Deep Vein Thrombosis Resolution Is Modulated by Monocyte CXCR2-Mediated Activity in a Mouse Model

Peter K. Henke, Andrea Varga, Sumit De, C. Barry Deatrick, Jonathon Eliason, Douglas A. Arenberg, Pasu Sukheepod, Porama Thanaporn, Steven L. Kunkel, Gilbert R. Upchurch, Jr, Thomas W. Wakefield

Objective—To determine the role of CXCR2, the receptor for cysteine-X-cysteine (CXC) chemokines, and its primary effector cell, the neutrophil (PMN), on deep venous thrombosis (DVT) resolution.

Methods and Results—DVT in BALB/c, anti-CXCR2 antibody-treated, and BALB/c CXCR2−/− mice were created by infrarenal inferior vena cava (IVC) ligation and the thrombus harvested at various time points over 21 days. The CXCR2−/− mice had significantly larger thrombi at early time points (days 2 to 8), and significantly decreased intrathrombus PMNs, monocytes, and neovascularization as compared with controls. Thrombus KC/CXCL1 was significantly higher at 2 days in CXCR2−/− mice, with significantly decreased 8 day MMP-2 activity, whereas MMP-9 activity was elevated as compared with controls. Similar impairment in DVT resolution was found at 8 days with anti-CXCR2 inhibition. However, systemic neutropenia, unlike CXCR2 deletion, did not increase the thrombus size as compared with controls.

Conclusions—Normal DVT resolution involves CXCR2-mediated neovascularization, collagen turnover, and fibrinolysis, and it is probably primarily monocyte-dependent. (Arterioscler Thromb Vasc Biol. 2004;24:1130-1137.)

Key Words: inflammation ■ chemokines ■ neutrophils ■ angiogenesis

Deep vein thrombosis (DVT) remains a significant clinical problem in hospitalized patients despite increased use of DVT prophylaxis.1 Although pulmonary embolism (PE) can be immediately fatal, chronic venous insufficiency (CVI) is more common and presents as limb swelling, pain, and often ulceration for which palliative therapy is primary. The morbidity that results from CVI is significant, with 30% to 67% of patients severely impaired 3 to 8 years after extensive iliofemoral DVT.2 Furthermore, CVI associated with an unresolved DVT is associated with more severe clinical symptoms than primary valve dysfunction.3,4 Current therapies limit the propagation of DVT, but the host must still resolve the thrombus to allow DVT recanalization, dissolution, and return of prograde blood-flow.

The relationship between inflammation and thrombosis has been strengthened over the past decade. Inflammation promotes thrombosis and vice versa.5,6 Antiinflammatory strategies such as gene-transferred or exogenous interleukin-10 are effective in limiting thrombus extent if given before or at the time of thrombosis.7,8 However, a proinflammatory environment is essential for many physiological processes such as wound healing.9–12 The presence of neutrophils (PMNs) within the early thrombus,13,14 the known inflammation surrounding the DVT,5–9 and the likeness to other proinflammatory environments, such as wound healing,11 suggest a central role of chemokines in this process. Cysteine-X-cysteine (CXC) chemokines mediate PMN activation and chemotaxis and are further subclassified by the presence or absence of an amino acid sequence preceding the cysteine motif, Glu-Leu-Arg (ELR+) or ELR−. This distinction is important as the ELR+ CXC chemokines are directly proangiogenic, mediated through the G protein-coupled CXCR2 receptor, and include KC/CXCL1 and MIP-2/CXCL2/3 in the mouse.15–17 In contrast, the ELR− CXC chemokines are angio-static, bind to CXCR3 receptors, and include IP-10/CXCL10.16,17 Thrombus resolution also involves neovascularization and fibrinolysis. Histological studies have confirmed the appearance of intrathrombus clefts and neovascular channels that evolve mostly within the first 4 weeks.9,18 Restoration of venous blood flow seems to involve true neovascularization with functional flow channels as well as thrombus retraction.9,10,14 In addition to CXC chemokine’s angiogenic activ-
ity, common angiogenic growth factors released by PMNs and monocytes known to be present in the resolving thrombus include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). 19–21 Fibrinolysis in venous thrombi is thought to be primarily mediated by urokinase plasminogen activator (uPA) from monocyte cells, 22 as evidenced from impaired thrombus resolution in mice lacking uPA but not tPA. 23 Linked to the plasminogen activators are the matrix metalloproteinases (MMP), particularly MMP-2 and MMP-9, which modulate collagen and matrix turnover in vessel wall remodeling and early wound healing. 24–26

On the basis of these background data, the role of CXCR2 in a mouse model of stasis DVT was investigated. Significantly impaired DVT resolution was observed in those mice without CXCR2 receptor activity and by antibody blockade. However, selective depletion of PMN did not impair DVT resolution to the same degree, suggesting that CXCR2-mediated activity on leukocytes other than PMN may be more important in DVT resolution.

Methods

Mouse Model

The rodent model of DVT has been well described. 6–10,14 BALB/c (controls) and BABL/c-Cmc92 (CXCR2–/–) (Jackson Labs, Bar Harbor, Me) mice, weighing 20 to 30 g and between 4 to 6 weeks in age were used. The mice underwent general inhalational anesthesia and via a laparotomy the infrarenal IVC was ligated with a 6-0 polypropylene suture. Mice were euthanized at 2, 4, 8, 12, and 21 days. In a separate group of experiments, a group of BALB/c mice received either intraperitoneal rat thrombosed inferior vena cava (IVC) was snap-frozen and stored at −80°C. The University of Michigan Committee on Use of Laboratory Animals approved this research protocol.

Histological Analysis/Immunohistochemical Staining/Neovascular Channel Quantification

Immunohistochemical staining was performed on the paraffin-embedded tissue sections (10 μm) as described 8,10,14 Anti-PMN (1:1000; Accurate Chemical and Scientific Corp, Westbury, NY), anti-F4/80 (1:100; Serotec, Oxford, UK), anti-laminin (1:100; Santa Cruz Biotechnology, Santa Cruz, Calif), anti-von Willebrand factor (vWF) (1:100; Serotec), Anti-uPA (1:100; ICN Biomedicals, Costa Mesa, Calif), or anti-CXCR2 (1:100; cordially provided by Dr Robert Strieter, UCLA) by intraperitoneal injection or goat nonspecific serum as control and were harvested at 8 days. 28 In brief, at harvest, the thrombus was separated from the vein wall and homogenized. The supernatant was assayed for myeloperoxidase (MPO) activity using a spectrophotometric reaction with o-dianisidine hydrochloride (Sigma Chemical Co, St. Louis, Mo) at 490 nm. The results were determined by observing the change in optical density (ΔOD) per minute and corrected to thrombus weight as described. 7

Chemokine/Cytokine/Vascular Growth Factor Enzyme-Linked Immunosorbent Assay

After thrombus–vein wall separation, the thrombus was placed in complete lysis buffer at 0°C (Boehringer Mannheim, Indianapolis, Ind), homogenized, sonicated for 10 seconds, centrifuged at 10 000 g for 5 minutes, and the supernatant collected. Quantification of peptide mediators was normalized to total protein in the sample. Total protein quantitation was performed by a modified Bradford assay per manufacturer’s instructions (Pierce Inc, Rockford, Ill) with serial dilutions of bovine serum albumin (BSA) (Sigma Chemical, St. Louis, Mo) as standards. Tissue homogenate enzyme-linked immunosorbent assays (ELISAs) for mouse MIP-2, KC, and IP-10 were performed with species-specific primary antibodies quantified using a double ligand technique, as has been described for similar chemokines. 6–10,28,29 Mouse VEGF and bFGF were analyzed by using a commercial ELISA according to the manufacturer’s instructions (R & D, Minneapolis, Minn).

Collagen Assay

Vein wall collagen content was estimated by a commercially available kit according to manufactures instructions (Bicolor LTD, Belfast, North Ireland) as described. 14 Collagen amount was then corrected to thrombus weight for each sample (μg/mL per mg thrombus).

Real-Time Polymerase Chain Reaction

Expression of uPA and β-actin mRNA was determined using quantitative polymerase-chain reaction as described. 30 The uPA and β-actin primer sequences were derived using primer premier software (Premier Biosoft International, Palo Alto, Calif) based on primary cDNA sequences from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). The uPA sense is: 5′ GCC TAC TAC AAT GCC CAC AGA 3′ and antisense: 5′ TGG AAC CCT TGT TGG TCT A 3′. The β-actin sense is: 5′ CCA TAA GCC CAA CCG TGA A 3′; β-Actin anti: 5′ GGT GAA GGT CTC AAA CAT GAT C 3′. For quantification of mRNA levels, ΔCt values were calculated by the formula ΔCt = Ct target gene − Ct β-actin. Expression of the target gene in ratio to β-actin expression was calculated by the formula: target gene expression/β-actin expression = 2ΔCt.

Gelatin SDS-PAGE Substrate Zymography

As described, 31 precast 10% SDS-polyacrylamide gels containing 1 mg/mL of gelatin were used to determine MMP-2 and MMP-9 activity (unless otherwise stated, all zymography supplies were from Novex, San Diego, Calif). Samples containing human recombinant MMP-2 and MMP-9 (Oncogene, Boston, Mass) were included as standards. Densitometry analysis was performed using a FOTO/Analyst CCD Camera (Fotodyne, Hartland, Wis) and GEL-Pro Analyzer software version 3.1 (Media Cybernetics, Silver Springs, Md) and normalized to thrombus weights (mg).

Endothelial Chemotaxis Assay

The assay is based on a gradient effect of the potential angiogenic substance through a membrane as described. 32,33 Briefly, human microvascular endothelial cells (HMEC-1) were placed into each of the bottom wells (165 μL) of a 12-well Boyden chamber (Neuroprobe, Gaithersburg, Md) and allowed to adhere for 1 hour. Chambers were then reinverted and the thrombus homogenate or control placed in the upper well (120 μL). Chambers were reincubated for 2 hours. Fixed cells that had migrated through the membrane were totaled in 5 hpf (200×) and expressed per mg thrombus.

Fibrin Content Determination

Fibrin staining with antihuman fibrin antibody (Dako A/S, Glostrup, Denmark), which cross-reacts with rat fibrin, was used for quantitative fibrin content analysis as described. 29

Myeloperoxidase Assay

In brief, at harvest, the thrombus was separated from the vein wall and homogenized. The supernatant was assayed for myeloperoxidase (MPO) activity using a spectrophotometric reaction with o-dianisidine hydrochloride (Sigma Chemical Co, St. Louis, Mo) at 490 nm. The results were determined by observing the change in optical density (ΔOD) per minute and corrected to thrombus weight as described. 7
Statistical Analysis
All data are represented as mean±SE. All experiments were repeated more than once to confirm reproducibility. Unpaired Student t test was used as appropriate for comparison between the groups at their same individual time points (Sigma Plot; SPSS Inc. Chicago, Ill), and P<0.05 was assigned significance.

Results

CXCR2 Antigen Is Expressed in the Thrombus and Localizes to Mononuclear Cells.
To determine where and what morphological cell type expressed the CXCR2 antigen, control mice thrombus histologic sections were stained with anti-CXCR2 antibody (Figure 1). From analysis of sections at an early time point (4 days, N=3), the antigen was radially distributed diffusely within the thrombus periphery, present in both cells identified morphologically as mononuclear and PMN and within the vein wall. The diffuse nature likely represents free CXCR2 antigen from PMN lysis. Later time-point thrombi (21 days, N=3) showed less intense staining and were identified with distinctly mononuclear type cells distributed throughout the thrombus. No significant staining was noted in the CXCR2−/− thrombus sections (data not shown).

Thrombus Size Is Increased and Monocyte and PMN Content Are Decreased in CXCR2−/− Mice
The thrombus size (weight/length) is a reproducible assessment of thrombus dissolution.6–10,14 The thrombi were grossly larger at harvest and more extensive in the CXCR2−/− mice as compared with control mice. This difference was statistically significant in 2-, 4-, and 8-day-old thrombi with a 20%, 17%, and 19% increase in CXCR2−/− thrombus size was found as compared with controls, respectively. No significant differences were found at later time points (N=11 to 13, P<0.05) (Figure 2a).

Intrathrombus PMNs were significantly fewer in CXCR2−/− mice as compared with controls at 2 and 4 days

Figure 1. A, Immunohistologic CXCR2+/+ mouse 4-day thrombus (T) section stained with anti-CXCR2 antibody, 200×. Note the stain is present diffusely in the periphery of the thrombus. T=thrombus, W=vein wall. B, A thrombus section of a 2-day-old thrombus with anti-CXCR2 antibody, 1000×. Note stain is associated with both mononuclear cells (arrow) and PMNs (double arrow). C, A thrombus section of a 21-day thrombus, 100×. CXCR2 antigen is associated with distinct mononuclear cells (arrow). D, A thrombus section, 2-day, representative of negative control IgG antibody showing no appreciable staining.

Figure 2. A, Thrombus weight to length ratios (mg/cm) was greater at early time points in CXCR2−/− thrombi as compared with wild-type (WT) controls. A significant difference was observed at days 2, 4, and 8 (N=11 to 13, *P<0.05 by t test). B, Thrombus PMN were assessed by immunohistochemistry and were significantly fewer in 2- and 4-day CXCR2−/− thrombi as compared with WT controls. No significant difference in PMN number was found at day 8 (N=4 to 5, *P<0.01). C, To corroborate what was observed immunohistochemically, MPO activity per mg thrombus was assessed. Significantly less MPO activity was observed in 2- and 4-day thrombi (N=3 to 4, *P<0.05). D, Monocytes (F4/80 positive staining) were significantly less at all time points in CXCR2−/− as compared with WT controls (N=4 to 5, *P<0.01).
were significantly less at all time points in CXCR2−/− mice as compared with controls (N=4 to 5; P<0.05; data not shown). After day 8, neovascular channels stained positive for vWF in the thrombus and appeared larger and more well-formed than the laminin channels consistent with mature endothelial cells. These channels were primarily located in the peripheral but not the central portion of the thrombus. Positive vWF channels increased from day 8 through 21 in the control mice, whereas significantly fewer channels with no linear increase over time were observed in the CXCR2−/− mice (N=4 to 5; P<0.05) (Figure 3a).

To further corroborate our immunohistologic neovascularization findings, a separate group of control and CXCR2−/− mice had their thrombus homogenized and assessed for angiogenic chemotactic activity. Endothelial chemotactic activity has been shown to correlate with in vivo angiogenic activity.17,32,33 No significant amount of activity was able to be measured before 8 days. A significantly reduced amount of angiogenic activity in the CXCR2−/− mice as compared with controls was found at 8 through 21 days (N=5; P<0.05) (Figure 3b). A steady linear increase in thrombus endothelial chemotactic activity per mg thrombus was observed over time in the control mice, but not in CXCR2−/− mice, and mirrored what was observed immunohistologically.

**bFGF Is Decreased and KC/CXCL1 Is Elevated in CXCR2−/− Thrombi**

Given that thrombus neovascularization was impaired in CXCR2−/− mice, 2 common angiogenic growth factors, and were significantly less at all time points in the CXCR2−/− mice as compared with controls (N=4 to 5; P<0.05; data not shown).

Similar to what was observed with the intrathrombus PMNs, thrombus monocytes (F4/80 positive staining cells) were significantly less at all time points in CXCR2−/− mice as compared with controls (N=4 to 5; P<0.05) (Figure 2d).

**CXCR2−/− Mice Have Less Thrombus Neovascularization**

Thrombus neovascularization increases as the thrombus ages and resolves.9,10,18,21 In the current study, thrombus neovascularization was assessed by 2 separate methods. Previous work with FITC-dextran and colloidal carbon intravenous injection followed by histological analysis confirmed that channels identified by immunohistological staining communicate with the systemic venous circulation.9,10

Laminin is a basement membrane protein associated with early endothelial cells and angiogenic precursors.34 Laminin-positive channels appeared in thrombus sections after 4 days and were significantly less at all time points in the CXCR2−/− mice as compared with controls (N=4 to 5; P<0.05; data not shown). After day 8, neovascular channels stained positive for vWF in the thrombus and appeared larger and more well-formed than the laminin channels consistent with mature endothelial cells. These channels were primarily located in the peripheral but not the central portion of the thrombus. Positive vWF channels increased from day 8 through 21 in the control mice, whereas significantly fewer channels with no linear increase over time were observed in the CXCR2−/− mice (N=4 to 5; P<0.05) (Figure 3a).

To further corroborate our immunohistologic neovascularization findings, a separate group of control and CXCR2−/− mice had their thrombus homogenized and assessed for angiogenic chemotactic activity. Endothelial chemotactic activity has been shown to correlate with in vivo angiogenic activity.17,32,33 No significant amount of activity was able to be measured before 8 days. A significantly reduced amount of angiogenic activity in the CXCR2−/− mice as compared with controls was found at 8 through 21 days (N=5; P<0.05) (Figure 3b). A steady linear increase in thrombus endothelial chemotactic activity per mg thrombus was observed over time in the control mice, but not in CXCR2−/− mice, and mirrored what was observed immunohistologically.

**bFGF Is Decreased and KC/CXCL1 Is Elevated in CXCR2−/− Thrombi**

Given that thrombus neovascularization was impaired in CXCR2−/− mice, 2 common angiogenic growth factors,
VEGF and bFGF, were analyzed by ELISA. These angiogenic growth factors have been documented to be present in resolving thrombi in rats.  

No significant difference in VEGF levels in CXCR2−/− mice as compared with controls was demonstrated. In contrast, there was an increasing trend in bFGF from day 4 to day 21 in control mice but was not observed in the CXCR2−/− mice, and the difference achieved significance at day 21 (control 981±246 versus CXCR2−/− 228±96 pg/mg protein, N=5 to 6; P=0.03).

Significantly increased KC in the CXCR2−/− thrombi as compared with controls was observed in 2-day-old thrombi (control 114±19 versus 261±43 pg/mg protein, N=4 to 5; P=0.005) with a later trend of elevated levels through 21 days (days 8 to 21; N=4 to 5; P=0.07). The chemokines MIP-2/CXCL2/3 and IP-10/CXCL10 were not significantly different between the controls as compared with the CXCR2−/− mice (data not shown).

**CXCR2−/− Mice Have Increased Fibrin Content and Decreased uPA Gene Expression**

Total fibrin content as measured by staining intensity has been correlated with thrombus size and indirectly to dissolution in a rat model of DVT.  

Early time point analysis (days 2 to 4) showed no significant difference in fibrin content. However, later time point analysis showed 8 day CXCR2−/− thrombi had 17% greater intensity fibrin staining than controls (control 1.41±0.05 versus CXCR2−/− 1.53±0.11 I/I° ratio, P<0.05, N=5) and 20% greater fibrin staining intensity at day 21 (control 0.61±0.05 versus CXCR2−/− 0.76±0.03, P<0.05, N=5). As other investigators have shown, uPA is probably the primary fibrinolytic mechanism for DVT resolution.  

Staining for uPA was performed. Earlier time-point staining showed diffuse antigenic thrombus staining, suggesting uPA’s extracellular and matrix associated location in the process of DVT resolution. Less positive uPA staining was present in the CXCR2−/− thrombi after 8 days, and although this did not reach statistical significance, the difference was greatest in 21-day-old thrombi (controls 19±2 versus CXCR2−/− 14±3, cells/5 hpf, N=3 to 4; P=0.14). As the transition point between early and later thrombus maturation seemed to be at the 4 and 8 time points, uPA gene expression was analyzed. Significantly less uPA expression was observed in the CXCR2−/− rat thrombi at 4 days (ΔCt: control 183±29 versus CXCR2−/− 0.00±0.00; ×10−6, N=2 to 4; P<0.05) with similar expression at 8 days (ΔCt: control 293±60 versus CXCR2−/− 295±58; ×10−6, N=3 to 4; P=NS) as compared with control. Good β-actin peaks were observed in all samples.

**CXCR2−/− Mice Have Impaired Thrombus Fibrotic Maturation and Altered MMP Activity**

To assess if the altered DVT resolution in the CXCR2−/− mice affected the normal thrombus fibrotic process, total thrombus collagen was determined. In control mice, the greatest amount of collagen per mg of thrombus was at day 2, with a linear decreasing trend through day 21 (Figure 4a). However, collagen metabolism was altered in the CXCR2−/− mice, with 2-fold less present in 2-day-old thrombi as compared with controls, but a significantly greater amount at day 21 (N=2 to 4 pooled thrombi; P<0.01). Trichrome staining showed the fibrotic process to be concentrated in the thrombus periphery at early time points (days 2 and 4) and more diffusely distributed by day 21 (Figure 4c and 4d).

Collagen turnover in acute injury is mediated by collagenases. MMP-2 and MMP-9 are best described in acute wounds as well as being critical for angiogenesis.  

The 8-day time point thrombi was chosen, because this is when the transition between PMN and monocyte predominance occurs and most MMPs are produced by monocytes. Interestingly, MMP-2 was >8-fold less in the CXCR2−/− thrombi as compared with control (82±58 versus 657±187 U activity/mg thrombus, P=0.05, N=3 to 5) (Figure 4b). Con-
versely, MMP-9 activity was 1.7-fold greater in CXCR2−/− thrombi as compared with control (7717±700 versus 4515±155 U activity/mg thrombus, P=0.002, N=3 to 5).

**Antibody Inhibition of CXCR2 Impairs DVT Resolution**

To confirm the impaired DVT resolution that was suggested by deletion of CXCR2−/− activity, direct antibody inhibition of CXCR2 was performed in the same stasis DVT model. At 8 days, PMNs were low in both groups, but F4/80 cells were significantly reduced in the anti-CXCR2 group compared with controls (Figure 5). Similarly, vWF positive neovascular channels were significantly fewer in anti-CXCR2 group with a reduction similar to the CXCR2−/− mice (Figure 3). Larger thrombus size was observed, with a 20% increase in the anti-CXCR2 group (P=0.06, N=8).

**Neutrophil Depletion Does Not Impair DVT Resolution**

Neutrophils are the primary effector cells for CXC chemokines. To determine whether the observed impairment in DVT resolution was primarily caused by CXCR2 mediated activity on multiple leukocyte types or the PMN itself, a PMN antibody depletion strategy was used. In those mice receiving the anti-PMN antibody, a mean PMN decrease of 72% at 2 days after thrombus development was documented (P=0.002, N=4 to 5). Similarly, a significantly reduced number of PMN was observed in the thrombus of treated mice (N=2 to 4 sections, P<0.05) (Figure 6). However, unlike the CXCR2−/− mice, no significant difference in thrombus weights at 2, 4, or 8 days was found (N=4). Furthermore, no significant difference in F4/80 positive monocytes was found in the 2-, 4-, or 8-day-old thrombi (N=2 to 4 sections). Neovascularization, as assessed by laminin positive channels, was 4-fold less in 2-day-old thrombi in mice depleted of PMNs (N=3, P=0.001) but was not significantly different in 4- or 8-day thrombi as compared with controls.

**Discussion**

DVT resolution involves dissolution of the thrombus matrix by fibrinolysis, cellular influx, and neovascularization. By these parameters, DVT resolution was impaired in those mice who were CXCR2-deficient by 2 different experimental strategies and suggests that CXCR2 mediates, in part, both leukocyte neovascular and fibrinolytic activity. These findings are consistent with other inflammation models in which CXCR2 activity was impaired. For example, in a murine model of wound healing, CXCR2 deletion was associated with significantly fewer intrawound PMNs, decreased neovascularization, and impaired wound strength. Similarly, in a murine model of cerebral abscess, significantly greater bacterial counts, less PMNs, and higher mortality was observed in mice lacking CXCR2. Moreover, the data suggest that KC/CXCL1 may be the primary chemokine modulator in postsurgical wound healing, infection, and barotrauma.

The CXCR2-dependent resolution actions may be independent of the CXCR2 primary effector leukocyte, the PMN. Monocytes are known to express CXCR2 receptors, respond to CXC chemokines, and were significantly fewer in both CXCR2−/− and anti-CXCR2 groups, as compared with controls. Indeed, CXCR2-mediated activity in monocytes seems to be localization and activation at a specific inflammatory nidus (as shown in animal models of nephritis and atherosclerosis) that is not compensated for by CC chemokine redundancy. The lack of dependence on PMN in mediating early DVT resolution was surprising given that in a similar rat model of DVT in which PMN depletion was performed, larger thrombi at days 2 and 7, but not day 4, were found. However, this difference may be accounted for by the slightly different surgical techniques, rat-versus-mouse genetics, the physiological age of the thrombus (ie, older in the mouse as compared with the rat at the same harvest time points), and/or the intravenous versus intraperito-
However, given the lack of larger thrombi in PMN-depleted mice, it may be that the early contribution of PMNs is minor in established DVT. It is more likely that the monocyte is the primary leukocyte responsible for DVT dissolution, probably through uPA activity. From our data, we speculate that CXCR2 directs monocytes to the thrombus site and/or activates the monocytes to release uPA, although the later action may be more important as the absolute numbers of uPA staining cells was not significantly different between the groups. Our data suggest uPA gene expression is impaired early in CXCR2−/− mice and may explain the later time point increased fibrin content (days 8 and 21). Also, it is very likely that the vein wall plays a role in thrombus dissolution because uPA is known to be expressed on endothelium as well as similar late thrombus sizes and a nonsignificant decrease in uPA staining in CXCR2−/− mice were observed.

Figure 6. PMN depletion failed to impair DVT resolution. A, Thrombus PMNs were significantly less in those mice receiving anti-PMN serum as compared with control antibody (N=3 to 4, *P<0.05). B, Thrombus size (weight/length) in mice treated with anti-PMN antibody as compared with antibody controls. Note no significant difference in thrombus size at any time point (N=4 to 5). C, Neovascularization was assessed by laminin-positive channel staining. Significantly fewer channels were found in PMN-depleted mice at 2 days only, with no difference thereafter (N=3 to 4, *P<0.01).

Thrombus neovascularization was significantly reduced in the CXCR2−/− as well as anti-CXCR2–treated mice thrombi. However, no difference in thrombus size was noted at the later time points. This suggests that thrombus neovascularization is probably more important for DVT maturation, matrix remodeling, and allowing functional inroads for leukocytes and other cells rather than directly contributing to thrombus dissolution or conveying significant blood flow. It is suggested from this study that PMNs are important for early (day 2), but not later, neovascularization (after day 4), possibly by angiogenic growth factor production. However, monocytes synthesize most angiogenic factors, such as VEGF and bFGF, which predominate in maturing thrombi. It cannot be ruled out from this study that the angiogenic growth factors may come from the vein wall itself, because endothelial cells and smooth muscle cells synthesize bFGF and VEGF. Unlike other experimental systems, the CXC chemokines do not seem to have proangiogenic activity independent of leukocytes in resolving DVT. For example, although KC/CXCL1 was elevated in CXCR2−/− thrombi, no increase in thrombus neovascularization was observed, neither immunohistologically nor by thrombus angiogenic potential. However, other cofactors that allow primary chemokine angiogenic activity may not be present in the thrombus or unopposed IP-10 angiostatic activity may have predominated.

Fibrosis is an essential process of wound healing, is a dynamic process, and involves chemokines and neovascularization. Interestingly, collagen content is most extensive in the early thrombi and probably represents acute wound type III collagen. A greater amount of thrombus collagen in 21-day CXCR2−/− thrombi is consistent with the notion that CXCR2 plays a role in late collagen turnover, possibly through altered MMP production. CXCR2−/− thrombi had significantly less MMP-2 activity but increased MMP-9 activity at 8 days. Although both MMPs have similar substrates, MMP-2 may be more important for neovascularization and activation of growth factors, whereas MMP-9 has a greater role in collagen turnover. For example, MMP-9−/− deficient mice have less cardiac fibrosis after injury. The converse of an increase in MMP-9 as that was observed in the CXCR2−/− thrombi is consistent with this mechanism. Impaired thrombus fibrinolysis, as shown in the CXCR2−/− mice, also contributes to organ fibrosis. Similarly, uPA activates MMP-2 and MMP-9, both of which are involved with fibrin invasive activities of neovascularization and collagen turnover.
The findings presented in this study serve to underscore the complex process of DVT resolution and that a proinflammatory environment, conferred in part by CXCR2, is essential for normal DVT resolution. The apparent dissociation between increased CXCR2+/− thrombus size at early time points and later impaired CXCR2+/− thrombus matrix remodeling is an area that bears further investigation with in vitro experiments toward the ultimate goal of molecular targeting to accelerate DVT resolution without the hazards of anticoagulation.

References

Deep Vein Thrombosis Resolution Is Modulated by Monocyte CXCR2-Mediated Activity in a Mouse Model

Peter K. Henke, Andrea Varga, Sumit De, C. Barry Deatrick, Jonathon Eliason, Douglas A. Arenberg, Pasu Sukheepod, Porama Thanaporn, Steven L. Kunkel, Gilbert R. Upchurch, Jr and Thomas W. Wakefield

Arterioscler Thromb Vasc Biol. 2004;24:1130-1137; originally published online April 22, 2004; doi: 10.1161/01.ATV.0000129537.72553.73

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/24/6/1130

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/