Abstract

Mycobacterial infection-induced diseases, particularly tuberculosis (TB), cause more than 1 million deaths annually. Mycobacteria initially infect lungs, invade alveolar macrophages and can develop a systemic infection with high lethality. However, currently available drugs are only partially effective due to the development of drug-resistant mycobacteria.

The sphingomyelinase/ceramide system has been implicated to play many roles in mycobacterial infections. Thus, this study aims to identify the mechanisms of sphingomyelinase/ceramide-regulated mycobacterial infection and provide potential therapeutic strategies.

The present work investigated roles of neutral sphingomyelinase (Nsm) and acid sphingomyelinase (Asm)/ceramide system in the systemic infection of murine macrophages and mice with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG).

Nsm has been shown to allow mycobacterial persistence in mice by suppressing autophagy. The present study focused on the role of Nsm in the generation of granuloma, the hallmark of mycobacterial infections. The results in this thesis reveal a novel mechanism of Nsm-dependent granuloma formation upon mycobacterial infection. The results indicate that the infection of bone marrow-derived macrophages (BMDMs) with BCG leads to rapid activation of Nsm and activation of surface β1-integrin via phosphorylation of p38 mitogen-activated protein kinases (p38K) and c-Jun N-terminal kinase (JNK). Nsm-dependent β1-integrin activation results in the activation of small GTPase Rac1 and reorganization of the cytoskeleton. This leads to macrophage migration and granuloma-like clusters *in vitro*. Mice heterozygous for Nsm or mice treated with neutralizing antibodies against β1-integrin contain fewer macrophage clusters *in vitro*, fewer granulomas *in vivo* and, most importantly, fewer bacteria *in vivo*. These findings indicate that the Nsm/ceramide system plays an important role in mycobacteria-induced granuloma formation by regulating a signaling cascade via p38, JNK, β1-integrin and Rac1.

Furthermore, the current study suggests a novel mechanistic link between Asm and mycobacterial infection. The results show that BCG infection of BMDMs triggers activation of Asm and acid ceramidase (Ac), and elevated levels of sphingosine-1-phosphate (S1P) which increases reactive oxygen species (ROS) via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit $p47^{phox}$. ROS promote BCG degradation by the lysosomal enzyme, cathepsin D. BMDMs of Asm deficient mice abrogate these effects, and these mice are more susceptible to BCG infection than wild-type (Wt) mice. Transplantation of Wt BMDMs into Asm deficient mice confirmed the crucial role of Asm in macrophages. The transplantation partially reversed the susceptibility of Asm deficient mice to BCG infection. These findings indicate that Asm/ceramide system is important in the control of BCG infection.

These studies reveal the role of sphingomyelinases in mycobacteria-induced granuloma formation and bacterial elimination. The further study combines neutral and acid sphingomyelinases may provide a novel therapeutic strategy against mycobacterial infection.