TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Neutrophil Dysregulation in Rheumatoid Arthritis

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Background
RA is a systemic autoimmune disease that primarily affects synovial joints and affects ~1% of the UK population. Treatment options have improved dramatically in recent years but there is no cure. Autoantibodies, especially pathogenic anti-citrullinated protein antibodies (ACPA) are abundant in both serum and synovial fluid of RA patients. ACPA form immune complexes (ICs) which circulate in bodily fluids as soluble and insoluble ICs and precipitate onto synovial joint surfaces as immobilised ICs. The initiation of RA is dependent on T cells, but neutrophils are key once disease is established. A vicious cycle between B cells and neutrophils promotes disease progression [1]. Neutrophils are highly abundant, extremely short-lived circulating leucocytes that function in host immunity making use of specialised functions. Neutrophils are more activated in RA and inappropriate neutrophilic inflammation can drive important host tissue damage. Critical for inflammation, following their short lives, neutrophils undergo a pro- or anti-inflammatory cellular death [2]. ICs are powerful stimuli of neutrophils, that elicit both pro- and anti-inflammatory neutrophil functions and deaths. In RA, ACPA-ICs stimulate neutrophils, e.g. to generate highly inflammatory neutrophil extracellular traps (NETs). NETs are a major source of citrullinated proteins that trigger generation of new ACPA [1], feeding into the vicious cycle. While NET association with RA is well documented, how NETs (and indeed NET release) regulate neutrophils themselves remains poorly defined. NET release can be triggered via pathways with distinct molecular signatures. The best characterised 'NETosis' pathway is easily triggered using non-physiological agonists and results in lytic neutrophil death. In contrast, physiological agonists such as ICs result in viable neutrophils releasing 'vital NETs' the biology of which remains obscure. Neutrophilic inflammation is limited by neutrophil apoptosis, an anti-inflammatory cell death. Apoptotic cells promote their own efferocytosis (uptake) by macrophages, triggering macrophage polarisation from a pro-inflammatory to a pro-resolving phenotype. This results in the generation of pro-resolution cytokines by the macrophages. We have previously shown that ICs promote neutrophil apoptosis but also fast release of vital NETs [2 and unpublished]. This project will explore how these functions are altered in RA, identify molecular pathways involved, and trial the ability of existing small molecules targeting these pathways to reverse the changes observed.

Aims
This project will test how ICs promote neutrophil dysregulation in RA, in turn affecting macrophages polarisation and accelerating disease progression. RA, disease control and healthy volunteer neutrophils and monocyte-derived macrophages (MDMs) will be analysed. (i) NET release and induction of neutrophil apoptosis in response to IC-stimulation will be compared (ii) Effect of IC-induced NETs on MDMs and neutrophils themselves will be characterised, with a focus on particularly pertinent aspects (e.g. NETs clearance via internalisation; effects on IC-induced neutrophil apoptosis). Since clearance of pro-inflammatory material is vital for the resolution of inflammation, MDM polarisation and cytokine production following stimulation with NETs and apoptotic neutrophils will also be examined. (iii) Bulk transcriptomics/proteomics datasets will be generated from cells which were co-cultured with apoptotic neutrophils, NETs and suitable controls. Analysis of these datasets will map molecular pathways that are affected by immune dysregulation in RA. (iv) Existing inhibitors targeting key molecules identified in (iii) will be trialled, testing whether neutrophil dysregulation and knock-on effects on macrophages can be reversed, focussing once more on IC-induced NET release, induction of apoptosis and polarisation of MDMs. Plan B. Should (iii) not identify any suitable molecular pathways, (iv) will focus on pathways modulated via interferon signalling, which was found upregulated in previous studies [e.g. 3] that characterised synovial neutrophils.

Training and experience provided
This research project will provide the PhD student with a hands-on research experience and data analysis that will equip them well for their future career as a clinician scientist. The student will become part of the CIR postgraduate cohort. They will join a vibrant laboratory in the state-of-the-art IRR which is dedicated to the study of inflammation, resolution and tissue repair. The student will benefit from an expert supervisory team made up of a scientist (SV) and a clinician scientist (MG). They will gain expert understanding of autoimmune diseases, study design and statistics; they will become practical skills and data analysis and acquire valuable transferable skills.

Expected outcomes
The systematic analysis of dysregulation of neutrophils in RA, and follow-on effects this has on macrophages will help with identifying molecular pathways that are pertinent to disease progression in RA after the initial T cell-dependent initiation phase. By trialling existing compounds that modulate these pathways, the proposed work will build a platform for future clinical research aimed at reducing the morbidity and mortality of RA patients.

References
[1] Karmakar & Vermeren (2021) Crosstalk between B cells and neutrophils in rheumatoid arthritis. Immunology 164, 689. doi:10.1111/imm.13412 [2] Karmakar et al (2021) Immune complex-induced apoptosis and concurrent immune complex clearance are anti-inflammatory neutrophil functions. Cell Death Dis 12, 296. doi: 10.1038/s41419-021-03528-8 [4] Grieshaber-Bouyer et al (2022) Ageing and interferon gamma response drive the phenotype of neutrophils in the inflamed joint. Amm Rheum Dis 81, 805. doi:10.1136/annrheumdis-2021-221866