Understanding the role of decidual macrophages in preeclampsia

Original hypothesis:

In preeclampsia, the decidual immune system becomes dysregulated, and that decidual macrophage (DMφ) function is altered, including reduced ability to efferocytose trophoblast cells, and increased pro-inflammatory cytokine release. This contributes to the development of preeclampsia.

This report will focus on macrophage functional responses, however further data on immune cell populations in placental samples, maternal blood and cord blood have been obtained, but are waiting analysis.

Results

Demographics

To date we have recruited 12 full-term healthy control (HC) samples, and 4 preeclamptic (PET) samples. PET samples had clinician confirmed PET, with 3 samples also having fetal growth restriction. These babies were delivered at an earlier gestation >34weeks, and the babies were smaller by weight. All HC and PET samples were obtained from caesarean section without labour.

	Healthy control	PET
Maternal age	33.6 (3.9)	32.8 (1.5)
Gestational age (weeks)	39.2 weeks (0.4)	35.2 (2.5)**
Baby weight (g)	3596 (310)	2265 (500)**

Table 1 – Demographics. PET deliveries were significantly earlier then healthy control (35 vs 39 weeks), and babies had a smaller birth weight (2265g vs 3596g). Data shows mean (SD). **p<0.01.

Sample processing

From each sample, we collected maternal blood and cord blood, which underwent phenotyping by flow cytometry (data not shown). Alongside this the CD14- portion of placenta underwent immune cell analysis (data not shown).

CD14+DMφ were isolated from the decidua basalis (DBMφ) and decidua partietalis (DPMφ). Fresh macrophages underwent phenotyping by flow cytometry (Figures 3-6), while cells were also plated overnight to rest. On day 2, macrophages underwent efferocytosis of apoptotic trophoblasts, or phagocytosis of *Streptococcus agalactiae*, with uptake measured by flow cytometry (Figures 1-2), and supernatants stored for cytokine analysis by ELISA (Figure 7). Where cell numbers allowed, macrophages underwent confocal microscopy to confirm uptake of prey (Figure 8).

Decidual macrophage function

In PET placentas, elevated pro-inflammatory cytokines TNFα and IFNγ induce apoptosis of extra villous trophoblasts, which require removal by DM. However, the pro-inflammatory environment of the PET placenta may impair DMφ efferoytosis. **Our study is the first to show** that both DBMφ and DPMφ are able to efferocytose apoptotic trophoblasts (Figure 1). When the amount of trophoblasts efferocytosed is studied, both DBMφ (Figure 1b) and DPMφ (Figure 1d) have increased efferoytosis compared to HC. This suggests that PET DM are responding to increased trophoblast apoptotisis by attempting to clear these cells.

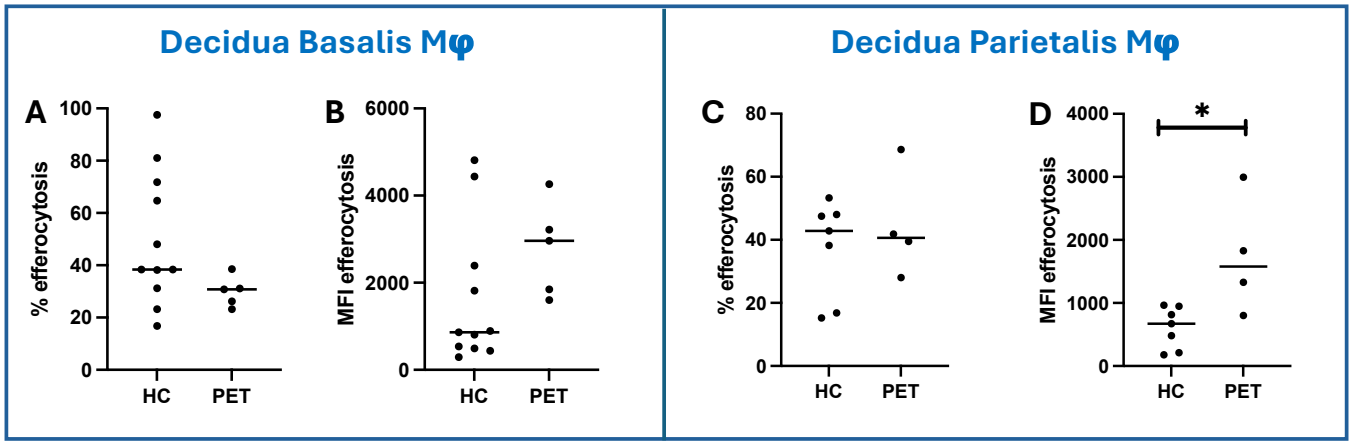

Figure 1 – Decidual macrophage efferocytosis.
Macrophages were isolated from placenta collected from healthy full-term placenta, or placenta with PET. In DBMφ, a similar amount of Mφ efferocytose apoptotic trophoblasts in HC vs. PET (50% vs 30%), however the amount of trophoblasts efferocytosed appears elevated (MFI 1616 vs 2777, p=0.1149), with further numbers required. In DPφ, a similar amount of Mφ efferocytose apoptotic trophoblasts in HC vs. PET (37.4% vs 44.5%), however the amount of trophoblasts efferocytosed is significantly elevated in PET (MFI 610 vs 1738, p=0.0154). HC n=7-11, PET n=4-5, analysed by t-test.

Phagocytosis of bacteria is a well known function of macrophages, but whether this is true of DMφ is yet to be determined. Our study is the first to show that both DBMφ and DPMφ are able to phagocytose *S. agalactiae* (Group B strep, Figure 2). When the amount of bacteria phagocytosed is studied, both DBMφ (Figure 2b) and DPMφ (Figure 2d) have increased phagocytosis compared to HC. This suggests that DMφ in PET are more activated and primed to respond to infection.

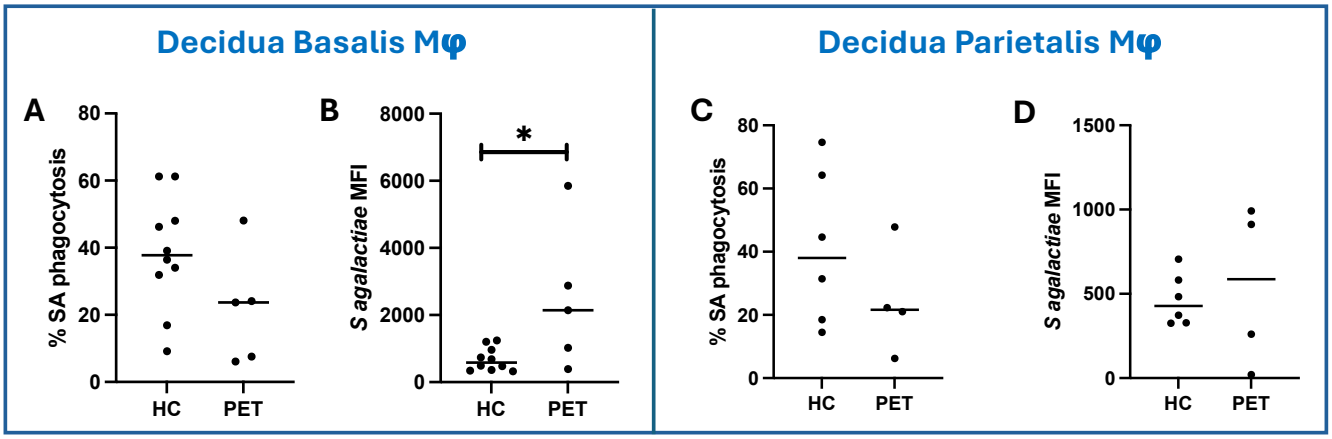

Figure 2 – Decidual macrophage phagocytosis.
Macrophages were isolated from placenta collected from healthy full-term placenta, or placenta with PET. In DBMφ, a similar amount of Mφ phagocytose *S. agalactiae* in HC vs. PET (38% vs 22%), however the amount of bacteria phagocytosed is significantly elevated in PET (MFI 683 vs 2458, p=0.0193). In DPφ, a similar amount of Mφ phagocytose *S. agalactiae* in HC vs. PET (41% vs 24%), as is the amount of trophoblasts efferocytosed (MFI 466 vs 547, p=0.0154). HC n=7-11, PET n=4-5, analysed by t-test.

Decidual macrophage phenotype

In healthy pregnancy, DMφ are reported to have an anti-inflammatory phenotype, which becomes more inflammatory with the onset of labour. However, conflicting reports exist as to the nature of DMφ phenotype during PET. Here, we have analysed DMφ phenotype by flow cytometry, and have expressed data as both the % cells expressing each receptor (Figure 3 and 5), and the amount of receptor expressed measured by MFI (Figure 4 and 6). These data are important, as they indicate different aspects of macrophage phenotype – both showing the amount of cells of a particular phenotype, and whether the cells are changing expression based on maternal condition. To date, there are no significant changes in receptor expression between HC and PET samples, for both DBMφ (Figure 3 and 4) and DPMφ (Figure 5 and 6), but there are trends of different phenotypes that require further samples to elucidate.

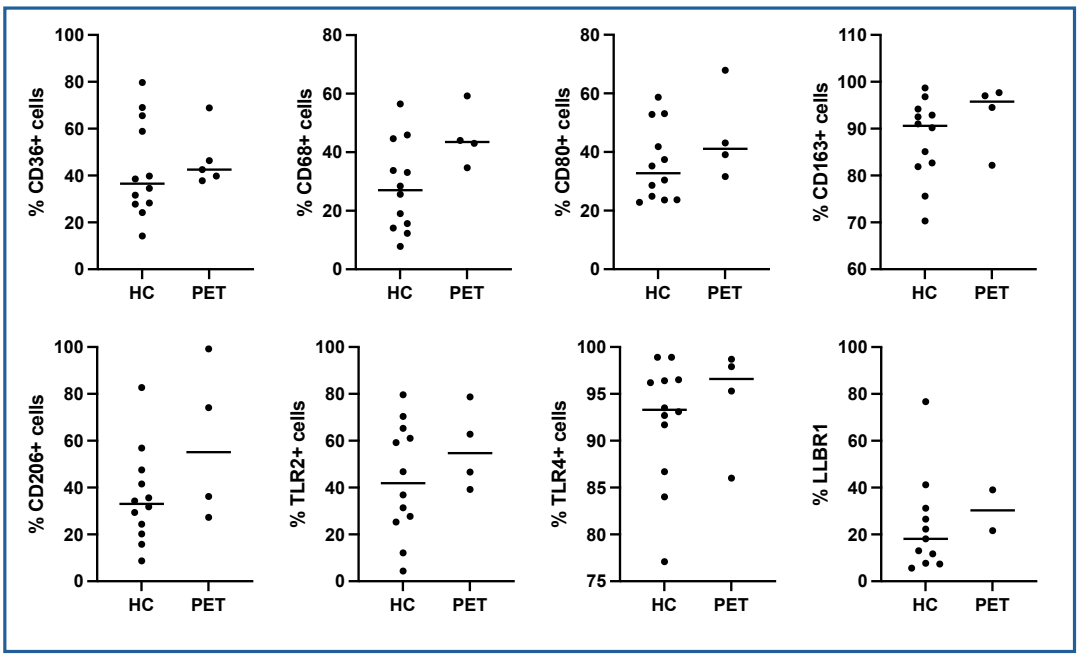

Figure 3 – Decidua Basalis Mφ phenotype, as % receptor expression
Cell surface expression of important macrophage phenotypic markers was analysed on freshly isolated DBM, by flow cytometry. There are trends for altered receptor expression in PET DBMφ, but further numbers are required to obtain statistical significance. HC n=12, PET n=4.

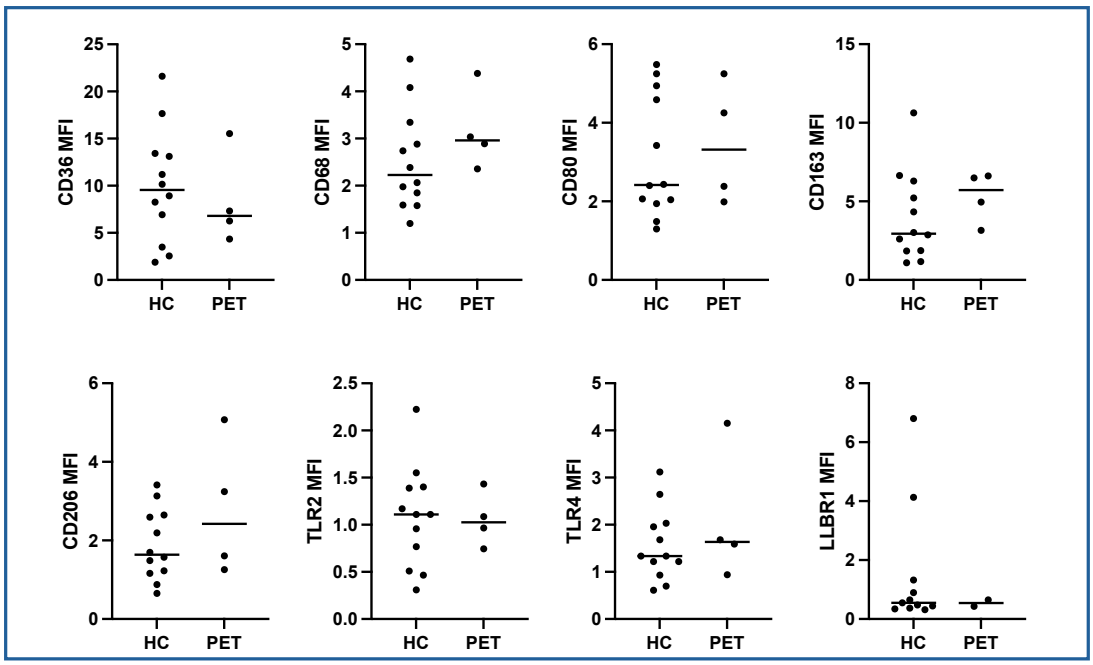

Figure 4 – Dedidua Basalis Mφ phenotype, as MFI receptor expression
The amount of receptors expressed on DBMφ, expressed as median fluorescence intensity (MFI) was analysed on freshly isolated DBMφ, by flow cytometry. There are trends for altered receptor expression in PET DBMφ, but further numbers are required to obtain statistical significance. HC n=12, PET n=4.

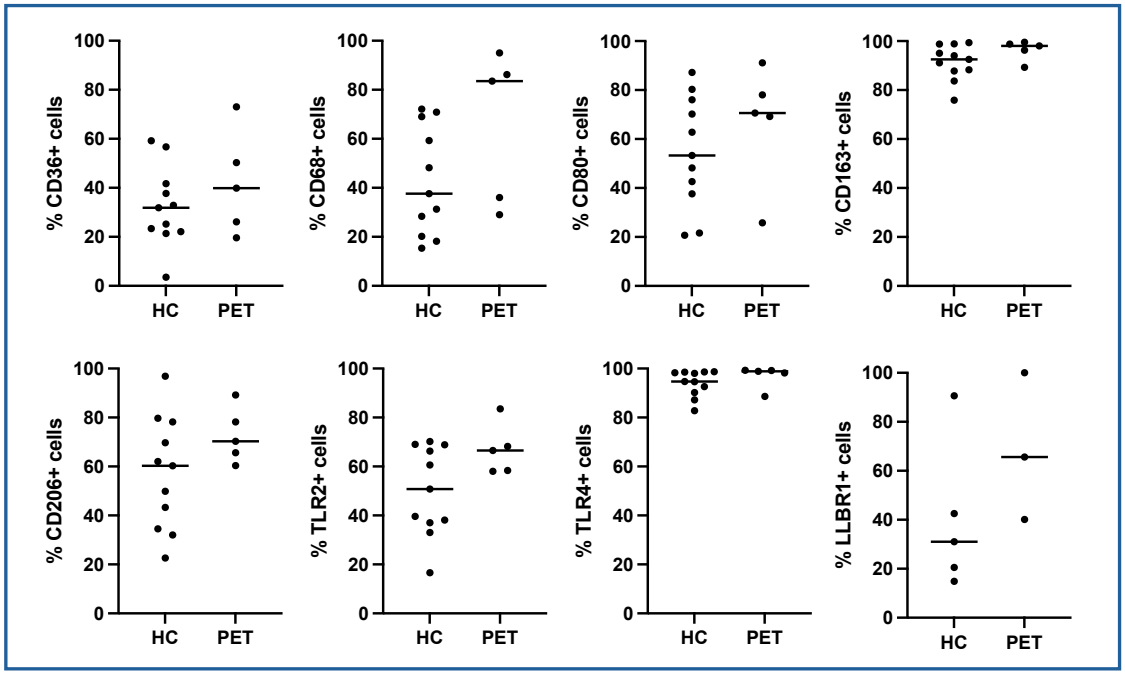

Figure 5 – Decidua Parietalis Mφ phenotype, as % receptor expression
Cell surface expression of important macrophage phenotypic markers was analysed on freshly isolated DPMφ, by flow cytometry. There are trends for altered receptor expression in PET DPMφ, but further numbers are required to obtain statistical significance. HC n=12, PET n=4.

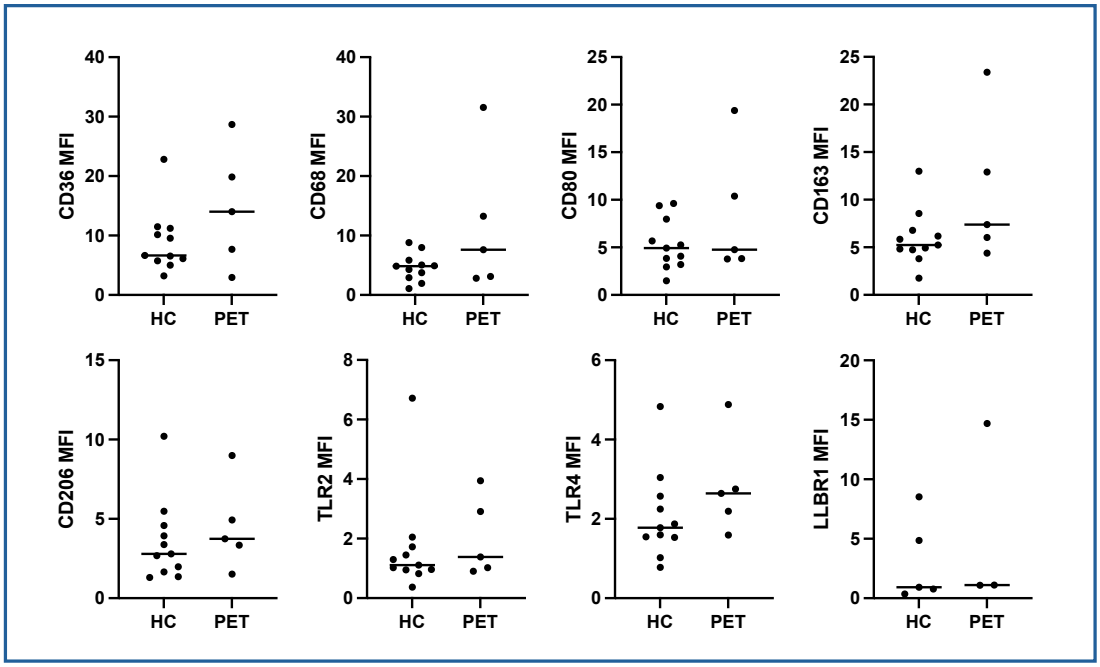

Figure 6 – Decidua Parietalis Mφ phenotype, as MFI receptor expression
The amount of receptors expressed on DPM, expressed as median fluorescence intensity (MFI) was analysed on freshly isolated DPMφ, by flow cytometry. There are trends for altered receptor expression in PET DBMφ, but further numbers are required to obtain statistical significance. HC n=12, PET n=4.

Decidual macrophage cytokine release

Supernatants from untreated (UT) DMφ, or after efferocytosis or phagocytosis were analysed for cytokine release by ELISA. To date, 6 HC samples have been analysed, with remaining supernatants stored for analysis. So far, data indicates that DBMφ and DPMφ release different cytokines in response to function (Figure 7), but further analysis is required to determine changes in PET. These samples are stored and will be batched analysed in future.

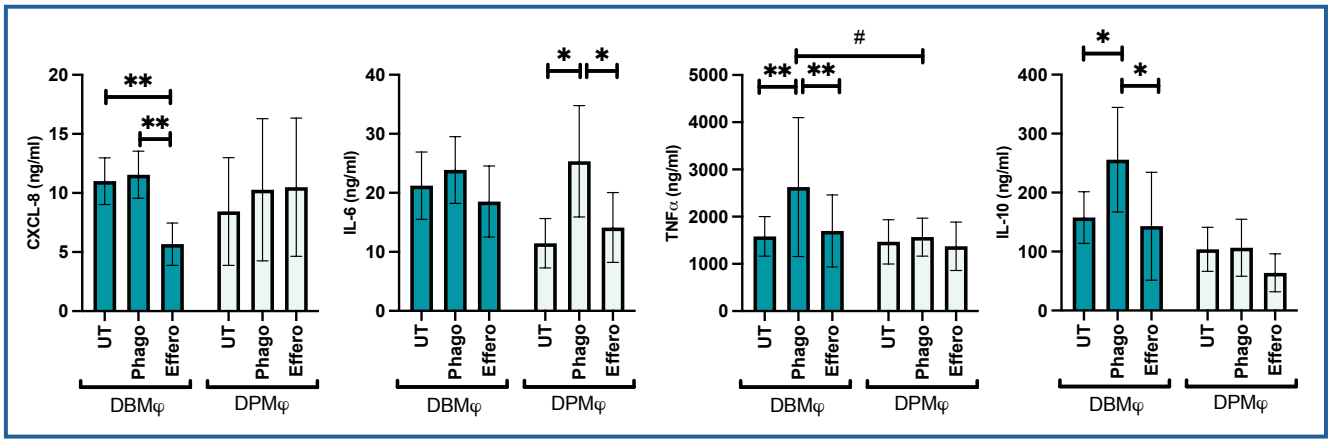

Figure 7 – Cytokine release by DMφ after phagocytosis/efferocytosis in healthy controls. In DBMφ, efferocytosis reduces CXCL-8 release ($p<0.01$), whereas phagocytosis elevates TNFα release ($p<0.01$) and IL-10 release ($p<0.05$). In DPMφ, phagocytosis increased IL-6 release ($p<0.05$). DBMφ n=6, DBMφ n=5. Further analysis of stored samples from HC and PET placentas are required to extend these observations.

Decidual macrophage microscopy

Confocal microscopy has been used to confirm internalisation of prey. Representative images from both efferocytosis (Figure 8a) and phagocytosis (Figure 8b) are shown below.

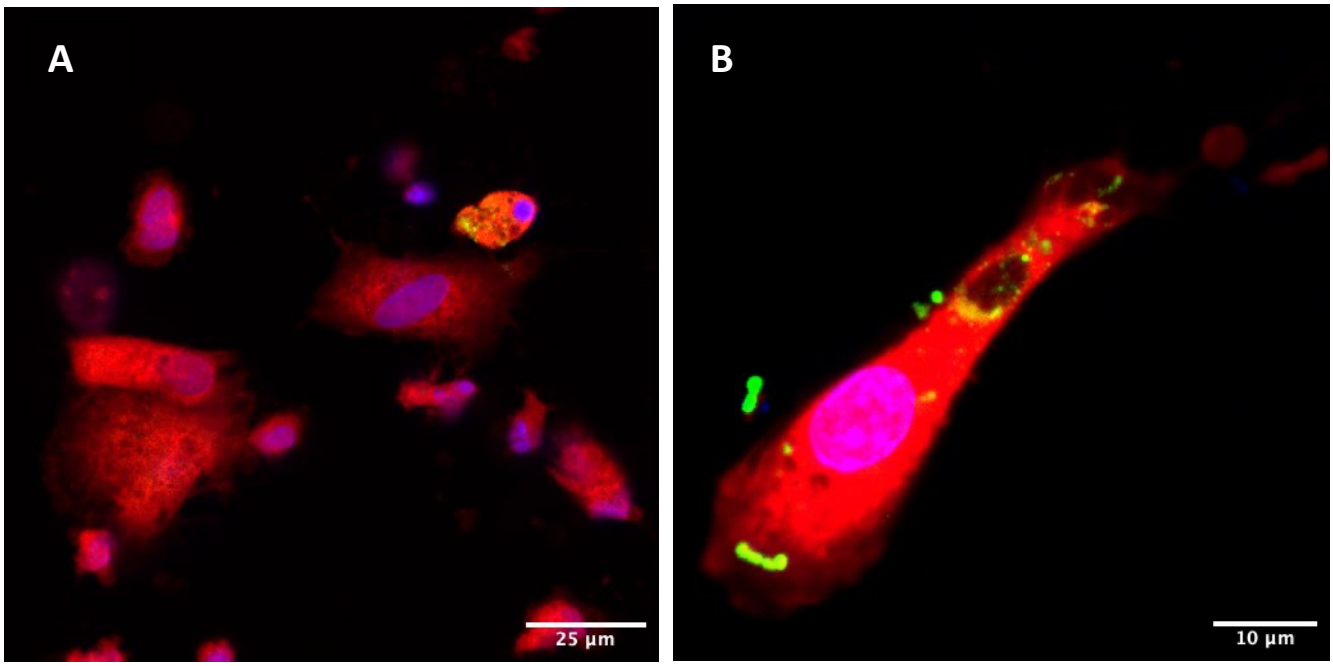

Figure 8 – Confocal microscopy of DMφ. A shows DBMφ (cell tracker red, DAPI nucleus blue) that has efferocytosed an apoptptic trophoblast (green). B shows DBMφ (cell tracker red, DAPI nucleus blue) that has phagocytosed S. agalactiae (green). Representative images.

Summary

To date, we have successfully analysed 12 HC samples, and 4 PET samples. We believe that the progress is successful so far, with PET samples proving more difficult to obtain due to emergency situations which can prevent consent to ethics. However, our team of midwives and obstetricians are working hard to identify suitable patients when they present to our hospital. The experimental work is progressing nicely and is starting to show interesting results which thus far match our hypothesis.

Limitations

We have so far struggled to recruit HELLP cases to this study. We have seen one patient with HELLP syndrome who was eligible, but they delivered as an emergency over the weekend, and as such we were unable to obtain samples due to staffing levels over the weekend. We remain focused to recruit HELLP pregnancies to this study, and will continue efforts to identify these patients.

Changes to progress going forwards

I have accepted a tenure-track Lectureship in Maternal and Fetal Health, within the Department of Maternal and Fetal Health at the University of Manchester, UK, and will take up this post in September 2024. This exciting opportunity was only possible due to the funding of this project by the Preeclampsia Foundation, which highlighted the importance of my research in the field. I am therefore very grateful for the foundation

for this support and look forward to ongoing research in this area. I believe this move will increase my research potential in the field, as I will have further access to samples from a range of pregnancy complications, but also will join a team of expert scientists and clinicians who can assist in this work, and who currently lack an immunologist to help in their studies.

This means there will likely be a pause in recruitment to this study between August – September 2024 as I move my reagents and equipment, and set up my new laboratory space. However, I am confident that I can then carry on recruitment in this new department, which sits on top of a large maternity hospital, and already has ethics in place that cover this study. There is also a team of research midwives who can recruit to this study.

I am therefore positive that I can complete this study, and hopefully that this may help in the recruitment of HELLP syndrome patients in this new site. I will keep the PET foundation up to date on the move and any obstacles that this brings.

Presentations of this work

1. British Immunological Society Conference – Belfast December 2023 – Preliminary data for this study was presented as a poster at the BSI conference, and lead to interesting discussions about the immunology of the placenta.
2. British Maternal and Fetal Medicine Society Conference – Liverpool April 2024 – Data from this study was presented as a poster at the BMDMS conference, and lead to discussions with obstetricians and midwives about the importance of collecting samples from pregnant women. This meeting also highlighted the potential for further types of samples that could be collected from women throughout pregnancy, including cervical swabs, stool and urine.