Project Details	
Project Code	MRCIIAR26Ca Raby
Title	Can Damage Associated Molecular Patterns be targeted to reduce susceptibility to infections in people with Chronic Kidney Disease?
Research Theme	IIAR
Project Type	Wet lab
Summary	People with chronic kidney disease (CKD) are more vulnerable to infections, in part because their immune systems don't function properly. One reason is that chronic inflammation in CKD leads to constant low-level activation of immune cells, which over time may exhaust the body's defences. We have identified certain molecules called Damage-Associated Molecular Patterns (DAMPs) that are released when tissues are damaged in CKD, and found that they trigger immune sensors to cause chronic inflammation. We now aim to evaluate whether blocking the activity of certain DAMPs could prevent immune exhaustion in CKD patients and help them fight infections better.
Description	**Background** Infections are a major cause of death in people with Chronic Kidney Disease (CKD). Higher susceptibility to infections in CKD is in part due to innate and adaptive immune dysfunctions that impair pathogen clearance and reduce vaccine effectiveness [1-2]. The concept that chronic inflammation in CKD leads to continuous low-level activation of immune cells and their subsequent lack of responsiveness has emerged as a potential driver of CKD-associated immunosuppression, but mechanisms are poorly understood. DAMPs are endogenous molecules released by cells following cellular stress and tissue damage. They are varied in nature, including notably intracellular proteins, cell-free nucleic acids and matrix components. Their detection by innate immune sensors called Toll-Like receptors (TLRs) leads to inflammation. Our work has shown that CKD leads to elevated plasma levels of DAMPs, and that their interaction with TLRs drives chronic inflammation [3-4]. **Hypothesis** We therefore hypothesise that DAMPs may promote CKD immune dysfunction by inducing continuous low-grade activation of immune cells. In support of this, our initial work has shown that: - Pre-exposure of monocytes to the CKD-associated DAMPs we previously identified led to reduced cytokine release following bacterial encounter - There was a negative correlation between a CKD patient's plasma levels of some DAMPs and their blood cells' responses to bacteria. - Pre-exposure of monocytes to CKD serum impaired their ability to release cytokines upon pathogen encounter. This effect was reduced by addition of a multi-TLR inhibitor previously identified by us (soluble TLR2, sTLR2 [3-5]). Aim: This project will assess the contribution of DAMPs to immune dysfunction in CKD, with the aim of evaluating therapies to limit background TLR activation by DAMPs, thereby reducing chronic inflammation and preventing immune dysfunction in CKD.

	Objectives and approaches Objective 1: Identify the DAMPs most associated with immune dysfunction in late-stage CKD Circulatory levels of DAMPs previously found elevated in CKD or not previously tested will be compared in late-stage CKD patients displaying more vs those displaying less immunosuppression. The degree of immunosuppression will be established based on either: Approach 1- Number of serious infections: Plasma from CKD patients who did not go on to have (assumed to be less immunosuppressed), versus those who did develop (assumed to be more immunosuppressed), serious infections will be obtained from the Wales Kidney Tissue Bank (n=100). For patients with infections, the last sample obtained prior to the first infection will be tested for DAMP levels. Non-infected samples will be matched for age, sex, co-morbidities, eGFR, as well as dialysis modality and time on dialysis if relevant. Student development: Mastery of core biochemistry techniques; Working with clinical samples (inc. HTA training); Interrogating large clinical data bases; Identifying and Controlling for confounding factors. Approach 2- Ex vivo responses to pathogenic challenge: Ex vivo responses to bacterial exposure will be compared in blood cells from late-stage CKD patients (n=30) and healthy donors (n=20). Typical functions associated with neutrophils, monocytes, T and B cells will be investigated and degree of immunosuppression will be determined using healthy donors as a non-immunosuppressed reference. DAMPs levels will be analysed in plasma from the blood sample prior to bacterial stimulation. Patients data will also be regularly checked for the development of a serious infection after this testing, to bridge Approach 1 and Approach 2 when possible. DAMPs found associated with immune dysfunction in both approaches, or the top two DAMPs for each approach, will be selected for further testing below. Contingency plan: The use of 2 approaches to measure immunosuppression, including one that has successfully been used in preliminary work (Approach 2), will increase our chances of identifying DAMPs associated with immunosuppression. Student development: Mastery of several tissue culture techniques and immune-focused functional assays; Decision-making about most promising DAMP targets. Objective 2: Verify the immunosuppression-inducing potential of the selected DAMPs Normal blood cells will be pre-exposed to the selected DAMP(s) prior to bacterial stimulation. Typical functions associated with neutrophils, monocytes, T and B cells will be assessed. Contingency plan: If single DAMPs fail to induce immunosuppression, a combination of the selected DAMPs will be tested Student development: Assay protocol optimization (e.g., DAMP concentration, duration of pre-exposure), decision-making about end of assessment for DAMPs displaying no immunosuppressive ability.

	Objective 3: Evaluate the ability of inhibitors of DAMP:TLR interactions to combat immune dysfunction in CKD mice A model of Aristolochic-Acid-induced nephropathy (AAN) that leads to immune dysfunction will be used. AAN mice will be administered with inhibitors for the selected DAMP(s)or soluble TLR2 for a month before infection or vaccination. Bacterial clearance and responses to vaccination will be assessed [6]. Student development: Work with animals, including obtention of personal licence and proficiency in a number of in vivo techniques. **References:** 1.Clin J Am Soc Nephrol 3(5),1487(2008) 2.Clinical and experimental nephrology 23(4),437(2019) 3.Front Immunol 14,1240679(2023). 4.Front Cell Infect Microbiol 13,1285193(2023) 5.J Immunol 183(1),506(2009) 6.Science translational medicine 5(185),185ra64(2013)
Supervisory Team	
Lead Supervisor	
Name	Dr Anne-Catherine Raby
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Email Address	rabya@cardiff.ac.uk
Co-Supervisor 1	
Name	Dr Kiki Cano-Gamez
Affiliation	Exeter
College/Faculty	Faculty of Health and Life Sciences
Department/School	Department of Clinical and Biomedical Sciences
Co-Supervisor 2	
Name	Dr Soma Meran
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	