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Complement-Dependent Antiviral Monospecific Antibody-Mediated Lysis of Murine Cells Coated with Sendai Virus or Its Envelope Component

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Complement-dependent antiviral antibody-mediated lysis of murine cells coated with Sendai virus or its envelope component (P815 mastocytoma cells and L929 cells) was studied with antiviral monospecific sera (anti-F and anti-HN sera). Three types of interactions different in terms of susceptibility to complement-dependent antibody-mediated lysis were distinguished: (i) fusion-positive Sendai viruses induced the susceptibility with both anti-F and anti-HN sera; (ii) fusion-negative envelope particles with F protein induced the susceptibility with only anti-F serum; (iii) noninfectious Sendai viruses with F_0 protein induced no susceptibility. The lack of complement-dependent antibody-mediated cytolysis susceptibility in case (iii) was found to be due neither to detachment of cell-associated noninfectious virus in the presence of antiserum nor to antibody-mediated particular redistribution of cell surface virus antigens. Differences in virus or envelope component-cell association in these three cases were discussed.

Sendai virus is supposed to initiate infection via envelope fusion with host cell membranes (2, 15, 19). Sendai virus-inoculated cells soon become susceptible to thymus-derived (T) immune lymphocyte-mediated lysis (7, 8, 21, 23, 24, 28, 32, 35), and also to complement-dependent antibody-mediated cytolysis (CDAMC) (6). The involvement of viral fusion activity in the formation of target cell susceptibility to anti-Sendai T cell-mediated lysis has been demonstrated (8, 32, 34). However, requirements for formation of target cell susceptibility to CDAMC are not yet known.

To investigate these requirements, we prepared physically and functionally different Sendai viruses and envelope components and analyzed their interactions with murine cell membranes (P815 mastocytoma and L929 cells) by CDAMC assay using monospecific antisera. Late harvested Sendai virus grown in eggs was used as fully functional Sendai virus. The virus had hemolytic activity, as demonstrated by envelope fragility (17). Since this hemolytic activity is regarded as an indication of envelope fusion activity (15), Sendai virus and envelope components with hemolytic activity were termed fusion-positive particles. Noninfectious Sendai viruses containing a precursor (F_0) of fusion protein, with the ability to adsorb to, but not fuse with, the membranes, were prepared in continuous tissue culture cell lines (2, 19). Membrane-bound and free particles with hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins, with and without fusion activity, respectively, were reassembled from Nonidet P-40-solubilized envelopes (14). The term "fusion-negative" in this study means no manifestation of hemolytic activity after treatment of virus or envelope components with freeze-thawing (11).

The present report describes results of analyses of the interactions of these viruses and envelope components with murine cells. The results suggest the involvement of F protein or fusion activity in formation of target cell susceptibility to CDAMC, depending on the system used.

MATERIALS AND METHODS

Virus. Sendai virus, Z strain, was used throughout the present investigation. Virus was grown in the chorioallantoic cavity of embryonated eggs for 72 h. The virus was purified by differential centrifugation of infected chorioallantoic fluid, followed by velocity sedimentation in sucrose gradients (12), and the resulting preparation is hereafter referred to as egg Sendai virus. Noninfectious Sendai viruses lacking fusion activity were produced in LLC-MK_2 cells, an established line of rhesus monkey kidney cells (LLC-SV preparation), and in L929 cells (L-SV preparation) inoculated with egg Sendai virus. LLC-SV and L-SV were usually used after partial purification by differential centrifugation.

Virus labeling. Egg Sendai virus was inoculated onto LLC-MK_2 cultures at a multiplicity of infection of 5 or 10. After an adsorption period of 30 min at 37°C, the cultures were washed three times and maintained in Eagle minimum essential medium supplemented with 2% heat-treated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) and containing one-

fourth the regular concentration of L-leucine and 5 μCi of L-[^{3}H]leucine (New England Nuclear Corp.; NET-135H) per ml. Fresh medium was added again after 3 days, and incubation was continued for 2 days further. The labeled virus was purified from the combined culture media by velocity sedimentation as described above.

Preparation of envelope components. Reassembled envelope particles (REP) were prepared from Nonidet P-40–solubilized virus envelopes by removal of the detergent by dialysis against phosphate-buffered saline through Spectrapor membrane tubing 2 (Spectrum Medical Industries, Inc., Los Angeles, Calif.) (14). The REP preparation used was a fraction of the dialyzed sample pelleted by centrifugation at 50,000 × *g* for 30 min.

Envelope glycoprotein particles were separated from Nonidet P-40–solubilized envelopes as a band with density of about 1.26 by CsCl equilibrium centrifugation (14). Envelope particles contained two glycoproteins, HN and F. Fusion-negative envelope particles were prepared by treatment with Emasol 1130 (Tween 20)-ether (13) or 10% ethanol (34).

Antisera. HN and F immunogens were separated using glutaraldehyde-treated chicken erythrocytes (our unpublished data). Briefly, the glycoprotein fraction of Sendai virus was obtained by the method of Scheid and Choppin (30) from virions solubilized with 1 M KCl + 2% Triton X-100, and the HN fraction was separated from the F fraction by treatment with chicken erythrocytes to which only the HN fraction was adsorbed. These immunogens were injected into rabbits repeatedly. The monospecificities of the antisera obtained were verified by examining the immune complexes made with the antisera in gel diffusion in 1% agarose A-37 (Nakarai Chemical Co., Kyoto, Japan). All the antisera were absorbed with P815 or L929 cells and heated at 56°C for 30 min before CDAMC tests.

Assays of infectivity, hemagglutination, and hemolytic activity. Assays were carried out as described previously (12).

Polyacrylamide slab gel electrophoresis. The method of Laemmli (26) was used with 7.5% gels for polyacrylamide slab gel electrophoresis. Often the stacking gel was omitted (10).

Cytotoxicity assay. P815 mastocytoma cells passaged in the peritoneal cavity of DBA/2 mice, kindly given by M. Igarashi (3rd Department of Internal Medicine, Osaka University School of Medicine, Osaka, Japan), and L929 cells maintained in tissue culture were used as target cells. They were labeled with $Na_2{}^{51}CrO_4$ (200 μCi/ml) at 37°C for 1.5 to 2 h, washed, and treated with Sendai virus at a multiplicity of infection of 100 hemagglutinating units (HAU) per 10^6 cells at 37°C for 30 min, unless otherwise mentioned. Then, the cells were washed and dispensed in Falcon Microtest II plates (3040), at 2×10^4 cells per 100 μl per well. For formation of target cells coated with envelope components, cells were similarly processed except with different multiplicities of infection, as described for individual experiments.

For CDAMC assay, 100-μl samples of antiserum dilutions were put into the wells of target cell cultures, kept at room temperature for 1 h, and then incubated with 100 μl of a 10-fold dilution of Low-Tox-M rabbit complement (Cedarlane Laboratories Ltd., Hornby, Canada) at 37°C for 1 h. Then 200-μl samples of the supernatants were removed for measurements of radioactivity in a gamma counter (Packard Scintillation Spectrometer, model 3002). Results were expressed as mean percent specific lysis of quadruplicate wells, calculated by the following formula: Percent specific lysis = [(^{51}Cr release with antiserum + complement) − (^{51}Cr release with normal serum + complement)]/[(total ^{51}Cr release with 1% Triton X-100) − (^{51}Cr release with normal serum + complement)] × 100. Spontaneous release of ^{51}Cr from target cells was less than 20% of the total release.

Staining of virus antigens with immunofluorescence. The indirect method was used with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories, Inc., Downingtown, Pa.). Sendai viruses were inoculated onto L929 cells in suspension at a high multiplicity of infection and incubated at 37°C for 30 min. Then the cells were washed and treated with 10-fold-diluted antiviral sera at 37°C for 30 min. They were washed again and incubated with fluorescein isothiocyanate-conjugated antibody at 37°C for 30 min. The cells were washed twice and examined for cell surface fluorescence under a Tiyoda fluorescence microscope (model FM200) (Tiyoda Kogaku Co., Japan). The reaction condition used was that expected to favor antibody-induced redistribution (cap formation, etc.) of cell surface viral antigens.

RESULTS

Fusion-negativeness of L-SV and LLC-SV grown in tissue cultures. Table 1 summarizes data on the infectivity, hemolytic activity, and composition of the fusion protein of egg Sendai virus, L-SV, and LLC-SV. The polypeptide patterns of the preparations on slab gel electrophoresis are seen in Fig. 1. LLC-SV has the lowest ratio of infectivity to HAU, and, consistent with this, it shows the faintest band of F and the strongest band of F_0 on the gel. LLC-SV has no detectable hemolytic activity.

L-SV has higher infectivity and a trace of hemolytic activity, which seems to be due to the presence of a significant amount of F protein. Egg Sendai virus has the highest infectivity and hemolytic activity, and contains predominantly F protein with little F_0 protein. The hemolytic activities of both L-SV and LLC-SV were found not to increase after they were treated with freezing and thawing (data not shown). These properties of L-SV are consistent with the results of Homma (16). These properties of L-SV and LLC-SV are explained by their reduced content of F protein (2, 15, 19). For convenience, L-SV and LLC-SV are called noninfectious Sendai virus.

Antibody dose dependency of CDAMC of egg Sendai virus-coated murine cells. Both

TABLE 1. *Comparison of infectivities, hemolytic activities, and fusion proteins of egg Sendai virus, L-SV, and LLC-SV*

Virus	Infectivity[a]	Hemolytic activity[b]	Fusion protein composition[c]
SV	10^6–10^7	0.5–1.0	F predominant
L-SV	$10^{2.3}$–$10^{3.0}$	0.04–0.07	F and F_0
LLC-SV	$10^{1.7}$–$10^{2.2}$	<0.02	F_0 predominant

[a] 50% egg infectious dose per HAU for egg Sendai virus, and hemadsorption-focus-forming units per HAU or L-SV and LLC-SV are expressed in logarithmic terms. Values are ranges of four samples.

[b] 500 HAU/0.1 ml of virus was incubated with 2 ml of 2% chicken erythrocytes at 37°C for 1 h, and release of hemoglobin was measured as increase in optical density at 540 nm. Value ranges are the same as in footnote *a*.

[c] See Fig. 1.

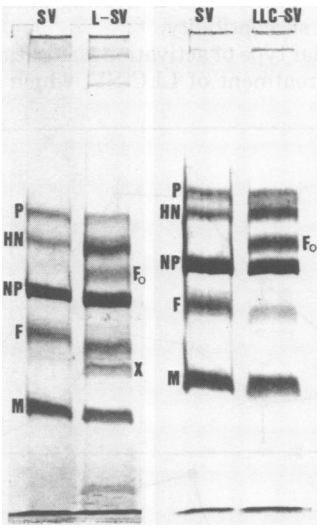

FIG. 1. *Slab gel electrophoretic pattern of SV, L-SV, and LLC-SV. Virions (200 HAU) were dissociated overnight with 2% sodium dodecyl sulfate and 5% 2-mercaptoethanol at 37°C overnight and subjected to electrophoresis in a 7.5% gel by the method of Hewitt and Nermut (10). Protein bands were stained with Coomassie brilliant blue. Designation of bands is by Choppin and Compans (2). X is unidentified but possibly actin, judging from its mobility.*

anti-F and anti-HN sera were found to be cytotoxic in the presence of rabbit complement to egg Sendai virus-coated (0.5 h after inoculation) P815 and L929 cells.

Figure 2 shows that the CDAMC of egg Sendai virus-coated P815 cells with anti-F serum is high over a wide range of the serum dilutions, but that with anti-HN serum it is highest at a 20-fold dilution of serum, tailing off at higher dilutions. Similar results were obtained on the CDAMC of egg Sendai virus-coated L929 cells, but in general the extent of CDAMC was slightly less than with egg Sendai virus-coated P815 cells.

These results were interpreted in the following way, inoculation of infectious Sendai virus onto P815 and L929 cells results in transfer of both F and HN antigens onto cell membranes, and consequently in formation of target cells susceptible to CDAMC with both anti-F and anti-HN sera. The extents of CDAMC seemed to vary depending on the cell lines and antisera used. In subsequent experiments, 20-fold dilutions of antisera were used for CDAMC assay, unless otherwise mentioned.

Viral dose dependency of CDAMC of infectious virus-coated murine cells. Figures 3A and 4A show CDAMC of egg Sendai virus-coated P815 and L929 cells, respectively. Inoculation of the virus rendered these cells susceptible to CDAMC, particularly with anti-F serum, over a wide range of viral doses. A minimum virus dose of 4 HAU/10^6 cells was required to make susceptible P815 targets displaying a definite value (around 20%) of CDAMC with anti-F serum. Assuming that 1 HAU represents 10^7 particles (17, 27), this dose is about 40 particles per cell.

Little or no CDAMC of noninfectious Sendai virus-coated murine cells. When noninfectious Sendai viruses were inoculated onto P815 and L929 cells, no significant CDAMC was observed (Fig. 3B and C; Fig. 4C), except in the case of L-SV-coated L929 cells, which showed slight but definite CDAMC with anti-F serum, but not with anti-HN serum (Fig. 4B). These

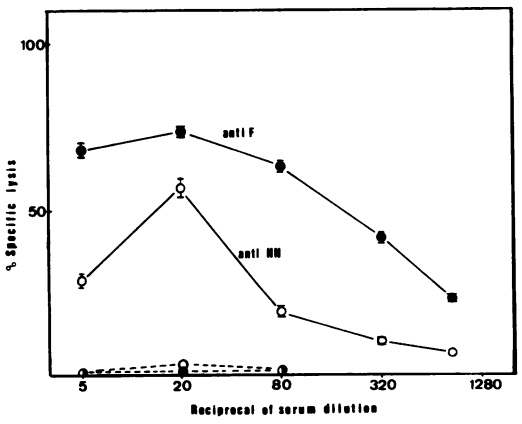

FIG. 2. *Antibody dose dependency of CDAMC of egg Sendai virus-coated P815 cells. Virus-coated* (——) *and uncoated* (-----) *target cells. Bars indicate standard errors of the mean in all the figures.*

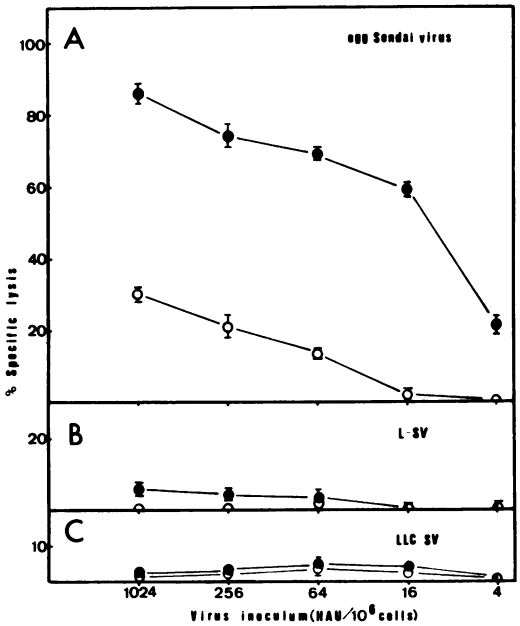

FIG. 3. *Virus dose dependency of CDAMC of infectious and noninfectious Sendai virus-coated P815 cells. CDAMC with anti-F (●) and with anti-HN (○) serum.*

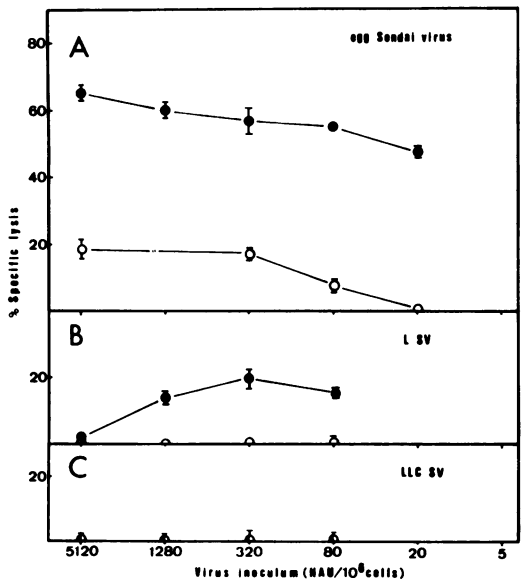

FIG. 4. *Virus dose dependency of CDAMC of infectious and noninfectious Sendai virus-coated L929 cells. CDAMC with anti-F (●) and with anti-HN (○) serum.*

results suggest the importance of viral fusion activity in induction of CDAMC susceptibility and supports the idea that CDAMC of Sendai virus-coated cells is affected by the cell line used. The susceptibility of L-SV-coated L929 cells to CDAMC was probably due to the trace of fusion activity associated with L-SV.

Effect of trypsinization of noninfectious Sendai viruses on their ability to induce CDAMC susceptibility. We examined whether the noninfectious Sendai viruses L-SV and LLC-SV, which were different in their original ability to induce CDAMC susceptibility (see Fig. 4), gained or increased in this ability when they were treated with trypsin (2, 19). Figure 5 shows activation, by treatment with various concentrations of trypsin, of L-SV ability to induce the CDAMC susceptibility of virus-coated L929 cells (Fig. 5A), and of L-SV infectivity and hemolytic activity (Fig. 5B). The optimal concentrations of trypsin for activation of the infectivity and hemolytic activity were the same, and were also the same as those for activating inducibility of CDAMC susceptibility.

A similar type of activation was obtained upon trypsin treatment of LLC-SV, which originally

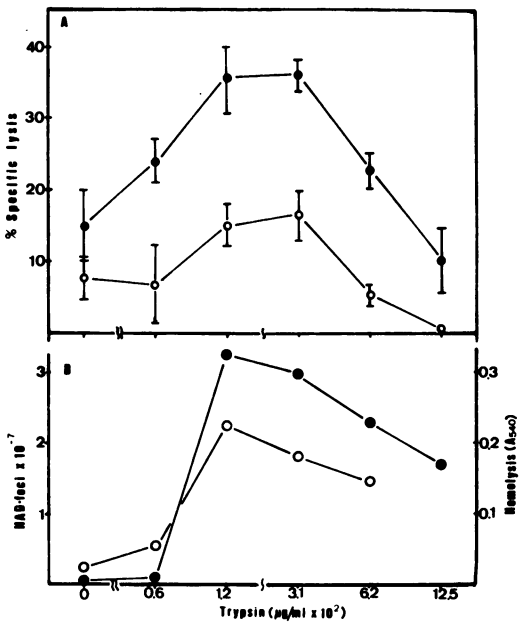

FIG. 5. *Effect of trypsinization of L-SV on its inducibility of CDAMC susceptibility (A) and infectivity and hemolytic activity (B). Samples (0.2 ml; 640 HAU) of L-SV were treated with the indicated final concentrations of trypsin at 37°C for 5 min, then diluted with 40 volumes of cold phosphate-buffered saline and centrifuged at 40,000 × g for 30 min. The precipitates were resuspended in their original volumes of phosphate-buffered saline, and 10- and 100-μl samples were used for measurements of infectivity (●) and hemolytic activity (○), respectively. The rest of the precipitates were inoculated onto 10^6 L929 cells for CDAMC assay with anti-F (●) and anti-HN (○) sera.*

could not induce CDAMC susceptibility (Fig. 6). The optimal concentrations of trypsin for activation of these viral activities in L-SV and LLC-SV were almost the same. Cleavage of F_0 to F in L-SV and LLC-SV by trypsin under a similar condition was demonstrated by slab gel electrophoresis (Fig. 7).

P815 cells coated with trypsin-activated LLC-SV or L-SV were also found to be susceptible to CDAMC with both anti-F and anti-HN sera (data not shown).

These results indicate that when noninfectious Sendai viruses are modified to fusion-positive viruses, they acquire or increase ability to induce CDAMC susceptibility with both anti-F and anti-HN sera of virus-coated murine cells. The results strongly suggest that the ability of Sendai virus depends on the virus F protein contents but not on any host-derived virus-associated component.

Effect of antisera on cell-attached noninfectious Sendai virus. It seemed possible that noninfectious Sendai viruses might have little or no ability to induce CDAMC susceptibility because they became detached from the cells in the presence of anti-F and anti-HN sera. To examine this possibility, we compared the detachment rate, in the presence of antisera, of [^{3}H]leucine-labeled LLC-SV attached to L929 cells with that of trypsin-activated infectious LLC-SV. Table 2 shows that more than 70% of the virus attached to L929 cells remained associated with the cells in both infectious and noninfectious viruses, after treatment with anti-F or anti-HN serum at room temperature for 30 min. Table 2 also shows that the attachment rates of inoculated LLC-SV and trypsin-activated infectious LLC-SV were similar (25 and 22%).

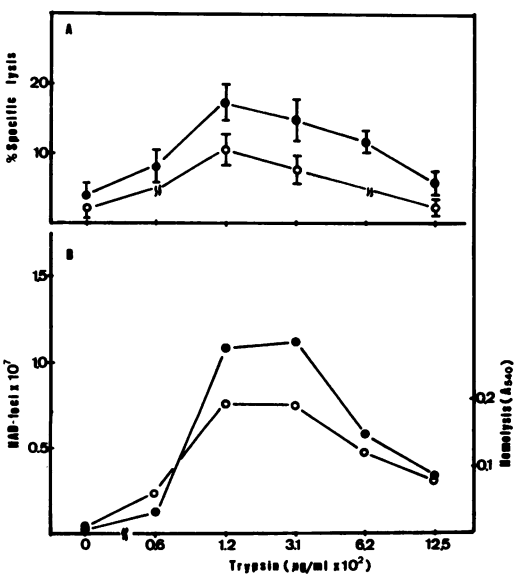

FIG. 6. *Effect of trypsinization of LLC-SV on its inducibility of CDAMC susceptibility (A) and infectivity and hemolytic activity (B). Conditions of trypsin treatment and symbols were the same as in Fig. 5.*

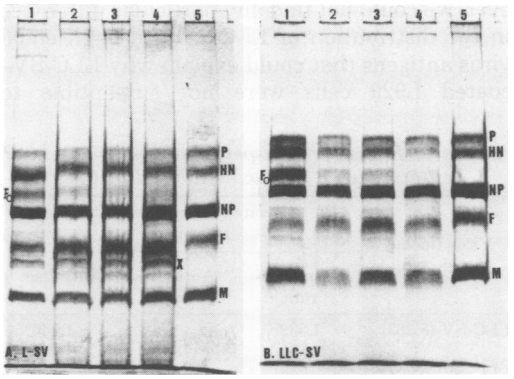

FIG. 7. *Slab gel electrophoretic patterns of trypsinized L-SV (A) and LLC-SV (B). Conditions for electrophoresis were the same as described in Fig. 1. Egg Sendai virus (lane 5) was used as a control. The trypsin concentrations used were (1) 0%; (2) 0.012%; (3) 0.031%; (4) 0.125%. Designation of bands is the same as described in the legend to Fig. 1.*

Antigen distribution pattern of Sendai virus-coated L929 cells observed by the fluorescein isothiocyanate-antibody technique. The redistribution, induced by antibody, of measles virus (20) and herpesvirus (29) antigens on the cell surface was reported to be correlated with resistance to CDAMC with antivirus sera, which varied depending on the cell line infected with virus. Therefore, we examined the redistribution of virus antigens on noninfectious Sendai virus-coated L929 cells with antiviral sera, somewhat specifically to noninfectious virus, and the resistance of these cells to CDAMC.

Figure 8 shows L929 cells coated with egg Sendai virus (Fig. 8A and B) and LLC-SV (Fig. 8C and D) and stained indirectly by the fluorescein isothiocyanate-conjugated antibody technique. The virus antigens in all the samples appeared mainly as fine granules and small patches, with a very few caps. Two differences were found between cells coated with egg Sendai virus and with LLC-SV: one was that the former cells were generally larger than the latter, with the larger cells being multinucleate, probably as the result of virus-induced cell fusion; the other difference was that the stained antigens on the cells coated with egg Sendai virus, and particularly the larger cells, looked brighter, possibly because on the larger cells more antigens reacted with antibodies and became redistributed. How-

ever, we could not find any particular difference in the distribution of LLC-SV and egg Sendai virus antigens that could explain why LLC-SV-coated L929 cells were not susceptible to CDAMC. Another thing to note was that no significant difference was observed in the antigen distribution patterns between HN and F (Fig. 8A and B; C and D).

Thus, the above two findings eliminated two possible causes of the lack of CDAMC susceptibility in noninfectious Sendai virus-coated cells; detachment of cell-attached virus in the presence of antiviral sera, and antibody-mediated particular redistribution of the cell surface virus antigens.

TABLE 2. *Effect of monospecific antiserum on L929 cell-attached [^{3}H]leucine-labeled LLC-SV*

Virus (inoculated cpm)	Cell-associated virus (cpm) after serum treatment[a]		
	Normal serum	Anti-HN serum	Anti-F serum
LLC-SV (5,363)	1,332 (100%)	976 (73%)	1,040 (78%)
LLC-SV; trypsinized[b] (3,000)	648 (100%)	472 (73%)	570 (88%)

[a] 0.2-ml samples of virus were inoculated onto L929 cell sheets (Falcon dish 3001) and incubated at 37°C for 30 min. The cells were washed and treated with 0.5 ml of 10-fold dilutions of sera at room temperature for 30 min. Then the cells were washed twice, detached with a rubber policeman, solubilized with Aquasol-2 (New England Nuclear Corp.), and counted in a liquid scintillation counter. Values are means of duplicate measurements.

[b] LLC-SV (2,000 HAU/ml) was treated with a final concentration of 0.012% trypsin at 37°C for 5 min, precipitated by centrifugation at 40,000 × *g* for 30 min, and suspended in its original volume of phosphate-buffered saline. The treated virus was used after clarification by low-speed centrifugation.

CDAMC of REP-coated murine cells. Next, we prepared envelope particles (REP) with fusion activity by reassembly from Nonidet P-40-solubilized egg Sendai virus, and examined their ability to induce CDAMC susceptibility. Table 3 shows that fusion-positive REP induced CDAMC susceptibility with anti-F and anti-HN sera of REP-coated P815 cells, although CDAMC values varied depending on REP preparations. This variation may be due to that of the hemolytic activity of REP preparations. REP also induced CDAMC susceptibility with both anti-F and anti-HN sera of REP-coated L929 cells (data not shown).

Induction of CDAMC susceptibility by fusion-negative particles containing F pro-

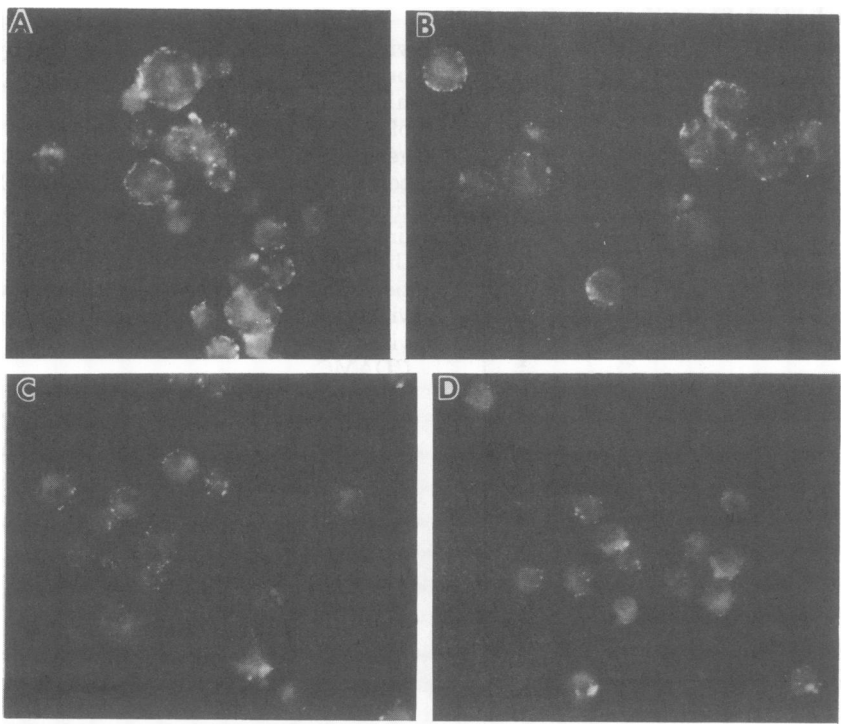

FIG. 8. *L929 cells coated with egg Sendai virus (A, anti-F; B, anti-HN serum), and LLC-SV (C, anti-F; D, anti-HN serum) stained indirectly with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G. See the text. Multiplicity of infection, 300 HAU/10^6 cells.*

tein. The previous results indicate the important role of viral fusion activity in induction of CDAMC susceptibility. Therefore, we examined whether viral fusion activity was essential for induction of the susceptibility, using fusion-negative envelope particles containing F protein.

Table 4 shows that membrane-free glycoprotein aggregates and two preparations of membrane-bound envelopes, all without fusion activity, induced susceptibility of P815 cells to CDAMC with anti-F serum, but not with anti-HN. These findings indicate that viral fusion activity was not necessary for induction of the susceptibility. However, this CDAMC was different from that of fusion-positive virus-coated cells in that it occurred with only anti-F serum, but not with both anti-F and anti-HN sera.

DISCUSSION

There have been several reports (3, 4, 6, 22) concerning CDAMC of cell cultures infected with Sendai virus, with anti-Sendai whole virus serum. The present study demonstrated that the anti-Sendai monospecific sera, anti-F and anti-HN, are both cytotoxic to murine cells coated with fusion-positive Sendai virus or REP in the presence of rabbit complement, although CDAMC of REP-coated murine cells was variable depending on the REP preparation. This finding seems to be consistent with the result that in the presence of rabbit complement both anti-HA and anti-hemolysin sera were cytotoxic to cells chronically infected with measles virus (5).

The capability of Sendai virions to induce CDAMC susceptibility with anti-F and anti-HN sera was found to be invariably associated with fusion activity. Viral replication was not involved in that, since the target susceptibility occurred during the stage of virus attachment. With envelope fusion, the two envelope antigens, F and HN, are supposed to be transferred onto cell membranes, and become target antigens to CDAMC.

A second type of CDAMC susceptibility with only anti-F serum was observed with cells coated with fusion-negative particles containing F protein. F protein was reported to have a strong hydrophobic NH_2 terminus (9). However, it is uncertain whether the possible biological function associated with this structure was involved in induction of CDAMC susceptibility, or alternatively, whether the antigenicity of attached F protein was sufficient. The lack of CDAMC with anti-HN serum might be due to cell dissociation of HN proteins with viral neuraminidase function.

The above two types of virus-cell interactions

TABLE 3. *CDAMC of REP-coated P815 cells*

Expt. no.	REP dose[a]	Antiserum	% Specific lysis of REP-coated cells[b]
1	512	Anti-F	45.8 ± 2.0
		Anti-HN	13.3 ± 1.8
	32	Anti-F	22.6 ± 2.0
		Anti-HN	8.0 ± 1.0
	No REP	Anti-F	4.3 ± 1.0
		Anti-HN	1.1 ± 0.8
2	320	Anti-F	13.3 ± 0.7
		Anti-HN	6.0 ± 0.5
	No REP	Anti-F	2.0 ± 0.9
		Anti-HN	1.0 ± 0.3

[a] HAU/10^6 cells.
[b] Mean ± standard error of the mean.

TABLE 4. *CDAMC of P815 cells coated with fusion-negative particles containing F protein*

Antiserum	Serum dilution	% Specific lysis[a] of P815 cells coated with:							
		Envelope glycoprotein particles		Tween-ether-treated virus[b]		Ethanol-treated virus[c]		Egg Sendai virus (250 HAU)	No virus
		2,000 HAU[d]	400 HAU	1,000 HAU	200 HAU	250 HAU	50 HAU		
Anti-F	1:20	32.8 ± 2.5	22.0 ± 0.9	20.8 ± 0.7	24.2 ± 0.6	20.5 ± 0.7	31.4 ± 3.0	66.8 ± 0.9	6.2 ± 1.2
	1:50	14.9 ± 1.4	10.7 ± 1.0	10.2 ± 1.1	11.2 ± 2.4	11.6 ± 3.0	9.6 ± 0.6	64.4 ± 1.1	3.6 ± 0.5
Anti-HN	1:20	−2.5 ± 0.7	−1.0 ± 0.7	0.6 ± 0.8	0.4 ± 1.3	−0.7 ± 1.0	0.5 ± 2.0	14.2 ± 2.2	0.5 ± 0.3
	1:50	2.6 ± 1.1	−1.4 ± 0.6	−0.2 ± 0.2	0.3 ± 0.8	2.0 ± 2.0	−0.3 ± 2.0	0.2 ± 2.0	−0.9 ± 0.8

[a] Mean ± standard error of the mean.
[b] Egg Sendai virus (20,000 HAU/ml) was treated with ether in the presence of 0.5% Tween 20, and the disrupted virus fraction was prepared by differential centrifugation at 60,000 × *g* for 30 min and at 2,000 × *g* for 15 min. This preparation and EP exhibited no hemolytic activity.
[c] Egg Sendai virus (20,000 HAU/ml) was treated overnight with 10% ethanol in the cold, and then sedimented by centrifugation at 40,000 × *g* for 30 min and suspended in PBS. This preparation occasionally exhibited residual and slight hemolytic activity.
[d] Virus titer (HAU)/10^6 cells.

were different from each other in terms of target susceptibility to anti-Sendai T lymphocyte-mediated lysis. Egg Sendai virus-coated P815 cells were recognized by anti-Sendai T lymphocytes, whereas P815 cells coated with envelope glycoprotein particles or ethanol-treated virus were not recognized (our unpublished data; 34). Assuming that a complex of viral and histocompatibility antigens (25, 31, 36) is the target antigen by virus-immune T cells, viral antigens are supposed to be inserted into cell membranes to result in association with histocompatibility antigens in the former cells, but not in the latter cells.

A third type of CDAMC susceptibility, lack of susceptibility, was observed on noninfectious Sendai virus-coated murine cells. The results by fluorescein isothiocyanate-antibody technique demonstrated that antigen-antibody complexes were formed on these cells. When enveloped viruses are treated with anti-viral serum and complement, they undergo lysis, called virolysis (1, 33). Such immune virolysis of attached noninfectious virus probably occurred on the cell-distal surface of the virus, under the present conditions. However, this virolysis did not affect the permeability of the cell membranes leading to CDAMC. We have no information as to how noninfectious Sendai virus remained cell-associated. Is the help of F_0 proteins necessary for cell-association of the virus? Furthermore, when noninfectious virus is fragmented, is its ability to induce CDAMC susceptibility unmasked? We are now studying these problems.

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LITERATURE CITED

1. **Berry, D. M., and J. D. Almeida.** 1968. Morphological and biological effects of various antisera on avian infectious bronchitis virus. J. Gen. Virol. **3**:97-102.
2. **Choppin, P. W., and R. W. Compans.** 1975. Replication of paramyxoviruses, p. 95-178. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Press, New York.
3. **Eaton, M. D., and A. R. Scala.** 1969. Further observation on the inhibitory effect of myxoviruses on a transplantable murine leukemia. Proc. Soc. Exp. Biol. Med. **132**: 20-26.
4. **Eaton, M. D., and A. R. Scala.** 1970. Species source of complement in viral-immune and other cytolytic reactions. Proc. Soc. Exp. Biol. Med. **133**:615-619.
5. **Ehrnst, A. C.** 1975. Characterization of measles virus-specific cytotoxic antibodies by use of a chronically infected cell line. J. Immunol. **114**:1077-1082.
6. **Fan, D. P., and B. M. Sefton.** 1978. The entry into host cells of Sindbis virus, Vesicular stomatitis virus and Sendai virus. Cell **15**:985-992.
7. **Finberg, R., M. Mescher, and S. J. Brakoff.** 1978. The induction of virus-specific cytotoxic T lymphocytes with solubilized viral and membrane proteins. J. Exp. Med. **148**:1620-1627.
8. **Gething, M.-J., U. Koszinowski, and M. Waterfield.** 1978. Fusion of Sendai virus with the target cell membrane is required for T cell cytotoxicity. Nature (London) **274**:689-691.
9. **Gething, M.-J., J. M. White, and M. D. Waterfield.** 1978. Purification of the fusion protein of Sendai virus: analysis of the NH_2-terminal sequence generated during precursor activation. Proc. Natl. Acad. Sci. U.S.A. **75**: 2737-2740.
10. **Hewitt, J. A., and M. V. Nermut.** 1977. A morphological study of the M-protein of Sendai virus. J. Gen. Virol. **34**:127-136.
11. **Hosaka, Y.** 1958. On the hemolytic activity of HVJ. I. An analysis of the hemolytic reaction. Biken J. **1**:70-89.
12. **Hosaka, Y., and Y. Hosokawa.** 1977. Purification of Sendai virions with glutaraldehyde-treated red blood cells. Intervirology **1**:70-89.
13. **Hosaka, Y., Y. Hosokawa, and K. Fukai.** 1959. A new device for preparing subunits of myxovirus. Biken J. **2**: 367-370.
14. **Hosaka, Y., and Y. K. Shimizu.** 1972. Artificial assembly of envelope particles of HVJ (Sendai virus). I. Assembly of hemolytic and fusion factors from envelopes solubilized by Nonidet P40. Virology **49**:627-639.
15. **Hosaka, Y., and K. Shimizu.** 1977. Cell fusion by Sendai virus, p. 129-155. *In* G. Poste and G. L. Nicolson (ed.), Virus infection and the cell surface. North-Holland Publishing Co., Amsterdam.
16. **Homma, M.** 1972. Trypsin action on the growth of Sendai virus in tissue culture cells. II. Restoration of the hemolytic activity of L cell-borne Sendai virus by tryspin. J. Virol. **9**:829-835.
17. **Homma, M., K. Shimizu, Y. K. Shimizu, and N. Ishida.** 1976. On the study of Sendai virus hemolysis. I. Complete Sendai virus lacking hemolytic activity. Virology **71**:41-47.
18. **Isaacs, A., and H. B. Donald.** 1955. Particle counts of hemagglutinating viruses. J. Gen. Microbiol. **12**:241-247.
19. **Ishida, N., and M. Homma.** 1978. Sendai virus. Adv. Virus Res. **23**:349-383.
20. **Joseph, B. S., and M. B. A. Oldstone.** 1974. Antibody-induced redistribution of measles virus antigens on the cell surface. J. Immunol. **113**:1205-1209.
21. **Junk, H., K. Pfizenmaier, A. Starzinski-Powitz, M. Röllinghoff, and H. Wagner.** 1978. Primary in vitro sensitization of virus specific cytotoxic T lymphocytes. Immunology **34**:763-769.
22. **Kieling, F., G. Bandlow, and R. Thomssen.** 1972. Cytotoxische Reaktionen an Sendai-Virus infizierten Zellen. Arch. Gesamte Virusforsch. **36**:123-132.
23. **Koszinowski, U., M.-J. Gething, and M. Waterfield.** 1977. T-cell cytotoxicity in the absence of viral protein synthesis in target cells. Nature (London) **267**:160-163.
24. **Kurle, R., M. Röllinghoff, and H. Wagner.** 1978. H-2-linked murine cytotoxic T cell response specific for Sendai virus-infected cells. Eur. J. Immunol. **8**:910-912.
25. **Kvist, S., L. Ostberg, H. Persson, L. Philipson, and P. A. Peterson.** 1978. Molecular association between transplantation antigens and cell surface antigen in adenovirus-transformed cell line. Proc. Natl. Acad. Sci. U.S.A. **75**:5674-5678.
26. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.

Nature (London) **227**:680–685.

27. **Okada, Y., S. Nishida, and J. Tadokoro.** 1961. Correlation between the hemagglutination titer and the virus particle number of HVJ. Biken J. **4**:209–213.
28. **Palmer, J. C., L. J. Lewandowski, and D. Waters.** 1977. Non-infectious virus induces cytotoxic T lymphocytes and binds to target cells to permit their lysis. Nature (London) **269**:595–597.
29. **Rawls, W. E., and W. A. F. Tompkins.** 1975. Destruction of virus-infected cells by antibody and complement, p. 99–111. *In* A. L. Notkins (ed.), Viral immunology and immunopathology. Academic Press, New York.
30. **Scheid, A., and P. W. Choppin.** 1973. Isolation and purification of the envelope proteins of Newcastle disease virus. J. Virol. **11**:263–271.
31. **Schrader, J. W., B. A. Cunningham, and G. M. Edelman.** 1975. Functional interactions of viral and histocompatibility antigens at tumor cell surfaces. Proc. Natl. Acad. Sci. U.S.A. **72**:5066–5070.
32. **Schrader, J. W., and G. M. Edelman.** 1977. Joint recognition by cytotoxic T cells of inactivated Sendai virus and products of the major histocompatibility complex. J. Exp. Med. **145**:523–539.
33. **Shimizu, Y. K., K. Shimizu, N. Ishida, and M. Homma.** 1976. On the study of Sendai virus hemolysis. II. Morphological study of envelope fusion and hemolysis. Virology **71**:48–60.
34. **Sugamura, K., K. Shimizu, and F. H. Bach.** 1978. Involvement of fusion activity of ultraviolet light-inactivated Sendai virus in formation of target antigens recognized by cytotoxic T cells. J. Exp. Med. **148**:276–287.
35. **Sugamura, K., K. Shimizu, D. A. Zarling, and F. H. Bach.** 1977. Role of Sendai virus fusion glycoprotein in target susceptibility to cytotoxic T cells. Nature (London) **270**:251–253.
36. **Zarling, D. A., I. Keshet, A. Watson, and F. H. Bach.** 1978. Association of mouse major histocompatibility and Rauscher murine leukemia virus envelope glycoprotein antigens on leukemia cells and their recognition by syngeneic virus-immune-cytotoxic T lymphocytes. Scand. J. Immunol. **8**:497–508.