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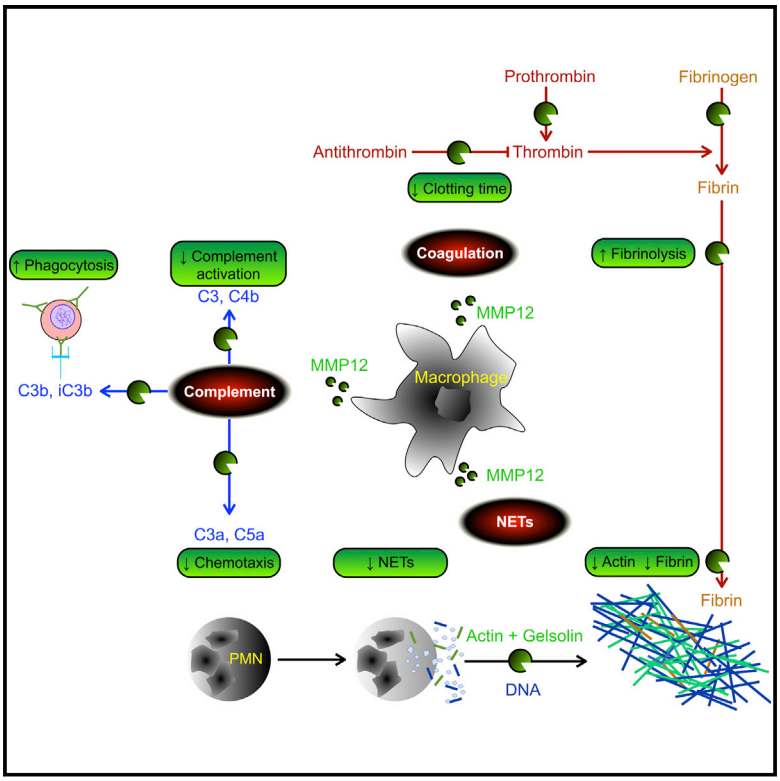
Macrophage Matrix Metalloproteinase-12 Dampens Inflammation and Neutrophil Influx in Arthritis

Graphical Abstract



Highlights

Macrophages resolve inflammation via matrix metalloproteinase 12 (MMP12)

MMP12 dampens neutrophil infiltration and clears actin and fibrin from NETs

MMP12 terminates complement activation and increases phagocytosis

By activation of prothrombin in vivo, MMP12 exhibits procoagulant activity

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In Brief

Using TAILS proteomics, Bellac et al. now demonstrate that macrophage MMP12 executes multiple protective roles in vivo in inflammation resolution. In arthritis, MMP12 counters neutrophil influx, terminates complement activation, accelerates coagulation, and clears NETs of actin and fibrin to dampen and prepare for the resolution of inflammation.

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Macrophage Matrix Metalloproteinase-12 Dampens Inflammation and Neutrophil Influx in Arthritis

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SUMMARY

Resolution of inflammation reduces pathological tissue destruction and restores tissue homeostasis. Here, we used a proteomic protease substrate discovery approach, terminal amine isotopic labeling of substrates (TAILS), to analyze the role of the macrophage-specific matrix metalloproteinase-12 (MMP12) in inflammation. In murine peritonitis, MMP12 inactivates antithrombin and activates prothrombin, prolonging the activated partial thromboplastin time. Furthermore, MMP12 inactivates complement C3 to reduce complement activation and inactivates the chemoattractant anaphylatoxins C3a and C5a, whereas iC3b and C3b opsonin cleavage increases phagocytosis. Loss of these anti-inflammatory activities in collagen-induced arthritis in *Mmp12*$^{-/-}$ mice leads to unresolved synovitis and extensive articular inflammation. Deep articular cartilage loss is associated with massive neutrophil infiltration and abnormal DNA neutrophil extracellular traps (NETs). The NETs are rich in fibrin and extracellular actin, which TAILS identified as MMP12 substrates. Thus, macrophage MMP12 in arthritis has multiple protective roles in countering neutrophil infiltration, clearing NETs, and dampening inflammatory pathways to prepare for the resolution of inflammation.

INTRODUCTION

The inflammatory response to tissue damage involves a fine interplay of different pathways and mediators involving the coagulation cascade, complement system, acute-phase reactants, and innate and adaptive immune cells. This coordinated process ensures that pathogens and damaged tissue are removed and healing commenced to restore tissue homeostasis. Unresolved inflammation contributes to chronic inflammatory diseases, such as rheumatoid arthritis. Macrophages play a dominant role in innate immunity (Galli et al., 2011; Geissmann et al., 2010; Houghton et al., 2009; Marchant et al., 2014). Early in inflammation, resident macrophages recognize foreign or damaged material and secrete proinflammatory mediators that recruit polymorphonuclear neutrophils. Infiltrating neutrophils phagocytize microorganisms and secrete chemokines. Later, cell debris and apoptotic neutrophils are cleared by macrophage phagocytosis. The influx of neutrophils is partially halted through chemokine inactivation by matrix metalloproteinase (MMP) proteolytic processing (Dean et al., 2008) and by phagocytosis-derived signals (Soehnlein and Lindbom, 2010). Apoptotic neutrophils display specific efferocytosis "eat-me" signals, whereas nonspecific opsonization by acute-phase reactants, including complement C3b and iC3b, enhances phagocytic removal of opsonized material and cells. Macrophage protease activity is crucial for phagocytosis and proinflammatory functions, but the in vivo roles of individual macrophage proteases over time in resolving inflammation are unclear.

Whereas MMPs are canonically considered destructive and proinflammatory, beneficial roles for the macrophage-specific matrix metalloproteinase-12 (MMP12) in inflammation and immunity are known: *Mmp12*$^{-/-}$ mice show exacerbated inflammation in lipopolysaccharide-induced lung inflammation (Dean et al., 2008) and increased mortality upon bacterial (Houghton et al., 2009) and viral infections (Marchant et al., 2014). In the absence of MMP12, development of experimental autoimmune encephalomyelitis is accelerated and is associated with cytokine and chemokine dysregulation (Goncalves DaSilva and Yong, 2009). However, MMP12 deficiency reduces neuroinflammation in aged mice by decreasing the recruitment of bone marrow-derived microglia to the brain (Liu et al., 2013). Thus, MMP12 can exhibit both proinflammatory and anti-inflammatory activity in a tissue- or disease context-dependent manner.

Previous studies have focused on the cleavage of extracellular matrix (ECM) substrates by MMP12 (Gronski et al., 1997). However, genetic overexpression introduces a protease at non-physiological levels or in tissues or cells where the protein is not normally found. In contrast, protease knockout tissues