The good, the bad and the ugly: killer cells in endometriosis

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What is the problem?

Endometriosis is a disorder characterised by the presence of endometrial-like (like the lining of the uterus) tissue or lesions outside the uterine cavity, usually within the pelvis (see Figure 1). It is a chronic, incurable, hormone-dependant inflammatory disorder associated with pain and decreased fertility. Inflammatory responses are the body's normal response to infection or tissue injury.

Endometriosis affects 176 million women word-wide and approximately 1.5 million women in the UK, costing the UK >£9 billion per year. It has a devastating impact on the health and wellbeing of patients.

Its cause is unknown - a better understanding of the condition might lead to therapies and/or prevention.

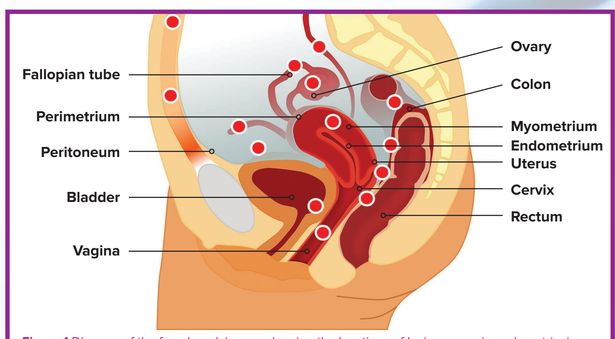

Figure 1 Diagram of the female pelvic area showing the locations of lesions seen in endometriosis (red and white circles). Image licensed under CC BY-SA 3.0 via Commons - https://commons.wikimedia.org/wiki/Category:Endometriosis#/media/File:Endometriosis_loc_en.svg

Who am I?

I carried out my Summer Vacation project in 2014, with the funding from Medical Research Scotland, and found it a very valuable experience, which gave me insight into research and ignited my interest in a career in this field. After graduating from the University of Edinburgh with my BSc (Hons) in Biomedical Sciences (Reproductive Biology) in 2015, I continued my studies and I am currently studying for an MScR in Genetics and Molecular Medicine at University of Edinburgh. I wish to continue working in research and hope to pursue a PhD in the future.

I would like to thank my supervisor Professor Philippa Saunders, my day-to-day supervisors Dr Erin Greaves and Dr Douglas Gibson, and to Medical Research Scotland for providing funding for my project.

What are we interested in?

The ectopic lesions attract a variety of cells of our immune system (which normally defends our bodies against infection and foreign bodies). We are interested in a particular class of immune system cell, called natural killer cells (NK cells). It has been suggested that a special NK cell population found in the uterus (womb) (uterine NK cells (uNK cells)) may not function correctly in women with endometriosis. My study focused on examining the uNK cells to see if we could learn more about their characteristics in endometriosis.

In healthy women, uNK cells are commonly found in the endometrium which lines the uterus. I wanted to find out whether

a) uNK cells are present in the endometriosis lesions; and

b) if so, are they different from the 'normal' uNK cells we have found in the endometrium of women without endometriosis?

What did we do?

I used tissue samples from the endometrium and peritoneum (the membrane which lines the cavity of the abdomen and abdominal organs, see Figure 1) of healthy women and tissue samples from the eutopic endometrium (endometrium within the uterus) and endrometriotic lesions from women with endometriosis to look at protein markers of NK cells and levels of mRNA for particular genes in order to characterise the cells. The presence of mRNA (an intermediate molecule in the process of gene transcription involved in making protein) indicates that the genes were being expressed.

What does this mean?

These data provide new insights into the characteristics of NK cells in the endometrium and differences between healthy control tissue and tissue from endometriosis sufferers. Further investigation of the differences may help explain the development or persistence of the condition and pave the way to new therapies modifying the activity of the NK cells.

What did we find?

Activation state of the NK Cells

i) CD16A is a marker of how active and effective NK cells are.Normally, uNK cells and peritoneal NK cells exhibit low levels of CD16A, however, I found that NK cells from ectopic lesions show high levels of CD16A mRNA and, therefore, may be making more CD16A protein and be more activated (see Figure 2). There are two possible explanations for this - the presence of a lesion triggers uNK cell activation and a change in their characteristics, or the lesion attracts a different kind of NK cells. At this point the precise mechanism is unknown.

ii) It is known that activated NK cells in other parts of the body kill cells by releasing enzymes such as GZMA (granzyme A), GZMB (granzyme B) and PRF1 (pore forming protein 1), which break down their target cells. I looked to see where, in NK cells in normal human decidua (endometrium altered during pregnancy), GZMB was located using a technique called immunohistochemistry. This technique uses stained antibodies that bind to a specific protein of interest to enable you to visualise the protein's location. The NK cells were identified using antibodies stained red for CD56 and green for GZMB (see Figure 3). The pattern of staining indicates that GZMB was located in vesicles (little sacs where the enzyme is stored before it is released to its target) inside the cells (in the cytoplasm), indicating these cells are not activated, and the CD56 was found at the cell surface, as expected.

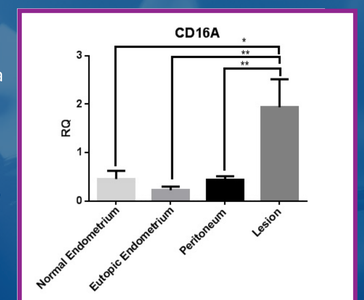

Figure 2 shows CD16A mRNA levels (RQ) were higher in the ectopic lesions (n=13) compared to the eutopic (uterine) endometrium (n=10) of those with endometriosis and the peritoneum (n=16) ($p<0.01$) and normal endometrium (n=7) ($p<0.05$) from healthy controls.

iii) I also looked at the levels of GZMA and PFR1 mRNA in the tissue samples from the healthy controls and those with endometriosis. mRNA levels of GZMA and PFR1 mRNA were low in lesions compared to normal endometrium (see Figure 4), suggesting a different activation state in lesions.

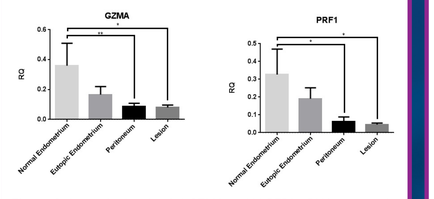

Figure 4 shows the levels of a) GZMA and b) PFR1 mRNA in normal endometrium (n=7), eutopic endometrium (n=10), peritoneum (n=16) and lesions (n=13).

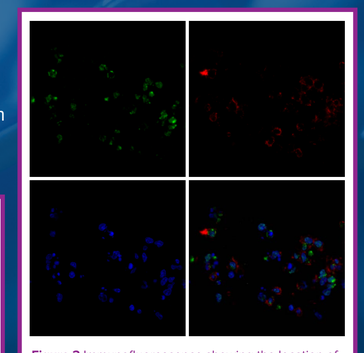

Figure 3 Immunofluorescence showing the location of **A)** GZMB (green) in vesicles inside the NK cells and **B)** CD56 (red) around the surface of the cells at the cell membrane in human decidua from a healthy control. **C)** DAPI (blue) was used to stain the nuclei and **D)** shows merged image.

Miss Sona Relovska was supervised during her Vacation Scholarship project, *"Investigations into the association between endometriosis and natural killer cells"*, by Professor Philippa Saunders.