Project Details	
Project Code	MRCIIAR25Ca Stanton
Title	Enhancing Immunological Control of SARS-CoV-2
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Neutralizing antibodies are key to preventing transmission of SARS-CoV-2, however the virus can rapidly mutate to avoid these. Furthermore, once infected, neutralizing antibodies cannot access intracellular virus. Therefore, antibodies that recognise cell-associated virus are critical. However, current vaccines fail to induce these. We will investigate how this activity can be induced, as the basis for next-generation and variant-resistant coronavirus vaccines.
Description	Studies of antiviral humoral immunity are dominated by neutralising antibodies, which inhibit cell-free virions. However, these are narrowly focussed on limited antigens, thus antigenic drift readily enables escape. In addition, viruses undergo direct cell-to-cell transmission, which protects from neutralising activity – as seen in the reduced efficacy of monoclonal SARS-CoV-2 antibodies when given after infection has become established(1). Consequently, antibodies that target cell-associated virus are also needed. These bind viral antigens on the infected cell surface and activate cellular immunity (antibody-dependent cellular cytotoxicity; ADCC) through Fc receptors (1). The importance of ADCC is underscored by several observations: (i) it correlates with natural and vaccine-mediated control of multiple viruses; (ii) enhancing or abrogating these functions in monoclonal antibodies alters their ability to control viruses in animal models; (iii) it is responsible for the efficacy of numerous clinically approved anti-cancer antibodies (e.g. trastuzumab, rituximab); (iv) viruses dedicate genomic space to antagonising it. Yet despite its importance, we found that current SARS-CoV-2 vaccines fail to induce ADCC (2). Spike vaccines generate potent neutralising antibodies, but poor ADCC. Instead, Nucleocapsid (N), ORF3a, and Membrane (M) are the dominant targets for this response. We therefore propose that next generation vaccines can be enhanced by including antigens that induce ADCC, enabling targeting of both cell-free and cell-associated virus. We hypothesize that this will reduce the need for boosters, reduce the waning of efficacy over time, and the broader polyclonal nature of ADCC will provide superior resistance to virus escape. We will determine: 1. Which antigens most efficiently induce ADCC in vitro. We have developed technologies to isolate human monoclonal antibodies (3), and have banked PBMC from >100 donors throughout the pandemic. This will be exploited to isolate antibodies targeting N, M, and ORF3a. Antibodies will be screened for ADCC capacity individually and in combination. We will also use blocking peptides and competition ELISAs to define critical epitopes. This will define which of our targets are naturally superior at controlling virus through ADCC. 2. Whether enhanced ADCC activity improves control in vivo. Mice will be vaccinated with Adenovirus (Ad) vectors expressing N, M, or ORF3a, then serum tested for ADCC capacity. K18-hACE2 Mice will be vaccinated (prime/boost), with Ads expressing our lead ADCC antigen, or

	Spike (generating neutralising responses), or both together, then challenged with SARS-CoV-2. Animals will be assessed for weight loss, survival, and viral loads in the lung, 72h later. This will define whether optimising ADCC enhances virus control in vivo, and whether it synergises with neutralising activity. 3. How broadening of antigenic targets beyond spike enables immune responses to better resist the evolution of VOCs. Variants of SARS-CoV-2 that are altered in neutralising epitopes on Spike are continuously being selected. However, since ADCC targets a wider range of antigens, it has the potential to render vaccines more resistant to this process. We will therefore screen the ADCC capacity of our banked polyclonal serum and/or mAbs (aim 1) against multiple VOCs, to determine whether this is the case. If it is, we will use our in vivo systems (aim 2) to investigate whether optimising ADCC induction limits the susceptibility of immune responses to evasion. 1.Yamin et al. Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2 efficacy. Nature (2021) 2.Fielding et al. SARS-CoV-2 host-shutoff impacts innate NK cell functions, but antibody-dependent NK activity is activated through non-spike antibodies. eLife (2022) 3.Vlahava et al. Monoclonal antibodies targeting nonstructural viral antigens can activate ADCC against human cytomegalovirus. JCI (2021)
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