**Mastering the monocyte monolayer assay**

**What is this research about?**

Serious adverse reactions to incompatible blood are caused by antibody–antigen interactions: if the recipient’s antibodies recognize an antigen on the surface of transfused blood cells, they bind to the antigen, which flags the blood cells for destruction by the recipient’s immune cells and can cause illness due to severe anemia. Similar reactions can occur in patients receiving other blood products, such as the plasma-derived drug intravenous immunoglobulin (IVIG).

It is difficult to predict which antibodies will cause a serious clinical reaction. The monocyte monolayer assay is a cell-based laboratory test used to predict the clinical significance of a patient’s antibodies – that is, the likelihood that the antibodies will cause red cell destruction. To perform the assay, monocytes, the immune cells thought to be responsible for destroying red blood cells during an adverse reaction, are isolated from blood and placed in a single layer (a monolayer) on a plate in the laboratory. Red blood cells are mixed with antibodies to prime them for destruction and added to the monocytes. Under a microscope, the number of monocytes with one or more red blood cells adhered to or inside (termed “phagocytosis”) the monocytes at the end of the assay is counted and used to calculate a “phagocytic index”. If the phagocytic index is low (e.g. less than five per cent) this indicates that the red blood cells likely won’t be destroyed by phagocytosis and that the patient won’t have a serious reaction. This assay was pioneered by Canadian Blood Services’ scientist, Dr. Don Branch, and has been used for over 35 years to predict the clinical outcome of incompatible blood transfusions. In recent years, it has been adapted to look at red blood cell destruction associated with IVIG treatment. Despite its long use, the optimal conditions for performing this assay have not been firmly established. In particular, the best way to prepare and store whole blood and monocytes for the monocyte monolayer assay has not been well studied. It is also not clear how using the patient’s monocytes (called “autologous” monocytes) or monocytes from someone else (called “allogeneic” monocytes) affects the results of the assay.

**What did the researchers do?**

Canadian Blood Services’ researchers systematically investigated the best conditions to successfully conduct the monocyte monolayer assay. To determine the best conditions to preserve monocyte function for the assay, the researchers drew whole blood from healthy donors into three different anticoagulants – ACD, EDTA and heparin – and stored the blood at room temperature or 4°C for up to two days. They also determined the best pH at which to carry out the assay. Special red blood cells that were susceptible to destruction by monocytes were used as positive controls to test the assay. The researchers examined blood from patients with and without clinically significant red blood cell destruction (hemolysis) reactions following treatment with IVIG to draw correlations between the assay results and clinical cases.

**What did the researchers find?**

- In a side-by-side comparison with EDTA and heparin, ACD was found to be the best anticoagulant to preserve monocyte function for the monocyte monolayer assay.

**In brief...**

The best conditions to successfully perform the monocyte monolayer assay—a laboratory-based test that predicts the severity of adverse reactions to blood products—were determined.
Monocyte function is well-preserved during storage of whole blood for up to 36 hours.

- Monocyte function is not well-preserved if whole blood is stored for up to 48 hours at room temperature.
- Storage of whole blood at 4°C greatly affects the number of monocytes that can be collected from blood.
- An optimal pH of 7.4 was determined.
- Monocyte monolayer assay results from stored and shipped patient whole blood correlated with clinical levels of red blood cell destruction.
- When patient (autologous) and healthy control (allogeneic) monocytes were compared, only the assays using autologous patient monocytes gave consistent results that corresponded to clinical outcome.

**How can you use this research?**

The results of this comprehensive investigation show that the monocyte monolayer assay can be reliably performed on samples in ACD anticoagulant, kept at room temperature for up to 36 hours, and when physiologic pH is maintained during the assay.

This study highlights the importance of using autologous rather than allogeneic monocytes in the monocyte monolayer assay. There have been concerns that using monocytes from someone other than the patient might give results that are less well able to predict clinical outcome. Despite this, many laboratories use monocytes from fresh whole blood from healthy volunteer donors rather than from the patient. In part, this is due to concerns over how well monocytes would survive the shipping and storage that might be required to get the patient’s blood to the laboratory to conduct the assay. Importantly, the optimal conditions described in this study show that the assay can be successfully conducted after storing and shipping patient blood samples. It also shows that using patient monocytes better reflects what may be happening in the patient and provides more accurate results.

This study focused on reactions to IVIG. Future studies will confirm whether use of the same assay conditions with patient monocytes can also provide accurate predictions of the significance of antibodies in patients requiring blood transfusion. Optimizing the potential of the monocyte monolayer assay to accurately predict whether IVIG treatment or transfusion will lead to a clinically significant reaction will help make the safest choice of blood product for the patient.

**About the research team:** This work was conducted in the laboratory of Dr. Don Branch, a Canadian Blood Services’ scientist, an associate professor in the departments of medicine and laboratory medicine and pathobiology at the University of Toronto and an affiliate scientist at the University Health Network, Toronto, ON. Dr. Branch pioneered the monocyte monolayer assay, an achievement recognized by two AABB awards: the 2003 Morten Grove-Rasmussen Award and the 2014 Sally Frank Memorial Award and Lectureship. Tik Nga (Cindy) Tong is a Canadian Blood Services’ supported graduate student, and Emeralda Burke-Murphy and Darinka Sakac are research assistants in Dr. Branch’s laboratory in Toronto, ON. Collaborators included Dr. Jacob Pendergrast and Dr. Christine Cserti-Gazdewich from the University Health Network, Toronto, ON, and Dr. Vincent Laroche from Université Laval, Quebec City, QC.

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