

Identification of Immunogenic Epitopes That Permit the Detection of Antigen-Specific T Cell Responses to Coxsackievirus B3

Troy Wesson¹, Adeeba Dhalech², Christopher M. Robinson²

¹Indiana University School of Medicine, ²Department of Microbiology and Immunology, Indiana University School of Medicine

Background and Hypothesis:

Coxsackievirus B3 (CVB3) is a non-enveloped RNA virus from the Picornaviridae family and is a primary cause of viral myocarditis in the United States. Approximately 5% of all symptomatic CVB3 infections are fatal. Therefore, there is a need to identify the mechanism(s) that regulate a protective immune response to CVB3. However, viral epitopes that stimulate T cell responses to CVB3 remain poorly characterized. To this end, we used a mouse model of CVB3 infection to identify the viral immunogenic CD8 T cell epitopes. We hypothesized that isolated antigen-experienced CD8 T cells from infected mice would be stimulated in the presence of predicted viral epitopes, confirming CVB3-specific T cells.

Experimental Design:

To identify novel CD8 T cell epitopes, predicted 9-mer MHC binding peptides from the CVB3-Nancy polyprotein were identified using the Immune Epitope Database (IEDB) analysis resource consensus tool. The top ten predicted peptides were synthesized for our assays. Splenocytes from CVB3-infected male and female IFNAR ^{-/-} mice were stimulated with each peptide in the presence of brefeldin A for 6 hours at 37°C. Following stimulation, cells were surface stained with antibodies specific for antigen-experienced CD8 T cells. Next, we performed intracellular staining for IFN-gamma. Cells were analyzed using flow cytometry. Candidate epitopes were identified as having results ≥ 2 standard deviations over the control.

Results:

Thus far, our analysis has revealed responses to three novel CD8 T cell epitopes within the peptide library, including the viral epitopes within VP1 protein and the RNA-dependent RNA polymerase.

Conclusion and Potential Impact

Overall, these data provide an advancement in CVB3 immunology. Further, these data generate new tools like MHC-tetramers to track endogenous T cell responses to CVB3 infection.

