL-4 and IL-13 were shown to mediate angiogenesis in rat corneas. Adding anti-Ab (2 mg) almost completely inhibited the angiogenic response induced by rat IL-4 in all three corneas tested (zero of three corneas were positive), while control IgG had a negligible effect on the angiogenic response in the contralateral corneas (three of three corneas were positive) (Fig. 8). Angiogenesis induced by murine IL-13 was also inhibited by the addition of anti-Ab (zero of three corneas were positive). However, anti-Ab did not inhibit the angiogenic response induced by bFGF (three of three corneas were positive).

54.3 ng/ml after the cells were exposed for 28 h to 20 ng/ml of EGF, 50 ng/ml of IL-4, and 50 ng/ml of IL-13, respectively. VCAM-1 protein levels in the cellular fraction also increased up to 4-fold over the controls after 20 h of stimulation and then decreased by an additional 8 h of incubation, suggesting that the membrane-bound form of VCAM-1 is converted to the soluble form and is secreted into the conditioned medium.

Inhibition of VCAM-1 or 4 integrin inhibits angiogenesis induced by IL-4 and IL-13

Because sVCAM-1 promotes angiogenesis (2), we examined whether the formation of tube-like structures induced by IL-4 or IL-13 is mediated through sVCAM-1 and its counter-receptor integrin 4/4. Vascular endothelial cells were exposed to 50 ng/ml of IL-4 and IL-13 with or without anti-VCAM-1 Ab that could block adhesion to integrin 4/4. Adding anti-VCAM-1 Ab did not inhibit tubular morphogenesis dependent on EGF. However, coinadministration of the anti-VCAM-1 Ab almost completely inhibited the formation of tube-like structures dependent on IL-4 and IL-13 (Fig. 7). To determine whether angiogenesis in the rat cornea induced by IL-4 and IL-13 is mediated through sVCAM-1 and integrin 4/4, we implanted a pellet of Hydron that had been impregnated with cytokines and either anti-rat 4 integrin mAb or IgG control into rat corneas. Adding anti-Ab (2 mg) almost completely inhibited the angiogenic response induced by rat IL-4 in all three corneas tested (zero of three corneas were positive), while control IgG had a negligible effect on the angiogenic response in the contralateral corneas (three of three corneas were positive) (Fig. 8). Angiogenesis induced by murine IL-13 was also inhibited by the addition of anti-Ab (zero of three corneas were positive). However, anti-Ab did not inhibit the angiogenic response induced by bFGF (three of three corneas were positive).

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IL-13 phosphorylated STAT6, and coadministration of anti-IL-4Rα Ab blocked STAT6 phosphorylation. IL-4 and IL-13 thus exerted their shared signaling pathways on angiogenesis through the common receptor on vascular endothelial cells.

Consistent with our previous report (10), IL-4 could induce neovascularization in both in vitro and in vivo (rat cornea) angiogenesis models. Toi et al. (9) previously reported that IL-4 was a potent mitogen for capillary endothelial cells. Moreover, expression of urokinase-type plasminogen activator was enhanced in vascular endothelial cells by the exogenous addition of IL-4 (6) as well as potent angiogenic factors, VEGF, bFGF, and TNF-α (17, 30, 31). Urokinase-type plasminogen activator is well known to facilitate proteolysis of extracellular matrix and cell migration by vascular endothelial cells (32, 33). In contrast, Saleh et al. (4) reported that vessel density in tumors was halved when tumor cells secreted IL-4, suggesting that IL-4 might inhibit neovascularization in tumors. The antiangiogenic effect by IL-4-producing tumor cells (4) might be mainly due to local eosinophil influx into the tumor, resulting in tumor regression and decreased production of various angiogenic factors from the tumor.

Volpert et al. (5) have reported that administration of human or mouse IL-4 blocks bFGF-induced angiogenesis in rat cornea. In their study human IL-4 (1–100 pg) or mouse IL-4 (1 ng) inhibited bFGF (1 ng)-induced angiogenesis in rat corneas (5). Moreover, cell migration by vascular endothelial cells was enhanced in response to a low concentration (10 pg/ml) of mouse or human IL-4, but was inhibited in the presence of higher concentrations of IL-4 (5). However, we observed no apparent inhibition of bFGF (100 ng)-induced angiogenesis in rat cornea when 1–1000 ng of human IL-4 was coadministered, and angiogenesis was weakly induced in rat cornea when 1000 ng of human IL-4 was administered (data not shown). In addition, we did not observe any apparent inhibition of cell migration of bovine or human vascular endothelial cells by human, rat, or mouse IL-4 at 0.1–100 ng/ml when assayed by a wound cell migration system (data not shown). Because the concentrations of bFGF and IL-4 employed for the in vivo assays by Volpert et al. (5) were much lower than those employed in our assays, it is possible that an antiangiogenic effect of IL-4 in vivo might be observed only when IL-4 and bFGF are used at very low concentrations. In our previous report (10) and our present study we used rat IL-4 for angiogenesis assay with rat corneas. The species specificity of cytokine and angiogenesis models could also be the basis of the discrepancy between the study by Volpert et al. (5) and our study. Understanding why IL-4 induces such dual effects of stimulation and inhibition of angiogenesis under different experimental conditions will require additional studies.

Both IL-4 and IL-13 induce the up-regulation of VCAM-1 in human vascular endothelial cells (7). VCAM-1 mediates adhesion between endothelial cells and activated leukocytes expressing αvβ3 integrin (7) and is closely associated with inflammation and angiogenesis. In human coronary atherosclerotic plaques, endothelial cells express VCAM-1 mainly in areas of neovascularization (34). Soluble VCAM-1 is chemotactic for vascular endothelial cells and is angiogenic in rat cornea (2), and the sVCAM-1/α4 integrin pathway plays a key role in angiogenesis induced by TNF-α (35). Consistent with these previous studies, we observed that human microvascular endothelial cells treated with IL-4 or IL-13 resulted in a significant up-regulation of VCAM-1 mRNA (data not shown), and a significant accumulation of sVCAM-1 proteins at a level of 50–55 ng/ml in cultured medium for vascular endothelial cells treated with cytokine. Anti-VCAM-1 Ab administered together with IL-4 or IL-13 almost completely blocked tubular morphogenesis by microvascular endothelial cells, and anti-αv integrin Ab blocked the angiogenic response in rat corneas induced by IL-4 or IL-13. The sVCAM-1/α4 integrin pathway thus appeared to be closely involved in angiogenesis dependent on IL-4 and IL-13. For example, rheumatoid arthritis is characterized not only by infiltration of inflammatory cells, but also by proliferation and neovascularization of the synovium. Increased levels of IL-4 and IL-13 and sVCAM-1 have been detected in the synovial fluid and synovium of persons with rheumatoid arthritis compared with levels in persons with osteoarthritis (36, 37). Therefore, IL-4/IL-13 might be responsible for angiogenic states in rheumatoid arthritis, possibly together with increased expression of sVCAM-1.

The conditioned media from activated macrophages or mast cells stimulate cell migration and tube formation by vascular endothelial cells in vitro (38, 39). Infiltration of activated macrophages is often associated with angiogenesis in tumors such as breast cancers, gliomas, and melanomas (27, 40–42). TNF-α and IL-1 are representative cytokines produced from monocytes and macrophages. TNF-α or IL-1 that shows angiogenic activity in vitro and in vivo also increases the production of the potent angiogenic factors, VEGF, bFGF, and IL-8, from vascular endothelial cells and tumor cells, indicating autocrine and/or paracrine control of angiogenesis (23, 26, 27, 43). However, vascular endothelial cells treated with IL-4 and IL-13 did not increase the production of the angiogenic factors, VEGF, bFGF, and IL-8. Angiogenesis caused by IL-4 and IL-13 appears to be specifically mediated by sVCAM-1 rather than by angiogenic factors such as VEGF, bFGF, and IL-8.

Increased expression of VCAM-1 by IL-4 and IL-13 in vascular endothelial cells is closely associated with adhesion of monocytes, lymphocytes, eosinophils, and basophils (44, 45, 46). IL-4 and IL-13 are chemotactic for monocytes (47) and also increase the expression of monocytic chemotactant protein-1 in vascular endothelial cells (14). Monocytes in blood thus should have an increased affinity to vascular endothelial cells, resulting in movement to the angiogenic foci, such as tumor or inflammatory loci, when IL-4 and IL-13 are present. In fact, a profound level of macrophage infiltration is observed in the IL-4-expressing tumors (48). In the angiogenic foci, infiltrating activated macrophages exert their angiogenic signaling by producing angiogenic factors and proteases (41, 42, 43, 49). Further study is needed if such an angiogenesis model together with blood cells is to be used for various diseases related to angiogenesis.

References


