Conjugation of specific antibodies to Sendai virus particles

A new tool for targeting of fusogenic vesicles

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1. INTRODUCTION

Infection of cells by enveloped viruses such as Sendai virus consists of two steps:

(1) Binding of the particles to cell surface receptors, mainly sialoglycoproteins and sialoglycolipids [1,2];

(2) Fusion of the viral envelopes with the recipient cell plasma membranes, with the concomitant injection of the viral nucleocapsid into the intracellular space of the cell [2].

Membrane vesicles made of phospholipid bilayers, in which Sendai virus envelope glycoproteins are incorporated, are fusogenic [3–5]. Such reconstituted envelopes of Sendai virus can serve, as do phospholipid liposomes [6], as a vehicle to introduce macromolecules into living mammalian cells. Indeed, loaded fusogenic vesicles have been used as a vehicle to introduce both proteins [5] and DNA molecules into the appropriate cells [7]. Since sialic acid residues, which serve as virus receptors, are present on plasma membranes of a wide variety of cells, virus particles or its reconstituted envelopes can serve almost as a 'universal syringe'.

For certain purposes, however, and especially for the future use of fusogenic vesicles for drug and gene therapy, it is of paramount importance to develop a fusogenic vesicle which will be able to attach and to fuse with only specific cells. Such 'targeted' fusogenic vesicles can probably be obtained by covalently binding specific antibodies to the virus surface. Attachment of specific antibodies to phospholipid liposomes [8] or to erythrocyte membranes [9] has been reported.

Here, we describe binding of specific antibodies to envelopes of intact Sendai virus particles. Virus particles to which anti-human erythrocyte antibodies are attached, agglutinate and hemolyse neuraminidase-treated human erythrocytes, namely, erythrocytes from which native virus receptors have been removed [1].

2. MATERIALS AND METHODS

Sendai virus was propagated in the allantoic fluid of 10-day-old embryos, and was purified and determined as in [10].

Human erythrocytes (type 0^+, aged 3–6 weeks) were washed with solution A (150 mM NaCl, 20 mM tricine–NaOH (pH 7.4)) as in [11].

Human erythrocytes were treated with neuraminidase (Boehringer, *Cl. perfringens*) as in [12]. Briefly, the cells were washed in isotonic solution containing 150 mM NaCl, 10 mM NaP_i (pH 7.4) then washed twice with a solution of 150 mM NaCl and 40 mM sodium acetate (pH 5.7) which contained 5 mM $CaCl_2$. The pellet obtained was suspended in the above acetate buffer to give a cell suspension of 35–40% (v/v). Then, to each 1 ml cell suspension, 15 μl neuraminidase solution (0.6 U/mg, 1 mg/ml) was added and the suspension was incubated for 2 h at 37°C with gentle shaking. After washing with solution A, the neuraminidase-treated

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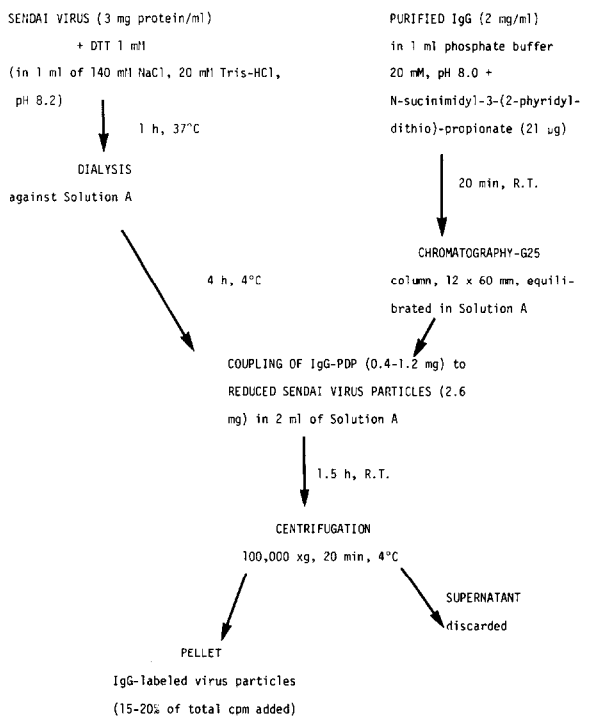

Fig.1. Schematic summary of the method for coupling IgG molecules to Sendai virus particles. See section 2 for composition of solution A

cells were suspended in solution A to give a cell suspension of 35–40% (v/v).

Reduction of Sendai virus with dithiothreitol (DTT) was performed as follows (see also fig.1): A virus suspension (3 mg/ml in solution A) was first sonicated briefly (2–3 min in bath sonicator) then centrifuged to remove large virus clumps (2000 rev./min, 10 min at room temperature). To the supernatant, DTT was added to 1 mM final conc. and the virus mixture was incubated for 1 h at 37°C with gentle shaking. The reduced virus was then dialysed at 4°C for 48 h in Spectrophore 2 tubing against solution A with 3 changes of the buffer.

The coupling procedure between reduced virus and immunoglobulin (IgG) is described in fig.1 and [9]. 21 µg or 42 µg *N*-succinimidyl-3-(2-pyridyl-dithio)-propionate (SPDP), dissolved in dimethyl sulfoxide (1 mg/ml), was mixed under vortexing with 2 mg purified IgG dissolved in 1 ml/20 mM sodium phosphate buffer (pH 8.0). After 20 min incubation, the mixture was chromatographed on Sephadex G-25 columns (12 × 60 mm) to remove the unreacted SPDP. Then, the IgG containing 2-pyridyl disulphide (IgG–PFP) moiety (0.4–1.2 mg), was incubated for 1 h at room temperature with the reduced virus (2.6 mg). The IgG-coupled virus particles were collected by centrifugation and the pellet obtained was suspended in solution A to give 2–3 mg protein/ml. Assays of agglutination, hemolysis and fusion of human erythrocytes promoted by Sendai virus particles were as in [11].

Rabbit human erythrocyte antisera and anti-Sendai virus antisera were prepared as in [13] and the IgG fraction was obtained by fractionation of DEAE-cellulose column [14]. IgG was radiolabeled by $Na^{125}I$ using chloramine T [15].

Human IgG and wheat germ agglutinin were from Miles Yeda, Rehovot; SPDP was from Pharmacia, Uppsala; DTT from Sigma, St Louis MO. Protein was determined by the Lowry method using bovine serum albumin as standard [16].

3. RESULTS

When ^{125}I-IgG–PDP was incubated with DTT-treated Sendai virus particles, a significant percentage of it remained covalently attached to the virus particles. Whereas, when it was incubated

Table 1

Coupling of ^{125}I-IgG–PDP to DTT-reduced Sendai virus particles

IgG:SPDP (molar ratio)	System	^{125}I-IgG coupled (cpm × 10^{-3})
1:10	Intact virus	0.1
	DTT-treated virus	0.1
1:5	Intact virus	0.2
	DTT-treated virus	42
1:10	Intact virus	0.2
	DTT-treated virus	57

In each experiment 1 mg ^{125}I-IgG–PDP (2.3×10^5 cpm/mg) in 1 ml 20 mM Tris–HCl buffer (pH 7.4) was coupled with 2.6 mg Sendai virus particles, as in section 2 and in fig.1. The ^{125}I-IgG coupled represents the radioactivity recovered in the pellet after centrifugation and washing of ^{125}I-IgG–PDP virus particles

Table 2

Biological activities of 'targeted' Sendai virus particles

Treatment of IgG-coupled virus	Human Erythrocytes				
	Untreated			Neuraminidase-treated	
	Agglutination	Lysis (%)	Cell–cell fusion	Agglutination	Lysis (%)
Virus (native)	+ +	60	+ +	–	–
Virus-SH	–	0	n.d.	–	0
Virus-SS–human IgG	–	0	n.d.	–	0
Virus-SS–anti-HE-Ab	+ +	73	–	+	58
Virus-SS-anti-HE-Ab					
Antiviral antiserum	–	0	n.d.	–	0
Phenylmethylsulphonyl fluoride (8 mM)	+ +	0	n.d.	+	0
Dithiothreitol (10 mM)	–	0	n.d.	–	0

Symbols: + Agglutination of 40–60% of the cells; + + Agglutination and fusion of 60–80% of the cells

Abbreviations: virus-SH, reduced and dialysed Sendai virus particles; virus-SS–human IgG, as above but coupled to human IgG; virus-SS–anti-HE-Ab, as above but coupled to anti-human erythrocyte antibody; n.d., not determined

Reduced and/or coupled Sendai virus particles (50 μg/system) were incubated with 2.5% (v/v) of human erythrocytes suspension at 37°C for 60 min. Treatments of IgG-coupled virus were performed by incubating 100 μg virus-SS–anti-HE-Ab suspended in solution A, for 1 h at 37°C, with the specified substances. Lysis of human erythrocytes by intact native virus particles was performed with 3 μg virus particles/system, while cell–cell fusion was induced with 20 μg/system. Cell agglutination and fusion were examined by phase microscopy [12]

with non-reduced virus particles, negligible amounts of IgG were conjugated with virus particles (table 1). In addition, no binding to the virus particles was observed when unmodified IgG was incubated with the virus. Significant binding was observed when, under the experimental conditions used, the IgG-PDP was obtained by incubation of SPDP and IgG at a molar ratio of IgG:SPDP 1:5–1:10.

The DTT-treated and dialysed virus particles did not agglutinate human erythrocytes (see table 2). The lectin, wheat germ agglutinin (WGA), was used to mediate binding of the DTT-treated virus to human erythrocytes [3,5] and, in its presence, hemolysis was induced when the DTT-treated virus was incubated with human erythrocytes at 37°C (fig.2). Induction of hemolysis under these conditions indicates the presence of a biologically active F-glycoprotein [2–5].

Table 2 shows that virus particles, to which anti-erythrocyte antibodies are attached, agglutinate neuraminidase-treated erythrocytes as well as untreated human erythrocytes. These results clearly indicate that the agglutination is mediated by the anti-erythrocyte antibody. No agglutination was observed when human erythrocytes were incubated with either DTT-treated virus or with virus particles to which human IgG was coupled. Virus particles to which human IgG was coupled were precipitated by mouse anti-human IgG antibody (not shown). Virus particles with attached anti-human erythrocyte antibodies hemolysed untreated or neuraminidase-treated human erythrocytes (table 2). Treatment of these virus particles with either phenylmethylsulfonyl-fluoride (PMSF) or DTT completely blocked the hemolytic activity.

As opposed to intact virus particles, IgG-coupled virus particles failed to induce cell–cell fusion

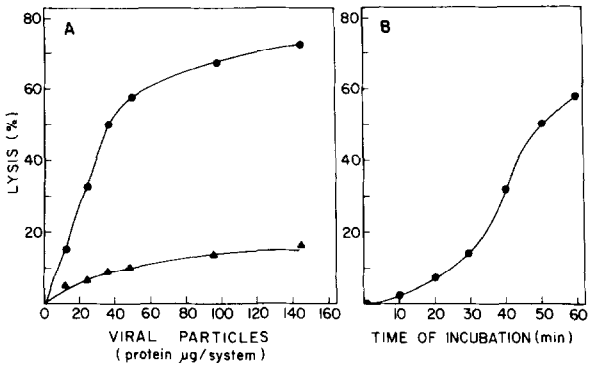

Fig.2. (A) Hemolysis as a function of viral particle concentration. WGA (2–4 µg) was added to a mixture of human erythrocytes and increasing amounts of DTT-reduced viral particles obtained after 3 days of dialysis, as in section 2 and fig. 1 (▲). Increasing amounts of viral particles coupled to antihuman erythrocytes antibody incubated with neuraminidase-treated human erythrocytes, as described in table 2 (●). (B) Hemolysis induced by Sendai virus particles coupled to anti-human erythrocyte antibodies as a function of time. Hemolysis was induced by 50 µg virus, as described in table 2 and estimated as in [11].

(table 2). 'Targeted' virus particles were less active in inducing hemolysis than intact virus particles. About 50 µg antibody-coupled virus particles were required to obtain almost the same degree of hemolysis than was induced by 3 µg intact virus particles (table 2).

Fig.2 shows that the hemolysis induced by virus particles to which anti-human erythrocyte antibodies were coupled, was dependent upon the concentration of the virus particles (fig.2A) and on the incubation time at 37°C (fig.2B). Addition of WGA to DTT-treated virus particles, although it caused agglutination of the cells, resulted in a much lower degree of hemolysis at 37°C than that obtained with the 'targeted' virus particles (fig.1A).

4. DISCUSSION

Virus-induced agglutination and hemolysis of human erythrocytes from which the virus-receptor has been removed by neuraminidase treatment, was used here as a model system to study the activities of Sendai virus particles to which specific antibodies were attached.

Agglutination reflects a process of virus–cell binding, while hemolysis occurs only after fusion of virus envelope with erythrocyte membrane [4,17]. Sendai virus particles containing an active hemagglutinin/neuraminidase glycoprotein (HN) but inactive fusion factor (F-protein), although they agglutinate human erythrocytes, they fail to hemolyse them [2,17,18]. Indeed, inhibitors of proteolytic enzymes such as PMSF were shown to completely inhibit Sendai virus-induced hemolysis and cell fusion but not agglutination of human erythrocytes [18].

Our results indicate that:

(i) Specific antibodies can be covalently be attached to Sendai virus outer surface;

(ii) In IgG-coupled virus particles, both the antibody and the virus remain at least partially active, as shown by their ability to agglutinate (bind to) and to hemolyse (fuse with) [2,17] human red blood cells from which the native virus receptor (sialic acid residues) was removed.

Incubation of Sendai virus particles with low concentrations of DTT resulted in reversible reduction of the F glycoprotein but irreversible reduction of HN [19,20]. This work shows that reduced virus particles from which excess DTT was removed by prolonged dialysis, failed to agglutinate human erythrocytes but hemolysed them in the presence of WGA, thus indicating the presence of active F but inactive HN.

We have used such DTT-reduced virus particles, i.e., virus with free SH (supposedly of the viral HN) for anchoring specific antibodies. This was done by first obtaining the PDP-derivatives of IgG [9] which were then coupled to DTT-treated virus particles.

Hemolysis of human erythrocytes observed by IgG-coupled Sendai virus particles, which is subject to inhibition by antiviral antibodies and by phenylmethylsulfonyl-fluoride, reflects probably a process of virus–cell fusion [17,18]. Experiments using electron microscopic techniques are underway to confirm this view. However, it should be emphasized that the 'targeted' virus exhibits ~1–10% (table 2; not shown) of the hemolytic activity of the native, untreated virus when incubated with normal human erythrocytes. This may be due to:

(i) Not all the reduced F molecules undergo reoxidation;

(ii) The HN molecules, besides mediating binding, also participate in the fusion process [17];
(iii) The size of IgG avoids close association between the viral envelope and the cell membrane.

Although the virus particles to which anti-human erythrocyte antibodies are attached promote agglutination and hemolysis, they fail to induce cell–cell fusion, a process which is promoted by native virus (table 2; [11]. This could also be due to the molecular size of the coupled IgG which may prevent tight contact between adjacent membranes, a step that is a prerequisite for the induction of cell–cell fusion [2,10].

Substituting IgG with $F(ab)_2$ may provide a 'targeted' virus which will be able to promote cell–cell fusion. However, it should be emphasized that for the use of virus envelopes as biological carriers, an ideal vehicle will be a fusogenic vesicle that will fuse with the cells plama membrane without the induction of cell–cell fusion.

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