ANCHOR SEQUENCE-DEPENDENT ENDOGENOUS PROCESSING OF HUMAN IMMUNODEFICIENCY VIRUS 1 ENVELOPE GLYCOPROTEIN gp160 FOR CD4⁺ T CELL RECOGNITION

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The recognition of protein antigens by CD4⁺ and CD8⁺ T cells requires a critical initial step in which the antigen is denatured and/or partially proteolyzed within the APC. Two major pathways for the processing of protein antigens have been identified (1–12). Exogenous antigens taken up from the extracellular environment by macrophages and other APC are processed in a low pH endocytic compartment (1–4). Fragments of processed antigen then associate with class II MHC gene products, and the resulting complexes are expressed on the cell surface for subsequent recognition by CD4⁺ T cells (13–15). In contrast, antigenic proteins that are synthesized within APC (as in the case of viral infection) are generally processed for association with class I MHC gene products and subsequent recognition by CD8⁺ T cells (4–12). The processing of these endogenous antigens for association with class I MHC molecules appears to require that the protein be synthesized within or otherwise gain access to the cytoplasm of the cell (5–12).

Viral envelope proteins present an interesting problem in terms of antigen processing because these proteins are translocated during biosynthesis into the lumen of the rough endoplasmic reticulum (RER).[1] Class I–restricted T cell responses to viral envelope proteins synthesized in infected cells have been observed for a number of viruses, including influenza (7), and are particularly prominent in the case of infection by the human immunodeficiency virus type 1 (HIV-1). HIV-1–infected individuals have high levels of circulating CD8⁺ CTL specific for the HIV-1 envelope glycoprotein gp160 (16–19). Interestingly, class II–restricted T cell responses to the

[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; RER, rough endoplasmic reticulum; VSV, vesicular stomatitis virus.

envelope proteins of influenza virus have also been observed (7, 20, 21). However, in some instances this appears to result from the uptake of exogenous, virion-associated envelope protein and subsequent processing by the normal class II pathway rather than the direct processing within the infected cells of endogenously synthesized envelope protein (7, 21). It is therefore unclear whether endogenously synthesized viral envelope proteins can generally be processed within infected cells for association with class II MHC gene products and subsequent recognition by CD4$^+$ T cells.

We present here a mechanistic analysis of the processing of the envelope glycoprotein gp160 of HIV-1 and show that endogenously synthesized gp160 can be processed for recognition by CD4$^+$ T cells. This processing requires that the protein, after synthesis on the RER and during subsequent cellular transport, remain attached to the luminal/extracellular membrane face by a hydrophobic anchor sequence.

Materials and Methods

Antigens. Recombinant HIV-1 gp120 produced in Chinese hamster ovary (CHO) cells transfected with a truncated form of the gene encoding the gp160 envelope precursor protein as previously described (22) was kindly provided by Drs. Tim Gregory and Phil Berman of Genentech, Inc. (South San Francisco, CA). Recombinant HIV-1 gp160 produced using the baculovirus expression system was kindly provided by Dr. Gale Smith, MicroGeneSys, Inc., New Haven, CT. A synthetic peptide representing amino acids 410–429 of gp160 of the PV22 strain of HIV-1 (GSDTITLPCRIKQFINMWE) was produced using standard solid-phase methods and was purified by reverse-phase HPLC as previously described (23).

T Cell Lines and Clones. The HIV-1 gp120-specific CD4$^+$ human T cell clone Een217 was isolated from an HIV-1 seronegative donor as previously described (23). The CD4$^+$ T cell clone ERT5.3 was derived from the same donor by limiting dilution cloning of PHA-activated PBMC. The gp160-specific CD8$^+$ human T cell clone SF1E91 was isolated from an HIV-1 seropositive donor as previously described (18). The gp160-specific human T cell line 15.2 was derived from a volunteer who received a recombinant gp160 subunit vaccine (MicroGene-Sys, Inc.) as part of a Phase I AIDS vaccine trial conducted by the Johns Hopkins University Center for Immunization Research. All T cell clones and lines were maintained in the presence of IL-2 with weekly restimulation with irradiated PBMC and antigen or mitogen.

Vaccinia Virus Vectors. The vPE7 vector for the expression of HIV-1 *env* gene has been described (24, 25). Higher levels of *env* gene expression were achieved using a related vector, vPE16, in which two vaccinia early transcriptional termination signals in the *env* sequence were eliminated by changes at the nucleotide level (Earl, P. L., and B. Moss, manuscript submitted for publication). The vPE8 vector was prepared by deleting the gp41 sequence and inserting a stop codon at the end of the gp120 coding sequence just 5' of the proteolytic processing signal sequence (REKR) at which gp160 is cleaved into gp120 and gp41 (Earl, P. L., and B. Moss, manuscript in preparation). To eliminate the signal peptide from gp160, a plasmid carrying the *env* gene, mpPE7 (25), was mutagenized at the junction of the signal peptide and gp160 with the oligonucleotide ATCTGTAGTGCTGATATCCACCATGACAG-AAAAATTG, adding an Eco RV site and an ATG codon. The 2.4-kb Eco RV fragment containing the gene was cloned into the Sma I site of pSC11 (24) to generate pPE11. To express the gp120 sequence attached to a vesicular stomatitis virus (VSV) G protein anchor sequence, the plasmid mpSCenv (24) was used as the parent of mpenvVSV. It was mutagenized at the 3' end of gp120 with oligonucleotide GAGAGAAAACCCGGGAAGAGCAGT to insert a Sma I site. This clone was cut with Sma I and Xho I to remove the gp41 coding sequence. A 250-bp Alu I–Xho I fragment from pMM37 (26) containing the VSV-G anchor sequence was then inserted into the Sma I–Xho I cut mpSCenv mutant. The clone, mpenvVSV, contains a hybrid gene between HIV-1 gp120 and the VSV-G anchor. The coding region was removed with Sph I and Sst I, blunted with T4 polymerase, and inserted into the Sma I site of pSC11 to make the plasmid pSC38. The recombinant viruses vPE11 and vSC38 were made by homologous recombination between wild-type vaccinia and the plasmids pPE11 and pSC38,

respectively, by standard techniques (27). The preparation of the vPE12 vector in which the proteolytic processing site has been deleted will be described elsewhere (Earl, P. L., and B. Moss, manuscript submitted). Viruses were grown in HeLa cells and purified by centrifugation through a 36% sucrose cushion and then by rate zonal centrifugation through a 25-40% sucrose gradient. vPE16 was obtained from the AIDS Research and Reference Reagent Program, NIH, Bethesda, MD.

Cytolytic Assays. Cytotoxicity was measured in a ^{51}Cr-release assay as previously described (23) except that the assay period was 8 h. Target cells were pulsed with the indicated antigens or infected with recombinant vaccinia viruses for 12-16 h at 37°C before labeling with ^{51}Cr and use in the cytolytic assay. Vaccinia infection of target cells was carried out at a multiplicity of infection (MOI) of 10. UV light inactivation was performed using a Blak Ray UV light at 6 cm for 15 min on ice.

Western Blots. Laz 509 cells were infected with each virus (MOI = 10) for 18 h at 37°C. Cells were washed three times, lysed in SDS sample buffer, and electrophoresed on 8% SDS-polyacrylamide gels. Supernatants from infections were precipitated with trichloroacetic acid, and precipitates were also electrophoresed. Proteins were transferred onto nitrocellulose and detected with a rabbit anti-gp160 antisera (generous gift of Dr. Ron Willey, National Institutes of Health, Bethesda, MD) and ^{125}I-labeled Protein A (Pharmacia Fine Chemicals, Piscataway, NJ).

Results

Processing of Endogenously Synthesized gp160. To investigate the mechanisms by which viral envelope proteins are processed for recognition by CD4$^+$ T cells, we examined human T cell recognition of the HIV-1 envelope glycoprotein gp160. This protein is synthesized on the RER of the infected cell and is cleaved by a cellular protease into a larger NH$_2$-terminal glycoprotein (gp120), which remains noncovalently associated with the smaller membrane-spanning COOH-terminal fragment (gp41) (28-31). CD4$^+$ human T cell clones specific for gp120 were isolated by stimulation of peripheral blood T cells from HIV-1 seronegative donors with soluble recombinant gp120 and autologous monocytes followed by soft agar cloning (23). The representative CD4$^+$ gp120-specific T cell clone used in these studies, Een217, recognized an epitope in gp120 (residues 410-429) in association with HLA DR4 (Dw10) (23). This clone showed a high level of antigen-specific cytolytic activity against autologous macrophages and B lymphoblastoid cells that had taken up and processed exogenous gp120 (23).

To determine whether Een217 also recognized endogenously synthesized envelope protein, we used recombinant vaccinia vectors to express the HIV-1 *env* gene within cells of an autologous EBV-transformed B lymphoblastoid cell line (Laz 509). As shown in Fig. 1 *A*, Een217 lysed autologous B lymphoblastoid cells that had been pulsed with exogenous gp160 or with a peptide representing the epitope in gp160 that is recognized by this clone (residues 410-429). Significantly, Een217 also showed a high degree of lytic activity against target cells infected with a recombinant vaccinia vector carrying the HIV-1 *env* gene (vPE16). In contrast, there was little lysis of target cells infected with a similar vaccinia vector lacking the HIV-1 *env* gene (vSC8). The lysis of vPE16-infected cells was not due to the uptake and processing of trace amounts of exogenous gp160 or gp120 associated with the sucrose gradient–purified recombinant virus preparation. Rather, as shown in Fig. 1 *B*, infectious virus was required since virus preparations inactivated with UV light did not efficiently render Laz 509 cells susceptible to lysis, whereas similarly treated intact gp160 protein was highly effective. Taken together, these results demonstrate that, when synthesized
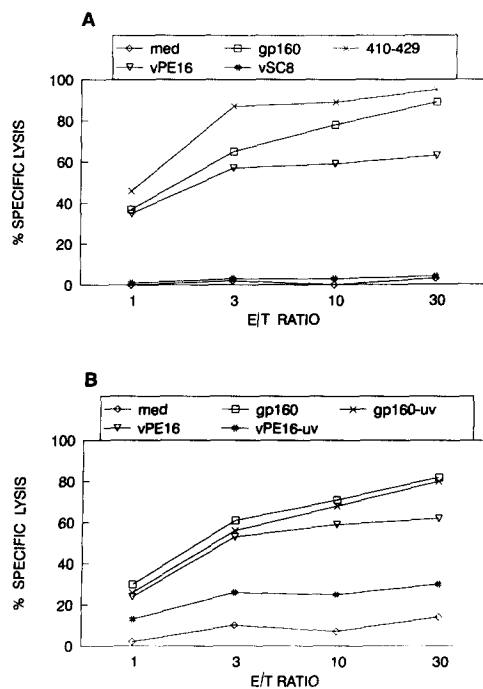
FIGURE 1.   Recognition of endogenously synthesized gp160/120 by the CD4$^+$ T cell clone Een217. (A) Lysis of the autologous EBV-transformed B lymphoblastoid cell line Laz 509 by clone Een217. Target cells were pulsed with media alone, recombinant gp160 (60 μg/ml), or peptide 410-429 (10^{-6} M), or infected with the recombinant vaccinia viruses vPE16 or vSC8. Cytotoxicity was measured in a standard ^{51}Cr-release assay. (B) Effect of UV irradiation on the ability of vPE16 to render Laz 509 cells susceptible to lysis. Aliquots of vPE16 and gp160 were UV irradiated before addition to Laz 509 cells. Lysis by Een217 was measured as described above.

in infected cells, the HIV-1 envelope protein gp160 can be processed for association with class II MHC gene products and subsequent recognition by CD4$^+$ T cells.

Several mechanisms for the processing of endogenously synthesized gp160 were considered. One possibility is that gp120/gp41 complexes expressed on the surface of infected cells undergo dissociation releasing soluble gp120, which is subsequently taken up nonspecifically by cells and processed by the normal class II pathway. Another possibility is that processing takes place after endocytic uptake of membrane gp120/gp41 complexes expressed on the surface of infected cells. Alternatively, the delivery of the envelope protein to compartments where processing and MHC association take place could occur by a completely intracellular route. It is also possible that cytoplasmic degradation of a small amount of newly synthesized gp160 that is not translocated into the lumen of the RER could generate the peptide fragments that ultimately associate with class II MHC molecules.

Expression of Altered Forms of the HIV-1 env Gene.   To distinguish among these alternatives, we used a series of vaccinia constructs with various alterations in the HIV-1 *env* gene (Figs. 2 and 3). The vPE16 and vPE7 vectors contain the entire *env* gene and express gp160, which is cleaved to give gp120 and gp41. The vPE11 vector has been modified to remove the NH2-terminal hydrophobic leader sequence of the envelope protein. This vector directs the synthesis of a form of gp160 that is rapidly degraded in the cytoplasm. Although gp160 expression by cells infected with vPE11 was not readily detectable by Western blotting analysis (Fig. 3), expression was detected by immunofluorescence analysis of permeabilized cells. In addition, target cells infected with vPE11 were readily lysed by class I–restricted CTL (see below).

vPE16, vPE7

MRVKEKYQHLWRWGWRWGTMLLGMLMICSATEK   TKAKRRVVQREKRAVGIG

vPE11
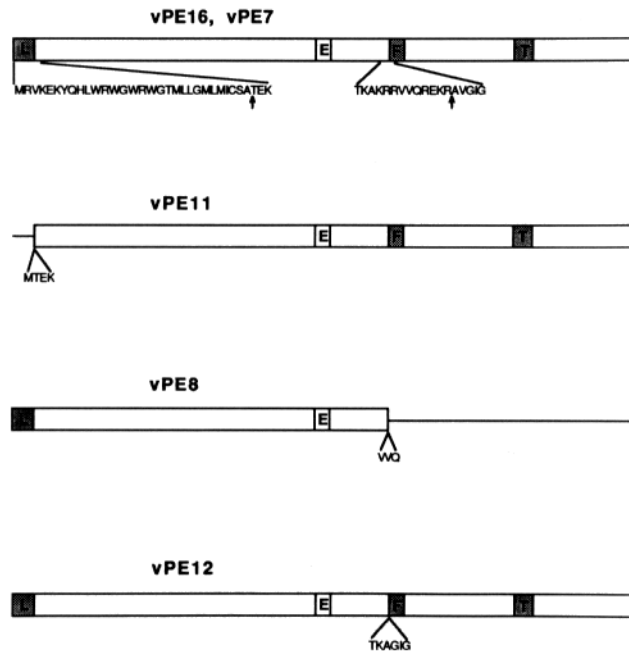

MTEK

vPE8

WQ

vPE12

TKAGIG

FIGURE 2. Vaccinia vectors for the expression of the HIV-1 *env* gene. Portions of the *env* gene inserted into the recombinant vaccinia expression system (27) are shown. Hydrophobic regions in the *env* sequence including the NH2-terminal leader sequence (L) of gp160 and the fusion (F) and transmembrane (T) anchor domains of gp41 are boxed. The proteolytic processing sites at which the signal peptide is cleaved and at which the gp160 is cleaved to gp120 and gp41 are indicated by arrows. The position of the epitope (E) in gp120 recognized by Een217 (residues 410–429) is also indicated. The vPE16 and vPE7 vectors contain the entire HIV-1 *env* gene (BH8 clone), starting at the putative translation initiation codon, under the control of a vaccinia virus promoter as previously described (24). The vPE16 vector has been modified by site-directed mutagenesis to remove two vaccinia early termination signal sequences present within the *env* gene. This alteration results in significantly greater *env* gene expression. In the vPE11 vector, the leader sequence of the *env* gene has been eliminated and a Met codon has been placed immediately 5' of the first residues of the mature protein (TEK. . .). In the vPE8 vector, the gp41 coding sequence has been removed and a stop codon has been placed at the end of the gp120 coding sequence just 5' of the proteolytic processing signal sequence (REKR), which directs the cleavage of gp160 into gp120 and gp41. In the vPE12 vector, the processing site has been removed. See Materials and Methods for details of vector construction.

vSC8   vPE16   vPE7   vPE11   vPE8   vPE12
C   S   C   S   C   S   C   S   C   S   C   S
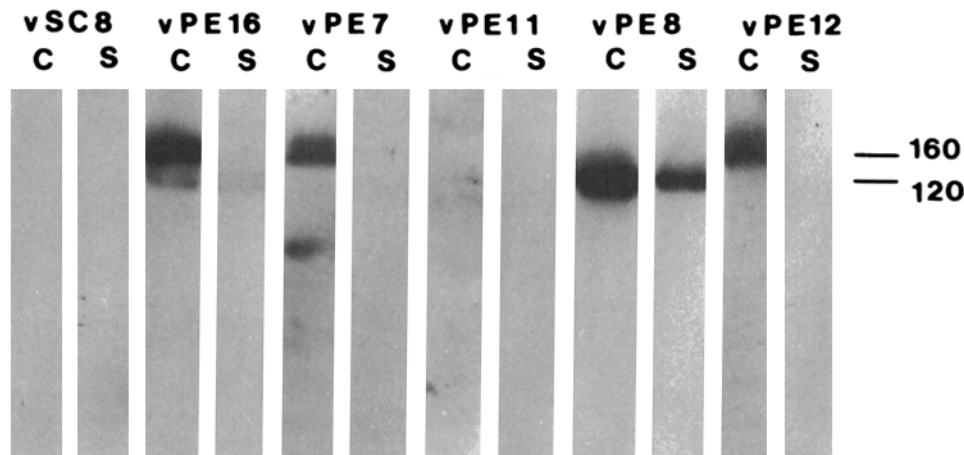

— 160
— 120

FIGURE 3. Expression of wild-type and mutant HIV-1 *env* genes in Laz 509 target cells. The expression of gp160 and gp120 was detected by Western blot analysis of cell lysates (*C*) and supernatants (*S*) after infection with recombinant vaccinia viruses.

The vPE8 vector lacks the gp41 coding sequence and has a stop codon within the *env* gene the near the gp120/gp41 proteolytic processing site. As shown by Western blot analysis (Fig. 3), this vector induced the production of large amounts of gp120, which was detected in infected cells and which was secreted into the medium. In the vPE12 vector, the *env* gene has been modified to remove codons for 12 residues (KRRVVQREKRAV) at the COOH terminus of gp120, including the proteolytic processing site at which gp160 is normally cleaved into gp120 and gp41. As shown in Fig. 3, this vector directed the synthesis of a gp160 protein that was not cleaved into gp120 and gp41. Consistent with the modifications described above, analysis of cell surface expression of gp160/120 by flow cytometry was positive on cells infected with vPE7, vPE16, and vPE12 but negative on cells infected with vPE11 and vPE8 (data not shown).

EBV-transformed B lymphoblastoid cells of appropriate MHC genotype were infected with these recombinant viruses and tested for susceptibility to lysis by envelope-specific CTL. In all experiments, cells infected with the vaccinia recombinant vSC8, which lacks the HIV-1 *env* gene, were used as negative controls. Cells infected with vPE7 or vPE16, which express the wild-type *env* gene, served as positive controls.

Processing of Signal Peptide–minus Envelope Protein.   To determine whether translocation of the newly synthesized envelope protein into the RER was required for processing, the signal peptide–minus vector vPE11 was used. As shown in Fig. 4, the lysis of vPE11-infected Laz 509 cells by the CD4$^+$ CTL clone Een217 was very weak compared with the lysis of vPE16-infected cells that express the wild-type envelope protein. In contrast, vPE11-infected B lymphoblastoid cells were readily lysed by a gp160-specific, MHC class I–restricted CD8$^+$ CTL clone (Fig. 5). These results suggested that gp160 synthesized in the cytoplasm of an infected cell was efficiently processed for class I–restricted, but not class II–restricted recognition. This may reflect the fact that insufficient amounts of the protein accumulated for class II–restricted processing. Alternatively, efficient processing for association with class II MHC molecules may require that the protein be translocated during synthesis into the RER, as indicated by the higher levels of lysis of vPE16-infected cells by Een217.

Processing of Anchor Sequence–minus Envelope Protein.   Although translocation of the newly envelope synthesized protein into the RER appeared to be necessary for efficient
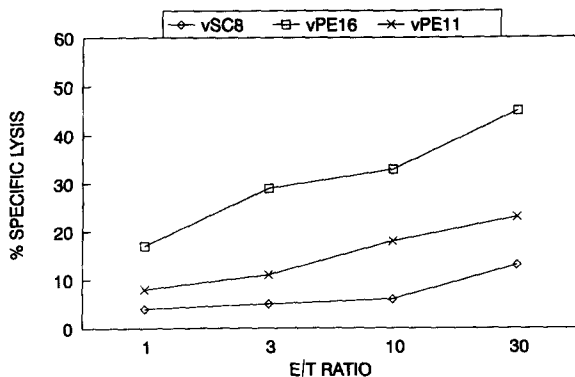

Figure 4. Lysis of target cells expressing signal peptide–minus gp160 by a CD4$^+$ CTL clone. Laz 509 cells were infected with the signal peptide–minus recombinant vaccinia virus vector vPE11 or the positive and negative control vectors vPE16 and vSC8, respectively. Lysis by clone Een217 was measured in an 8-h ^{51}Cr-release assay.
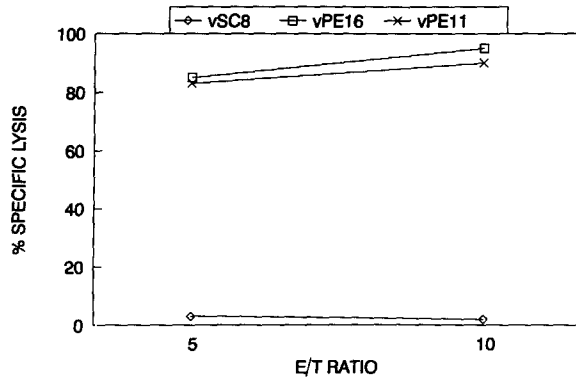
FIGURE 5. Lysis of target cells expressing signal peptide–minus gp160 by a CD8$^+$ CTL clone. Autologous EBV-transformed B lymphoblastoid cells were infected with vPE11, vPE16, or vSC8. Lysis by the CD8$^+$ CTL clone SF1E91 was measured in a 4-h ^{51}Cr-release assay.

class II–restricted processing, translocation was not a sufficient condition for class II–restricted processing since cells infected with the vPE8 vector, which induced the production of an anchor sequence–minus, secreted form of gp120, were not lysed (Fig. 6 A). The gp120 protein produced by vPE8-infected cells contains the epitope recognized by Een217 (Fig. 2). The failure of Een217 to lyse vPE8-infected cells was particularly noteworthy in light of the fact that vPE8-infected cells produced a large amount of envelope protein compared with cells infected with the wild-type vector vPE7 (Fig. 3). Even cells infected with vPE8 at very high multiplicities (30:1) were not lysed. As shown in Fig. 6 B, these results were not unique to clone Een217 since the gp160-specific CD4$^+$ T cell line 15.2, which was obtained from a different donor and which recognized epitopes within gp120, also recognized endogenously synthesized gp160 when the protein was synthesized normally (vPE16), but not when it was made as a secreted protein (vPE8).

Two important conclusions can be drawn from these experiments. First, lysis of cells expressing the normal HIV-1 *env* gene by CD4$^+$ CTL was not simply due endocytic uptake and processing the exogenous envelope protein released by other cells. There was no lysis of vPE8-infected B lymphoblastoid cells that released into the supernatant a much larger amount of gp120 than did vPE16- or vPE7-infected cells (Fig. 3). These results argue strongly that endogenously synthesized envelope protein is processed directly for class II–restricted recognition at a single cell level without a requirement for release into the fluid phase and subsequent endocytic uptake and processing. Second, the results indicate that transport of significant amounts of envelope protein into the RER is not sufficient for processing. Rather, it appears that processing of endogenously synthesized gp160 for class II–restricted recognition requires that after synthesis on the RER and during subsequent intracellular trafficking, the protein remain attached to the luminal/extracellular membrane face by a hydrophobic anchor sequence.

Processing of Membrane-anchored Forms of the Envelope Protein. To test this hypothesis, we used two other recombinant vaccinia vectors that directed the synthesis of forms of the envelope protein that remained anchored to the luminal/extracellular membrane face by a hydrophobic anchor sequence. In cells infected with vPE12, gp160 is not cleaved into gp120 and gp41 as a result of deletion of the proteolytic cleavage
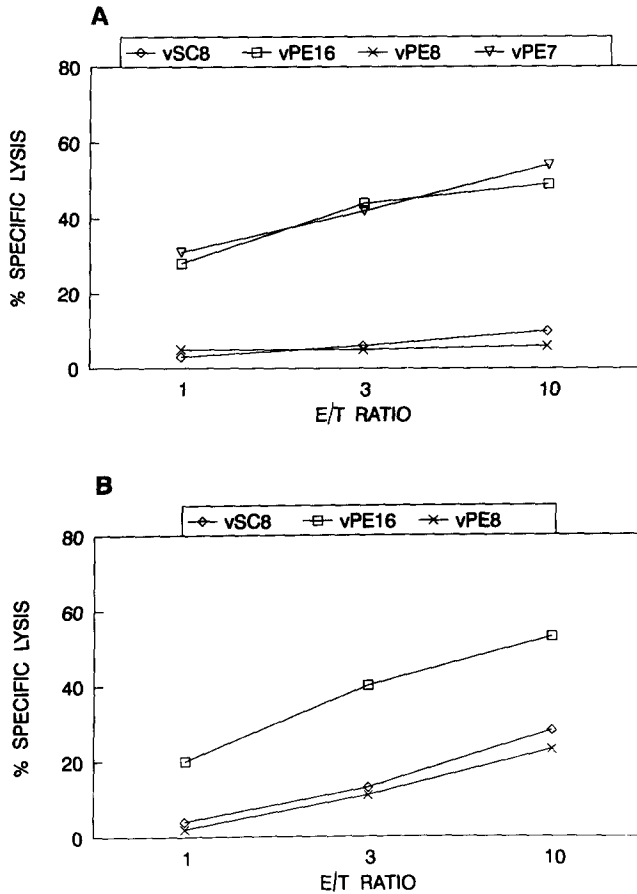
Figure 6. Lysis of target cells expressing anchor–sequence minus envelope protein by CD4^{+} CTL clones and cell lines. (A) Laz 509 cells were infected with the anchor sequence-minus recombinant vaccinia virus vector vPE8 or the positive control vectors vPE7 and vPE16, or the negative control vector vSC8. Lysis by clone Een217 was measured in an 8-h ^{51}Cr-release assay. (B) Autologous EBV-transformed B lymphoblastoid cells were infected with vPE8, vPE16, or vSC8. Lysis by the CD4^{+} CTL line 15.2 was measured in an 8-h ^{51}Cr-release assay.

signal sequence (REKR). Therefore, gp120 cannot dissociate from the transmembrane gp41 protein and remains membrane associated. As shown in Fig. 7 A, vPE12-infected cells were readily lysed, indicating that cleavage of gp160 into gp120 and gp41 was not required for antigen processing. The vSC38 vector, in which the NH2-terminal portion of the *env* gene including the gp120 coding sequence was fused to the membrane anchor and COOH-terminal cytoplasmic domain of the vesicular stomatitis virus glycoprotein G, directs the synthesis of a fusion protein which is expressed on the surface of infected cells as shown by flow cytometry (Chakrabarti, S., and B. Moss, unpublished results). Cells infected with vSC38 were lysed to the same extent as cells infected with the vPE7 vector that expresses the unaltered *env* gene (Fig. 7B). These results demonstrate that it is the presence of a membrane anchor sequence and not the nature of the anchor sequence that allows endogenously synthesized envelope protein to be processed for class II–restricted antigen recognition.

Discussion

In this study, we examined whether endogenously synthesized HIV-1 envelope glycoproteins could be processed for association with class II MHC gene products
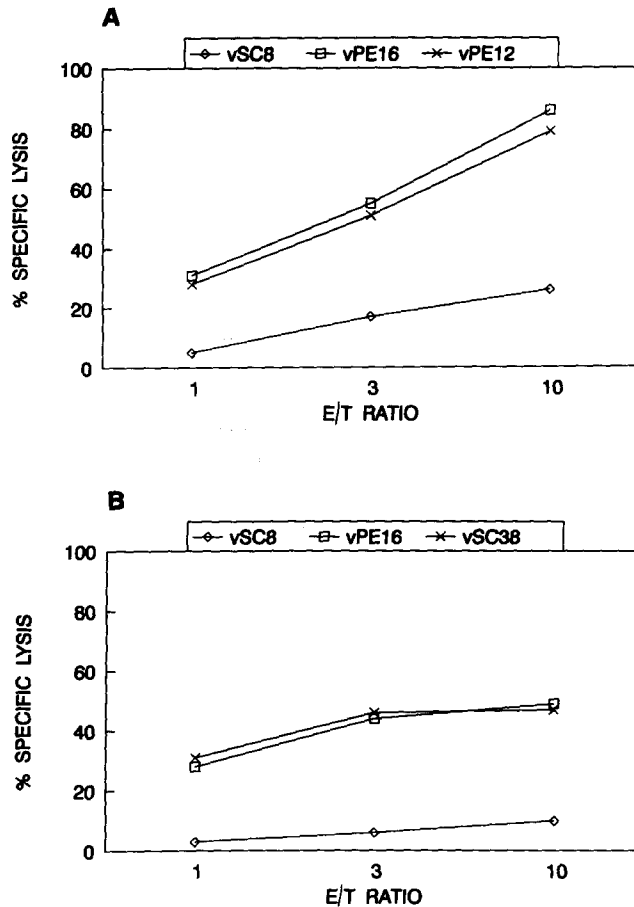
A

B

FIGURE 7. Lysis of target cells expressing membrane-anchored forms of the HIV-1 envelope glycoprotein by the CD4$^+$ CTL clone Een217. (A) Laz 509 cells were infected with the gp120/gp41 cleavage site– minus recombinant vaccinia virus vector vPE12, the positive control vector vPE16, or the negative control vector vSC8. Lysis by clone Een217 was measured in an 8-h ^{51}Cr-release assay. (B) Laz 509 cells were infected with the VSV-G anchored gp120 expression vector vSC38, the positive control vector vPE16, or the negative control vector vSC8. Lysis by clone Een217 was measured in an 8-h ^{51}Cr-release assay.

and subsequent recognition by CD4$^+$ T cells. To detect such a processing event, we used CD4$^+$ T cell clones generated in response to soluble forms of the envelope glycoprotein. These clones recognized exogenous gp160 that had been taken up and processed by APC. To determine whether endogenously synthesized gp160 could also be processed for recognition by these clones, we used recombinant vaccinia virus vectors to express the HIV-1 *env* gene in autologous B lymphoblastoid cells. Our data demonstrate that in this system, endogenously synthesized gp160 is processed for class II–restricted recognition by at least some CD4$^+$, gp160-specific T cell clones. Lysis of cells expressing the *env* gene results from the processing of endogenously synthesized envelope protein rather than processing of exogenous envelope protein taken up by target cells. This was demonstrated by showing that vPE8-infected cells that release large amounts of a soluble form of the envelope protein into the medium were not themselves lysed. Rather, processing requires that the protein remain cell associated. It should be pointed out that B lymphoblastoid cells can take up and process soluble exogenous gp120, but only when the protein is present at a concentration (10^{-7} M), which greatly exceeds the concentration of gp120 secreted by vPE8-infected cells under these conditions. In contrast, activated CD4$^+$ T cells can

take up and process very low amounts of gp120 (23), and these cells are lysed by Een 217 after coculture with vPE8-infected B lymphoblastoid cells (Siliciano, R. F., unpublished results). Thus vPE8-infected cells produce a form of the envelope protein that contains the epitope recognized by Een217, but do not directly process the protein. In contrast, cells infected with vectors expressing membrane-anchored forms of the envelope protein did show processing for class II–restricted recognition. These results indicate anchoring of the protein to the luminal/extracellular membrane face is required for delivery of the gp160 to the appropriate cellular compartments where processing takes place.

Several mechanisms could explain the anchor-sequence dependence of the processing of gp160 for class II–restricted recognition. One possibility is that the processing requires the expression of the gp120/41 complex on the plasma membrane followed by endocytic uptake of the complex and subsequent processing by the normal class II pathway for processing of exogenous antigens. Such a model is consistent with the finding that leupeptin partially inhibits processing of both exogenously added and endogenously synthesized gp160 (data not shown). It is also possible that an anchor sequence is required for the intracellular delivery of gp160 to the compartments where processing and MHC association take place. In this regard, it is interesting to note that Willey and colleagues have recently shown that only a small fraction of the newly synthesized gp160 produced in HIV-1–infected cells is cleaved to gp120 and gp41, and that the uncleaved gp160 is sorted to lysosomes for degradation (31).

It is of interest to consider these findings in light of results obtained with other viral proteins that are processed for class II–restricted recognition. In contrast to the results described above, class II–restricted recognition of the hemagglutinin (HA) of influenza appears to involve uptake from the extracellular fluid of HA present in the virus preparation (7) or released from cells expressing the HA gene (22). The recognition of the influenza neuraminidase by some T cell clones appears to require viral gene expression (21) and may occur by a mechanism similar to that used by HIV-1 gp160. Class II–restricted recognition of the HSV type 1 glycoprotein D expressed in HSV-infected or recombinant vaccinia virus–infected cells has been reported (32). Processing of endogenously synthesized Ig by B lymphoma cells has also been described (33). The anchor-sequence dependence of processing in these systems is as yet unclear. Class II–restricted recognition of endogenously synthesized measles virus matrix and nucleocapsid proteins (34) and hepatitis B surface antigen (35) also occurs, but the mechanism is likely to be different since these viral proteins are not integral membrane proteins.

The results described here are of general interest for three reasons. First, they demonstrate that endogenously synthesized viral envelope proteins can be directly processed at the level of individual cells for class II–restricted as well as class I–restricted recognition. Second, they provide insight into possible mechanisms for the processing of viral envelope proteins for class II–restricted reocognition. Finally, with respect to AIDS vaccine development, our results indicate that CD4$^+$ CTL induced by immunization with purified envelope protein vaccines may be capable of destroying HIV-1–infected cells expressing processed envelope protein. Recent studies indicate that these CTL can lyse HIV-1–infected autologous CD4$^+$ T cell clones

(Siliciano, R. F., unpublished results). Thus, in addition to mediating potentially detrimental effects through the destruction of noninfected, activated CD4$^+$ T cells that have taken up and processed free gp120 (23), such CTL may also serve to limit the spread of HIV-1 infection by lysing infected macrophages and activated T cells.

Summary

Human CD4$^+$ T cell clones and cell lines were shown to lyse recombinant vaccinia virus–infected cells that synthesize the HIV-1 envelope glycoprotein gp160. The processing of endogenously synthesized gp160 for recognition by CD4$^+$ T cells required that the protein, after synthesis on the rough endoplasmic reticulum and during subsequent cellular transport, remain attached to the luminal/extracellular membrane face by a hydrophobic anchor sequence.

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