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Difference in Capacity of Sendai Virus Envelope Components to Induce Cytotoxic T Lymphocytes in Primary and Secondary Immune Responses

YASUO FUKAMI,* YASUHIRO HOSAKA, YUKO YASUDA, AND JOSÉ A. BONILLA

Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Suita City, Osaka 565, Japan

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Studies were made on the abilities of Sendai virus envelope components to induce primary and secondary generations of virus-specific cytotoxic mouse T lymphocytes (CTL). The primary CTL response in BALB/c mice was induced by reassembled envelope particles that had fusion activity but not by envelope glycoproteins without fusion activity, although both preparations induced a humoral immune response. Reconstitution of membrane-bound envelope proteins from envelope glycoproteins with lipids restored the fusion activity and the capacity to induce CTL. Target cells susceptible to virus-specific CTL could be induced by reassembled envelope particles, but not by envelope glycoproteins or LLC-MK_2 cell-grown Sendai virus, neither of which had fusion activity. On the other hand, all the viruses and envelope components tested were found to stimulate a virus-specific CTL response in the in vitro secondary generation of CTL from virus-primed spleen cells. These results suggest that Sendai virus fusion activity is involved in primary induction of the CTL response as well as in target cell formation, but that it is not essential for secondary stimulation of the CTL response.

Murine cytotoxic thymus-derived lymphocytes (CTL) specific for virus-infected syngeneic target cells can recognize both viral and histocompatibility antigens on target cells (4). The mechanism of recognitition of antigenic determinants is unknown, but physical association between viral and histocompatibility antigens has been demonstrated (12, 16), and this complex is thought to be the target antigen recognized by CTL (17). In the case of Sendai virus, ultraviolet (UV) light- or β-propiolactone-inactivated virus can induce the target antigens recognized by CTL (11, 14, 17-19), indicating that infectivity of the virus is not necessary for formation of target antigens. Recently, it was reported that the fusion activity of Sendai virus was involved in target cell formation (6, 18, 19).

Inactivated Sendai virus has also been shown to induce primary and secondary CTL responses (17-19). However, it is not known whether fusion activity is required for induction of the CTL response. In this study, we examine this problem using Sendai virus envelopes, the fusion activity of which can be reconstituted reversibly by association of envelope glycoproteins without fusion activity and lipids. In vivo primary sensitization with viral components was assayed by measuring the secondary generation of CTL from primed spleen cells in culture. Using envelope components with and without fusion activity, we showed that only those with fusion activity were effective in primary induction of the CTL response and in target cell formation. Differences between the primary generation of CTL in vivo and the secondary generation of CTL in vitro by envelope components were also investigated.

MATERIALS AND METHODS

Virus. Sendai virus, Z strain, was used throughout the present investigation. The virus was grown in the chorioallantoic cavity of embryonated eggs. The virus was purified by differential centrifugation of infected chorioallantoic fluid and then velocity sedimentation in sucrose gradients (7). Noninfectious Sendai virus with no fusion activity was produced in LLC-MK_2 cells, an established line of rhesus monkey kidney cells, inoculated with egg-grown Sendai virus. This virus, abbreviated as LLC-SV, was used after partial purification by differential centrifugation at 2,000 $\times$ g for 20 min and 40,000 $\times$ g for 30 min.

Irradiation of virus with UV light. The purified virus (10,000 hemagglutination units [HAU] per ml) was placed in a plastic dish (Falcon 3002) (fluid depth, 1 to 2 mm) and irradiated from a distance of 20 cm with UV light from a 15-W germicidal lamp for 15 to 20 min. The infectivity after irradiation was less than

10 50% egg infectious doses per ml. This virus was abbreviated UV-SV.

Preparation of envelope components. Virus envelopes were solubilized with 0.25% Nonidet P-40 (NP-40). Reassembled envelope particles (REP) were prepared from the solubilized envelopes by dialysis of the preparation in Spectrapor membrane tubing 2 (Spectrum Medical Industries, Inc.) against phosphate-buffered saline for 3 days (10) to remove the detergent. The preparation was then centrifuged at 50,000 × *g* for 30 min, and the precipitate was used for experiments. Envelope glycoproteins (EP) and top lipids were separated from the NP-40-solubilized envelopes by CsCl equilibrium centrifugation (10). Envelope particles with fusion activity were reconstituted by dialysis of a mixture of EP and the top lipids. The preparation was then centrifuged at 50,000 × *g* for 30 min, and the precipitate was used as reconstituted envelope (EP + lipids). REP and EP both contained two Sendai virus glycoproteins, hemagglutinin-neuraminidase and fusion protein (2), as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8, 10).

Infectivity assay. The 50% egg infectious dose value was determined by measuring the number of hemagglutinin-producing eggs 3 days after inoculation of 10-fold serial dilutions of virus samples. Hemadsorption focus-forming units were determined by counting foci formed by guinea pig erythrocytes on L929 cell sheets (Falcon dish 3001) 20 to 24 h after inoculation of serial dilutions of virus.

Hemagglutination assay. Hemagglutination was measured in HAU by Salk's pattern method (15).

Hemagglutination inhibition assay. Immunized mouse serum was diluted twofold serially, and the reciprocal of the greatest dilution capable of inhibiting hemagglutination by virus (4 HAU) was taken as the hemagglutination inhibition titer. Before the hemagglutination inhibition test, sera were treated with trypsin and KIO_4 to destroy nonspecific inhibitors (3).

Hemolytic activity assay. Samples of 0.1 ml of virus or envelope components were mixed with 2 ml of 2% chicken erythrocyte suspension and incubated at 37°C for 60 min. The hemolytic activity was expressed as the release of hemoglobin measured in terms of the optical density at 540 nm.

Target cells. Samples of 3×10^6 to 7×10^6 mastocytoma P815 cells grown in the peritoneal cavity of DBA/2 (H-2^d) mice were labeled in vitro with ^{51}Cr (200 μCi/ml) at 37°C for 1.5 to 2 h. The cells were then washed and infected with virus at a multiplicity of about 50 HAU/10^6 cells, unless otherwise stated. After adsorption at 37°C for 30 min and washing, the infected target cells were suspended in RPMI1640 (Flow Laboratories) supplemented with penicillin and streptomycin, containing 10% heat-inactivated fetal calf serum (Flow Laboratories) (RPMI10), and dispensed in flat-bottomed wells (Microtest II, Falcon 3040) at 2×10^4 cells/100 μl per well. Uninfected target cells were prepared similarly. Preparation of target cells inoculated with UV-SV, LLC-SV, or envelope components was done with the inoculation doses shown in Table 5.

Immunization of mice and in vitro secondary generation of cytotoxic effector cells. Male BALB/c (H-2^d) mice, 6 to 20 weeks old, were inoculated intraperitoneally (i.p.) with virus or envelope components. The immunized mice were killed 6 to 8 days later, and the primary generation of CTL was measured in terms of cytotoxicity of primed spleen cells, after treating the cells with 0.15 M NH_4Cl to remove erythrocytes.

Spleen cells of mice primed with virus or envelope components, without NH_4Cl treatment, were cultured in vitro in RPMI1640 with penicillin and streptomycin, containing 15% heat-inactivated fetal calf serum and supplemented with 5×10^{-5} M 2-mercaptoethanol and 10 mM HEPES (*N*-2-hydroxylethylpiperazine-*N'*-2-ethanesulfonic acid), in Linbro 24 flat-bottomed wells (Linbro Chemical Corp.) at 4×10^6 to 6×10^6 cells/2 ml per well, at 37°C in a CO_2 incubator. Since HEPES was found not to be essential for the cultures, it was omitted in later experiments. Stimulating antigens were added to the cultures of spleen cells at the beginning of incubation. After 5 days, the stimulated spleen cells were harvested and washed, and their cytotoxicity was assayed for secondary CTL generation.

Cytotoxicity assay. Sensitized spleen cells in 200 μl of RPMI10 were added to target cell cultures in a ratio of effector to target cells of 20:1, unless otherwise indicated. The effector number was adjusted on the basis of viable cells, which were measured by the trypan blue exclusion method. After incubation at 37°C for 6 h, 200 μl of culture fluid was carefully removed from the wells, and its radioactivity was counted in a gamma scintillation counter. The percentage of specific lysis was calculated from the following formula: % specific lysis $[E - N]/[T - N] \times 100$, where E represents ^{51}Cr release from experimental wells, N represents ^{51}Cr release from wells with normal BALB/c spleen cells, and T represents the maximum ^{51}Cr release from wells treated with 1% Triton X-100. The percentage of specific lysis was calculated from average values for four wells.

Incubation of target cells with effectors started 1 h after inoculation of virus or envelope components on target cells and ended at 7 h. The extent of spontaneous ^{51}Cr release after the incubation period from virus-infected and uninfected target cells was approximately 25 and 21%, respectively.

Treatment of immune spleen cells with antiserum and complement. Immune spleen cells (2×10^7) were incubated in 2.5 ml of RPMI1640 with 10% rabbit anti-mouse T cell serum (Cedarlane Laboratories Ltd.) or rabbit anti-mouse immunoglobulin serum (Capel Laboratories Inc.) at room temperature for 1 h, and then 2.5 ml of 10-fold-diluted rabbit complement (Miles Laboratories Inc.) was added. The reaction mixture was incubated at 37°C for 1 h. The treated cells were then washed and suspended in RPMI10 for cytotoxicity assay.

RESULTS

Fusion activity of viral antigens used. Table 1 shows the infectivities and hemolytic activities of the preparations of Sendai virus and envelope components used for primary immunization and secondary stimulation. Since hemolytic activity of Sendai virus is thought to be

an expression of the envelope fusion activity of the virus (9), viruses and envelope components with hemolytic activity are described as having fusion activity. Thus, egg-grown Sendai virus had fusion activity, even after its infectivity was abolished by UV irradiation. LLC-MK_2 cell-grown Sendai virus had low infectivity and no detectable fusion activity. REP had fusion activity. EP had no fusion activity, but when they were reconstituted with lipids into membraneous particles, fusion activity was regained.

In vivo immunization with envelope components, followed by in vitro stimulation. BALB/c mice were immunized i.p. with REP or EP, and 8 days later their spleen cells were separated. Part of the preparation was assayed for cytotoxicity against Sendai virus-infected P815 target cells, whereas the rest was cultured in vitro in the presence of REP or EP to stimulate secondary CTL generation. As positive controls, some mice were immunized with live or UV-SV and then treated similarly.

TABLE 1. *Infectivities and hemolytic activities of the Sendai viruses and envelope components used*

Viral antigens	HAU/ml	Infectivity/ml		Hemolytic activity[a] (OD_{540})
		HAD-FFU[b]	EID_{50}[b]	
Sendai virus	2,000	$>6 \times 10^8$	$10^{9.5}$	0.32
UV-SV	2,400	ND[c]	$<10^1$	0.50
LLC-SV	3,200	2×10^5	ND	0.01
REP	6,400	ND	$<10^1$	1.07
EP	3,200	ND	$<10^1$	0.01
(EP + lipids) A[d]	1,200	ND	$<10^1$	0.20
(EP + lipids) B[d]	1,200	ND	$<10^1$	0.10

[a] Hemolytic activity was measured as described in the text. OD_{540}, Optical density at 540 nm.

[b] HAD-FFU, Hemadsorption-focus-forming units; EID_{50}, 50% egg infectious dose.

[c] ND, Not determined.

[d] (EP + lipids), Envelope particles reconstituted from a mixture of EP and lipids. A and B were preparations made from mixtures of EP plus 0.5 and 2 times the equivalent amount of lipids, respectively. The amounts of total lipids and glycoproteins of NP-40-solubilized envelopes of Sendai virus were regarded as equivalent (8).

Table 2 shows results on the primary generation of CTL. High CTL activity was induced by live virus, low CTL activity was induced by UV-SV and REP, and scarcely any CTL activity was induced by EP. Table 2 also shows results on the secondary generation of CTL. Live virus and UV-SV both induced high CTL activity, REP induced moderate CTL activity, whereas EP induced no activity. We also examined the humoral immune responses with the viruses and envelope components by measuring the hemagglutination inhibition titers of immunized mice sera. Mice were immunized i.p. with live Sendai virus (200 HAU per mouse), UV-SV (2,400 HAU per mouse), REP (200 μg per mouse), or EP (200 μg per mouse). Two weeks later, the hemagglutination inhibition titers were found to be 256 with all the antigens used, indicating that all the antigens, including EP, were immunologically competent. Therefore, the failure of EP to induce CTL is not due to degradation or inactivation of its antigenicity. The results suggest that only viruses and envelope components with fusion activity can prime the cell-mediated immune response in BALB/c mice. To confirm this, we reconstituted membrane-bound glycoproteins from EP and lipids (10), thus restoring their fusion activity (Table 1), and then we tested their ability to induce cell-mediated immune response. In this experiment, Sendai virus,

TABLE 2. *In vivo primary CTL generation and in vitro secondary CTL generation with Sendai virus or its envelope components*[a]

Antigens for primary immunization	Dose per mouse (HAU)	Specific lysis[b] of Sendai virus-infected P815 targets (%)	Antigens for secondary stimulation	Dose per well	Specific lysis[c] of Sendai virus-infected P815 targets (%)
Sendai virus	200	74.2 ± 2.3[d]	Sendai virus	4 HAU	67.2 ± 3.7
				40 HAU	75.5 ± 3.4
UV-SV	2,400	14.9 ± 3.0	UV-SV	16 HAU	67.9 ± 2.7
				160 HAU	70.7 ± 4.4
REP	4,800 (200 μg)[e]	10.4 ± 1.6	REP	10 μg	12.8 ± 3.8
				40 μg	44.5 ± 2.6
EP	2,000 (200 μg)[e]	2.0 ± 0.7	EP	10 μg	5.8 ± 2.7
				40 μg	4.7 ± 4.3

[a] BALB/c mice were immunized i.p. with virus or envelope components, and 8 days later their spleen cells were prepared. Some of the cells were used for assay of primary CTL generation and the others were cultured in vitro in the presence of the homologous antigen for 5 days for assay of secondary CTL generation.

[b] Effector-to-target ratio of 40:1.

[c] Effector-to-target ratio of 20:1.

[d] Mean ± standard error of the mean.

[e] Protein content in parentheses.

a more effective stimulator than envelope components, as shown below (see Fig. 1 and 2), was also used as stimulating antigen for secondary CTL generation. Table 3 shows that high virus-specific CTL activity was induced secondarily with Sendai virus from spleen cells primed with REP or reconstituted envelopes (EP + lipids), but no significant virus-specific CTL was induced from spleen cells primed with EP or lipid alone. When unprimed spleen cells were cultured with Sendai virus, no significant virus-specific CTL was generated, indicating that in vitro priming with Sendai virus did not occur in this experiment. These results strongly support the idea that only preparations with fusion activity can prime the cell-mediated immune response.

In vitro secondary CTL generation. We next examined the abilities of Sendai viruses and envelope components to induce secondary CTL generation from virus-primed spleen cells. BALB/c mice were primed with live Sendai virus, and 3 weeks later their spleen cells were cultured for 5 days with various amounts of live Sendai virus, UV-SV, LLC-SV, REP, or EP. Figure 1 shows that a wide range of doses of live virus, UV-SV, and LLC-SV induced secondary CTL with a high cytotoxicity to Sendai virus-infected P815 cells. The presence or absence of virus infectivity and fusion activity did not seem to influence this secondary CTL stimulation. Similarly, both REP and EP induced CTL with a lower, but definite, cytotoxicity, irrespective of whether they had fusion activity (Fig. 2). These results demonstrate that Sendai virions are good inducers of secondary CTL generation in vitro, like virus-coated cells (5, 11, 18), and that Sendai virus envelope components can also induce secondary CTL generation. The cytotoxicities against uninfected P815 targets were much lower than those against Sendai virus-infected target cells (Fig. 1 [inset] and 2). Influenza virus (PR8 strain, 10 HAU per well) did not stimulate cytotoxicity against either uninfected cells or Sendai virus-infected cells (data not shown). Thus the CTL induced with Sendai virions or envelope components was specific for Sendai virus.

Table 4 shows that the cytotoxicity induced with Sendai virus was largely abolished by treating the effector cells with anti-T cell serum and complement, but not with anti-immunoglobulin serum and complement, implying that the cytotoxic cells generated in this system were largely T lymphocytes. This result is consistent

TABLE 3. *In vitro secondary CTL generation from mouse spleen cells primed with reconstituted envelope particles*[a]

Primary immunization	Dose per mouse (HAU)	Secondary stimulation	E:T[b]	Specific lysis of P815 cells (%)	
				Sendai virus-infected	Uninfected
EP	3,400 (40 μg)[c]	EP	20:1	−0.4 ± 1.1[d]	3.8 ± 0.8
		SV	5:1	11.4 ± 1.0	8.3 ± 1.0
			20:1	25.9 ± 0.5	16.9 ± 4.9
(EP + lipids) A[e]	580 (40 μg)	A[e]	20:1	3.6 ± 0.4	5.0 ± 0.5
		SV	5:1	59.0 ± 1.6	4.5 ± 0.7
			20:1	80.3 ± 1.7	12.5 ± 0.9
(EP + lipids) B[e]	200 (40 μg)	B[e]	20:1	16.1 ± 1.7	4.1 ± 0.7
		SV	5:1	78.0 ± 1.5	3.9 ± 1.1
			20:1	89.4 ± 2.7	16.2 ± 1.4
REP	570 (40 μg)	REP	20:1	15.5 ± 1.6	1.3 ± 0.2
		SV	5:1	45.2 ± 2.1	7.4 ± 1.6
			20:1	80.7 ± 2.3	16.2 ± 1.6
Lipids	1,200 (virus eq.)[f]	Lipids[g]	20:1	5.5 ± 1.2	7.4 ± 0.8
		SV	20:1	20.0 ± 1.2	14.2 ± 0.6
Unprimed		SV	5:1	7.1 ± 0.8	3.5 ± 1.3
			20:1	17.0 ± 1.9	6.6 ± 0.4

[a] BALB/c mice were immunized i.p. with envelope components, and 6 days later their spleen cells were prepared and cultured in the presence of the homologous antigen (10 μg per well) or purified Sendai virus (1 HAU per well) for 5 days.

[b] Effector-to-target ratio.

[c] Protein content in parentheses.

[d] Mean ± standard error of the mean.

[e] (EP + lipids), Reconstituted envelopes. See Table 1, footnote *d*, for details.

[f] 1,200 HAU virus equivalent (virus eq.) indicates the amount of top lipid (10) prepared from 1,200 HAU of Sendai virus.

[g] 240 HAU virus equivalent was added per well.

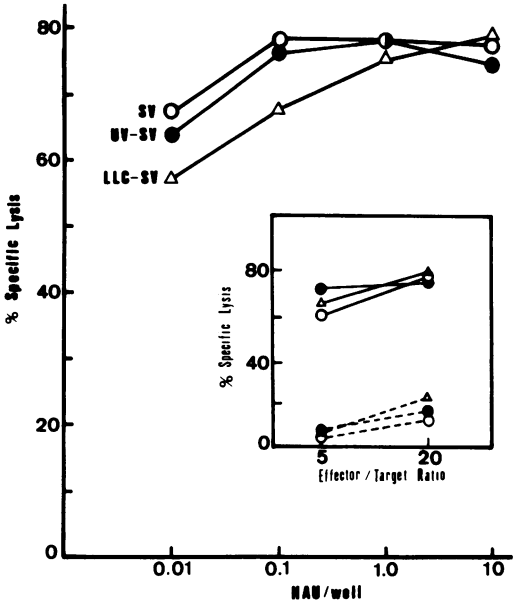

FIG. 1. *In vitro secondary CTL generation with Sendai viruses from virus-primed mouse spleen cells. BALB/c mice were immunized i.p. with live Sendai virus (80 HAU per mouse), and 3 weeks later their spleen cells were isolated and cultured in vitro for 5 days in the presence of various amounts of purified Sendai virus, UV-SV, and LLC-SV (UV irradiated), respectively. Cytotoxicity assay was carried out as described in the text. Values are for percentage of specific lysis of Sendai virus-infected P815 cells. The effector-to-target ratio was 20:1. (Inset) Cytotoxicities of spleen cells stimulated by 1 HAU of virus per well were assayed on Sendai virus-infected (solid line) or uninfected (broken line) target cells at two different effector-to-target ratios. Cytotoxicities of unstimulated spleen cells were 3.8 and 2.9% for SV-infected and uninfected target cells, respectively, at an effector-to-target ratio of 20:1. Symbols: ○, live Sendai virus; ●, UV-irradiated Sendai virus; △, LLC-MK$_2$ cell-grown Sendai virus. Standard errors of the means were less than 2.2%.*

with those of other investigators (6, 11, 17–19).

Formation of target cells with Sendai viruses and envelope components. The preparations of Sendai virus and envelope components used for CTL generation were tested for ability to make target cells susceptible to cell-mediated lysis. Table 5 shows that viruses and envelope components with fusion activity induced susceptible targets, but those without fusion activity (LLC-SV and EP) did not.

DISCUSSION

Using Sendai virus envelope particles reconstituted from viral glycoproteins and lipids, we showed that envelope particles with fusion activity could induce a primary cell-mediated immune response in BALB/c mice.

EP with no fusion activity did not induce either susceptible targets or the primary CTL response, although it induced a humoral immune response. This is probably because the viral antigens of EP did not become integral parts of cell membranes, since EP has no fusion activity. If the integration of viral antigens into the host cell membrane results in a physical association between viral and histocompatibility antigens as reported (12, 16), this complex may be required for both primary CTL induction and formation of target cells. Thus, the cell-mediated immune responses in mice induced by UV- or β-propiolactone-inactivated Sendai virus (5, 14, 17–19) or even by β-propiolactone-inactivated rabies virus (21) seem to be explained on the basis of virus-associated envelope fusion activity. Rhabdoviruses, to which rabies virus belongs, are supposed to fuse with the cell membrane at the beginning of infection (20).

On the other hand, in in vitro secondary CTL generation from Sendai virus-primed spleen

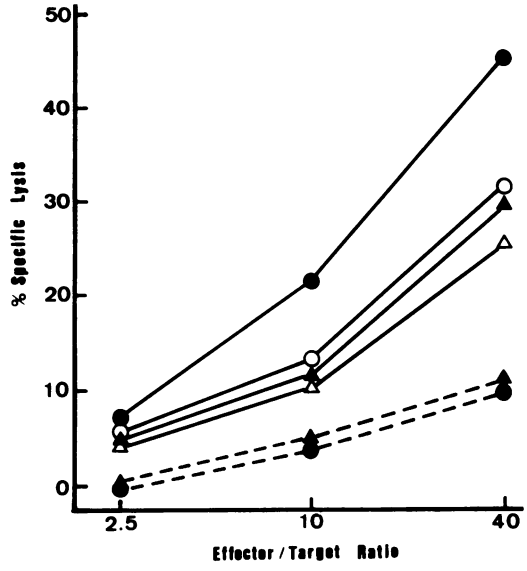

FIG. 2. *In vitro secondary CTL generation with envelope components from virus-primed mouse spleen cells. Immune spleen cells were prepared and assayed as in Fig. 1, except that in vitro stimulation was done with different doses of REP and EP. Symbols: solid line, cytolysis of Sendai virus-infected P815 cells; broken line, cytolysis of uninfected P815 cells; ●, REP (5 μg per well); ○, REP (0.5 μg per well); ▲, EP (5 μg per well); △, EP (0.5 μg per well). Standard error of the means were less than 0.84, 1.34, and 1.49% at the effector-to-target ratio of 2.5:1, 10:1, and 40:1, respectively. Cytolysis of virus-infected target cells was significantly higher than that of uninfected cells ($P < 0.01$ at 2.5:1 and 10:1; $P < 0.001$ at 40:1).*

TABLE 4. *Specification of cytotoxic effectors secondarily generated with Sendai virus from virus-primed spleen cells*[a]

Treatment of effectors	E:T[b]	Specific lysis of P815 targets (%)	
		Virus-infected	Uninfected
None	20:1	83.9 ± 2.1[c]	29.2 ± 0.8
	5:1	81.4 ± 1.2	8.2 ± 0.7
Anti-T cell serum + complement	20:1	2.8 ± 0.6	ND[d]
Anti-IgG[e] serum + complement	20:1	78.4 ± 1.7	ND

[a] Spleen cells were obtained from BALB/c mice primed i.p. with live Sendai virus (80 HAU per mouse) 3 weeks previously. The cells were cultured in vitro for 5 days in the presence of Sendai virus (1 HAU per well). Treatment of spleen cells with antiserum plus complement was carried out as described in the text.

[b] Effector-to-target ratio. Viabilities of effector cells after the treatments were 76.5% for anti-T + complement, and 52.4% for anti-IgG + complement taking that of untreated effectors as 100%.

[c] Mean ± standard error of the mean.

[d] ND, Not determined.

[e] IgG, Immunoglobulin G.

TABLE 5. *Cytolysis of target cells inoculated with Sendai viruses or envelope components*[a]

Inoculum	Dose (HAU/10^6 cells)	Specific lysis of P815 target cells (%)
Expt 1		
Sendai virus	200	64.0 ± 0.1[b]
UV-SV	200	59.6 ± 3.5
LLC-SV	50	1.1 ± 0.4
	200	−0.4 ± 0.9
Expt 2		
Sendai virus	200	73.2 ± 1.2
REP	200	5.4 ± 1.4
	800	10.8 ± 1.8
	3,200	18.2 ± 3.5
EP	800	7.3 ± 0.5
	3,200	7.1 ± 0.5

[a] Effectors were induced secondarily in vitro by live Sendai virus from spleen cells primed with virus, under similar conditions to those in Table 4, and added to P815 target cell cultures at an effector-to-target ratio of 5:1. Percentage of specific lysis of normal P815 cells (2.3% in experiment 1 and 8.2% in experiment 2) was subtracted.

[b] Mean ± standard error of the mean.

cells, all the preparations of Sendai virus and envelope components tested were effective, irrespective of whether they had fusion activity (Fig. 1 and 2). These results suggest that the fusion activity is not essential for in vitro secondary CTL stimulation. Similar results have been reported for influenza virus with no fusion activity (1): inactivated influenza virus induced in vitro secondary CTL generation from live virus-primed mouse spleen cells, but not in vivo primary CTL generation. Furthermore, the hemagglutinin of influenza virus, an envelope component, has been shown to induce hemmagglutinin type-specific secondary CTL from virus-primed mouse spleen cells (23).

Recently, Finberg et al. (5) reported that in vitro secondary stimulation of virus-specific CTL was restricted by viral and H-2 histocompatibility antigens. Assuming that H-2 compatibility is required for the secondary generation of virus-specific CTL, it seems possible that viral antigens with no fusion activity attach to the cell membrane and interact in some way with H-2 antigens to stimulate secondary CTL generation. However, it is still possible that in vitro secondary CTL generation is not restricted by H-2 antigens. At present we do not know which of these possibilities is the better explanation for our finding that LLC-MK_2 cell-grown Sendai virus and EP without fusion activity could induce secondary CTL generation (Fig. 1 and 2).

Our results raise the problem of why UV-inactivated Sendai virus and REP were less effective inducers than intact virus for primary generation of CTL (Table 2), although they had high fusion activity (Table 1). Recently, Zinkernagel et al. (22) demonstrated that antigen-presenting cells of the peripheral lymphoreticular system triggered mature T cells and H-2I-restricted helper T cells. It seems likely that these processes generally occur in the cell-mediated immune responses by viruses. Inoculation i.p. of infectious Sendai virus probably results in virus replication in mouse peritoneal cells, to follow these steps. However, UV-SV and REP, which must function as antigen-transferring particles, may not be so efficient in making antigen-presenting cells and inducing helper T cells as intact virus.

REP induced target cells susceptible to cell-mediated lysis (Table 5), although it was a less effective inducer than virions. This result is in agreement with the idea that fusion activity of Sendai virus is involved in formation of target antigens recognized by CTL (6, 11, 17–19). The reason why REP was less effective than virions for target cell formation may be attributable to the differences in physical size, integrity (REP has glycoprotein spikes on both sides of its membrane), and membrane fragility (8–10). The finding that noninfectious Sendai virus produced in LLC-MK_2 cells could not make susceptible target cells is consistent with the results of Gething et al. (6) on noninfectious Sendai virus grown in MDBK cells.

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