Scientific summary (Scientific report)

a. What were the main scientific objectives of the grant?

Main scientific objectives:

1) Assess total dendritic cells (DCs), T cells and their subsets in blood, spleen, lymph node (LN) and synovial tissue from EPCR knockout (-/-) and wild type (WT) mice in a collagen-induced arthritis (CIA) model, and then correlate cell numbers and their localization with the severity of CIA;

2) Determine whether loss of EPCR inhibits the maturation of DCs in vitro.

b. *What were the main scientific achievements of the grant?*

Our data so far showed that EPCR knockout mice display reduced arthritis incidence and disease severity in CIA when compared to matched wild type mice. Analysis of cell populations in blood circulation, spleens and in lymph nodes showed that EPCR knockout mice have significantly higher numbers of DC, myeloid-derived-suppressor cells, and lower numbers of total T cells, Th cells and cytotoxic T cells. In the joint synovium, although there was no different in DC cell numbers, but the subpopulations of DC cells such as pDC and mDC were different between EPCR knockout and wild type mice. DC were more mature in blood and joint synovium, and less mature in lymph nodes from EPCR knockout mice, when compared to wild type mice. In vitro, after induction, there were much less DC cells either from bone-marrow or spleen of EPCR KO mice, these cells are more resistant to mature in response to inflammatory stimulation in cultures, when compared to WT. In spleen and thymus of EPCR mice, numbers of Th1 and Th17 cells were considerably less; in plasma, EPCR KO mice had 40% less transforming growth factor-β1 and 30% higher IL-17 when compared to WT mice. In EPCR mice, spleen cells and mature DC produced less matrix metalloproteinase-9 than WT cells. These data indicate that the reduction in arthritis in EPCR knockout mice is likely achieved via modulating the function of these specific immune cells.

c. What problems, if any, did you encounter in achieving the project's objectives, and how did you address them?

The Covid-19 pandemic limited our laboratory access, particularly to the animal laboratory, however, by working flexibly, outside of normal hours, we were able to conduct most of our proposed experiments.

d. *Have you disseminated, or plan to disseminate, the results of this research? Please tell us about:*

- *References for peer-reviewed papers that have been published (please provide pdf copies of papers if possible)*
- *Papers that have been submitted and/or accepted for publication*
- *Meetings/conferences at which you have presented this research, or are due to present it (please provide abstracts if possible)*
- *Any other ways in which you may have disseminated the research, including to the public and the media (please provide urls to relevant press releases or media articles)*

Part of this work was submitted to 2020 Australian Rheumatology Association Annual Scientific Meeting, scheduled in May but was cancelled due to COVID-19. This abstract is published in the Supplementary Issue of the Internal Medicine Journal 2020 (see below). We have submitted another abstract associated with this grant to 2021 Australian Rheumatology Association Annual Scientific Meeting, are currently using the data to apply for 2021 NHMRC Ideas grant.

One publication supported by a previous Arthritis Australia project grant:

Meilang Xue PhD, Haiyan Lin MD, Helena Liang PhD, Kelly McKelvey PhD, Ruilong Zhao MD, Lyn March MD, PhD and Christopher Jackson PhD, Deficiency of protease-activated receptor (PAR)1 and PAR2 exacerbates collagen-induced arthritis in mice via differing mechanisms. Rheumatology (Oxford). 2020 Dec 1;keaa701.doi: 10.1093/rheumatology/keaa701. was accepted for publication by Rheumatology (Oxford) on 21st Sept 2020 ()

Two abstracts have been submitted from this grant:

ARA abstract 2020 :
Inhibition of EPCR suppresses inflammatory arthritis in mice via inhibition of inflammatory T cells

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Aim: Endothelial protein C receptor (EPCR) is highly expressed in synovial tissue of rheumatoid arthritis (RA), but the functions of this receptor remains unknown in RA. In this study, we investigated the effect of EPCR on the onset and development of inflammatory arthritis and its underlying mechanisms.

Methods: Collagen-induced arthritis (CIA) was induced in EPCR gene knockout (KO) and matched wild type (WT) mice. The onset and development of arthritis was monitored clinically and histologically. T cells and cytokines were detected by flow cytometry and enzyme-linked immunosorbent assay.

Results: EPCR KO mice displayed more than 40% lower arthritis incidence and 50% less disease severity when judged by clinical and histological scores, when compared to WT mice. Flow cytometrical analysis showed that EPCR KO mice had significantly higher number of myeloid-derived suppressor cells and lower number of total T cells in blood and spleen, and

higher numbers of Treg and cytotoxic cells in lymph nodes, when compared to WT mice. In spleen and thymus of EPCR mice, numbers of Th1 and Th17 cells were considerably less; in plasma, EPCR KO mice had 40% less transforming growth factor-β1 and 30% higher IL-17 when compared to WT mice.

Conclusion: Deficiency of EPCR prevents inflammatory arthritis in CIA via inhibition of immune cells, particularly Th cells. Our data suggests that blockade of EPCR may be a potential target for prevention or early treatment of inflammatory arthritis.

ARA 2021 abstract:

Endothelial cell protein C receptor ameliorates inflammatory arthritis in mouse collagen-induced arthritis via suppressing the migration and maturation of dendritic cells

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Aim: Dendritic cells (DC) are the most potent antigen-presenting cells and play a critical role in rheumatoid arthritis (RA). Increased total DC in circulation and plasmacytoid (p) DC in periphery can prevent RA autoimmunity, while mature DC and myeloid (m) DC in synovium involve in RA pathogenesis. Endothelial protein C receptor (EPCR) is an anticoagulant receptor expressed by DC. However, the functions of EPCR in DC remain unknown. This study used EPCR knockout (KO) mice and collagen-induced arthritis (CIA) to investigate the regulatory role of EPCR in these cells.

Methods: CIA was induced in EPCRKO and wild type (WT) mice, and arthritis incidence and severity monitored clinically and histologically. Mature DC were obtained by incubation of mouse barrow (BM) derived cells with GM-CSF and LPS in vitro**.** Total DC, mDC and pDC, and DC maturation were detected by flow cytometry, the production of matrix metalloproteinase (MMP)-9 by zymography.

Results: EPCRKO mice displayed significantly lower arthritis incidence and less severe CIA when compared to WT mice. In EPCRKO mice, total DC number was higher in blood, but lower in synovium; DC were less mature in spleen and lymph node, when compared to WT mice. mDC were significantly lower in synovium, while pDC were higher in blood, spleen and synovium of EPCRKO mice, when compared to WT mice. In vitro, mature WT DC expressed higher levels of CD40, CD80 and CD86, showing a more mature status when compared to EPCRKO DC.

Furthermore, in contrast to EPCRKO DC, WT DC produced increased MMP-9, a crucial factor for DC migration.

Conclusion: EPCR deficiency ameliorates CIA. This is likely achieved via increasing total DC in blood, suppressing DC migration and maturation, resulting in increased pDC and decreased mDC in synovium, and inhibition of MMP-9 production.