Inhibition of blood group antibodies by soluble substances

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The presence of multiple alloantibodies or an antibody to a high-prevalance antigen in a patient sample can pose challenges in antibody identification. The pattern of reactivity seen on an antibody panel may show various strengths of reactivity by different methods of testing or same strength of reactivity at one or more phases of testing. To ensure proper identification, multiple investigative tools may be used. We review one of these methods—inhibition by soluble substances—which has become an expansion of our toolbox within the past 10 years. Alloantibodies can be inhibited using specific soluble substances. These soluble substances occur naturally in various fluids or can be manufactured. When a patient sample contains multiple antibodies, clinically significant or not, inhibition of one may help determine specificities of others. Specific inhibition of a particular antibody will also help to confirm its presence. ***Immunohematology* 2019;35:19–22.**

Key Words: soluble blood group substance, dilution control, neutralization, soluble peptide, inhibition, recombinant blood group proteins

Reagents/Supplies

Reagents	Supplies
▪ Reactive blood sample (plasma or serum) ▪ Soluble substance (antigen, natural, or synthetic) ▪ Inert substance, such as albumin or saline ▪ Other reagents as appropriate, such as anti-IgG, IgG-coated RBCs	▪ Test tubes (gel cards if that method is used) ▪ Pipettes ▪ 37°C incubator ▪ Centrifuge

RBCs = red blood cells.

Procedural Steps

- Prepare soluble substance for use.
- Mix soluble substance with test sample.
- Prepare a dilution control containing equal volumes of test sample and an inert substance, such as albumin or saline.
- Incubate at optimal temperature and time.
- Test the sample plus the substance and the sample plus the inert substance (dilution control) against RBCs that previously gave a positive reaction.

RBCs = red blood cells.

Background

When performing pretransfusion testing, serologic results may indicate the presence of one or more alloantibodies. There are many methods that can be used to identify and separate specificities.[1] One such method is based on the principle of inhibition. The ability to specifically inhibit one antibody may help identify that antibody and allow other antibody specificities to also be identified. Inhibition can aid in the identification of an antibody to an antigen that shows variable expression among individuals, such as anti-P1. Some antibodies can be inhibited by soluble substances such as sugars, proteins, and peptides; examples include ABH, Lewis, P1, Sd^a, Chido/Rodgers, and I^D. Human saliva, hydatid cyst fluid, pigeon egg white, human or guinea pig urine, human serum, and human milk have been used as soluble substances to inhibit red blood cell (RBC) antibodies before the 1990s.[1–4]

Since then, recombinant blood group proteins (rBGPs) have also been shown to be effective in the identification of antibodies to high-prevelance antigens that are single pass and glycosylphosphatidylinositol-linked proteins, thus leading to the detection of underlying alloantibodies.[5–8] Unlike traditional soluble substances that are found naturally in human and other animal sources, rBGPs are manufactured.[5–7] The result of the manufacturing is a very specific rBGP that could aid in antibody identification.[7] Gone are the days when the only tools available to the investigational immunohematologist were RBCs, polyclonal antibodies, lectins, and natural inhibitory substances.[9] Recombinant peptides and proteins represent the latest addition to our growing toolbox.

Principle

Inhibition takes place when plasma or serum containing an antibody is incubated with a soluble substance (natural or synthentic) of corresponding specificity. Subsequent testing (hemagglutination) reveals the lack of reactivity with RBCs that tested positive before inhibition. Other antibodies, if

present, in treated plasma or serum should remain unaffected and can be identified. The principle of this reaction is based on the inhibition of the antibody by the corresponding soluble protein.

Indications

Inhibition of blood group antibodies by soluble substances can aid in the identification of specific antibodies. Antibody activity of known specificity can be selectively "removed" by using the inhibition method, thus leaving behind other antibodies to be identified. Other indications for inhibition are to determine ABH secretor status and immunoglobulin class of anti-A and/or anti-B. Inhibition using rBGP has also helped classify a new blood group antigen, CD59.[6] One may consider using rBGPs based on serological clues of the specimen and the availability and specificity of the rBGP.

Soluble Substances

One must determine the appropriate soluble substance to use. Today, the choices are many (Table 1). Blood group substances in water-soluble form in tissue fluids and secretions of the body have been known since the 1930s.[10] Agglutination inhibition tests using A and B substances and boiled saliva were being used as early as 1940.[11,12] In 1996, soluble CR1 produced by recombinant DNA techniques was used to identify Knops system antibodies.[13]

The latest development is the use of soluble proteins to inhibit drugs in the plasma and serum of patients who are treated with monoclonal antibodies. Besides the surface proteins on the target cells, some of this novel drug binds to RBCs. Examples are anti-CD38[14,15] and anti-CD47[16] monoclonal antibody therapy. Both drugs can be inhibited by recombinant soluble proteins, CD38[17] and CD47 (unpublished results), although their high titers in patients may preclude the effective inhibition in neat plasma samples. A recent article by Velliquette et al.[18] evaluated anti-CD47 (Hu5F9-G4) interference in pretransfusion testing and offered mitigation strategies. Covering the target blood group antigens on the RBC surface is another approach. This novel and attractive alternative will add to our toolbox and should eventually become the topic of another review.

Procedure

Obtain plasma or serum sample for testing. If necessary, process the substance (Table 2). Once ready for testing, the most common procedure starts with labeling two test tubes: one for the sample and one for the dilution control. Combine test sample and soluble substance into the tube labeled "sample." To the tube labeled "dilution control," combine test sample and inert substance. Incubate both tubes for a specific time and temperature determined by the known "ideal" for the target specificity. After incubation, test samples against previously reactive RBCs selected by phenotype. Inhibition has occurred when the "sample" is nonreactive and the "dilution control" is still reactive. These results confirm that inhibition of antibody has taken place and the lack of reactivity was not caused by dilution. If the "dilution control"

Table 1. Known soluble substances and their use

Blood group system	ISBT number	Antibody inhibited	Immmunoglobulin class	Soluble substance
ABO	001	Anti-A; anti-B	IgM; IgG	Human saliva (secretor)
H	018	Anti-H	IgM	Human saliva (secretor)
Lewis	007	Anti-Le^a; anti-Le^b	IgM	Human saliva (secretor)
P1PK	003	Anti-P1	IgM	Hydatid cyst fluid, pigeon egg albumin
901 series	901	Anti-Sd^a	IgM>IgG	Human urine or saliva, guinea pig urine
Chido/Rodgers	017	Anti-Ch; anti-Rg	IgG	Pooled plasma
Cromer	021	Most Cromer antibodies	IgG	Human serum, plasma, or concentrated urine
Scianna	013	Anti-Sc1 (Seltsam et al.[5])	IgG	Recombinant protein
CD59, Lutheran, Yt, Dombrock, Chido/Rodgers, Cromer, Knops, JMH	035, 005, 011, 014, 017, 021, 022, 026	See Anliker et al.[6] and Seltsam et al.[7]	Usually IgG	Soluble recombinant proteins from eukaryotic expression systems

ISBT = International Society of Blood Transfusion.

Table 2. Preparation of soluble substances

Source material	Preparation
Guinea pig urine	After collection of urine, boil for 10 minutes. Dialyze for 48 h at 4°C against multiple changes of pH 7.4 PBS. Centrifuge, aliquot, and freeze.[19]
Human milk	Collect milk from lactating women, centrifuge at 1000*g* (serofuge at 3400 rpm) for 10 minutes, remove and discard the cream layer, incubate milk in boiling water for 10 minutes, mix 1 volume of milk with 1 volume of PBS. Aliquot and freeze.[2]
Human saliva	Collect 2 mL saliva, boil for 10 minutes, centrifuge at 1000*g* (serofuge at 3400 rpm) for 10 minutes, harvest supernate, aliquot, and freeze.[2]
Human urine	Collect urine from three individuals, pool, centrifuge, dilute with equal volume of distilled water, check pH (dialysis with PBS may be needed to obtain pH between 6 and 8.5), aliquot, and store frozen until needed.[2]
Hydatid cyst fluid	Incubate HCF (with scolices) from animal or human sources at 56°C for 1 hour. Dilute 1 volume of hydatid cyst fluid with 9 volumes of PBS. Aliquot and freeze.[2]
Pigeon egg albumin	Separate egg white from the yolk. Prepare dilutions of 1:100 to 1:1000 in PBS. Test the dilutions using a potent anti-P1 to determine the best dilution for inhibition studies. Make aliquots of appropriate dilution and freeze.[2]
Pooled plasma	Purchase commercial pooled plasma or prepare pooled plasma from six or more donors. Aliquot and freeze.[3]
Recombinant protein	May be commercially available.[5–7,15]

PBS = phosphate-buffered saline.

is nonreactive, the test is invalid. The hemagglutination test can be done in tubes as described here or by other methods such as column agglutination or solid phase if recommended by the manufacturer or validated in-house.

Limitations

A positive reaction when testing the sample plus soluble substance may indicate that additional alloantibodies are present in the sample. Testing additional RBCs using the sample plus soluble substance may be indicated to determine possible additional antibody specificities. It may not be possible to test low-titer antibodies because of the required dilution that cannot be achieved. If one wants to use specific rBGPs, they are commercially available if the molecular and genetic basis are known.[20] It may not always be possible to inhibit high-titer antibodies, particularly monoclonal antibody drug formulations, because the required high concentration of the soluble substance cannot be achieved.

Quality Control

The dilution control containing the sample plus inert substance should result in a positive reaction when tested against an RBC positive for the corresponding antigen to the antibody under investigation. The lack of reactivity in the dilution control indicates dilution of weakly reactive low-titer antibody and invalidates the test.

Precautions

Use caution when using any of these soluble substances. Follow the procedure as written and test using the recommended test system, such as tube or gel method. For example, if one chooses to change from testing in tube to gel method, the nonstandard test method should be validated before use. Immunoglobulin class (IgG versus IgM) should be considered when evaluating an unexpected result, such as a false positive.

Summary

Inhibition has proven to be useful in separating, identifying, and detecting alloantibodies that may be present in a patient's sample. Traditional sources (saliva, plasma, and urine) of soluble substances and anti-drug proteins can be used to inhibit antibodies, allowing for their detection and identification. The use of rBGPs has expanded our ability to inhibit a greater number of antibody specificities and can be used in different assays to detect and identify distinct antibodies.

Acknowledgments

We thank Debrean A. Loy, Marina U. Bueno, and Kshitij Srivastava for providing the inhibition of anti-CD47 by soluble protein (unpublished results).

This article was authored as a group writing assignment as part of the Specialist in Blood Bank (SBB) training program at the National Institutes of Health (NIH) Clinical Center, Department of Transfusion Medicine (www.cc.nih.gov/dtm/education.html).[21]

The authors are federal employees. This work was supported by the Intramural Research Program (project ID Z99 CL999999) of the NIH Clinical Center. There were no nonfederal sources of support.

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