M3
Thrombin Fine Tunes Innate Immune Cell Function in Models of Localised Antigen

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Abstract
Introduction: Thrombin is the main effector protease in the coagulation cascade. It can also affect a wide array of cell types by signalling via protease activating receptors (PAR). The presence of these receptors on the surface of innate immune cells has been well reported but the functional consequence of activation has yet to be defined. This study aims to investigate the role thrombin has on innate immune cell function using contact hypersensitivity (a response to localised antigen) as a model of transplantation.

Methods: Mice were sensitised with oxazolone on the shaved abdomen, 5 days later they were re-challenged with oxazolone on the right ear. Ear thickness difference vs control was measured at 24 and 48 hours. In vitro experiments were conduced with bone marrow macrophages (BMM) cultured with thrombin then analysed by flow cytometry

Results: Inhibiting thrombin signalling via transgenic expression of hirudin on murine CD31 cells significantly reduced ear thickness swelling versus WT. This phenotype was shown to be due to inhibition of thrombin signaling on the monocytes, as WT recipients of transgenic bone marrow had a reduction in ear swelling, reduced CD68 infiltration, granuloma and iNOS expression. This was not seen in transgenic recipients of WT bone marrow.
In Vitro, stimulating mature BMM with thrombin did not affect gross markers of macrophage polarisation (iNOS or CD206) but did increase surface expression of CD69 & MHC II and reduced ABCA1. Thrombin stimulated cell supernatants had increased Interferon gamma and reduced IL10. Thrombin treated cells had increased lipid rich microdomains by Cholera Toxin B staining and increased co-localisation of the LPS receptor within the lipid raft. The thrombin stimulated cells were highly sensitive to low dose M1 polarising stimuli.

Conclusion: Thrombin, as well as being a key mediator of coagulation, provides a proinflammatory signal and provides an important target for future cytotopic therapies in transplantation.

Categories
1. Basic and translational science