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ELICITATION OF ANTI-VIRAL CYTOTOXIC T LYMPHOCYTES WITH PURIFIED VIRAL AND H-2 ANTIGENS[1]

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The minimal molecular requirements for elicitation of secondary anti-Sendai virus CTL were investigated. The hemagglutinin-neuraminidase (HN) glycoprotein of Sendai virus and the H-2K^k glycoprotein of YAC tumor cells were purified and incorporated into phospholipid vesicles. These unilamellar liposomes were then tested for the ability to elicit H-2 restricted secondary anti-Sendai virus CTL. The results indicate that these well-defined vesicles were capable of eliciting secondary anti-Sendai virus CTL which lysed only target cells possessing the H-2K^k haplotype and modified with inactivated Sendai virus.

Determination of the minimal molecular requirements for elicitation of cytotoxic T lymphocytes (CTL)[3] has been an important goal in the study of viral and tumor immunology. Recent advances using cell-surface membrane components incorporated into artificial lipid vesicles have provided an important approach to investigating these questions (1, 2). Finberg *et al.* (3) and Loh *et al.* (4) have shown that H-2-restricted secondary anti-viral effector cells can only be elicited when partially purified viral antigens and cell surface membrane components obtained from tumor cells of the appropriate H-2 haplotype are incorporated together into the same liposomes. The effector cells elicited with these liposomes specifically lysed only target cells possessing the appropriate viral antigen and an H-2 haplotype identical to the H-2 antigens incorporated into the liposomes. Although these results shed significant light on the nature of the eliciting antigen, it has not been conclusively established whether the serologically defined H-2K or H-2D antigens in association with the viral antigens are the sole requisites for the elicitation of anti-viral CTL. In this communication, we have purified the hemagglutinin-neuraminidase (HN) protein of Sendai virus and the H-2K^k of YAC tumor cells, and by incorporating them into unilamellar phospholipid vesicles have elicited anti-Sendai virus CTL specific for virus-modified H-2K^k-containing target cells.

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[3] Abbreviations used in this paper: CTL, cytotoxic T lymphocytes; HN, hemagglutinin-neuraminidase protein of Sendai virus; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; NMS, normal mouse serum.

MATERIALS AND METHODS

Mice. Male and female mice of the following strains were used: BALB/c (d,d); BALB·HTG(d,b); BALB·K (k,k); B10·A (k,d); and (BALB/c × BALB·K)F1. These were either purchased from Cumberland View Farms (Clinton, Tenn.) or produced in our own breeding colony from breeding stock obtained from Herman Eisen (Massachusetts Institute of Technology). Letters in parentheses indicate the H-2K and H-2D alleles.

Virus. Sendai virus was grown in embryonated eggs and used to elicit anti-Sendai virus CTL or to render cells susceptible to lysis by anti-Sendai virus CTLs (5–7). The virus was inactivated by ultraviolet (UV) light as described previously (6, 7).

Purification of HN glycoprotein. Purified Sendai virus was extracted and HN was purified as previously described (5–7). Purity was monitored by sodium dodecylsulfate polyacrylamide gel electrophoresis (8, SDS PAGE).

Purification of H-2K^k. H-2K^k was purified as described by Herrmann and Mescher (9) from YAC tumor cells. The purity of the H-2K^k gene product was monitored by SDS PAGE (8). H-2K^k molecules were eluted from Sepharose 4-B columns conjugated with monoclonal anti-H-2K^k (clone 11-4.1, Becton Dickinson), with 0.2% sodium deoxycholate (DOC) in 140 mm NaCl (8).

Iodination. Purification of HN and H-2K^k was monitored by SDS PAGE (8) and autoradiography. YAC tumor cells or purified Sendai virus were iodinated with ^{125}I in the presence of lactoperoxidase and H_2O_2 (10). The cells or virus were washed by centrifugation and each protein was purified as described above. Samples were taken, run on SDS PAGE (8), and identified by autoradiography.

Production of CTL. Secondary anti-Sendai virus CTL were elicited by using spleen cells from mice primed 4 to 6 weeks earlier with 100 μg of UV-inactivated Sendai virus (protein determination by the method of Lowry *et al.* 11) by an i.p. injection.

When Sendai virus glycoproteins incorporated into liposomes were used to elicit a secondary anti-Sendai virus CTL response, 8.0 × 10^6 Sendai virus-primed responder spleen cells were incubated in 0.5 ml of supplemented RPMI 1640 (10% heat-inactivated fetal calf serum, 0.03% glutamine, (50 μm) 2-mercaptoethanol, penicillin, and streptomycin (12)) containing liposomes for 1.0 hr at 37°C with continual agitation. After the 1.0-hr incubation, the mixture was diluted to a final volume of 2.0 ml and plated in 1.7 × 1.6 cm wells (Linbro, Hamden, Conn.)

for 5 days. After 5 days incubation, the cells were harvested, washed by centrifugation (450 × G), counted, and resuspended in supplemented RPMI 1640 at various cell concentrations for the cytotoxicity assay (12).

Target cells. P815 (H-2^d) mastocytoma and EL-4 (H-2^b) lymphoma cells were maintained by weekly i.p. transfers of 1.0 × 10^6 ascites cells in mice of the strains of origin, DBA/2 and C57BL/6, respectively. YAC, a T cell lymphoma induced by Maloney leukemia virus in A/SN mice, was maintained in culture. $R1^+$ and $R1^-$, C58 mouse lymphoma lines were also maintained in culture. The $R1^-$ variant was originally obtained by selection among $R1^+$ cells from variants that lack β-2 microglobulin. The $R1^-$ variant lacks H-2K and H-2-D gene products, as well as the TL antigen (13). BALB/c, BALB·K, BALB·HTG, and B10·A spleen cells were stimulated with concanavalin A (Con A) as described previously (12). Target cells were then incubated with $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, Mass.) in supplemented RPMI 1640 as described previously (12). Target cells were made susceptible to lysis by anti-Sendai virus CTL by incubation at 4°C for 60 min with 10 μg of UV-inactivated Sendai virus per 10^6 target cells in RPMI 1640 (5–7).

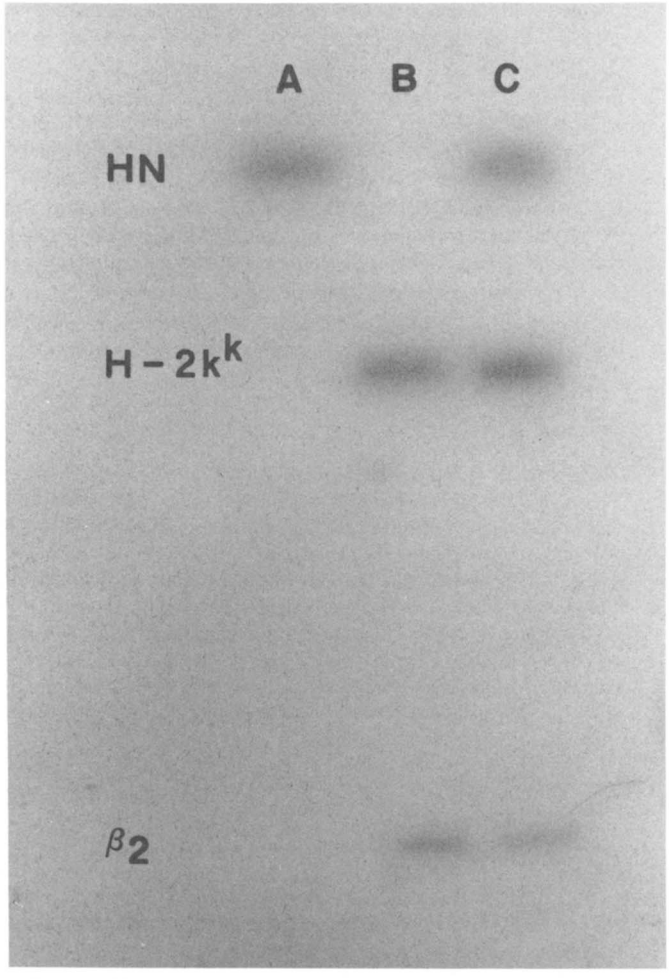

Figure 1. Electrophoretic characterization of the purity of the HN (69,000 daltons) glycoproteins (*A*) and the H-$2K^k$ (heavy chain 47,000 daltons and light chain 12,000 daltons) glycoproteins (*B*). Tumor cells (YAC) or purified Sendai virus were iodinated with ^{125}I in the presence of lactoperoxidase and H_2O_2 (13). The H-$2K^k$ and HN molecules were purified as described in *Materials and Methods* and each fraction was analyzed for purity on SDS PAGE (8). Purified H-$2K^k$ and HN molecules after incorporation into liposomes were extracted with electrophoresis sample buffer (8) and also analyzed on SDS PAGE (C).

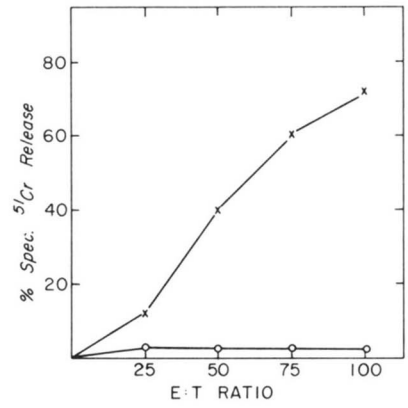

Figure 2. Spleen cells (8.0 × 10^6) from B10·A mice primed 4 to 6 weeks earlier with UV-inactivated Sendai virus (100 μg) were incubated with HN-H-$2K^k$-containing liposomes (total protein per culture = 1.0 μg) for 5 days. Cells were harvested and tested at different effector to target ratios (E:T ratio) for ability to lyse ^{51}Cr-labeled B10·A Con A-stimulated spleen cells. Spleen cells incubated with inactivated Sendai virus (10.0 μg/10^6 target cells) were lysed (×) whereas cells not incubated with the virus were not lysed (○). Percent ^{51}Cr release for B10·A spleen cells incubated with Sendai virus was 19.7 whereas the unmodified spleen cells had a spontaneous ^{51}Cr release of 15.9%. These background levels were obtained by incubating the target cells with primed spleen cells that were not incubated with HN-H-$2K^k$ liposomes.

Reconstitution of viral and H-2 antigens into liposomes. Reconstitution of liposomes was done essentially as described before (4). Briefly, dimyristoylphosphatidylcholine (DMPC) suspended in cholic acid by sonication was mixed with purified HN and H-$2K^k$ glycoprotein at a lipid to protein ratio of 1:1 (w/w). The protein ratio (w/w) of H-$2K^k$ to HN was 1:1. This solution was then dialyzed for 36 to 48 hr against phosphate-buffered saline at room temperature. The dialysate was recovered as an opalescent solution with greater than 80% of the protein associated with the vesicles. The resultant liposomes were further purified by flotation isopycnic centrifugation to ensure that proteins not associated with liposomes were removed from stimulator fractions. The final liposome fraction contained unilamellar structures 400 to 850 Å (determined by transmission electronmicroscopy) in diameter with a density that ranged between 1.05 and 1.11 g/cm^3.

RESULTS

Analysis of the purity of the isolated HN and H-$2K^k$ glycoproteins by SDS PAGE is shown in Figure 1. After incorporation of these purified preparations into liposomes, analysis of these phospholipid vesicles by SDS PAGE demonstrated that both the HN (69,000 daltons) and the H-$2K^k$ (heavy chain 45,000 and light chain 12,000 daltons) molecules were incorporated into the liposomes (Fig. 1).

These liposomes were tested for their ability to elicit secondary anti-Sendai virus effector cells. The results indicated that only target cells modified with UV-inactivated Sendai virus and possessing the H-$2K^k$ haplotype were susceptible to lysis by these effector cells (Fig. 2, Table I). It is clear from the data that both the HN glycoprotein and the H-$2K^k$ molecules incorporated into a single liposome were required for elicitation of effector cells.

An analysis of the identity of anti-Sendai virus effector cells

TABLE I

Elicitation of anti-Sendai virus CTL with purified HN + H-2K^k glycoproteins[a]

Stimulator Liposomes	% Specific ^{51}Cr Release (E:T Ratio 25:1)[b] of Con A-stimulated spleen cells											
	R1$^+$-SV (k,k)	R1$^+$	P815-SV (d,d)	P815	YAC-SV (k,d)	YAC	B10·A-SV (k,d)	B10·A	BALB·K-SV (k,k)	BALB·K	BALB·B-SV (b,b)	BALB·B
HN	0.7	−0.8	0.8	0.0	2.0	0.4	2.1	−0.9	−0.4	−1.1	−1.8	−2.0
H-2K^k	0.4	1.3	1.4	0.7	1.2	−0.7	2.0	0.8	1.4	1.8	1.0	0.7
HN-H-2K^k	39.7	1.7	0.9	−1.2	64.7	1.2	48.7	2.1	39.7	1.5	2.4	−0.4
HN + H-2K^k	0.9	1.8	−0.9	1.1	1.2	0.9	0.0	1.0	0.7	1.2	−2.6	0.8

[a] Spleen cells (8.0×10^6) from (BALB/c × BALB·K)F$_1$ mice primed 4 to 8 weeks earlier with UV-inactivated Sendai virus were incubated with 0.5 μg HN incorporated into liposomes. H-2 antigens, when incorporated into the HN liposomes, were added at a final concentration of 0.5 μg of the purified H-2K^k per 2.0 ml of culture medium. Each determination represents the mean of six assays. The standard error of all determinations was lesss than 2.8%. "HN + H-2K^k" indicates that the viral antigens and the H-2K^k glycoproteins were incorporated into separate liposomes; "HN-H-2K^k" indicates that both proteins were incorporated into the same liposomes. "Target-SV" indicates that the cell was incubated with UV-inactivated Sendai virus (10 μg/10^6 cells) before testing for susceptibility to lysis. Letters in parentheses represent the H-2 haplotype at the H-2K + H-2D end of the MHC.

[b] The percent release of ^{51}Cr from R1$^+$-SV, R1$^+$, P815-SV, P815, YAC-SV, YAC, B10·A-SV, B10·A, BALB·K-SV, BALB·K, BALB·B-SV, and BALB·B were 6.2, 2.1, 11.7, 6.2, 6.7, 4.3, 18.7, 15.6, 16.7, 10.7, 18.7, and 15.8, respectively.

TABLE II

Susceptibility of anti-Sendai virus effector cells elicited with HN-H-2K^k liposomes to anti-thy 1.2, anti-Ly-1+ and anti-Ly-2+ serum[a]

Treatment	% Specific ^{51}Cr Release (E:T Ratio 100:1) of B10·A-SV[b]
None	39.7
NMS	40.1
Anti-thy 1.2	38.4
Anti-Ly-1	41.2
Anti-Ly-2	43.4
NMS + C	37.6
Anti-thy 1.2 + C	2.7
Anti-Ly-1 + C	37.7
Anti-Ly-2 + C	3.1

[a] (BALB/c × BALB·K)F$_1$ spleen cells (8.0×10^6) incubated with HN-H-2K^k liposomes (see Table I) were treated with anti-thy 1.2, anti-Ly-1 or anti-Ly-2 antiserum in the presence of C (Cedarlane Laboratories, Ontario, Canada). Effector cells (1×10^6) were incubated with a predetermined optimal concentration of each antiserum. After 30 min incubation at room temperature, the cells were washed by centrifugation ($500 \times g$) and rabbit C was added at a predetermined dilution. These cells were incubated for 20 min at 37°C, washed by centrifugation and tested for cytolytic activity against B10·A-SV target cells. The B10·A target cells had a spontaneous ^{51}Cr release of 12.8%.

[b] Each number represents the average of six determinations with a standard deviation for all measurements of not greater than 3.8%.

elicited by these liposomes is shown in Table II. The effector cells were T cells that were Ly 1$^-$, 2$^+$.

DISCUSSION

It is clear from our results that anti-Sendai virus CTL can be elicited with purified HN and H-2K^k molecules incorporated into a phospholipid vesicle. The observation that spleen cells from Sendai virus-primed (BALB/c × BALB·K) F1 mice stimulated with HN-H-2K^k liposomes elicit anti-Sendai virus CTL that specifically lyse only VSV-infected H-2K^k target cells, suggests that antigen presentation does not occur by cells within the culture. Furthermore, it is implied that interaction with HN and H-2K^k molecules is both necessary and sufficient to elicit anti-Sendai virus CTL. These data do not exclude the possibility, however, that antigen presentation by cells within the culture could occur under different conditions.

More importantly, these data indicate that the serologically defined H-2K^k molecule is capable of interacting in some unknown way with the purified HN molecules of Sendai virus to elicit anti-Sendai virus CTL. Recent results suggest that the ability of HN-H-2K^k liposomes to elicit secondary anti-Sendai virus CTL is not unique to this system. We have observed that liposomes containing vesicular stomatitis virus (VSV) major envelope glycoprotein and H-2K^k have similar ability to elicit CTL specific for VSV-infected H-2K^k target cells (manuscript submitted). This suggests that the serologically defined H-2K and H-2D gene products are indeed the same molecules that are important for syngeneic *in vivo* CTL responses (anti-viral anti-tumor, etc.).

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