Investigating the immunomodulatory impact of low-dose IL-2 on atherosclerotic plaque-resident lymphocyte populations and vascular smooth muscle cell phenotype

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Background and Abstract

The BDIAP Student Elective Award has provided me the opportunity to undertake an 8-week research placement at the Mallat laboratory situated in the Victor Phillip Dahdaleh Heart and Lung Research Institute on the Cambridge Biomedical Campus. During my time there, I contributed to ongoing investigations into immunomodulatory strategies to promote protective immune responses in atherosclerosis. Specifically, previous work by the lab on mice suggests Interleukin-2 (IL-2) inhibits atherosclerotic lesion development and inflammation via expansion of the inducible regulatory T cell population (Treg) (Newland, personal communication, July 31, 2023) and increases plaque stability by promoting alternative macrophage activation and increased collagen deposition by vascular smooth muscle cells (VSMC) via activation of type 2 innate lymphoid cells (ILC2) (Newland at al., 2017). My research project involved further experimental testing of these hypotheses, particularly by investigating the proposed role of the ILC2 population in mediating such phenotypic changes.

Introduction

Interleukin-2 (IL-2) plays a critical role in the survival, expansion, and suppressive function of Tregs, holding potential as an immunomodulatory therapy for patients with atherosclerotic cardiovascular disease and acute ischaemic syndromes. The Mallat lab recently completed an investigator-initiated, prospective, randomised, double-blind, placebo-controlled, dose-escalation, phase 2a clinical trial (LILACS), which displayed that low-dose IL-2 expanded Tregs at the expense of T effector cells by exploiting high-affinity IL-2 (CD25) receptors (Zhao et al., 2018).

Previous work by the lab has also classified IL-2 as a prominent upstream regulator of ILC2 using RNAseq analysis. Accordingly, 4 weeks after experimentally induced myocardial infarction in mice, scar size was significantly larger in ILC2 knockout mice compared to controls, with Sirius red staining demonstrating a significantly lower per unit area of collagen deposition by VSMCs in each section (Yu at al., 2021). Additionally, inflammatory monocytes and macrophages were increased in ILC2 knockout mice, severely impacting cardiac function, suggesting that ILC2s skew macrophages towards an alternatively activated and potentially reparative phenotype, contributing to less intrusive remodelling during scar formation (Yu at al., 2021). Furthermore, pilot data from work in mice has demonstrated greater collagen deposition by VSMCs from an ILC2 wildtype mouse cultured in a cytokine cocktail (containing IL-25, IL-33 and TSLP) compared to VSMCs from an ILC2 knockout mouse, yet in the presence of the same cytokines (Newland, personal communication, August 17, 2023). In the context of an atherosclerotic plaque, this could advantageously promote greater plaque stability, and is thought to occur via ILC2-derived IL-13 (Newland et al., 2017).

Therefore, the question remained whether low-dose-IL-2 could have such desired effects on both T cells and VSMCs in human atherosclerotic plaques which could directly alter pathophysiology and further, whether these desired effects were mediated via IL-2’s expansive effect on ILC2.

My project aims were to:

1. Line 1 investigation: Isolate ILC2 from apheresis cones and culture them to produce ILC2-conditioned media for application to a human cell line derived-VSMC culture. The ILC2 culture would be differentially stimulated either in the presence or absence of IL-2 to investigate the following research question**: "Can human ILC2 affect human VSMCs in culture to replicate the pilot mouse data?"**

2. Line 2 investigation: Conduct whole tissue culture of atherosclerosis-affected human tissues such as carotid plaques and incubate the samples for 96 hours either in the presence of IL-2 (ILC2- and T-cell activating) or IL-2 and ICI 118,551 (selective inverse agonist of the β2 adrenergic receptor on ILC2 or T cells and thus ILC2- and T cell-inhibitory). After tissue digestion, the samples will be analysed by flow cytometry to study lymphocyte population subsets at single-cell resolution thereby answering the question: **"Can whole tissue culture influence lymphocyte populations embedded in atherosclerosis-affected tissues?"** 3. Conduct a cytometric bead array of the ILC2 culture supernatants (ILC2 +/- IL-2) and smooth muscle co-culture supernatants (VSMC alone, VSMC + ILC2, VSMC + ILC2 + IL-2) from the Line 1 investigation and of peripheral blood mononuclear cell (PBMC) and whole tissue culture supernatants (PBMCs/carotid plaques in media, IL-2 and IL-2 + ICI 118,551) from the Line 2 investigation to detect cytokine secretions that would be indicative of T helper cell phenotype.

My personal objectives were to:

1. Acquire a range of key experimental techniques.

2. Experience how clinical practice interacts with academic research whilst working within the scientific community.

Conclusion

Investigating lymphocyte activation in atherosclerosis-affected human tissues such as carotid plaques has never been attempted due to practical challenges such as the rarity of such tissues for research purposes, given the difficulty of obtaining these tissues from affected individuals. I show that after isolating these tissues from surgery and bathing them in either complete medium, medium + IL-2 or medium + IL-2 + ICI 118,551 for 96 hours, I can modify plaque-resident lymphocytes using aldesleukin, a recombinant form of human IL-2 that is clinically used to treat renal cell carcinoma and malignant melanoma due to its proliferative and maturational effects on T cells. I show that even after considerable manipulation (tissue explant, maceration and digest) and flushing of plaque fragments with PBS, viable plaque-resident cells remain which can be studied at single-cell resolution to assay any phenotypic changes. I have also investigated the supernatants within which the plaque tissue was incubated in a cytometric bead array to study secreted cytokines that would be indicative of T helper cell phenotype. Therefore, I can conclude that the macerated carotid plaque tissue survives and as the constituent T cells change phenotype (alteration in naïve and effector cell proportions as well as surface marker expression) under control, ILC2- and T cell-activating and inhibitory conditions, cytokine secretion that is itself indicative of T helper cell phenotype is altered. Additionally, the VSMC-ILC2 co-cultures as part of the Line 1 investigation provided the lab pilot data on human VSMC lines, successfully replicating results obtained from

previous studies in mice. Nevertheless, further refinements and repetitions of the experiments conducted are warranted before further conclusions are formed.

To conclude, this pilot study has provided the Mallat lab an investigational tool which can be used to further explore the immunomodulatory effects of differential IL-2 dosage, a considerable challenge given time restraints and limited plaque availability. This work can now be built upon through modulation of dose and culture conditions. Specifically, what remains to be investigated is whether a higher or lower IL-2 dose is more beneficial for Treg proliferation. It is currently thought that a low IL-2 dose preferentially expands the Treg population whilst a higher dose, in this case, unfavourably increases effector T cell activation (via downregulation of CTLA4) in a dose-dependent response. In future whole tissue culture experiments, studying starting lymphocyte populations in the carotid plaque prior to incubation with various agonists is also recommended. I would also like to investigate whether cells resuspended in the same agonists but from unaffected donor artery tissues behave similarly to atherosclerosis-affected tissue or if these observed phenotypic changes are unique to pathological tissue.

Personal development and value of this research placement

This research placement has been an invaluable experience that has developed both my scientific knowledge as well as my practical skillset. I have learnt the importance of scrutinising experimental evidence supporting scientific theories thoroughly before accepting any information for plain fact and I have simultaneously appreciated the applications of medical knowledge that I have learnt in my pre-clinical years of study. Being an active member of the lab, I learnt how to succinctly present the experimental results I had obtained with confidence and formulate propositions for such data to contribute to group discussions. The frequent communication I had with my supervisors as well as with other members of the research group for advice on experimental troubleshooting or optimisation has provided me an insight into the collaborative nature of academic research. This collective approach to overcoming practical challenges and contributing towards the generation of novel scientific knowledge that supplements clinical practice has cemented my desire of a clinical career involving academic research. I would therefore like to thank the BDIAP once again for supporting this truly enlightening experience.