

How does anti-D prevent hemolytic disease of the fetus and newborn? Lessons from a mouse model

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What is this research about?

Antibodies are critical elements of an immune response to combat pathogens such as harmful bacteria and viruses. Interestingly, certain antibodies can also stop an immune response - a phenomenon known as antibody-mediated immune suppression (AMIS). AMIS is used in transfusion medicine to prevent an unwanted immune response, for example, the immune response that leads to hemolytic disease of the fetus and newborn (HDFN). HDFN occurs when there is an incompatibility between a mother and her fetus' blood type — for example, when the mother is Rh-negative and the fetus is Rh-positive. If, during the pregnancy, the mother is exposed to fetal red blood cells, her immune system recognizes the Rh-antigen as foreign and produces antibodies against it. These antibodies cross the placenta and bind to the Rh-antigen on the fetal red blood cells, triggering their destruction. HDFN can be life-threatening for the fetus or newborn. HDFN can be prevented through AMIS with an anti-D drug that suppresses the mother's immune response against the fetal Rh-antigen. However, this treatment has limitations.

There are several mechanisms by which anti-D is thought to suppress the mother's immune response. Clarifying which mechanisms are most important for the clinical effectiveness of anti-D could help in developing improved treatments not only for HDFN, but also for other serious immune conditions. Researchers conducted a series of studies to evaluate the contribution of each mechanism in suppressing an immune response.

What did the researchers do?

Researchers studied immune suppression using a mouse model that simulates the mother's immune system following treatment with an antibody therapy like anti-D. The mice were injected with 'foreign' red blood cells that were genetically modified to add antigens that stimulate an immune response. This mimics exposure of a mother to fetal red blood cells. In this model, the antigens on the 'foreign' red blood cells were engineered to present different sites for antibody recognition. In addition, different AMIS antibodies were studied. This allowed the researchers to study different mechanisms of immune suppression.

In one study, the researchers examined four different antibodies that trigger immune suppression activity: two that cause 'foreign' red blood cell clearance from the blood stream and two that do not. They examined how immune suppression was influenced by sugar molecules present on these antibodies. Mice were treated with either unmodified antibodies or antibodies that had been treated with an enzyme to remove sugar molecules attached in a region of the antibody that interacts with immune cells (the Fc domain). This modification impairs the antibodies' ability to interact with Fc receptors on immune cells.

In another study, the researchers used an antibody that causes 'foreign' red blood cell clearance and that allows other antibodies generated by the immune system to bind to the red blood cell antigen portion that causes an immune response. The immune response was assessed by measuring antibody levels in the blood.

What did the researchers find?

Findings from the first study imply that 'foreign' red blood cell clearance may not have a dominant role in AMIS:

- For the two antibodies that cause immune suppression and do not cause 'foreign' red blood cell clearance, immune suppression activity was not altered by the removal of sugar molecules.
- For the two antibodies that caused 'foreign' red blood cell clearance, removing sugar molecules prevented or reduced clearance. However, one antibody still caused full immune suppression despite the enzyme treatment that removed its ability to affect clearance.

In brief...

Researchers are learning more about how red blood cell antibodies can suppress the immune system, to develop better treatments for immune disorders.

Confirming findings from the first study, the second study shows that 'foreign' red blood cell clearance is not always required for AMIS:

- The researchers identified a threshold effect with antibody treatment. Immune suppression only occurred when the mice were given enough antibody to saturate all of the antigen sites on the 'foreign' red blood cells.
- Steric hindrance – that is, the antibody physically blocking access to the 'foreign' red blood cell antigen – did not appear to be a major mechanism of action in affecting the immune recognition of the 'foreign' red blood cells.
- Two hours after transfusion, mice treated with the antibody had lower amounts of detectable antigen on the 'foreign' red blood cells (antigen loss). This suggests that the antibody may suppress the immune reaction by removing the antigen from the 'foreign' red blood cells.
- Complete immune suppression was seen at all three antibody doses that caused antigen loss. 'Foreign' red blood cell clearance varied among these three doses, suggesting that clearance is likely not the major mechanism needed for immune suppression.

How can you use this research?

In the context of HDFN, researchers have thought for many years that anti-D interactions with Fc receptors on immune cells are critical for inducing fetal red blood cell clearance in the mother's circulation and drive antibody-mediated immune suppression. In the first study, although interfering with Fc glycosylation impaired or prevented red blood cell clearance, it did not completely prevent immune suppression. The second study supports this conclusion, showing that red blood cell clearance is not always required for antibody-mediated immune suppression.

These two studies can help shape future research to develop alternatives to anti-D drugs for preventing HDFN. Anti-D drugs are purified from plasma donated by a small number of people who produce large amounts of anti-D antibodies. The reliance on donated plasma makes the product vulnerable to shortages and introduces a theoretical safety risk, although anti-D products are very safe. To avoid these limitations, it would be beneficial to replace donor-derived anti-D with an alternative therapy such as laboratory-designed antibodies. By learning how an antibody suppresses the immune system, researchers can design new antibodies that target those mechanisms, making it easier to assure a consistent supply of safe and effective treatment for patients with immune disorders like HDFN. Furthermore, if antibody saturation is a critical element of immune suppression as this research suggests, this information could be used to improve current treatment regimens and to guide the development of new therapeutic strategies.

About the research team: The senior author of both papers, **Dr. Alan Lazarus**, is a Canadian Blood Services scientist, a professor of medicine at the University of Toronto, and a member of the Toronto platelet immunology group at the Keenan Research Centre for Biomedical Science at St. Michael's Hospital, Toronto. Other authors include a graduate student (D Marjoram), three postdoctoral fellows (Y Cruz-Leal, L Bernardo and X Yu), and collaborators from the U.K. (M Crispin and NPL Le) and Japan (M Uchikawa).

This ResearchUnit is derived from the following publications:

1. Marjoram D, Cruz-Leal Y, Bernardo L, Le NPL, Crispin M, Yu X, Uchikawa M, Lazarus AH. Immunoglobulin g Fc glycans are not essential for antibody-mediated immune suppression to murine erythrocytes. *Blood* 2017; 130: 2902-5.
2. Cruz-Leal Y, Marjoram D, Lazarus AH. Erythrocyte saturation with IgG is required for inducing antibody-mediated immune suppression and impacts both erythrocyte clearance and antigen-modulation mechanisms. *J Immunol* 2018; 200: 1295-305.

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