THE ROLE OF IGF-1 IN MURINE MACROPHAGES AGAINST MYCOBACTERIUM TUBERCULOSIS

Julius E Chia[1,2], Mumin Ozturk[1,2], Reto Guler [1,2] , Suraj P. Parihar[1,2] and Frank Brombacher[1,2]

[1]International Centre for Genetic Engineering and Biotechnology (ICGEB), Cape Town Component, Cape Town 7925, South Africa

[2]University of Cape Town, Institute of Infectious Diseases and Molecular Medicine (IDM), Division of Immunology and South African Medical Research Council(SAMRC) Immunology of Infectious Diseases, Faculty of Health Sciences, University of Cape Town, Cape Town 7925, South Africa.

Background

The ability of macrophages to modulate their phenotype in response to environmental signals from local tissues make them a crucial mediator of immune response, *Mtb* has several ways of subverting host immune responses; understanding the mechanisms and the genes involved is required for the development of novel strategies to combat the disease. Insulin-like growth factor 1(IGF-1) is one of the genes identified to be upregulated during alternative activation of macrophages in deepCAGE transcriptomics atlas on murine macrophages. IGF-1 in macrophages is downregulated upon infection with *Mtb* clinical and H37Rv strains. Lentivirus-mediated knockdown of IL-4 regulated IGF1 results in decrease in bacterial burden in bone marrow derived macrophages, and decrease pro-inflammatory cytokines. Chemical blocking of IGF-1 also led to decrease in bacteria growth, overall these results suggest that IGF-1 may play a role in the control of *Mtb* in vitro.

Methods

Primary macrophages, Bone marrow derived macrophages(BMDMs) were derived from 8-12week old BALB/C male mice, cultured for 10 days at 37°C in M-CSF supplemented medium for macrophage differentiation. BMDMs were harvested and plated overnight in the presence or absence of activators (100U/ml IL-4, IL-13 and IFNy) after 24hours of stimulation, the BMDMs were either left uninfected or infected with live logarithmic phase hyper virulent Mtb HN878 strain or H37Rv strain at a MOI 5:1 (bacilli: macrophage). Cells were transduced with shRNA containing lentivirus against IGF-1 gene for 10days, stimulated with Il-4 to induce expression of IGF-1, after 24 hours cells were replenished with medium containing gentamicin. Following 4 hours, 3 days and 6 days infection, macrophages were lysed to determine bacterial growth, CFUs and cytokines were measured using ELISA. BMDMs IGF-1 receptor were chemically blocked with tyrophostin and CFUs evaluated.

Results

We validated the DeepCAGE by RTqPCR. We observed an increase expression of IGF-1 expression upon alternative activation with no change in the classically activated BMDMs. Upon infection with both strains there was a down regulated expression of IFG-1. Knockdown of IFG-1 was confirmed with targeted shRNA containing lentivirus against IgF-1 with a corresponding reduction in CFUs at day 3 relative to the vector. IGF-1 chemical blocking resulted in reduction in CFU also. There was also a reduction in pro-inflammatory cytokines in the IGF-1 knockdown and blocked BMDMs.

Conclusion

We have shown that IGF-1 plays a role in the control of *Mtb* growth in primary macrophages. Further investigation on the mechanisms involved would suggest new strategies that can be used to design more effective drugs against Tuberculosis.