Research Unit

Improving the usefulness of a laboratory tool to match donors and patients

Collected in July 2018

What is this research about?

Exposure to cells or tissues that are genetically different to your own, for example through blood transfusion, a tissue or organ transplant, or pregnancy, comes with a risk. If the immune system identifies these cells and tissues as foreign, it will mount an immune response against the transfused or transplanted cells or tissues. To avoid these serious reactions, the blood and tissue type of recipients and donors is determined before transfusion or transplantation. To ensure compatibility, cross-matching can also be done, using laboratory tests to determine if a specific donor is compatible with a specific recipient.

In brief…

Using cells that blood operators discard during the manufacture of platelet products increases the usefulness of an assay to find blood for hard-to-match patients.

The monocyte monolayer assay is a laboratory test pioneered by Canadian Blood Services scientist, Dr. Donald Branch. This assay predicts the clinical significance of a patient's antibodies — that is, the likelihood that the patient's antibodies will cause the destruction of the transfused red cells and a serious transfusion reaction. The assay uses monocytes, immune white blood cells obtained from peripheral blood mononuclear cells that are isolated from whole blood immediately before the assay. These blood cells can be from the patient (called "autologous" monocytes) or from someone else (called "allogeneic" monocytes). However, getting monocytes to do the assay can be logistically challenging, and has limited its usefulness.

In this study, the researchers investigated an alternative source of monocytes – using peripheral blood mononuclear cells from "buffy coats". Buffy coats are produced from whole blood donations as part of the process to make platelet components. Buffy coats from several matched donors are pooled together and the platelets are isolated to make a platelet product for transfusion. However, not all buffy coats are suitable or can be pooled for platelet production, and those that are not used are usually discarded. Buffy coats are highly enriched in monocytes and other white blood cells. The researchers investigated whether monocytes isolated from buffy coats might be suitable for use in the monocyte monolayer assay.

What did the researchers do?

To conduct the monocyte monolayer assay, monocytes are placed in a single layer (a monolayer) on a plate. Red blood cells are mixed with antibodies from a patient and added to the monocytes. Under a microscope, the number of monocytes with one or more red blood cells adhered to or inside the monocytes (called "phagocytosis") at the end of the assay is counted. This number indicates whether the patient's antibodies reacted with the red blood cells, and thus whether the patient will have a serious transfusion reaction.

They developed a freezing (cryopreservation) technique to preserve and store peripheral blood mononuclear cells from buffy coats at extremely low temperatures for years or decades without any significant loss in cell function. The researchers compared three sources of monocytes:

- Monocytes isolated following freezing of cells from pooled buffy coats;
- Fresh monocytes isolated from pooled buffy coats;
- Fresh monocytes isolated from pooled, freshly drawn blood.

The research team looked at two important features of monocyte function: their ability to phagocytose cells in the monocyte monolayer assay; and their ability to secrete substances that can regulate the immune response (called "cytokines"). They investigated the function of frozen monocytes when challenged by red

blood cells that were exposed to three different antibodies previously implicated in transfusion-associated immune reactions in recipients.

What did the researchers find?

- After freezing, thawing, and washing, the percentage of monocytes recovered from buffy coats was similar to percentages recovered from fresh buffy coats and fresh blood. However, 95 per cent of cells isolated from buffy coats had intact membranes versus 100 per cent for the other sources.
- There was no significant difference in phagocytosis ability between monocytes that were frozen for three weeks versus those frozen for over six months.
- There was no significant difference in the phagocytosis ability of monocytes from the three sources.
- Levels of cytokine secretion were significantly different between fresh and frozen monocytes.

How can you use this research?

Practical restrictions, such as the need for fresh blood and the labour-intensive methods needed to isolate monocytes, have limited the usefulness of the monocyte monolayer assay in both hospital and research laboratories. Here, the researchers showed that monocytes could be isolated from buffy coats, and that the monocytes' ability to phagocytose was not affected by careful freezing. Using frozen buffy coat-derived monocytes increases the usefulness of this assay to determine clinically significant antibodies, while creating a novel use for currently unused buffy coat components. Further advantages include the reduced amount of time needed to perform the assay, and lower variability, as monocytes are from pooled buffy coats, providing more consistent results, and standardized experimental conditions.

Clinically significant antibodies are frequently associated with serious immune reactions, such as hemolytic transfusion reactions. For patients with rare blood types, it can be difficult to find compatible blood products to prevent the risk of these reactions. Using frozen buffy coat-derived monocytes could broaden the uptake of this assay by blood operators, hospital laboratories, and research laboratories. For example, Canadian Blood Services is currently working to implement the monocyte monolayer assay in its diagnostic service laboratories, using frozen monocytes from buffy coats. This will improve patient safety by helping to determine which red blood cell units are compatible and can be safely transfused to hard-to-match patients with rare blood types.

About the research team: This work was led by **Jelena Holovati**, a Canadian Blood Services adjunct scientist, laboratory director of the Edmonton stem cell manufacturing program, and an associate professor in the department of laboratory medicine and pathology at the University of Alberta, Edmonton. First author **Betty Kipkeu** is an MSc student at the University of Alberta, Edmonton, who's work on this project was funded by a Canadian Blood Services Intramural grant. The work was conducted with Canadian Blood Services scientists **Jason Acker**, also a professor in the department of laboratory medicine and pathology at the University of Alberta, and **Donald Branch**, a professor in the department of medicine, University of Toronto, Ontario.

This ResearchUnit is derived from the following publication(s):

[1] Kipkeu BJ, Shyian ML, da Silveira Cavalcante L, et al.: Evaluation of the functional properties of cryopreserved buffy coat-derived monocytes for monocyte monolayer assay. Transfusion 2018; doi:10.1111/trf.14650.

Acknowledgements: This work was funded by the Intramural Research Grant Program from Canadian Blood Services, funded by the federal government (Health Canada) and the provincial and territorial ministries of health. L. da Silveira Cavalcante was supported by a Canadian Blood Services Graduate Fellowship. The views herein do not reflect the views of the federal government of Canada. Canadian Blood Services is grateful to blood donors for making this research possible.

Keywords: Red blood cell, transfusion, alloimmunization, laboratory, rare blood.

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