INVOLVEMENT OF FUSION ACTIVITY OF ULTRAVIOLET LIGHT-INACTIVATED SENDAI VIRUS IN FORMATION OF TARGET ANTIGENS RECOGNIZED BY CYTOTOXIC T CELLS*

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There is increasing evidence for a critical role of the major histocompatibility (H-2) gene complex in cell-mediated immunity. Cytotoxic thymus-derived lymphocytes (T_c)[1] specific for virus infected cells can be generated in vivo by infection with various viruses (1–4), or in vitro in a primary or secondary immune response (5, 6). The specificity of killing by these virus-specific T_c requires the sharing of H-2K and/or D histocompatibility antigens between effector T_c and target cells (7). Similar requirements have also been demonstrated for the killing of chemically modified cells (8), tumor cells (9), and cells which differ in their minor histocompatibility antigens (10, 11). The observed H-2 restriction in these cytotoxic reactions has been explained by the nature of the receptor on T_c (one receptor or two receptor hypothesis), and also by the nature of the target antigen structure (altered self or dual antigenic recognition) (12).

The structural association of H-2 and viral antigens on the target cell surface has been suggested by co-capping and co-patching experiments using anti-H-2 and anti-murine leukemia virus gp70 sera (13), as well as by the observation that H-2 antigens are incorporated into mature Friend virus particles (14). Studies in several laboratories have asked whether a preformed virion component(s) alone is enough for the association of antigens to form the target antigen on the target cell surface. Noninfectious viruses, ultraviolet light (UV)-inactivated Sendai virus (6), β-propiolactone-inactivated rabies virus (15), and formalin-inactivated influenza virus (16) have all been shown to induce T_c, suggesting that infectivity of the virus is not necessary for the induction of cytotoxicity. Furthermore, in the cytotoxic effector phase, inactivated Sendai

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[1] *Abbreviations used in this paper*: F glycoprotein, fusion glycoprotein; HA, hemagglutinin; HAU, hemagglutinating units; 2-ME, 2-mercaptoethanol; NA, neuraminidase; SVuv, ultraviolet light-inactivated Sendai virus; T_c, cytotoxic thymus-derived lymphocytes; UV, ultraviolet light.

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virus can serve as the target antigen on the cell surface without de novo viral protein synthesis (6); in addition, an isolated Sendai virus envelope fraction was able to form the target antigen on cells coated with this fraction (17). For other viruses it is not clear whether certain virion component(s) are able to present the target antigen on the cell surface without viral genome expression, since it has been observed that some viral protein synthesis in the target cells was required for lysis by T_c specific for ectromelia virus-infected cells (18) or influenza virus-infected cells under the experimental conditions cited (19).

In a previous study, we demonstrated that fusion (F) glycoprotein of the Sendai virus envelope plays an essential role in the formation of target antigen on cells coated with UV-inactivated virus (20). The requirement of F glycoprotein for cytotoxicity can be explained by the following possibilities: (*a*) the F glycoprotein itself can directly become a part of the target antigen, or (*b*) the biological fusion activity of F glycoprotein may be primarily required for the association of H-2 and viral antigenic determinants. In the present study, we demonstrate that the essential role of F glycoprotein is due primarily to its fusion activity, not to its antigenicity. This suggests that the effective association of H-2 and viral antigens for cytotoxicity takes place through Sendai virus fusion activity, and that the simple adsorption of the virion components to the cell surfaces is not sufficient for the formation of target antigen(s).

Materials and Methods

Mice. Adult male BALB/cJ, DBA/2J, C57BL/6J, and CBA/J were purchased from The Jackson Laboratory, Bar Harbor, Maine. Occasionally, serum antibody activity against Sendai virus was observed in these animals, but it was always shown to be <8 hemagglutinin inhibition units per ml.

Virus. A Nagoya strain of Sendai virus was grown in 10-day-old embryonated chicken eggs. The virus from the allantoic fluids was purified by sequential differential centrifugations at 3,000 *g* for 15 min, 55,000 *g* for 30 min, and subsequently by linear sucrose gradient (from 20 to 50% wt/vol) centrifugation at 75,000 *g* for 3 h. 2-ml samples of the virus collected from the virus-layer of the sucrose gradient were sonicated for 3 min by a B-12 sonicator (Branson Sonic Power Co., Danbury, Conn.) to disrupt the aggregation of virus; they were then placed in plastic Petri dishes (model 25010; Corning Glass Works, Corning, N.Y.) and exposed to UV at 800 $\mu W/cm^2$ for 20 min on a continuous-rocking platform. The UV-inactivated Sendai virus (SVuv) was resuspended in phosphate-buffered saline (PBS), pH 7.2, at the final concentration of 6,400 hemagglutinating units (HAU)/ml. Purified SVuv gave 16 plaque-forming units (PFU)/ml in a primary rhesus monkey kidney cell culture; an equal number of virions which were not UV-treated resulted in 1.9×10^9 PFU/ml. Aliquots of SVuv were stored at −80°C.

Treatment with Ethanol. Allantoic fluids harvested from Sendai virus-inoculated embryonated chicken eggs were centrifuged at low speed to remove blood cells and aggregates. Absolute ethanol was added directly to the allantoic fluid at a final concentration of 10%, and the mixture was incubated at room temperature for 1 h. After incubation, Sendai virus in the mixture was purified and inactivated by UV under the same procedure as described above. As a control virion preparation, PBS instead of ethanol, was employed.

Alkali-Tween 20 Treatment. To prepare solubilized virus envelope, the purified Sendai virions from various preparations were disrupted in 0.02 M bicarbonate buffer (pH 10) containing 1.0% Tween 20 at room temperature for 1 h as described previously (21). Nucleocapsids and partially disrupted virions were removed by centrifugation at 140,000 *g* for 1 h.

Hemagglutination Assay. Hemagglutination titration was carried out by Salk's pattern method (22).

Neuraminidase Assay. Cell-associated (as detected on virus-coated cells) and free Sendai virus neuraminidase activities were assayed using fetuin type IV (Sigma Chemical Co., St. Louis, Mo.) as substrate. Virus-coated cells were washed twice with and resuspended in PBS. To 0.05 ml

of the free virus or washed cell suspension was added 0.05 ml of 0.2 M sodium acetate buffer, pH 5.0, and 0.1 ml of fetuin in water (10 mg/ml). The mixture was incubated at 37°C for 20 min and the *N*-acetyl neuraminic acid liberated was assayed by the thiobarbituric acid method (23). The neuraminidase unit was calculated according to the formula described previously (21).

Hemolytic Assay. 0.1 ml of Sendai virus suspensions in test tubes was admixed with 2 ml of a 5% suspension of chicken erythrocytes in PBS. After incubation at 37°C for 20 min, the tubes were centrifuged, and the supernatant fluids were measured for hemolysis according to their optical density at 540 nm.

Cell Fusion Assay. Replicate cultures of HeLa cells which were seeded 1 day previously at 2×10^6 cells per plastic Petri dish (Corning 25010), were inoculated with 1.0 ml of the Sendai virus preparations. After a 1-h virus adsorption with gentle shaking every 15 min at 37°C, the cultures were overlaid with 5.0 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and subsequently incubated for 2 h at 37°C in humidified 5% CO_2 in air. The remaining total cell numbers in the cultures were measured after trypsinization. The cell fusion activity was expressed as the percent decrease in the total number of cells in the virus-treated cultures compared to the untreated cultures.

Antisera. Sendai virus (1.2×10^3 HAU) envelopes solubilized by alkali-Tween 20 treatment were emulsified in an equal volume of Freund's complete adjuvant, and the emulsion was injected intramuscularly into a rabbit at weekly intervals for 4 wk. The serum was collected 1 wk after the last injection.

In Vitro Generation of Cytotoxic Effector Cells. Secondary stimulation of spleen cells against Sendai virus was performed in vitro according to the modified method of Schrader and Edelman (6). Mice inoculated intraperitoneally with SVuv at a dose of 500 to 1,000 HAU per mouse were sacrificed 1–2 wk later, and a suspension of spleen cells was prepared. The spleen cells, suspended in EHAA medium (24) supplemented with 5% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol (2-ME), and antibiotics (EHAA complete medium), were used as the responder cells in the mixed leukocyte culture. Stimulator cells were prepared from spleens of normal mice syngeneic to the responder strain which were treated with Tris-buffered NH_4Cl to remove erythrocytes. Subsequently, the stimulator cells were incubated with 200 HAU of SVuv per 1×10^7 cells in 1 ml of PBS at 37°C for 1 h. After washing twice, the cells were resuspended in EHAA complete medium and irradiated with 2,000 R. The in vitro stimulation culture consisted of 5×10^7 responder cells and 3×10^7 stimulator cells in a total of 20 ml of EHAA complete medium in a tissue culture flask (model 3013; Falcon Plastics, Div. BioQuest, Oxnard, Calif.), and was cultivated at 37°C for 5–6 days in a humidified incubator with 5% CO_2 in air. As the experimental control, the same stimulator cells treated only with PBS (untreated cells) were employed instead of SVuv-coated stimulator cells. Alloreactive effector cells were also prepared under a similar procedure in which 5×10^7 normal responder spleen cells were co-cultivated for 5 days with 5×10^7 normal allogeneic stimulator spleen cells which were irradiated with 2,000 R.

Target Cells. The three target cell lines used in the present experiments differed in their H-2 haplotypes. DBA/2 (H-2^d) mastocytoma P815 cells were cultivated in a spinner culture flask with RPMI-1640 supplemented with 10% fetal calf serum, 25 mM Hepes buffer, and antibiotics (RPMI medium). C57BL/6 (H-2^b) lymphoma EL-4 cells, obtained from Dr. M. Sopori, The University of Wisconsin, were maintained in C57BL/6 mice, and a few days before use as target cells, they were cultivated in vitro with RPMI medium containing 5×10^{-5} M 2-ME in a spinner culture flask. C3H (H-2^k) fibroblast L929 cells obtained from Flow Laboratories, Inc. (Rockville, Md.), were maintained as a monolayer in a tissue culture flask (Falcon model 3024) with RPMI medium. Just before use, a single cell suspension was prepared by trypsinization of the L929 monolayer culture. These three lines of target cells were labeled with ^{51}Cr as described elsewhere (25) and 1×10^7 cells of each line were incubated with 400 HAU of the UV-inactivated Sendai virus preparations at 4°C for 1 h with gentle shaking every 15 min, and the cells were then washed twice with PBS.

Cytotoxicity Assay. ^{51}Cr-labeled target cells (1×10^4 per well) were incubated in EHAA complete medium with various numbers of effector cells in round-bottom well microplates (Linbro Chemical Co., Hamden, Conn.) for 6 h with P815 and L929 cells, or for 4 h with EL-4 cells at 37°C in humidified 5% CO_2 in air. Plates were then centrifuged, and the radioactivity released in the supernate was measured. Percent cytotoxicity was calculated as previously described (20). In addition, specific cytotoxicity was calculated as follows:

$$\frac{I - N}{Max - N} \times 100,$$

in which I = the mean count released by spleen cells cultured with virus-coated stimulator cells, N = the mean count released by spleen cells cultured with untreated stimulator cells, and Max = the mean count released by detergent. The standard deviation was always <5%.

Results

Secondary in Vitro Stimulation. We first examined the kinetics for in vitro secondary stimulation of precursor T_c specific for SVuv. Spleen cells from BALB/cJ (H-2^d) mice which had been primed in vivo with SVuv were co-cultivated with irradiated syngeneic spleen cells coated with SVuv. Fig. 1 shows cytotoxic activity of effector cells from the secondary cultures against SVuv-coated or untreated P815 target cells which are H-2 identical with the effector cells. Killing activity of the restimulated cells against the virus-coated target cells increases linearly with time until day 5. Highly specific killing of the SVuv-coated cells was observed from days 4 to 7, while very little increase in the lysis of untreated target cells was seen during this period. The specificity of recognition by the cytotoxic effector cells for Sendai virus was also examined as follows. BALB/cJ spleen cells primed in vivo with SVuv were co-cultivated for 5 days with either SVuv-coated or untreated syngeneic spleen cells. Alloreactive BALB/cJ (H-2^d) spleen cells were also prepared against C57BL/6J (H-2^b) spleen cells. Fig. 2 shows the cytotoxic activities of these effector cells against SVuv-coated P815 target cells. Only effector cells primed in vivo and restimulated in vitro with SVuv-coated syngeneic cells could significantly kill the virus-coated target cells. In vivo primed effector cells which were not restimualted in vitro, and alloreactive (anti-H-2^b) effector cells did not significantly kill the virus-coated P815 (H-2^d) target cells; these same alloreactive effector cells could lyse the EL-4 (H-2^b) target cells (data not shown). Therefore, cytotoxic activity against virus-coated target cells is due to virus-specific recognition by effector cells and not to nonspecific killing caused by effector cells binding to target cells via Sendai virus bridges. Similar observations have already been reported using DBA/2 effector cells specific for Sendai virus (20).

The susceptibility of the effector cell population to treatment with anti-thy 1.2 and complement was examined. BALB/cJ effector cells from a 6-day restimulated culture were treated with anti-thy 1.2 (AKR anti-C3H ascitic fluid purchased from Bionetics Laboratory Products, Kensington, Md.) and guinea pig complement. Sendai virus-specific killing activity of the effector cells was greatly decreased after treatment with anti-thy 1.2 and complement (data not shown). These results indicate that the Sendai virus-specific effector cells generated in the secondary stimulation culture are primarily T lymphocytes.

H-2 Restriction of Sendai Virus-Specific Cytotoxicity. Virus-primed spleen cells resensitized against Sendai virus in vitro were derived from BALB/cJ (H-2^d), DBA/2J (H-2^d), CBA/J (H-2^k), and C57BL/6J (H-2^b) mice. Each of these restimulated cells was employed as effector cells against target cells of three different haplotypes, P815 (H-2^d), L929 (H-2^k), and EL-4 (H-2^b), which were either untreated or coated with SVuv. Table I shows the specificity of cytotoxicity for various combinations of effector and target cells. Effector cells derived

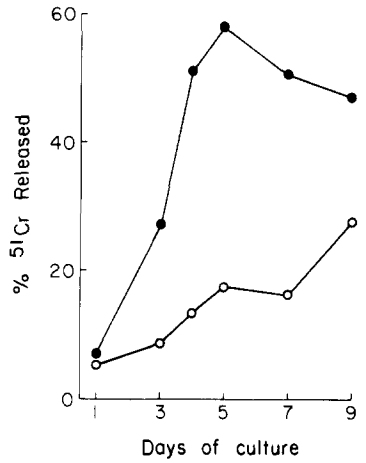

FIG. 1. Percent lysis of SVuv-coated (●) and untreated (○) P815 target cells by BALB/cJ spleen cells at various times after in vitro restimulation with SVuv-coated syngeneic stimulator cells. Effector:target cell ratio was 30:1. The spontaneous release of all targets ranged from 12 to 20% of the maximum release.

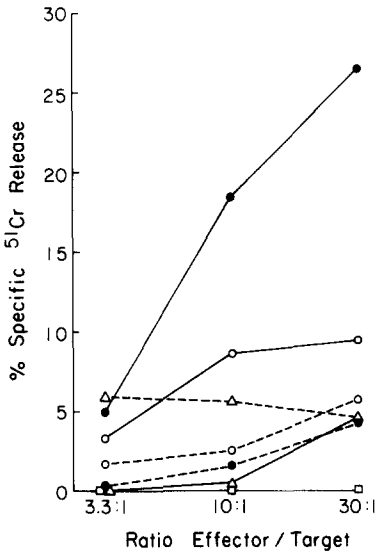

FIG. 2. Specific lysis of P815 target cells for Sendai virus. BALB/cJ effector cells were prepared from mixed leukocyte cultures at day 5. Effector cells were in vivo SVuv-primed and in vitro restimulated with SVuv-coated syngeneic cells (●), in vivo SVuv-primed and in vitro cultivated with normal syngeneic cells (□), in vivo unprimed and in vitro stimulated with SVuv-coated syngeneic cells (○), and in vivo unprimed and in vitro stimulated with allogeneic (C57BL/6) spleen cells (△). P815 target cells were coated with SVuv (——), or untreated (- - -). For calculation of the specific lysis, effector cells which were unprimed in vivo and in vitro cultivated with normal syngeneic cells were employed as normal effector cells.

from BALB/cJ, DBA/2J, and CBA/J strains could significantly kill the virus-coated H-2 syngeneic target cells, but they were not appreciably cytolytic to any of the allogeneic target cells. On the other hand, C57BL/6 effector cells significantly killed the virus-coated syngeneic EL-4 cells but not the untreated

TABLE I
*H-2 Restriction of Sendai Virus-Specific Cytotoxicity**

Effectors‡	Effector: target ratio	P815 ($H\text{-}2^d$) targets		Difference§	L929 ($H\text{-}2^k$) targets		Difference§	EL-4 ($H\text{-}2^b$) targets		Difference§
		Untreated	SVuv-coated		Untreated	SVuv-coated		Untreated	SVuv-coated	
BALB/CJ	6:1	1.7	15.0	13.3	−0.1	−0.1	0.0	0.0	−0.8	−0.8
($H\text{-}2^d$)	30:1	4.6	32.5	27.9	4.2	5.5	1.3	0.0	7.5	7.5
DBA/2J	6:1	0.2	3.3	3.1	1.1	2.2	1.1	0.0	−6.5	−6.5
($H\text{-}2^d$)	30:1	2.2	11.3	9.1	4.3	1.6	−2.7	0.0	4.7	4.7
CBA/J	6:1	1.5	1.4	−0.1	4.9	15.3	10.4	1.1	0.0	−1.1
($H\text{-}2^k$)	30:1	5.0	6.2	1.2	20.1	41.3	21.3	1.5	3.0	1.5
C57BL/6J	10:1	17.9	18.5	0.6	–	–	–	9.4	16.8	7.4
($H\text{-}2^b$)	30:1	36.0	38.7	0.7	–	–	–	−5.4	25.4	30.8

* Cytotoxicity values are represented as percent specific lysis.
‡ Effector cells were prepared from day 5 in vitro restimulation cultures.
§ Difference = (% specific lysis of virus-coated target cells) − (% specific lysis of untreated target cells).
Spontaneous releases of untreated and SVuv-coated P815, L929, and EL-4 target cells ranged from 13 to 22%, 16 to 29%, and 9 to 36%, respectively.

TABLE II
Effects of Ethanol Treatment on Sendai Virus Envelope Activities

Sendai virus	HAU/ml	NAU/ml*	Cell fusion	Hemolysis (A_{540})
			%	
Ethanol-treated	6,400	188	4.2	0.00
Untreated	6,400	200	100	1.89

* NAU, neuraminidase units.

EL-4 cells; these effector cells also lysed allogeneic P815 target cells independent of the presence or absence of Sendai virus. In these experiments, we could detect Sendai virus-specific cytotoxicity only in combinations in which effector and target cells were syngeneic.

Unsuccessful Formation of Target Antigen by Using Fusion-Inactivated Virions. Ethanol-treatment of Sendai virus resulted in the complete inactivation of fusion activity of the virus envelope. Sendai virus in allantoic fluids was either treated with ethanol, at a final concentration of 10%, or PBS (as an untreated control), and then inactivated by UV as described in Materials and Methods. The ethanol-treated virions were compared with the untreated virions with respect to their envelope biological activities. After treatment with ethanol, the virions completely lost hemolytic activity as well as ≅95% of cell fusion activity (Table II). Both of these activities are known to reside on the F glycoprotein of the Sendai virus envelope (26). However, hemagglutinin (HA) and neuraminidase (NA) activities which are associated with the HANA glycoprotein (26) were mostly retained (Table II).

Effects of ethanol treatment on the antigenicity of the virus envelope were also examined. Envelope fractions from the ethanol-treated and untreated virions solubilized by alkali-Tween 20 treatment each produced two precipitin bands with rabbit antiserum prepared against the untreated solubilized Sendai virus envelope in an Ouchterlony immunodiffusion plate (Fig. 3). These two bands give a reaction of identity between the ethanol-treated and untreated

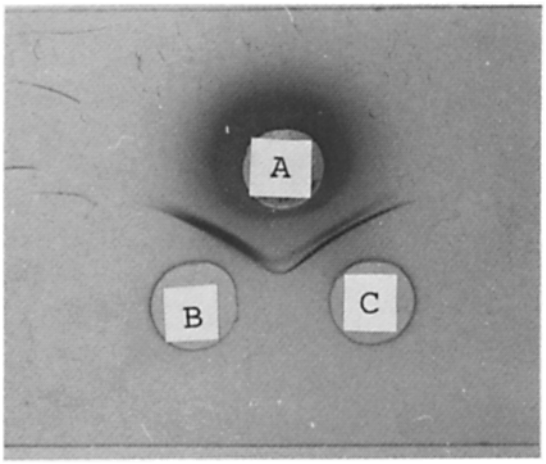

FIG. 3. Effect of ethanol-treatment on the antigenicity of Sendai virus envelopes. 0.9% agarose (Bio-Rad Laboratories, Richmond, Calif.) in PBS was used for the double immunodiffusion. Solubilized envelope fractions were obtained from untreated Sendai virions (B), and ethanol-treated virions (C) by alkali-Tween 20 treatment. (A), rabbit antiserum against alkali-Tween 20-disrupted Sendai virus envelopes. Precipitin bands were stained with amido black 10B.

virus envelopes. The two antigenic components of the Sendai virus envelope have been recognized as the F and HANA glycoproteins (21). A semi-quantitative assay of these two antigens was also performed on the immunodiffusion plate. Serial dilution of the envelope fractions resulted in no detectable precipitin line at 32-fold and 16-fold dilution for F and HANA antigens with no apparent difference between the ethanol-treated and untreated fractions. Thus, ethanol treatment did not appear to affect the antigenicity of F and HANA glycoproteins of the Sendai virus envelope.

Fig. 4 shows the cytotoxicity observed on P815 target cells by Sendai virus-specific T_c (derived from BALB/cJ) when target cells were coated with various doses of ethanol-treated or untreated virus. Lysis of untreated virus-coated target cells was dependent on the dose of virus used for coating the cells. At least 5 HAU of the input virus per 1×10^7 target cells appears to be sufficient for formation of target antigen and the detection of significant cytolysis. The target cells coated with ethanol-treated virus were far less susceptible to lysis at the various coating doses of virus used. These results indicate that assuming the ethanol-treated virions adsorb to the cell surface, they have lost the ability to form target antigens.

We thus assayed whether or not there was a difference in the amount of adsorbed virions on the target cells coated with either ethanol-treated or untreated virions. P815 cells were coated with ethanol-treated or untreated virions and then incubated in complete medium for at least 6 h, which corresponded to the cytotoxicity reaction time. After incubation, cell-associated virions were indirectly quantitated by determining the viral NA activity associated with the cells. Only minor differences in the cell-associated NA activities were detected betwen ethanol-treated and untreated virion-coated P815 cells during the indicated incubation periods (Table III). Therefore, the

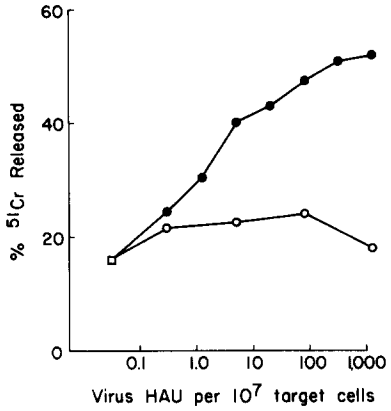

FIG. 4. ^{51}Cr-labeled P815 target cells were untreated (□), or coated with untreated Sendai virus (●) or ethanol-treated virus (○) at the doses indicated. The percent cytotoxicity of BALB/c effector cells from day 5 in vitro restimulation cultures is given against these target cells at the effector:target cell ratio of 30:1. Spontaneous release of the targets ranged from 12 to 24%.

TABLE III
Neuraminidase Activities of Sendai Virus-Coated P815 Cells

Time after virus coating	Virus dose	Neuraminidase activity (A_{549}) from cells coated with:		
		Ethanol-treated virus	Untreated virus	No virus
h	*HAU/10^7 cells*			
0	200	0.429	0.494	0.129
	20	0.372	0.383	
1	200	0.352	0.350	0.132
	20	0.276	0.284	
3	200	0.350	0.330	0.113
	20	0.247	0.267	
6	200	0.301	0.311	0.117
	20	0.204	0.233	

P815 cells were treated with ethanol-treated virus in PBS, untreated virus in PBS, or PBS (no virus) at the two different doses of the viruses in the same manner as described for the target cell preparation. After washing, the cells were incubated at 37°C in the EHAA complete medium for the indicated times. After incubation, 4×10^6 cells were pelleted and assayed for cell-associated viral neuraminidase activity.

inability of the ethanol-treated virions to induce target antigen could not be accounted for by a quantitative difference in the number of surface-adsorbed virions on the target cell.

Discussion

Sendai virus-specific T_c could be generated secondarily in a mixed leukocyte culture consisting of mouse spleen responder cells primed in vivo with the UV-inactivated virus and restimulated in vitro with irradiated syngeneic spleen

cells precoated with this virus (6, 20). The present study illustrates the time course of secondary, in vitro stimulation with highly significant killing observed on days 4–7 in the secondary stimulation culture. This time course was quite similar to that seen in allogeneic stimulation systems using a similar protocol (27). The cytotoxic cell population generated in our system was largely comprised of T lymphocytes as characterized by the removal of effector cells with anti-thy 1.2 ascitic fluid and complement. The possibility that Sendai virus via its HA and fusion activities acts nonspecifically to bring effector cells together with Sendai virus-coated target cells to result in killing has been ruled out (6, 20). Our results, given in Fig. 2, are consistent with this conclusion in that alloreactive BALB/c cytotoxic effector cells (anti-H-2^b) were unable to lyse P815 (H-2^d) target cells coated with Sendai virus.

It has been observed that the major histocompatibility complex plays a role in killing by Sendai virus-specific T_c (6) as in other virus sytems (7). Examination of H-2 haplotype restriction in Sendai virus-specific cytotoxicity performed in our laboratory also shows that significant and virus-specific killing by effector cells generated in vitro against SVuv-coated syngeneic stimulator cells, was detected only when target cells expressed the same H-2 haplotype as the effector cells and were coated with Sendai virus (Table I). Although C57BL/6 effector cells were able to kill allogeneic P815 target cells, there was no specificity with regard to the presence or absence of virus on the P815 target cell surface (Table I). Similar observations of P815 cell lysis by SVuv-immune C57BL/6 effector cells have been reported by Schrader and Edelman (6).

In a previous study (20) we demonstrated that Sendai virus stripped of F glycoprotein by trypsin digestion could not serve as or induce the target antigen upon adsorption to target cells. These results strongly suggest that the F glycoprotein plays an essential role in the formation of the target antigen which is recognized by the Sendai virus-specific T_c. One explanation for this role is that a trypsin-digestible structure of the F glycoprotein itself may participate directly in target antigen formation on the target cell surface. Alternatively, the biological fusion activity of F glycoprotein may be involved primarily in the formation of a target antigen which may or may not include the F glycoprotein. To discriminate between these two hypotheses, we used ethanol-treated Sendai virions which lack cell-fusion and hemolytic activities, but still retain the serologically defined antigenic structure of F glycoprotein. The structure and biological activities of the HANA glycoprotein of the envelope are also left intact after ethanol treatment.

Target cells coated with ethanol-treated Sendai virions were not susceptible to Sendai virus-specific T_c cytotoxicity despite the lack of a difference in the amount of cell-associated virus between untreated virus- and ethanol-treated virus-coated target cells. These observations strongly support the hypothesis that fusion activity is a crucial function of F glycoprotein for expression or presentation of target antigen on target cells coated with SVuv.

Two models have been proposed to explain H-2 restriction in virus-specific T-cell-mediated cytotoxicity. A single receptor on T_c may recognize the physical association of H-2 and viral antigens or the virus-modified H-2 antigen on the target cell surface. Alternatively, there may be two receptors on T_c which recognize H-2 and viral antigens separately (12). Studies using cold target

inhibition techniques argue against the two receptor hypothesis, since blocking cells required preinfection of virus (28). On the other hand, a recent study of influenza virus-HA-specific T-cell-mediated cytotoxicity has yielded evidence supporting dual recognition of H-2 and viral antigens (29). Although no definitive evidence exists that differentiates between these two models, it has been suggested that H-2 and viral antigens might associate physically with each other as, for instance, in the murine leukemia virus systems (13, 14). It is therefore conceivable that Sendai virus fusion activity can promote effective association of antigens in the target cell membrane since it has been shown that virion components are quickly dispersed throughout the cell membrane after fusion with the virus envelope (30). This version of an active role for Sendai virus fusion activity is consistent with the observation that choleragenoid-specific T-cell-mediated cytotoxicity could not be successfully generated against target cells coated with choleragenoid, even though choleragenoid could bind stably to cell surface receptors and such cells could be lysed by treatment with anti-choleragenoid serum and complement (31). The need for an active association between viral and H-2 antigens is not necessarily contradicted by the finding that isolated influenza HA glycoprotein can induce secondary T_c (32), since the activity of influenza HA in the formation of target cells is unknown. It may be that isolated influenza HA glycoprotein itself, separate from NA glycoprotein which breaks HA receptors, associates stably with H-2 antigens on the cell surface, since H-2 molecules contain the HA receptor (33). In the case of Sendai, it is thus possible that fusion activity may serve to allow the formation of a target antigen consisting of H-2 and the F and/or HANA moieties in a stable form of association.

The need for fusion-like activity, or recognition between H-2 and viral products that exists when inactivated virus or viral components are used to coat the target cell, may not exist for target formation when live, infectious virus is used. Under these conditions, the physical association of viral and H-2 antigens could occur through de novo viral protein synthesis in cells infected with the live virus; in such a system, as has been shown in the influenza virus sytem, viral antigenic components may already be associated with H-2 antigens as matrix proteins when they appear on the cell surface (19).

Summary

Mice inoculated with ultraviolet light-inactivated Sendai virus mount a cell-mediated immune response to the virus. Cytotoxic T cells specific for Sendai virus can be obtained by in vitro secondary stimulation of primed spleen cells with syngeneic stimulator cells coated with UV-inactivated Sendai virus. Neither in vivo nor in vitro stimulation alone is sufficient to generate specific cytotoxic T cells. Sharing of the H-2 haplotype between cytotoxic T cells and target cells is required for the Sendai virus-specific lysis to occur.

The fusion (F) glycoprotein of Sendai virus has been implicated in target antigen formation (20). Ethanol treatment of Sendai virus causes complete inactivation of the cell-fusion and hemolytic activities of the envelope, but does not affect the antigenicity of the F glycoprotein; furthermore, hemagglutinin and neuraminidase activities of the envelope HANA glycoprotein are also left intact after ethanol treatment. Target cells can be prepared by coating them

with various numbers of UV-inactivated Sendai virus that have been treated with ethanol or, as a control, phosphate-buffered saline (PBS). The amount of virus adsorbed to target cells during the cytotoxicity reaction time using either ethanol-treated or untreated (PBS "treated") virions is essentially identical, but target cells coated with ethanol-treated Sendai virus fail to serve as targets for cytotoxic T cells. These results indicate that fusion activity of the Sendai virus envelope is essential to the formation of the target antigen and that virus adsorption to cell surfaces without fusion of the envelope with cell membranes is not sufficient to allow killing by virus-specific cytotoxic T cells.

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