Project Details	
Project Code	MRCIIAR26Ca McLaren
Title	Immune modulation of tonsillar Natural Killer cell immunity by Group A Streptococcus
Research Theme	IIAR
Project Type	Wet lab
Summary	The human tonsils are crucial for producing neutralizing antibodies against infection yet are a breeding ground for Group A Streptococcus (GAS) which causes localized illnesses (tonsillitis) but also life-threatening, invasive infections (>500,000 global deaths/year). Children prone to recurrent tonsillitis generate poor levels of neutralizing antibodies but the reasons why are unclear. Natural killer (NK) cells, immune cells that are typically protective, can suppress antibody production in lymphoid organs during virus infections. The student will examine if NK cells are functionally altered in children with recurrent GAS-associated tonsillitis, whilst identifying if and how tonsillar NK cells mediate impaired, GAS-specific antibody production.
Description	Background: Group A Streptococcus (GAS) is a Gram-positive bacterium that is a major cause of global mortality, particularly in low- and middle-income countries. Also known as Streptococcus pyogenes, GAS typically colonises the human skin and throat and can cause mild, localized infections such as tonsillitis which occurs in 600 million individuals globally each year. However, invasive, severe GAS infections can occur (e.g. bacteraemia, osteoarticular infections, toxic shock syndrome) which can be life-threatening, causing >500,000 deaths per year globally. Whilst antibiotics can clear GAS infections, autoimmune manifestations can persist, such as rheumatic heart disease which can lead to permanent heart valve damage and has a disability burden equivalent to a quarter of all forms of cancer. Currently, there is no licenced GAS vaccine which is partly due to our incomplete understanding of the immune response to GAS infection. Accordingly, the World Health Organisation has made it a priority for scientists to develop an efficacious vaccine that is protective against GAS infections. The human tonsils are a breeding ground for GAS, despite being secondary lymphoid organs that are anatomically poised in the throat to generate protective mucosal immune responses against pathogens. The production of neutralizing antibodies by B cells within germinal centres of the tonsils is critical for counteracting the threat of GAS, which is crucially supported by the actions of CD4+ follicular helper T (TFH) cells. However, certain individuals, especially children, are prone to recurrent bouts of GAS-associated tonsillitis which is associated with a lack of neutralizing antibodies. It has been described that natural killer (NK) cells, specialist immune cells that typically protect the host against virus infections, can suppress germinal centre CD4+ TFH and B cell responses in in response to vaccination or chronic virus infections. Such negative regulation of CD4+ TFH and B cells in tonsils by NK cells could help explain why certain individuals generate poor levels of GAS-specific neutralizing antibodies. It also could rationalise why NK cell-depleted mice are more resistant to GAS infection, have better survival rates and slower development of GAS-associated diseases. However, our

	knowledge of how NK cells respond to GAS infection, especially within human lymphoid organs is poor. Significance: Both GAS and Staphylococcus aureus have evolved the ability to produce "superantigens", highly potent exotoxins which drive life-threatening complications of invasive infection, such as toxic shock syndrome and bacteraemia. The inability to produce sufficient levels of neutralizing antibodies against superantigens correlates with disease severity, whilst children experiencing recurrent bouts of GAS-associated tonsillitis show lower titres of anti-superantigen antibodies. These enterotoxins typically act by forcing T cells to over-proliferate and produce excessive amounts of pro-inflammatory cytokines yet there is evidence they also regulate NK cell function. Indeed, we have recently shown that superantigens from Staphylococcus aureus promote the expansion of highly proliferative, interferon-□ producing NK cell subsets expressing the inhibitory receptor, NKG2A. However, very little is known about whether GAS-encoded superantigens affect peripheral and tonsillar NK cells, and if their effects contribute to the promotion of impaired antibody production in tonsils. Research Question: Can NK cells negatively regulate the production of GAS-specific neutralizing antibodies in human tonsils and do superantigens produced by GAS (e.g. SpeA) promote functional and phenotypic changes in tonsillar NK cells to promote this? Objectives: The student will gain a comprehensive understanding of how NK cells respond to GAS-associated superantigens, their impact on germinal centre-elicited antibody production and if alterations in NK cell functionality are prevalent in children with recurrent GAS-associated tonsillitis. These specific objectives are to understand: • The functional and phenotypic alterations elicited by GAS-encoded superantigens within peripheral blood and tonsillar NK cells • What impact NK cells have on the production of GAS-specific neutralizing antibodies in human tonsils • Whether children with recurrent GAS-associated tonsillitis possess altered NK cell-based immunity Research Training and Added value: The student will receive expert training in in vitro-based immunological, microbiological and next-generation sequencing techniques to address these objectives. They will gain experience in culturing tonsillar organoids, using GAS strains with genetic deletions in superantigens (e.g. SpeA) and measuring GAS-specific antibody production. They will learn to use advanced flow cytometric approaches to assess differences in human NK cell phenotype (e.g. expression of activating and inhibitory receptors) and functionality (cytokine production, degranulation) whilst using RNA sequencing to map transcriptomic alterations in tonsillar NK cells from children with recurrent GAS-associated tonsillitis. The project combines biological and mathematical approaches and has been designed with flexibility to enable the student to steer the project and align it with their interests, whilst enabling them to benefit from established local and international collaborations in Cardiff, Bristol and Australia. Furthermore, the student will be encouraged to publicise their

	research to specialist and/or public audience(s) through peer-reviewed publications, presentations at institutional seminars and scientific conferences or outreach events involving using social media, institute websites and public engagement opportunities.
Supervisory Team	
Lead Supervisor	
Name	Dr James McLaren
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Email Address	mclarenje@cardiff.ac.uk
Co-Supervisor 1	
Name	Dr Anu Goenka
Affiliation	Bristol
College/Faculty	Faculty of Health & Life Sciences
Department/School	School of Cellular and Molecular Medicine
Co-Supervisor 2	
Name	Dr Alice Halliday
Affiliation	Bristol
College/Faculty	Faculty of Health & Life Sciences
Department/School	School of Cellular and Molecular Medicine
Co-Supervisor 3	
Name	Professor Eddie Wang
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine