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Mast cell-mediated signaling in reduction of *Francisella tularensis* SchuS4 intramacrophage replication and apoptosis

A. R. Rodriguez¹, J.-J. Yu¹, J. P. Chambers¹, K. E. Klose¹, M. N. Guentzel¹, M. T. Berton², B. P. Arulanandam¹

¹University of Texas at San Antonio, South Texas Center for Emerging Infectious Diseases, Biology Department, San Antonio, United States, ²University of Texas Health Science Center at San Antonio, Microbiology Department, San Antonio, United States

Aims: To determine the effector mechanism(s) by which mast cells mediate the inhibition of intramacrophage *F. tularensis* subsp. *tularensis* SchuS4 or LVS replication and induction of apoptosis.

Methods: An in vitro bone-marrow derived mast cell/macrophage co-culture system, in addition to confocal and scanning electron microscopy were utilized. Flow cytometry was used for analysis of activation markers and expression of apoptotic proteins.

Results: Macrophages infected with *F. tularensis* SchuS4 or LVS and co-cultured with mast cells exhibited significant reduction of intramacrophage growth and caspase-3 expression compared to macrophages cultured alone. This reduction was dependent on mast cell secreted products, including IL-4, and contact-dependent events. Mast cells co-cultured with macrophages exhibited up-regulation of MHC II (12.7% to 87.1%), and c-Kit (17.0% to 61.0%) expression associated with cellular activation. Since mast cells express toll-like receptor-2 (TLR2), which is important for pathogen recognition and IL-4 production, TLR2 deficient (TLR2^{-/-}) mast cells additionally were analyzed. TLR2^{-/-} mast cells exhibited a significant increase (2-3.0 log₁₀) in LVS replication and enhanced expression of caspase-3 (10% to 28%) in contrast to wild type (WT) mast cells. Whereas, LVS-infected TLR2^{-/-} mast cells did not produce IL-4; these cells cultured in the presence of WT macrophages restored IL-4 production and inhibition of apoptosis and *Francisella* intramacrophage growth.

Conclusions: The inhibitory effect of mast cells on intramacrophage bacterial replication and apoptosis also is evident with a type A human strain. Moreover, the TLR2 signaling pathway within mast cells may be an important component in control of *Francisella* replication within the mast cell itself. Together, our results demonstrate that cross-activation events are critical for mast cell production of IL-4 and control of *Francisella* intramacrophage replication and induction of apoptosis.

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