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Use of heteropolymeric monoclonal antibodies to attach antigens to the C3b receptor of human erythrocytes: A potential therapeutic treatment

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ABSTRACT **We have prepared bispecific, cross-linked monoclonal antibodies (heteropolymers) with specificity for both targeted antigens and the human erythrocyte (RBC) complement receptor. These heteropolymers facilitate binding of target antigens (human IgG and dinitrophenylated bovine γ globulin) to human RBCs under conditions that either allow or preclude complement activation. Quantitative analyses of this binding agree well with the number of complement receptors per RBC. *In vitro* "whole-blood" model experiments indicate heteropolymer-facilitated binding of antigens to RBCs is rapid and stable at 37°C. It may be possible to extend these prototype experiments to the *in vivo* situation and use heteropolymer-attached RBCs for the safe and rapid binding, neutralization, and removal from the circulation of pathogenic antigens associated with infectious disease.**

In 1953 Nelson demonstrated many details of the immune adherence phenomenon (1, 2), which involves binding of complement-opsonized antibody/particulate antigen immune complexes (IC) to human erythrocytes (RBCs) through a receptor (CR1) (3) specific for C3b, C3bi, and C4b (3, 4). He provided intriguing evidence that this reaction plays an important role in the body's defense against microorganisms by facilitating their phagocytosis after adherence and immobilization on RBCs. Recent studies in the nonhuman primate by Hebert and coworkers (5, 6) as well as reports from other laboratories (7–9) indicate that immune adherence of soluble complement-opsonized IC to RBCs via CR1 provides a vehicle for safe and rapid clearance of potentially pathogenic IC from the circulation. Defects in this RBC-based clearance reaction have been shown in a number of diseases (10–12) and are believed to presage disease activity, such as enhanced deposition of inflammatory IC in susceptible tissues and organs (13).

For RBCs to function in either immunological role (enhancement of phagocytosis or IC transport), the "target" antigen or IC must be opsonized with C3b (14, 15) to allow recognition and binding by CR1. If targeted antigens could be bound to RBCs via CR1 in the absence of complement, it would be possible to "use" RBCs therapeutically in a more general capacity in immunological defense. Toward this goal we have prepared bispecific, cross-linked monoclonal antibody (mAb) heteropolymers with specificity for CR1 and target antigen. We describe *in vitro* prototype experiments using heteropolymers to promote binding of antigens to RBC CR1 in the absence of complement activation.

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MATERIALS AND METHODS

mAbs and Heteropolymer Preparation. mAbs to CR1 (12, 16, 17), human IgG (14), and the dinitrophenyl (DNP) group (6) were purified from ascites fluid by treatment with octanoic acid and precipitation with 50% saturated ammonium sulfate (14) or by affinity chromatography on Sepharose-linked protein G (Pharmacia LKB). Cross-linked heteropolymers were prepared using *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (18–20). A 10% excess of anti-ligand mAb was complexed with anti-CR1 mAb. SDS/PAGE analyses and gel-permeation chromatography indicated that ≈50% of the proteins were polymerized (molecular mass of 320 kDa or greater). Heteropolymers analyzed under reducing conditions revealed bands at 50 kDa and 25 kDa, in agreement with reports (19) using SPDP. Several heteropolymer mixtures were purified by gel-permeation chromatography on an Ultrogel AcA 34 column (20), and the highest molecular mass material (corresponding to trimers and larger species) was used in the studies reported here.

Antigens. Dinitrophenylated bovine γ globulin [$(DNP)_{55}$-BGG] was prepared and characterized according to Eisen and coworkers (21). Human IgG (Sigma), $(DNP)_{55}$-BGG, and two heteropolymers were iodinated with Iodo-Gen (22) to specific activities of 200–1000 cpm/ng. Human IgG and a mouse mAb to human IgM (15) were individually bound to Sepharose 4B by using cyanogen bromide (23). The latter protein was dinitrophenylated after coupling.

RBCs. Blood was obtained from normal volunteers, and RBCs were isolated by standard procedures (16) and reconstituted in 1% bovine serum albumin/phosphate-buffered saline, pH 7.4 (BSA/PBS). "Whole-blood" experiments were done in freshly drawn blood anti-coagulated with EDTA or citrate. Alternatively, blood was drawn into Alsever's solution (15) and used immediately or within 2 days. Assays for CR1 levels on isolated RBCs followed standard RIA methods (11, 16) and revealed 200–400 epitopes per RBC, as defined by anti-CR1 mAbs 1B4, 3D9, and HB8592.

RIA. Binding of ^{125}I-labeled antigens to RBCs "franked" (18) with heteropolymers was determined as follows:

Direct sensitization and binding isotherm analyses. Between 0.1 and 1.0 ml of a 10–50% dispersion of washed RBCs (in BSA/PBS) were treated for 1 hr at room temperature, with shaking, with 10–50 μl of a dilution of one or more of the heteropolymers. The RBCs were washed three times (to remove unbound heteropolymer) and, after reconstitution in

Abbreviations: RBC, erythrocyte; BGG, bovine γ globulin; $(DNP)_{55}$-BGG, dinitrophenylated BGG; BSA/PBS, 1% bovine serum albumin/phosphate-buffered saline, pH 7.4; CR1, complement receptor type 1 (with specificity for C3b, C3bi, and C4b); DNP, dinitrophenyl; IC, immune complexes; mAb, monoclonal antibody; SPDP, *N*-succinimidyl-3-(2-pyridyldithio)propionate; HIV, human immunodeficiency virus.

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BSA/PBS or normal human serum (for experiments with ^{125}I-labeled human IgG and $(DNP)_{55}$-BGG, respectively), mixed with a small volume of ^{125}I probe. After another incubation (1 hr at room temperature, with shaking), RBC-bound and free ^{125}I-labeled antigens were separated by either of two procedures: RBCs were spun through oil (16) or washed. RBC-associated ^{125}I counts were measured in a Beckman 5500 γ counter.

"Whole-blood" binding kinetics. (*i*) In most procedures blood was drawn into Alsever's solution and centrifuged. Some supernatant was removed, and after blood cells were redispersed to a hematocrit of 50%, ^{125}I-labeled $(DNP)_{55}$-BGG antigen was added. Various amounts of heteropolymer were added to these whole-blood dispersions containing ^{125}I-labeled $(DNP)_{55}$-BGG and incubated with shaking at 37°C. RBC-associated ^{125}I counts were determined at various times after centrifugation. Some reaction mixtures were centrifuged through Percoll (5, 8) to confirm that only RBCs bound the ^{125}I-labeled antigen. In some whole-blood experiments, blood was drawn into 0.01 M EDTA or 0.1 M citrate and used at once. Alternatively, washed human RBCs were redispersed in fresh homologous serum as a source of complement (11). Comparable whole-blood experiments used ^{125}I-labeled human IgG as target antigen, except RBCs were dispersed in BSA/PBS to avoid competition due to serum-associated IgG. (*ii*) In other kinetic experiments 1 vol of RBCs was franked with saturating amounts of heteropolymer and after washing was added to 10 vol of anti-coagulated blood containing ^{125}I-labeled $(DNP)_{55}$-BGG and incubated at 37°C. Aliquots of the dispersions were processed to determine RBC-associated ^{125}I counts.

Direct binding of ^{125}I-labeled heteropolymers to a number of matrices was determined: Duplicate aliquots of 100 μl of ^{125}I-labeled heteropolymer 4 (see below) were incubated for 1 hr at room temperature, with shaking, with 100 μl of a 50% dispersion of human RBCs or 100 μl of a 33% dispersion of human IgG-Sepharose. Samples were washed twice, and matrix-bound ^{125}I counts measured. Direct binding to human RBCs of ^{125}I-labeled heteropolymers was determined as a function of time at 37°C. Control experiments verified the requirement for CR1. These experiments included use of heteropolymer-treated sheep RBCs [which lack CR1 (16)], untreated human RBCs, and excess homologous monomeric mAbs (in ascites fluid) to block action of the heteropolymers.

RESULTS

Preparation and Initial Characterizations of Heteropolymers. We prepared heteropolymers by SPDP cross-linking and examined their abilities to react with washed human RBCs and facilitate antigen binding (Tables 1 and 2). Use of saturating amounts of unfractionated material (containing heteropolymers and non-cross-linked monomers) demonstrated specific RBC-associated binding of ^{125}I-labeled antigens. An excess of ^{125}I-labeled antigen was used to determine the maximum number of ligands bound per RBC. For each heteropolymer the results agree with the number of CR1 epitopes recognized by anti-CR1 mAbs.

Heteropolymer mixture 1 can facilitate binding via two noncompeting mAbs to CR1: 1B4 and HB8592. This mixture can place approximately twice as many anti-IgG heteropolymers on the RBCs as a heteropolymer containing one anti-CR1 mAb. The maximum ^{125}I-labeled human IgG bound to such doubly franked RBCs is nearly equal to the sum of the ^{125}I-labeled IgG bound to RBCs franked with individual components of the mixture (Table 2); this situation illustrates the principle of additivity. Dose–response experiments with heteropolymers (Table 2, and see below) confirm that binding of heteropolymer and antigen is saturable. "Background" binding of antigen to naive RBCs is low, and use of heteropolymers with "irrelevant" specificities for the target ligands [e.g., 8E11 (anti-C3b) × HB8592] gave no binding. Finally, the multivalent nature of the $(DNP)_{55}$-BGG may explain why less binding is seen for this protein than for human IgG (Table 1). The $(DNP)_{55}$-BGG may engage several anti-DNP mAbs on the RBC, and this would lead to a lower number of molecules bound compared with the monovalent (14) HB43–IgG system.

Table 1. Survey of cross-linked mAb heteropolymer mixtures in facilitating antigen binding to washed human RBCs

Heteropolymer	mAb 1 × mAb 2*	Molecules bound/RBC†
	Binding of ^{125}I-labeled human IgG	
1	1B4 and HB8592 × HB43	1150
2	HB43 × HB8592	601
3	HB43 × 1B4	492
4	HB43 × HB8592	357
5	HB43 × 1B4	479
6	HB43 × 3D9	355
7	HB43 × 57F	387
Control	8E11 × HB8592	−2
	Binding of ^{125}I-labeled $(DNP)_{55}$-BGG	
8	3D9 × 2A1	191
9	3D9 × 23D1	243
10	23D1 × HB8592	129
11	23D1 × 1B4	255
12	23D1 × 3D9	196
13	HB8592 × 2A1	95
14	HB8592 × 23D1	133
15	1B4 × 23D1	279
16	1B4 × 2A1	236
Control	8E11 × HB8592	−11

*For each heteropolymer listed, the first mAb was reduced with dithiothreitol [after reacting with SPDP (18)] and then coupled to the second SPDP-treated mAb. Specificities of the mAbs are as follows: anti-CR1: 1B4, 57F, HB8592, and 3D9; anti-human IgG: HB43; anti-DNP: 2A1 and 23D1; anti-C3b: 8E11. Heteropolymers 2 and 4 represent preparations with HB8592 purified via protein G and octanoic acid/50% saturated ammonium sulfate, respectively. Heteropolymer 1 was prepared by treating a mixture consisting of both SPDP-coupled and reduced 1B4 and SPDP-coupled and reduced HB8592 with SPDP-coupled HB43. CR1 epitopes per RBC for the donor RBCs were 350 for HB8592, 290 for 1B4, and 280 for 3D9.
†Binding was determined by centrifuging RBCs through oil. Background binding to naive human RBCs was 40 human IgG and 60 $(DNP)_{55}$-BGG per RBC, respectively, and was subtracted to give net specific binding reported. Predetermined saturating inputs of both heteropolymer and ^{125}I-labeled antigen were used.

Binding Isotherms with Isolated Heteropolymers. Heteropolymer mixtures were purified by gel-permeation chromatography, and the highest-molecular-mass fractions were used to quantitate binding (Figs. 1 and 2). Binding of ^{125}I-labeled antigens was determined by direct counting of washed RBC pellets. At saturating input of heteropolymer, the maximum number of antigen molecules bound per RBC is in good agreement with our results using unfractionated

Table 2. Demonstration of saturation of binding with heteropolymer 1 and ^{125}I-labeled IgG

Relative heteropolymer concentration	Relative ^{125}I-labeled IgG concentration	Molecules of IgG bound/RBC*
5	5	994
5	1	868
5	0.2	343
1†	1‡	777
0.2	1	205

Binding was determined as for Table 1.
*A different donor was used than for Table 1. CR1 epitopes per RBC for this donor were 380 for HB8592, 330 for 1B4, and 300 for 3D9.
†One corresponds to heteropolymer at 3.0 μg/ml in a 12.5% hematocrit.
‡One corresponds to ^{125}I-labeled human IgG at 0.92 μg/ml in a 12.5% hematocrit.

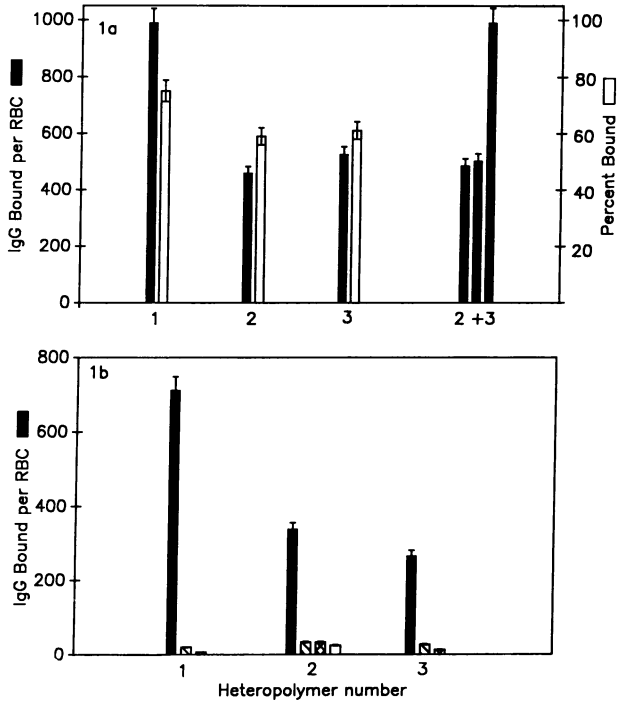

FIG. 1. Binding of ^{125}I-labeled human IgG to heteropolymer-franked RBCs from two normal donors with moderate (*a*) and low (*b*) CR1 levels (≈380 and 220 molecules of HB8592 bound per RBC, at saturation, respectively). Results are reported after subtraction of nonspecific binding for untreated human RBCs. Heteropolymers are defined in Table 1. Solid bars represent the maximum number of antigens bound per RBC at antigen excess. At low antigen inputs (open bars in *a*) % of input antigen bound is displayed (background binding was <5%). The three solid bars over 2 + 3 in *a* represent, respectively, binding after franking with heteropolymer 2, heteropolymer 3, or a mixture containing both heteropolymers. Controls in *b* indicate almost complete loss of specific binding upon inhibition with ascites fluid containing homologous anti-CR1 (▨), anti-IgG (□) (shown for heteropolymer 2 only), or upon substitution of sheep RBCs for human RBCs (▩).

heteropolymer mixtures and centrifugation through oil. These experiments confirm that binding is saturable, since excess heteropolymer or ^{125}I-labeled antigen does not increase binding beyond the saturation level (200–1000 antigens per RBC, Figs. 1 and 2). Results with blood from two donors (Fig. 1 *a* and *b*) demonstrate that maximum binding reflects the number of CR1 epitopes per RBC of the donor. The principle of additivity is illustrated in experiments in which RBCs were franked with two heteropolymers (Fig. 1*a*). Combined action of two heteropolymers in facilitating binding of ^{125}I-labeled antigen is close to the sum of the action of each species individually.

Bispecificity of the heteropolymers was shown in inhibition experiments with excess homologous monomeric mAbs. Our goal was to block binding of heteropolymer to RBCs by using either an anti-CR1 mAb or a monomeric anti-DNP mAb. More than 90% of specific binding was eliminated (Fig. 1*b*). Heteropolymers directed against CR1 do not facilitate binding of ^{125}I-labeled antigen to sheep RBCs (Fig. 1*b*), which lack CR1. The dual specificities of two of the heteropolymers were confirmed by labeling with ^{125}I and examining binding to human RBCs and to a Sepharose 4B matrix containing their respective target antigens (Table 3). The results also indicate that direct binding to human RBCs is rapid at 37°C.

Kinetic Studies of Binding. *In vivo* and *in vitro* experiments have established that IC formation and immune adherence are rapid (8, 25). For example, antibody/double-stranded DNA IC can form, fix complement, and bind to primate

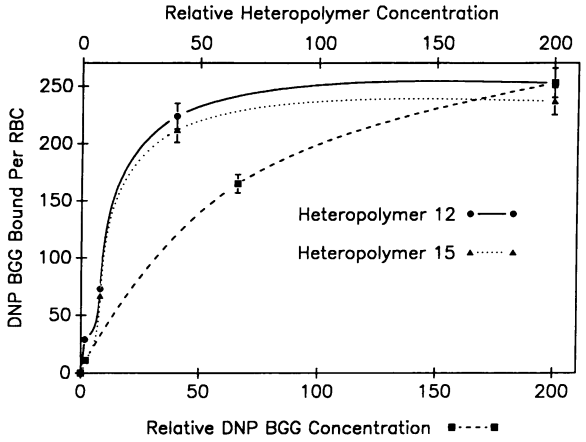

FIG. 2. Binding isotherms in which either the input of franking heteropolymer is varied before washing and probing with excess ^{125}I-labeled antigen (— and · · ·) or input of antigen is varied for RBCs franked with excess heteropolymer 12 (---). One hundred corresponds to an input heteropolymer concentration of 6.5 μg/ml in a 50% hematocrit (—, · · ·); alternatively, 100 corresponds to an input concentration of ^{125}I-labeled $(DNP)_{55}$-BGG at 1.4 μg/ml in a 50% hematocrit of franked RBCs (---).

RBCs in 2 min at 37°C (8). For a heteropolymer to be therapeutically useful, it should be effective over a comparable time frame in the circulation. We conducted *in vitro* kinetic whole-blood experiments in which we added the heteropolymers to anti-coagulated blood (in Alsever's solution) containing ^{125}I-labeled antigens. We find interesting dose–response curves for addition of heteropolymers to whole blood, which resemble quantitative precipitation curves (Fig. 3). Presumably the curves reflect titration of CR1 sites as well as binding of antigen. At high input of heteropolymer (an excess relative to CR1 sites) the majority of the reagent must be free in solution (only a small fraction can be RBC-bound), and binding of ^{125}I-labeled antigen to RBCs is low. However, intermediate inputs of heteropolymer increased binding considerably (≈50% or more). Centrifugation through Percoll (5, 8) confirmed that the only cell type that binds the ^{125}I-labeled antigens is indeed the RBC (results not shown).

Table 3. Binding of ^{125}I-labeled heteropolymers to human RBCs or to Sepharose-coupled ligands

		Binding, % bound*	
Heteropolymer	Mixture	Human RBCs	IgG (or DNP)-Sepharose
4	Unfractionated mixture	45 ± 5	55 ± 5
4	Isolated polymer fraction	65 ± 3	86 ± 5
11	Unfractionated mixture	34 ± 6†	86 ± 5
11	Isolated polymer fraction	72 ± 2‡	85 ± 5

*% bound after incubation (with excess of binding matrix) for 1 hr at room temperature (Sepharose samples) and/or 37°C (RBCs were examined at both temperatures). IgG–Sepharose was used as binding matrix for heteropolymer 4, and DNP–Sepharose (containing a dinitrophenylated mAb to IgM) was used for heteropolymer 11. All samples were corrected for background binding (5% or less) to sheep RBCs or naive (untreated) Sepharose.

†Binding was 30% and 32%, respectively, after incubation for either 2 or 5 min at 37°C.

‡Binding was 57% and 69%, respectively, after incubation for either 2 or 5 min at 37°C.

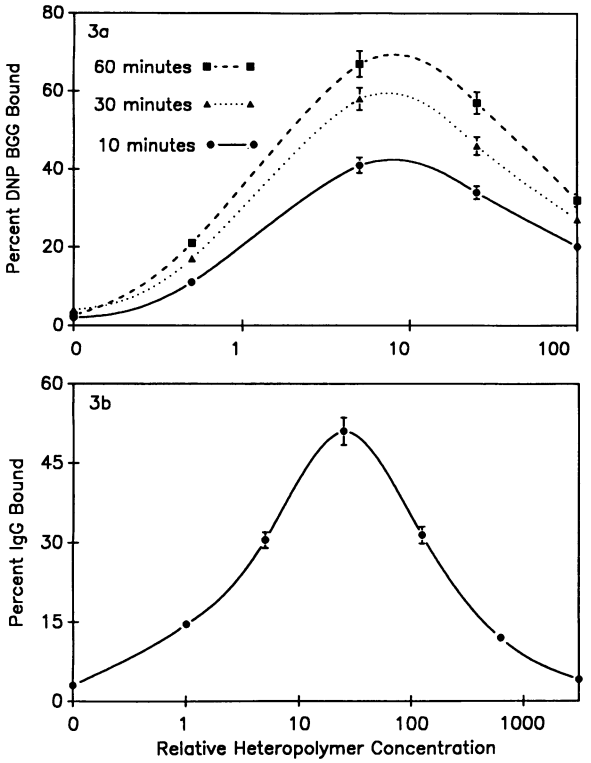

FIG. 3. Binding of trace amounts of ^{125}I-labeled antigens to RBCs in whole blood sensitized with various inputs of heteropolymer. (*a*) RBCs were dispersed in Alsever's solution, and binding at 37°C was determined. Maximum binding was achieved for heteropolymer 12 (used exclusively in *a*) for an extrapolated input concentration of 10, which corresponds to 0.8 μg/ml in a 50% hematocrit. (*b*) Binding was determined after 1 hr at room temperature for heteropolymer 1 (unfractionated). The maximum (at 25) corresponds to 0.5 μg/ml for a 50% hematocrit of RBCs dispersed in BSA/PBS.

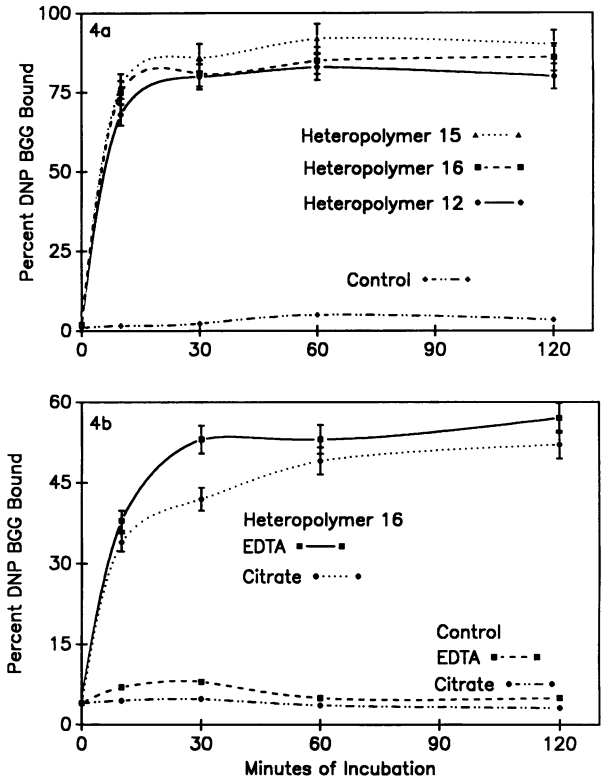

FIG. 4. Kinetic studies of binding of ^{125}I-labeled $(DNP)_{55}$-BGG to RBCs at 37°C in whole blood after sensitization with optimal inputs (≈1 μg/ml) of different heteropolymers. (*a*) RBCs were at a 50% hematocrit in Alsever's solution. (*b*) Fresh whole blood was anticoagulated in 0.01 M EDTA or 0.1 M citrate. Control samples (no heteropolymer) were otherwise treated and processed identically.

Kinetic studies in whole blood indicate that heteropolymer recognition and binding of target antigen and CR1 on the RBC are rapid and comparable in rate to *bona fide* immune adherence (Figs. 3 and 4). Binding is stable; the ^{125}I-labeled antigen remains RBC-bound for >2 hr at 37°C under conditions in which C3b-opsonized IC would be released due to action of factor I (26). Heteropolymer-facilitated binding does not require opsonization with C3b and, therefore, the potential release reaction catalyzed by factor I should not be operative in this system.

Whole-blood experiments were done in anti-coagulated blood (Fig. 4*b*) in 0.01 M EDTA (which prevents complement activation) or in 0.1 M citrate [which allows complement activation and immune adherence of antibody/double-stranded DNA IC (25)]. Alternatively, the experiment was done with washed RBCs in fresh serum [to allow complement activation (11)] and binding levels of 80–90% of $(DNP)_{55}$-BGG were achieved at optimum heteropolymer inputs. Although binding of the ^{125}I label varied in the different media, the results provide strong evidence that binding of antigens to heteropolymer-franked RBCs can occur in the circulation. Our RIA procedure (14) revealed no C3b bound to RBCs (above background for naive RBCs) when the whole-blood experiment was conducted in fresh serum. Other experiments used an alternative model to introduce a heteropolymer into the circulation. One volume of washed RBCs franked with heteropolymer was added to 10 vol of whole blood containing ^{125}I-labeled antigen. After 20 min at 37°C, 50% of the antigen was RBC bound (data not shown).

DISCUSSION

A voluminous literature has established that cross-linked mAbs with dual specificities can simultaneously bind both of their respective antigens (18–20, 27, 28). Heteropolymers can be used both *in vitro* and *in vivo* to destroy tumor cells by linking them to different cytotoxic cells (18–20, 27, 28). One of the key observations of this work is that a biologically relevant site on the cytotoxic cell must be engaged by the heteropolymer. Such sites include Fc receptors on monocytes or T-cell receptors on lymphocytes. By analogy, and in view of the biological properties of CR1 (3, 4, 6, 24, 29–33), we have selected CR1 as the most reasonable (and possibly only) biologically relevant site for using RBCs to facilitate safe and rapid clearance (and perhaps phagocytosis) of target antigens in the circulation. CR1 is found in clusters on human RBCs (29, 30) and provides discrete patches for multivalent binding of "natural" ligands (C3b-containing IC) or heteropolymers. Once bound, these heteropolymers have the potential to bind targeted ligands via multiple contacts. Recent work of Emlen *et al.* (33) suggests that IC binding to CR1 helps organize IC for rapid transfer to acceptor monocytes. A similar mechanism may be operative for targeted antigens bound to heteropolymer-franked RBCs.

Our experiments establish the specificities of the prepared heteropolymers (Tables 1–3). Quantitative saturation analyses of binding are consistent with the number of CR1 epitopes per RBC for individual donors (Figs. 1 and 2). Experiments showing the principle of additivity (Table 1 and Fig. 1*a*) indicate that choice of a mixture of heteropolymers (with specificities for nonoverlapping epitopes on both CR1 and the target antigen) can increase the potential effectiveness of this technique. *In vitro* kinetic experiments reveal that heteropolymer-facilitated binding is sufficiently rapid to be effective in the circulation (Figs. 3 and 4).

Binding of the heteropolymers to RBCs may have another positive benefit because the number of molecules bound per RBC will be limited. Transfusion of one unit of RBCs

saturated with heteropolymer (presumed to be a trimer) would introduce only 0.5 mg of protein, which is less than is used in most mAb-based therapies (34–36). If the mAb in the heteropolymer is of high avidity ($\approx 10^{11}$ liter/mol or higher), then heteropolymer inputs 10–100 times lower should be possible. The concentration of franked RBCs will still be higher than the level of most infectious agents. Such low inputs of mouse mAbs may not be easily recognized as foreign, and this may reduce the problem of the host's immune response to mAb therapy. It is also possible to reduce immunogenicity of the mouse mAbs through construction of mouse–human chimeric mAbs (37).

We do not know whether, after heteropolymer-mediated RBC binding, target antigens will be cleared from the circulation and detoxified by the liver or spleen. The mechanism by which *bona fide* complement-fixing IC are cleared from the circulation after binding to CR1 on RBCs is controversial (6, 9, 11, 26). If, as suggested, there is a proteolytic step or recognition and binding by fixed Fc receptors of the reticuloendothelial system, then safe and rapid clearance should occur. Certainly we expect that heteropolymer-mediated binding to RBCs of particular antigens (viruses and bacteria) should inhibit their free movement in the circulation and decrease their pathogenic potential. If needed, alternative therapies could involve selective removal from the body of RBCs that bound the pathogenic agents. The procedure we describe may find applications in facilitating clearance of "pathogenic" species that are not foreign antigens, such as low density lipoproteins or IgE. It is unlikely the franked RBCs would be removed from the circulation. The maximum amount of mouse IgG bound to the RBCs (500–1000 IgG/RBC) is less than is seen when IC are bound (14), and primate studies demonstrate that such RBCs containing IC are not cleared (5, 6, 9).

The quantitative aspects of this work are quite favorable with respect to the concentration of RBCs and infectious pathogenic particles in the circulation. For example, at extremes of disease activity in AIDS, the level of human immunodeficiency virus (HIV) that circulates freely in the blood ranges between 1000 and 50,000 virus particles per ml (38, 39). These reports suggest that it is this form of HIV (free in the circulation) that is most cytopathic; in fact, high levels in the circulation correlate with disease activity. Because the concentration of RBCs in the circulation is many orders of magnitude greater than reported HIV titers, there would appear to be ample numbers of RBCs [perhaps enhanced by a blood transfusion for patients with low levels of CR1 on their RBCs (12)] available to bind HIV in the presence of a mixture of cross-linked mAbs to CR1 and HIV. Patients might develop an immune response to the mouse mAb heteropolymers, but human mAbs against HIV are available (40), and the danger of an immune response to the mouse mAbs may be outweighed by the advantage of neutralization and clearance of a large fraction of freely circulating HIV.

Further studies will be required to answer the questions we have raised with respect to the potential therapeutic use of heteropolymer-franked RBCs in processing pathogenic factors in the circulation. If these questions can be resolved, then the original optimistic report of Nelson (1, 2) with respect to the potential role of RBCs in the body's immunological defense will be confirmed and considerably enhanced.

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