Research Highlight

Macrophage Metalloelastase: Stretching Therapeutic Opportunities

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While tissue macrophages are at the first line of microbial host defense, they are also convenient hideouts for pathogens escaping immune attack. Houghton et al. discovered that alveolar macrophage mobilizes macrophage metalloelastase to destroy bacteria present inside the cell.

Owing to their low numbers in peripheral blood and lymphoid organs, tissue resident macrophages have not always received the level of attention they deserve. Only recently did tissue macrophages take center stage based on their implication in a wide range of disease indications including tumor growth and metastasis, autoimmunity and wound healing (Gordon, 2007). These indications are often secondary to the macrophages’ prime mission to protect host against pathogens. As innate responders, tissue macrophages are equipped with a large repertoire of germline-encoded receptors including scavenger, complement and Fc-receptors to rapidly detect, capture and destroy microbial threats that are trying to invade the host (Gordon, 2007). Upon recognition by phagocytic receptors, pathogens are internalized into phagosomes, an intracellular membrane-bound organelle that serves to transport phagocytosed particles to the lysosomes, the destruction center of the cell. Macrophages then proceed to eradicate the pathogens through mobilization of oxidative and non-oxidative antibacterial responses (Flannagan et al., 2009). The oxidative mechanism involves production of reactive oxygen and nitrogen species inside the macrophage that can lead to impairment of bacterial metabolism and ultimately inhibition of replication. The non-oxidative mechanisms include degradation of the microbes in acidic phagolysosomes and in phagosomes by antimicrobial proteins such as endopeptidases and hydrolases. Despite being equipped with this heavy artillery, macrophages are unable to destroy some species of pathogens like Listeria monocytogenes that manage to escape the phagosome. Other pathogens such as Legionella pneumophila and Mycobacterium tuberculosis have developed strategies to evade the antimicrobial mechanisms in the phagosome by perturbing phagosomal maturation. These pathogens that have survived inside the macrophage are able to avoid recognition by NK cells, T cells and neutrophils that are surveilling the immune environment in the circulation, and thus, remain unnoticed by the adaptive arm of the immune system.

Houghton et al. (2009) identified macrophage metalloelastase (MMP12) as a lead player in the direct killing of bacteria sequestered in the phagolysosomes. They demonstrated that mice lacking MMP12 have impaired ability to destroy bacteria in the phagolysosomes and die as a result of uncontrolled spread of the infection. Intriguingly, the increased mortality was observed when the bacteria were injected through the airway, but not when the bacteria were injected into the blood. This can be explained by the preferential expression of MMP12 in lung alveolar macrophages when compared with macrophages resident in other tissues. Alveolar macrophages are a subset of tissue macrophages that reside along the alveolar wall and are the first innate immune responders to bacteria that enter the host via the respiratory tract (Figure 1A). In contrast, when pathogens are injected intravenously, they are efficiently cleared by Kupffer cells in the liver that employ a different strategy to eliminate the microbes (Blizier et al., 2006).

Once the bacteria are internalized into the alveolar macrophages, the authors provided evidence that the ‘ready-to-go’ intracellular pool of MMP12 aggregates onto the bacteria that are trapped inside the phagolysosomes and disrupts the cell wall constituents, resulting in bacterial death (Houghton et al., 2009). In this sense, MMP12 is unique among MMPs, in that it can act intracellularly rather than extracellularly. How the intracellular pool of MMP12 is recruited to and activated in the phagolysosomes is unknown. Since a majority of MMP12 is secreted, it remains possible that MMP12 binds to the bacteria outside the cell prior to phagocytosis.

MMP12 is a member of the MMP family, a large group of structurally related matrix-degrading proteinases whose activity depends on zinc ions (Parks et al., 2004). Like most of the MMPs, MMP12 is produced and secreted as a latent pro-enzyme containing an amino-terminal pro-domain, a catalytic domain and a carboxy-terminal hemopexin-like domain (CTD) (Shapiro et al., 1992) (Figure 1B). The pro-domain keeps pro-MMP12 in a catalytically inactive state. After cleavage of the pro-domain, MMP12 becomes proteolytically active. The active MMP12 is thought to undergo
further processing leading to CTD shedding, resulting in the mature active form of MMP12. Since MMP12 without CTD is still enzymatically active, it is believed that MMP12 CTD is not required for substrate catalysis. In their study, Houghton et al. have determined the domain in MMP12 that is responsible for its bactericidal activity. Since the catalytic domain is essential for the substrate-converting activities of MMPs, one would expect this domain to be involved in the bacterial cell wall-destroying activity of MMP12. Unexpectedly, the authors demonstrated that the antimicrobial activity of MMP12 is embedded in the CTD, and not in the catalytic domain. Notably, in in vitro bacteria growth assays, recombinant MMP12 or MMP12 CTD inhibits the growth of several bacterial strains, except *L. monocytogenes*. This suggests that MMP12 has a direct bactericidal activity but is unable to kill certain bacteria such as those that have the ability to escape the phagosome. Whether MMP12-deficient mice are susceptible to *L. monocytogenes* infection has yet to be shown. By mutational and functional analysis, Houghton et al. narrowed down the bactericidal activity to a unique four amino acid sequence (Lys-Asp-Glu-Lys) within MMP12 CTD. This sequence is not conserved in other MMPs which may explain the distinct antimicrobial property found in MMP12 but not in most of the other MMPs. In a three-dimensional homology model for MMP12 CTD, the authors localized this four amino acid sequence to a surface accessible loop that flanks and connects the two beta strands of the CTD which could potentially interact with bacteria. Conclusively, the current study by Houghton et al. (2009) has uncovered an important unexpected function for MMP12 CTD in host defense.

The coincidental observation of aggregated MMP12 on disrupted bacterial cell wall within phagolysosomes begs the question of how MMP12 disintegrates the network of cell wall components. Matrilysin (or MMP7) is the only other MMP that has been implicated in bacterial killing. It acts indirectly by cleaving and activating alpha-defensins, an antimicrobial peptide (Wilson et al., 1999). Previous work from Belaaouaj et al. (2000) has demonstrated that neutrophil elastase elicits bactericidal activity in the phagolysosome by degrading outer membrane protein A (OmpA) localized on the surface of Gram-negative bacteria and, therefore, disrupts the cell wall integrity. MMP12, through the catalytic domain, can cleave a variety of substrates in addition to elastin such as type IV collagen, fibronectin, and gelatin in *in vitro* assays (Lagente et al., 2009). However, Houghton et al. have shown that the catalytic domain is not required for the bactericidal property of MMP12. Recombinant MMP12 CTD alone is able to inhibit bacteria growth in culture, but this domain has not been reported to have enzymatic activity. Possibly, MMP12 CTD has an antibiotic-like effect on the cell wall. How the CTD disrupts bacterial cell wall integrity and mediates bactericidal activity is a question of great interest.

Next to their antimicrobial activity, MMPs have been extensively studied for their role in tissue remodeling during development, growth and tissue repair. Genetic studies have further implicated MMPs in inflammatory diseases such as ‘vanishing bone’ syndrome and arthritis as well as in tumor angiogenesis and progression (Brinckerhoff and Matrisian, 2002). Different from other MMPs, MMP12 is predominantly produced by airway epithelia and alveolar macrophages, and therefore, its pathology is
observed primarily in the respiratory tract. MMP12 has been implicated in inflammatory respiratory disorders such as asthma, pulmonary fibrosis and chronic obstructive pulmonary disease (COPD) (Lagente et al., 2009). A number of studies have defined a critical role of MMP12 in the development of emphysema in response to long-term exposure to cigarette smoke. Emphysema is a type of COPD resulting from excessive degradation of elastin by MMP12 and recruitment of neutrophils and macrophages into the airways that exacerbates lung inflammation. Recently, MMP inhibitors were applied in animal models of emphysema and were successful in reducing lung inflammation (Lagente et al., 2009). Although in a cell-free system, MMP12 CTD is autolytically cleaved from the catalytic domain, it is unknown if this also occurs endogenously in macrophages. Therefore, MMP inhibitors that can potentially interact with or interfere with functions of the CTD domain will need to be used with caution to treat inflammatory respiratory disorders because they may inhibit the bactericidal activity of MMP12.

The exciting discovery by Houghton et al. has stretched the function of MMP12 from extracellular matrix remodeling to bactericidal activity. By demonstrating that MMP12 can act in the phagolysosome, employing the carboxy-terminal domain to destroy bacteria, the authors have provided new insights into the biology and pathophysiology of MMPs. This will undoubtedly open up new avenues of research that can lead to improved therapeutics targeting antibiotic-resistant bacterial strains.

References


