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CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR VESICULAR STOMATITIS VIRUS RECOGNIZE THE MAJOR SURFACE GLYCOPROTEIN OF VSV

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The major surface glycoprotein (G) of vesicular stomatitis virus (VSV) was purified and incorporated into lipid vesicles containing the purified hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins of Sendai virus. These lipid vesicles were then used to modify and render target cells susceptible to lysis by anti-VSV cytotoxic T lymphocytes (CTLs). This result provides direct evidence that the G protein is a target antigen of anti-VSV CTLs.

anti-VSV CTLs target antigen G protein

INTRODUCTION

Establishment of the minimal molecular requirements for elicitation of cytotoxic T lymphocytes (CTLs) has been an important goal in the study of viral and tumor immunology. Incorporation of cell surface membrane components into artificial lipid vesicles has provided a means to investigate these questions [1, 9]. Several investigators [2, 4, 6, 10] have shown that H-2-restricted secondary antiviral effector cells can only be elicited when partially purified viral antigens and partially purified H-2 glycoproteins of the haplotype syngeneic to the viral-primed spleen cells are incorporated together into the same liposome. The resultant antiviral CTLs lyse only target cells possessing viral and H-2 antigens identical to those incorporated into the eliciting liposomes. Although these studies have enabled an evaluation of the minimal requirements for elicitation of antiviral CTLs, they do not provide an acceptable approach for identifying antigens recognized by CTLs.

Abbreviations: CTLs, cytotoxic T lymphocytes; DOC, sodium deoxycholate; F, fusion glycoprotein of Sendai virus; G, major surface glycoprotein of VSV; HN, hemagglutinin-neuraminidase glycoprotein of Sendai virus; MDBK, Madin–Darby bovine kidney cells; MHC, major histocompatibility complex; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SV, Sendai virus; UV, ultraviolet light; VSV, vesicular stomatitis virus.

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Recently, we have developed a method of liposome modification of cell surfaces to render unsuitable target cells susceptible to lysis by antiviral cytotoxic T lymphocytes (CTLs). Liposomes containing the hemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins of Sendai virus as well as purified H-2K^k antigens were shown capable of binding to the surface of H-2-negative cells, and rendering those cells susceptible to lysis by B10·A anti-Sendai virus or anti-H-2K^k CTLs. The absence from the modifying liposomes of the HN or F proteins or the H-2K^k antigens eliminated the ability of the target cells to be recognized and lysed by either effector cell population. Vesicles containing HN, H-2K^k molecules and inactive fusion protein (Fo) were not capable of increasing the susceptibility of H-2-negative target cells to lysis by either anti-H-2K^k or anti-Sendai virus CTLs. These results indicated that the purified H-2K^k glycoprotein is a target antigen for anti-H-2K^k CTLs and that B10·A anti-Sendai virus CTLs recognize in an H-2-restricted manner the HN and/or F glycoproteins of Sendai virus in the context of the purified H-2K^k glycoproteins [5].

In this report we have incorporated the major surface glycoprotein (G protein) of vesicular stomatitis virus (VSV) into liposomes containing the HNF glycoproteins of Sendai virus. These liposomes were then tested for their ability to render target cells susceptible to lysis by anti-VSV CTLs. The results indicate that the G protein is recognized by anti-VSV CTLs.

MATERIALS AND METHODS

Mice

Male and female mice of the following strains were used: BALB/c (*d,d*); BALB·HTG (*d,b*); and BALB·B (*b,b*). These were produced in our own breeding colony from stock obtained from Herman Eisen (Massachusetts Institute of Technology). Letters in parentheses indicate the H-2K and H-2D alleles.

Virus

Sendai virus with the inactive fusion glycoprotein (Fo) was grown in Madin–Darby bovine kidney (MDBK) cells, the virus was purified, and Fo was activated by treatment with trypsin (5 μg/ml, TPCK-trypsin, Worthington, Freehold, NJ) as described by Scheid and Choppin [11]. Viral infectivity was inactivated by ultraviolet light (UV) as described previously [4, 12]. Stocks of VSV were prepared as described previously [7].

Antisera

Anti-Thy-1.2, anti-Ly-1$^+$, anti-Ly-2$^+$ sera were obtained from Cedarlane Laboratories (Hicksville, NY).

Purification of G protein

The envelope glycoprotein (G protein) of VSV was extracted from sucrose gradient-purified virus with Nonidet P40 (NP-40) [12] as described previously [7].

Purification of Sendai virus glycoproteins

The glycoproteins of MDBK-grown Sendai virus with and without trypsin treatment were isolated by extraction of the purified virus with Triton X-100 (final concentration of 2.0%) as described by Scheid and Choppin [11]. The HNF, HNFo, HN and F glycoproteins were purified as described by Hale et al. [4]. Purification of HNF, HNFo, HN, F and G protein was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) [14] and autoradiography. Purified Sendai virus or VSV were iodinated with ^{125}I in the presence of lactoperoxidase and H_2O_2 [8]. The virus was washed by centrifugation and each protein was purified as described above. Samples were taken, run on SDS PAGE [11] and identified by autoradiography. The purified proteins (G, HNF, HN and F) were single bands on SDS PAGE as reported previously [4, 5, 7].

Cultured lines

The following cell lines were maintained in culture: P815 (d,d), a mastocytoma of DBA/2 origin, and MC57G (b,b), a macrophage-like line of C57 BL/6 origin.

Media

RPMI 1640 was supplemented with 10% fetal calf serum (FCS) (heat-inactivated, 56°C, 45 min), 0.03% glutamine, 2-mercaptoethanol (2-ME; 50 μM) and penicillin and streptomycin (Flow Laboratories, Inc., McLean, VA) [4].

Generation of anti-VSV CTLs

Mice were immunized by an intravenous injection of 2.0×10^6 plaque-forming units (p.f.u.) of Indiana VSV. Six days later spleen cells were harvested and serial dilutions of lymphocytes were incubated with target cells (see below).

Reconstitution of viral and H-2 antigens into liposomes

In a typical preparation, 200 μg of egg lecithin plus cholesterol (30% w/w, Calbiochem, San Diego, CA) dissolved in chloroform was dried in the form of a film under N_2. This film was then dissolved into a solution containing 0.2% DOC, purified G protein, and Sendai virus glycoproteins at a lipid to protein ratio of 1 : 1 in a final volume of 4 ml. The protein ratio (w/w) of G protein to HNF or HNFo, or G to isolated HN or F was 1 : 1. This solution was then dialyzed for 36–48 h 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 [1, 4, 5, 9].

Target cells

Target cells (1.0×10^6) were labeled with 500 μCi of $Na_2\ ^{51}CrO_4$ in 200 μl of 0.15 M NaCl for 20 min at 37°C. This suspension was then diluted 1 : 3 with supplemented RPMI 1640 and incubated for another 60 min at 37°C in an atmosphere of 94% air and 6% CO_2. ^{51}Cr-labeled target cells (1.0×10^5) were rendered susceptible to lysis by CTLs, by incubating reconstituted viral liposomes (100 μg of protein) in RPMI 1640 for 15 min

at 4°C and 30 min at 37°C. The cells were then washed by centrifugation at 450 × *g* and suspended with effector cells in a total volume of 100 μl of supplemented RPMI 1640.

Cytotoxic assays

^{51}Cr-labeled target cells (1.0×10^3) were mixed with serial dilutions of effector spleen cells in a total volume of 200 μl of supplemented RPMI 1640. This mixture was incubated 4–6 h at 37°C in an atmosphere of 6% CO_2 and 94% air. The assay was stopped by diluting with cold phosphate-buffered saline and the amounts of radioactivity in the supernatant and pellet were determined [4, 7]. Percent specific release was calculated as 100 $(E-C/1-C)$ where E is the fraction of ^{51}Cr released by antigen-stimulated effector cells and C is the fraction of ^{51}Cr released by a mock-stimulated responder population. Percent release was calculated 100 (E/T), where E is the amount of radioactivity released by target cells incubated with antigen-stimulated effector cells and T represents the total amount of radioactivity in each assay tube.

RESULTS

Modification of P815 or EL-4 tumor cells with lipid vesicles

Incorporation of purified G protein into lipid vesicles containing HNF and incubation of these vesicles with P815 tumor cells resulted in rendering the tumor cells susceptible to lysis by BALB/c anti-VSV CTLs (Table 1). Tumor cells modified with only HNF lipid

TABLE 1

Modification of P815 tumor cells with G protein incorporated into HNF-containing lipid vesicles[a,b]

BALB/c anti-VSV (E : T ratio)	% Specific ^{51}Cr release				
	P815	VSV–P815	G–HNF–P815	HNF–P815	G+HNF–P815
100 : 1	0.1	65.8	42.7	1.1	0.8
50 : 1	0.7	60.8	37.8	-0.9	1.3
25 : 1	-1.3	52.7	22.3	-0.4	1.4
12 : 1	1.2	40.8	12.7	1.2	-0.7
6 : 1	0.8	21.3	3.1	1.3	-0.2
0 : 1	0.6	-0.9	-0.7	-0.6	1.0

a BALB/c mice were infected with 1.0×10^6 p.f.u. of VSV. Six days later the spleen cells were harvested and serial dilutions of cells were tested for ability to lyse ^{51}Cr-labeled target cells. P815 cells were modified by VSV infection (m.o.i. = 50, VSV–P815), and by incubation with lipid vesicles (100 μg of protein/10^5 target cells) containing purified G protein and HNF glycoproteins (G–HNF–P815), or only HNF glycoproteins (HNF–P815), or G protein (50 μg of protein/10^5 target cells) and HNF (50 μg of protein/10^5 target cells) incorporated into separate lipid vesicles (G+HNF–P815). Each determination represents the average of triplicate measurements with the standard deviation never exceeding 2.9%.

b The spontaneous ^{51}Cr release for any of the target cells never exceeded 21.1%.

TABLE 2

Lysis of G protein-modified tumor cells is H-2-restricted[a,b]

Effector cells	% Specific ^{51}Cr release						
	E : *T* ratio	P815	VSV–P815	G–HNF–P815	MC57G	VSV–MC57G	G–HNF–MC57G
BALB/c α VSV	100 : 1	1.1	72.7	38.7	1.1	4.1	-0.7
	50 : 1	0.7	67.8	25.6	0.8	1.1	-0.8
	25 : 1	-1.2	52.7	16.7	2.1	0.7	0.4
BALB·B α VSV	100 : 1	0.8	6.1	1.1	0.7	84.2	49.7
	50 : 1	-0.7	3.1	1.2	0.3	72.3	39.8
	25 : 1	-0.1	2.1	0.8	0.4	67.8	31.6

[a] BALB/c or BALB·B mice were injected (i.v.) with 1.0×10^6 p.f.u. of VSV. Six days later the spleen cells were harvested and serial dilutions of cells were tested for their ability to lyse ^{51}Cr-labeled target cells. MC57G or P815 cells were modified by VSV infection (m.o.i. = 50, VSV–MC57G or VSV–P815) and by incubation with lipid vesicles (100 μg of protein/10^5 target cells) containing purified G protein and HNF glycoproteins (G–HNF–P815). Each determination represents the average of triplicate measurements with the standard deviation never exceeding 3.2%.

[b] The spontaneous ^{51}Cr release for any of the target cells never exceeded 17.7%.

vesicles or with G proteins and HNF incorporated into separate lipid vesicles (G + HNF) were not susceptible to lysis by anti-VSV CTLs. P815 tumor cells modified with V.-U. inactivated Sendai virus (10 μg of virus protein/10^6 cells) were not lysed by the anti-VSV CTLs (data not shown) [4]. As shown in Table 2, G–HNF modified tumor cells were lysed by H-2^b or H-2^d anti-VSV CTLs in an H-2-restricted manner. That is, BALB/c anti-VSV lysed primarily G–HNF-modified P815 tumor cells and not MC57G tumor cells modified with G–HNF. The reciprocal experiment was also true; BALB·B anti-VSV CTL lysed primarily only G–HNF-modified MC57G cells.

Requirements for G protein modification of tumor cells

Incorporation of G protein alone into lipid vesicles was not capable of modifying target cells (Table 3). In addition, G protein incorporated into lipid vesicles containing purified HN or F glycoproteins was not effective for rendering targets susceptible to lysis by anti-VSV CTLs. The inability of G–HN or G–HNFo lipid vesicles to render targets susceptible to lysis by anti-VSV CTLs suggests that an active fusion protein is important for modification of target cells. The inability of G–F lipid vesicles to modify target cells suggests a requirement for HN; possibly for binding to the cell surface [5, 14].

TABLE 3

Molecular requirements for modification of tumor cells with lipid vesicles[a,b]

Lipid vesicles	Lipid vesicles/10^5 cells (μg of protein)	% Specific ^{51}Cr release $E : T$ ratio = 100 : 1
G	100	0.7
	1000	-0.2
G–HNF	100	37.7
	50	16.7
	25	4.7
	10	1.2
	0	0.8
G–HN	100	1.1
G–F	100	-0.9
G–HNFo	100	2.1
	1000	8.1
G+HNF	50 + 50	0.6
	100 + 100	1.0

[a] BALB/c mice were infected by i.v. injection with 1.0×10^6 p.f.u. of VSV. Six days later spleen cells were harvested and assayed for anti-VSV CTL activity. Each determination represents the average of triplicate assays with the standard deviation never exceeding 2.4%.

[b] The P815 tumor cells were modified with different lipid vesicles: G protein (G), G protein in liposomes containing HNF (G–HNF), G protein in liposomes containing only HN (G–HN), G protein in liposomes containing only F (G–F), G protein in liposomes containing HNFo (G–HNFo) and G protein incorporated in a separate liposome from HNF (G + HNF). All protein fractions G–HN, G–F, G–HNF, or G+HNF were at a G : Sendai virus glycoprotein ratio of 1 : 1 by weight.

DISCUSSION

A major theme in the study of CTLs is to identify the antigenic moieties recognized by these effector cells. Various indirect methods have been used: 1) antibody-blocking experiments; 2) genetic mapping data; and 3) correlations between cytolysis and expression of known antigens. In previous work, we have studied CTLs which recognize and lyse target cells infected with VSV [7]. These anti-VSV CTLs recognize and lyse target cells in an H-2-restricted fashion and require the expression of G protein on the surface of the target cells for effective lysis [7, 14]. In this report, we have purified G protein, incorporated it into uninfected cells, and rendered those cells susceptible to lysis by anti-VSV CTLs. This result indicates that indeed the G protein is recognized by anti-VSV CTLs and is at least one antigen recognized by anti-VSV CTLs. We cannot eliminate the possibility that other antigens (i.e., matrix) might also be recognized by anti-VSV CTLs. Recently, Kosinowski et al. [8] demonstrated by a similar technique that the hemagglutinin glycoprotein (HA) of influenza virus was recognized by anti-influenza CTLs and that serologically distinct HA molecules are recognized by cross-reactive anti-influenza CTLs.

In addition, we identified some of the parameters required for target cell modification. The lack in any type of G-containing liposomes of HN and/or a functional F protein resulted in a reduced capacity of those liposomes to modify target cells in a way which rendered them susceptible to lysis by anti-VSV CTLs. This suggests, albeit does not prove, that an active fusion process is required for target cell modification [3, 4, 11, 12].

Recently, Volsky et al. [13] reported that the anion transport protein from erythrocyte membranes could be incorporated into leukemic cells through the use of liposomes containing Sendai virus glycoproteins and the transport protein. We have confirmed this technique and shown it to be applicable to analysis of the antigenic specificity of CTLs. These techniques may be potentially important in the development of immunotherapeutic measures to control many viral and neoplastic diseases.

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