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REVIEW ARTICLE

Immune cell–mediated venous thrombus resolution

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Abstract

Herein, we review the current processes that govern experimental deep vein thrombus (DVT) resolution. How the human DVT resolves at the molecular and cellular level is not well known due to limited specimen availability. Experimentally, the thrombus resolution resembles wound healing, with early neutrophil-mediated actions followed by monocyte/macrophage–mediated events, including neovascularization, fibrinolysis, and eventually collagen replacement. Potential therapeutic targets are described, and coupling with site-directed approaches to mitigate off-target effects is the long-term goal. Similarly, timing of adjunctive agents to accelerate DVT resolution is an area that is only starting to be considered. There is much critical research that is needed in this area.

KEYWORDS
deep vein thrombosis, fibrinolysis, inflammation, leukocytes, venous thrombosis

Essentials

- How a deep vein thrombus resolves is a complex and understudied process.
- Immune cells mediate the resolution process in a coordinated and time-dependent fashion.
- Fibrinolysis, neovascularization, and retraction are all potentially modifiable processes.
- Targeted therapies to promote venous thrombus resolution without anticoagulation are needed.

1 | INTRODUCTION

Venous thromboembolism (VTE) is currently one of the largest sources of morbidity and mortality, comparable in scope to Alzheimer's disease and diabetes [1]. This disease encompasses both deep vein thrombosis (DVT) and the sequela, including pulmonary embolism (PE) and postthrombotic syndrome (PTS). Recent studies suggest the age- and sex-adjusted incidence of VTE to be 1.22 to 2.39 per 1000 patient-years, rising to 5 to 11 per 1000 patient-years by the eighth decade of life [2–7]. Of these patients with VTE, 27% to 56% present with PE, putting them at imminent risk of death, while roughly another 20% to 50% of patients with VTE later develop PTS [8], which reduces quality of life through loss in mobility, leg swelling, and skin ulcers.

Current treatment for VTE is primary anticoagulation, but bleeding risks remain, even with safer anticoagulants [9,10]. Anticoagulation serves to prevent DVT extension and embolization. As the thrombus evolves from an acute fibrin-rich structure to a chronic collagen-rich structure, it induces inflammation, resulting in valvular damage. In fact, it is thought that the DVT may develop in valve pockets [11,12], with thrombus as propagation and subsequent valve damage.

Venous thrombi form and resolve in a fairly well-characterized process, and are similar to those in humans with extrinsic and intrinsic coagulation pathways, which is reviewed in detail elsewhere [13].

Residual chronic thrombus obstructs the return of blood. Both of these phenomena culminate in venous hypertension, the primary

mechanism of PTS. While mechanical and pharmacologic clot–debulking therapies exist, they come at the risk of additional bleeding and do not address valvular damage, and in fact, they may not be all that effective [14]. Therefore, a major gap exists for therapies that can accelerate DVT resolution without impairing hemostasis and potentially decrease PTS.

2 | INSIGHTS FROM MURINE MODELS AND DIFFERENCES FROM HUMAN DVT

Due to the difficulty of examining human thrombus and vein specimens, several experimental murine models of DVT are commonly used [15,16]. The 2 most common models involve complete ligation (stasis) or partial ligation (stenosis) of the mouse inferior vena cava (IVC). While the ligation model yields more consistent thrombi, partial stenosis is believed to more closely mimic the hemodynamic environment of venous valve pockets where most DVTs initiate [11]. Other models include electrolytic injury and laser induced injury. Both human and murine DVTs develop from masses of erythrocytes and platelets, enmeshed with fibrin, and finally to collagen; changes directed primarily by various leukocytes (neutrophils and macrophages) [17].

There are several critical differences between human DVT and murine DVT models. First, the hematological parameters vary between humans and mice [18]. For example, mice have smaller erythrocytes, more lymphocytes, and fewer neutrophils, as well as shorter lived platelets. The basic coagulation pathways are the same as humans. Second, DVT resolution in the mouse is typically measured by directly assessing the physical and histological characteristics of the thrombosed IVC, which are taken as surrogates for the clinically relevant outcomes of DVT and PTS, and does not recapitulate the primary occurrence. Second, the surgical creation of these experimental models may mimic DVT as a complication of major surgery [19], but likely produces a different immune response when compared to those induced by immobility or hypercoagubility [20,21]. Third, there is no component of venous hypertension in these mouse models due to nonbipedal position of rodents, although it can be simulated by creation of an arteriovenous fistula [22]. Lastly, there are significant biochemical and cellular differences in the innate immune system of mice compared to humans [23,24]. For example, as compared with humans, the mouse has a higher lymphocyte–neutrophil (PMN) ratio than humans [25] and altered circadian rhythms [26].

3 | FIBRINOLYSIS

Fibrin breakdown begins soon after DVT development, depending on experimental conditions. In mice, roughly 55% of clot area is observed to be fibrin and 35% to be collagen (around the periphery) prior to 2 weeks postligation [27]. By week 4, only 20% of the thrombus area is fibrin, with collagen comprising the rest [28]. Indeed, fibrin content can be quantified to better define venous thrombus (VT) amenable to lysis by Gd-based magnetic resonance imaging (MRI) [27].

The liver-secreted zymogen plasminogen, following activation and conversion to plasmin, degrades fibrin [29] into an E-fragment and D-dimer [30]. Tissue plasminogen activator (tPA), retained on the surface of endothelial cells [31], appears essential to typical thrombus resolution, as evidenced by both blood values in the context of DVT and genetics [32–34]. Urokinase-type plasminogen activator (uPA) is efficient at resolving thrombi in murine models [35,36], possibly because it is secreted by both endothelial cells and monocytes as they infiltrate the thrombus and differentiate into macrophages [37,38], which in turn promotes additional circulating monocyte recruitment as the thrombus matures [39,40].

Several proteins can also inhibit the fibrinolytic activity of plasmin directly or indirectly. Within circulating blood, α_2-antiplasmin (α2AP) rapidly binds to plasmin, thereby halting fibrinolysis [41,42]. Deficiencies in α2AP are linked to rare bleeding disorders and increased mortality in models of PE [43,44]. Thrombin-activable fibrinolysis inhibitor (TAFI) operates primarily by removing the C-terminal lysine from fibrin polymers, thereby preventing several amplification mechanisms of fibrinolysis that occur when plasminogen binds to fibrin [34,45,46]. It may also act in a profibrotic way during PE resolution experimentally [47]. Factor XIII, which crosslinks fibrin to render it more stable [48], also crosslinks α2AP to fibrin, where α2AP prevents fibrinolysis by serving as a shield against plasmin [49]. FXIII is also critical for thrombogenesis and resolution, maintaining the thrombus consistency and ultimate contraction [50].

Plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis by cleaving tPA and uPA via the serine protease mechanism [51]. PAI-1 gene expression is strongly linked to VTE within large genome-wide association studies, and mice overexpressing PAI-1 form 50% larger thrombus than mice with PAI-1 deficiency [52–54]. Gene deletion of PAI-1's cofactor vitronectin, an extracellular matrix glycoprotein that stabilizes PAI-1, also results in impaired thrombus resolution [53]. However, animal studies have been mixed as evidenced by dose dependence of tiplaxtinin, a PAI-1 inhibitor [55–57].

PAI-2 also cleaves tPA and uPA, but much more slowly than PAI-1 [58]. Experimental models of PAI-2 deficiency exhibit accelerated DVT resolution without significantly affecting fibrinolysis [59,60]. PAI-2 is primarily retained intercellularly but can be abundantly secreted by proinflammatory macrophages [61,62] and acts to regulate many aspects of circulating monocyte and macrophage behavior [63,64]. Within apolipoprotein E gene–deleted mice, hyperlipidemia resulted in a significantly elevated level of PAI-1 and an undetectable uPA concentration and was associated with impaired VT resolution [65]. Rosuvastatin can reverse these effects in mice and with potential benefit in reduced incident VTE in patients [66,67]. Certainly, enhancing the thrombus lysis most effectively with low bleeding risk is the overarching goal.

4 | COLLAGENOLYSIS

Fibrotic damage to the vein wall seems to drive the development of PTS [68]. Venous segments measured by duplex ultrasound from

patients with chronic PTS are over twice as thick as those in comparable controls [69], and some inflammatory markers are predictors of PTS such as intracellular adhesion molecules-1 (ICAM-1), interleukin-6 (IL-6), and IL-10 [70,71]. The thrombus as it matures creates 3 mechanically distinct vein wall surfaces: the entrapped wall (through which immune cells migrate to reach the thrombus), the fresh/intra-thrombus wall, and the remaining sections of original vascular wall [72]. Collagen is deposited both within the remodeling thrombus and the vein wall, peaking at 12 days in a mouse model [73]. In human postthrombotic femoral vein sections, this material is >80% type I collagen, with the remainder consisting of type III collagen [74].

Extensive collagen turnover occurs within the resolving VT, with type III early and later type I [72]. Collagenolysis is carried out interstitially by matrix metalloproteases (MMPs) and neutrophil elastase [75]. Neutrophil elastase and MMP2 appear ineffective toward type III collagen [76,77], while MMP9 can digest both type I and type III collagen [78]. Within patients with DVT, most of the MMP enzymes appear elevated (with the exception of MMP3) [79]. Mouse models recapitulate this elevation, and further suggest that while MMP9 is elevated throughout DVT remodeling, MMP2 and membrane type-1 MMP are elevated primarily at later stages [73]. The decreasing ratio of MMP9/MMP2 can be used to estimate thrombus age [80].

In contrast to fibrinolysis, paradoxically, inhibition of collagenolysis yields less collagen at late DVT resolution. Mice with global MMP2$^{-/-}$ demonstrate larger venous thrombi, decreased vein wall collagen, greater monocyte influx, and fewer von Willebrand's factor (vWF)–tagged neovascular channels [81]. MMP9 genetic knockout mice also display impaired thrombus resolution at 21-day time point, with vein walls that are significantly stiffer, likely due to the biomechanical components of both the extracellular matrix and collagen/elastin [82].

MMP2 transcription is dependent on the presence of the intracellular tumor suppressor p53 [83]. Expression of p53 stimulates PAI-1 secretion in endothelial cells (among other genes in endothelial cells). The effect of reducing fibrinolysis outweighs that of increasing collagenolysis because overexpression of p53 increases thrombus size [84] and, in aged mice, an endothelial knockout of p53 protects against DVT [85]. However, genetic or pharmacologic inhibition of p53 impairs DVT resolution in younger adult mice, whereas the p53 agonist quinacrine accelerates resolution [86]. The direct mediators of both collagen synthesis and breakdown are thus an area of active investigation.

5 | NEUTROPHILS AND EARLY THROMBOGENESIS

Although the focus of this review is on VT resolution, the early polymorphonuclear leukocytes' (neutrophils, PMN) actions set the stage for later VT resolution mechanisms. PMNs are among the first immune cells to infiltrate the thrombus, initially outnumbering Mo/MΦ cells at a ratio of 7:1 at 24 hours and then steadily decreasing in number by roughly 50% each week [87]. PMNs possess the ability to degrade fibrinogen internally, potentially giving them the ability to infiltrate the forming thrombus [88]. For example, neutropenia in rats yields larger thrombi and increased collagen deposition [89]. Similarly, in humans, a decreased PMN to monocyte ratio confers an increased risk for DVT [90].

An example of a prothrombotic PMN mechanism is NETosis; neutrophil extracellular traps (NETs), or chromatin released by self-destructing PMN [91]. This fine-webbing also acts as a scaffold onto which erythrocytes and platelets can aggregate, thereby contributing to VT formation [92,93]. Among other things, activated platelets expressing High Mobility Group Box 1 (HMGB1) trigger the immune responses of PMN recruitment to the site of a developing thrombus, NET formation, and monocyte infiltration [94,95]. P-selectin is also a driver of NETs and is present in early murine VT [96,97]. Immunoglobin from patients with antiphospholipid syndrome also triggers NETosis and amplifies thrombosis [98]. These physical matrix structures may be important for later immune cell responses and direct how the VT resolves.

NET-related extracellular DNA is recognized by the toll-like receptor 9 (TLR9) on both monocytes and PMNs [99,100], and a mutation in this receptor has been linked to VTE in patients [101,102]. TLR9$^{-/-}$ mouse VT models have demonstrated larger VT, decreased collagen and fibrin deposition, and an increase in PMNs at 2 days and Mo/MΦ cells after 1 week [103]. VT resolution can be restored by transferring the TLR9$^{+/+}$ bone marrow–derived monocytes into a TLR9$^{-/-}$ mouse, although this does not rescue vein wall fibrosis [104]. This suggests that TLR9 primarily allows Mo/MΦ cells to recognize the products of NETs and then dispose of them. Consistently, citrullinated histones, a marker of NETs, are elevated in TLR9$^{-/-}$ mice [105]. Moreover, PMN depletion inhibits thrombogenesis in TLR9$^{-/-}$ mice, although these thrombi are still larger than those in wild type mice. Investigation of COVID-19–related VT has also shown that autoantibodies to NETs in patients may contribute to thrombogenesis [106].

6 | MONOCYTES/MACROPHAGES AND THROMBUS MATURATION

Monocyte-derived macrophages (Mo/MΦ) are the primary effectors of immune-directed VT resolution with infiltration of the VT over the first week [17,48,107,108]. Interestingly, elevated circulating monocyte counts are linked to nearly 3-fold higher risks of DVT, but whether this translates to increased or decreased Mo/MΦ in human DVT is unclear [109].

Activation patterns for Mo/MΦ cell phenotypes are now designated proinflammatory and proresolving [110–112]. However, significant complexity arises from differential expression of surface markers *in vivo* and *in vitro* exposure to interferon gamma (IFNγ) or lipopolysaccharide (LPS) [112,113]. In general, proinflammatory Mo/MΦ cells are functionally specialized to kill pathogenic or cancerous cells via phagocytosis or reactive oxygen and nitrogen species with subsequent inflammatory damage [114,115].

The source of thrombus Mo/MΦ cells remain uncertain [17], and bone-marrow–derived endothelial progenitors with monocyte

features are present in the developing VT [116]. Additionally, tissue-resident macrophages may be able to self-renew, and may behave differently than influxing Mo/MΦ cells [17,117]. Proinflammatory Mo/MΦ cells tend to be short-lived and are rapidly recruited to sites of inflammation, whereas proresolving Mo/MΦ cells may arise from the proinflammatory monocytes and patrol noninflamed tissues over prolonged periods [118–121]. Subpopulations of monocytes can rapidly cocoon themselves with fibrin deposits [122] and crosslink that fibrin by secreting FXIII.

In the context of pathologic DVT in patients, there is a polarization toward proinflammatory Mo/MØ as sampled from the blood [123]. These proinflammatory human Mo/MΦ cells are characterized by increased IL-6, TNFα, and cellular adhesion marker, such as ICAM-1 expression. Multiple other markers for proinflammatory Mo/MΦ cells are described and differ between humans and mice [111,112,124]. For example, 95% of circulating human monocytes are proinflammatory as identified by $CD16^{-}$, compared to 50% of mouse monocytes that are proinflammatory as identified by $Ly6C^{hi}$ [125].

Proresolving Mo/MΦ cells also directly contribute to the process of DVT resolution through a variety of mechanisms. They phagocytize erythrocytes, platelets, matrix debris, and other cellular remains [94,126–128]. They also process the thrombus iron, as measured by MRI [129]. Proresolving Mo/MΦ cells directly express fibrinolytic and collagenolytic enzymes that allow them to invade the thrombus tissue, particularly uPA and MMP9 [37,130–132]. After proresolving Mo/MΦ cells burrow tunnels through fibrin and collagen, it has been shown in other disease models that fibroblasts backfill these paths with collagen [130,133]. Neovascularization or angiogenesis is also promoted by proresolving Mo/MΦ cells [134–136], and although evidence on whether neovascularization directly speeds up DVT resolution is mixed [137,138]; this phenomenon is present in PTS in humans [74].

Mo/MΦ likely are the main mediator cells of VT resolution. Although the monocyte phenotypes were not defined, peritoneal macrophage injection decreased experimental thrombus size by 5-fold and exogenous monocyte chemotactic protein-1 (MCP-1) decreased size by 6-fold [139], a strong effect. Directly depleting mice of $LysM^{+}$ cells improves VT resolution in mice, and knocking out the transcription factor T-bet (linked to M1 activation) has the same effect [140]. IFNγ-deficient mice displayed a phenotype characteristic of proresolving Mo/MØ activation, with enhanced MM9 and VGEF expression [141]. Consistently, depletion of proresolving Mo/MΦ cells significantly impairs DVT resolution [142].

Recent data suggest that directly stimulating the Mo/MΦ Nr4a1 pathway with cytosporin B (CsnB) can both preemptively and post-thrombogenesis accelerate VT resolution, as defined by duplex ultrasonography in a stenosis model of VT [143]. Similarly, a proinflammation resolution pathway can affect VT resolution. Resolvin D4 reduces PMN infiltration and antagonizes NET formation, in addition to recruiting proresolving monocytes and reducing the thrombus size [144]. Thus, directly modulating the many Mo/MØ functions to accelerate VT resolution is ripe for further investigation.

7 | THROMBUS CYTOKINES AND THROMBUS RESOLUTION

A recent review of cytokine and chemokine in VT has been published and is reviewed elsewhere [145]. However, 3 primary examples are presented. In patients, elevated circulating IL-6 level correlates with incident DVT [71,146]. In mice, IL-6 contributes to thrombogenesis [147,148] and is expressed on both proinflammatory and proresolving Mo/MΦs [111,149,150]. This may partly explain the lack of a significant difference in early thrombus size between control and $IL\text{-}6^{-/-}$ mice [151], as both inflammatory and prohealing Mo/MΦ functions are impaired. Mice treated with anti-IL-6 antibodies have decreased Mo/MΦ influx and accelerated DVT resolution, which is tied to reduced cysteine–cysteine ligand-2 expression, the primary chemoattractant for Mo/MØ [152].

As stated, the proresolving Mo/MΦ drives VT resolution [153]. Consistently, exogenous administration of IL-10, an anti-inflammatory cytokine, accelerates VT resolution by decreasing general and leukocyte influx inflammation [154]. In a rat model of stasis VT, local expression of viral IL-10 was associated with significant reduction in inflammation [138]. As a reflection of the activity of proresolving Mo/MΦ cells, IL-10 levels are significantly elevated in patients with DVT and PTS, but in nonthrombotic patients, high IL-10 levels appear protective [71,146]. Hypoxia-inducible factor-1 alpha (HIF1-α) is present in the local venous environment due to hypoxia. HIF-1α agonism accelerates VT resolution, with increased local leukocyte counts [155].

8 | LYMPHOCYTES CAN DIRECT MONOCYTE/MACROPHAGE ACTIONS IN THROMBUS RESOLUTION

Other immune cells may modulate the proinflammatory/proresolving Mo/MΦ polarization in the context of resolving DVT [115]. For instance, T-lymphocytes appear to infiltrate the thrombus early in its development [87], and the largely effector-memory T-cells (Tem) produce IFNγ without antigen stimulation, thereby triggering early inflammation [156]. However, if the T-cells recognize antigens, secretion of IFNγ increases dramatically [157]. IFNγ completely suppresses the proresolving Mo/MΦ phenotype and converts these cells back to proinflammatory expression [158]. Similarly, T-cell depletion speeds VT resolution [156], and in fact, Tem cell depletion is associated with fewer thrombus proinflammatory Mo/MΦ, with an increase in MMP9, but no change in uPA levels.

Supplanting a particular type of engineered or T-cell against a specific antigen that mediates thrombus resolution is a promising potential therapy. For instance, engineered CAR-T cells yielded a significant reduction in cardiac fibrosis and could be investigated for their potential regarding VT [159], but defining the best target antigen for the T-cells requires careful study.

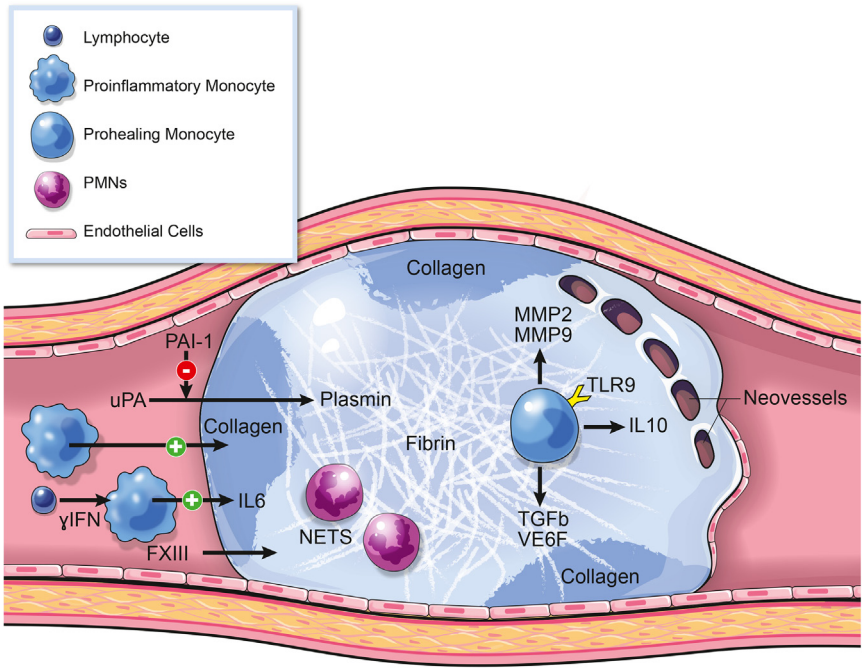

FIGURE Global mechanism of thrombus resolution via mediators and the immune system cells. Early neutrophils invade the thrombus, promoting thrombogenesis and then early lysis. T-cells trigger the activation of proinflammatory macrophages via IFNγ, inhibiting macrophages from switching into the proresolving phenotype. Over time, proinflammatory macrophages likely transition to become proresolving macrophages, at which point they promote fibrinolysis, angiogenesis, and collagenolysis via various mediators. IFNγ, interferon gamma; IL, interleukin; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor; TGFb, transforming growth factor-beta; TLR, toll-like receptor; uPA, urokinase plasminogen activator; VEGF, vascular endothelial growth factor.

9 | ENDOTHELIAL CELLS AND VT RESOLUTION

Endothelial cells also play a role in VT resolution. Thrombus neovascularization in mice models has been well described. For example, early work showed that these channels are both laminin and vWF+ cells, with later CD31+ channels. Stimulating neovascularization may accelerate VT resolution, although it depends on the model and agent used [136,160,161]. Endothelial cells can directly modulate leukocyte actions by expressing such proteins as ICAM-1, which is also expressed by PMNs [162] and proinflammatory Mo/MΦ cells [123], and vascular endothelial growth factor (VEGF), which triggers proresolving Mo/MΦ activation [111,141].

Cell adhesion molecules, involved in venous thrombogenesis [163], may also be associated with postthrombotic resolution [146,164,165]. These include P-selectin [166–168] and E-selectin [169,170], which are receptors on endothelial cells that specifically bind and activate immune cells in early thrombogenesis and are elevated in acute DVT [171,172]. Experimentally, inhibition of these selectins both prophylactically and as a postthrombotic treatment improved VT resolution [173,174].

In contrast, the absence of another cell-to-cell signaling molecule, platelet endothelial cell adhesion molecule 1 (PECAM-1 or CD31) [175], inhibits thrombus resolution. PECAM-1 may promote thrombosis via platelet adhesion and aggregation, transducing high shear stress into inflammatory signals [176]. PECAM-1 can change conformation and promote anti-inflammatory actions: suppression of Mo/MΦ cells' proinflammatory phenotype and inflammatory cytokines, sealing the endothelial vascular wall at endothelial cell junctions, and preventing apoptosis of endothelial cells [177,178].

The resolving VT becomes covered with endothelium by day 4 in a murine model of VT, as measured by intravital imaging on the

TABLE 1 Leukocyte's role in VTE resolution.

Cell type	Role in VTE resolution
Neutrophils (PMN)	Infiltrate the early developing thrombus; also maintain thrombus stability via NETosis and fibrinolysis.
Monocyte-derived macrophages (Mo/MΦ)	Proinflammatory monocytes are recruited to the thrombus, promote early inflammation, and then convert to proresolving monocytes/macrophages. Proresolving macrophages phagocytize erythrocytes, conduct both fibrinolysis and collagenolysis, and promote thrombus neovascularization.
T-cells	Secrete IFNγ among other immune factors that trigger proinflammatory Mo/MΦ cell recruitment and inhibit proresolving monocyte phenotype.
Endothelial cells	Produce surface proteins that recruit immune cells to the site of the developing thrombus and allow them to adhere. Proliferate to coat the early thrombus in endothelium or transdifferentiate into mesenchymal-like cells.

IFNγ, interferon gamma; Mo/MΦ, monocyte-derived macrophage; VTE, venous thromboembolism.

TABLE 2 Targets to accelerate DVT resolution

Molecule	Actions	Translational potential
PAI-1, Vn, PAI-2	Inhibition allows increased lysis	+++
FXIII	Inhibition of thrombus organization	++
Mo/MØ proresolving CsnB, R_vD_v	Promotes prohealing monocyte actions	++
MMPs	Promotion may accelerate matrix thrombus breakdown	+
p53	Quinacrine inhibits p53 to increase resolution	++
Cytokines IL6, IL10, γIFN	Inhibition to decrease inflammation or promote resolution	+
TAFI	Inhibition promotes thrombolysis	++
TLR9	Agonism may promote sterile clearance and resolution	++

FXIII, factor XIII; IL, interleukin; MMP, matrix metalloproteinase; PAI-1/2, plasminogen activator inhibitor; TAFI, tissue activatable fibrinolysis inhibitor; TLE, toll-like receptor; Vn, vitronectin.

abluminal side. The endothelialization can be measured indirectly by exposure of fibrin FTPIII binding, and thus, exogenous fibrinolysis is more effective prior to complete abluminal endothelization [179]. Recent work has also shown a very time-dependent nature of lysis using a clip-unclip IVC thrombus model in mice [180]. Endothelial progenitor cells accelerate DVT resolution [181], and proliferation and migration are promoted by VEGF, angiopoietins [182], low-molecular-weight heparin [183], and various endogenous micro-RNAs [184–187]. Homocysteine [188], Phosphatase and Tensin homolog (PTEN) [185], the tumor necrosis factor ligand FASLG [186], SRC Kinase Signaling Inhibitor 1 (SRCIN1) [187], and serum response factor (SRF) [184] delay re-endothelialization, which may have therapeutic utility by lengthening the window of opportunity for use of common anticoagulants, or thrombolytic agents.

Another significant process in VT resolution is endothelial–mesenchymal transition (EndMT), in which endothelial cells transdifferentiate into mesenchymal-like cells [189], an essential process in embryonic heart development [190]. Within a resolving VT in mice, cells that have undergone EndMT no longer express endothelial proteins such as PECAM-1 and instead produce proteins such as α-smooth muscle actin and both type I and type III collagen [191]. The EndMT may impair VT resolution, as the overall antithrombotic endothelial cells are replaced with fibrotic mesenchymal-like cells [192]. EndMT is triggered primarily by extracellular transforming growth factor-β (TGFβ), and this signal is transduced through endothelin-1 expression, as shown in a stenosis model of VT [193].

10 | WHAT MIGHT BE THE NEXT LOGICAL TARGETS TO ACCELERATE VT RESOLUTION?

The primary processes and cells that mediate them in VT suggest multiple targets for therapeutic benefit (Figure and Table 1). Fibrinolysis and collagenolysis are both required to fully resolve the VT. Neutrophils are more important in early thrombus resolution, with some fibrinolytic capacity, but are countered by NETosis. Mast cells may also drive thrombogenesis [194] and this subsequent resolution, although no later role has yet been shown. In later VT resolution, proinflammatory Mo/MØ infiltrate the developing thrombus and likely transition to proresolving phenotype. Although T-cells promote the proinflammatory response, inflammation eventually gives way to proresolving macrophages that mediate fibrin removal for collagen and retract the thrombus into the vascular wall. Endothelial cells present throughout the resolution process, most notably via proliferation to "coat" the stabilized thrombus, and may also undergo endothelial–mesenchymal transition.

It is unclear if these pathobiological mechanisms will translate into human therapies. However, targeted agents that could enhance local thrombus resolution without off-target effects are likely possible with many advances in nanotechnology. There exist a vast array of potential targets, and much investigation remains to be done (Table 2). One such area is administering agents at specific times in the VT resolution process; for example, targeting PAI-1 to increase local fibrinolysis, early use of DNAase1 to breakdown NETs, and CsnB to promote accelerated Mo/MØ–mediated healing and lumen establishment. This also embraces a multitarget time-dependent approach that is rarely tested in basic biologic experimental papers but may be useful clinically.

Finally, there is a great need for more scientists and clinicians to investigate the pathobiology of VT resolution. We acknowledge that the models are not perfect, but these do provide histologically similar specimens to late human vein postthrombotic specimens. We also acknowledge that late-stage postinflammation fibrosis is challenging to study, but strides have been made in liver, kidney, and lung fibrosis. Per our review, we found no abstracts at the 2022 ISTH meeting on experimental phase of VT resolution. Similarly, from review of the 2022 American Society of Hematology meeting, no experimental VT abstracts were found, and most recently, no plenary or oral talks at 2023 Vascular Discovery meeting were found. Thus, this is a critical gap in research activity, as much remains to be done from the basic pathobiology to new therapeutic translation for human benefit.

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RELATIONSHIP DISCLOSURE

The authors have no competing interests to disclose.

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