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Affinity targeting of Sendai virions to desialized human erythrocytes using hybrid antibody molecules

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F(ab′) fragments obtained from anti-Sendai virus antibodies were chemically coupled to F(ab′) fragments obtained from anti-human red blood cell antibodies (anti-hRBC-Ab). This led to the formation of hybrid antibody molecules (anti-SV-anti-hRBC($F(ab')_2$) each of whose F(ab′) fragment possessed different binding specificity. The anti-SV(F(ab′)) part of the hybrid molecule interacted specifically with Sendai virus particles, while the anti-hRBC(F(ab′)) part interacted with the surface of hRBC. These hybrid antibodies were able to mediate binding and fusion of SV to hRBC, from which the virus receptors were removed by treatment with neuraminidase (desialized hRBC). Neither anti-SV-anti-SV($F(ab')_2$) nor anti-hRBC-anti-hRBC($F(ab')_2$) possessed the same ability. Thus, it is shown that soluble, hybrid antibody molecules can effectively mediate functional binding of Sendai virus to virus-receptor-depleted cells.

Introduction

Infection of cells by animal viruses involves two successive steps: binding of the virus particles to specific cell receptors, and the subsequent introduction of the viral nucleocapsid into the cell cytoplasm [1,2]. Entry of enveloped viruses, especially those belonging to the paramyxovirus group such as Sendai virus particles, occurs following fusion of the viral envelope with the plasma membrane of the recipient cell [2,3]. Sialic acid residues of glycoproteins and glycolipids have been shown to serve as specific receptors for Sendai virus [3,4]. Binding to sialic acid-containing membrane components is mediated by a specific viral envelope polypeptide, the hemagglutinin/neuraminidase (HN) glycoprotein [2,3]. Fusion between the two adjacent membranes, namely, the viral envelope and the recipient cell plasma membrane, is promoted by a second viral polypeptide, the F (fusion) glycoprotein, whose mechanism of action is still obscure [2,3].

It is not yet entirely clear whether membrane components which serve as virus receptor play an active and specific role in the entire process of virus penetration and infection. It is possible that the only function of the viral binding protein, the HN glycoprotein in the case of Sendai virus (SV), and its respective membrane receptor is to bring the viral envelope and the cell plasma membrane into close proximity. This may be sufficient to activate the viral fusion factor which will then promote the fusion between the two adjacent membranes.

In order to elucidate the function of the SV-HN glycoprotein and its membrane receptor in the process of virus-cell fusion and infection, we have recently studied the question whether anti-SV-antibody (anti-SV-Ab) can serve as a functional membrane virus receptor. Anti-SV-Ab were cova-

lently coupled to human red blood cells (hRBC) from which sialic acid residues were removed by treatment with neuraminidase [5]. Our experiments showed that Sendai viruses were able to attach to and fuse with the neuraminidase-treated human erythrocytes bearing anti-SV-Ab [5]. Similarly, it has recently [6] been shown that attachment and fusion of Senadi virus to human erythrocytes can be mediated by the specific complex formed between avidin and biotin. Avidin molecules were chemically coupled to hRBC membranes, while biotin molecules were coupled to Sendai virus envelopes. Sendai virions bearing biotin molecules were able to interact and to fuse with human erythrocytes bearing avidin molecules in the presence of anti-SV-HN antibody (F(ab') fragment), which was added in order to neutralize the native viral binding protein [6]. However, it should be emphasized that the use of anti-SV-Ab [5] and avidin molecules [6] for mediating attachment of virus particles necessitated chemical modification of either the recipient cell plasma membrane or of both the cell and the viral membrane, respectively. this may cause a high degree of cell killing, especially when cells in culture are used, and inactivation of the viral fusogenic factor.

In order to avoid the chemical modification of the recipient cells and the viral membranes but still be able to use molecules of specific binding properties for mediating binding of virus particles to receptor-depleted cells, we have studied the function of hybrid antibody molecules. Hybrid antibody molecules are $F(ab')_2$ molecules each of whose F(ab') fragment possesses a different binding specificity [7]. In the present work we have constructed hybrid antibody molecules by coupling F(ab') fragments obtained from anti-SV-Ab to F(ab') fragments obtained from anti-hRBC-Ab. These specific hybrid antibodies were formed after reoxidation of chemically reduced anti-SV and anti-hRBC $F(ab')_2$ fragments [7]. As a model system we have studied the ability of such hybrid antibodies to mediate binding and fusion of Sendai virus to neuraminidase-treated human erythrocytes. It is conceivable that the anti-SV part (F(ab')) of the hybrid antibody will specifically interact with Sendai virus, while the anti-hRBC part (F(ab')) of the hybrid will interact with the erythrocyte surface. Indeed, the results of the present work show that such hybrid antibody mediated the functional binding of Sendai virus to neuraminidase-treated human erythrocytes.

Materials and Methods

Cells. Human blood type O, Rh^+, recently outdated, was washed three times in Solution A (150 mM NaCl, 20 mM Tris, pH 7.4), and the final pellet was resuspended in Solution A to give 2.5% (v/v), The washed human erythrocytes were desialized by treatment with neuraminidase, as described before [8].

Virus. Sendai virus (SV) was propagated, harvested and suspended in Solution A, to give 10% (v/v), and its hemagglutinating units were determined as previously described [8]. SV were radiolabeled by the use of chloramine-T and $Na^{125}I$, as described before [9].

Preparation of anti-SV and anti-hRBC antibodies. Anti-SV-Ab were prepared in rabbit by subcutaneous injection of reconstituted SV envelopes, as previously described [10]. Anti-hRBC-Ab were prepared in rabbit by subcutaneous injection of neuraminidase-treated human erythrocytes. The IgG fraction of the above antisera and from serum of non-immunized rabbits was obtained by ammonium sulfate precipitation (33%, v/v) [9]. Anti-hRBC-Ab were assayed by their ability to promote agglutination of neuraminidase-treated human erythrocytes, while anti-SV-Ab were evaluated by their ability to inhibit SV-induced agglutination and hemolysis.

Preparation of anti-SV-anti-hRBC ($F(ab')_2$) hybrid molecules. The IgG fraction (10 mg/ml in Solution A) of the two antisera was dialysed against a buffer containing 50 mM sodium acetate (pH 4.0) for 5 h, at the end of which 5 ml of the dialysate was incubated with pepsin (250 μg/10 mg of IgG), as previously described [11]. The $F(ab')_2$ fragments obtained were separated from the Fc fragments by chromatography on Sephacryl-300 column (2 cm $\times$ 30 cm), pre-equilibrated with 10 mM phosphate buffer (pH 8.0). The various fractions were lyophilized and the dry material obtained was dissolved in distilled water and then dialysed at 4°C against Solution A. The molecular weight of the fragments was analyzed by acrylamide gel electrophoresis (7.5% acrylamide).

Fractions containing the $F(ab')_2$ fragments, obtained from anti-SV-Ab and anti-hRBC-Ab (see scheme in Fig. 1) were mixed at a ratio of 2 : 1 (w/w), respectively. After adjusting the protein concentration to 3 mg/ml with Solution A, the $F(ab')_2$ fragments were reduced (to give F(ab')) with 2 mM β-mercaptoethanol, for 60 min at 37°C, under nitrogen atmosphere. Excess, free β-mercaptoethanol was removed by loading 1 ml of the above mixture on a column (5 ml syringe) containing 5 ml swollen Sephadex G-25. The column was then centrifuged (500 × *g*, 5 min) and the eluent containing F(ab') fragments was dialysed against Solution A for 12 h at 4°C. During the dialysis period, the reduced F(ab') fragments were reoxidized to give $F(ab')_2$ molecules (see scheme in Fig. 1). At the end of the dialysis period, the solution containing the $F(ab')_2$ molecules was divided into small aliquots and stored at −20°C. F(ab') fragment of anti-SV-Ab coupled to F(ab') fragment obtained from normal rabbit IgG were prepared exactly as described above for anti-SV-anti-hRBC ($F(ab')_2$) molecules. Three kinds of $F(ab')_2$ molecules should be obtained: the original anti-SV and anti-hRBC and hybrid molecules consisting of anti-SV F(ab') fragments coupled to anti-hRBC F(ab') fragments by dithio bonds (anti-SV-anti-hRBC ($F(ab')_2$)). The relative ratio between the various kinds of $F(ab')_2$ molecules is highly dependent upon the weight ratio between the anti-SV and anti-hRBC $F(ab')_2$ fragments in the reaction mixtures. Under the experimental conditions used (anti-SV $F(ab')_2$/ anti-hRBC $F(ab')_2$, w/w = 2 : 1), a relatively small amount of anti-hRBC molecules should be formed. Fig. 1 schematically summarizes the way by which the hybrid antibody molecules were constructed.

Incubation of $F(ab')_2$ antibody preparations with neuraminidase-treated human erythrocytes. Neuraminidase-treated human erythrocytes ($(3.7–8)\cdot 10^7$) were incubated with the $F(ab')_2$ fragments, in a final volume of 200–250 µl, for 60 min at 37°C. At the end of the incubation period the suspension was washed once with Solution A, containing 1 mg/ml of bovine serum albumin (BSA) (Solution-BSA) and twice with Solution A.

Incubation of neuraminidase-treated human erythrocytes bearing $F(ab')_2$ hybrid antibody preparations with Sendai virus. Washed neuraminidase-treated human erythrocytes ($(3.7–8)\cdot 10^7$), with or without attached hybrid antibody molecules, were incubated with Sendai virus in a final volume of 200 µl for 60 min at 37°C. At the end of the incubation period, the degree of cell-cell agglutination, hemolysis and cell-cell fusion was determined as previously described [8].

Materials. Pepsin (porcine pancreas) was obtained from Sigma; neuraminidase from *Vibrio cholera* was purchased from Boehringer; Sephacryl-S-300 was obtained from Pharmacia. All other chemicals were of analytical grade.

Results

Use of the hybrid anti-SV-anti-hRBC ($F(ab')_2$) preparations to mediate binding of Sendai virus particles to neuraminidase-treated human erythrocytes

The results in Fig. 2 (A and B) clearly show that hybrid molecules (anti-SV-anti-hRBC, $F(ab')_2$) can bind to neuraminidase-treated human erythro-

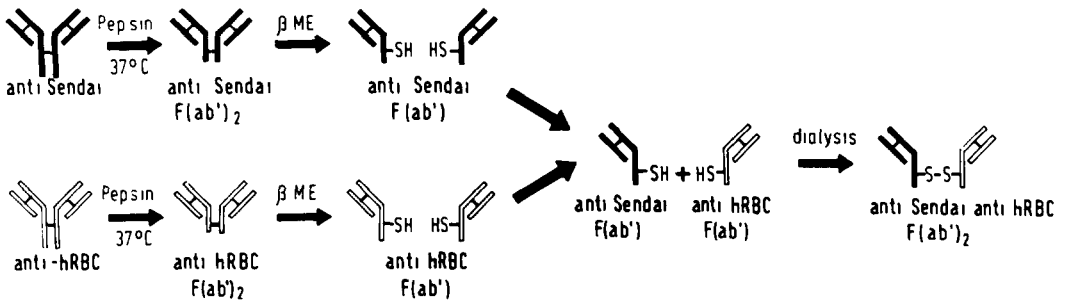


Fig. 1. Construction of hybrid anti-SV-anti-hRBC $F(ab')_2$ molecules Schematic representation Hybrid molecules were constructed according to the method of Nisonoff and Rivers [7]. For experimental details, see Materials and Methods βME, β-mercaptoethanol

cytes. As can be seen, anti-hRBC-Ab competitively inhibit binding of the hybrid molecules to the erythrocyte membrane (Fig. 2B). Inhibition was not observed when anti-SV-Ab were used instead of anti-hRBC-Ab (not shown). In these experiments only the anti-SV (F(ab')) part of the hybrid molecule was radiolabeled.

Fig. 3 (A and B) shows that the hybrid anti-SV-anti-hRBC preparations can mediate binding of ^{125}I-labeled Sendai virus particles to virus-receptor-depleted cells, namely, neuraminidase-treated human erythrocytes. The degree of binding was dependent upon the amount of hybrid molecules present in the system (Fig. 3A) as well as on the amount of virus particles added (Fig. 3B). It is

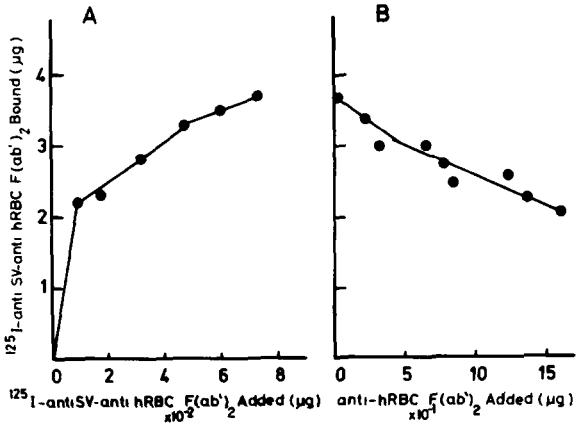


Fig 2 Binding of ^{125}I-anti-SV-anti-hRBC F(ab')$_2$ to desialized human erythrocytes. F(ab')$_2$ fragments from anti-SV-Ab were prepared as described in Materials and Methods, and then radiolabeled with chloramine T and Na^{125}I, as previously described [16], reaching a specific activity of (2.5–5)·10^7 cpm/mg protein ^{125}I-anti-SV F(ab') and anti-hRBC F(ab') fragments were reoxidized to give a hybrid molecules of ^{125}I-anti-SV-anti-hRBC (F(ab')$_2$), as described in Materials and Methods (A) Dependency on hybrid F(ab')$_2$ fragments' concentration. Increasing concentrations of hybrid molecule preparations were incubated at 37°C for 60 min with neuraminidase-treated human erythrocytes (5·10^7 cells), suspended in a final volume of 200 μl of Solution A. At the end of the incubation period, the cells were washed twice with Solution A-BSA and the erythrocyte-associated radioactivity was estimated by a gamma-counter (B) Inhibition of binding by anti-hRBC Human erythrocytes (5·10^7 cells) were incubated with 740 μg of hybrid antibody preparations (^{125}I-anti-SV-anti-hRBC F(ab')$_2$) and increasing amounts of anti-hRBC F(ab')$_2$ All other experimental conditions as described in (A) above Anti-hRBC, F(ab')$_2$ fragment, was obtained from pepsin-digested anti-hRBC-Ab as described in Materials and Methods

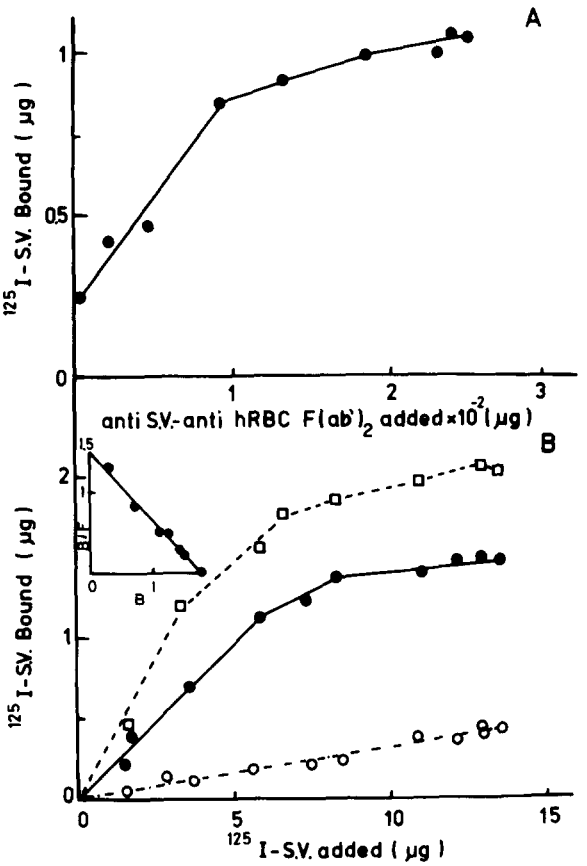


Fig 3 Hybrid antibody-mediated binding of Sendai virus particles to desialized human erythrocytes. (A) Dependency on the F(ab')$_2$ hybrid concentration Hybrid antibody preparations were incubated with desialized human erythrocytes, as described in Fig 1, except that the erythrocytes were washed only once with Solution A-BSA and then twice with Solution A Erythrocytes (0.3 10^7 cells) bearing hybrid molecules were incubated with 100 μg of ^{125}I-labelled Sendai virus (^{125}I-S V) (4 10^5 cpm/mg viral protein) for 60 min at 37°C, in a final volume of 250 μl of Solution A At the end of the incubation period the erythrocytes were washed twice with Solution A-BSA, and erythrocyte-associated radioactivity was estimated by gamma-counter. Unadsorbed ^{125}I-labeled Sendai virus was separated from cell-associated virions by centrifugation through 0.3 M sucrose, as described before [10]. (B) Dependency on virus concentration Hybrid antibody molecules were first attached to desialized human erythrocytes by incubating 280 μg of anti-SV-anti-hRBC F(ab')$_2$ with 0 33 10^7 human erythrocytes, as described in Fig. 2 Desialized human erythrocytes with (●———●) or without (○- - -○) hybrid molecules, or normal untreated erythrocytes (□- - -□) in the absence of hybrid molecules were then incubated with increasing concentrations of ^{125}I-labeled Sendai virus, as described in A. Inset gives the Scatchard plot determined from the curve showing the binding of Sendai virus to desialized human erythrocytes bearing hybrid molecules (●———●) B = μg of added virus bound per 0 33·10^7 cells, B/F = μg of added virus bound per 0 33 10^7 cells per μg of free viral particles

evident from the results in Fig. 3B that the maximum amount of Sendai virus which can be attached to neuraminidase-treated human erythrocytes, via the hybrid antibody preparations, is very close to the amount of virus particles bound at saturation to control, untreated human erythrocytes, A Scatchard plot analysis (insert in Fig. 3B) revealed that maximally about 5.4 μg virus particles can attach to 10^7 neuraminidase-treated cells in the presence of the hybrid molecules. Based on a viral molecular weight of $5 \cdot 10^8$ (see Ref. 9), this will give a maximum of 650–700 viral particles per desialized human erythrocyte. Normal non-treated human erythrocytes bind maximally 800–1200 virus particles/cell (not shown, see Ref. 9). The apparent association constants obtained were about $7 \cdot 10^{-1}$ and $11 \cdot 10^{-1}$ M for binding to neuraminidase-treated human erythrocytes (in the presence of hybrid molecules) and to untreated hRBC, respectively (inset in Fig. 3B and not shown).

The ability of the hybrid anti-SV-anti-hRBC preparations to mediate virus-cell and cell-cell fusion

Previously it has been shown that virus-induced hemolysis of erythrocytes of various origins reflects a process of virus-cell fusion [3,12]. Thus, hemolysis of human erythrocytes by Sendai virus particles, under different conditions, may be considered as a semiquantitative estimation of the fusion process occurring between the virus envelope and the erythrocyte membrane.

Fig. 4 (A and B) and Table I confirm previous observations showing that very little, if any, hemolysis was induced by Sendai virus particles in neuraminidase-treated human erythrocytes, thus indicating that these cells are resistant to fusion with Sendai virus particles [3,12]. Evidently, hybrid antibodies attached to the human erythrocyte membranes greatly enhanced the susceptibility of the neuraminidase-treated human erythrocytes to Sendai virus particles (Fig. 4, Table I).

As can be seen in Table I, about 8% and 60% hemolysis was induced in neuraminidase-treated human erythrocytes in the absence and presence, respectively, of hybrid antibody molecules. The degree of virus-induced hemolysis was highly dependent on the amount of hybrid molecules present in the system (Fig. 4A) as well as on the

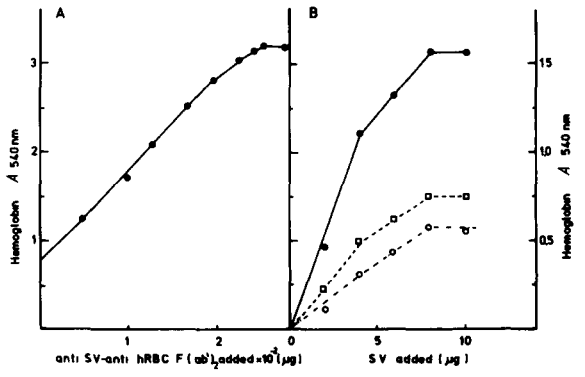


Fig 4. Virus-induced hemolysis of desialized human erythrocytes. (A) The effect of increasing concentrations of anti-SV-anti-hRBC $F(ab')_2$ hybrid molecules. (B) Dependency on virus concentration. Hemolysis was determined by estimating the amount of hemoglobin which was released from the erythrocytes at 540 nm, as described before [8]. All other experimental conditions of attachment of hybrid molecules to desialized human erythrocytes and incubated with Sendai virus particles, as described in Figs 2 and 3. □- - -□, human erythrocytes incubated without or with the following $F(ab')_2$ fragments: anti-SV-anti-hRBC (●———●); anti-SV-anti normal rabbit-IgG ($F(ab')_2$) (○- -○).

amount of virus particles added (Fig. 4B). Stimulation of hemolysis in neuraminidase-treated human erythrocytes by Sendai virus particles was specifically dependent on the presence of anti-SV-anti-hRBC hybrid molecules (Fig. 4B, Table I). Neither hybrid molecules obtained after reoxidation of a mixture containing anti-SV F(ab') and F(ab') of rabbit IgG, nor $F(ab')_2$ fragments consisting of anti-SV-anti-SV or anti-hRBC-anti-hRBC or a mixture of the latter could stimulate virus-induced hemolysis in neuraminidase-treated human erythrocytes (Table I).

The involvement of the viral fusion factor in hybrid-mediated viral-induced hemolysis is evident from the results showing that trypsinization of the virus particles abolished its hemolytic activity (Table I). Proteolytic digestion of Sendai virus particles by trypsin specifically affects the viral fusion factor [13]. Trypsinization of the Sendai virus particles did not affect their ability to agglutinate neuraminidase-treated human erythrocytes bearing attached hybrid antibody molecules (Table I).

Further support for the specific function of the bound hybrid molecules is obtained from the results in Fig. 5. As can be seen, both the anti-hRBC

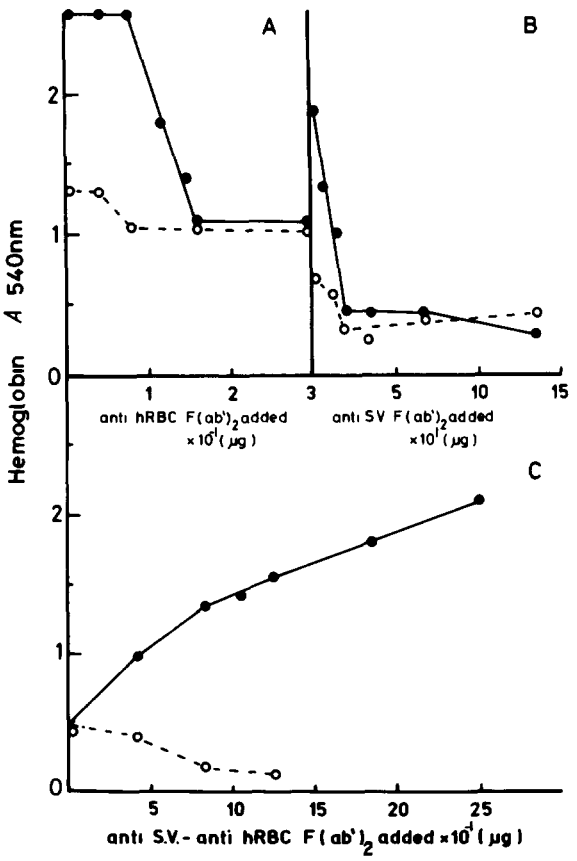
TABLE I

SENDAI VIRUS-INDUCED AGGLUTINATION, HEMOLYSIS AND CELL-CELL FUSION OF DESIALIZED HUMAN ERYTHROCYTES DEPENDENCE ON THE PRESENCE OF anti-SV-anti-hRBC $F(ab')_2$ PREPARATIONS

All the $F(ab')_2$ molecules used were obtained after reoxidation of the appropriate reduced F(ab'), as described under Materials and Methods for obtaining anti-SV-anti-hRBC ($F(ab')_2$) molecules. The various $F(ab')_2$ preparations were incubated with $3.5 \cdot 10^7$ desialized human erythrocytes (see Fig 1 and Materials and Methods) After washing, the different systems were incubated with 5.5 μg Sendai virus (SV) particles. The Sendai virus particles were trypsinized essentially as described before [13]. Agglutination and cell-cell fusion were estimated by observation in a phase microscope [8]. Hemolysis was determined at 540 nm [8].

System	Agglutination	Hemolysis (% of total)	Cell-cell fusion
SV	–	8.1	–
SV + anti-hRBC-anti-hRBC	+ [a]	7.5	–
SV + anti-SV-anti-SV	–	3.5	–
SV + anti-SV-anti-SV + anti-hRBC-anti-hRBC	–	3.8	–
SV + anti-SV-rabbit-IgG	–	9.0	–
SV + anti-SV-anti-hRBC	+	60.0	+
Anti-SV-anti-hRBC	–	1.3	–
Trypsin-SV	–	1.3	–
Trypsin-SV + anti-SV-anti-hRBC	+	1.2	–

[a] Agglutination is due to the presence of the anti-hRBC $F(ab')_2$ fragments



$F(ab')_2$ (Fig. 5A) or the anti-SV $F(ab')_2$ (Fig. 5B), greatly inhibited virus-induced hemolysis of desialized hRBC bearing attached hybrid anti-SV-anti-hRBC $F(ab')_2$ molecules. Fig. 5C shows that in order to mediate functional binding of Sendai virus particles, the hybrid antibody molecules must

Fig. 5. Hybrid-mediated virus-induced hemolysis of desialized human erythrocytes. Inhibition by anti-SV or anti-hRBC $F(ab')_2$ fragments (A) Desialized human erythrocytes were incubated with increasing concentrations of anti-hRBC $F(ab')_2$ in the presence (●———●) or absence (○– –○) of hybrid antibody preparations (228 μg) After washing (for experimental details, see Fig 1), the erythrocytes ($6.25 \cdot 10^7$ cells) were incubated with 10 μg of Sendai virus for 60 min at 37°C Anti-hRBC ($F(ab')_2$) fragment was obtained as described in Fig. 1. Hemolysis was estimated at 540 nm [8] (B) Desialized human erythrocytes were incubated in the presence (●———●) or absence (○–·–○) of hybrid antibody preparations (350 μg). After washing, the erythrocytes (3.75 10^7 cells) were incubated with 15 μg of Sendai virus and increasing concentrations of anti-SV $F(ab')_2$ fragments for 60 min at 37°C Anti-SV $F(ab')_2$) fragments were obtained as described for anti-hRBC $F(ab')_2$ in Fig 1. Hemolysis was determined at 540 nm [8] (C) Human erythrocytes were incubated with increasing concentrations of hybrid antibody preparations. The erythrocytes (1 10^8 cells) were then either incubated immediately with (●———●) or without (○–·–○) washing (for experimental details, see Fig. 1) with 25 μg of Sendai virus particles Hemolysis was determined after 60 min of incubation at 37°C [8]

TABLE II

RSVE-INDUCED HEMOLYSIS OF DESIALIZED HUMAN ERYTHROCYTES

RSVE were obtained after solubilization of intact Sendai virus particles by Triton X-100, as described before [15] All other experimental conditions of attachment of hybrid antibody molecules to desialized human erythrocytes, as well as incubation of erythrocytes bearing antibody with RSVE or Sendai virus (SV) particles, were as described for intact Sendai virus particles in Table I (5.5 μg of viral protein and 3 5 10^7 desialized human erythrocytes)

System	Agglutination	Hemolysis (% of total)	Cell-cell fusion
SV	–	9 3	–
SV + anti-SV-anti-hRBC	+	59.2	+
RSVE	–	11 7	–
RSVE + anti-SV-anti-hRBC	+	71 8	+

be attached to the desialized human erythrocytes prior to the addition of the virus particles. When the anti-SV-anti-hRBC hybrid preparations were added to the neuraminidase-treated human erythrocytes, together with the virus particles, hemolysis was not induced. (Some inhibition of the basal level of hemolysis was noted, Fig. 5C.) This is probably due to the immunoprecipitation of the virus particles by the anti-SV-Ab present in the hybrid molecule preparation.

The results in Table II demonstrate that also reconstituted viral envelopes (RSVE), which are phospholipid vesicles containing only the viral envelope glycoproteins [14], were able to effectively hemolyse neuraminidase-treated human erythrocytes bearing attached hybrid antibody molecules (Table II). Similar to the observation with intact Sendai virus, the induction of hemolysis by RSVE was highly and specifically dependent on the presence of anti-SV-anti-hRBC hybrid molecules.

Discussion

The results of the present work demonstrate that hybrid antibody molecules (anti-SV-anti-hRBC $F(ab')_2$) can mediate functional binding of Sendai virus particles to neuraminidase-treated human erythrocytes. By functional binding we mean that the bound virus particles are able to fuse with the erythrocyte membrane as reflected by the process of virus-induced hemolysis, and to promote cell-cell fusion. These experiments support the view [5,6] that close contact between the Sendai virus envelope and the recipient cell plasma membrane is sufficient to allow the viral fusion factor to exert its effect and to promote fusion between these two membranes. Evidently, the specific cell membrane virus receptor and the viral binding protein (HN glycoprotein) are not essential for functional association between the virus particles and the erythrocyte membranes.

Fig. 6 summarizes schematically the way by

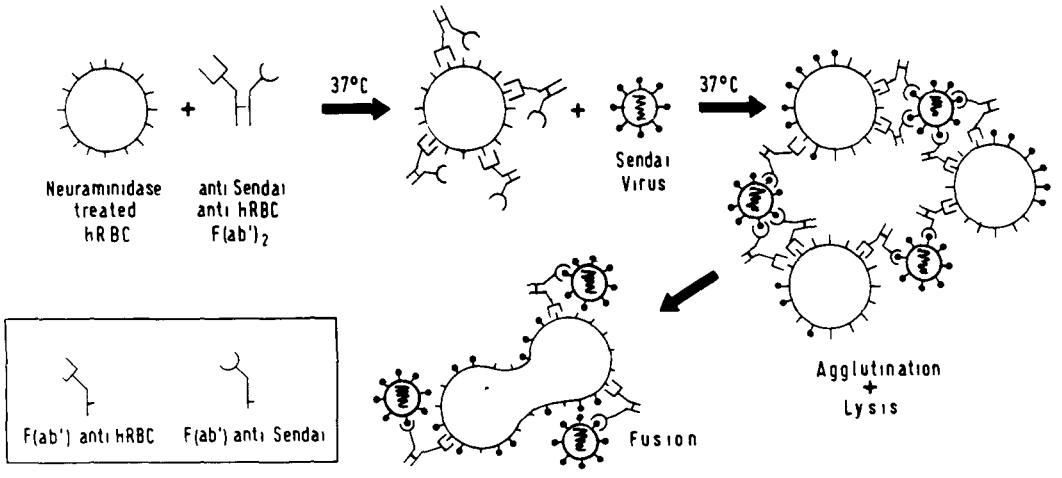


Fig 6. Schematic representation of the use of hybrid anti-SV-anti-hRBC $F(ab')_2$ molecules to mediate functional binding of Sendai virus particles to neuraminidase-treated human erythrocytes.

which hybrid antibody molecules mediate functional binding of Sendai virus particles to the neuraminidase-treated human erythrocytes:

(1) Hybrid antibodies were able to attach to neuraminidase-treated erythrocytes through the anti-hRBC F (ab') portion of the molecule. Binding of the hybrid molecules was competitively inhibited by anti-hRBC $F(ab')_2$.

(2) Cell associated hybrid antibody molecules were able to mediate binding of Sendai virus particles to neuraminidase-treated human erythrocytes. $F(ab')_2$ fragments obtained from anti-SV-Ab or anti-hRBC-Ab did not support binding of Sendai virus particles to these virus-receptor-depleted human erythrocytes.

(3) Virus-induced hemolysis and promotion of cell-cell fusion was competitively inhibited by anti-hRBC $F(ab')_2$.

Functional binding of Sendai virus particles to neuraminidase-treated human erythrocytes was obtained only, if the hybrid antibody molecules were first attached to the erythrocyte surface prior to the addition of the virus particles. In this way most of the free anti-SV $F(ab')_2$ fragments present in the population are removed, leaving the hybrid antibody molecules attached to the erythrocyte membranes.

Obviously, free anti-SV $F(ab')_2$ antibodies, if present, interfere with the binding of the SV particles to the hybrid molecules by immunoprecipitating the added Sendai virus particles. This can be inferred from the experiments showing that simultaneous addition of the reoxidized population of the antibodies ($F(ab')_2$ molecules) and Sendai virus particles to neuraminidase-treated human erythrocytes did not result in induction of hemolysis and promotion of cell-cell fusion.

Potentially, hybrid antibody molecules can be used to mediate the binding of any virus or other molecule to the membrane of living cells lacking receptors for these ligands. The only condition required is that the ligand as well as the cell membrane be antigenic and able to raise specific antibody of high affinity. The present method offers a convenient and interesting tool to study the interaction between animal viruses as well as other macromolecules such as various toxins or polypeptide hormones and eukaryotic cells, without the need for chemical modification of the ligand or the cell surface membrane. Preliminary experiments in our laboratory have already shown that hybrid anti-SV-Ab-anti-cell membrane $F(ab')_2$ molecules could efficiently mediate infection of primary cultured cells by Sendai virus particles.

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