What is this research about?

Dengue is the most common virus spread by insects. Typically transmitted through the bite of Aedes mosquitoes (like its close cousins West Nile virus and Zika virus), symptoms range from mild flu-like symptoms to, in rare cases, severe hemorrhagic fever, which can be fatal. The global burden of dengue virus is considerable: Approximately 2.5 billion people are at risk and almost 400 million people are infected every year — about 25,000 die. Although mostly in tropical and subtropical regions, travel and increasing globalization means dengue can be found worldwide. Like all viruses, dengue needs another living organism — a host — in order to reproduce and survive. Viruses enter the host’s cells and use the cell’s internal machinery to replicate their own genetic material (in the case of dengue, single-stranded RNA). New viruses are then released from the infected cell, continuing the cycle of infectivity.

Dengue virus infections have a three to 14-day window period before symptoms appear. Approximately half of those infected with dengue never develop symptoms, despite having high levels of virus in their blood (up to a million or more viruses per milliliter of blood). This makes dengue a concern for blood operators because blood for transfusion could be collected from a donor who is infected with the virus but is not displaying symptoms. Dengue also affects blood cells. A common symptom in more severe cases is thrombocytopenia or loss of platelets. Platelets are small blood cells that lack a nucleus and are important for blood clotting and immunity. Platelets from patients with dengue have been shown to contain virus-like particles; however the involvement of platelets in dengue virus infections is unclear. It is also unknown how well dengue virus survives in blood components during storage before transfusion. Platelets and other cells lacking a nucleus (including red blood cells) have never been considered possible contributors to the virus lifecycle — merely thought of as innocent bystanders.

What did the researchers do?

The researchers investigated the hypothesis that dengue virus enters platelets and, once inside, uses each platelet's cellular machinery to produce viral proteins and replicate the virus' genetic material — its genome. They used a technique called quantitative real-time polymerase chain reaction (qRT-PCR) to quantify the amount of viral genetic material bound to and contained within platelets. They examined viral protein amounts using a technique called immunoblotting, in which specific antibodies are used to detect a protein of interest.

Next, the researchers investigated what would happen if blood components became infected with dengue virus. They used a smaller amount of the virus than in the first study to mimic the level of virus typically found in a potential donor who is infected with dengue and does not have symptoms. They added the virus to platelet and red blood cell units, and then stored the units in standard blood banking conditions. They measured the amount of dengue virus by qRT-PCR and by an assay in which the virus is grown and measured directly.

What did the researchers find?

- Dengue virus binds to platelets at both 37°C (body temperature) and 25°C (room temperature and blood bank storage temperature). The binding efficiency is lower at 25°C. Two platelet surface proteins (called DC-SIGN and HSP) are dengue virus receptors through which the virus can bind.
Dengue virus causes platelets to become activated. Stimulation of platelets with thrombin, a substance that initiates blood clotting, enhances the binding of dengue virus to platelets by about two-fold.

The dengue virus genome is replicated and dengue viral proteins are synthesized by platelets. Live, infectious dengue virus offspring are produced by infected platelets.

Even at lower levels of virus that mimic an asymptomatic donor, infectious dengue virus persists and its genome can replicate in stored platelet and red blood cell units.

Infectious dengue virus can continue to reproduce in stored platelet and red blood cell units. However, the virus production in these units did not offset overall virus decay. This means that older units have less infectious dengue virus.

**How can you use this research?**

This research advances the fundamental understanding of how dengue virus replicates. Although platelets lack a nucleus, these tiny blood cells contain all the machinery needed to synthesize proteins and replicate RNA. The findings of this study are unprecedented and show that dengue hijacks blood platelets, commandeering the cell’s machinery to produce viral proteins and replicate its own genome. How this links to thrombocytopenia normally seen with dengue virus is still unclear. The possibility that viral antigens are exposed directly on platelets is now being considered for the first time. Dengue virus replicating in red blood cell units was a surprising finding as these cells do not have replication machinery. The involvement of other residual cells found in red blood cell units is suspected.

Blood donor systems worldwide are vulnerable to dengue virus. There have been five documented cases of dengue transmission by blood transfusion, all occurring in regions where dengue is present or endemic. It is likely many other cases have been overlooked. Increasing travel, globalization and global warming, which expands mosquito-friendly habitats, mean dengue is an emerging infectious threat to the blood system in Canada. This research shows that platelets or red blood cells collected from an asymptomatic donor carrying dengue could harbor the virus. Importantly, the virus remains infectious and can continue to replicate in stored blood components. Current deferrals for donors who have traveled to malaria risk zones also include areas where dengue is endemic and provide a level of protection to the blood supply. New pathogen inactivation techniques that destroy viruses in blood might also be effective against dengue and are being investigated.

**About the research team:** This research was conducted in the laboratory of Dr. Ed Pryzdial, a Canadian Blood Services research scientist, associate director of the centre for blood research and a clinical professor in the department of pathology and laboratory medicine, University of British Columbia, Vancouver B.C. In his laboratory, Dr. Michael Sutherland, a senior research associate and clinical assistant professor, and Dr. Ayo Simon, as postdoctoral fellow, now assistant professor, Ahmadu Bello University, conducted the studies. The study looking at dengue virus in blood products was in collaboration with Canadian Blood Services colleagues: Dr. Katherine Serrano and Dr. Peter Schubert, both Centre for Innovation research associates and clinical associate professors in the department of pathology and laboratory medicine at the University of British Columbia; and Dr. Jason Acke r, a Centre for Innovation senior development scientist and a professor in the department of laboratory medicine and pathology at the University of Alberta.

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