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Persistence of Vesicular Stomatitis Virus in Cloned Interleukin-2-Dependent Natural Killer Cell Lines

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We have investigated virus-lymphocyte interactions by using cloned subpopulations of interleukin-2-dependent effector lymphocytes maintained in vitro. Cloned lines of H-2-restricted hapten- or virus-specific cytotoxic T lymphocytes (CTL) and alloantigen-specific CTL were resistant to productive infection by vesicular stomatitis virus (VSV). In contrast, cloned lines of natural killer (NK) cells were readily and persistently infected by VSV, a virus which is normally highly cytolytic. VSV-infected NK cells continued to proliferate, express viral surface antigen, and produce infectious virus. Furthermore, persistently infected NK cells showed no marked alteration of normal cellular morphology and continued to lyse NK-sensitive target cells albeit at a slightly but significantly reduced level. The persistence of VSV in NK cells did not appear to be caused by the generation of temperature-sensitive viral mutants, defective interfering particles, or interferon. Consequently, studies comparing the intracellular synthesis and maturation of VSV proteins in infected NK and mouse L cells were conducted. In contrast to L cells, in which host cell protein synthesis was essentially totally inhibited by infection, the infection of NK cells caused no marked diminution in the synthesis of host cell proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoprecipitates of viral proteins from infected cells showed that the maturation rate and size of VSV surface G glycoprotein were comparable in L cells and NK cells. Nucleocapsid (N) protein synthesis also appeared to be unaffected in NK cells. In contrast, the viral proteins NS and M appeared to be selectively degraded in NK cell extracts. Mixing experiments suggested that a protease in NK cells was responsible for the selective breakdown of VSV NS protein. Finally, VSV-infected NK cells were resistant to lysis by virus-specific CTL, suggesting that persistently infected NK cells may harbor virus and avoid cell-mediated immune destruction in an immunocompetent host.

von Pirquet (50) classically demonstrated that infection by measles virus abrogates delayed-type hypersensitivity reactions to tuberculin. Subsequently, it was shown that infection with a variety of both nononcogenic and oncogenic viruses leads to altered immune responses (15, 20, 36, 54). This is best exemplified by the current epidemic of acquired immunodeficiency syndrome. Acquired immunodeficiency syndrome, the most devastating disease of the adult immune system, is caused by the infection of helper T lymphocytes by a recently discovered human retrovirus, variously referred to as lymphadenopathy-associated virus (5) or human T-cell lymphotropic virus type III (41).

The ability of viruses to alter immune responses led numerous investigators to examine virus-lymphocyte interaction in vitro (8, 16, 32, 38, 53). Evidence obtained in these studies suggested that the ability of virus infection to suppress immune responses may be related to the ability of certain viruses to replicate in lymphocytes activated by T-cell mitogens. This finding was exemplified by the studies of Bloom et al. (9) and Nowakowski et al. (38) which demonstrated that vesicular stomatitis virus (VSV) could infect a small percentage (less than 6%) of resting T lymphocytes but required lymphocyte activation before infectious virus was produced.

At present we are aware of the complexity and variety of lymphocyte subpopulations involved in mediating immune responses. Since most of the earlier studies concerning the effect of viral infection on immune responses used bulk populations of poorly characterized lymphocytes or transformed lymphoblastoid cell lines, we decided to examine virus-lymphocyte interaction by using homogeneous cloned lines of well-characterized effector lymphocytes maintained in vitro in interleukin-2 (IL-2).

Our results demonstrate that cloned lines of H-2-restricted virus- or hapten-specific cytotoxic T lymphocytes (CTL) or alloantigen-specific CTL were resistant to productive infection by VSV. In contrast, cloned lines of IL-2-dependent natural killer (NK) cells were readily and persistently infected by VSV. Furthermore, the ability of VSV, normally a highly cytolytic virus, to persist in cloned NK cells appears to be caused by the inability of the virus to shut off host protein synthesis and the production of viral proteins at a level compatible with both host cell survival and limited virus replication. Our results also demonstrate that, despite the surface expression of VSV G glycoprotein and the production of nucleocapsid (N) protein, persistently infected NK cells were resistant to lysis by virus-specific CTL.

MATERIALS AND METHODS

Cloned CTL and NK lymphocyte lines. Cloned lines of H-2-restricted, virus-specific CTL were generated and maintained in vitro as previously described (43). The clones were restimulated every 7 to 10 days with irradiated syngeneic VSV-infected macrophages and continuously proliferated in IL-2-containing medium. Phenotypically these cells are Thy

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1.2^+ Lyt 2.2^+. These cells lyse targets in an H-2-restricted, VSV-specific manner and do not display NK cell activity (43). Similarly, H-2-restricted, hapten-specific CTL clones directed against the hapten acetyl-*N*-(5-sulfonic-1-naphthyl)ethylenediamine and alloantigen-specific CTL clones were produced, maintained, and characterized as previously described (3, 39, 40). Additionally, a number of cloned NK cell lines were used in our investigations. The origin and characteristics of cell lines HY-1 (2), HY-3 (2), NKB6IA2 (51), NKB61B10 (51), and BALB/c NK (14) were described previously.

These cloned NK lines efficiently lyse NK-sensitive target cells (YAC-1) in a non-H-2-restricted, non-antigen-specific manner. The cloned NK cell lines are IL-2 dependent and morphologically are large granulated lymphocytes (7, 42). Most recently, all five of these cloned NK cell lines were found to have rearrangement and expression of the β-chain gene of the T-cell antigen receptor (55).

Cloned CTL and NK cell lines were routinely maintained in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% heat-inactivated fetal bovine serum, 10% conditioned medium from concanavalin A-stimulated rat spleen cells as a source of IL-2, 5×10^{-5} M 2-mercaptoethanol, L-glutamine, and antibiotics.

Generation of IL-2-containing supernatants. Supernatants of concanavalin A-stimulated rat spleen cells were prepared and assayed by the method of Gillis et al. (19). Briefly, rat spleen cells were cultured at a density of 5×10^6/ml in RPMI 1640 medium containing 5 μg of concanavalin A (Pharmacia, Uppsala, Sweden) per ml. The supernatant was recovered 48 h after culturing was initiated and after centrifugation at $1,000 \times g$ for 20 min. Supernatants were then filtered for sterility and stored at −20°C until used. Additionally, conditioned medium from a subclone (EL4.E1) of murine T lymphocytes described by Farrar et al. (17) was used as a source of IL-2. Culture supernatant, harvested 16 h after the incubation of these cells with 10 μg of phorbol myristic acetate per ml in serum-free medium, contained IL-2 which was devoid of detectable interferon activity.

Cells and virus. Mouse L cells were maintained in alpha-minimal essential medium (GIBCO) supplemented with 5% fetal bovine serum, 0.075% $NaHCO_3$, and antibiotics at 37°C. VSV Indiana was grown and titrated as previously described (10, 44).

Immunofluorescence. Hybridoma clone 60-4 produces immunoglobulin G1 class monoclonal antibody specific for the surface G protein of VSV as previously described (43). Uninfected or VSV-infected cloned CTL or NK cells (1×10^6) were incubated with 250 μl of culture supernatant from hybridoma 60-4 for 30 min at room temperature. Cells were then washed and reacted with 100 μl of a 1:15 dilution of fluorescein-conjugated goat anti-mouse immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) for an additional 30 min. After cells were washed, they were suspended in a drop of Tris-glycerol, mounted under a cover slip, and examined for fluorescence with an Orthoplan microscope (Leitz Opti/Metric Div. of E. Leitz Inc., Rockleigh, N.J.). Cells (200 to 300) were counted and scored for positivity.

Transmission electron microscopy. Approximately 5×10^6 cloned infected NK cells were centrifuged and fixed with 2% gluteraldehyde and 0.8% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2). The cells were postfixed in an aqueous solution of 1% osmium tetroxide–1.5% $K_4Fe(CN)_6$ and preembedded in agar. Small blocks of agar were dehydrated in increasing concentrations of ethanol and embedded in Epon. Ultrathin sections (approximately 50 nm) were contrasted with uranyl acetate and lead citrate and examined with a Philips model 300 electron microscope.

Radiolabeling of cells and immunoprecipitation. ^{35}S-methionine-labeled, infected NK or L-cell extracts were prepared in RIPA buffer as previously described (6). Briefly, VSV was absorbed to cloned NK cells or mouse L cells at 37°C for 30 min at a multiplicity of infection of 20 PFU per cell. Cells were then washed with warm phosphate-buffered saline and incubated for various lengths of time in Joklik modified minimal essential medium minus methionine, with or without 10% rat concanavalin A supernatant, plus ^{35}S-methionine (Amersham Corp., Arlington Heights, Ill.) at 30 Ci/ml. The labeled cells were lysed as described above and immunoprecipitated by mixing cell extracts with heat-inactivated hyperimmune anti-VSV serum (6) and protein A-Sepharose CL 4B beads (Pharmacia) with rotation for at least 4 h. After absorption, the beads were centrifuged at $300 \times g$ for 1 min, and the supernatant was removed. The beads were then washed at least four times with cold RIPA buffer, eluted, and analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE. SDS-PAGE was carried out by the method of Laemmli (30). Briefly, samples were applied to gels composed of 10% acrylamide and 0.4% *N,N'*-methylene bisacrylamide. Electrophoresis was carried out at 200 V until the bromophenol blue marker reached the bottom of the gel. The gel was removed, fixed in methanol-water-acetic acid (50:50:7), dried onto Whatman filter paper (Whatman, Inc., Clifton, N.J.) by low-pressure dessication at 100°C, and then exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) for the appropriate length of time.

Interferon assay. Monolayers prepared by seeding 10^4 L929 cells per flat-bottomed microtiter well were incubated with 100 μl of twofold dilutions of culture supernatants for 24 h at 37°C. Subsequently, 100 PFU of VSV Indiana was added, and the cytopathic effects were assessed 24 h later. The limit of sensitivity of this assay was 2 IU of interferon.

^{51}Cr release assays. Cloned effector cells were tested for cytotoxicity by using a modification of the ^{51}Cr release assay described previously (44). Briefly, tumor target cells were labeled with $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass.) for 90 min at 37°C. Cells were then washed and counted. Then 5×10^4 ^{51}Cr-labeled target cells in 50 μl of medium were inoculated into each well of a 96-well, round-bottomed microtiter plate (Nunc, Roskilde, Denmark). Washed effector cells were then added at the noted effector-to-target cell ratios, generally 6:1, 3:1, and 1:1. The microtiter plates were then centrifuged at $300 \times g$ for 3 min and were incubated at 37°C for the indicated lengths of time.

RESULTS

Interaction of VSV with cloned effector lymphocyte lines. A variety of cloned IL-2-dependent CTL and NK cell lines were infected in vitro with VSV at 1 to 10 PFU per cell and examined for cell viability, viral antigen expression, and virus production. VSV infection of cloned H-2-restricted virus- or hapten-specific CTL or cloned alloantigen-specific CTL lines did not affect cell viability nor growth relative to uninfected controls. Further, none of the infected cloned lines of CTL expressed VSV surface antigen (Table 1) or produced infectious virus progeny. Thus, cloned lines of H-2-restricted, antigen-specific CTL and alloantigen-specific CTL were resistant to productive infection by VSV.

In contrast, VSV was found to rapidly infect cloned IL-2-dependent NK cell lines in vitro. Our results demon-

TABLE 1. Expression of VSV surface glycoprotein antigen on infected cloned CTL and NK cell lines[a]

Clone	Function	% Immunofluorescence positive[b]
A	CTL BALB/c anti-VSV	<2
5/10-13	CTL C57BL/10 anti-AED[c]	<2
5/10-20	CTL C57BL/10 anti-AED	<2
8/10-2	CTL C57BL/10 anti-AED	<2
3F9	CTL BALB/c anti-C57BL/6	<2
14D3	CTL C57BL/10 anti-B10.D2	<2
HY-1	NK	>95
HY-3	NK	>95
NKB61A2	NK	>95
NKB61B10	NK	>95
BALB/c NK	NK	>95

[a] Cells were infected at a multiplicity of infection of 1 PFU per cell and 24 h later were examined for surface immunofluorescence by using a monoclonal antibody specific for the G glycoprotein of VSV.

[b] Immunofluorescence was determined as described in the text. Uninfected controls were negative.

[c] anti-AED, Anti-acetyl-*N*-(5-sulfonic-1-naphthyl) ethylenediamine.

strate that essentially all cells of each of five distinct cloned NK lines expressed VSV surface G glycoprotein (Table 1). Furthermore, despite the fact that VSV is normally a highly cytolytic virus, after an initial drop in viable cell number of approximately 50 to 75%, VSV-infected NK cells continued to proliferate and produce infectious VSV at relatively stable levels.

Established VSV-infected NK cells showed no marked alteration of normal cellular morphology (Fig. 1), and budding VSV particles were observed at the plasma membrane of persistently infected NK cells (Fig. 2). Furthermore, persistently infected NK cells continued to lyse NK-sensitive tumor target cells albeit at a slightly but significantly reduced level compared with uninfected NK clones (Fig. 3).

We have maintained cultures of VSV-infected NK cells in vitro for over a year, and these cells continue to grow, express VSV surface antigen, and produce infectious VSV at approximately 1×10^6 to 5×10^6 PFU/ml of culture supernatant. Thus, cloned lines of IL-2-dependent NK cells were rapidly and persistently infected by VSV, a virus which is normally highly cytolytic.

Possible mechanism of VSV persistence in cloned NK cells. Characterization of the viruses produced by persistently infected NK cells demonstrated that these viruses were not temperature sensitive and that defective interfering particles were not responsible for the establishment or maintenance of the persistent state (data not shown). Further, the cloned NK cell lines did not produce detectable endogenous levels of interferon either before or after VSV infection, and culturing cloned NK cells in IL-2-containing medium devoid of interferon had no effect on the establishment of VSV persistence in cloned NK cells (data not shown).

Since none of these mechanisms appeared to be responsible for VSV persistence in cloned NK cells, we compared the intracellular synthesis and maturation of VSV proteins in cloned NK cells with that in infected mouse L cells. In contrast to infected mouse L cells (Fig. 4, lane 4) in which host cell protein synthesis was essentially totally inhibited, VSV infection of cloned NK cells caused no marked diminution in the synthesis of host cell proteins (Fig. 4). The inability of VSV infection to shut off NK cell protein synthesis was evident despite the presence of VSV G, N, and M proteins in the gel profile of infected NK cells (Fig. 4, lane 2). Furthermore, a new band migrating below the VSV matrix (M) protein band appeared in extracts of infected NK cells (Fig. 4, lane 2, arrow). We refer to this low-molecular-weight band as the sub-M band.

SDS-PAGE analysis of immunoprecipitates of virus proteins from infected cells (Fig. 5) showed that the amount and maturation to full-sized VSV surface G glycoprotein were similar in L cells (Fig. 5, lanes 4 through 6) and NK cells (lanes 1 through 3). Furthermore, these results confirm our immunofluorescence data, which demonstrated surface expression of VSV G glycoprotein in infected NK cloned lines (Table 1). N protein synthesis also appeared to be comparable in NK and L cells (Fig. 5). Some differences were seen in the immunoprecipitated virus proteins from NK cells when compared with those from L cells (Fig. 5). First, no significant amount of NS protein was seen in immunoprecipitates of VSV-infected NK cells. Second, the level of M protein was reduced and did not significantly change during the chase period in NK cells. Third, a prominent sub-M band was immunoprecipitated from infected NK cells (Fig. 5).

Since transcription of the VSV genome is sequential from the 3′ end (4) and results in decreasing molar concentrations of viral proteins in the order N > NS > M > G > L (1, 49), the absence of NS and decreased amounts of M proteins in VSV-infected NK cells were most likely caused by a post-transcriptional event.

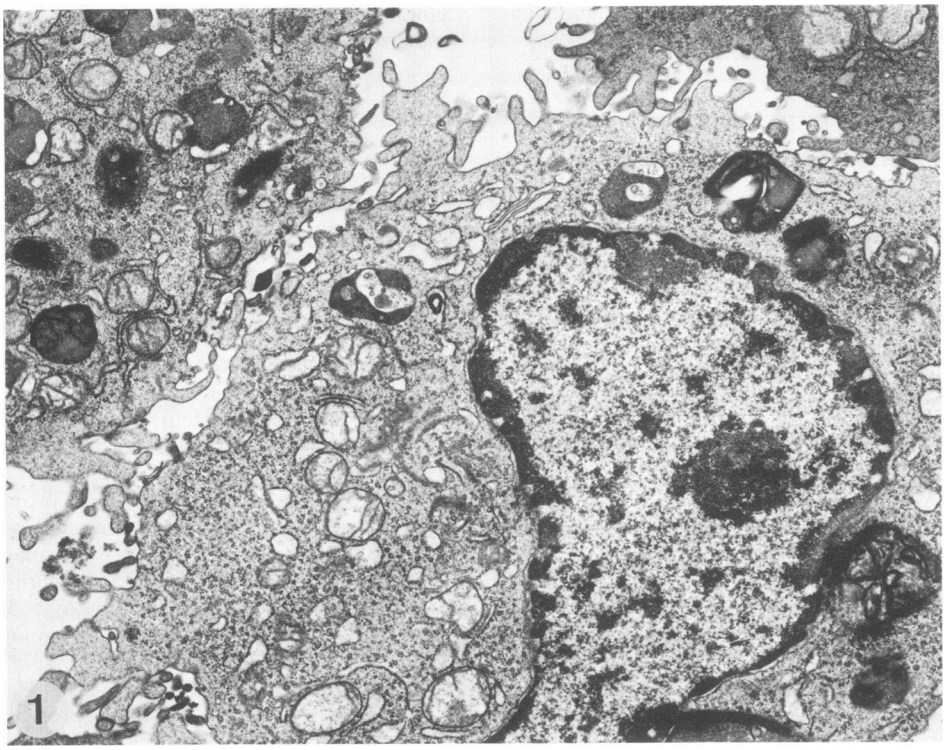
The possible occurrence of a protease in the NK cell extracts, producing the selective breakdown of NS and possibly M protein and the concomitant appearance of the low-molecular-weight sub-M band, was examined by mixing experiments in which labeled extracts from VSV-infected L cells were mixed with unlabeled extracts of either infected or uninfected NK cells (Fig. 6). Both total proteins (Fig. 6, lanes 2 and 3) or immunoprecipitates (lanes 8 and 9) of labeled infected-L-cell extracts demonstrated a loss of NS and the appearance of the sub-M protein band after mixing with unlabeled extracts of either infected or uninfected NK cell extract (Fig. 6).

These results suggest that NK cells may selectively degrade viral gene products to a level compatible with host cell survival and limited virus production, thus leading to a state of viral persistence.

Susceptibility of persistently infected NK cells to antiviral cell-mediated immunity. Since persistently infected NK cells expressed VSV surface antigen, it was of interest whether these cells could serve as targets for CTL directed against VSV. Syngeneic spleen cells generated during the primary infection of mice with VSV were unable to lyse NK cells persistently infected with VSV, despite their ability to lyse standard syngeneic VSV-infected MC57 tumor target cells (Table 2). The inability to lyse persistently infected NK cells could not be attributed to a lack of H-2 antigen expression or general insensitivity to lysis, since anti-H-2^b-specific alloantigen-reactive CTL were able to lyse VSV-infected HY-1 cells (Table 2). Thus, persistently infected NK cells were resistant to lysis by virus-specific CTL. These results suggest that NK cells could harbor persistent virus and avoid cell-mediated immune destruction in an immunocompetent host.

DISCUSSION

Results presented here demonstrate that a variety of cloned CTL lines, including H-2-restricted virus or hapten-specific CTL and alloantigen-specific CTL, were resistant to productive infection by VSV. In contrast, cloned lines of IL-2-dependent NK cells were readily and persistently in-



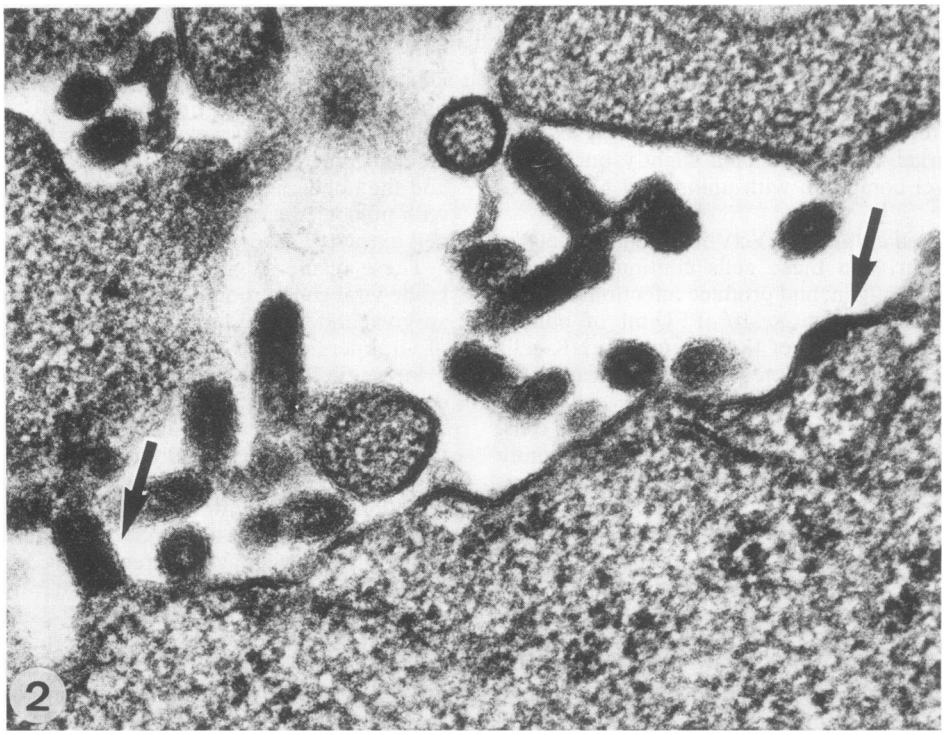


FIG. 1. Electron micrograph of persistently infected, cloned IL-2-dependent NK cells (HY-1). Magnification, ×12,000.
FIG. 2. Electron micrograph of budding (arrows) and free VSV particles at the surface of persistently infected NK cells. Magnification, ×94,000.

fected by VSV, a virus which is normally highly cytolytic. VSV-infected NK cells continued to proliferate, express mature surface viral G glycoprotein, and produce infectious virus. Further, persistently infected NK cells showed no marked alteration of normal cellular morphology and continued to lyse NK-sensitive tumor target cells albeit at slightly but significantly reduced levels. These results corroborate recent results showing that measles virus infection of human peripheral blood lymphocytes can diminish NK cell activity in the absence of virus-induced cytocidal effects (11).

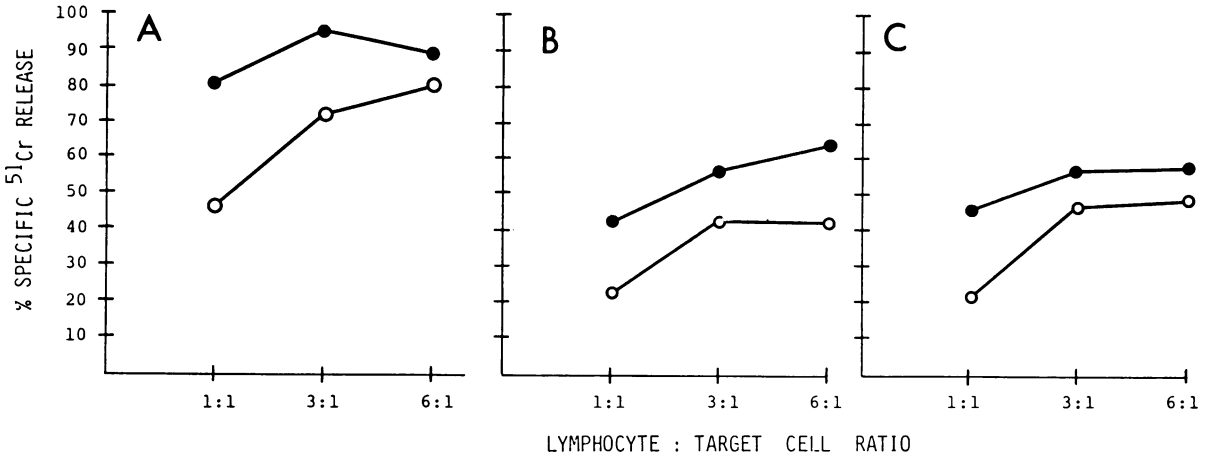


FIG. 3. Lysis mediated by VSV-infected cloned NK cells. HY-1 cells which were infected with VSV for at least 7 days (○) were compared with uninfected HY-1 cells (●) for lysis of YAC-1 (A), EL-4 (B), and P815 (C) tumor target cells. The assay was performed for 3 h at the indicated effector/target cell ratios. Spontaneous release from all targets was <20%.

The interaction between VSV and lymphocytes was first investigated by Bloom and co-workers (8, 9, 38). They used the infectious-center assay to demonstrate that VSV could infect a small percentage (less than 6%) of resting T cells but that these cells required lymphocyte activation before infectious virus was produced. Thus, they demonstrated that VSV could persist in lymphocytes. Furthermore, despite the low percentage of lymphocytes infected by VSV, Nowakowski et al. (38) were able to characterize these cells as large atypical lymphocytes. Interestingly, NK cells have

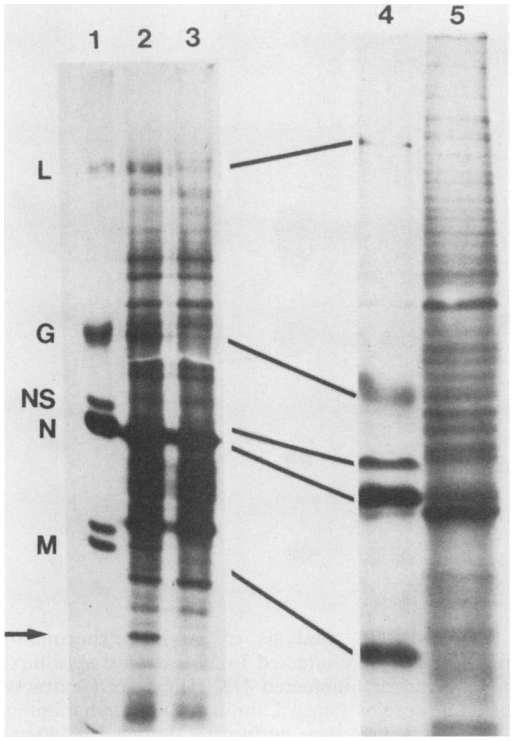


FIG. 4. SDS-PAGE analysis of proteins synthesized in VSV-infected cloned NK cells. Infected and uninfected NK (HY-1) and L cells were labeled with ^{35}S-methionine for 1 h at 4 h postinfection, and total cell extracts were then electrophoresed. Lanes: 1, VSV marker proteins indicated at left; 2, VSV-infected NK cell extract; 3, uninfected NK cell extract; 4, VSV-infected L-cell extract; 5, uninfected L-cell extract. The low-molecular-weight sub-M band which appeared in the VSV-infected NK cell extracts (lane 2) is indicated by the arrow at the left.

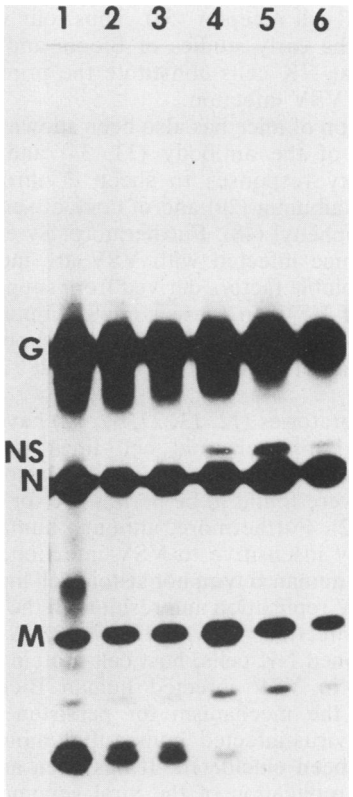


FIG. 5. Analysis of VSV polypeptides immunoprecipitated from infected NK and L cells. Cloned NK (HY-1) and L cells were pulse-labeled with ^{35}S-methionine for 1 h at 4 h postinfection and chased for 1.5 or 3 h at 37°C. Rabbit hyperimmune anti-VSV serum was used to immunoprecipitate infected-cell extracts, which were then electrophoresed. Viral proteins were immunoprecipitated from pulse-labeled infected NK cells (lane 1) followed by a 1.5- (lane 2) or 3-h (lane 3) chase. Similarly, proteins were immunoprecipitated from pulse-labeled infected L cells (lane 4) followed by a 1.5- (lane 5) or 3-h (lane 6) chase.

TABLE 2. Resistance of persistently infected cloned NK cells to virus-specific cell-mediated lysis

Effector cells[a] (effector/target cell ratio)	% Specific ^{51}Cr release[b]				
	MC57 (VSV)	HY-1		NKB61A2	
		Uninfected	VSV	Uninfected	VSV
C57BL/6 anti-VSV					
40:1	51	<1	<1	<1	<1
12:1	26	<1	<1	<1	<1
4:1	12	<1	<1	<1	<1
C3H anti-C57BL/10					
40:1	35	69	45	NT	NT
12:1	28	47	16		
4:1	21	16	2		

[a] Effector spleen cells were obtained from VSV-infected mice on day 6 after infection as described previously (44). Alloantigen-specific CTL were generated in a 5-day mixed-lymphocyte culture.

[b] Assay time, 6 h. Spontaneous release for all targets was <20%. NT, Not tested.

been characterized as large granulated lymphocytes (25). Indeed, all five cloned NK cell lines used in this investigation have been characterized as large granulated lymphocytes, and all the cells of each line are Thy positive (7, 42). Additionally, we recently demonstrated that all five NK cell lines demonstrate rearrangement and expression of the β chain of the T-cell receptor (55). Thus, our work confirms and extends the early studies of Bloom and suggests that IL-2-dependent NK cells constitute the primary lymphoid target cell for VSV infection.

VSV infection of mice has also been shown to result in an enhancement of the antibody (33, 34) and delayed-type hypersensitivity responses to sheep erythrocytes (28) or bovine serum albumin (29) and of contact sensitivity to the hapten dinitrophenyl (48). Furthermore, Sy et al. (47) have shown that mice infected with VSV are incapable of responding to soluble factors derived from suppressor T cells. The effects of VSV on these complex immune responses have been interpreted as indicating that suppressor T cells serve as the target for VSV replication (28, 29, 33, 34, 47, 48).

Several laboratories (12, 13, 27, 37, 52) have used permanent human lymphoblastoid cell lines to study VSV-lymphocyte interaction. Human lymphoblastoid cell lines of T-cell origin were found to be permissive for lytic infection by VSV (9, 12). Furthermore, although human B-cell lines were relatively insensitive to VSV infection, Epstein-Barr virus-infected human B lymphoblastoid cell lines were found to restrict VSV replication and resulted in the establishment of persistent infections (12, 13, 27, 37, 52). As we observed in infected cloned NK cells, host cell protein synthesis was not inhibited in VSV infected human B-cell lines (12). Despite this, the mechanism for persistence of VSV in Epstein-Barr virus-infected human B lymphoblastoid cell lines has not been elucidated. It has been argued that the block was in replication of the viral genome (37) or viral morphogenesis (52), whereas Creager et al. (13) have suggested a role for Epstein-Barr virus in establishing the persistence.

Clearly, the study of virus-lymphocyte interaction by using cloned lines of well-characterized lymphocytes in vitro has many advantages. Use of cloned lymphocyte lines circumvents the difficulties inherent in characterizing the low percentage of cells in bulk lymphocyte populations susceptible to any given virus and avoids the problem of second guessing viral tropism based on the outcome of complex immune responses. Since cloned effector lymphocytes are growth factor dependent, they may more truly reflect in vivo lymphocytes than permanently transformed lymphoblastoid cell lines. Further, virus-susceptible cloned lymphocyte lines provide an excellent system to investigate virus-lymphocyte interaction at a molecular level.

Although VSV is normally a highly cytolytic virus, persistence has been achieved in certain cells in vitro by coinfection with defective interfering particles, the use of virus mutants with reduced cytopathogenicity, or infection of interferon-treated cells (45). Since none of these mechanisms appeared to be responsible for VSV persistence in the NK cells, we compared the intracellular synthesis and maturation of VSV proteins in infected mouse L cells, which undergo normal lytic infection, and cloned IL-2-dependent NK cells. In contrast to L cells, in which host cell protein synthesis was essentially totally inhibited, infection of cloned NK cells with VSV caused no diminution of host cell protein synthesis. A similar inability of VSV to inhibit host cell protein synthesis was observed in human B lymphoblastoid cell lines (12). Although we do not yet know what accounts for the inability of VSV to shut off host cell protein synthesis in NK cells, it appears that the minimal requirements for VSV to shut off host protein synthesis are transcription of part of the viral genome producing leader RNA

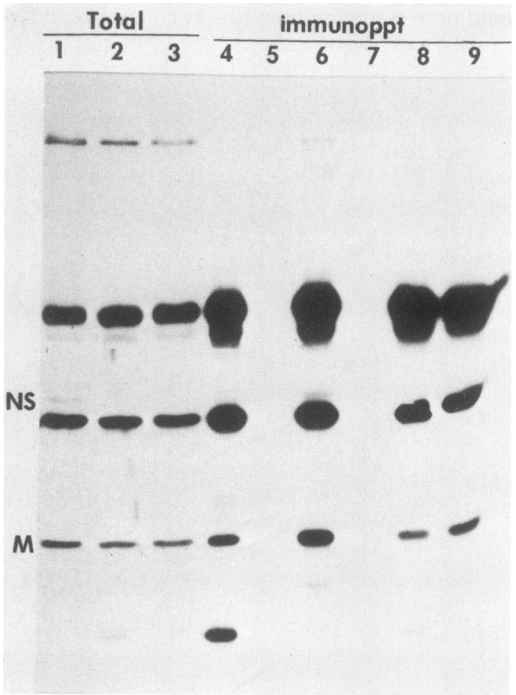


FIG. 6. SDS-PAGE analysis of mixing experiments. ^{35}S-methionine-labeled, VSV-infected L-cell extracts were mixed with unlabeled infected or uninfected NK (HY-1) cell extracts. SDS-PAGE of total proteins (lanes 1 through 3) or immunoprecipitates (lanes 4 through 9) was then performed. Labeled infected L cells were either not mixed (lane 1) or mixed with unlabeled infected (lane 2) or uninfected (lane 3) NK cell extract. Immunoprecipitates of labeled infected (lane 4) or uninfected (lane 5) NK cells and labeled infected (lane 6) or uninfected (lane 7) L cells were run as controls. Immunoprecipitates, from mixing experiments, of labeled infected L-cell extracts with unlabeled infected (lane 8) or uninfected (lane 9) NK cell extracts demonstrate that NK cells contained a protease capable of selectively degrading VSV NS protein with the concomitant appearance of the sub-M band.

and N and NS mRNAs and subsequent production of at least minimally functional N and NS proteins (31).

SDS-PAGE analysis of immunoprecipitates of viral proteins from infected cells demonstrated that the amount and maturation rate of VSV surface G glycoprotein were comparable in L cells and NK cells. This observation supported our immunofluorescence data, which indicated that essentially all infected NK cells expressed surface VSV G glycoprotein. Similarly, N protein synthesis appeared to be unaffected in infected NK cells.

In contrast to L cells, no nonstructural NS phosphoprotein was seen in either immunoprecipitated or total cell extracts of VSV-infected cloned NK cells. Furthermore, the level of VSV M protein was reduced in NK cells, and a prominent sub-M protein band was observed. These alterations in viral proteins most likely occurred at a posttranscriptional level. Our observations might be explained by intracellular proteases characteristic of NK cells which could lead to degradation of NS and the appearance of the sub-M band. Mixing experiments with labeled L-cell extracts and unlabeled extracts of either infected or uninfected NK cells confirmed that a protease in the cloned NK cells, active in the presence of serine protease inhibitors, was responsible for the loss of NS protein. This loss was accompanied by the appearance of the low-molecular-weight sub-M band. Whether this protease is active in the NK cell cytoplasm or was simply released and activated by cell disruption remains to be determined.

The selective degradation of viral proteins coupled with the inability of VSV to shut off host protein synthesis in infected NK cells would favor the establishment of a persistent infection. Although this is a novel mechanism for the persistence of VSV, it has been shown that the persistence of measles virus in subacute sclerosing panencephalitis (SSPE) is accompanied by the absence or reduction of viral M protein in cultured brain cells from SSPE patients (23). Furthermore, patients with SSPE have a selective deficiency of antibodies to M protein (24). Recently, it was shown that M protein was selectively degraded in an SSPE cell line (46). Thus, the selective breakdown of viral proteins may be a common and relevant mechanism in persistent infections.

For a virus to persist in vivo, it must infect a cell in a way that will permit both the survival of the cell and limited virus replication. But additionally, the persistently infected cell must avoid immune destruction. For this reason, we decided to investigate the susceptibility of persistently infected cloned NK cells to virus-specific, cell-mediated immune destruction. Our results demonstrate that persistently infected NK cells were resistant to lysis by H-2-restricted, VSV-specific CTL. The inability of cytotoxic T cells to kill VSV-infected NK cells could not be the result of a lack of expression of mature VSV G glycoprotein at the cell surface since both immunofluorescence and SDS-PAGE analysis indicated that the size and amount of VSV G were similar in infected L and NK cells. Similarly, the resistance of VSV-infected NK cells to anti-VSV CTL lysis could not be attributed to a lack of H-2 antigen expression or general insensitivity to lysis since alloantigen-specific CTL were able to lyse VSV-infected cloned NK cells. Thus, although persistently infected NK cells expressed detectable surface viral antigen, they were resistant to lysis by anti-viral cell-mediated immunity.

These results are surprising in light of studies which have established that VSV G protein is the target for recognition by anti-VSV CTL (21, 22, 57). Although we do not yet understand the resistance of persistently infected NK cells to lysis by virus-specific CTL, a few possibilities can be suggested. The most likely is that VSV G protein in and of itself does not constitute the target for anti-VSV CTL. This hypothesis is supported by a demonstration that cells transfected with VSV G protein are not lysed by NK cells (35). More recently, Yewdell et al. (56) used recombinant vaccinia viruses containing cloned VSV genes to demonstrate that N protein serves as the major target antigen for VSV-specific CTL. In light of our past studies (18), it is also possible that VSV G protein may not associate properly with H-2 molecules at the surface of infected NK cells. Alternatively, the selective degradation of VSV NS and possibly M proteins may affect the conformation of surface G protein or the transport of N protein to the cell surface.

These results also suggest that an individual need not be tolerant or unable to mount an immune response to a particular virus for a persistent infection to be established. Indeed, our results demonstrate that VSV, normally a highly cytolytic virus, can readily establish a persistent infection in a particular subpopulation of lymphoid cells and that these infected cells could potentially avoid immune destruction in an immunocompetent host. Our findings may be relevant to understanding acquired immunodeficiency syndrome, since it was recently demonstrated (26) that human T-cell lymphotropic virus type III or lymphadenopathy-associated virus can establish a persistent noncytopathic infection in normal human helper T cells. It is hoped that further studies with cloned effector lymphocytes will enhance our understanding of virus-lymphocyte interaction and the pathogenesis of persistent virus infection.

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