A CRISPR-Cas9 knockout screen identifies interferon-induced regulators of *Toxoplasma gondii*

Anton Gossner[1], Anna Raper[1], Musa A. Hassan[1]

[1]The Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom.

Abstract

A third of the world's human population is thought to be chronically infected with *Toxoplasma gondii* despite the robust immune response to the parasite in immune competent individuals. As with many intracellular pathogens interferon gamma (IFNγ) production has been shown to be central to the immune response to *Toxoplasma* and control of infection of this protozoan parasite, which is able to infect almost all warm-blooded vertebrates. Murine studies of innate immunity to *Toxoplasma* have identified pathways involved in the IFNγ response to parasite infection. Despite this the response in humans is less defined and there are fundamental differences between the human and murine innate immune systems, such as TLR11 and TLR12, both involved in resistance to the parasite in mice are absent in humans. Despite these differences IFNγ has a major role in the induction of cell-autonomous responses by the human innate immune system, through activation of a large transcriptional sequence of events resulting in the heightened expression of interferon stimulated genes (ISGs). Among the many ISGs expressed by IFNγ stimulated cells, GTPases such as guanylate-binding proteins (GBPs) and immunity-related GTPases (IRGs) have been shown to be important in the immune response to *Toxoplasma*. While the role of IFNγ, a type II IFN, has been extensively studied and shown to be important in immunity against *Toxoplasma*, other IFNs are less well studied. IFNβ, a type I IFN, has been shown to restrict *Toxoplasma* growth in both mouse and human macrophages. Mice lacking the functional Interferon alpha and beta receptor subunit 1 (*IFNAR1*), a type I IFN receptor, have increased susceptibility to *Toxoplasma* infection. While there is activation of overlapping pathways by type I and II IFNs there are also specific type I and II ISGs, resulting in the activation of different signalling pathways. A potential role for type I IFNs in response to the parasite was investigated using a type I IFN inducible ISG-Knockout library. Several genes restricting *Toxoplasma* growth in type I IFN stimulated THP-1 cells, a human monocytic cell line, were identified. These IFN inducible genes included sorting nexin-5 (*SNX5*), coagulation factor II receptor-like 2 (*F2RL2*), myc-associated factor X (*MAX*) and small RNA Binding Exonuclease Protection Factor La (*SSB*), all previously unknown restrictors of *Toxoplasma* growth. To characterise their mode of action we generated CRISPR knockout THP1 cell clones for each gene. These THP1 KO cells will be used to functionally characterise how the selected ISGs restrict *Toxoplasma* growth in THP-1 cells in a type I IFN-induced response.