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Is it Time to Reconsider Neutrophil Antibody Testing of Platelet Donors?

David F. Stroncek[1] and **Mary Clay**[2]

[1]The Department of Transfusion Medicine, Clinical Center, NIH, Bethesda, MD

[2]The Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN

The original research article in this month's ***Transfusion*** by Angelika Reil and colleagues[1] is the third in a series of landmark articles that represent a major advancement in the field of neutrophil immunology. This same group of investigators has recently characterized the molecular basis of the human neutrophil antigen (HNA)-3 system. They found that HNA-3a and HNA-3b result from a single nucleotide polymorphism in the choline transporter-like protein-2 gene[2] and their results have been confirmed by Curtis and colleagues.[3] The manuscript in this issue of ***Transfusion*** described a HNA-3a and -3b genotyping method using sequence-specific primers (SSP) and PCR and measured HNA-3a and -3b gene frequencies in a large number of Caucasians. It also assessed the risk of alloimmunization associated with pregnancy. These three publications mark the completion of the characterization of the 5 described neutrophil antigen systems: HNA-1, -2, -3, -4, and -5[1,4–8] and will likely lead to improvements in HNA genotyping and antibody detection methodologies.

For many Human Leukocyte Antigen (HLA) applications, molecular genotyping and solid phase antibody detection assays have replaced cellular phenotyping and antibody screening. These advances in testing methodology have led to the development of highly automated genotyping and solid phase antibody detection assays that lend themselves to high throughput, low cost testing and have resulted in more precise and rapid typing. Similar advances will likely take place for HNA testing.

HNA antigens were initially characterized on a serological level using cellular assays. These cellular assays are, however, difficult and laborious to perform. Genotyping assays are now available for HNA-1, HNA-3, HNA-4 and HNA-5 systems.[9–11] Although HNA-2a has been found to be located on CD177, HNA-2a antigen is an isoantigen and the lack of expression of HNA-2a is the result of gene misplicing[7] and a simple genotyping method for HNA-2a is not yet available. However, CD177 monoclonal antibodies specific to HNA-2a are commercially available which can be used to detect antigen null subjects.

Typing of all HNA antigens can now be readily performed and in the future HNA antibody detection methods will likely be widely available. The availability of monoclonal antibodies to the glycoproteins bearing HNA antigens permits the use solid phase assays to detect antibodies specific to these antigens. These monoclonal antibodies can be used in the monoclonal antibody immobilization of neutrophil antigens assay (MAINA) and to isolate

Please Address all correspondence to: David Stroncek, MD Department of Transfusion Medicine Clinical Center, National Institutes of Health Building 10, Room 1C711 Bethesda, Maryland 20892-1184 Phone: 301-402-3314 dstroncek@cc.nih.gov.

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the glycoprotein bearing HNA antigens in order to develop ELISA and related solid phase alloantibody detection assays. In addition to CD177, monoclonal antibodies are also available to CD16, CD11b and CD11a which carry HNA-1, HNA-4 and HNA-5 antigens respectively.[4,8] The availability of these monoclonal antibodies make solid phase testing of HNA-1, -2, -4 and -5 antigen possible, but prior to the molecular characterization of HNA-3, solid phase testing for antibodies specific to HNA-3a was not possible.

Traditionally, HNA-3a antibodies have been detected by testing samples against freshly isolated neutrophils from phenotyped donors in cellular assays: Granulocyte Agglutination (GA) and Granulocyte Immunoflourescence (GIF) assays. HNA-3a phenotyping is best preformed with GA.[12] These assays, however, require highly trained staff and are laborious to perform. Phenotyping of the panel donors is also problematic because the availability of anti-HNA-3a for phenotyping is limited and anti-HNA-3b is very difficult to obtain. A method for determining HNA-3 genotypes is now available thus eliminating the need for HNA-3 alloantisera and, hopefully, monoclonal antibodies will soon be available that are directed to the choline transporter-like protein-2 to facilitate its isolation for solid phase HNA-3a alloantibody detection assays. Monoclonal antibodies specific to the choline transporter-like protein-2 could be used by reference laboratories for MAINA assays to detect antibodies specific for HNA-3a and -3b on a small scale or by companies to isolate the choline transporter-like protein-2 to produce HNA-3a and -3b antibody detection kits that could used on standard platforms and would allow for high throughput testing. Commercial solid phase assays that detect antibodies specific to HNA-1a, -1b, -1c, -2a, -3a, -4a, and -5a are under development and are going through the final regulatory requirements for release, but it may be several months or even longer before these assays are available.

The availability of the HNA-3a/b genotyping assay described by Reil[1] and a solid phase anti-HNA-3a detection assay are of particular importance for transfusion reaction evaluation and prevention. HNA antibodies are an important cause of alloimmune neonatal neutropenia, autoimmune neutropenia, and transfusion reactions, but antibodies specific to HNA-1a, HNA-1b, and HNA-2a are most often responsible for alloimmune neonatal neutropenia[13,14] and those specific to HNA-1a and HNA-1b for autoimmune neutropenia.[15–17] Antibodies specific for HNA-1a, -1b, -2a and -3a can all cause transfusion reactions but the transfusion of products containing large volumes of plasma with anti-HNA-3a seems to be an especially important cause of transfusion related acute lung injury (TRALI). Case reports, series of case reports and look-back studies suggest that among all leukocyte antibodies, anti-HNA-3a may be the most potent at causing severe and fatal TRALI.[18–21] Hopefully, the molecular characterization of HNA-3a will provide needed information about the immunologic properties and mechanisms associated with this unique antigen.

A recent survey of US blood centers found that 91% have implemented measures to reduce the incidence of TRALI associated with the transfusion of plasma products.[22] To reduce the risk of TRALI due to transfusion of plasma products most centers are using male only whole blood to manufacture plasma for transfusion, male only apheresis plasma, predominantly male plasma, or male and never pregnant female plasma. For TRALI risk reduction associated with the transfusion of platelet components 41 of 47 centers have implemented a strategy to reduce the risk of TRALI. The most commonly used strategy is to increase the number of apheresis platelets collected from male donors (70%). The second most common method is to test platelet donors for HLA antibodies (43%). No centers are testing donors for HNA antibodies.

It is likely that the lack of availability of high throughput solid phase assays of detecting HNA antibodies accounts for the lack of use of HNA antibody screening as a TRALI risk

reduction measure. However, the new understanding of the molecular basis of HNA-3a and -3b will lead to advances that make anti-HNA-3a screening and confirmation assays more precise and reliable. As a result, the current practice of not testing platelet apheresis donors for anti-HNA-3a and other HNA antigens will become unsupportable. If a reagent is added to a commercial HLA antibody screening assay that allows for the detection of antibodies to HNA-3a, then centers collecting platelets from female donors should consider testing for HNA antibodies. If reagents are available that permit reliable testing of other HNA antibody implicated in TRALI, donors should also be tested for these antibodies.

Female platelet donors could be tested for both HNA and HLA antibodies. However, if a center can only test for either HNA or HLA antibodies, it may be better to test female donors for HNA antibodies rather than HLA antibodies since relative to HLA antibodies the prevalence of HNA antibodies in female donors is very low and the TRALI risk associated with the transfusion of HNA antibodies is high compared to HLA antibodies. Although antibodies to HNA are more potent at causing transfusion reactions, it is not certain if the portion of TRALI reactions that are caused by HNA antibodies is larger or smaller than those caused by HLA antibodies. It may well be that more reactions are due to HNA antibodies than HLA antibodies. In any case, transfusion safety would be improved by eliminating HNA antibodies from plasma containing blood products, especially anti-HNA-3a.

While it is still not practical to screen platelet donors for anti-HNA-3a or other HNA antibodies, the genotyping assays described by Reil[1] will allow centers to screen platelet donors in order to identify those homozygous for HNA-3b and hence who could produce anti-HNA-3a. Multiparous donors could be genotyped for HNA-3a and -3b and those homozygous for HNA-3b could be excluded from donating. Excluding HNA-3b homozygous multiparous females would lead to the loss of 5.5% of those tested.[1] The number of donors deferred could be reduced by testing for anti-HNA-3a since Reil[1] found that the immunization rate for HNA-3a during pregnancy was only 7%.

It is not yet time to begin to screen donors for HNA antibodies, however, the work by Reil and colleagues provides new tools for eliminating donors who could produce a clinically important neutrophil antibody, anti-HNA-3a.

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