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Coagulation and Immunity in the Peritoneum: Caught in the Fibrin Web

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Summary

In this issue of *Immunity*, Vega-Perez et al. (2021) reveal the formation of a dynamic multicellular aggregate within a fibrin scaffold consisting of large peritoneal macrophages, B1 cells, neutrophils and monocytes during antibacterial immunity in the peritoneum. Anticoagulants targeting thrombin or peritoneal macrophage depletion by clodronate impaired efficient control of *E.coli* infection.

Coagulation and inflammation are integrated biological processes that have co-evolved to protect from mechanical, environmental or pathological challenges and ultimately ensure survival. Inflammation promotes coagulation by enhancing the initiation and propagation of hemostatic mechanisms, as well as suppressing the resolution of blood clots (Esmon, 2003). Conversely, blood coagulation factors induce pathogenic activation of innate immune cells, including macrophages, monocytes and microglia in the brain and periphery (Davalos and Akassoglou, 2012). The COVID-19 pandemic has illustrated the detrimental effects when the intricate interdependence between inflammation and coagulation is tilted toward pathogenic thrombosis resulting in life-threatening thromboinflammation. Bacterial pathogens have also evolved to engage and activate components of the coagulation cascade of their hosts. In peritonitis, bacterial clearance depends on activation of innate immune responses and immune cell recruitment in the peritoneal cavity. While immune cells are free in a fluidic environment in the peritoneal cavity in homeostasis, there is a strong reduction of large peritoneal macrophages in response to inflammation, a physiological phenomenon described as macrophage disappearance reaction (MDR). Studies in the early 1960s showed that inhibition of coagulation and adhesion abolished the MDR in peritoneal inflammation (Nelson, 1963). This has raised the possibility of an extracellular stromal support to spatially and temporally organize innate immune responses. Vega-Perez et al. (2021) now report that activation of the blood coagulation protein thrombin results in the formation of a dynamic

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fibrin scaffold anchoring large peritoneal macrophages (LPM) that limit dissemination of peritoneal bacterial infection.

Vega-Perez et al. (2021) used sublethal *E.coli* intraperitoneal inoculation of mice to study how resident peritoneal immune cells function during infection without stromal support. Whole mount immunofluorescence, confocal and electron microscopy revealed mesothelium-bound LPMs within a fibrin scaffold and a stepwise recruitment of B1 cells, neutrophils and monocytes forming a multicellular complex, termed resident macrophage (resMØ)-aggregates (Figure 1). While LPMs were primarily involved in the clearance of *E. coli* and underwent pyroptosis at the early stages, *E. coli* was detected in neutrophils at later stages of infection. LPMs were detected in resMØ -aggregates as early as 30 minutes after infection, suggesting that LPMs transition from the peritoneal cavity to the mesothelium-bound fibrin scaffold during the MDR. Four hours after infection, omental organized milky spots with a specialized vascular and lymphoid tissue also harbored LPM aggregates. At late stages of infection, resMØ-aggregates with disintegrated fibrin networks harbored LPMs and recruited monocytes removing dead cells and degraded fibrin. During resolution of inflammation, LPM aggregates temporally correlated with degradation of the fibrin scaffold, reduction of neutrophils and inflammatory markers, and eventually initiation of tissue repair. These findings identified the formation of a fibrin matrix as a dynamic scaffold for stromal support for resident macrophages during peritoneal *E. coli* clearance.

Which components of the resMØ-aggregate are necessary for bacterial clearance? Deletion of the transcription factor GATA6 in *LysM-Gata6*$^{-/-}$ mice did not alter bacterial clearance. While LPMs were reduced in *LysM-Gata6*$^{-/-}$ mice, neutrophil recruitment to resMØ-aggregates was substantially higher, suggesting compensatory host defense by neutrophils. However, when neutrophils were depleted in either wildtype or *LysM-Gata6*$^{-/-}$ animals, resMØ-aggregate formation remained intact after inoculation with *E. coli*. Similarly, depletion of B1 cells using the Xid mouse model, which carries a point mutation in Bruton's tyrosine kinase, did not alter the formation of resMØ-aggregates. resMØ aggregate formation was also unaffected in *Gsdmd* deficient mice, which are protected from macrophage pyroptosis. Collectively, these results suggest that B cells, neutrophils, and pyroptosis of LPMs are dispensable for the clearance of peritoneal bacterial infection in this model. In contrast, depletion of LPMs by clodronate abolished resMØ-aggregate formation and increased bacterial dissemination and mortality rates. These results illustrate that local LPMs are necessary for bacterial clearance, as they are required for the formation of the resMØ-aggregates and host survival after sublethal *E. coli* peritonitis.

Are coagulation and the formation of a fibrin scaffold required for macrophage aggregation in *E.coli* peritonitis? The blood coagulation factor fibrinogen is a 340 kDa protein that upon the activation of the coagulation cascade is proteolytically cleaved by thrombin and converted to insoluble fibrin (Figure 1). In addition to fibrinogen, thrombin also has multiple proteolytic targets that regulate inflammation (Esmon, 2003; Luyendyk et al., 2019). Thrombin proteolytically cleaves protease-activated receptors (PARs), which are expressed by macrophages and regulate immune responses during infection (Coughlin, 2000). Thrombin also induces generation of activated protein C that inhibits thrombosis and inflammation. Fibrin is essential for normal hemostasis, but excessive coagulation

increases fibrin formation leading to pathogenic thrombosis. Fibrin deposition in tissues is a potent proinflammatory and pro-oxidant activator of innate immunity causally linked with autoimmune, inflammatory and traumatic diseases in brain and periphery (Davalos and Akassoglou, 2012; Petersen et al., 2018). Mice genetically deficient for fibrinogen or other coagulation factors are not immunocompromised and can be housed in conventional animal facilities without opportunistic infections. Fibrinogen deficient humans (afibrinogenemic patients) cannot form fibrin clots and have excessive bleeding, but without any reported increase in opportunistic infections. Studies in infection models in fibrinogen mutant mice have revealed beneficial or detrimental contributions to bacterial infection depending on the pathogen, path of infection and modulation of hemostatic or immune effector functions (Davalos and Akassoglou, 2012; Luyendyk et al., 2019). Vega-Perez et al. (2021) tested the consequences of prophylactic intraperitoneal administration of two anticoagulants inhibiting thrombin in *E.coli* clearance in the peritoneum. Heparin, an inhibitor of thrombin and Factor Xa, abolished resMØ-aggregate formation leading to failure of bacterial clearance and increased mortality rate. Similar to heparin, direct thrombin inhibition by hirudin reduced bacterial clearance and increased mortality. These findings indicate that thrombin activation is required for LPM adhesion to initiate resMØ-aggregate formation and subsequent bacterial clearance. These results are in line with detection of LPMs in fibrin aggregates after zymosan administration and the detrimental effects of inhibition of Factor V, a key cofactor for thrombin activation, in bacterial peritonitis (Zhang et al., 2019). As expected, inhibition of thrombin either by heparin or hirudin decreased fibrin formation in *E.coli* peritonitis. Thrombin has multiple proteolytic targets and the role of coagulation factors depends on the pathogen and path of infection. Future loss-of-function studies using mice deficient for thrombin proteolytic targets and other coagulation factors are needed to determine their contribution in *E. coli* peritonitis.

Is monocyte recruitment to resMØ-aggregates necessary for immune response to bacterial infections? Vega-Perez et al. (2021) showed that recruitment of $CCR2^+$ monocytes expressing the plasminogen receptor PlgRTK was associated with degradation of the fibrin network during resolution of inflammation at the late phase of infection (Figure 1). *Ccr2* deficient mice exhibited prolonged peritoneal inflammation and persistent fibrin scaffold after *E. coli* infection with increased inflammatory cytokines, chemokines and an abundance of neutrophils. Fibrin uptake was detected in the cytoplasm of both monocytes and monocyte-derived LPMs. Since PlgRTK plays a role in inflammation and fibrinolysis during wound healing, future studies should explore a potential role for PlgRTK in immune functions and tissue repair in *E. coli* peritonitis. Fibrin degradation depends on conversion of the inactive zymogen plasminogen to active plasmin by urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA) (Figure 1). Excessive fibrin deposition due to impaired plasmin degradation is inhibitory to tissue repair including wound healing and nervous system regeneration (Bugge et al., 1996; Petersen et al., 2018). Indeed, plasminogen deficient mice have persistent fibrin deposition in all tissues, severe thrombosis and early lethality that are rescued by fibrinogen deficiency, suggesting that fibrin is the main physiological substrate for plasmin in vivo (Bugge et al., 1996). Overall, these findings are in line with previous data showing proteolytic fibrin degradation and fibrin endocytosis by $CCR2^+$ cells regulating inflammatory responses (Luyendyk et al., 2019). Future studies

to test the role of the plasminogen activation system that controls fibrin degradation will elucidate the mechanisms of fibrin scaffold remodeling and resolution of inflammation in bacterial peritonitis.

The findings by Vega-Perez et al. (2021) shed new light on the dynamic remodeling of the extracellular stromal support that orchestrates the innate immune responses regulating bacterial clearance. The study is based on a multipronged experimental design including whole-mount immunofluorescence, confocal and electron microscopy, genetically encoded fluorescent reporters, genetic depletion and pharmacologic treatments. Vega-Perez et al. (2021) elegantly describe activation of thrombin and the formation and resolution of a fibrin matrix that contains the large peritoneal macrophages required to clear bacterial infection in the peritoneum. The fibrin matrix is an established spatial signal for innate immune cell clustering and initiation of pathogenic inflammation in the brain and peripheral tissues (Davalos and Akassoglou, 2012; Davalos et al., 2012; Luyendyk et al., 2019). The results of this study support the aggregation of the peritoneal macrophages within the fibrin scaffold as an explanation for the MDR in acute peritonitis. Although several of the pathways tested were dispensable for bacterial clearance, clodronate and thrombin-targeting anticoagulants impaired *E. coli* clearance in the peritoneal cavity and host survival. Future studies will explore the interdependence of coagulation and innate immunity in infection and resolution of inflammation in other body cavities. These exciting results open new directions for future research to decipher the cellular and molecular players controlling innate immune activation at the nexus of coagulation and immunity.

REFERENCES

Bugge TH, Kombrinck KW, Flick MJ, Daugherty CC, Danton MJ, and Degen JL (1996). Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. Cell 87, 709–719. [PubMed: 8929539]

Coughlin SR (2000). Thrombin signalling and protease-activated receptors. Nature 407, 258–264. [PubMed: 11001069]

Davalos D, and Akassoglou K (2012). Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol 34, 43–62. [PubMed: 22037947]

Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, Petersen MA, Deerinck TJ, Smirnoff DS, Bedard C, Hakozaki H, et al. (2012). Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun 3, 1227. [PubMed: 23187627]

Esmon CT (2003). Inflammation and thrombosis. J Thromb Haemost 1, 1343–1348. [PubMed: 12871267]

Luyendyk JP, Schoenecker JG, and Flick MJ (2019). The multifaceted role of fibrinogen in tissue injury and inflammation. Blood 133, 511–520. [PubMed: 30523120]

Nelson DS (1963). Reaction to antigens in vivo of the peritoneal macrophages of guinea-pigs with delayed type hypersensitivity. Effects of anticoagulants and other drugs. Lancet 2, 175–176. [PubMed: 13938029]

Petersen MA, Ryu JK, and Akassoglou K (2018). Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. Nat Rev Neurosci 19, 283–301. [PubMed: 29618808]

Vega-Perez A, Villarrubia LH, Godio C, Gutiérrez-González A, Feo-Lucas L, Ferriz M, Martínez-Puente N, Alcain J, Mora A, Sabio G, et al. (2021). Dynamic immune scaffolds drive anti-bacterial defense in the peritoneal cavity. Immunity.

Zhang N, Czepielewski RS, Jarjour NN, Erlich EC, Esaulova E, Saunders BT, Grover SP, Cleuren AC, Broze GJ, Edelson BT, et al. (2019). Expression of factor V by resident macrophages boosts host defense in the peritoneal cavity. J Exp Med 216, 1291–1300. [PubMed: 31048328]

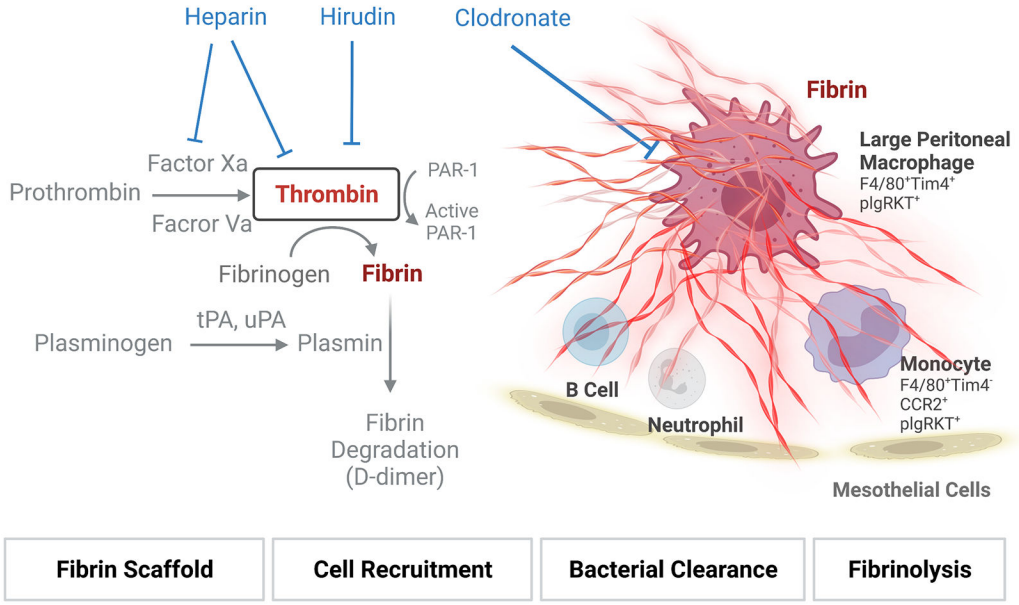

Figure 1. Dynamic intersection of coagulation and innate immunity in peritonitis.

Using a sublethal *E.coli* bacterial peritonitis model, Vega Perez et al. (2021) describe the aggregation of large peritoneal macrophages ($F4/80^{+}Tim4^{+}$) on mesothelial cells with recruited B cells and neutrophils forming a multicellular complex within a fibrin scaffold. Fibrin formation depends on the activation of the coagulation cascade and the proteolytic cleavage of fibrinogen by thrombin. Thrombin also has additional proteolytic targets that regulate inflammation including PAR-1 expressed in macrophages. Depletion of large peritoneal macrophages by clodronate or inhibition of thrombin by heparin or hirudin decreased bacterial clearance and host survival. Fibrin is a provisional matrix proteolytically degraded by plasmin, derived from the inactive zymogen plasminogen, upon cleavage by uPA or tPA. At late stages of infection monocyte recruitment ($CCR2^{+}F4/80^{+}Tim4^{-}$) expressing plgRKT orchestrate removal of degraded fibrin and initiate resolution of inflammation and tissue repair. Created with BioRender.com