Targeting the chemokine network in cardiometabolic inflammation

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Project outline

Background: Leucocyte recruitment by redundant and synergistically acting CC- and CXC-chemokines drives metabolic inflammation of organs such as adipose tissue, liver, and blood vessels[4]. Existing therapeutic strategies to combat such inflammation are associated with adverse effects secondary to immunosuppression[5]. The causal role of the chemokine network in metabolic and atherosclerotic inflammation has been demonstrated by human genetic and mouse pharmacological approaches[6]. The chemokine network is thus a validated therapeutic target and provides the opportunity to *precisely* modulate the signals recruiting leucocytes *without* affecting their function. No therapeutic yet developed overcomes chemokine redundancy, which arises from multiple CC and CXC chemokines being expressed at the site of inflammation. The idea that overcoming chemokine network redundancy will suppress *human* inflammation arises from a remarkable "*experiment-of-nature*". Ticks have evolved over 50 distinct chemokine-interacting proteins that bind and inhibit *multiple* CC and CXC-class chemokines respectively, overcoming redundancy, and creating an inflammation-free zone at the bite site[7]. Evasins are therapeutically unsuitable as they are foreign proteins with risk of immunogenicity, and evasin therapy requires use of both CC and CXC-binding evasins. We have recently identified a lead 16-mer peptide from a tick evasin that remarkably binds and inhibits *both* CC and CXC class human chemokines[1]. We have extensively characterised the lead candidate's pharmacophore using phage-display, saturation mutagenesis and AlphaFold modelling[1].

Pilot data: Using combinatorial saturation mutagenesis of the lead peptide we have improved binding affinity and potency to develop a peptide that can efficiently neutralise the monocyte, neutrophil and T-cell chemotactic activity of a pool of 24 chemokines known to be present in the human atherosclerotic plaque. We have converted the peptide to a peptibody (IgGFc-fusion), a well-established therapeutic development method that is known to improve both pharmacokinetic and pharmacodynamic activity [8]. We are now able to take our lead peptibody forward for in depth *in vitro* and *in vivo* characterisation. We **hypothesise** that targeting the chemokine network will reduce the adverse impacts of metabolic and cardiovascular inflammation. The **aims** of this project are to develop a combinatorially mutated peptibody as a therapeutic candidate to target the chemokine network in metabolic and cardiovascular inflammation.

Experimental approaches:
Aim 1: Human *ex vivo* cardiometabolic inflammation models. We will characterise human atherosclerotic plaque chemokine burden using O-link proximity extension assay. We will use equivalent data from the Leducq CHECKPOINT ATHERO networks[9] and from published proteomic[10]

and RNASeq studies[11] to complement these experiments and use such information to create synthetic chemokine pools that model the plaque chemokine expression pattern, for monocyte, neutrophil and T-cell chemotaxis assays. In parallel we will use cultured human plaque supernatants as described by us[10] and supernatants from human iPSC-derived vascular organoids[12] (exposed to pro-atherogenic stimuli (oxLDL, TNFα and oxidative stress[13]) for chemotaxis assays. Similarly, we will use an adipose inflammation model (human pre-adipocytes exposed to TNFa[14]) and use supernatants in chemotaxis assays.

Aim 2: Peptibody characterisation. Peptibodies will be produced using mammalian CHOK1 expression system, and human chemokine binding affinity characterised using biolayer interferometry and in-solution methods (microscale thermophoresis or fluorescent polarisation)[1, 15]. We will then test peptibody potency in the *ex vivo* multi-chemokine chemotaxis assay models developed above, and against chemokines which are known to be beneficial in inflammatory resolution.

Aim 3: Animal models The peptibody lead will first be studied in a mouse air-pouch inflammation model[15] to establish dose and frequency, and then taken forward in a mouse model of cardiometabolic inflammation (*Ldlr-/-* mice fed with a high sucrose-fat-cholesterol diet, with which it develops diet-induced obesity, insulin resistance, adipose inflammation, adipose chemokine expression, liver inflammation, and atherosclerosis within 12-16 weeks[16]). The ability of our lead peptibody to reduce adipose tissue (gonadal fat), liver and atherosclerotic plaque inflammation will be assessed by flow cytometry. Insulin resistance will be studied using glucose tolerance tests and analysis of insulin signalling pathways in liver and skeletal muscle. Atherosclerosis plaque burden will be characterised by histological approaches.

References

1. Vales, S. et al. Discovery and pharmacophoric characterization of chemokine network inhibitors using phage-display, saturation mutagenesis and computational modelling. *Nat Commun* **14**, 5763 (2023).
2. Engelen, S.E., Robinson, A.J.B., Zurke, Y.X. & Monaco, C. Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed? *Nat Rev Cardiol* **19**, 522-542 (2022).
3. Douglas, G. et al. A key role for the novel coronary artery disease gene JCAD in atherosclerosis via shear stress mechanotransduction. *Cardiovascular research* **116**, 1863-1874 (2020).
4. Wu, H. & Ballantyne, C.M. Metabolic Inflammation and Insulin Resistance in Obesity. *Circ Res* **126**, 1549-1564 (2020).
5. Chaffey, L., Roberti, A. & Greaves, D.R. Drug repurposing in cardiovascular inflammation: Successes, failures, and future opportunities. *Front Pharmacol* **13**, 1046406 (2022).
6. Yan, Y., Thakur, M., van der Vorst, E.P.C., Weber, C. & Doring, Y. Targeting the chemokine network in atherosclerosis. *Atherosclerosis* **330**, 95-106 (2021).
7. Bhattacharya, S. & Nuttall, P.A. Phylogenetic Analysis Indicates That Evasin-Like Proteins of Ixodid Ticks Fall Into Three Distinct Classes. *Front Cell Infect Microbiol* **11**, 769542 (2021).
8. Shimamoto, G., Gegg, C., Boone, T. & Queva, C. Peptibodies: A flexible alternative format to antibodies. *MAbs* **4**, 586-591 (2012).
9. Lutgens, E., Mulder, W.J.M. & consortium, C.A. CHECKPOINT ATHERO: developing immune checkpoint-based therapeutics for atherosclerosis. *Eur Heart J* **44**, 1010-1012 (2023).
10. Shalhoub, J. et al. Multi-analyte profiling in human carotid atherosclerosis uncovers pro-inflammatory macrophage programming in plaques. *Thromb Haemost* **115**, 1064-1072 (2016).
11. Bazan, H.A. et al. A pro-inflammatory and fibrous cap thinning transcriptome profile accompanies carotid plaque rupture leading to stroke. *Sci Rep* **12**, 13499 (2022).

12. Wimmer, R.A. et al. Human blood vessel organoids as a model of diabetic vasculopathy. *Nature* **565**, 505-510 (2019).
13. Zhang, X. et al. Modeling early stage atherosclerosis in a primary human vascular microphysiological system. *Nat Commun* **11**, 5426 (2020).
14. Tourniaire, F. et al. Chemokine Expression in Inflamed Adipose Tissue Is Mainly Mediated by NF-kappaB. *PLoS One* **8**, e66515 (2013).
15. Darlot, B. et al. Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. *JBC* **295**, 10926-10939 (2020).
16. Neuhofer, A. et al. An accelerated mouse model for atherosclerosis and adipose tissue inflammation. *Cardiovasc Diabetol* **13**, 23 (2014).

Supervisor's recent relevant publications:

Professor Shoumo Bhattacharya

1. Vales, S., Kryukova, J., Chandra, S., Smagurauskaite, G., Payne, M., Clark, C.J., Hafner, K., Mburu, P., Denisov, S., Davies, G., Outeiral, C., Deane, C.M., Morris, G.M. & **Bhattacharya, S**. Discovery and pharmacophoric characterization of chemokine network inhibitors using phage-display, saturation mutagenesis and computational modelling. *Nat Commun* 14, 5763 (2023).
2. Singh, K., Fang, H., Davies, G., Wright, B., Lockstone, H., Williams, R.O., Cihakova, D., Knight, J.C. & **Bhattacharya, S.** Transcriptomic Analysis of Inflammatory Cardiomyopathy Identifies Molecular Signatures of Disease and Informs in silico Prediction of a Network-Based Rationale for Therapy. *Front Immunol* **12**, 640837 (2021).
3. Darlot, B., Eaton, J.R.O., Geis-Asteggiante, L., Yakala, G.K., Karuppanan, K., Davies, G., Robinson, C.V., Kawamura, A. & **Bhattacharya, S.** Engineered anti-inflammatory peptides inspired by mapping an evasin-chemokine interaction. *J Biol Chem* **295**, 10926-10939 (2020).
4. Lee, A.W., Deruaz, M., Lynch, C., Davies, G., Singh, K., Alenazi, Y., Eaton, J.R.O., Kawamura, A., Shaw, J., Proudfoot, A.E.I., Dias, J.M. & **Bhattacharya, S.** A knottin scaffold directs the CXC-chemokine-binding specificity of tick evasins. *Journal of Biological Chemistry* **294**, 11199-11212 (2019).
5. Eaton, J.R.O., Alenazi, Y., Singh, K., Davies, G., Geis-Asteggiante, L., Kessler, B., Robinson, C.V., Kawamura, A. & **Bhattacharya, S.** The N-terminal domain of a tick evasin is critical for chemokine binding and neutralization and confers specific binding activity to other evasins. *J Biol Chem* **293**, 6134-6146 (2018).

Associate Professor Gillian Douglas

1. Wood A, Antonopoulos A, Chuaiphichai S, Kyriakou T, Diaz R, Al Hussaini A, Marsh AM, Sian M, Meisuria M, McCann G, Rashbrook VS, Drydale E, Draycott S, Polkinghorne MD, Akoumianakis I, Antoniades C, Watkins H, Channon KM, Adlam D, **Douglas G (2022)**. PHACTR1 modulates vascular compliance but not endothelial function: a translational study. ***Cardiovasc Res***. Jun 2:cvac092. PMID: 35653516, DOI: 10.1093/cvr/cvac092
2. **Douglas G**, Mehta V, Al Haj Zen A, Akoumianakis I, Goel A, Rashbrook VS, Trelfa L, Donovan L, Drydale E, Chuaiphichai S, Antoniades C, Watkins H, Kyriakou T, Tzima E, Channon KM **(2020)**. A key role for the novel coronary artery disease gene JCAD in atherosclerosis via shear stress mechanotransduction. ***Cardiovasc Res***. 2020 Sep 1;116(11):1863-1874. PMID: 31584065, DOI: 10.1093/cvr/cvz263
3. Chuaiphichai S, Rashbrook VS, Hale AB, Trelfa L, Patel J, McNeill E, Lygate CA, Channon KM, **Douglas G** (2018). Endothelial Cell Tetrahydrobiopterin Modulates Sensitivity to Ang (Angiotensin) II-Induced Vascular Remodeling, Blood Pressure, and Abdominal Aortic Aneurysm. ***Hypertension***. Jul;72(1):128-138. PMID: 29844152, DOI: 10.1161/HYPERTENSIONAHA.118.11144
4. **Douglas G**, Hale, AB, Patel J, Chuaiphichai S, Haj Zen A, Rashbrook VS, Trelfa L, Crabtree MJ, McNeill E, Channon KM (**2018**). Roles for endothelial cell and macrophage Gch1 and

tetrahydrobiopterin in atherosclerosis progression. ***Cardiovascular Research***. 2018: 114 (10) 1385-1399. PMID: 29596571, DOI: 10.1093/cvr/cvy078

5. Chuaiphichai S. Crabtree MJ, McNeill E, Hale AB, Trelfa L, Channon KM, **Douglas G (2017)**. A Key Role for Tetrahydrobiopterin-Dependent Endothelial NOS Regulation in Vascular Resistance Arteries: Studies in Endothelial Cell Tetrahydrobiopterin-Deficient Mice. ***British Journal of Pharmacology*** 2017;174 (8): 657-671. PMID: 28128438, DOI: 10.1111/bph.13728

Professor Claudia Monaco

1. **Monaco, C.,** Gregan, S.M., Navin, T.J., Foxwell, B.M., Davies, A.H. & Feldmann, M. Toll-like receptor-2 mediates inflammation and matrix degradation in human atherosclerosis. Circulation 120, 2462-2469 (2009).
2. Shalhoub, J., Viiri, L.E., Cross, A.J., Gregan, S.M., Allin, D.M., Astola, N., Franklin, I.J., Davies, A.H. & **Monaco, C.** Multi-analyte profiling in human carotid atherosclerosis uncovers pro-inflammatory macrophage programming in plaques. Thromb Haemost 115, 1064-1072 (2016).
3. Cole, J.E., Park, I., Ahern, D.J., Kassiteridi, C., Danso Abeam, D., Goddard, M.E., Green, P., Maffia, P. & **Monaco, C.** Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity. Cardiovascular research 114, 1360-1371 (2018).
4. Kassiteridi, C., Cole, J.E., Griseri, T., Falck-Hansen, M., Goddard, M.E., Seneviratne, A.N., Green, P.A., Park, I., Shami, A.G., Pattarabanjird, T., Upadhye, A., Taylor, A.M., Handa, A., Channon, K.M., Lutgens, E., McNamara, C.A., Williams, R.O. & **Monaco, C.** CD200 Limits Monopoiesis and Monocyte Recruitment in Atherosclerosis. Circ Res 129, 280-295 (2021).
5. Park, I., Goddard, M.E., Cole, J.E., Zanin, N., Lyytikainen, L.P., Lehtimaki, T., Andreakos, E., Feldmann, M., Udalova, I., Drozdov, I. & **Monaco, C.** C-type lectin receptor CLEC4A2 promotes tissue adaptation of macrophages and protects against atherosclerosis. Nat Commun 13, 215 (2022).