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T cell Epitope Identification in *F. tularensis*

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Aims: *F. tularensis* is a facultative intracellular pathogen that infects a range of vertebrate hosts. The innate immune system is critical for the resistance to infection and the adaptive immune system is required for bacterial clearance. We have defined T cell epitopes derived from *F. tularensis* to facilitate the study of the adaptive T cell immune response. We developed a series of T cell hybridomas from *F. tularensis* infected animals using standard hybridoma technology. These hybrids have been used to define *F. tularensis* proteins able to stimulate T cells.

Methods: Recently we have developed a novel method to screen nearly all open reading frames from *F. tularensis*. We recloned the Venter Institute SchuS4 ORF library into the pBAD-DEST49 expression vector. We expressed and purified each product and arrayed the recombinant proteins in a high throughput 96 well arrayed format.

Results: We expanded this method by producing a sub-array of 45 gene products identified by a combination of seroreactivity on western blots and previously described immune targets. This set of expression constructs has been dubbed "Greatest Hits." We used these to screen 6 hybridomas derived from infected B6 mice and identified one hybridoma reactive with pathogenicity island encoded protein, IgIB.

In addition we have described four epitopes in B6 mice. Two map in LpnA (Tu4) and two in IgIC. We have also described one epitope in BALB/c mice mapping in L3 ribosomal subunit protein. Finally we have prepared hybrids from transgenic HLA-DR4, IA^{-/-} mice. We have identified approximately 60 hybridomas that respond to *F. tularensis* extracts presented on DR4⁺ but not DR4⁻, B6 APC, indicating they are human DR4 restricted.

Conclusions: We have devised a novel method for the identification of *F. tularensis* epitopes and have been able to use it to provide information on T cell reactivity.