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Functional expression of the CD4 protein after cross-linking to red blood cells with a bifunctional reagent

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We used a bifunctional reagent for the design of a new therapeutic agent constructed by cross-linking a soluble form of the CD4 protein to red blood cell membranes. CD4 is a member of the immunoglobulin gene superfamily and is the receptor for the AIDS virus, HIV. We produced soluble CD4 in eucaryotic cells transfected with a soluble CD4 expression vector, and purified it by cation-exchange chromatography. Flow cytometry studies demonstrated that CD4-coated red blood cells were specifically stained with an anti-CD4 monoclonal antibody, whereas intact red blood cells and intermediates obtained during the coupling procedure were not stained. By comparison, with CD4 + lymphoid cells, the number of soluble CD4 molecules per CD4-expressing red blood cells was estimated to be approx. 100000. We present evidence that, unlike the classical chromium chloride coupling method, large amounts of soluble CD4 were efficiently and uniformly coupled to RBCs. CD4 red blood cells bind specifically HIV particles, and inhibit the binding of HIV particles to target cells, the initial step of HIV life cycle. The anti-HIV activity of CD4-bearing red blood cells was found to be at least 20-times higher than that of free soluble CD4. Our results demonstrate that proteins can be efficiently coupled to red blood cells using bifunctional reagents. They also suggest that CD4-coated RBC are promising CD4-based anti-HIV agents.

Introduction

Chemical cross-linking of proteins has been widely used to study cytosolic or membrane-associated protein–protein interactions [1,2]. For this purpose, chemical bifunctional reagents that can be covalently linked to different reactive groups have been designed [3,4]. Recently, this technique has been used for the construction of therapeutic agents. For example, 'immunotoxins' were constructed by coupling bacterial toxins to monoclonal antibodies directed against tumors cells [5–8]. These molecules retained both the ability to bind and kill target cells. We used a bifunctional reagent for the design of new therapeutic agents constructed by coupling molecules of interest to red blood cell (RBC) membranes. As a model, we used the CD4 protein. This molecule is a member of the immunoglobulin gene superfamily and consists of four tandem Ig-like domains (denoted V1J1-V4J4), a membrane spanning region and a cytoplasmic domain [9]. CD4 is also the receptor for HIV, a human lentivirus causing acquired immune deficiency syndrome (AIDS) [10–12].

The binding of a viral particle to its receptor expressed on the surface of its target cell is an essential step of the viral life cycle. Thus, blocking the virus–receptor interaction represents a potential target for antiviral agents. In the case of HIV, soluble forms of CD4 comprising its four external domains (denoted sT4) were first shown to potently inhibit HIV infection in vitro [13–17], then in vivo in infected rhesus-monkeys

Abbreviations: AIDS, acquired immune deficiency syndrome; CHO cells, Chinese hamster ovary cells; DHFR, dihydrofolate reductase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; FPLC, fast protein liquid chromatography; HIV, human immunodeficiency virus; Ig, immunoglobulins; kDa, kilodaltons; Leu2a-PE, phycoerythrin conjugated anti-CD8 monoclonal antibody; Leu3a-PE, phycoerythrin conjugated anti-CD4 monoclonal antibody; mAb, monoclonal antibody; Mes, 2-(*N*-morpholino)ethanesulfonic acid; MTX, methotrexate; PBS, 5 mM sodium phosphate buffer, 145 mM NaCl (pH 7.4); RBC, red blood cell(s); sT4, soluble human CD4 comprising the four V1J1-V4J4 external domains of human CD4; sSMCC, sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; BSA, bovine serum albumin; PBA, sodium azide.

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[18]. However, sT4 may not be the ultimate anti-HIV agent, mostly because of its short half-life in humans which ranges from 2 to 10 h. Researchers have recently begun modifying the sT4 molecule in order to enhance its antiviral capabilities. Initial attempts at modifying sT4 are aimed at increasing its serum half-life. In addition, increasing the valency of sT4-based compounds, as well as adding new functions to the molecule, are modifications currently being pursued. For example, truncated forms of CD4 were genetically coupled to toxin [19], IgG [20] and IgM [21] and shown to have good antiviral effects on HIV infection in vitro. Because the life span of RBCs is 120 days, constructing CD4 coated RBC's might be an efficient way of simultaneously increasing the valency and the half-life of CD4-based therapeutics. Recently, native CD4 was inserted at low pH in the membranes of liposomes [22] and intact red blood cells [23].

In this communication, we describe an alternative method for coupling proteins to RBC. We constructed and characterized sT4-RBC obtained by chemical cross-linking of modified RBC to purified recombinant soluble CD4, using a bifunctional reagent. Evidence is presented that, unlike previous methods, large amounts of sT4 can be coupled to RBC and that these molecules retain their capabilities of reacting with monoclonal antibodies or HIV gp120. Finally, these sT4-RBC display very potent anti-HIV activities.

Materials and Methods

Chemicals

Glutamine, gentamycin and dialyzed fetal calf serum were from Gibco (Grand Island, NY, U.S.A.). Ham F12 medium was from Specialty Media (Lavalette, NJ, U.S.A.). Chromium chloride, 2-(*N*-morpholino)ethanesulfonic acid (Mes), methotrexate (MTX), sodium phosphate, sodium chloride, and L-cysteine were from Sigma (St. Louis, MO, U.S.A.). 5,5′-Dithio-bis(2-nitrobenzoic acid) (DTNB), 2-iminothiolane and sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) were from Pierce Chemical (Rockford, IL, U.S.A.).

Electrophoresis chemicals and standard proteins were from Bio-Rad (Richmond, CA, U.S.A.). Phycoerythrin conjugated Leu-3a and Leu-2a were from Becton Dickinson (Mountain View, CA, U.S.A.). The biotinylated sheep anti-mouse antibody was from Amersham (Buckinghamshire, U.K.) and streptavidine phycoerythrin conjugate was from Southern Biotechnology Associate (Birmingham, AL, U.S.A.). The '110-4' anti-gp120 monoclonal antibody was from Genetic Systems (Seattle, WA, U.S.A.).

The LAV-Bru strain of HIV-1 was kindly provided by Pasteur Vaccin (Paris, France), and was heat-inactivated 1 h at 56 °C before use.

Production and purification of sT4

We produced soluble CD4 essentially as described earlier [16]. We used a DHFR negative Chinese hamster ovary (CHO) cell line [24] transfected with a plasmid vector expressing both a soluble form of CD4 comprising its four external domains, and a gene coding for the enzyme dihydrofolate reductase (DHFR). After selection of the transformants in nucleoside free medium, we amplified sT4 expression through rounds of increasing methotrexate (MTX) concentration.

For purification, we produced sT4 in Ham-F12 nucleoside free medium containing 1% glutamine and 1% gentamycin. 500 ml of clarified medium were concentrated 10-times with a FILTRON concentrator using a 10000 M_r cut-off membrane. After appropriate dilution, sT4-containing material was applied to a Mono-S column (Pharmacia, Uppsala, Sweden) connected to an FPLC (Pharmacia) equilibrated with 50 mM Mes (pH 6.5). Bound material was eluted by a gradient of 0–1 M NaCl, made in the buffer.

Isolation of red blood cells

Blood samples from O Rh^+ healthy donors were collected in heparin lithium. After centrifugation on self-forming Ficoll gradients, the RBC were pelleted and washed three times with isotonic NaCl.

Construction of sT4-RBC

Modification of sT4. Prior modification, sT4 (1 mg/ml) eluted from the Mono-S column was loaded on a PD-10 column (Pharmacia, Uppsala, Sweden) equilibrated with 0.2 M $NaHPO_4$ (pH 8.0) in order to change the buffer. A 5-fold molar excess of 2-iminothiolane was then added and the mixture was incubated 1 h at 37 °C with intermittent shaking [8]. Unreacted reagent was removed by passing the mixture on a PD-10 column equilibrated with 50 mM $NaHPO_4$, 50 mM NaCl (pH 7.0). The number of free SH groups generated during coupling were determined by the DTNB method [7].

Modification of the RBC. RBC were washed three times with 50 mM $NaHPO_4$, 50 mM NaCl (pH 7.0). Pelleted RBC were brought to 25% hematocrit in the cross-linking buffer. Sulfo-SMCC was added (10^{-3} M final concentration) to 8 ml of the RBC suspension and the reaction mixture was agitated at 15 °C for 30 min. The reaction was stopped by centrifugation and washing the cells three times with the cross-linking buffer. Packed modified RBC were brought back to 25% hematocrit in cross-linking buffer.

Construction of ST4-RBC. SH coupled sT4 (sT4-SH) was added to the modified RBC solution at a final ratio of 1 mg sT4-SH/ml packed cells. Cross-linking was performed by rotating the mixture 3 h at 15 °C. The reaction was stopped by washing the cells three times with PBS. To block unreacted maleimide groups re-

maining at the surface of modified RBC, the cells were incubated with L-cysteine, (1.5 mM final concentration) 15 min at 15°C. The cells were finally washed three times with cold PBS. As controls, intact or modified RBC were used and subjected to the same protocol without adding sT4.

Chromium chloride coupling procedure. Intact sT4 was conjugated to RBC according to the method of Goding [25], with minor modifications. Briefly, sT4 was added to packed RBC equilibrated with 0.85% sterile NaCl at a final ratio of 1 mg sT4/ml of packed cells. One volume of the chromium chloride solution (1 mg/ml of chromium chloride dissolved in 0.85% NaCl and adjusted at pH 5.0 with sodium hydroxide) was added dropwise to the suspension containing both sT4 and RBC. For cross-linking, the mixture was allowed to rotate 10 min at room temperature. The reaction was stopped by centrifugation and coupled cells were finally washed three times with cold PBS.

Immunofluorescence labelling

$5 \cdot 10^5$ sT4-RBC, intact or modified RBC, or CEM cells were incubated at 4°C for 90 min with the appropriate concentration of phycoerythrin conjugated anti-CD4 (Leu3a-PE) or anti-CD8 (Leu2a-PE) monoclonal antibodies in 100 µl of PBS containing 0.5% bovine serum albumin (BSA) and 0.05% sodium azide (PBA). After 3 washes with cold PBA, resuspended stained cells were analyzed for fluorescence with a FACS analyser (Becton Dickinson). Single cells were gated according to their volume and forward light scatter signals. FACS settings remained constant for all samples.

For assessing HIV binding on sT4-RBC, $5 \cdot 10^5$ intact RBC or sT4-RBC were incubated with pelleted HIV particles (1 µg of viral proteins) for 60 min at 37°C. After washing with cold PBA, bound HIV particles were revealed by indirect immunofluorescence staining using a monoclonal antibody directed to HIV gp120 (110-4, Genetic System), at a 1:100 dilution in PBA. After three washes in cold PBA, cell labelling was revealed by incubation with a biotinylated sheep anti-mouse immunoglobulin and phycoerythrin-streptavidin.

sT4-RBC anti-HIV activity was assessed by measuring sT4-RBC capability to inhibit HIV binding to CEM cells. $0.5 \cdot 10^6$ or $20 \cdot 10^6$ sT4-RBC or intact RBC were rotated with 1 µg of inactivated HIV, in 200 µl of PBA, for 1 h at 37°C. RBC, sT4-RBC and control (1 µg

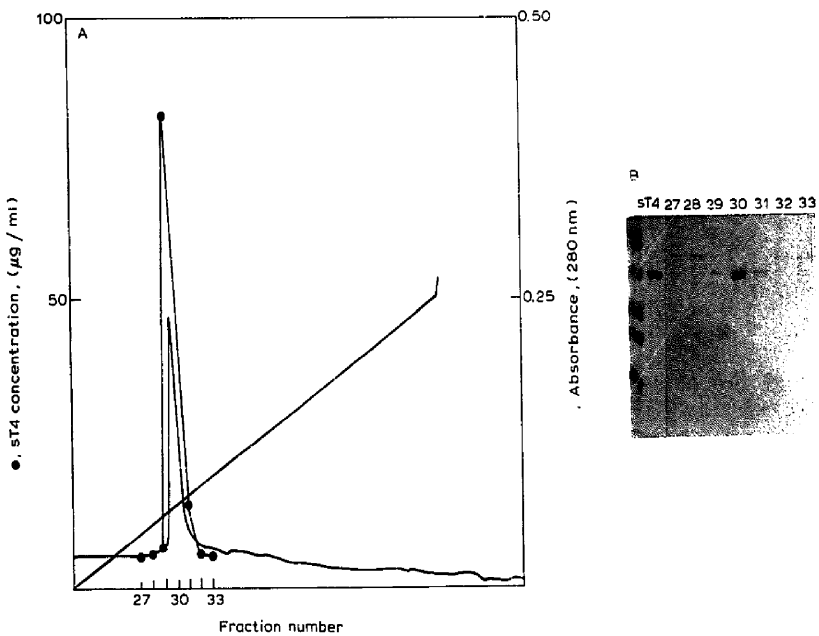

Fig. 1. Purification of sT4 by ion-exchange chromatography. (A) 10 ml of concentrated sT4-containing CHO culture medium was diluted 4-fold with 50 mM Mes (pH 6.5) and loaded on a Mono-S column equilibrated in buffer at 4°C. Elution was carried out with a 0–1 M linear NaCl gradient in buffer at a flow rate of 1.0 ml·min^{-1}. Absorbance at 280 nm (thick line) and NaCl concentration (thin line) were recorded. 1 ml fractions were collected and aliquots were analyzed for sT4 concentration (●). (B) SDS-polyacrylamide gel electrophoresis analysis. Aliquots from fractions eluted from the Mono-S column were submitted to SDS-PAGE analysis according to Laemmli [32]. Sample, control and standard proteins were run on a 10% acrylamide gel and Coomassie blue-stained. Prestained standard proteins were: phosphorylase B (M_r = 110000), bovine serum albumin (M_r = 84000; ovalbumin (M_r = 47000), carbonic anhydrase (M_r = 33000), soybean trypsin inhibitor (M_r = 24000) and lysozyme (M_r = 16000). Purified sT4 (0.5 µg) was run as control.

inactivated HIV in 200 µl PBA) were briefly centrifuged at 800 × *g* and 100 µl of the supernatant was incubated with $5 \cdot 10^5$ CEM cells for 1 h at 37°C. The cells were then washed with PBA and bound HIV was revealed as described above. For comparison of sT4-RBC and sT4 capabilities to inhibit HIV binding to CEM cells, 0.01 µg and 10 µg of sT4 were used in the same assay under the same experimental procedures.

sT4 ELISA assay

We used a previously described ELISA [26] for the semi-quantitative determination of sT4 concentration during supernatant collection and purification. Briefly, sT4 was coated onto ELISA plates and detected with Leu-3a monoclonal antibody (mAb) and peroxidase labelled anti-mouse IgG.

Results

Production and purification of sT4

DHFR-negative CHO cells were transfected with a plasmid coexpressing sT4 and DHFR. In stable transformants, we amplified sT4 production by adding to the culture medium increasing concentration of MTX, a tetrahydrofolate competitive inhibitor. Prior to purification, the cells were grown in the absence of fetal calf serum. Using this procedure, we selected clones producing approx. 30 mg/l of sT4, which represents more than 40% of total proteins present in the culture medium (data not shown).

For purification, clarified culture medium was loaded on a cation-exchange column. A major and sharp absorbance peak was eluted from the column at 125 mM NaCl (Fig. 1). This peak contained the soluble CD4 protein as demonstrated by its reactivity with specific anti-CD4 mAb (Fig. 1). sT4 concentration in each chromatography fraction was determined by an ELISA assay using purified sT4 as a standard. Proteins contained in each fraction were coated to ELISA plastic plate and coated sT4 was detected by Leu-3a, a monoclonal antibody directed against the V1 domain of human CD4. This mAb recognizes an epitope which overlaps with the gp120 binding site [27], and thus its reactivity gives an indication of both the concentration and the bioreactivity of the purified product. 90% of the bioreactivity was recovered in a single fraction (Fig. 1). Using SDS denaturing electrophoresis, this fraction was shown to contain a major protein of 47 kDa, migrating at the apparent K_d value of recombinant soluble CD4 (Fig. 1). The purity of the purified material was estimated at 95% by Coomassie blue staining.

Preparation sT4-RBC

Coupling. Purified sT4 was cross-linked to RBC as described in Materials and Methods. The specificity of the coupling procedure was checked by flow cytometry analysis of sT4-RBC, intact or modified RBC, stained either with an anti-CD4 (Leu3a-PE) or an irrelevant (Leu2a-PE) mAb. As shown in Fig. 2, staining of sT4-RBC with Leu3a-PE resulted in a unique peak of high specific fluorescence intensity as compared to unstained cells, indicating that all the RBC were coupled with sT4. The sharpness of the peak indicates a uniform staining of the erythrocytes. The absence of a fluorescence shift with modified RBC (sSMCC-RBC) demonstrates that Leu3a-PE does not bind non-specifically to the chemical arm coupled to the erythrocyte (Fig. 2G), nor does sT4-SH bind to unmodified RBC (Fig. 2I). Finally, the specificity of our coupling technique was demonstrated by the absence of fluorescence staining after incubating sT4-RBC, modified and intact RBC, with an irrelevant monoclonal antibody, Leu2a-PE (Fig. 2D, F, H and J). For comparison, sT4 was also coupled to RBC with the

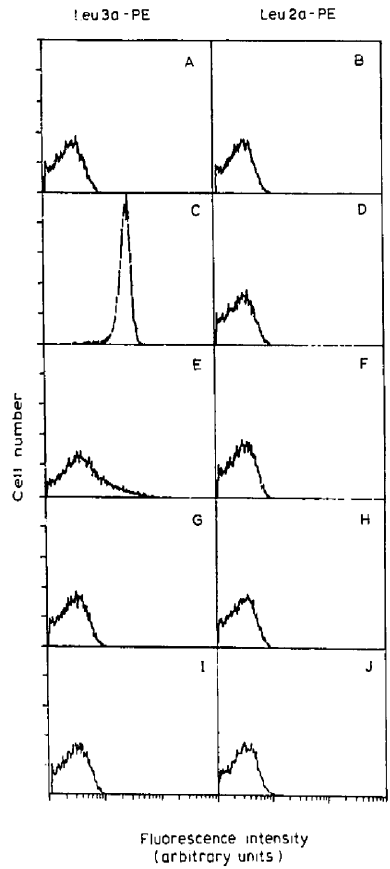

Fig. 2. Characterization of sT4-RBC. Intact RBC (A, B), sT4-RBC (C, D), chromium chloride coupled sT4-RBC (E, F), modified sSMCC-RBC (G, H) and intact RBC preincubated with modified sT4-SH (I, J) were incubated with Leu3a-PE anti-CD4 mAb, (A, C, E, G, I) or Leu2a-PE anti-CD8 mAb (B, D, F, H, J) and submitted to FACS analysis.

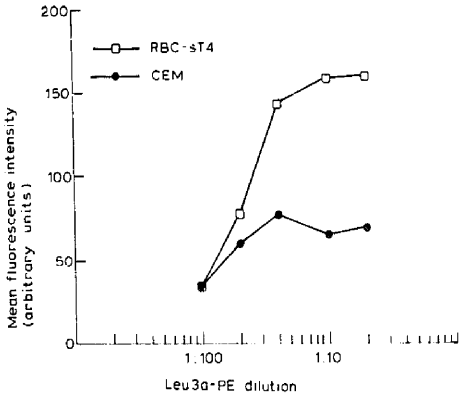

Fig. 3. Determination of the number of sT4 coupled to RBC. $5 \cdot 10^5$ sT4-RBC (□) or CEM cells (●) were incubated with a serial (1:5, 1:10, 1:25, 1:50 and 1:100) dilution of Leu3a-PE and subjected to FACS analysis. FACS settings remained constant for all samples.

widely used chromium chloride technique. As seen in Fig. 2E, only a small fraction of the RBC's are weakly and broadly stained using this technique. This indicates that the chromium chloride procedure does not cross-link sT4 efficiently and uniformly onto the RBC membrane. In summary, our technique provides a very efficient and specific procedure for coupling soluble CD4 to red blood cells. In addition, the reactivity of our sT4-RBC with Leu-3a indicates that the epitope recognized by this mAb is accessible at the cell surface.

Determination of the number of sT4 molecules coupled to RBCs

The number of sT4 coupled to RBCs was indirectly estimated by comparison with the level of CD4 expression on the surface of the CD4+ CEM cells (Fig. 3).

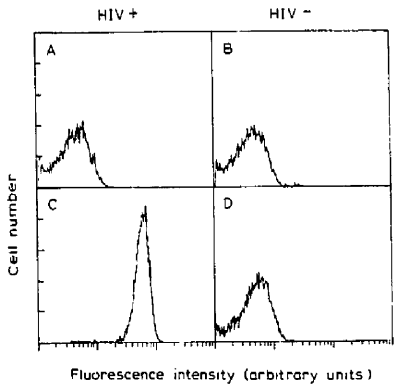

Fig. 4. HIV binding to sT4-RBC and intact RBC. Intact RBC (A, B) and sT4-RBC (C, D) were incubated in the presence (A, C) or the absence (B, D) of HIV particles. Viral particles attached to cells were then detected using anti-HIV mAb, biotinylated sheep anti-mouse-IgG and phycoerythrin-streptavidin.

TABLE I

Inhibition of HIV binding to CEM cells by sT4-RBC and sT4

HIV particles were incubated in the presence of either intact RBC ($20 \cdot 10^6$), sT4-RBC ($20 \cdot 10^6$), or sT4 (10 μg), as described in Materials and Methods. After centrifugation, supernatants were incubated with CEM cells. Viral particles bound to CEM cells were detected as described in Fig. 3. Results were expressed as percent inhibition of controls corresponding to the absence of inhibitor in each of these three independent expriments.

	Exp. 1	Exp. 2	Exp. 3
RBC	12	18	0
sT4-RBC	80	94	54
sT4	92	95	60

We stained both our sT4-RBC and CEM cells with increasing amounts of Leu3a-PE to reach staining saturation. We obtained a concentration-dependent staining of sT4-RBC and CEM cells, reaching a plateau at a 1:25 dilution of Leu3a-PE. At the plateau, sT4-RBC and CEM cells display a mean fluorescence intensity of 160/170 and 70/80 arbitrary units, respectively. This indicates that sT4-RBC are 2- to 2.5-fold more stained than CEM cells. The number of CD4 molecules on the surface of CEM 13 cells is about 45000 [28], and thus we can estimate the number of sT4 coupled at around 100000 per RBC.

HIV binding to sT4-RBC

We next determined the ability of the sT4-RBC to bind HIV. After incubation of intact RBC or sT4-RBC with heat-inactivated HIV, the binding of HIV particles on RBC was detected by staining with an anti-HIV gp120 mAb. A sharp peak with a high fluorescence intensity (Fig. 4) was observed with sT4-RBC, indicating that all the cells were uniformly coated with HIV. There was no detectable non-specific binding of the anti-gp120 mAb on sT4-RBC or intact RBC (Fig. 4A, 3, D). Our results demonstrate that sT4-RBC efficiently and specifically binds inactivated HIV.

Inhibition of HIV binding to CEM cells

We then compared the anti-viral properties of sT4-RBC to those of standard sT4. As seen in Table I, sT4-RBC and sT4 both inhibit HIV binding to CD4-positive CEM cells. Preincubation of HIV with $20 \cdot 10^6$ sT4-RBC lead to a mean of 76% inhibition of HIV binding. Similarly, a mean of 82% inhibition of HIV binding was observed when 10 μg of sT4 were substituted to sT4-RBC in these assays. No significant inhibition was observed with intact RBC.

Discussion

Red blood cells have been widely used as tools for in vitro experiments or as therapeutic agents. For example, by coupling mAbs to their surface, RBC have been used

to isolate cellular clones expressing surface molecules synthetized after transfection of exogenous DNA [9]. In this case, coupling of proteins to RBC membranes was performed by the classical chromium chloride procedure. Recently, low-pH association of proteins with the membranes of RBCs has been described [23]. We have developed a novel method to efficiently couple proteins to red blood cells, using bifunctional reagents for cross-linking. This method provides better results than previously described ones. First, the coupling is efficient and results in a homogeneous labelling of RBCs. Under similar conditions, the amount of coupled protein is at least 20-times higher using our method than the chromium chloride coupling procedure. Second, the staining obtained with our method is more homogeneous, as indicated by the sharpness of the fluorescence intensity profiles of cross-linked RBC stained with a specific mAb. Unlike the chromium chloride coupling, all RBCs used for cross-linking are uniformly stained. The heterobifunctional reagent we are using is able to bind covalently free SH groups on one side and free NH_2 groups on the other side, and therefore has the potential of cross-linking together RBCs. However, less than 1% RBC's multimers are generated during the coupling procedure under our experimental conditions (data not shown).

The chemical cross-linking method does not alter the protein bioreactivity. This has already been shown when this technique was used for immunotoxin construction [8]. In our case, the creation of free SH groups at the surface of sT4 does not lower the bioreactivity of the molecule. We have previously shown that sT4-SH binds HIV gp120 and inhibits HIV infectivity at the same doses as unmodified sT4 (Idziorek, T. and Klatzmann, D., unpublished data). After coupling to RBCs, the bioreactivity of our protein remained unaffected. It could still bind two of its high affinity ligands, HIV gp120 and Leu-3a, a monoclonal antibody that recognizes an epitope present in the V1 domain of CD4 [27]. This indicates that the conformation of the protein was not lost during the coupling procedure, and that the SH group created on the surface of sT4 was probably not introduced on the V1 domain of the CD4 molecule. It is noteworthy that we could easily detect by immunofluorescence sT4 on the surface of RBCs, while this was not possible using the low-pH association method [23]. This is probably not due to the number of molecules expressed per cell, since with both methods the number of CD4 expressed at the RBC membrane surface is in the same order of magnitude.

In a series of nine independent experiments, we obtained sT4-RBC with a maximum number of sT4 per cell of approx. $5 \cdot 10^5$. The number of sT4 cross-linked to the RBC membrane is dependent on the concentration of sT4-SH and modified RBC during the final coupling procedure. It is also dependent on the number of bifunctional reagent molecules coupled to the surface of RBC, and to a lesser extent on the number of free SH groups per sT4. We used a concentration of 2-iminothiolane which introduces a mean of one SH group per molecule of sT4 (data not shown). The number of NH_2 groups at the red blood cell surface which are accessible for protein coupling might be one of the limiting factors in this coupling procedure. If necessary, this could be overcome by amino groups supplementation of surface glycoproteins on intact cells with covalently bound free alkylamino groups [29].

The CD4 protein is the receptor for HIV [10–12]. Thus, blocking CD4–HIV interaction represents a potential therapeutical target for controlling HIV infection. Soluble forms of CD4 comprising its four external domains are strong inhibitors of HIV infection in vitro [13–17]. At a concentration of 10 μg per ml, sT4 reduces the infectivity of HIV by at least four logs [16]. Phase I studies with escalating doses have not shown any toxicity of sT4 [30,31]. Unfortunately, the half-life of sT4 ranges only from 2 to several hours [30,31]. Different approaches have been pursued to improve sT4 antiviral capabilities, i.e., increasing its serum half-life or the valency of sT4-based compounds. Adding new functions to the molecule is also currently being pursued. For example, truncated forms of CD4 were genetically coupled to toxin [19], IgG [20] and IgM [21] and shown to have good antiviral effects on HIV infection in vitro. We have used our coupling method to construct CD4-coated RBC, because it might be an efficient way of simultaneously increasing the valency and the half-life of CD4-based therapeutics. We first checked the ability of viral particles to bind its receptor onto the surface of sT4-RBC. Our results indicate that inactivated HIV binds to sT4-bearing RBC as well as it binds to CEM CD4 + cells. We next compared the ability of sT4 and sT4-RBC to inhibit HIV binding to CD4 + target cells, the initial step of HIV life cycle. sT4-RBC efficiently inhibit such binding. By comparing the amount of sT4 molecules added in the assay, either as free or RBC expressed molecules, sT4-RBC works approx. 20-times better than free sT4. Preliminary results also indicate that sT4-RBC inhibit HIV infection of CEM cells at least 10-times better than free sT4 (data not shown). This is probably due to the valency of our sT4-RBC, since decavalent CD4-IgM molecules have also been found to be more efficient than sT4. We are now investigating if the anti-HIV properties of sT4-RBC can be improved by varying the amount of sT4 coated per cell.

Our results suggest that sT4-RBC are very promising CD4-based antiviral agents. It remains to be determined whether sT4-RBC will retain the long life-span of RBCs. We are currently pursuing these studies.

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References

1 Bäumert, H.G. and Fasold, H. (1989) Methods Enzymol. 172, 584–609.
2 Staros, J.V. and Anjaneyulu, P.S.R. (1989) Methods Enzymol. 172, 609–628.
3 Wold, F. (1972) Methods Enzymol. 25, 623–651.
4 Ji, T.H. (1983) Methods Enzymol. 91, 580–609.
5 Ghose, T.I., Blair, A.H. and Kulkarni, P. (1983) Methods Enzymol. 93, 280–337.
6 Cumber, A.J., Forrester, J.A., Foxwell, B.M.J., Ross, W.C.J. and Thorpe, P.E. (1985) Methods Enzymol. 112, 207–225.
7 Fitzgerald, D.J. (1987) Methods Enzymol. 151, 139–145.
8 Fitzgerald, D.J., Idziorek, T.I., Batra, J., Willingham, M. and Pastan, I. (1990) Bioconjugate Chem. 1, 264–268.
9 Maddon, P.J., Littman, D.R., Godfrey, M., Maddon, D.E., Chess, L. and Axel, R. (1985) Cell 42, 93–104.
10 Dalgleish, A.G., Beverley, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R. (1984) Nature 312, 763–767.
11 Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J.-C. and Montagnier, L. (1984) Nature 312, 767–768.
12 Klatzmann, D.R., McDougal, J.S. and Maddon, P.J. (1990) Immunodeficiency Rev. 2, 43–66.
13 Smith, D.H., Byrn, R.A., Marsters, S.A., Gregory, T., Groopman, J.E. and Capon, D.J. (1987) Science 238, 1704–1707.
14 Fisher, R.A., Bertonis, J.M., Meier, W., Johnson, V.A., Costopoulos, D.S., Liu, T., Tizard, R., Walker, B.D., Hirsch, M.S., Schooley, R.T. and Flavell, R.A. (1988) Nature 331, 76–78.
15 Hussey, R., Richardson, N.E., Kowalski, M., Brown, N.R., Chang, H.-C., Siciliano, R.F., Dorfman, T., Walker, B., Sodroski, J. and Reinherz, E. (1988) Nature 331, 78–81.
16 Deen, K.C., Mc Dougal, J.S., Inacker, R., Folena-Wasserman, G., Arthos, J., Rosenberg, J., Maddon, P.J., Axel, R. and Sweet, R.W. (1988) Nature 331, 82–84.
17 Traunecker, A., Lüke, W. and Karjalainen, K. (1988) Nature 331, 84–86.
18 Watanabe, M., Reimann, K.A., Delong, P.A., Liu, T., Fisher, R.A. and Letvin, N.L. (1989) Nature 337, 267–270.
19 Chaudhary, V.K., Misukami, T., Fuerst, T.R., Fitzgerald, D.J., Moss, B., Pastan, I. and Berger, E.A. (1988) Nature 335, 369–372.
20 Capon, D.J., Chamow, S.M., Mordenti, J., Marsters, S.A., Gregory, T., Mitsuya, H., Byrn, R.A., Lucas, C., Wurm, F.M., Groopman, J.E., Broder, S. and Smith, D.H. (1989) Nature 337, 525–531.
21 Traunecker, A., Schneider, J., Kiefer, H. and Karjalainen, K. (1989) Nature 339, 68–72.
22 Cudd, A., Noonan, C.A., Tosi, P.-F., Melnick, J.L. and Nicolau, C. (1990) J. AIDS 3, 109–114.
23 Arvinte, T., Schulz, B., Cudd, A. and Nicolau, C. (1989) Biochim. Biophys. Acta 981, 51–60.
24 Urlaub, G., Kas, E., Carothers, A.M. and Chasin, L.A. (1983) Cell 33, 405–412.
25 Goding, J.W. (1976) J. Immunol. Methods 10, 61–66.
26 Chams, V., Jouault, T., Fenouillet, E., Gluckman, J.-C. and Klatzmann, D. (1989) AIDS 2, 353–361.
27 Peterson, A. and Seed, B. (1988) Cell 54, 65–72.
28 Poncelet, P. and Carayon, P. (1985) J. Immunol. Methods 85, 65–74.
29 Schweizer, E., Angst, W. and Lutz, H. (1982) Biochemistry 21 6807–6818.
30 Shooley, R.T., Merigan, T.C., Gaut, P., Hirsch, M.S., Holodniy, M., Flynn, T., Liu, S., Byington, R.E., Henochowicz, S., Gubish, E., Spriggs, D., Kufe, D., Schindler, J., Dawson, A., Thomas, D., Hanson, D.G., Letvin, B., Liu, T., Gulinello, J., Kennedy, S., Fisher, R. and Ho, D.D. (1990) Ann. Intern. Med. 112, 247–253.
31 Khan, J.O., Allan, J.D., Hodges, T.L., Kaplan, L.D., Arri, C.J., Fitch, H.F., Lo, A.E., Mordenti, J., Sherwin, S.A., Groopman, J.E. and Volberding, P.A. (1990) Ann. Intern. Med. 112, 254–261.
32 Laemmli, U.K. (1970) Nature 227, 680–685.