**RAMP Project Abstract: BIRMINGHAM, ALABAMA**

**Project Title:** Evaluation of HLA-E restricted SARS-CoV-2 specific responses in following COVID-19 infection and vaccination

**Project Type:** Short-term Project 8-10 weeks On-site

**Proposed Project Dates:** Flexible 8 -10 weeks On-site between June 2023 – August 2023

**Project Site:** University of Alabama at Birmingham

**Project Overview:**

The prevailing dogma of adaptive immunity posits that endogenously processed peptides (self and pathogen derived) are commonly presented to CD8 T cell via major histocompatibility antigen (MHC)-class Ia or the so-called classical alleles. The contribution of MHC- class Ib or non-classical alleles has been examined mainly in context of innate immunity i.e., natural killer cells. In humans and macaques, the MHC alleles are designated as HLA and Mamu alleles, respectively. In humans, the classical alleles include HLAs-A, B and C and the non-classical alleles are represented by HLAs-E, F and G. Of the non-classical, HLA-E is the most well-studied. This allele has limited polymorphism and its dimorphic alleles E01:01 and E01:03 together cover >99% of all human populations and is thus an attractive antigen presenting mode for vaccines.

Recent studies have shown E allele restricted CD8 T cell responses to several bacterial and viral pathogens including SIV and HIV. Seminal work from Picker and colleagues showed that Mamu-E restricted CD8 T cells are essential for protection from establishment of SIV infection in a rhesus macaque model. Our recent data in humans showed that HLA-E restricted responses are commonly detected in chronic HIV infection and importantly these responses can be readily primed from HIV uninfected donors. The latter has implications for HIV-1 vaccine design.

Our recent data shows that HLA-E restricted responses can also be detected in COVID-19 infected individuals. While these are important preliminary findings, our understanding of HLA-E restricted CD8 T cells in COVID-19 disease pathogenesis is extremely limited. For instance, we do not know the frequency of such responses elicited following COVID-19 infection and how it relates to disease outcome. In addition, information is lacking on the role of E restricted responses post COVID vaccination and/or what role HLA-E restricted CD8 T cells play in the prevention and/or control of infection.

**Project Summary:**

Several lines of evidence indicate that myeloid cells present foreign peptides through binding to MHC-E and presentation to CD8 T cells. These findings would suggest that SARS-CoV-2 infection could induce such responses, as myeloid cells are readily infected by this virus. Our preliminary data supports this observation. In contrast, COVID-19 vaccination via the intramuscular route largely presents antigens via muscle cells. We, therefore, anticipate that HLA-E restricted CD8 T cells would not be readily detected following COVID-19 vaccination. In order to test this hypothesis, we will do the following:

1. Use stored peripheral blood mononuclear cells (PBMC) from 25 individuals each who have
    a. recovered from COVID-19 infection
    b. received two-dose mRNA based COVID-19 vaccinations
    c. received two-dose mRNA based COVID-19 vaccinations post infection

2. Use in vitro based ICS assay to examine CD8 T cell function by coculturing purified CD8 T cells (effector) with single HLA-E allele (E01:01/E0:03) expressing and peptide pulsed cell lines (targets). Based on HLA-class I typing information, in select samples we will also compare the HLA-E restricted CD8 T-cell responses with those induced against peptides presented by single classical HLA-A or -B expressing cell lines.
3. Evaluate ICS data using FlowJo analytic software to compare CD8 T-cell responses between the three groups and also between classical and non-classical alleles.

These methods will allow us to determine the frequency of HLA-E restricted CD8 T cells induced following COVID-19 infection and vaccination and lead to new hypothesis driven studies to examine the contribution of these responses in detail. Research staff in our laboratory will teach and assist the RAMP scholar to perform the assays and help acquire the samples via flow cytometry; however, if COVID-19 restrictions prevent travel by the RAMP scholar, all of these studies will be performed by lab personnel and the data analysis performed remotely by the RAMP trainee. We also anticipate weekly in person/virtual meetings to discuss the experimental design and execution, data acquisitions and its analysis and presentation. By the end of the rotation, it is expected that the RAMP scholar will have a comprehensive understanding of the research done, generate important data and be able to present the findings to the HVTN.

**Regulatory requirements for the project and plans for completing them:**

All the samples for the current project have already been collected in site from over 2,000 samples from COVID-19 infected and vaccinated individuals under an IRB approved protocol. We only need to add the RAMP scholar to the protocol after they have undergone IRB training which generally takes a few days.

**Expected Deliverables:**

HVTN-presentation
Preliminary data for a manuscript submission

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