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

Preferred Scholar On-Site Project Dates: Anytime between June-August 2024 (8-10 weeks)

Project Site: Birmingham, Alabama (Alabama Vaccine Research Clinic at University of Alabama)

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 classical alleles. The contribution of MHC- class Ib or non-classical alleles has been mainly examined 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 MHC-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 CD8 T-cell responses (HLA-E/CD8s) 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.

A recent publication by Yang et al (PMID: 37390223; DOI: 10.1126/sciimmunol.abl8881) showed that HLA-E responses can be detected in SARS-CoV-2 infected individuals. This work showed that during SARS-CoV-2 infection although HLA class I was downregulated, HLA-E expression was unaltered. Furthermore, the study found that HLA-E restricted SARS-CoV-2 specific T cell responses were observed at similar frequency as responses restricted by the classical HLA class I alleles. In addition, HLA-E/CD8s from convalescent patients were able to suppress viral replication and HLA-E/CD8s showed a diverse TCR repertoire. These data suggest that HLA-E restricted CD8 T cell responses could be an important contributor to viral control, working in conjunction with those responses restricted by the classical HLA-I alleles. Preliminary data from our laboratory also shows that HLA-E restricted CD8 T-cell responses can also be detected in COVID-19 infected individuals.

Despite these observations, our understanding of how HLA-E restricted CD8 T cells compare in functionality to those restricted by classically HLA-I restricted CD8 T cells and how HLA-E/CD8s targeting relates to COVID-19 outcome is not known. In addition, information is lacking on the role of HLA-E restricted CD8 T-cell responses post COVID vaccination and/or what role HLA-E restricted CD8 T cells play in the prevention and/or control of infection. Finally, whether HLA-E/CD8s continue to be targeted in individuals with long COVID will be important to determine.

Project Summary: HLA-E/CD8 responses capable of suppressing SARS-CoV-2 virus have been reported in a recent publication indicating that these T cells may be playing an important role. However, several questions remain unanswered. These include the following:

a) Does the functionality of HLA-E/CD8s compared to the classically restricted CD8 T-cell responses in terms of exacting viral control?
b) Does the targeting of HLA-E/CD8s relate with COVID-19 outcome (mild, severe, hospitalized)?
c) Does the targeting of HLA-E/CD8s play an important role in long COVID?
d) Are HLA-E/CD8s elicited by mRNA based COVID vaccinations? If yes,

a. Does this targeting prevent breakthrough infections or impact symptomatic disease once infected?

e) Are infection induced HLA-E/CD8s, expanded post COVID vaccination.

To address this, we will perform the following:

1. Synthesize 29 peptides from SARS-CoV-2 that are predicted to bind HLA-E (PMID: 37390223) for use in immune assays.
2. Use archived peripheral blood mononuclear cells (PBMC) from 50 individuals each who,
   a. recovered from COVID-19 and had acute infection (with/without hospitalization), mild infection and those with long COVID.
   b. Despite receiving full booster doses of mRNA vaccines either got infected or not.
   c. Got vaccinated following symptomatic infection.
3. Develop select tetramers based on immunogenicity data (#2 above) and use them for isolation of HLA-E/CD8s.
4. Use in vitro based flow cytometry assays to examine phenotype and function by coculturing purified CD8 T cells (effector) and single HLA-E allele (E01:01/E0:03) or classical HLA-I expressing and peptide pulsed cell lines (targets). These will include both ICS and AIM assays.
5. Evaluate all flow cytometry-based data (#3 and 4 above) by using Flowjo software.
6. Perform single cell(sc) based sortig of HLA-E and classically restricted CD8 T cells (N=10 pairs) and examine the scRNA and TCR seq profile using 10X genomics based 5' gene expression system.

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. ***The scholar will first complete all laboratory work and human subject related trainings that are needed to work in the laboratory. The scholar will then learn immune assays and get training on data acquisition and analysis from our research staff who will later also assist the RAMP scholar (if needed) to perform the assays and acquire the samples via flow cytometry. We also anticipate weekly in person/virtual meetings to discuss the experimental design and execution, data acquisition and its analysis and presentation. The RAMP scholar will also attend other weekly seminars/lectures at UAB in virology and immunology to enhance their educational experience. In addition, they will be able to do some clinical shadowing in the 1917 HIV clinic where Dr. Goepfert provides care to patients living with HIV. By the end of the rotation, it is expected that the RAMP scholar will have a comprehensive understanding of the research done, be able to work independently, generate important and quality data and be able to present the findings to the HVTN.*** *In addition, data generated will be assembled into a manuscript and submitted for publication.*

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:

The assay protocols and samples will be shared with interested investigators. Once completed, the work will be presented at an HVTN meeting. A manuscript based on the final data will be submitted for publication.

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