The use of polyethylene glycol (PEG) to enhance the adsorption of autoantibodies

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The use of polyethylene glycol (PEG) to enhance the adsorption of warm autoantibodies on red blood cells (RBCs) was evaluated in our laboratory in an effort to reduce the time and cost associated with routine differential adsorptions. Sera from 19 patients with warm autoantibodies were tested. Fourteen of these sera contained alloantibodies or additional autoantibody specificities underlying the dominant autoantibody. The sera were differentially adsorbed using equal volumes of serum, reagent RBCs, and PEG for 15 minutes at 37°C. The PEG/serum mixture was harvested and used for testing. Six drops of the PEG/serum mixture were tested against reagent RBCs for 15 minutes at 37°C. An antiglobulin test was then performed using anti-IgG. The PEG adsorption technique took a total of 10 hours to completely eliminate autoantibody reactivity in all 19 samples. The reference method required a total of 59.5 hours to adsorb the autoantibodies in all 19 samples. Two weak alloantibody specificities (anti-K, anti-Jk^b), known to be present in the serum, were not detected in the PEG tests. Four specificities were weaker with the PEG adsorbed serum. All other alloantibody specificities (13) were detected with equal or greater strength in the PEG adsorbed serum. The use of PEG to enhance the adsorption of autoantibodies should be considered as an option to reduce the time and cost of labor-intensive differential adsorptions. Laboratories should be cautioned that weak alloantibodies may not be detected using this method. ***Immunohematology*** 1997;13:119–122.

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Patients with warm autoimmune hemolytic anemia (WAIHA) present a special problem in the blood bank. Broadly reactive autoantibodies can mask the presence of underlying alloantibodies. Removal of autoantibodies often require adsorption using allogeneic, fully phenotyped reagent red blood cells (RBCs). This is a costly and time-consuming process, which can take many hours to complete and may delay transfusion for the patient.

Liew and Duncan[1] outlined a procedure using polyethylene glycol (PEG) for enhanced autoadsorption of these autoantibodies. Champagne and Moulds[2] reported that, using a PEG additive solution, the procedure was effective at removing an autoantibody, but underlying alloantibody reactivity may be diminished using the technique described. Our laboratory evaluated this technique to determine if it could be used to perform differential adsorptions without reducing the detection of underlying alloantibodies.

Materials and Methods

Sera from 15 patients with RBC autoantibodies were initially selected at random to determine the method to be used in this study. We evaluated different volume ratios of serum and PEG used for adsorption, along with different serum to RBC ratios used upon testing the adsorbed serum. It was determined that Method B (the PEG method; see below) gave the best combination of adsorption ability and underlying alloantibody detection when compared to control Method A (the standard method; see below).

Nineteen sera were tested using the techniques outlined in Method B. Sera with known underlying alloantibodies were chosen preferentially, in order to determine the effect of PEG adsorptions on the detection of underlying alloantibodies. These sera had demonstrated autoantibody reactivity in manual tube antiglobulin tests ranging from weakly reactive to 4+ agglutination. Fourteen of the 19 samples contained alloantibody or an additional autoantibody specificity. Samples were first evaluated using Method B. Method A testing was performed by one of six technologists; Method B testing was performed by a single technologist.

Reagent RBCs were prepared in-house from AS-1 Red Blood Cell units. These cells of known phenotype were group O, with Rh phenotypes R_1R_1, R_2R_2, or rr. This set of RBCs was used to perform the adsorptions. Prior to use, aliquots of the RBCs were washed x 4 with 0.9% sodium chloride. After the last wash, the cells were packed and residual saline was removed.

PEG (20% w/v) was prepared in-house. 100 g of PeG (Sigma Chemical Company, St. Louis, MO) was placed in

a 500 mL volumetric flask. Phosphate buffered saline, pH 7.3 (Sigma Chemical Company), was added to the 500 mL mark. The solution was mixed and stored at 4°C.

In Method A, aliquots of the reagent RBCs were treated with papain (American Red Cross, Philadelphia, PA) for 10 minutes at 37°C. The enzyme-treated cells were washed x 4 with 0.9% sodium chloride and packed; residual saline was then removed. To perform the adsorptions, 1 mL of a patient's serum was added to 1 mL of each of the papain-treated RBCs (R_1R_1, R_2R_2, or rr). Each aliquot was incubated in a 37°C water bath for 45–60 minutes, with frequent mixing. The aliquots were then centrifuged, and the adsorbed serum was removed. This procedure was repeated until all autoantibody reactivity was eliminated as measured by testing the adsorbed serum versus the reactive adsorbing RBCs. The adsorbed serum was then ready to test to determine if underlying alloantibodies were present. Two drops of adsorbed serum were tested following a 15-minute incubation at 37°C with selected reagent RBCs, using a LISS enhancement medium (BCA, Durham, NC). All tests were read at the antiglobulin phase, using polyspecific anti-human globulin serum (BCA).

In Method B, aliquots of reagent RBCs were used without enzyme treatment. Equal volumes of washed, packed RBCs, serum, and PEG were combined. The mixture was incubated in a 37°C water bath for 15 minutes. The aliquots were then centrifuged, and the PEG/serum mixture was removed from the packed RBCs. This procedure was repeated until all autoantibody reactivity was eliminated—the additional adsorptions were performed with new aliquots of RBCs—but additional PEG was not added. To perform testing to detect underlying alloantibodies, 6 drops of the PEG/serum mixture was added to 1 drop of selected reagent RBCs. No additional enhancement was added. This mixture was incubated at 37°C for 15 minutes. All tests were read at the antiglobulin phase, using monospecific IgG anti-human globulin serum (BCA).

Results

Table 1 shows the strength of the autoantibody in the 19 serum samples, the number of differential adsorptions necessary to eliminate autoantibody activity using both standard and PEG-enhanced adsorptions, and the total time required for adsorption. Each adsorption using Method A took a total of 85 minutes to complete (10 minutes for treatment with papain, 10 minutes for washing, 60 minutes for incubation time, and 5 minutes to spin and remove the adsorbed serum). Each adsorption using Method B took a total of 20 minutes to complete (15 minutes for incubation time and 5 minutes to spin and remove the adsorbed serum). Samples adsorbed using Method A required an average of 1.9 adsorptions, whereas samples adsorbed using Method B required an average of 1.5 adsorptions. Thus, the average time needed to remove autoantibody activity using Method A was 161.5 minutes (2.7 hours) versus 30 minutes required for Method B.

A total of 13 of the specimens contained underlying alloantibodies or an additional autoantibody specificity. A

Table 1. Autoantibody strength and the number of adsorptions needed

Sample number	Autoantibody strength	Number of adsorptions (Method A)	Time required for completion (85' per adsorption)	Number of adsorptions (Method B)	Time required for completion (20' per adsorption)
1	3+	4	340	2	40
2	4+	2	170	1	20
3	1+	2	170	2	40
4	2+	3	255	2	40
5	3+	3	255	1	20
6	1+	1	85	1	20
7	w+	1	85	1	20
8	2+	4	340	4	80
9	1+	1	85	1	20
10	3+	3	255	2	40
11	1+	3	255	1	20
12	w+	2	170	1	20
13	1+	1	85	1	20
14	w+	1	85	1	20
15	3+	4	340	2	40
16	w+	1	85	1	20
17	1	1	85	1	20
18	w+	1	85	1	20
19	3+	4	340	4	80
Total time required for adsorption		3,570 minutes (59.5 hours)		600 minutes (10 hours)	

* = minutes

Table 2. Alloantibody and additional autoantibody specificity found in 13 of the 19 samples tested and strength of reactivity

Specimen number	Underlying antibody specificity	Strength of reactivity* Standard adsorption	PEG adsorption
1	Anti-C	m+*	1+
	Anti-E	2+	2+
	Anti-Fy^a	1+	1+
	Auto-anti-e	1+	1+
6	Auto-anti-e	w+	1+
7	Anti-K	3+	2+
	Anti-Jk^b	m+	Not detected
9	Anti-E	1+	w+
	Anti-Jk^a	1+	1+
10	Auto-anti-e	1+	2+
11	Anti-D	3+	3+
	Anti-C	3+	3+
	Anti-V	2+	2+
	Anti-Js^a	2+	2+
	Auto-anti-e	w+	Not detected
12	Anti-C	m+	w+
	Anti-K	2+	2+
13	Anti-c	2+	2+
	Anti-E	3+	2+
	Anti-K	w+	Not detected
14	Anti-C	w+	1+
15	Anti-C	1+	1+
	Auto-anti-E	1+	1+
	Auto-anti-e	Not detected	m+
16	Anti-E	2+	3+
18	Anti-c	2+	2+
	Anti-Jk^b	w+	1+
19	Anti-E	2+	2+
	Anti-Fy^b	3+	2+

*m+ = microscopically positive; w+ = weakly positive macroscopically

total of 29 specificities were contained in these specimens. Table 2 lists the antibody specificities and their relative strength using both standard and PEG-enhanced adsorptions. Three antibodies were detected using the standard method that were not detected using the PEG-enhanced method of adsorption. Of these three, one was subsequently shown to be an autoantibody. Thus, two alloantibodies were not detected using the PEG-enhanced adsorptions. One anti-K (in sample 13) reacted weakly in the standard adsorbed serum but was nonreactive with the PEG-adsorbed serum when tested with RBCs presumed to have double (homozygous) as well as single (heterozygous) expression of the K antigen. One anti-Jk^b (sample 7) reacted microscopically positive with Jk(a–b+) RBCs using the standard method; however, it was not detected in the PEG-adsorbed serum. Four antibodies (one each anti-K and anti-Fy^a, and two examples of anti-E) were weaker with the PEG-adsorbed serum. Five specificities showed stronger reactions in the PEG-adsorbed serum (one each anti-E and anti-Jk^b, and three examples of anti-C). One example of autoanti-e was detected in the PEG-adsorbed serum that was not detected by the standard method (sample 15).

Discussion

PEG has been shown to be a valuable enhancement media to aid in the detection and identification of alloantibodies. PEG potentiates antigen/antibody reactions by increasing the formation of immune complexes through steric exclusion.[3] Antibody reactions are often stronger and titration scores higher when PEG is used as compared to LISS and Polybrene enhancement.[4] PEG is also an excellent potentiator of autoantibodies, which is one of the biggest disadvantages of the media in routine use. However, the enhancement of autoantibodies by PEG can be used to minimize the length and number of adsorptions used to remove autoantibody reactivity from serum.

The addition of PEG greatly enhanced the speed at which an autoantibody could be adsorbed from serum. For this study, a time savings of 49.5 hours was noted when using the PEG technique versus the routine adsorption method (see Table 1). Although we did not specifically address cost issues, the decrease in the time used to perform adsorptions would lead to a more efficient and cost-effective operation, and ultimately may provide more timely transfusions to the patient.

It does appear that the reactivity of very weak alloantibodies may be lost when performing these adsorptions. The use of 6 drops of the PEG/serum mixture should theoretically minimize the dilutionary effects of using the modified procedure; however, some antibodies still showed decreased reactivity. These diminished reactions may be due to nonspecific binding of immunoglobulin promoted by the PEG enhancement. However, at this time, the mechanism for this phenomenon is unclear. Other examples of similar specificities showed increased reactivity (see Table 2).

The use of PEG to enhance adsorption of autoantibodies should be considered as an option in order to reduce the time and cost of differential adsorptions. However, some weak alloantibodies may not be detected using the PEG method. If nondetection is suspected, the patient's serum should be evaluated using standard procedures.

References

1. Liew YW, Duncan N. Polyethylene glycol in autoadsorption of serum for detection of alloantibodies (letter). Transfusion 1995;35:713.
2. Champagne KJ, Moulds M. Autoadsorptions for the detection of alloantibodies—should polyethylene

glycol be used? (letter). Transfusion 1996;36:384.
3. Wenz B, Apuzzo J. Polyethylene glycol improves the indirect antiglobulin test. Transfusion 1989;29:218–20.
4. Nance SJ, Garratty G. A new potentiator of red blood cell antigen-antibody reactions. Am J Clin Pathol 1987;87:633–5.

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