MHC I-dependent antigen presentation is inhibited by poliovirus protein 3A

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The effects of poliovirus 3A protein expression and poliovirus infection on the presentation of hepatitis C virus antigens in cultured chimpanzee cells were examined. Expression of poliovirus 3A protein inhibits protein secretion when expressed in isolation and was sufficient to protect chimpanzee cells from lysis by hepatitis C virus-specific cytotoxic T cells in standard ^{51}Cr-release assays. Poliovirus infection also inhibited antigen presentation, as determined by decreased cytotoxic T cell activation. A mutation in 3A that abrogates the inhibition of protein secretion also abolished the effects of poliovirus on antigen presentation. These results demonstrate that the inhibition of secretion observed in poliovirus-infected cells substantially reduces the presentation of new antigens on the cell surface. These observations may reflect a general mechanism by which nonenveloped viruses such as poliovirus and other viruses that do not require a functional protein secretory apparatus can evade detection by the cellular immune response.

Picornaviruses are extremely prevalent and successful viruses, replicating abundantly in organisms ranging from insects to humans. Diseases caused by picornaviruses include paralytic poliomyelitis (polioviruses), the common cold (rhinoviruses), chronic and acute heart disease (coxsackieviruses), lethal encephalitis of newborns (echoviruses), and the economically devastating foot-and-mouth disease of livestock.

Picornaviruses are nonenveloped viruses that encode no known glycosylated or transmembrane proteins. However, poliovirus, the most extensively studied picornavirus, encodes at least three nonstructural proteins that drastically affect host intracellular-membrane structure and function. Specifically, poliovirus protein 2C induces membrane vesiculation (1–3), whereas proteins 2B and 3A are each sufficient to inhibit protein traffic through the host secretory pathway (4, 5). In isolation, protein 3A interacts with endoplasmic reticulum (ER) membranes to inhibit protein transport from the ER to the Golgi apparatus (4, 5).

One possible role for these membrane perturbations is to construct a structural scaffold for the viral-RNA-replication complex. Poliovirus RNA replication occurs on the cytoplasmic surface of double-membraned vesicles that proliferate in virally infected cells (6–8). All the viral proteins required for RNA replication (2B, 2BC, 3A, 3AB, 3CD, and 3D) are physically associated with these vesicles in infected cells (7). In combination, viral proteins 2BC and 3A mimic the morphology and biochemistry of the membrane vesicles formed during poliovirus infection (9).

Several lines of reasoning led us to believe that inhibition of secretion is probably not required for vesicle formation. A cold-sensitive mutation in poliovirus 3A, 3A-2, inhibits secretion to a much lesser degree than does wild-type virus, even at the permissive temperature for RNA replication (5, 10). Furthermore, although all picornaviruses replicate on membranous vesicles, 3A proteins from some other picornaviruses do not inhibit secretion, suggesting that this aspect of 3A is not a requirement for viral RNA replication (D.A.D. and K.K., unpublished data).

What is the purpose of inhibiting secretion if it is not required for viral RNA replication? There is a growing body of literature that describes the various mechanisms used by viruses to evade detection by the cellular-immune response. $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize virally infected cells by the presence of viral antigens that are presented in the context of class I MHC proteins. Pathogens such as herpesvirus, adenovirus, cytomegalovirus, and Epstein–Barr virus interfere with antigen presentation by such disparate mechanisms as down-regulation of MHC gene expression, inhibition of antigen peptide processing and translocation into the ER, and sequestration of MHC proteins in the ER (reviewed in refs. 11–13). In other picornaviruses, rhinovirus is known to inhibit antigen-induced T cell proliferation via interactions with intercellular adhesion molecule-1 (14), and the L* protein of Theiler's virus reduces CTL-mediated lysis of infected cells by an unknown mechanism (15).

MHC I-dependent antigen presentation requires a functional secretory pathway. Therefore, it is possible that a virus that does not require a functional secretory pathway during its infectious cycle could effectively hide from the cellular immune system by inhibiting bulk secretion. To test this hypothesis directly, we have expressed poliovirus protein 3A and full-length poliovirus in cell types that are amenable to studying CTL activity. We have found that both isolated 3A protein and poliovirus infection can inhibit functional antigen presentation; for poliovirus infection, this activity is localized to the 3A region of the poliovirus genome.

Materials and Methods

Chimpanzee Cell Lines and Vaccinia Expression Vectors. The chimpanzee B lymphoblastoid cell lines, CTL cell lines, and recombinant vaccinia that express hepatitis C virus (HCV) proteins used in this study have been described (16). B lymphoblastoid cell lines were grown in RPMI medium 1640 with 10% (vol/vol) FBS (GIBCO/BRL). CTLs were grown in a T cell medium composed of RPMI medium 1640, 100 units/ml recombinant IL-2 (a generous gift from Chiron), 5% (vol/vol) human T-Stim (Collaborative Biomedical Products, Bedford, MA), and 10% (vol/vol) FBS. CTL were restimulated for growth every 10–14 days with irradiated human-peripheral-blood-mononuclear cells as described (17).

To make recombinant vaccinia viruses (rVV) that express poliovirus 3A and green fluorescent protein (GFP) from a dicistronic mRNA (rVV-3A,GFP), the wild-type poliovirus 3A-coding sequence, the poliovirus internal ribosome entry (IRES), and the coding sequence for an enhanced GFP (18) were

Abbreviations: CTL, cytotoxic T-lymphocyte; ER, endoplasmic reticulum; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorter; HCV, hepatitis C virus; IRES, internal ribosome entry; pfu, plaque-forming units; rVV, recombinant vaccinia viruses.

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amplified by PCR, ligated together, and inserted into a plasmid termed pTRE-3A,GFP (D.A.D. and D. Suhy, unpublished data). The 3A-IRES-GFP fragment was excised from the cloning vector with *Eco*RI and *Xba*I and inserted into *Eco*RI–*Nhe*I-digested pRB21, a shuttle vector for vaccinia recombination (19). BSC-1 cells were transfected with pRB21–3A,GFP and infected with the plaque-deficient vaccinia virus, vv-RB12 (20). Individual plaques of recombinant vaccinia were isolated, and expression of poliovirus 3A protein and GFP was confirmed by immunoblot. A recombinant vaccinia that expresses GFP in the absence of 3A protein was constructed by using a similar strategy except that the 3A-coding sequences were omitted. Expression of 3A was tested by immunoblot as described (5).

Cell-Surface Expression of MHC Class I. Chimpanzee lymphoblastoid 497LCL cells (8×10^7) were infected with rVV-GFP or rVV-3A,GFP at a multiplicity of infection of 10 plaque-forming units (pfu) per cell for 3.5 h, collected by centrifugation, incubated in PBS for 20 min at 37°C, collected by centrifugation, resuspended in 4 ml of RPMI medium lacking cysteine and methionine (Mediatech, Herndon, VA) containing 700 μCi/ml [^{35}S]methionine and [^{35}S]cysteine (1 Ci = 37 GBq; Express Label, New England Nuclear), and incubated for 15 min at 37°C. Cold chase was performed by the addition of 4 ml of complete RPMI medium supplemented with 10% (vol/vol) FBS followed by continued incubation at 37°C. Cells in each 1-ml aliquot were harvested at various times by transfer to ice, washed three times with ice-cold PBS (pH 8.0), and resuspended in 200 μl of PBS (pH 8.0). To isolate surface-expressed MHC class I molecules, 0.5 mg/ml EZ-Link Sulfo-NHS-SS-Biotin (Pierce) was added to each aliquot, and samples were incubated for 30 min on ice, then washed three times with cold PBS (pH 8.0). All samples were lysed by resuspension in 1 ml of RSB + Nonidet P-40 (10 mM Tris, pH 7.5/10 mM NaCl/1.5 mM $MgCl_2$/1% Nonidet P-40, pH 7.5) supplemented with 1 mM PMSF and incubated on ice for 10 min. Cell debris were removed by centrifugation at 4°C for 10 min at 10,000 $\times$ *g*. Supernatants were precleared by incubation with 250 ng/ml irrelevant mouse monoclonal antibody [anti-human interferon-β (Research Diagnostics, Flanders, NJ)] and 25 μl of prepared protein A-agarose (Santa Cruz Biotechnology) for 16 h at 4°C followed by centrifugation. The supernatants from each biotinylated sample were incubated with 30 μl of a 50% (vol/vol) solution of streptavidin agarose (Life Technologies, Rockville, MD) at 4°C for 2 h. Pellets were collected by centrifugation, washed three times with RSB-Nonidet P-40, and eluted by incubation with 30 μl of PBS (pH 8.0) supplemented with 50 mM DTT for 10 min at room temperature. Then, 1 ml of RSB-Nonidet P-40 was added to each supernatent, and MHC class I molecules were precipitated from all samples by incubation with 3 ng of W6/32 antibody (Sigma) for 1.5 h at 4°C followed by incubation with 50 μl protein A-agarose solution for 1.5 h. Immunoprecipitates were collected by centrifugation, washed three times with RSB-Nonidet P-40, resuspended in 30 μl of a solution containing 62 mM Tris·HCl (pH 6.8), 1% SDS, 5 M urea, 10% (vol/vol) glycerol, 12 mM dithiothreatol, 100 mM β-mercaptoethanol, and 0.06% bromophenol blue, incubated at 100°C for 5 min, and subjected to SDS/PAGE in a 9% gel. ^{35}S-labeled proteins were visualized by Phosphorimager analysis. Consistent with a report that some biotinylated MHC class I molecules are not recognized by the W6/32 antibody (20), we found that effective visualization of biotinylated MHC class I molecules depended highly on the particular biotinylation reagent used (data not shown).

^{51}Cr-Release Assays. Chromium-release assays for cytolytic activity were performed as described (21) with minor modifications. Target cells were infected with vaccinia-expression vectors at a multiplicity of infection of 10 pfu per cell. When cells were preincubated with synthetic peptide GAVQNEITL, 10 mg/ml peptide was incubated with the cells for 12 h at 37°C before incubation with $Na_2{}^{51}CrO_4$. At 12 h after infection at 37°C, 5×10^5 infected cells were harvested, washed in medium, resuspended in 200 μl of medium, and labeled with 50 μCi $Na_2{}^{51}CrO_4$ (Amersham Pharmacia) for 1 h. Labeled cells were washed three times in medium and resuspended at 50,000 cells per ml in T cell medium (21). For each assay, 5,000 target cells were incubated with the indicated number of CTLs in round-bottomed 96-well plates in a final volume of 200 μl. At 4 h, 50 μl of supernatant was removed and transferred to 96-well LumaPlates (Packard) and allowed to dry overnight. Radioactivity was assayed by reading LumaPlates in a scintillation-microplate reader (Perkin–Elmer). Spontaneous ^{51}Cr release was determined by incubating labeled cells in the absence of CTLs. Total label was measured by substituting 5% (vol/vol) Triton X-100 for the CTLs in T cell medium.

Granzyme A Secretion Assays. Granzyme A secretion assays were modified from those described by Kane *et al.* (22). Target cells were infected as for the ^{51}Cr-release assays. At 12 h after infection, infected cells were harvested and washed in medium. For each assay, 1×10^5 target cells were combined with 1×10^5 CTLs in a total volume of 250 μl in round-bottomed 96-well plates. After a 4-h incubation, 5 μl of supernatant was added to 245 μl of enzyme-reaction mixture [0.2 mM *N*-benzyloxycarbonyl-L-lysine thiobenzylester/0.22 mM 5,5′-dithiobis(2-nitrobenzoic acid); Sigma] in flat-bottomed 96-well plates. Light absorbance at 405 nm was measured at 30-min intervals in a microplate reader (Bio-Tek, Winooski, VT). The amount of enzyme was determined from the rate measured during the linear phase of the reaction. One unit of granzyme A was defined as the amount of enzyme required to convert the reaction mixture to $OD_{405} = 1$ in 60 min.

Virus Infection and Fluorescence-Activated Cell Sorter (FACS) Analysis. Chimpanzee B lymphoblastoid cells were infected with rVV-GFP or rVV-3A,GFP at 10 pfu per cell unless otherwise indicated. Wild-type Mahoney-type-1 poliovirus and 3A-2 mutant poliovirus (10) infections were 20 pfu per cell. At 12 h after infection, 10^6 cells were washed with PBS and resuspended in 200 μl of PBS. Cells were labeled with w6/32 anti-human HLA mouse mAb (Sigma) and visualized with a phycoerthryin-conjugated anti-mouse secondary antibody (Sigma). Cells were analyzed by FACS with a FACScan flow cytometer (Becton Dickinson).

Results

To study the effects of poliovirus 3A protein expression and poliovirus infection on MHC I-dependent antigen presentation, we used cell lines generated during a study of HCV-infection in chimpanzees (16). This system was chosen, because chimpanzee cells can be infected by poliovirus (23) and the cell biology and biochemistry of poliovirus has been studied extensively in primate cells. CTL lines were derived from liver biopsies from an HCV-infected chimpanzee named Todd, who resolved the infection 11 weeks after inoculation (16). B lymphoblastoid cell lines were derived from the same individual for use as target cells to measure the cytolytic activity of the CTL lines. For these studies, we used several CTL lines that were all characterized according to MHC I allotype and HCV-epitope specificity (16).

Poliovirus 3A Slows the Rate of MHC Class I Transport to the Cell Surface and Protects Cells from CTL-Mediated Lysis. To express poliovirus 3A protein in the chimpanzee B lymphoblastoid target cells, vaccinia-expression vectors were constructed that encoded either rVV-GFP alone or both 3A and GFP from a dicistronic mRNA (rVV-3A,GFP). To coexpress specific HCV antigens, the target cells were infected with previously characterized rVVs that expressed various portions of the HCV polyprotein (16). Expression of 3A in rVV-3A, GFP-infected chimpanzee cells could be

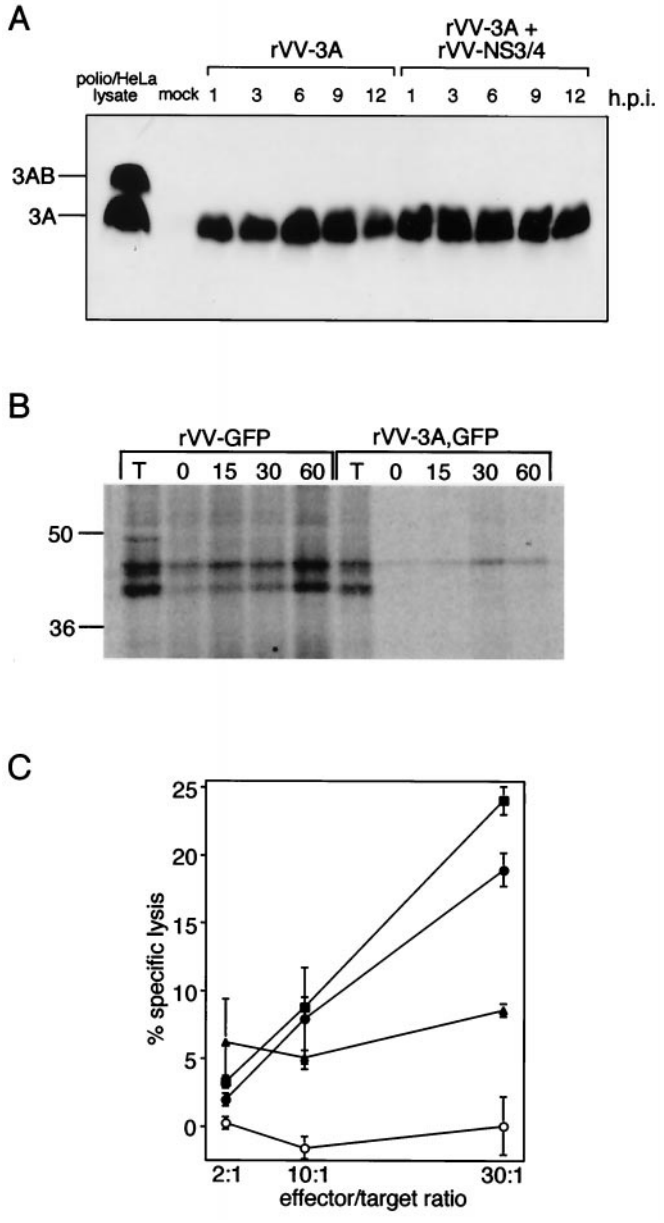


Fig. 1. (*A*) Expression of poliovirus 3A from rVV-3A,GFP. Immunoblot analysis of lysates from chimpanzee lymphocytes that were mock-infected, infected with rVV-3A/GFP, or coinfected with rVV-3A/GFP and rVV-E2/NS3. Time courses in hours after infection (h.p.i.) are shown. Lysate from poliovirus-infected HeLa cells shows both 3A and its precursor 3AB. (*B*) Effect of 3A protein on cell-surface expression of newly synthesized MHC class I. Chimpanzee B lymphoblastoid cells were infected with rVV-GFP or rVV-3A,GFP, labeled with [^{35}S]methionine and [^{35}S]cysteine for 15 min, and subsequently incubated with nonradioactive amino acids for various periods of time. Molecules on the cell surface were subjected to biotinylation and recovered by use of streptavidin agarose. Total (T) MHC class I molecules after 60 min of chase and MHC class I molecules that appeared at the cell surface after the indicated times (min) were isolated by immunoprecipitation with anti-MHC class I antibody w6/32. Molecular masses (kDa) of marker proteins are indicated. (*C*) Effect of poliovirus 3A on target-cell lysis by CTL. Chimpanzee B lymphoblastoid target cells were infected with wild-type vaccinia (○), infected with rVV-E2/NS3 alone (■), or coinfected with rVV-E2/NS3 and rVV-GFP (●) or rVV-E2/NS3 and rVV-3A,GFP (▲). At 12 h postinfection, target cells were labeled with ^{51}Cr and incubated for 4 h with T91 CTL at the indicated effector/target ratios. Specific lysis was expressed as the amount of ^{51}Cr released into the supernatant as a percentage of the total counts per sample. Data are presented as the mean of triplicate samples, and the standard deviations are indicated.

detected as early as 1 h after infection in the presence or absence of coinfecting vaccinia viruses that expressed HCV epitopes (Fig. 1*A*).

To determine whether the expression of poliovirus 3A protein inhibited transport of MHC class I molecules to the cell surface in the lymphoblastoid target cells, surface proteins were subjected to biotinylation at various times after pulse labeling. Labeled proteins that had reached the cell surface were recovered via their affinity for streptavidin-coated beads and MHC class I molecules were recovered from this population by immunoprecipitation. MHC class I molecules isolated from the chimpanzee lymphoblastoid cells (Fig. 1*B*) reflected the known heterogeneity in this population (E. Adams, unpublished data). All MHC class I molecules showed a reduced rate of transport to the cell surface in the presence of poliovirus 3A protein (Fig. 1*B*), as expected from the known activity of 3A protein in inhibiting ER-to-Golgi transport (4, 5).

To test the effect of poliovirus 3A expression on CTL-mediated lysis, target cells were infected with rVV-E2/NS3, which encodes HCV amino acids 264–1,618 (16). These cells were coinfected with rVV-GFP, rVV-3A,GFP, or no additional virus. At 12 h after infection the infected cells were labeled with ^{51}Cr and incubated with the CTL cell line T91, which specifically recognizes an HCV peptide from E2 (TINYTIFKI) in the context of the MHCPatr-B*2001 MHC I haplotype (16).

CTL-mediated lysis was observed in reactions containing target cells that were either infected with rVV-E2/NS3 alone or infected with both rVV-E2/NS3 and rVV-GFP (Fig. 1*C*). The observed lysis was antigen dependent, because target cells infected with a wild-type vaccinia virus that encoded no HCV proteins were unaffected by incubation with the CTLs. However, cells infected with both rVV-E2/NS3 and rVV-3A,GFP were substantially protected from CTL-mediated lysis even at high CTL-to-target ratios (Fig. 1*C*).

The Effects of 3A on Antigen Presentation Are Not Antigen- or MHC I-Specific. To test the effects of poliovirus 3A protein on other combinations of CTL and antigens, target cells infected with rVV-E2/NS3 and coinfected with either rVV-GFP or rVV-3A,GFP were challenged with CTL lines that recognize HCV antigens in the context of a second MHC class I allele, Patr-A*0601. The CTL line T4 is specific for an epitope from HCV P7 (KWVPGAVYTFY), and independently isolated CTL cell lines T73 and T84 are both specific for the same epitope from HCV E2 (RCDLEDRDRSELSPL). As with T91 CTL (Fig. 1*C*), lysis by T4, T73, and T84 was antigen dependent, only occurring in rVV-E2/NS3-infected target cells (Fig. 2). Target cells were efficiently lysed only when infected with rVV-E2/NS3 alone or when coinfected with rVV-GFP. Coinfection with rVV-3A,GFP resulted in reduced lysis with all three CTL lines. Therefore, protection from antigen-dependent CTLs by poliovirus 3A protein was not specific to any one antigen or MHC I haplotype.

Turnover of Cell-Surface MHC I Is Unaffected by Poliovirus Protein 3A. To test any effect of 3A protein expression on previously synthesized MHC class I molecules already at the plasma membrane, target cells were infected with either rVV-GFP or rVV-3A,GFP for 12 h, and the relative amounts of MHC class I on the surfaces of infected and uninfected target-cell surfaces were measured by FACS analysis. Infections were performed at relatively low multiplicities of infection to allow the direct comparison of uninfected and infected GFP-expressing cells within each sample. No differences were observed in the amounts of total MHC class I present on uninfected, rVV-GFP-infected, or rVV-3A,GFP-infected cells (Fig. 3*A*). Therefore, expression of poliovirus 3A protein did not significantly change the amount of total MHC class I on the surface of target cells, even though it slowed the transport rate of newly synthesized

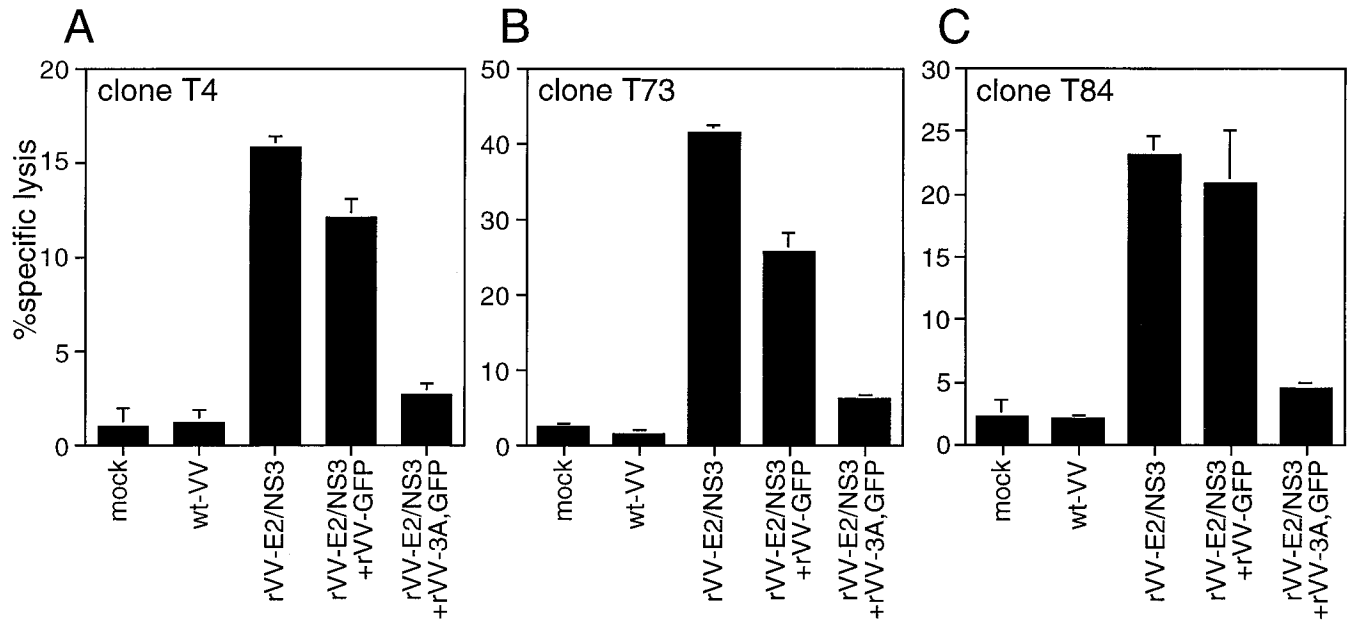


Fig. 2. Effect of poliovirus 3A expression in target cells on lysis by three different CTL lines. Target cells were infected with rVV-E2/NS3 and incubated for 4 h with CTL T4 (*A*), T73 (*B*), or T84 (*C*) at CTL/target-cell ratios of 20:1. Specific lysis was determined as in Fig. 1. Data are presented as the mean of triplicate samples, and the standard deviations are indicated. wt-VV, wild-type vaccinia.

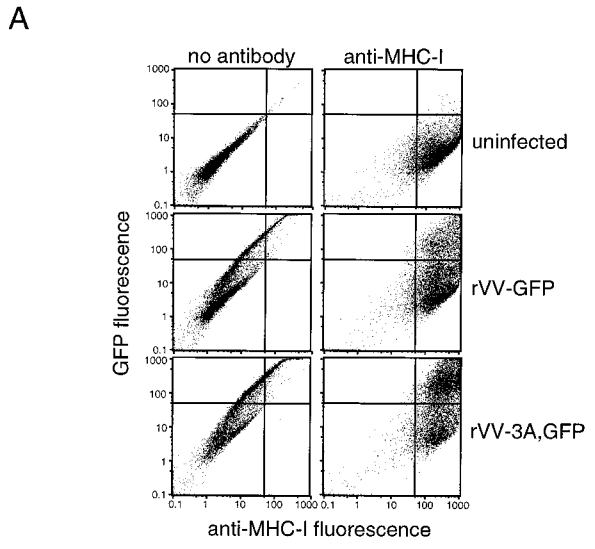
MHC class I (Fig. 1*B*). The lack of change of total MHC class I on the cell surface is not surprising, even over a 12-h period, because MHC class I complexes at the cell surface are often relatively stable. The physical half-life of peptide-bound MHC I on the cell surface is allele-specific (24). One study with the w6/32 antibody used here, which recognizes human HLA-A, -B, and -C, and chimpanzee Patr-A, -B and -C (25), measured the half-life of surface-labeled MHC class I in a human line as 8–10 h (26). In any case, the lack of change in MHC class I on the cell surface, even after several hours of expression of poliovirus 3A, argues that the protection from CTL-mediated lysis conferred by 3A to the target cells was caused by inhibition of new antigen presentation rather than increased internalization or degradation of MHC I on the cell surface.

3A-Expressing Cells Remain Susceptible to CTL-Mediated Lysis in the Presence of Exogenous Peptide. As a functional test for the presence of previously synthesized MHC class I on the surface of the chimpanzee target cells, cells that expressed HCV epitopes in the presence and absence of 3A were externally loaded with peptide, and their susceptibility to CTL-mediated lysis was measured. Target cells were coinfected with rVV NS3/4, which expresses HCV amino acids 1,590–2,050, and with rVV-3A,GFP in the presence and absence of the exogenously loaded peptide GAVQNEITL. CTL line T14, known to recognize this peptide in the context of Patr-A*0601, efficiently lysed cells even when they expressed poliovirus protein 3A in the presence of the peptide (Fig. 3*B*). In fact, 3A-expressing cells were more efficiently lysed at low effector/target ratios than control cells, suggesting that some feature of the altered cell biology of 3A-expressing cells (5) increased their susceptibility to CTL-mediated lysis. Therefore, the protective effects of 3A protein seen in Figs. 1*C* and 2 do not result from a reduction of total surface MHC I (Fig. 3*A*) or intrinsic resistance to CTL-mediated lysis (Fig. 3*B*). Instead, these data are consistent with a role for the inhibition of protein secretion by 3A protein in inhibiting the presentation of antigen by newly synthesized MHC class I molecules.

Wild-Type 3A Function Is Required for the Inhibition of Antigen-Dependent CTL Activation by Poliovirus. To determine whether the effects of 3A protein on MHC I presentation were relevant to poliovirus infection, chimpanzee target cells were infected with wild-type and mutant polioviruses. An alternative assay from the conventional ^{51}Cr-release assay was used, because the increased permeability of the cell membranes of poliovirus-infected cells (4, 27) led to poor retention of the ^{51}Cr label. We used an assay for functional antigen presentation, the secretion of granzyme A from the CTL line, which has been demonstrated to be an effective indicator of antigen-dependent CTL activation (28).

To test the effects of poliovirus infection on CTL activation, chimpanzee target cells were coinfected with rVV-NS3/4 and rVV-GFP, rVV-3A,GFP, or Mahoney-type-1 wild-type poliovirus, and the infections were allowed to proceed for 12 h. After 12 h of poliovirus infection in this cell line, no cell lysis was observed, and the abundance of 3A protein was approximately half that observed in the rVV-3A,GFP-infected cells (data not shown). The infected target cells were incubated with T14 CTL for 4 h, and the amount of granzyme A secreted into the medium was determined by enzymatic assay (Fig. 4). Consistent with its effects in ^{51}Cr-release assays, expression of 3A protein inhibited CTL activation. Furthermore, infection with wild-type poliovirus also inhibited CTL activation (Fig. 4), an effect that did not result from a reduction in the total amount of MHC class I molecules on the cell surface (Fig. 5).

If the inhibition of CTL activation during poliovirus infection were the result of the inhibition of ER-to-Golgi traffic by viral 3A protein, a mutation in the 3A-coding region that allowed host-protein secretion should also allow increased CTL activation. A previously characterized mutant poliovirus, 3A-2, replicates normally at 37°C in several lines of tissue-culture cells (10) and in the target cells used here (data not shown). When expressed in isolation, the 3A-2 protein exhibits very little inhibition of cellular-protein secretion compared with wild-type 3A protein (6). Unlike cells infected with wild-type poliovirus, target cells infected with 3A-2 mutant poliovirus showed no inhibition of CTL activation (Fig. 4). Again, total MHC class I



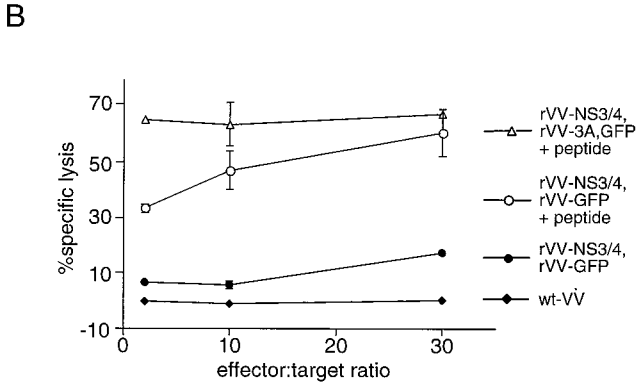


Fig. 3. (*A*) MHC I abundance on target-cell surfaces in the presence and absence of poliovirus 3A protein expression. Chimpanzee target cells were either uninfected or infected with rVV-GFP or rVV-3A,GFP at a multiplicity of infection less than 1 pfu per cell. At 12 h after infection, the cells were stained with anti-MHC I antibodies and analyzed by FACS. For each sample, 10^4 cells were plotted for red (MHC I) versus green (GFP) fluorescence. (*B*) Effect of exogenously loaded peptide on CTL-mediated lysis of 3A-expressing target cells. Chimpanzee target cells were infected with wild-type vaccinia (wt-VV) or coinfected with the viruses indicated in the presence or absence of the antigenic peptide GAVQNEITL as shown. Standard deviations of duplicated CTL assays are indicated when possible given the symbol size.

levels on the surface of the 3A-2 infected cells were unaffected (Fig. 5).

Discussion

We have shown that MHC class I-dependent antigen presentation in chimpanzee cells is inhibited during poliovirus infection. The mechanism by which poliovirus inhibits antigen presentation seems to be the inhibition of traffic through the host secretory pathway. A mutation in 3A that disrupts the ability of the protein to inhibit secretion (5) abolishes the effects of poliovirus on antigen presentation (Fig. 4), and the expression of poliovirus protein 3A alone is sufficient to inhibit presentation (Figs. 1*C* and 2). Many mechanisms have been described by which viruses and other pathogens interfere with antigen presentation and T cell activation. Virtually every step of antigen processing and presentation is a target for attack by one pathogen or another (see 11–13). However, this finding is the first example of immune evasion by the induction of a general

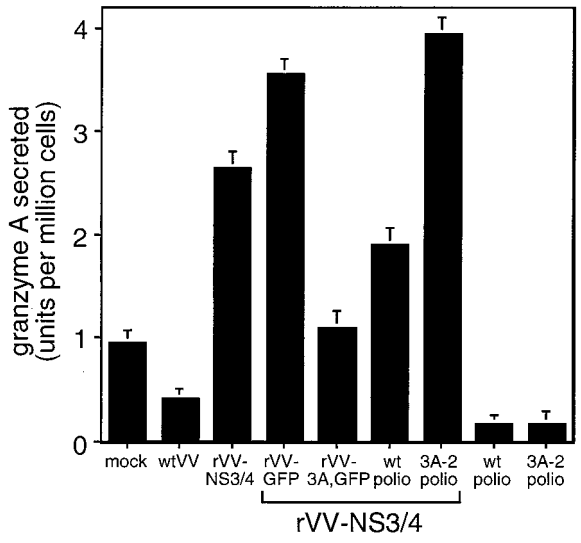


Fig. 4. Effects of wild-type and 3A-2 mutant poliovirus infections on antigen presentation to CTL. Chimpanzee target cells were infected with wild-type vaccinia (wt-VV), rVV-NS3/4, wild-type (wt) poliovirus, 3A-2 mutant poliovirus, or coinfected with rVV-NS3/4 and rVV-GFP; rVV-NS3/4 and rVV-3A,GFP; rVV-NS3/4 and wild-type poliovirus, or rVV-NS3/4 and 3A-2 poliovirus. At 12 h after infection, target cells were incubated for 4 h with T14 CTL at an effector/target ratio of 1:1. Granzyme A secretion into the supernatants was determined by enzymatic assay. Data are presented as the means of triplicate samples, and the standard deviations are indicated.

shutdown in host secretion, a strategy that is available only to pathogens such as nonenveloped viruses that can survive without a functional host secretory apparatus.

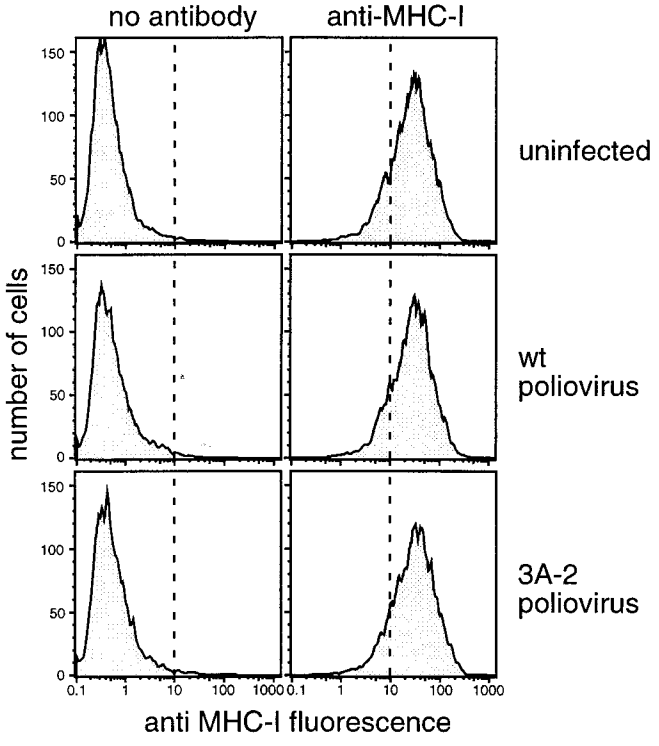


Fig. 5. Effect of wild-type and 3A-2 mutant poliovirus infections on MHC I-cell-surface turnover. Chimpanzee B lymphoblastoid cells were infected with either wild-type (wt) poliovirus or 3A-2 mutant poliovirus. At 12 h after infection, cells were stained with anti-MHC I antibody and analyzed by FACS.

Although new antigen is not efficiently presented in the presence of 3A protein, previously loaded MHC I molecules remain on the cell surface, making it unlikely that cells infected with poliovirus would be targets for natural-killer-cell destruction because of depletion of MHC I from their surface. Furthermore, the known inhibition of clathrin-dependent endocytosis during poliovirus infection (29) could extend the cell-surface longevity of MHC class I molecules in infected cells.

During poliovirus infection or expression of poliovirus 3A protein in isolation, cellular protein secretion is not absolutely inhibited, but its rate is decreased by approximately 3- to 5-fold (refs. 4 and 5; Fig. 1*B*). This decrease in rate should exert a large quantitative effect on the abundance of newly synthesized MHC I molecules on the surface of cells that could in turn cause a significant, but not absolute, reduction in the amount of CTL-mediated lysis of infected cells.

The literature is silent on the issue of $CD8^+$ T cell responses to poliovirus infection and clearance in humans, perhaps because of the technical difficulty of labeling poliovirus-infected cells with ^{51}Cr. In contrast, the humoral response to poliovirus infection, in the form of neutralizing IgG and mucosal IgA, is well documented and is the basis for the highly successful poliovirus vaccines (30). Whatever the role of $CD8^+$ CTLs in natural infection, the observation that more than 95% of wild-type poliovirus infections do not progress to paralytic disease argues that the immune system is generally effective in clearing this pathogen. The impact of viral 3A protein on antigen presentation during infection in humans is not known. It could either decrease the severity of disease symptoms by reducing inflammatory responses or prolong infection by reducing the killing of infected cells by $CD8^+$ CTLs.

Poliovirus infection has been studied in mice that express the poliovirus receptor, CD155 (31–33), and cytolytic T cell activity has been described. Two reports attributed the cytolytic activity to $CD4^+$, not $CD8^+$, T cells (34, 35). $CD8^+$ CTLs' responses to foreign antigens expressed from recombinant poliovirus genomes and to a poliovirus epitope have been described (36–38). In at least one of these studies (37), it was uninfected, professional antigen-presenting cells that acted to stimulate the $CD8^+$ CTL response. It has not yet been determined whether activated $CD8^+$ CTLs can recognize poliovirus-infected tissues *in vivo* and to what extent this recognition may be affected by the wild-type function of 3A protein.

Given the immune-privileged nature of the central nervous system (39), it is uncertain whether additional immune evasion by poliovirus would contribute to the pathogenesis of human poliomyelitis. However, the vast majority of poliovirus infections remain localized to the intestine (40), and immune evasion may be an important component of the virus–host interaction. Furthermore, the inhibition of secretion and antigen presentation observed during poliovirus infection may reflect a general mechanism by which viruses that do not require a functional protein secretory pathway can avoid detection by the cellular immune response.

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