Polyethylene glycol–coated red blood cells fail to bind glycophorin A–specific antibodies and are impervious to invasion by the *Plasmodium falciparum* malaria parasite


This study was designed to assess the binding of glycophorin A–specific antibodies to polyethylene glycol (PEG)-modified red blood cells (RBCs) and evaluate their resistance to invasion by *Plasmodium falciparum* malaria parasites. RBCs were conjugated with a range of concentrations (0.05 to 7.5 mM) of activated PEG derivatives of either 3.35 or 18.5 kd molecular mass. The binding of glycophorin A–specific antibodies was assessed by hemagglutination and flow cytometry. PEG-modified RBCs were assessed for their ability to form rosettes around Chinese hamster ovary (CHO) cells transiently expressing the glycophorin A binding domain of EBA-175, a *P falciparum* ligand crucial to RBC invasion. PEG-RBCs were also tested for their ability to be invaded by the malaria parasite. RBCs coated with 3.35 and 18.5 kd PEG demonstrated a dose-dependent inhibition of glycophorin A–specific antibody binding, CHO cell rosetting, and *P falciparum* invasion. These results indicate that glycophorin A epitopes responsible for antibody and parasite binding are concealed by PEG coating, rendering these cells resistant to *P falciparum* invasion. These studies confirm the effectiveness of PEG modification for masking RBC-surface glycoproteins. This may provide a means to prevent alloimmunization in the setting of RBC transfusion and suggests a novel method to enhance the effectiveness of exchange transfusion for the treatment of cerebral malaria.

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Introduction

The red blood cell (RBC) membrane is architecturally complex and is characterized by significant biochemical diversity. Protein-, lipoprotein-, glycoprotein-, and carbohydrate-rich structures play an important role in ensuring the physical integrity of the cell and carrying out its physiologic functions. However, the presence of these diverse RBC-surface molecules can also have adverse consequences. Several of these structures carry defined polymorphic epitopes, recognized serologically as blood group antigens, which can stimulate alloimmune responses (alloimmunization) after red blood cell transfusion or pregnancy. A second exposure to the same antigen may lead to serious clinical consequences, including hemolytic transfusion reactions and hemolytic disease of the newborn. Alloimmunization is particularly problematic in patient groups who receive multiple transfusions. For example, up to 30% of chronically transfused patients with sickle cell disease become sensitized to one or more blood group antigens.

Aside from alloimmunization, a more recently recognized consequence of the presence of diverse molecules on the surface of RBCs is that microorganisms have adapted to use certain ones as “Docking points” in the initiation of disease. As an example, parvovirus B19 (a prominent cause of pure red cell aplasia) homes to erythroid precursors in the marrow through an interaction with the P blood group antigen. In addition, the Duffy and glycophorin A blood group glycoproteins have been shown to play important roles as receptors for human malaria parasites. *Plasmodium vivax* has an absolute requirement for a Duffy protein interaction in order to successfully invade and parasitize human RBCs. Consequently, red cells of the Fya<sup>a</sup>-b<sup>b</sup> phenotype are resistant to invasion. *Plasmodium falciparum* malaria parasites use glycophorin A epitopes to achieve the efficient invasion of RBCs. The essential biochemistry of the complex invasion process of this parasite is not as well understood as that for *P vivax*. However, the gene encoding an important glycophorin A binding ligand of *P falciparum*, EBA-175, has been cloned and is being intensively studied.

There are circumstances in which it would be desirable to remove some of the antigenic epitopes or potential binding sites from the RBC surface, either to prevent alloimmunization or make RBCs resistant to microbial attack. Enzymatic removal of specific protein or carbohydrate structures is one possible approach. By using a specific exoglycosidase, this technique has been used successfully to cleave the B blood group antigen, allowing for the conversion of group B RBCs to group O before transfusion. However, this general approach is limited by the availability of enzymes of the required specificities. Of the more than 200 known blood group antigens, B is the only antigen that has been specifically and effectively removed enzymatically. Although other less-specific proteases and glycosidases will certainly remove many antigens, they will also compromise the structural and functional integrity of the RBCs. For example, it is a simple matter...
RBCs are resistant to invasion by PEG modification of the RBC membrane and that these modified parasitic ligand, EBA-175, with PEG-modified RBCs was also evaluated. The interaction of the malaria parasite or a specific antigens was assessed by hemagglutination and flow cytometric methods. The advantage of PEG coating, in contrast to the enzymatic approach, is that a single treatment will simultaneously mask most, and possibly all, RBC-surface molecules and their associated blood group antigens or attachment sites.

We undertook the current study to evaluate the effect of PEG modification of RBCs on a specific blood group carrying glycoprotein, glycophorin A. This is an important molecule in transfusion medicine, as the alloimmune response to antigens carried by this protein has been implicated in hemolytic transfusion reactions, but of potentially greater clinical importance is the fact that glycophorin A serves as a critical receptor for RBC invasion by \textit{P falciparum}, a major global cause of morbidity and mortality.

In this study, RBCs were PEG coated with a range of concentrations of activated PEG derivatives of either 3.35 or 18.5 kd molecular mass. The binding of glycophorin A–specific antibodies was assessed by hemagglutination and flow cytometric methodologies. The interaction of the malaria parasite or a specific parasitic ligand, EBA-175, with PEG-modified RBCs was also assessed. Our findings indicate that glycophorin A epitopes responsible for antibody and parasite binding are effectively concealed by PEG modification of the RBC membrane and that these modified RBCs are resistant to invasion by \textit{P falciparum} malaria parasites.

Materials and methods

Preparation of dichloro-s-triazine difunctional derivatives of polyethylene glycol

Derivatization of either 3.35 kd molecular mass PEG (Sigma Chemical Company, St Louis, MO) or 18.5 kd molecular mass PEG (Polysciences, Warrington, PA) with cyanuric chloride (2,4,6-trichloro-1,3,5-triazine; Sigma Chemical Company) was performed using a modification of the method of Abuchowski et al. The time of syntheses for the 3.35 kd dichlorotriazine (DT) and 18.5 kd DT PEG derivatives was 24 and 72 hours, respectively. The reactive polymers were precipitated from the reaction mixture, and unreacted cyanuric chloride was removed by repeated washing with cyclohexane.

Polyethylene glycol coating of red blood cells

Whole blood, anticoagulated with EDTA, was washed twice with Dulbecco phosphate-buffered saline (PBS) at 1750g for 10 minutes. The RBCs were resuspended to a 10% hematocrit in triethanolamine buffer (30 mM triethanolamine, 110 mM NaCl, 4 mM KCl, 5 mM D-glucose, 0.25% human serum albumin, pH 8.6, 270 mM osm/kg). Fresh stock solutions of the PEG-DT derivatives were prepared at a concentration of 100 mg/mL in cold 50 mM hypotonic phosphate buffer (pH 5.5, 220 mM osm/kg) approximately 1 to 2 minutes before the addition of blood. Various volumes of the reactive PEG stock were added to 1 mL blood samples and immediately mixed to yield the additions of 1 to 25 mg of reactive polymer. The samples were placed on a rocker and incubated at room temperature for 60 minutes. After 2 washes (PBS at 200g for 10 minutes), the RBCs were resuspended in PBS containing 5 mM D-glucose to a final hematocrit of 10% and stored at 4°C before use.

Hemagglutination studies

PEG-modified RBCs were assessed for concealment of glycophorin A epitopes through hemagglutination studies using a high-titer murine monoclonal antibody, 6A7, which recognizes the M-allele of glycophorin A. An irrelevant murine monoclonal antibody, L.5.1 (recognizes the human transferrin receptor), was also used as a control to assess for nonspecific agglutination. Each reaction was undertaken in a borosilicate test tube containing 50 μL of a 5% suspension of RBCs and 100 μL of antibody. After a 15-minute incubation at room temperature, the tubes were centrifuged for 15 seconds at 1500g, and agglutination was assessed. The relative strengths of agglutination were scored, as previously described.

Untreated RBCs and sham-treated RBCs (same conditions used with PEG derivatization but with no PEG added) were used as controls. Each antibody was assessed with PEG-modified RBCs (3.35 kd and 18.5 kd) treated with varying concentrations of the PEG-DT derivatives (0.05 to 7.5 mM).

Flow cytometry

Aliquots (40 μL) of fluorescein-isothiocyanate–labeled mouse anti-human glycophorin A IgG1 (FITC–anti-GpA; purchased from Immunotech, Westbrook, ME) prediluted 1:10 with PBS were added to 1 mL aliquots of blood at a 0.01% hematocrit. The samples were incubated for 30 minutes at room temperature in the dark with gentle mixing, centrifuged, and washed twice with PBS at 500g for 2 minutes. They were then resuspended with 1 mL of PBS. The fluorescence signal of the FITC–anti-GpA–labeled cells was measured using a FACSCAN flow cytometer (Becton Dickinson, San Jose, CA). Ten thousand cells were counted from each sample. Appropriate gating was established using FITC–anti-GpA–labeled uncoated RBCs as a positive control.

Red blood cell rosetting assay

The Lec 2 variant of Chinese hamster ovary (CHO) cells was transiently transfected using lipofectamine (Gibco BRL, Gaithersburg, MD) according to the manufacturer’s recommendations. A plasmid encoding the glycophorin A binding domain of malarial protein EBA-175 (a generous gift from Dr K. L. Sim, EntreMed, Inc, Rockville, MD) was introduced by this method. Lec 2 CHO cells have a metabolic defect in the ability to add sialic acid residues to proteins and were chosen for these studies because of their enhanced ability to promote RBC rosette formation in comparison to wild-type CHO cells (D.P.B., unpublished data, February 1998). Forty-eight hours after transfection, RBCs were prepared for the rosetting assay. Untreated, sham-treated, and PEG-modified RBCs were washed twice in PBS and resuspended to a hematocrit of 1%. One milliliter of the appropriate suspension was then applied to a 60-mm transfection plate. The RBCs were incubated for 2 hours at 37°C in a humidified 5% CO2 incubator. After incubation, the CHO cells were washed gently with PBS to remove unbound RBCs. Each plate was viewed microscopically under low power. Rosettes were counted on 50 consecutive microscopic fields.

Malaria reinvasion assay

The FCR-3 strain of \textit{P falciparum} was a gift from Dr H. Rubin (University of Pennsylvania School of Medicine, Philadelphia, PA). The parasite was cultured in RPMI-1640 media (Gibco BRL) supplemented with 10% human serum, 20 mM D-(+)-glucose, 25 mM HEPES, 50 μg/mL hypoxanthine, and 40 μg/mL gentamycin, as described. Parasites were synchronized to the ring-stage of development by using a 5% D-sorbitol solution. After 36 hours in culture, schizont-infected RBCs were isolated by banding on a 60% Percoll cushion. A parasitemia of 50% schizonts was routinely achievable by this method. After washing with unsupplemented media, the parasites were diluted with untreated, sham-treated, or PEG-modified RBCs to a parasitemia of approximately 2%. Individual cultures of the various RBCs and parasites were maintained in 35-mm petri dishes (5% final hematocrit in complete media). After a 16-hour incubation,
Giemsa-stained thin smears were made from each dish, and ring-form parasites were counted. A percentage parasitemia was established for each reinvaded red cell type, based on the ring-forms seen in 1000 RBCs.

**Results**

RBCs that underwent PEG modification were evaluated for concealment of glycophorin A epitopes by a test tube-based hemagglutination assay. RBCs treated with increasing concentrations of either 3.35 kd or 18.5 kd PEG derivatives exhibited a dose-dependent decrease in the strength of hemagglutination (Table 1). A well-characterized, high-titer murine monoclonal antibody (6A7) with specificity for human glycophorin A was used in these studies. This antibody recognizes a glycopeptide epitope encoding the M-allele of glycophorin A. It is sialic acid dependent and has an absolute requirement for a glycine residue at amino acid position 5 of the glycophorin A molecule. An irrelevant murine monoclonal antibody (L5.1), which recognizes the human transferrin receptor, was studied in parallel as a negative control for hemagglutination.

To further assess glycophorin A epitope exposure after PEG modification, antibody binding studies were performed. Control and PEG-modified RBCs were assayed by flow cytometry using a FITC-labeled murine anti-human glycophorin A antibody. There is a dose-dependent inhibition of antibody binding to RBCs treated with varying concentrations of both the 3.35 kd PEG derivative and the 18.5 kd PEG derivative (Figure 1). Error bars indicate the standard deviation of 3 determinations. Percentage inhibition of binding was determined by comparing peak mean fluorescence of PEG-modified RBCs to control RBCs as follows: Control RBCs – PEG RBCs/Control RBCs × 100.

![Figure 1. PEG-modified RBCs are resistant to antibody binding.](image1)

**Table 1. Effect of PEG derivitization on red blood cell agglutination**

<table>
<thead>
<tr>
<th>PEG concentration (mg/mL)</th>
<th>3.35 kd derivative</th>
<th>18.5 kd derivative</th>
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<tr>
<td>0</td>
<td>4+</td>
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<td>1</td>
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PEG = polyethylene glycol; RBCs = red blood cells; Neg = negative (no macroscopic agglutination). RBCs expressing the M blood group antigen were derivatized with 0 to 25 mg/mL of either 3.35 kd or 18.5 kd dichlorotriazine-activated PEG. This was followed by agglutination studies using murine monoclonal antibody 6A7 (anti-M). Agglutination reactions were scored according to the method of Marsh.

Figure 2. RBCs bind to CHO cells expressing a *P falciparum* malarial protein. CHO cells were transiently transfected with the glycophorin A binding domain of the *P falciparum* protein ligand, EBA-175. Control and PEG-modified RBCs were evaluated for their ability to form rosettes with CHO cells transiently expressing the glycophorin A binding domain of the EBA-175 malarial protein. An example of rosette formation is shown in Figure 2, and Figure 3 demonstrates a dose-dependent inhibition of rosette formation. This effect was seen with both the 3.35 kd PEG derivative and the 18.5 kd PEG derivative. However, as with the data obtained by flow cytometry (Figure 1), the 18.5 kd PEG derivative appeared to more effectively mask the glycophorin A epitopes responsible for anti-M antibody binding than the 3.5 kd PEG derivative.

After completion of the hemagglutination and glycophorin A-specific antibody binding studies, PEG-RBCs were evaluated for their ability to form rosettes with CHO cells transiently expressing the glycophorin A binding domain of the EBA-175 malarial protein. EBA-175 is a well-characterized malarial ligand that is essential for efficient parasite invasion of RBCs. This large protein has specificity for glycophorin A, and the specific domain responsible for high-affinity binding has been identified. "EBA-175 is known to bind glycophorin A in a sialic acid-dependent manner, but recent evidence suggests that the amino acid backbone of glycophorin A is also required for binding. Untreated and PEG-modified RBCs were evaluated for their ability to bind a P falciparum malarial protein, EBA-175. To further assess glycophorin A epitope exposure after PEG modification, antibody binding studies were performed. Control and PEG-modified RBCs were assayed by flow cytometry using a FITC-labeled murine antihuman glycophorin A antibody. There is a dose-dependent inhibition of antibody binding to RBCs treated with varying concentrations of both the 3.35 kd PEG derivative and the 18.5 kd PEG derivative (Figure 1). Error bars indicate the standard deviation of 3 determinations. Percentage inhibition of binding was determined by comparing peak mean fluorescence of PEG-modified RBCs to control RBCs as follows: Control RBCs – PEG RBCs/Control RBCs × 100.

Finally, PEG-derivatized RBCs were assessed for their ability to be invaded by malaria parasites in the context of an RBC reinvasion assay. This assay assesses the ability of the merozoite form of the organism to invade RBCs. Late-stage *P falciparum*...
schizonts were isolated and diluted with untreated, sham-treated, or PEG-modified RBCs. After a 16-hour incubation, during which time-free merozoites are released from schizont-infected RBCs, ring-stage parasites are counted on a thin smear. As shown in Figure 4, both PEG derivatives were associated with a dose-dependent inhibition of RBC invasion in comparison to control RBCs, with almost complete inhibition seen at the highest PEG concentration. Of note, there was no difference in malaria invasion efficiency when untreated RBCs and sham-treated RBCs were compared (data not shown).

Discussion

PEG is a nonionic, biocompatible, strongly hydrophilic polymer, which has a large exclusion volume in aqueous solution. More than 20 years ago, Abuchowski et al developed a technique to modify proteins by the covalent attachment of PEG. They showed that PEG-modified bovine albumin was less immunogenic than its unmodified counterpart and had a prolonged circulation time in rats that had been presensitized with unmodified bovine albumin. The covalent attachment of PEG is now commonly used to modify a variety of proteins, enzymes, drugs, and artificial surfaces. As examples, PEG-adenosine deaminase (PEG-ADA) is used for the treatment of severe combined immunodeficiency syndrome. PEG-modified hemoglobin is being developed for use as a blood substitute, and PEG-modified cytokines (eg, interleukin 2 [IL-2] and granulocyte-macrophage colony-stimulating factor [GM-CSF]) are being evaluated to provide for a longer time-free merozoites are released from schizont-infected RBCs.

Despite these varied applications, RBCs have only recently been considered for use as substrates for PEG modification. When bound to the RBC surface, the strongly hydrated PEG molecules create a steric barrier that prevents large molecules (eg, plasma proteins) from reaching the RBC surface. However, small molecules (sugars, amino acids) and dissolved gases can freely diffuse through the PEG-water barrier, allowing for the preservation of RBC function and viability. Studies by ourselves and other investigators have shown that PEG coating can successfully conceal blood group antigens from specific antibodies with no apparent adverse consequences for RBC function or in vivo survival.

The ability to camouflage RBC-surface proteins and glycoproteins prompted us to investigate whether PEG coating could protect RBCs from invasion by an intraerythrocytic parasite such as malaria. P falciparum was chosen as a model for addressing this question because of the significance of the clinical disease that it causes on a worldwide basis (estimated 2 000 000 deaths annually), and because it is readily adaptable to in vitro culture and experimental manipulation. The successful invasion of RBCs by P falciparum requires a complicated coordination of multiple receptor-ligand interactions across the red cell membrane. Although the precise biochemistry of these molecular interactions is not well understood, glycophorin A, a heavily sialylated glycoprotein, is an important early receptor in the invasion process. The malarial protein ligand for glycophorin A, EBA-175, is a well-studied sialic acid–dependent, cysteine-rich protein critical for efficient invasion by the parasite. Our data show that glycophorin A epitopes responsible for antibody and P falciparum binding are effectively blocked by PEG modification of the RBC membrane. This blockade occurred in a dose-dependent manner with PEG derivatives of 2 different molecular masses (3.35 kd and 18.5 kd). The exact mechanism for this blockade is not known, but it is presumed that the PEG molecules sterically block the antigenic epitopes, thereby preventing antibody binding. In a similar manner, the parasite’s specific adhesion ligands are unable to bind glycophorin A, and possibly other RBC-surface glycoproteins, because the recognition sites on these molecules are also obstructed by the bulky PEG molecules.

In the antibody binding, red blood cell rosetting and malaria reinvasion assays, the 18.5 kd PEG derivative demonstrated a relative potency of about 2.5 times that of the 3.35 kd derivative. This strongly suggests that the larger PEG derivative more effectively masked relevant glycophorin A epitopes. This conclusion rests on the assumption that similar numbers of molecules of...
each PEG derivative are bound to the RBC surface under the same conditions. If there was any difference, it is more likely that the smaller PEG derivative would bind more efficiently under the same conditions, simply by virtue of faster reaction kinetics. However, studies that use radiolabeled PEG derivatives will be necessary to completely resolve this issue.

For the hemagglutination studies, the relative effectiveness of the larger PEG derivative appeared to be much more than 2.5-fold. We speculate that the higher molecular mass PEG derivative may be large enough to sterically prevent the close approximation of adjacent RBC surfaces, particularly as PEG coating is present on both surfaces. Close contact between adjacent RBCs is a prerequisite for effective antibody cross-linking and hemagglutination. It is thus possible that the hemagglutination assay amplifies the apparent effectiveness of the larger PEG derivative.

The ability to covalently couple PEG to the RBC membrane and the consequent success at preventing antibody and malarial protein binding and parasite invasion suggest that PEG-modified RBCs may have a useful place in clinical medicine. If the immunogenic portions of proteins and carbohydrates defining human blood group antigens could be concealed successfully, this could provide a novel method to prevent alloimmunization (and its attendant complications) in heavily transfused, high-risk patient populations. A prime example is the patient with sickle cell disease. These patients, as a group, are heavily transfused and have a higher rate of alloimmunization (as high as 30% in some studies) than other transfused patients.7

An additional potential benefit of the use of PEG-modified RBCs is that PEG reduces or abolishes RBC-RBC aggregation, which results in a dramatic reduction of blood viscosity at low shear rates.38 In patients with sickle cell disease, this effect could significantly enhance the benefits of transfusion for both the management of acute complications (eg, acute chest syndrome) and chronic transfusion therapy. Finally, PEG-modified RBCs may also prove to be useful in those patients already sensitized to a blood group antigen or antigens in whom it may be difficult or impossible to find compatible units of blood, particularly in emergent situations. This same benefit could extend to those patients with red cell autoantibodies who are actively hemolyzing and clinically symptomatic. The ability to provide a blood product that is null or neutral with respect to blood group antigen composition and cell-surface molecular phenotype could provide for improved survival of red blood cells and the possibility of improved clinical outcome.

In addition to improved transfusion safety, PEG modification of the RBC membrane may represent a novel method for the treatment and/or prevention of human malaria. Malaria incurs its greatest toll on those with minimal exposure to the parasite and who have poorly developed immunity. In malaria endemic areas, children younger than age 4 are at greatest risk of death, secondary to either overwhelming parasitemias or end-organ damage (eg, cerebral malaria).37 Travelers to malarious areas are also at increased risk of severe infection.38 Exchange transfusion has proven to be beneficial as a supportive measure in those with clinically overwhelming malaria infections.39 The ability to exchange parasitized RBCs with PEG-modified RBCs may provide an additional important benefit, as PEG-coated RBCs, unlike normal unmodified RBCs, would be impervious to further invasion by the malaria parasite. Red blood cell exchange with PEG-modified RBCs would have the dual effect of acutely lowering dangerously high parasitemias and keeping the parasitemia at lower levels. As with the ischemic end-organ damage that may ensue with sickle cell disease, the same can be seen with severe \textit{P falciparum} infections. PEG modification may also have the benefit of reducing blood viscosity and improving flow to ischemic tissues. As a strategy to actually prevent malaria infection, however, a method for the in vivo modification of RBCs would likely have to be developed.

Our in vitro data suggest that PEG-coated RBCs could be useful in selected clinical situations. However, any benefits will only be realized if the PEG-coating technique also proves to be effective and safe in vivo. On the basis of existing experience with the administration of PEG in other applications, there is good reason for optimism in this regard. Free (ie, unconjugated) PEG has not been shown to have significant toxicity when administered intravenously or subcutaneously.15,40 PEG-conjugated enzymes have been approved for clinical use and appear to be safe. For example, clinical trials, followed by several years' clinical experience with PEG-ADA and PEG-asparaginase, have not shown any toxicity because of the PEG component.15,31,41

The amount of PEG that would be transfused with PEG-RBCs is relatively modest. We have estimated (by gel permeation chromatography of the supernatant) that less than 10% of the PEG in the incubation medium actually attaches to the RBC surface (unpublished results). This would correspond to an exposure of approximately 1 g of PEG per unit of transfused packed red blood cells previously incubated in 20 mg/mL PEG-DT. It is difficult to make a direct comparison with PEG-conjugated enzymes because the amount of PEG contained in these preparations is not known; literature values only state the dosage in units of enzyme activity without noting the specific activity or stoichiometry of the PEG conjugation. However, on the basis of the number of available sites for PEG attachment, it may be roughly estimated that each dose of PEG-ADA contains somewhere from a few tens of milligrams to 100 mg of PEG. Thus, the amount of PEG administered with each unit of PEG-RBCs would be greater than with a single dose of PEG-ADA, but lower than the total exposure to PEG during long-term, weekly PEG-ADA therapy.

It is noteworthy that PEG-hemoglobin–based blood substitutes would expose the recipient to substantially greater amounts of PEG than would PEG-RBCs. A PEG-modified bovine hemoglobin currently in development (PEG-Hb) has approximately 12 molecules of 5 kD PEG bound to each hemoglobin molecule.53 Assuming a hemoglobin concentration of 65 g per unit, each unit of PEG-Hb would contain about 60 g of conjugated PEG. Nevertheless, even with this high PEG content, preclinical studies in animal models have shown no evidence of significant toxicity that can be ascribed to the PEG component.33 The only abnormality demonstrated was vacuolation in the renal proximal tubule cells after PEG-Hb administration in rats. However, this histologic change was not associated with any apparent impairment of renal function.33

Most PEG-conjugated proteins are, by design, too large to be filtered by the glomerulus and are presumably removed from the circulation via pinocytosis by cells of the mononuclear phagocytic system (MPS). Unfortunately, little is presently known about the disposition of the PEG that is taken up by these cells, although there is no evidence that it accumulates in the body. PEG-coated liposomes are cleared by macrophages via phagocytosis.37,42 It therefore seems reasonable to assume that PEG-RBCs would also be cleared by phagocytosis through cells of the MPS, after which the PEG-modified surface proteins would be handled in the same manner as any other PEG-conjugated protein (with no adverse consequences).

Apart from the PEG component, the other potential source of
REFERENCES


Acknowledgments

We thank Dr Dennis Carrigan and Ms Moira Donnell of the University of Tennessee, Memphis, for expert technical assistance. We also thank Dr George Garratty of the American Red Cross, Southern California Region, for helpful advice and suggestions.
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