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Innate Immunity, Hemostasis and Matrix Remodeling: PTX3 as a Link

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Abstract

Innate immunity is evolutionarily connected with hemostasis. PTX3 is an essential fluid-phase pattern recognition molecule of the innate immune system that acts as a functional ancestor of antibodies. PTX3 by interacting with defense collagens and fibrinogens amplifies effector functions of the innate immune system. At wound sites, PTX3 regulates the injury-induced thrombotic response and promotes wound healing by favoring timely fibrinolysis. Therefore, PTX3 interacts with ancestral domains conserved in innate immunity, hemostasis and extracellular matrix and exerts functions related to both antimicrobial resistance and tissue repair. These findings strengthen the connection between innate immune system and hemostasis, and suggest that recognition of microbes and extracellular matrix are evolutionarily conserved and integrated functions of the innate immune system.

Keywords

innate immunity; inflammation; hemostasis; extracellular matrix; pattern recognition molecule; pentraxin 3 (PTX3)

1 Introduction

Innate immunity and hemostasis share common ancestry[1]. In the horseshoe crab, an integrated coagulation-complement system is a key component of antimicrobial defense and of prevention of wound bleeding via hemolymph clotting. These functions are exerted by amoebocytes, similar in morphology to mammalian platelets. Molecular phylogenetic analysis indicates that the ancestral functions of fibrinogen (FG)- and collagen (COL)-domain-containing proteins in invertebrates are related to immunity rather than to coagulation, a role subsequently acquired in vertebrates[2]. Throughout evolution, functions related to hemostasis and innate immunity have evolved and specialized in specific cell types and molecules, but proteins containing FG and COL motifs, such as collectins and ficolins, still retain ability to mediate immune recognition of microbes[3].

Innate immunity and inflammation are processes related to hemostasis, which includes platelet activation and fibrin deposition, and is resolved through fibrinolysis or activation of anticoagulant pathways[4, 5]. Innate immunity and hemostasis also impact tissue repair affecting inflammatory responses and as well as promoting tissue regeneration[6, 7]. Platelet functions affect hemostasis, innate immunity and extracellular matrix (ECM)[4, 6, 8, 9].

Involvement of the ECM in the innate immune response represents a recurrent theme. Although generally considered as separate from innate immune responses, evasion of pathogens from host defense comprises mechanisms mediated by their interaction with the ECM, as well as of hemostasis[10, 11]. In specific contexts, interaction of pathogens with the same molecules is instead a disadvantage for infection occurrence. Actually, elements of the ECM act as integral part of inflammatory and innate immune responses[12–14], or have opsonic activity for certain microbes[15–17]. Discontinuous evidence indicate that ECM elements interact with humoral pattern recognition molecules (PRMs)[18, 19], although a paucity of information exists on possible effector functions resulting from these interactions. For instance, C1q, collectins and ficolins interact with fibrin[20, 21], whereas pentraxins interact with fibronectin (FN), laminin (LAM) and COL[18, 22–24].

PTX3 is an essential component of humoral innate immunity, which plays evolutionarily conserved antibody-like functions including microbial recognition and opsonic activity via Fc γ R[25]. PTX3 by interacting with ancestral FG[26, 27] and COL[28, 29] domains conserved in the innate immune system and hemostasis[2], as well as in ECM, amplifies antimicrobial effector functions, regulates thrombosis and ECM fibrinolysis ensuring an appropriate tissue repair.

Here we will review collaborative responses between the innate immune system, hemostasis and ECM in infection and in tissue repair, focusing in particular on the role of PTX3, an emerging paradigm molecule at intersection between these systems.

2 Interplay Between Innate Immunity, Hemostasis and ECM in Antimicrobial Defense

2.1 Microbes interact with elements of hemostasis and ECM

The pathogenic potential of microbes includes immune evasion strategies based on interaction with elements of hemostasis and ECM, through the expression of a variety of surface proteins and specific proteases (Figure 1). Some examples are given here. *Staphylococcus aureus* (*S. aureus*) interacts with FN that allows invasion in several cell types via $\alpha 5\beta 1$ integrin[11]. In fungi, interaction with FN is also important in initiation and development of invasive aspergillosis[30]. *S. aureus* also interacts with FG, and through the action of staphylocoagulase, it forms a procoagulant complex with prothrombin allowing to protect themselves from recognition of opsonins and phagocytosis, a crucial determinant in bacteremia, endocarditis, septic arthritis and in initiation of infections associated with wounds[31, 32, 11]. In the pathogenesis of *S. epidermidis*, FG recognition is critical to initiate biofilm formation[11]. Generation of a robust antibody response to COL and FG binding proteins of *S. aureus* is an interesting target for vaccination[11]. *S. pneumoniae*, *S.*

pyogenes and a variety of Gram-negative bacteria recruit vitronectin (VN), which is crucial for attachment to host and for preventing complement activation[33].

During bacteremia, FG acts as a bridge for the interaction of pathogens with platelets or endothelial cells (ECs)[34]. Fc γ RIIa/IgG complex-mediated platelet activation causes serious complications including endocarditis, disseminated intravascular coagulation, immune thrombocytopenia, and increased risk of myocardial infarction and stroke[10].

Fibrinolysis is an integral part of the hemostatic response to infection and tissue injury[35]. Several invasive gram-positive and gram-negative bacteria exploit host plasminogen (Plg) through expression of surface binding proteins or bacterial Plg activators (PAs)[36]. *S. aureus* binds and activates Plg, which in turn cleaves C3b and IgG preventing phagocytosis[11]. Group A streptococci interact with Plg conferring increased bacterial invasiveness, therefore contributing to morbidity and mortality[37]. Interaction of *Yersinia pestis* with Plg is crucial for plague and is a determinant of high virulence through inactivation of complement C3[38]. Plg also contributes to pathogenicity of highly pathogenic viruses[39] and fungi[40] in lung.

2.2 Hemostasis influences innate immune responses

Fibrin deposition is an integral part of inflammatory and innate immune responses (Figure 1) [5], exerting direct antimicrobial function that limits bacterial spreading[41] and regulating local inflammation by acting as a scaffold for innate immune cell activation via its integrin receptor CD11b/CD18 ($\alpha_M\beta_2$)[42–44]. Genetic inactivation of FG and of FG interaction with immune cells are associated with impaired *S. aureus* clearance and increased microbial virulence in experimental peritonitis[44], and with increased mortality after subcutaneous inoculum of group A streptococci[37]. Absence of fibrin deposition is associated with impaired cytokine and chemokine production, decreased neutrophil recruitment and decreased host survival after intraperitoneal injection of *Y. enterocolitica*[45]. FG cleavage as a consequence of airway proteases triggers a TLR4-mediated allergic and antifungal immunity[13].

Signaling pathways coupling coagulation and inflammation regulate the outcome of infection. Cytokine-induced tissue factor (TF) in immune cells represents a key mediator of coagulation associated to thrombosis[4, 5] and is a hallmark of systemic inflammatory response syndromes in bacterial sepsis and viral hemorrhagic fevers[46]. Activation of PARs through the action of coagulation proteases (e.g. thrombin, Factor Xa, TF-Factor VIIa complex) contributes to coagulation-induced inflammation[47,48,49] inducing the expression of pro-inflammatory cytokines, chemokines and growth factors in ECs, mononuclear phagocytes and platelets [9, 50]. In certain viral infections, thrombin/PAR1- and TLR3-mediated coagulative and inflammatory responses protect mice against tissue injury[51]. However, in different conditions, such as endotoxemia, deletion of PAR1 has a beneficial role attenuating late-stage coagulation and ongoing inflammation and hence protecting mice from lethality[47]. Similarly, deletion of TF or inhibition of thrombin is associated with reduced inflammation and mortality [52]. Injection of protein C prevents coagulopathic, hepatotoxic, and lethal effects caused by infusion of lethal concentrations of *E. Coli* in baboons [53], and ameliorates disease progress and prolongs survival after high-

dose lethal injection of Ebola virus in macaques [54]. In humans, an abnormal TF- and PAR-mediated coagulation response in combination with microvascular failure occurs in systemic inflammatory response to severe viral hemorrhagic fever[54] and sepsis and contributes to multiple organ dysfunction[5]. Therefore, local coagulation supports the host antimicrobial response, but in different contexts, such as severe sepsis and viral hemorrhagic fever, pathogens benefit from host procoagulant signaling to drive microbial virulence.

In the circulation, platelets promote coagulation in response to pathogen-associated molecular pattern (PAMPs) and are sentinels effective against blood-borne pathogens. Platelets express several PRMs and phagocytic receptors (e.g. Fc γ RII, Fc ϵ RI) that allow them to sense and internalize pathogens in a process reminiscent of leukocyte phagocytosis[9]. Platelets, both at sites of microbial invasion and tissue damage, activate important mechanisms of innate resistance, including direct secretion of antimicrobial peptides, bacterial presentation, and enhancement in innate immunity effector functions[4, 9]. Platelets themselves have also been reported to generate reactive oxygen species (ROS) via PAR4 activation[55, 56]. Interaction of platelets with pathogens induces release of the kinocidin CXCL4, which increases neutrophil phagocytosis and pathogen killing[57]. Platelet interaction with neutrophils induces activation of integrins and chemokine receptors, hence favoring intravascular migration and generation of neutrophil extracellular traps (NETs), thus contributing to enhanced inflammatory response at the site of vascular injury[58,59]. In the liver, a collaborative immune response between platelets and macrophages is critical in eradicating blood-borne bacterial infection, and platelets are essential to resist *S. aureus* infection[14]. Thrombocytopenia, which occurs in certain viral and bacterial infections or as a result of autoimmune processes, represents an independent risk for increased incidence and severity of infections in several clinical settings, including bloodstream infections caused by methicillin-resistant *S. aureus* [8].

Hemostasis and the complement system interact at multiple levels[60, 61]. Platelets specifically interact with several complement components and activate the alternative or classical pathways, contributing to enhance innate resistance against microbes[61, 62]. Coagulation proteinases potentiate initiation of the complement cascade. In C3-deficient mice a direct enzymatic action of thrombin induces formation of C5a, which has chemotactic activity for neutrophils[63]. Similarly, coagulation Factor XIIa activates the complement complex C1, leading to the initiation of the classical complement pathway[64].

Fibrinolysis elements have a major role in inflammation-initiated coagulation by controlling thrombotic complications associated with bacterial sepsis[46] or affecting recruitment and activation of innate immune cells[65, 66]. In lung infection caused by *S. pneumoniae*, urokinase PA receptor (uPAR)-deficient mice show reduced neutrophil accumulation in lungs and enhanced bacterial dissemination and reduced survival[67], suggesting that uPAR is important to coordinate appropriate inflammatory and innate immune responses to the infection. Plasmin induces several pro-inflammatory mediators in innate immune cells via AP-1- and NF κ B [68], and promotes phagocytic activity in macrophages[69]. Plg has a protective role against *S. aureus*-induced arthritis[70] or systemic infection[71, 72] by activating inflammatory cells, thus facilitating killing bacteria, removing necrotic tissue and enhancing cytokine production, which results in lower bacterial load and reduced mortality.

Furthermore, genetic variants of Plg influence susceptibility to invasive aspergillosis[40]. In contrast, in *S. aureus*-induced severe sepsis, deficiencies in Plg or PAs result in increased survival[72], therefore suggesting that Plg-plasmin is beneficial for the host in the infection, but detrimental in severe sepsis, possibly due to its role in protease-mediated tissue destruction[65] and promotion of inflammation[68].

2.3 ECM influences innate immune response

Innate immune cells migrate into tissues interacting with ECM elements, which can affect inflammatory and innate immune responses (Figure 1). Endogenous ECM components (e.g. FN, FG, hyaluronan breakdown fragments) act as TLR ligands[73]. FN induces adhesion of neutrophils and monocytes and enhances innate immune effector functions[74]. FN fragments[74] generated by neutrophil elastase and osteopontin (OPN)[17] are chemoattractants for immune cells. In an *in vivo* model of endotoxin-induced acute lung injury, VN mediated neutrophil recruitment and cytokine production[12]. Interaction of neutrophils with fibrin, COL, vitronectin (VN), laminin (LAM) and thrombospondin (TSP) leads to release of antimicrobial enzymes and enhancement of respiratory burst[75].

Some ECM elements have direct opsonic activity for certain microbial species. FN and mindin (MIN) released at inflammatory sites recognize pathogens, promote phagocytosis and bactericidal activity of neutrophils and macrophages[15, 74, 76]. VN binds lipopeptide, lipoteichoic acid, and zymosan, and initiates a TLR2-mediated innate immune response via integrin $\beta 3$ [16]. Finally, OPN specifically binds to all known serotypes of *S. agalactiae* and *S. aureus* and opsonizes these bacteria for phagocytosis via integrin CD11c/CD18, identified as a novel OPN receptor in macrophages[17].

3 Involvement of Hemostasis in Tissue Repair

3.1 Coagulation and fibrinolysis influence tissue repair

The hemostatic response occupies a central role in the tissue repair cascade and primarily involves platelet activation and coagulation through action of a complex serine proteases cascade[6, 77–79]. As blood fills the damaged site, platelets interact with exposed COL and other ECM elements and consequent release of clotting factors results in fibrin formation, which is primarily involved in tissue repair by regulating cell migration and granulation tissue formation. In fact, FG-deficient[80], Factor VII-deficient[81] and hemophilic[82] mice show profound defects in cutaneous healing. In contrast, thrombin and thrombin receptor-activating peptide promote healing acting on vascularization and ECM deposition[83].

Platelets affect wound healing by regulating inflammatory and tissue responses through the release of a wide range of growth factors, chemokines and cytokines stored in their α -granules[6, 78, 84]. Release of TGF- β , PDGF-A/B/C, IGF-1, VEGF, CTGF, EGF, angiopoietin and ECM proteases provide signals for angiogenesis, stromal cell proliferation and ECM deposition, hence promoting wound healing[84]. TGF- β , particularly abundant in platelet granules, is a master regulator of activities of smooth muscle cells (SMCs) and mesenchymal cells (MSCs), by regulating integrin expression and by promoting ECM

deposition and limiting ECM breakdown[78]. Through release of wound-active signals, including CXCL1, CXCL2, CXCL4, CXCL5, CXCL7, and CCL3, CCL5, CCL7, CD40L, and a non-identified ligand for triggering receptor expressed on myeloid cells (TREM)-1, platelets directly regulate the recruitment and effector functions of innate immune cells involved in tissue repair[6, 9]. Proteomic analysis conducted on the content of granules suggests that platelets could have opposing and complex regulatory roles in several mechanisms of inflammation and wound healing. Indeed, platelets release growth factors and ECM proteases favoring inflammation and angiogenesis[9, 84]. In contrast, they also store inhibitors of activities of ECs and MSCs, including TSP, angiostatin, endostatin, and inhibitors of ECM proteases[85]. Similarly, besides having a crucial role in mediating blood clot formation, activated platelets release molecules actively involved in fibrinolysis, including Plg and PAs, but also their inhibitors such as PA inhibitor-1 (PAI-1) and α 2-antiplasmin[85].

Abnormal and persistent coagulation, as a consequence of altered expression of natural anticoagulants or defective fibrinolysis, impairs repair. Premature contraction and augmented COL deposition are among defects in skin of mice deficient for the natural anticoagulant thrombomodulin[86], recently described also as a receptor for Plg[87]. In the lungs, thrombin inhibition[88] or administration of protein C[89] reduces fibrosis. Activation of PARs represents the most important hemostasis-related pathway involved in tissue repair[50,90]. In liver, lung and cardiac injury, PARs induce potent mitogenic and pro-fibrotic effects on a variety of cell types, including MSCs, SMCs and ECs[91, 92].

Functions of Plg and PA in tissue repair are documented in several pathological processes involving ECM degradation and cell migration, inflammation, angiogenesis, and wound healing[35, 66]. In skin, deficiencies for Plg and PAs are commonly associated with delayed wound closure, defective cell migration and formation of COL rich-granulation tissue ascribed to defective proteolytic activity[93, 94]. Local release of platelet PAI-1 also impacts wound healing and fibrosis[95]. In general, a central paradigm in the field is that persistence of fibrin in the ECM promotes excess fibrosis in the tissue. Fibrin clotting is indeed centrally involved in platelet aggregation and activation and subsequent release of platelet-derived factors, which are potent stimulators of fibroblast activity and COL deposition[6, 77, 78]. Moreover, COL synthesis is enhanced in fibroblasts grown within fibrin, and defective pericellular fibrinolysis is associated with augmented COL production[96]. In general, a balanced expression of coagulation and fibrinolytic factors is critical for maintaining a proper tissue repair process.

4 Humoral Innate Immune System

4.1 Cellular and humoral PRMs

Innate immunity represents the first line of sensing and resistance against pathogens and plays important roles in maintenance of tissue integrity and in activation of reparative responses in wound repair[25, 73]. The innate immune system consists of a cellular and a humoral arm[25]. The innate immune response is activated when PAMPs or danger-associated molecular patterns (DAMPs) are recognized by cell-associated PRMs, with production of inflammatory mediators and cell recruitment at damaged sites[73]. Cellular

PRM belong to different molecular classes and include TLRs, nucleotide binding and oligomerization domain (NOD)-like receptor (NLR) family, retinoic acid-inducible gene (RIG) I-like RNA helicases, AIM2 like receptors (ALRs), C-type lectins and scavenger receptors[7, 25].

Humoral PRMs are functional ancestors of antibodies[25]. They are diverse in molecular structure but share common actions, including discrimination of self versus non-self and modified-self, regulation of complement activation, opsonisation, agglutination and neutralization. In damaged tissues, humoral PRMs play functions related to clearance of tissue debris and to sustaining the inflammatory response by regulating complement activation[3, 61, 97]. Fluid phase PRMs include elements of the complement cascade, ficolins, collectins and pentraxins[3, 25, 61].

4.2 Pentraxins

The pentraxin family is an ancient group of multimeric proteins evolutionarily conserved from arachnids and insects to humans that share a ~200 amino acids long domain with a distinctive primary motif, the so called “pentraxin signature” (His-x-Cys-x-Ser/Thr-Trp-x-Ser, where x is any amino acid)[98]. C-reactive protein (CRP) and serum amyloid P component (SAP) are prototypic short pentraxins produced by hepatocytes mainly in response to IL-6[99, 100]. The human CRP gene is located in chromosome 1 and comprises two exons, the first coding for the leader peptide (1-18) and the initial two amino acids of the mature protein (19-20), the second coding the remaining 21-224 sequence. CRP is made of five identical non-glycosylated subunits, whose tertiary structure is stabilized by an intra-chain disulfide bond (Cys36-Cys97), highly conserved among pentraxins[100]. The human SAP gene shares the same exon/intron architecture and protein structure[99]. Pentraxins were originally identified as prototypic soluble PRMs that recognize evolutionarily conserved pathogen molecules, such as C-polysaccharide, and self-derived components, such as apoptotic cells, nuclear antigens and chromatin subunits, and initiate immune response[25, 101]. CRP binds various microorganisms including fungi, yeasts, bacteria and parasites through common moieties such as PC and carbohydrate structures and to promote phagocytosis and therefore ensuring protection against infections[98, 100]. Similarly, SAP binds a wide range of microorganisms through structures present in their surface such as LPS, PC and terminal mannose or galactose glycan residues inhibiting their infectivity[99]. CRP and SAP share several functional properties with antibodies, acting as opsonins and, attached or aggregated on microbial pathogens, they interact with C1q and Fc γ receptors activating both the classical pathway of the complement cascade and Fc γ receptors[98, 102, 103]. Modeling studies of the SAP/FcRIIa complex has provided a general structural frame for the interaction of short pentraxins with FcRs, and highlighted similarities to antibody recognition by these receptors[102, 103]. Moreover, both CRP and SAP interact with self and modified-self elements. CRP localizes in human atherosclerotic lesions where it binds to modified low-density lipoproteins (LDL) via PC and cholesterol moieties in LDL[104]. SAP binds to chromatin and apoptotic cells and aid their clearance[99]. SAP has a role in amyloidosis where, through binding and consequent stabilization of amyloid fibrils, it promotes their persistence[105]. CRP and SAP regulate complement activation by

interacting with members of the classical complement pathway, and with the complement regulators factor H (FH) and C4-binding protein (C4BP)[98–100, 106].

PTX3 is a prototype of the long pentraxin family originally identified as cytokine-inducible gene [98] and will be discussed in the subsequent section.

5 PTX3

5.1 Gene and protein

PTX3 differs from CRP and SAP in gene chromosomal location and regulation, as well as in protein structure and cell sources[25, 98, 107]. PTX3 gene in mouse and human is localized on the chromosome 3 and organized in three exons with the first and second coding the signal peptide (aa 1-17) and the N-terminal domain (aa 18-178), and the third exon coding the C-terminal pentraxin domain (aa 179-381). A unique N-linked glycosylation site is located in the C-terminal domain of PTX3 at Asn220. Proximal promoter of both murine and human PTX3 gene include numerous potential binding sites for transcription factors such as Pu1, AP-1, NF κ B, SP1 and NF-IL-6. PTX3 consists of a multimeric glycoprotein of eight identical protomers assembled to form an elongated octamer with a molecular weight of 344KDa stabilized by intermolecular disulphide bonds mainly localized in the N-terminal domain. PTX3 is highly conserved among different species (e.g. 92% of conserved amino acid residues in murine and human PTX3). C-terminal domain of PTX3 contains the pentraxin signature and is up to 57% homologous to CRP and SAP. In contrast, the N-terminal domain is unrelated with any known protein structure[25, 107].

Innate immune cells and stromal cells locally release PTX3 in response to proinflammatory cytokines, PAMPs and DAMPs or following stimulation with intact microorganisms[25]. PTX3 is stored in secondary neutrophil granules and is promptly released upon their activation or it localizes on neutrophil extracellular traps (NETs)[108]. In both sterile damage and infection, IL-1 receptor and TLRs/MyD88 signaling centrally regulate PTX3 production in immune and stromal cells[109–111]. Induction of PTX3 in MSCs and epithelial cells is also mediated by the TNF- α /JNK axis[112]. In myeloid cells, the production of PTX3 in response to pro-inflammatory stimuli is amplified by IL-10[25]. PTX3 is among genes selectively induced by thrombin in monocytes[113]. Glucocorticoids (GCs) inhibit PTX3 production in myeloid dendritic cells (DCs), but induce and enhance it in stromal cells under inflammatory conditions[25]. In selected tumors, which include esophageal squamous cell carcinoma, leiomyosarcomas and colorectal cancer, PTX3 expression is epigenetically repressed due to hypermethylation of the gene promoter and of a putative enhancer, which has important implications in carcinogenesis[110].

5.2 Role of PTX3 in infection and inflammation

Studies conducted in genetically modified mice and genetic evidences in humans prove an essential role of PTX3 in innate resistance to pathogens and regulation of inflammation[114–117], prompting the ongoing clinical development of PTX3 as a candidate therapeutic agent for opportunistic infections in immunocompromised patients. PTX3 also represents an important serological marker rapidly reflecting local tissue

inflammation and damage, under several clinical inflammatory and infectious conditions including cardiovascular diseases, sepsis and cancer [117].

PTX3 is a soluble PRM with functional evolutionary conservation and a key player of humoral innate immunity in response to microbes[25]. PTX3 interacts with a variety of bacteria, fungi and viruses, such as *Klebsiella pneumoniae*[118], *Pseudomonas aeruginosa*[119], uropathogenic *Escherichia coli*[109], *Aspergillus fumigatus*[114], human and murine cytomegalovirus (CMV)[120], influenza viruses (IVs) type A[121]). It acts as an opsonin via Fc γ receptor engagement[122]. In humans, PTX3 genetic polymorphisms are associated with susceptibility to fungal infections[115], tuberculosis[123], *P. aeruginosa* infections[124] and urinary tract infections caused by uropathogenic *E. coli*[109].

In viral infections, PTX3 plays a protective role by neutralizing virus infectivity[120, 121]. PTX3 by interacting with human and murine CMV reduces viral entry into permissive cells and DCs and activates an IRF3-mediated response[120]. Similarly, PTX3 interacts with hemagglutinin and neuraminidase glycoprotein on IV envelope and prevents viral spread and infection[121]. In contrast, a pathogenic role for PTX3 in arthritogenic alphavirus infections has been recently reported, due to increased viral entry in cells[125, 126].

PTX3 regulates inflammatory reactions in infectious and sterile tissue damage[127–130]. The ability of the glycosidic moiety of PTX3 to interact with P-selectin dampening neutrophil recruitment to damaged sites represents a molecular mechanism underlying some of these phenotypes[131].

PTX3 interacts with complement components[132]. It interacts with the globular head of C1q and can activate the classical complement cascade[133]. Moreover, PTX3 interacts with COL-domain of MBL[28] and ficolin-M[29], and with FG-domain of ficolin-1[27] and ficolin-2[26], and synergistically amplifies the complement-mediated innate response to *A. fumigatus* and *Candida albicans* (Figure 2). PTX3 can also regulate the complement cascade by interacting with FH[134] and C4BP[135]. In an unexpected twist PTX3 was recently found to act as an extrinsic oncosuppressor in two models of carcinogenesis[110]. Genetic inactivation of PTX3 unleashes complement-driven, chemokine-amplified recruitment of tumor-associated macrophages which promoted tumor growth and genetic instability. In selected human tumors (e.g. colorectal cancer) the PTX3 gene was found to be silenced by methylation[110, 136, 137]. Thus PTX3 is a bona fide cancer gene.

5.3 Role of PTX3 in tissue repair

A hemostasis-related function of PTX3 in tissue repair has recently emerged[111, 138]. In different mouse models of non-infectious tissue damage, which include excisional skin wounding and chemical-induced liver and lung injury, by interacting with Plg and fibrin PTX3 promoted pericellular fibrinolysis in damaged site, thus ensuring appropriate tissue repair (Figure 2). Common defects associated with PTX3-deficiency in these models include altered thrombotic response to injury, increased fibrin deposition and COL accumulation in the wound site. Wound acidification sets PTX3 in a tissue repair mode. PTX3 specifically interacted with Plg and fibrin at acidic, but not at near-neutral pH, however retaining the capability to recognize microbes and to interact with complement. Along the same line,

PTX3-deficiency associates with augmented susceptibility to arterial thrombosis[139]. Administration of PTX3 rescues these phenotypes and attenuates arterial thrombosis in wt mice[111, 139]. Therefore, in agreement with previous reports[140, 141], PTX3 plays an essential role in the orchestration of the tissue injury response.

In the wound site, several cell types produce PTX3 in response to TLR activation and IL-1 amplification, including macrophages, MSCs and SMCs. PTX3 localizes in the clot, as a reasonable consequence of a neutrophil degranulation[108], and in damaged dermis where it borders the pericellular ECM of aligned macrophages and MSCs collectively migrating towards the wound site, as well as the epithelial outgrowth. Major defects in skin wound healing in PTX3-deficient mice include increased clotting response, excessive fibrin accumulation, premature wound contraction and delayed re-epithelialization at early time points, followed by augmented collagen deposition, epithelial hyperplasia and defective mature tissue formation at healing. Delayed re-epithelialization followed by epithelial hyperplasia are signs of defective repair, which have been associated to impaired degradation of the ECM by leading edge keratinocytes and excessive keratinocyte proliferation, respectively[79, 93, 94].

Interaction of PTX3 with fibrin and Plg results in amplification of pericellular fibrinolysis at acidic, but not neutral, pH. Actually, in wounds, as well as *in vitro*, PTX3-deficient macrophages and MSCs fail to create passages within fibrin matrix, hence indicating that PTX3 contributes to confer an increased invasive phenotype in a fibrin matrix. This property of PTX3 could also be the basis of *in vitro* increased invasiveness of certain cancer cells[142]. Moreover, PTX3-deficient wounds show higher intact fibrin, lower content of Plg and plasmin formation associated with low levels of D-dimer[111]. These results are strengthened by a subsequent *in vivo* study that shows the essential role of MSC-derived PTX3 in skin repair, through its ability to promote pericellular fibrinolysis and migration within fibrin-rich wound site[138].

PTX3 interaction with fibrin and Plg occurred via different sites at its N-terminal domain, and PTX3 did not interfere with fibrin interaction with Plg. In mapping experiments, PTX3 did not bind to Plg kringle (KR)1 domain, which is involved in the recruitment of the proenzyme to the C-terminal lysine moieties of fibrin or of cell surface receptors[143]. PTX3 specifically bound to Plg through its KR 5 domain, which is involved in triggering the conformational changes essential for the conversion to plasmin operated by PAs[143]. This suggests that fibrin-bound PTX3 could favor the opening of Plg, hence affecting its activation in the wound site.

The short pentraxin SAP has been reported to regulate fibrosis. SAP was reported to inhibit alternative activation of macrophages and fibrocyte differentiation via Fc γ Rs[144] and to reduce pulmonary fibrosis through modulation of immune cell activities via DC-SIGN[145]. In a clinical trial, use of recombinant human SAP (PRM-151) reduced the number of fibrocytes and improved lung function in patients affected by idiopathic pulmonary fibrosis[146].

6 Concluding Remarks

Components of the humoral arm of innate immunity are structurally related to extracellular matrix proteins, being constituted by fibrinogen (ficolins) or collagen (ficolins and collectins) domains[3, 25]. Moreover, as shown here for PTX3 interacting with FG, fluid phase PRM interact with selected matrix components. On the other hand, matrix components including fibrinogen/fibrin[13, 44], fibronectin[74], mindin[15], vitronectin[16] and osteopontin[17] recognize microbes and facilitate their disposal. In this respect it is noteworthy that the ancestral function of fibrinogen was defense[2]. Thus, recognition of extracellular matrix proteins and microbial moieties are evolutionary linked processes related to resistance and tissue repair.

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Highlights

- Innate immunity and inflammation are functionally connected to hemostasis and extracellular matrix.
- PTX3 is an essential soluble pattern recognition molecule of innate immune system.
- PTX3 plays hemostasis-related role in tissue repair.
- Recognition of microbes and extracellular matrix components are related functions in innate immunity.

Figure 1.

Interplay between innate immunity, hemostasis and ECM in infections. The mechanisms of hemostasis and of ECM involved in the innate immune response, as well as in the initiation of infection, are depicted here and described in the text.

Figure 2.

Role of PTX3 at intersection between innate immunity, hemostasis and ECM. Stimuli and cell sources of PTX3 are depicted. By interacting with FG and COL domains conserved in innate immune system, hemostasis and ECM, PTX3 retains antimicrobial effector functions and has a hemostasis-related role in tissue repair.