Natural killers: when cells go wrong

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What is this research about?

Fetal and neonatal alloimmune thrombocytopenia, or FNAIT, is a life-threatening disease affecting approximately 1 in 1,000 live births, but what is it? To break down its name: fetal and neonatal – affecting newborns and babies still in the womb; alloimmune – an immune response from the mother against the baby; thrombocytopenia – resulting in low platelet counts. FNAIT is characterized by severe bleeding, brain hemorrhage, growth restriction and, in some cases, death of the fetus or newborn. The bleeding symptoms are explained by the low platelet counts and other identified factors, but it is not yet clear why FNAIT results in growth restriction or miscarriage.

In brief…

This study clarifies how maternal antibodies can cause fetal growth restriction and miscarriage, and introduces a new therapeutic target: natural killer cells.

FNAIT occurs when the mother's immune system generates antibodies which cross the placenta and attack platelets in the fetus. In many cases of FNAIT, the antibodies target slightly different (paternally inherited) forms of β3 integrin, a protein on the surface of platelets. The β3 integrin protein is also found on other cell types, such as the endothelial cells that line blood vessels and the trophoblast cells that form the placenta and help the embryo connect to the lining of the uterus.

In addition to trophoblasts, maternal immune cells such as natural killer (NK) cells are important for the proper formation and function of the placenta. Unlike NK cells elsewhere in the body, NK cells in the uterus are believed to be non-cytotoxic (non-killing). During early pregnancy, NK cells in the uterus promote immune tolerance and control the migration of trophoblast cells from the placenta to the uterus. This invasion of trophoblast cells is critical for the proper formation of the complex blood vessel network that allows nutrient exchange in the placenta.

To clarify the effects of FNAIT on the placenta, researchers at Canadian Blood Services investigated the mechanisms of growth restriction and miscarriage in a mouse model of FNAIT.

What did the researchers do?

The researchers used a mouse model of the disease, generated by transfusing female mice lacking the β3 integrin gene with mouse platelets to generate an immune response, then breeding the mice with normal male mice. FNAIT-related symptoms in these pregnancies were compared with control pregnancies (female mice lacking the β3 integrin gene that were bred with normal males but not transfused with platelets). Some of the parameters measured included fetal survival, weight, size, platelet count and heart rate; immune cell numbers and NK cell characteristics in the placenta; and placental blood vessel development, nutrient exchange and structure.

The researchers also evaluated treatments on the pregnant mice, including intravenous immunoglobulin (IVIG, a plasma protein product distributed by Canadian Blood Services that is a common therapy for FNAIT) and antibodies that either targeted NK cells for destruction or prevented their activation.

What did the researchers find?

- The maternal immune response to β3 integrin impaired development of the placenta, as shown by abnormal structure, inflammation and decreased blood flow. The faulty placentas led to fetal growth restriction and, in many cases, miscarriage.
- The abnormal placenta development was caused by increased numbers of activated uterine NK cells, which reduced migration of trophoblast cells and induced trophoblast cell death. This may be through interaction of activated NK cells with trophoblast cells that have β3 integrin on their surface.

- Depleting NK cells or preventing their activation rescued the pregnancies, restoring normal growth of the fetuses and preventing miscarriage.

How can you use this research?

Most clinical research has focused on the bleeding symptoms of FNAIT, particularly brain hemorrhages. There is a lack of knowledge regarding the effects of FNAIT on the placenta and whether placental dysfunction contributes to the growth restriction and miscarriage often seen in FNAIT. This study establishes a relationship between placental dysfunction, growth restriction and miscarriage in FNAIT.

Our researchers discovered that NK cells in the uterus can be activated by maternal antibodies, and that these activated NK cells destroy trophoblast cells, resulting in a dysfunctional placenta. This is an important shift in our understanding of miscarriage that could help researchers learn more about pregnancy failure, even outside the context of FNAIT.

This study suggests that IVIG treatment, may improve FNAIT symptoms by preventing activated NK cells from attacking trophoblast cells. However, IVIG is extremely expensive and in limited supply, so new therapeutic options with lower cost or higher efficacy would be very useful.

This study identified NK cells as potential targets for therapeutic intervention in FNAIT and possibly also other antibody-related causes of pregnancy loss. Additional research is needed to determine whether NK cells have a similar role in human FNAIT and whether NK cell interference has a beneficial effect. If proven safe and effective, NK-targeting therapies could be a useful alternative to IVIG and other existing drugs for FNAIT patients.

About the research team: This study was conducted in the laboratory of **Dr. Heyu Ni**, a Canadian Blood Services Center for Innovation scientist and a professor in the departments of laboratory medicine and pathobiology, physiology and medicine at the University of Toronto. Dr. Ni is the platform director for hematology, cancer and immunologic diseases at St. Michael's Hospital. The research team included members of Dr. Ni's laboratory (Issaka Yougbaré, Wei-She Tai, Darko Zdravic, Brigitta Elaine Oswald, Sean Lang, Guangheng Zhu), and collaborators from Toronto (Howard Leong-Poi, Dawei Qu, Lisa Yu, Caroline Dunk, Jianhong Zhang, John G. Sled, Stephen J. Lye, Jelena Brkić, Chun Peng, Xiao-Yan Wen, S. Lee Adamson, John Freedman), Kingston (B. Anne Croy), Ottawa (Duncan Stewart) and Sweden (Petter Höglund).

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1. Yougbaré I, Tai W, Zdravic D, Oswald BE, Lang S, Zhu G, Leong-Poi H, Qu D, Yu L, Dunk C, Zhang J, Sled JG, Lye SJ, Brkić J, Peng C, Höglund P, Croy BA, Adamson SL, Wen X, Stewart DJ, Freedman J, Ni H. Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. *Nature Communications* 2017; 8:224.

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