Trojan Horse Lymphocytes: a Vesicular Stomatitis Virus-Specific T-Cell Clone Lyses Target Cells by Carrying Virus

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We have isolated a vesicular stomatitis virus (VSV)-specific CD4⁺ CD8⁻ murine T-cell clone. This clone proliferates only in response to VSV and lyses infected tumor cells bearing class II major histocompatibility antigens in short-term chromium release assays. In addition, the cell has VSV antigens on its surface and is capable of killing uninfected tumor cells without major histocompatibility antigen restriction in a 2-day assay. This latter cytolytic activity is eliminated by anti-VSV antibody, indicating that its lytic activity is provided by the virus. [³⁵S]methionine labeling and immunoprecipitation experiments demonstrated that viral protein translation is initiated after incubation of the clone with a tumor target cell, defining this as the mechanism of its cytolytic activity.

Virus-specific T-lymphocyte responses are usually restricted by recognition of viral antigens in the context of the major histocompatibility complex (MHC) antigens (31). Such cells can have cytolytic or helper/inducer activities. MHC class I-restricted cytolytic cells have been shown to be involved in the clearance of acute or persistent viral infections in mice (14, 16). Generally, cytolysis by CD4⁺ or CD8⁺ T cells is mediated by cells which recognize viral antigens in association with class II or class I MHC antigens, respectively (2, 4, 6, 13, 24, 25, 27), although a few exceptions have been reported (12, 23). The lytic event is initiated by activation of the mechanisms of cytolysis via T-cell-receptor recognition of the viral antigens and MHC proteins. It has recently been demonstrated by Schwimbeck et al. (22) and Tishon et al. (29), however, that lymphocytic choriomeningitis virus can become associated with lymphocytes without T-cell-receptor-mediated binding and can be recovered from CD4⁺ cells by cocultivating them with permissive cells. The significance of these observations in terms of viral pathogenesis is not clear.

Vesicular stomatitis virus (VSV) specifically kills activated T cells (8). We and others have noted that the simultaneous addition of live VSV in vitro will eliminate a virus-specific or allogeneic mixed-lymphocyte culture by killing the activated T cell. This finding is paradoxical since T cells are necessary for providing help to B cells in producing the antibodies and engaging in cytolytic and delayed-type hypersensitivity responses to the virus. If normal activated T cells were killed by live virus, there could never be a virus-specific response. This is not unlike the situation seen in humans with human immunodeficiency virus (HIV) infections in which, despite the presence of a virus which kills helper T cells, some CD4⁺ cells must survive infection at least long enough to help produce antibodies to HIV.

In this work, we have investigated the question of what T cells might survive VSV infection by repeated in vitro stimulation with live VSV and have characterized the surviving clone. We report a murine CD4⁺ CD8⁻ VSV-specific T-cell clone which is cytolytic to VSV-infected tumor target cells which express class II MHC antigens in short-term chromium release assays. This clone is also capable of killing

uninfected target cells unrestricted by MHC in 2-day-incubation assays. Experiments indicate that this clone, unlike other T-cell clones, is capable of withstanding live VSV infection. Furthermore, these cells are capable of killing uninfected nonhistocompatible cells by transmitting the lethal virus. A possible role for such cells in viral pathogenesis is discussed.

MATERIALS AND METHODS

Mice. BALB/cBy mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, and were used at 6 to 8 weeks of age.

Cell lines. A20 (*H-2^d Ia^d*), P815 (*H-2^d Ia⁻*) (American Type Culture Collection [ATCC]), BALB 3T3 (*H-2^d Ia⁻*) (ATCC), AJ9 (*K^k Ia^k D^d*), and L cells (*H-2^k*) (ATCC) were maintained in Dulbecco modified Eagle medium (Hazelton) supplemented with 10% fetal calf serum (Hazelton) heat inactivated at 56°C for 1 h, 100 U of penicillin per ml (GIBCO), 100 µg of streptomycin per ml, 1 mM sodium pyruvate (GIBCO), 0.1 mM nonessential amino acids (GIBCO), 10⁻⁴ M 2-mercaptoethanol (Sigma), and 2 mM L-glutamine (GIBCO). The cells were incubated in a 10% CO₂ incubator at 37°C.

Viruses. VSV (Indiana strain) was prepared by incubating a confluent monolayer of L cells with virus at a multiplicity of infection (m.o.i.) of 0.01 overnight. The source of the virus was the cell-free supernatant. The titer was determined by using a standard plaque assay. Herpes simplex virus (HSV; mP strain) was obtained from David Knipe (Department of Microbiology and Molecular Genetics, Harvard Medical School); the titer was determined by using Vero cells. The reovirus and VSV were gifts from Robert Kauffman (Beth Israel Hospital, Boston, Mass.). UV-irradiated VSV (UV-VSV) was prepared by irradiating the virus at 0°C by using a 30-W UV source (G30T8; General Electric) for 0.5 h at a distance of 5 cm.

Generation of VSV-specific T-cell clones. Immune spleen cells were obtained from mice injected 2 weeks earlier with 10⁸ PFU of VSV via the intraperitoneal route. The clone 1H10 was generated from a bulk culture prepared from the spleen of a VSV-immunized BALB/c mouse at a density of 4 × 10⁶ cells per well with an m.o.i. of 0.1 PFU per cell with live VSV on a 24-well plate (Costar, Cambridge, Mass.). To enrich for virus-specific T cells, the bulk culture was restimulated with antigen weekly for 4 weeks, in the absence of any

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exogenous source of T-cell growth factor, by using naive irradiated syngeneic feeder cells (4,000 rads by using a ^{137}Cs source) and UV-VSV at the same m.o.i. The clone was obtained by limiting dilution at the end of this period at a density of 1 cell per well on a 96-well plate with 2.5×10^5 feeder cells per well and UV-irradiated virus in the presence of 30% mixed-lymphocyte culture supernatant (21). The cells were further subcloned at a density of 0.1 cells per well by using the same density of feeder cells along with UV-VSV. The clone was split and restimulated once a week with feeder cells, antigen, and 30% mixed-lymphocyte-conditioned media, followed by the addition of another 0.3 ml of the supernatant 3 to 4 days later to each milliliter of cell culture. The cells were used in proliferation or cytotoxicity assays 6 days later.

Antibody against VSV. Antiserum against VSV was prepared by immunizing BALB/c mice with 10^7 PFU of VSV three times over the course of 3 months. The mice were bled, and serum was obtained, diluted in medium to a 1:50 ratio, and adsorbed over normal BALB/c spleen cells in order to remove any nonspecific binding prior to use. The monoclonal antibody against the G glycoprotein of VSV was a generous gift from Michael Mathieu.

Cytotoxic T-lymphocyte assay. Tumor target cells (A20, P815, AJ9, or BALB 3T3) were diluted to 10^6 cells per ml and incubated overnight with 150 μCi of $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear) in the media described above at 37°C and 10% CO_2 . The following day, half of each sample of cells was infected with either VSV or HSV for 2 h at an m.o.i. of 10. Reovirus-infected cells were infected 24 h before the assay. The target cells were washed to remove unbound virus. Five thousand target cells per well were incubated with effector T cells at various effector/target cell ratios in a total volume of 0.15 ml per well in a 96-well U-bottom plate (Costar). After 6 h of incubation, 0.09 ml of supernatant was harvested from each well to determine the amount of radioactivity released by using a gamma radiation counter (model 1185; Searle). Total release of ^{51}Cr was determined from wells incubated with 1% Triton X-100 (Sigma) instead of effector cells. Four replicate wells were used to obtain the ^{51}Cr release at each effector/target cell ratio. The percent ^{51}Cr release was computed as follows: % ^{51}Cr release = $100 \times [(\text{cpm released by sample} - \text{cpm released by targets alone}) / (\text{cpm released by sample} - \text{cpm released by 1\% Triton X-100} - \text{cpm released by sample})]$. Spontaneous release of ^{51}Cr was typically under 20%.

Proliferation assays. IH10 clone cells at 5,000 cells per well with 2.5×10^5 irradiated naive syngeneic stimulator spleen cells per well were incubated in quadruplicate in a total volume of 0.15 ml. UV-irradiated virus was incubated with the cells in an m.o.i. of 1. Control cultures without added virus were prepared in parallel. The cells were incubated for 2 days before being pulsed with 1 μCi of [^3H]thymidine (New England Nuclear) for 6 h and harvested by using a Skatron cell harvester. The samples were counted for 1 min on a beta particle counter (BetaTrac 6895; TM Analytic).

Inhibition of tumor proliferation. Inhibition of tumor replication by the clone was assayed by adding 10^4 IH10 clone cells irradiated with 2,000 rads to 2,000 tumor cells (A20, P815, AJ9, and BW5147) in the absence of any added antigen or in the presence of UV-VSV (10^6 PFU/ml) in a 96-well U-bottom tissue culture plate in triplicate. Another set of wells was prepared with murine anti-VSV antiserum (1:300 final dilution). Background proliferation of the irradiated IH10 clone alone was determined by adding medium or mixed-lymphocyte-culture medium (30% final concentra-

tion). The proliferation of the tumor cells was determined on the basis of incorporation of tritiated thymidine as described above. Cells containing infectious VSV were used on day 8 after stimulation with both UV-VSV, live VSV, and antigen-presenting cells. Another set of experiments was performed by using IH10 cells cured of infectious virus by incubation for 2 weeks in a 30% mixed-lymphocyte-culture supernatant with a 1:100 dilution of murine anti-VSV serum.

Fluorescence-activated cell sorter (FACS) analysis. To demonstrate the dual expression of VSV antigen on the surface of the clone with L3T4, the cloned T cells were incubated with VSV-immune antiserum (versus naive control serum) at a titer of 1:100 and washed, followed by incubation with (FITC)-labeled rabbit anti-mouse immunoglobulin at 0°C , washed, and finally stained with 0.1 ml of a 1:50 dilution of phycoerythrin-labeled L3T4 antibody (Becton Dickinson and Co., Mountain View, Calif.). The samples were sorted on an EPICS V cell sorter (Coulter) by using single (FITC) or double fluorescence (FITC and phycoerythrin).

Cell surface labeling. Restimulated IH10 and A20 cells infected overnight with live VSV at an m.o.i. of 0.05, separated from dead cells with lymphocyte separation medium (Organon Teknika) and washed with phosphate-buffered saline (PBS), were used for cell surface labeling. Cell surface iodination was carried out by the lactoperoxidase method (26). Cells (3×10^6 to 5×10^6) were suspended in 1 ml of PBS containing 5 mM glucose. Iodination was effected by the successive addition of Na^{125}I (1 mCi/ml) (New England Nuclear), lactoperoxidase (20 $\mu\text{g}/\text{ml}$), and glucose oxidase (0.65 units/ml) (Sigma). The reaction mixture was incubated at 20°C for 20 min with swirling. The cells were washed twice in PBS and then lysed in buffer containing 10 mM Tris hydrochloride (pH 7.4), 0.15 M NaCl, 2 mM EDTA, 2 mM ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA), 20 IU of aprotinin per ml, 1% Triton, and 1 mM freshly prepared phenylmethylsulfonyl fluoride (Sigma). After 1 h of incubation at 0°C , the lysate was clarified by centrifugation at $45,000 \times g$ for 1 h.

[^{35}S]methionine labeling. IH10 cells restimulated 1 week earlier and P815 cells were washed three times in medium followed by one wash in methionine-free minimum essential medium (GIBCO). P815 cells alone (2×10^6) or mixed with 2×10^6 IH10 cells or live VSV (m.o.i., 0.05 PFU per cell) and 2×10^6 IH10 cells alone were suspended in 10 ml of methionine-free minimum essential medium supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, 10 U of recombinant murine interleukin-2 per ml (Genzyme), and 20 μCi of [^{35}S]methionine per ml (New England Nuclear) and incubated overnight at 37°C in 10% CO_2 . Cells were washed twice in PBS and lysed in 1 ml of buffer containing 25 mM Tris hydrochloride (pH 7.4), 0.15 M NaCl, Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 20 IU of aprotinin per ml, and 1 mM phenylmethylsulfonyl fluoride. After 1 h of incubation at 0°C , the lysate was clarified by centrifugation at $45,000 \times g$ for 1 h at 4°C .

Immunoprecipitation. The iodinated cell lysates were pre-cleared by incubation with protein A-Sepharose CL-4B (Sigma) coated with rabbit anti-mouse immunoglobulin (Cappel). [^{35}S]methionine-labeled cell lysate was used directly. Immunoprecipitation was effected by incubating 400 μl of cell lysate with 5 μl of anti-VSV immune serum or 5 μl of naive mouse serum. The antigen-antibody complexes were precipitated by adding 100 μl of protein A-Sepharose CL-4B and were harvested after incubation overnight at 4°C . The precipitates were washed several times in the lysing buffer and solubilized in buffer containing 5% sodium dode-

FIG. 1. The IH10 clone responds only to VSV. Responses to UV-VSV, UV-HSV, and UV-irradiated reovirus were tested by incubating the clone cells with the antigen and irradiated syngeneic feeder cells; VSV-specific T-cell response was demonstrated by incorporation of tritiated thymidine.

cyl sulfate, 0.125 M Tris (pH 6.8), and 10% β -mercaptoethanol and boiled for 3 min. Solubilized samples were separated on 10% polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Laemmli (9). Gels were stained by using Coomassie brilliant blue, destained in 7.5% methanol–5% acetic acid, washed in water, dried, and exposed to Kodak XAR-5 film. [35 S] methionine-labeled sample gels were incubated with Auto-fluor (National Diagnostics) before they were dried. The experiment was also performed by using a murine anti-glycoprotein G monoclonal antibody.

RESULTS

IH10 is a VSV-specific T clone. The VSV-antigen-specific T-cell clone, IH10, was derived after limiting dilution from bulk cultures of VSV-challenged spleen cells from BALB/c mice immunized with VSV. It was found to be responsive only to UV-VSV, not to HSV or reovirus, as shown by its enhanced proliferative response to VSV but not to HSV or reovirus, indicating the exquisite specificity of the clone for VSV (Fig. 1). An HSV-specific CD4⁺ CD8[−] T-cell clone from A/J mice was obtained in a similar manner. This clone proliferates in response to UV-HSV, not to UV-VSV (data not shown). The IH10 clone was found to be positive for L3T4 (murine CD4) (4, 5), Lyt-1 (10), 145-2C11 (ϵ chain of murine CD3) (11), and F23.1 (protein encoded by the V β 8 region of the murine T-cell receptor) (26) but negative for Lyt-2 (murine CD8) (10). The supernatants of the IH10 T-cell clones were found to contain infectious virus 1 week after the last stimulation when cocultured with L cells. Gamma interferon was not detected in the supernatant.

Antibody directed against CD4 inhibits the proliferative response of IH10. In order to determine the role played by CD4 in VSV-induced proliferation of the IH10 clone, we added antibody to L3T4, the murine CD4 (GK1.5), and Lyt-2, the murine CD8 (53-6.72), with a medium alone as a control. Proliferation was inhibited by antibody to CD4 (GK1.5) but not by anti-CD8 antibody (53-6.72) (data not shown).

The IH10 clone kills target cells bearing class II MHC antigens. Cytolytic activity of IH10 was tested at ratios ranging from 12.5:1 to 1:1. The clone was found to be capable of lysing target cells at a 6:1 ratio of effector cells to target cells in a 6-h cytolytic assay with a greater than 60% 51 Cr release. The targets were chosen on the basis of the differences in expression of class I and class II MHC antigens in order to delineate the MHC restriction of the clone. The target used in Fig. 2a was A20, a histocompatible ($H-2^d$) BALB/c lymphoma cell line expressing both class I and class II MHC antigens. The killing was VSV specific; no HSV- or reovirus-infected target cells were killed during the assay (Fig. 2a). The clone was incapable of significant killing of VSV-infected P815 cells ($H-2^{d+} I-A^d-$) (Fig. 2b) in a 6-h assay. Furthermore, the antibody M5/114.15.2 (1), which can bind to I-A^d and I-E^d MHC antigens, blocked the killing of A20 cells (Fig. 2c). The clone did not lyse AJ9 ($I-A^k$) or BALB 3T3 ($H-2^d I-a^-$) cells (data not shown).

Unrestricted lysis of target cells by IH10 is associated with VSV. In short (4- to 6-h) assays, IH10 lysed only histocompatible target cells. Prolonged incubation (48 h) of IH10 with UV-VSV and tumor target cells, however, resulted in lysis of all tumor cell lines tested (irrespective of histocompatibility type). The lytic effect was of long duration and therefore not amenable to quantitation with a chromium release assay. For this reason, we decided to quantitate the killing of the target cells by inhibition of tritiated-thymidine incorporation. Incubation of IH10 cells with tumor target cells, even in the absence of any added UV-VSV, resulted in the elimination of target cell proliferation despite the MHC type or antigen specificity of the target (Fig. 3a). Because of the absence of target cell specificity, we postulated that IH10 might be releasing lymphotoxin or other toxic lymphokines. However, assays in the presence of anti-VSV sera revealed a loss of this effect, suggesting the presence of virus-induced cytotoxicity (Fig. 3b). Sera from unimmunized mice had no effect on the inhibition of tumor proliferation by the T-cell clone. Similar results were obtained when rabbit anti-VSV sera (Lee BioMolecular) were used instead of mouse anti-VSV sera. In a 48-h assay in the presence of both UV-VSV and antiserum against VSV, irradiated IH10 cells were also found to be capable of a greater than 95% inhibition of proliferation of A20 cells (MHC-compatible cells expressing class II antigens) but not of P815, AJ9, or BW5147 target cells (data not shown).

The IH10 clone cured of virus does not mediate nonspecific lysis. In order to determine whether the lytic effect against nonhistocompatible target cells was mediated by virus, IH10 cells were carried in the presence of serum from syngeneic mice immunized with VSV. After passage of cells in antiserum, the clone behaved like a prototypic virus-specific class II-directed CD4 cell (Fig. 4). Of note is the observation that only in the histocompatible class II MHC-bearing cell line A20 ($H-2^d I-A^d$) did we find a reduction in the proliferation of the tumor when incubated with the clone cured of VSV. This surprising finding has been verified in repeat experiments. A possible explanation of this finding is that low levels of viral antigens not detectable by antibody (see below) are passed on to the syngeneic class II MHC-bearing cells and that the antigen may be of a concentration sufficient to trigger cell-mediated cytotoxicity, even in the presence of antibody (Fig. 4).

The IH10 clone expresses VSV antigens. Because of the effect of the anti-VSV sera on the lytic ability of the clone, we examined the clone for evidence of virus on its surface. After being deprived of antigen for 8 days, the IH10 clone

FIG. 2. Cytolysis by IH10 cells is VSV specific and class II MHC restricted. (a) Cytolysis of A20 cells and A20 cells infected with VSV, HSV, or reovirus by IH10. The clone at separate effector/target cell (E:T) ratios was incubated for 6 h with 5,000 target cells that were uninfected or infected with VSV, HSV (mP strain), or reovirus. (b) Cytolysis of A20 and P815 cells. The clone was incubated at various effector/target cell ratios for 6 h with A20 and P815 tumor target cells at 5,000 cells per well with or without prior infection with VSV. (c) Role of class II MHC antigen in the cytolytic response of IH10. M5/114.15.2 (anti-IA^{b,d,q} IE^{d,k} framework antibody) supernatant at a dilution of 1:2 was incubated with IH10 and 5,000 target cells per well at three effector/target cell ratios for 6 h.

was stained with anti-CD4 (GK1.5) and anti-VSV serum separately and was found to have a single peak for each of these two antibodies. Over 30% of these cells stained with antibodies to both L3T4 and VSV, indicating the persistence of viral antigen on the surfaces of these cells (Fig. 5). Similar results were obtained from cells stimulated with live VSV instead of UV-VSV. The IH10 clone cells which were cured of virus with antiserum did not demonstrate any VSV antigens on their surfaces. The clone has maintained its phenotype (surface expression of the virus and CD4) for greater than 8 months in the absence of any anti-VSV serum.

VSV antigens can be immunoprecipitated from the surface of the IH10 clone. Immunoprecipitation with anti-VSV serum of lysates from iodinated cell surfaces of IH10 and VSV-infected A20 cells showed specific precipitation of a 65-kilodalton protein which corresponds to the VSV G glycoprotein (Fig. 6) from both IH10 and virus-infected A20 cells. Sera from naive mice were incapable of this.

Incubation of target cells with IH10 results in expression of VSV proteins. Both FACS sorting and cell surface labeling

and immunoprecipitation revealed the presence of VSV antigens on the surfaces of IH10 cells. In order to determine whether the incubation of the IH10 clone resulted in a productive VSV infection, we labeled IH10 cells and tumor target cells (P815) with [³⁵S]methionine in the presence and absence of VSV in order to determine whether viral protein synthesis occurred in the IH10 cells. Virus-specific proteins were immunoprecipitated by using anti-VSV serum after a 5-h incubation (Fig. 7). Viral protein synthesis was minimal in IH10 cells cultured alone (lane 4), but viral protein synthesis did occur when IH10 cells were cocultivated with the P815 cells. The same pattern of precipitation of viral proteins was seen in P815 cells infected with live virus (lane 1) or P815 cells cocultivated with IH10 cells without added virus (lane 3). These proteins were not precipitated from P815 cells grown in the absence of virus or IH10 (lane 2). Nonimmune mouse serum did not precipitate any of these proteins (data not shown). A murine anti-VSV G glycoprotein monoclonal antibody caused specific precipitation of the VSV G glycoprotein from P815 infected with VSV or cocul-

FIG. 3. Effects of IH10 cells on MHC-compatible and non-MHC-compatible tumor cells. (a) Irradiated VSV-specific T cells kill both MHC-compatible and non-MHC-compatible tumor cell lines. A20 ($H-2^d I^a$), P815 ($H-2^d I^a$), AJ9 ($H-2^a I^a$), and BW5147 ($H-2^k$) tumor cells were cultured in 96-well plates in 0.2 ml of medium with or without IH10 in the absence of any added virus for 48 h before being pulsed with tritiated thymidine and were then harvested. The background proliferation of the irradiated IH10 clone was assessed by the addition of either rat concanavalin A supernatant-conditioned medium at 30% as a source of T-cell growth factor or medium alone in the absence of any tumor cells and was found to be under 200 cpm. Assays were performed in triplicate. The error bars represent one standard deviation. (b) Incubation with anti-VSV serum eliminates the lysis of non-MHC-compatible tumor target cells. In a similar experiment, the cells were incubated in the presence of 1:100 anti-VSV serum obtained from multiple immunizations of naive BALB/c mice.

tivated with IH10 but not from IH10 or P815 alone (results not shown).

DISCUSSION

In this report, we have described a T-cell clone with a $CD4^+ CD8^-$ phenotype which is specific for VSV. The clone proliferates in response to antigen and exhibits class II-restricted cytotoxicity, as demonstrated by its ability to kill VSV-infected syngeneic cells which express class II MHC antigens but not cells which express class I MHC antigens alone. Interestingly, these cells are capable not only of mediating the functional activities described above but also of carrying live VSV on their surfaces, as shown by FACS staining and surface iodination and immunoprecipitation experiments. These cells by themselves are not rapidly

FIG. 4. VSV-cured IH10 no longer lyses non-MHC-compatible tumor cells. The irradiated IH10 T cells were incubated with 30% mixed-lymphocyte culture supernatant and a 1:100 dilution of anti-VSV serum for 2 weeks prior to this assay in order to cure the cells of the virus. The assay was set up as described in the legend to Fig. 3, without the addition of any antibody (ab) to the 96-well plate.

translating viral proteins, as determined by [35 S]methionine-labeling experiments. Tumor cells cocultured with the IH10 cells do translate VSV proteins (without the addition of exogenous virus). The IH10 T cells are able to kill tumor cells in a non-MHC-restricted fashion by using the virus they carry on the surface. Since VSV is lytic to most cell lines and activated T cells (8), the mechanism by which this T-cell

FIG. 5. The IH10 clone has viral antigens on its surface. Eight days after it had been exposed to antigen, the IH10 clone was washed three times to eliminate free virus and stained with anti-VSV antibody. The clone was stained sequentially with murine anti-VSV antiserum, FITC-labeled rabbit anti-mouse immunoglobulin, and then phycoerythrin (PE)-labeled L3T4 antibody. Fluorescence measured along the vertical axis is due to phycoerythrin, while fluorescence measured along the horizontal axis is due to FITC.

FIG. 6. Surface iodination and immunoprecipitation by VSV-immune sera demonstrate the presence of VSV antigen on the surface of IH10. Sera from naive and VSV-immunized mice were used to immunoprecipitate VSV antigen from the surfaces of IH10 and VSV-infected A20 cells. Lanes 1 and 2 are immunoprecipitations of IH10, while lanes 3 and 4 are immunoprecipitations of VSV-infected A20 cells. Lanes 2 and 4 are immunoprecipitations obtained by using VSV-immune sera, while lanes 1 and 3 were done by using naive sera. Migration of molecular mass markers is indicated on the right. The letter G on the left is for the G glycoprotein of VSV. The molecular masses are expressed in kilodaltons. Experimental details are described in Materials and Methods.

clone evades cytolysis by VSV remains uncertain. Experiments with cloned alloreactive T cells and T-cell lines specific against reovirus and Sendai virus indicate that these cells are killed when VSV is added to cultures (unpublished data), confirming the findings of Kano et al. (8). It is possible that the virus is unable to enter the VSV-specific T cell because of an endogenous defense mechanism such as interferon. Murine interferon has been found to inhibit the penetration of VSV into L cells without affecting the adsorption of the virus (30). The role of possible defective interfering viral particles in the induction of interferon production in T cells is uncertain, although there is evidence that it occurs in persistently infected L cells (15). It is clear that the failure of the virus to kill the T-cell clone is not due to inability of the clone to bind virus (Fig. 5). Others have noted that certain tumor cells may be persistently infected with low levels of virus without detectable proviral nucleic acid and that such cells could be cured of VSV (7).

In these experiments, we have found that most of our cloned T cells expressed viral antigen on their surfaces and that our cells could also be cured of the virus. Rosenthal et al. demonstrated that interleukin-2-dependent murine cells with natural killer cell-like activity can be persistently infected with VSV (18). Our clone, IH10, is a class II V β 8-expressing cytotoxic T lymphocyte which lyses targets in an antigen-specific MHC-restricted manner in a ^{51}Cr release assay.

Human lymphoblastoid cell lines of B-cell origin infected with VSV can maintain a persistent infection (3), while human T-cell tumor cell lines are permissive to viral replication, with cell death following within 24 h after infection.

FIG. 7. [^{35}S]methionine labeling demonstrates that IH10 incubated with P815 cells results in viral protein expression. Lane 1 represents immunoprecipitation following [^{35}S]methionine labeling of VSV-infected P815 cells, lane 2 is that of uninfected P815 cells, lane 3 is that of IH10 cocultured with P815 cells in the absence of any added VSV, and lane 4 is the immunoprecipitation of IH10 alone. Migration of molecular mass markers is indicated on the right. The molecular masses are expressed in kilodaltons. The letters on the left represent the VSV proteins; L and NS proteins are components of the ribonucleoproteins, G is the envelope G glycoprotein, N is the nucleocapsid protein, and M is the matrix protein. Experimental details are described in Materials and Methods.

Kano et al. demonstrated that a virus plaque assay can detect activated T lymphocytes derived from mixed-lymphocyte cultures (8). More recently, Sy and colleagues have shown that VSV infection results in the functional elimination of subpopulations of suppressor T cells *in vivo* (28), resulting in an inability to downregulate immune responses. Furthermore, Romano et al. have demonstrated that incubation of T cells may selectively eliminate clones capable of mediating graft versus host disease (17a). Rup and Scott have demonstrated that suppressor T cells will replicate VSV upon either antigenic or mitogenic activation (20). However, they did not assess viability or functional activities of these cells after challenge with VSV. None of these investigators have addressed the paradox of how, if the virus kills and activates T cells, the host can generate a virus-specific response. In addressing this question, we described a clone that not only resists viral infection but also carries the virus in a functionally important manner, i.e., is able to spread the virus to other cells and kill them.

It is unlikely that the T cells obtained in this work mediate target cell lysis by secretion of any toxic agents (e.g., lymphotoxin, perforin, serine esterase, etc.) because the non-MHC-restricted tumor lysis by the IH10 clone is inhibited if the clone is either cured of the VSV several days before coculturing with the tumor target cells or added to tumor target cells simultaneously with the anti-VSV sera. This has been verified by the observation that plaques may

be formed on L-cell (*H-2^k*) layers by infected T-cell clone cells in an infectious center assay (unpublished data).

Our clone is unique in that it mediates lysis of target cells by carrying virus on the surface but is not itself lysed by the virus. Lysis of the tumor target cell is clearly mediated by infectious virus, as was demonstrated in the [³⁵S]methionine cocultivation experiments (Fig. 7), where P815 expressed VSV antigens when cocultured with the IH10 clone. It is intriguing to speculate that such Trojan horse lymphocytes may be involved in the pathogenesis of viral infections by harboring viruses and then spreading the infection to susceptible tissue before neutralizing antibodies could be produced. In vivo experiments by Oldstone and colleagues have defined the association of infectious lymphocytic choriomeningitis virus particles with T cells (17). The implications of these findings in terms of disease pathogenesis have not yet been established.

Cytolytic T cells are known to be serial killers capable of killing multiple target cells. Cinematic studies of such cells reveal that they wander from one cell to the next, bypassing target cells not recognized by their T-cell receptor prior to giving the lethal hit to an antigen and MHC-compatible cell (19). This wandering from one cell to another requires cell-to-cell contact. In this case, the cell-to-cell contact results in the lysis of any cell which the IH10 cell contacts. While viruses, unlike bacteria, usually cannot survive extracellularly in the blood for extended periods, they may be able to hitchhike around the body by taking advantage of the circulating cells. Cytolytic T lymphocytes are ideal for this purpose since they travel around the body contacting one cell after another. In the case of an infected lymphocyte, the phenomenon of cell-to-cell contact may result in the lysis of the target cell by a hitherto unreported mechanism: release of live virus from the cell membrane. Such a mechanism may be operative in certain viral or even so-called autoimmune effects which follow viral infections.

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