Adsorption of autoantibodies in the presence of LISS to detect alloantibodies underlying warm autoantibodies

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BACKGROUND: The safe transfusion of patients with warm autoimmune hemolytic anemia requires an efficient and time-saving assay to detect alloantibodies underlying autoantibodies. Methods used include RBCs treated with ZZAP reagent, proteolytic enzyme, or untreated RBCs in the presence of PEG. We propose a method using LISS, which presents some advantages over previous methods.

STUDY DESIGN AND METHODS: We evaluated the effectiveness of autoantibody adsorption with papain-treated and untreated RBCs in the presence of LISS for removal of autoantibodies, without affecting alloantibodies. 

RESULTS: One-hundred twenty sera containing autoantibodies were adsorbed with our routine method, which uses papain-treated allogeneic RBCs. Seven-hundred twenty adsorptions (mean, 6 per sample) and 21,600 minutes (mean, 180 min per sample) were required to remove autoantibodies. Fifty sera were adsorbed with our routine method that uses papain-treated allogeneic RBCs in the presence of LISS. The number of adsorptions and the completion time were, respectively, 144 (mean, 2.9 per sample) and 2,880 minutes (mean, 57.6 min per sample). Twenty sera were evaluated with untreated autologous RBCs, in the presence of LISS; 58 adsorptions (mean, 2.9) and 1,160 minutes (mean, 58 min per sample) were required. Three adsorptions with antigen-negative allogeneic RBCs were performed on 8 sera containing alloantibodies with weak reactivity (anti-K [1], anti-D [1], anti-Fya [2], anti-S [2], anti-E [1], and anti-Jka [1]). Alloantibodies were detected with the LISS procedure with papain-treated and untreated RBCs and the routine papain method. Alloantibodies with weak reactivity, tested against the same antibody-detection RBCs, remained unchanged following adsorption of 5 sera. An anti-S, with very weak reactivity, was no longer detected, and an anti-Jka became weaker (1+), regardless of the procedure used. One anti-D became weaker only with the LISS adsorption method.

CONCLUSION: Autoantibodies can be adsorbed more efficiently in the presence of LISS.

When, autoimmune hemolytic anemia patients need a blood transfusion, detection of alloantibodies can be difficult because autoantibodies can mask the presence of clinically significant alloantibodies. To prevent hemolytic transfusion reactions, it has been suggested that autoantibodies should be removed by adsorption to detect any alloantibodies that are present.

The selected method should take into consideration safety, cost, and time aspects. Two approaches are widely used: adsorption with autologous RBCs (autoadsorption) and adsorption with allogeneic RBCs (alloadsorption). Theoretically, autoadsorption is the safest technique to eliminate autoantibodies from the serum without affecting the level of clinically significant alloantibodies, even when considering antibodies against high-frequency antigens. But when the patient has received transfusion within the previous 3 months, or when an adequate amount of autologous RBCs is not available (e.g., because of anemia), alloadsorption can be used. However, this technique presents some disadvantages, such as the adsorption of alloantibodies against high-incidence antigens.

Different methods of alloadsorption or autoadsorption include RBCs treated with ZZAP reagent or with proteolytic enzymes or untreated RBCs in the presence of PEG. The ZZAP procedure is not commonly used in France and in our preliminary studies, we found that column agglutination technology (CAT) was not suitable for

ABBREVIATION: CAT = column agglutination technology.

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testing PEG-adsorbed serum (data not shown). In addition, some investigators have reported that alloantibody activity may be diminished following the PEG adsorption technique.\(^6\) Our routine method uses adsorptions with papain-treated RBCs. Considering the enhancing properties for antigen-antibody interaction of LISS,\(^7,9\) we evaluated the effectiveness of adding LISS to our routine methods to enhance adsorption of autoantibodies, with papain-treated and untreated RBCs.

The first goal of this work was to consider the use of LISS with untreated RBCs when autoadsorption is performed. To use only one sample of adsorbing RBCs, the second goal was to evaluate the use of LISS with papain-treated RBCs when autoadsorption is required. The determination of FY and MNS phenotypes in patients with positive DATs can be difficult because, except for anti-S, specific IgM MoAbs are not available. However, because papain treatment destroys these antigens, only RH, KEL, and JK phenotypes of patient are required for the selection of RBCs used for adsorption studies.

**MATERIALS AND METHODS**

This investigation consisted of three parts.

**Evaluation of the effectiveness of removing autoantibodies with the LISS alloadsoption procedure with papain-treated RBCs**

Retrospective analysis of the number of adsorptions required to remove autoantibodies with papain-treated RBCs (our routine method). We evaluated 120 samples, processed from August 2000 through June 2001. These samples were found to contain warm autoantibodies by our routine screening procedure with LISS IAT by a CAT technique (BioVue system, Ortho Clinical Diagnostics, Raritan, NJ). The routine adsorption procedure in our laboratory is to mix equal parts of the patient’s serum and phenotypically (RH and JK) matched papain-treated RBCs and to incubate at 37°C for 30 minutes. The adsorbed serum was tested against the same RBCs that were used for the adsorption by LISS IAT: 10 μL of RBCs, a 4 percent suspension of RBCs, 40 μL of LISS, and 40 μL of adsorbed serum incubated for 15 minutes at 37°C and centrifuged for 5 minutes. If reactivity persisted, the adsorption procedure was repeated until the serum was no longer reactive. Antibody identification was performed by LISS IAT with CAT. Reactions were graded according to the manufacturer’s directions (0-4+).

Papain treatment of RBCs was performed according to the manufacturer’s instructions (Nantes Blood Bank, Nantes, France): Selected allogeneic RBCs (group O RBCs matching the patient’s RH and JK phenotypes) were washed once and centrifuged. One vol of prewarmed (37°C for 15 min) papain was added to 1 vol of RBCs, mixed, and incubated at 37°C for 10 minutes. The RBCs were washed three times with saline and the last wash was removed as completely as possible to prevent serum dilution.

**Comparison of our routine method and the proposed LISS allogeneic adsorption method.** Sera from nine patients with autoantibodies, with or without alloantibodies, were selected to compare our routine method with the LISS alloadsorption method. Equal parts of patient’s serum, LISS, and phenotypically (RH and JK) matched papain-treated RBCs were mixed and incubated at 37°C for 20 minutes. The adsorbed serum was tested against the same RBCs used for adsorption by LISS IAT with CAT with 2 vol of adsorbed serum and no additional LISS: 10 μL of RBCs in 4 percent suspension and 80 μL of mixed LISS adsorbed serum incubated for 15 minutes at 37°C and centrifuged for 5 minutes. If reactivity persisted, the adsorption procedure was repeated until the serum was no longer reactive. Antibody identification was performed by LISS IAT with CAT. The LISS used for these adsorption procedures was the solution used for antibody detection and/or identification testing protocol, namely, Formula 735: 239.78 mM glycocoll (glycine), 30.80 mM sodium chloride (NaCl), 1.31 mM dibasic sodium hydrogen phosphate (NaH_2PO_4), and 1.5 mM dibasic phosphate dibasic (Na_2HPO_4) provided by B. Braun Medical (Bologne, France). The papain treatment of RBCs was performed by the same method described above.

**Analysis of the number of adsorptions required to remove autoantibodies with the proposed LISS allogeneic adsorption procedure with our routine method.** We evaluated 50 samples with warm autoantibodies processed since May 2001 with the LISS adsorption method. The proposed LISS allogeneic adsorption procedure is described in the previous paragraph and the papain treatment cells is performed according to the same method described for our routine method.

**Evaluation of the effectiveness of LISS autoadsorption procedure in removing autoantibodies with untreated autologous RBCs**

RBCs and sera from 20 patients with autoantibodies were studied. For the proposed autoadsorption method, equal parts of patients’ sera, LISS, and untreated patients’ RBCs were mixed and incubated in sealed glass tubes, at 37°C for 20 minutes. The adsorbed sera were tested against antibody detection RBCs by LISS IAT with CAT with 2 vol of adsorbed serum and no additional LISS: 10 μL of RBCs in 4 percent suspension and 80 μL of mixed LISS adsorbed serum incubated for 15 minutes at 37°C and centrifuged for 5 minutes. If reactivity persisted, the adsorption procedure was repeated until the serum showed some difference in reactivity with the antibody detection RBCs. When this profile was obtained, an antibody identification was performed by LISS IAT with CAT.
Evaluating the effect of the LISS alloadsorption on alloantibody activity

Eight sera containing alloantibodies of clinical importance (anti-K, -D, -Fy\(^a\), -S, -C, -Jk\(^a\)) were adsorbed three times with papain-treated RBCs and with the LISS procedures with papain-treated and untreated RBCs. The strength of these antibodies in unadsorbed sera with the CAT ranged from 1+ to 3+.

RESULTS

Evaluation of the effectiveness of removing autoantibodies with the LISS alloadsorption procedure with papain-treated RBCs

Retrospective analysis of the number of adsorptions required to remove autoantibodies with conventional alloadsorption procedure. The number of adsorption steps required to remove autoantibodies with the conventional alloadsorption procedure routinely used in our laboratory is summarized in Table 1. The total number of adsorptions and the assay completion time (for all 120 samples) were 720 and 21,600 minutes, respectively (mean per sample, 6 adsorptions and 180 min).

Comparison of our routine adsorption method and the proposed LISS allogeneic adsorption method. The results are shown in Table 2. With our routine method, the total number of adsorptions and the completion time were 48 and 1440 minutes, respectively, with a mean of 5.3 adsorptions and 160 minutes per sample. When LISS was added in the adsorption procedures, autoantibodies of all nine patients were removed with a mean of 2.8 adsorptions and 57.7 minutes per sample. Both methods were capable of removing autoantibody. In addition, an alloanti-C and an alloanti-S in Samples 4 and 7 were detected by both methods.

Analysis of the number of adsorptions required to remove autoantibodies with the proposed LISS adsorption procedure (now routinely used in our laboratory). The total number of adsorptions and the assay completion time (for all 50 samples) were 144 and 2880 minutes, respectively (mean per sample, 2.88 adsorptions and 57.6 min) (see Table 1). These results are in agreement with the previous comparative study. In addition, 15 known alloantibodies were detected by this method: some alloantibodies were determined after adsorptions with conventional methods, and others were known in the absence of autoantibodies. Samples containing alloantibodies are listed in Table 3.

Evaluation of the effectiveness of LISS autoadsorption procedure in removing autoantibodies with untreated autologous RBCs

As indicated in Table 1, with untreated autologous RBCs, the LISS autoadsorption procedure required 58 adsorptions and 1160 minutes, respectively (mean per sample, 2.9 adsorptions and 58 minutes). The strength of DATs on the autologous RBCs was graded between 3+ and 4+ by CAT.
Evaluating the effect of the LISS alloadsorption procedure on the activity of alloantibody

As shown in Table 4, similar reactivity was obtained in five sera tested against the same antibody detection RBCs after the third adsorption.

The loss of anti-S and the diminution of strength (1+) of anti-Jk^a in Sera 4 and 8, respectively, seemed not to be due to the dilution effect of the added LISS, as this occurred in all three procedures. The anti-D in Serum 2 became weaker only with the LISS adsorption procedure. This may have been due to dilution because the antibody was only weakly reactive. However, except for a very weak anti-S, all the specificities were recovered.

**DISCUSSION**

The safe transfusion of patients with warm autoimmune hemolytic anemia, who have autoantibodies reacting with all RBCs tested, requires an efficient and time-saving assay to detect alloantibodies underlying autoantibodies.1

Because of the potentiator effect of LISS for antigen-antibody reactivity,7-9 we studied the use of LISS for increasing the efficiency of the adsorption of autoantibodies. As shown in Tables 1 and 2, LISS adsorption was more effective than our routine procedure. Sera from 129 patients with warm autoantibodies were adsorbed with the routine method, sera from 59 patients were adsorbed with the proposed LISS alloadsorption method, and 20 sera were adsorbed with the proposed LISS autoadsorption method. The number of adsorptions for autoantibody removal was reduced from a mean of 5.8 to 2.8 adsorptions, and the completion time was shortened from a mean of 160 to 58 minutes when using the LISS method. There was a 52 percent increase in efficiency in the number of adsorptions and 35 percent decrease in completion time when LISS was used. The LISS autoadsorption method with untreated RBCs and the LISS alloadsorption method with papain-treated RBCs gave similar results. Moreover, after three LISS adsorptions with papain-treated allogeneic RBCs and untreated autologous RBCs, the reactivity of weak alloantibodies tested against the same antibody detection RBCs remained unchanged for 5 sera, and only one very weak specificity (anti-S) disappeared regardless of the procedure used. The recovery of known alloantibodies in 15 samples after alloadsorption with LISS (Table 3) confirmed these results.

The processing time of samples with LISS was longer than that in other techniques. However, this is only an apparent disadvantage,4,5 since this time increase is partly compensated by the absence of the washing phase of IAT with CAT. Hence, the LISS method with CAT can be considered as an efficient tool to adsorb autoantibodies without affecting alloantibodies. Further studies should be performed to complete these results and to propose a standardized protocol for this method.

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**REFERENCES**

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