[Applicant name]

Identifying the NKG2D ligands that activate γδ T cells and characterising mechanisms of the γδ T cell immune response during SARS-CoV-2 infection

Aims and Objectives

This project aims to demonstrate the benefits harnessing γδ T cells could have in fighting SARS-CoV-2 infection by using the following objectives:

1. Identify the expression of molecules that might act as ligands for γδ T cells in SARSCoV-2 infected cells that activate the immune response by γδ T cells
2. Determine the molecular basis of how γδ T cells detect and kill infected cells
3. Investigate whether the identified ligands and immune responses are present in SARS-CoV-2 infected non-human primate tissue.

Background

Over the last two years severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 5 million deaths globally during the COVID-19 pandemic. Despite the rapid production of successful spike protein-based vaccines, variants of concern (VOC) continue to arise containing new mutations which confer increased resistance to vaccines. For instance, the delta variant has arisen due to independent evolution of mutations in the SARS-CoV-2 spike glycoprotein. Mutations such as these can strengthen SARS-CoV-2 receptor binding whilst reducing the ability of vaccine-induced antibodies to bind to this mutated spike glycoprotein. Although SARS-CoV-2 is a relatively stable virus, vaccine evasion is likely to become more prevalent due to the abundance of SARS-CoV-2 replication occurring worldwide. There is a requirement to explore additional novel antiviral therapies to combat vaccine evasion and bolster the response to COVID-19. Furthermore, low and middle income countries (LMICs) currently struggle with the cost and logistics of COVID-19 vaccine roll out. Vaccines in LMICs cannot be obtained easily due to their high price and cannot be distributed as effectively because of a lack of infrastructure.

□□ T cells are an overlooked arm of the immune system which could ameliorate the armamentarium in the fight against SARS-CoV-2 (1). These unconventional T lymphocytes are one of the first immune cells to react to viral entry, and have been shown to be critical in the clearance of certain viral infections such as influenza (1). Their early detection of 'danger signals', quick activation and rapid cytotoxic response appear vital in fighting infection. □□ T cells are activated by receptors such as the natural killer group 2-member D (NKG2D) receptors that detect danger signals (2). MHC class I related molecules A/B (MICA/MICB) are examples of danger signals that are upregulated in response to viral infection (2). Detection of MICA/MICB by NKG2D receptors on cells results in a swift □□ T cell response to infection (2). During the 2003 SARS-CoV outbreak, □□ T cells were selectively expanded in health care workers who survived infection (1). In these health care workers, □□ T lymphocytes were responsible for clearance of SARS-CoV in an interferon-□ (IFN-□) dependent manner (1).

Priming □□ T cells could be used to 'train' the innate immune response to treat SARS-CoV-2 (2). □□ T cells offer a source of immunity which have been shown to protect against other pathogens and could do the same against SARS-CoV-2 (2,3). Treatment with the Bacille Calmette-Guérin (BCG) vaccine primes □□ T cells, which can train the immune system to protect against other respiratory infections such as influenza and respiratory syncytial virus (1,2). The BCG vaccine could potentially be used as an affordable treatment of SARS-CoV-2 in LMICs who cannot afford the current vaccines. Furthermore, VOC will be susceptible to the less specific immune response mounted by □□ T cells. Future work in this field is currently limited by the scarcity of information about the □□ T cell interaction with SARS-CoV2. Identifying the mechanisms underlying □□ T cell detection and response against SARSCoV-2 is a pivotal step towards using □□ T cells in treatment (4).

[Applicant name]

I am passionate about researching □□ T cells and their response to SARS-CoV-2 infected cells. This has led me to choose the project at St. George's University of London (SGUL). I have been fortunate enough to meet with Dr. Bodman-Smith online to discuss potential research regarding □□ T cells in the context of SARS-CoV-2 infection. There is little known about the role of NKG2D ligands in the detection by □□ T cells during infection with SARS-CoV-2 and the subsequent cytotoxic response to these danger signals. Elucidating the mechanisms that mediate detection of danger signals and killing of infected cells is of paramount importance. My research question therefore would be **'How can we characterise the mechanisms through which □□ T cells detect and kill SARS-CoV-2 infected cells?'**

Methodology

1. Characterisation of detection and immune response by □□ T cells *in vitro*

The first part of the project will take place over one year to identify NKG2D ligands on cell surfaces and the response to activation of □□ T cells by these ligands. Blood products will be obtained from blood transfusion donors to source □□ T cells (3). Human epithelial cell lines will be infected with replication competent SARS-CoV-2 under the expert guidance of [deleted] in [deleted]. Confirming expression of NKG2D ligands on the surface of these infected human epithelial cells will be integral here. Flow cytometry will be used to detect NKG2D ligands expressed on cell surfaces (1,3). Quantitative assessment of intracellular NKG2D ligand expression will be carried out using Western blotting.

Having confirmed NKG2D ligand cell surface expression, I plan to expand □□ T cells using the BCG vaccine or zoledronic acid with interleukin-2. Cells will be isolated using magnetic bead kits and these cells will be co-cultured with SARS-CoV-2 infected epithelial cells *in vitro*. I will observe if activated □□ T cells kill infected cells by using flow cytometric killing assays such as CD107 expression assays and degranulation assays. Flow cytometry and fluorescence activated cell sorting (FACS) analysis will be used to observe immune cell changes during the □□ T cell response to infection. My undergraduate degree in [programme deleted] at [institution deleted] has given me experience in working with flow cytometry. I will analyse the upregulation of various products such as IFN-□ and tumour necrosis factor alpha (TNF-α) involved in killing infected cells. Cytokines and other molecules such as granulysin, granzymes and perforin are associated with cytotoxicity (3,4). These molecules in the supernatants of co-cultures will be analysed using multiplex bead immunoassays (Legendplex). Intracellular cytokine staining will be used to collect more data on the cytokines produced by □□ T cells at different stages of infection. I will use antibodies to block the cytokines identified as being involved in this mechanism to determine their roles in this pathway. If this first part of the project is successful I will characterise NKG2D ligand expression, the detection of danger signals such as MICA/MICB by □□ T cells during SARSCoV-2 infection and the immune response of □□ T cells to this infection *in vitro*.

2. Using archived tissue as an *ex vivo* model to identify danger signals and responses by □□ T cells

I will attempt to identify the ligands, signals and responses initially found in the *in vitro* system in non-human primate (NHP) tissue. This experiment will be performed *ex vivo* using SARS-CoV-2 infected [deleted] *macaque* lung tissue. I will look for NK2GD ligand expression and MICA/MICB danger signals in infected lung tissue. I will also look for presence of □□ T cells in infected tissue and observe if they are close to the expression of NK2GD ligands. Immunohistochemistry will be used to identify the antigens for signals and

responses that will have been characterised in the first stage of the project. I will use fluorescence *in situ* hybridization (RNAscope) to detect target nucleotide sequences from the components I identified in the first stage of the project. Here I will work closely with [deleted]. I will utilise quantitative image analysis throughout this project for analysis. NHP tissue is the only model aside from humans in which □□ T cells can be used as the V□2 population, which is of great importance for research.

Intended research outcomes

The research produced will result in a detailed understanding of the □□ T cell response to SARS-CoV-2. Elucidating the mechanisms that underlie this immune response will greatly help when analysing the effect of using □□ T cells as a treatment against SARS-CoV-2 (4). The overarching outcome of this research is to facilitate the production and administration of antiviral therapies that can help in the pandemic, especially with VOC (4). This will be of particular benefit in LMICs where vaccine roll out is inefficient. Furthermore, this will work synergistically with current vaccine strategies in place in other countries.

Applicant background

Studying [deleted] at [institution deleted] developed a curiosity in the co-evolutionary arms race between humans and viruses. This fascination led to host immune responses to viruses becoming my primary area of research interest. As a result of this interest, I completed a [applicant-specific experience deleted]. Completing this project garnered a further interest in researching viruses in doctoral studies and contributing to the fight against viruses. I am currently studying [programme deleted] at [institution deleted]. I have just finished [deleted] in which I performed [deleted]. The project entailed analysing [deleted]. This [deleted] will lead to further research in the form of [deleted].

I aspire to be a part of the high quality research in □□ T cells that Dr. Bodman-Smith and his colleagues are known for. In light of the COVID-19 pandemic, there is an increasing need for subject specialists to tackle SARS-CoV-2. It is my goal to use my scientific interest, knowledge and expertise in virology to be a valuable member of the co-evolutionary arms race against viruses. By completing a PhD in the project offered at SGUL with Dr. BodmanSmith and his colleagues, I will be able to achieve this objective.

References

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2. Morrison AL, Sharpe S, White AD, Bodman-Smith M. Cheap and Commonplace: Making the Case for BCG and γδ T Cells in COVID-19. Frontiers in Immunology. 2021:3612.

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