

Defining T Cell Epitopes in Francisella tularensis

L. Hensley, M. Woolard, A. Cawlfeld, T. Kawula, S. Abdul-Alim, J.G. Frelinger, J.A. Frelinger

Background Francisella tularensis (FT) is the causative agent of the disease tularemia. FT has significant potential as an agent of bioterrorism due to its virulence and its ability to persist in the environment. Despite this potential, the immune response to FT has not been well characterized. The goal of this study is to begin defining the T cell epitopes in FT in order to determine the quantity and quality of the T cell response to Francisella infection. In order to accomplish this it is necessary to have a reliable source of FT specific T cells. We are in the process of developing a large library of T cell hybrids from mice immunized with killed or live FT. The epitope specificity of these hybrids will be determined by a high throughput epitope mapping method (T cell antigen discovery, T-CAD). Methods C57BL/6 mice were immunized with ethanol-fixed FT-LVS by intradermal, subcutaneous or intraperitoneal injection or by intradermal injection with a sublethal dose of live FT-LVS. T cell hybrids were produced by standard fusion techniques using the parental cell line BWZ-36 which expresses the lacZ gene under control of the NFAT element. Clones were screened for lacZ activity after stimulation with FT antigen or FT infected syngeneic spleen cells. Results We screened 592 hybrids to identify 239 T cell hybrids that recognize syngeneic spleen cells (APC) which have been pulsed with FT extract, but not APC alone. We also screened 217 hybrids made from FT infected mice of which 30 recognized syngeneic APC which had been infected in-vitro with live FT-LVS. Using antibody blocking we have mapped the MHC restriction to class II in 57 of 64 hybrids tested. Seven of the hybrids were inhibited by both class I and class II specific antibodies, possibly representing a mixture of uncloned lines or restriction by non-classical MHC. 6 clones have been tested and shown to be negative for cross reactivity to E. coli. Conclusions We have developed a library of T cell hybridomas specific for FT. The majority of these clones are class II restricted. We will use these clones to determine the epitope specificity of the T cell response to FT.

"Lucinda L. Hensley" <hensley@med.unc.edu>