Induction of a Major Histocompatibility Complex Class I-Restricted Cytotoxic T-Lymphocyte Response to a Highly Conserved Region of Human Immunodeficiency Virus Type 1 (HIV-1) gp120 in Seronegative Humans Immunized with a Candidate HIV-1 Vaccine

R. PAUL JOHNSON,^{1*} SCOTT A. HAMMOND,² ALICJA TROCHA,¹ ROBERT F. SILICIANO,²
AND BRUCE D. WALKER¹

Infectious Disease Unit, Massachusetts General Hospital, and Harvard Medical School, Charlestown, Massachusetts 02129,¹ and Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205²

Received 12 November 1993/Accepted 31 January 1994

Efforts to induce broadly reactive immunity against human immunodeficiency virus type 1 (HIV-1) have been impaired by the extent of sequence variation exhibited by this lentivirus. Cytotoxic T lymphocytes (CTL) specific for other viruses such as influenza virus have been shown to mediate immunity against divergent viral strains, a property that is related to the ability of CTL to recognize processed antigen derived from conserved viral proteins. A recent candidate HIV-1 vaccine regimen has been described in which subjects receive a primary immunization with a recombinant vaccinia virus expressing gp160 and then a booster immunization with recombinant gp160. Volunteers immunized with this regimen have exhibited augmented humoral responses and have also developed CD4⁺ and CD8⁺ CTL specific for gp160. In this report, we have identified the epitopes recognized by CD4⁺ and CD8⁺ CTL obtained from two vaccinees. An immunodominant CD8⁺ CTL response was HLA-A3.1 restricted and recognized a 10-amino-acid epitope (gp120/38-47) in a highly conserved region of gp120. CTL specific for the epitope gp120/38-47 were able to lyse targets sensitized with peptides corresponding to all known natural sequence variants in this region. In addition, other HLA class I-restricted CTL epitopes were identified in relatively conserved regions of gp120 and gp41, and CD4⁺ CTL were shown to recognize two different regions of gp120. Thus, in these two volunteers, immunization with a single strain of HIV-1 induced CD4⁺ and CD8⁺ CTL that are specific for multiple conserved regions of HIV-1 and would be expected to recognize a broad range of viral isolates.

The continued spread of the human immunodeficiency virus type 1 (HIV-1) pandemic has prompted intensive research directed toward the development of an effective vaccine. Although the immune responses that correlate with protection against infection with HIV-1 have not been defined, the capacity to elicit both neutralizing antibodies and cytotoxic T lymphocytes (CTL) will likely be an important characteristic of a successful vaccine (1, 6). CTL mediate protective immunity against many viral infections and play an important role in the host response to HIV-1 infection as well (31, 45). Infected individuals mount a vigorous HIV-1-specific CTL response, which is directed against multiple viral proteins, including the envelope glycoprotein gp160 (30, 32, 34, 40, 43, 54, 56). CTL directed against HIV-1 appear to be able to play a role in the control of HIV-1 infection *in vivo*, as supported by the detection of CTL associated with a decline in viral titer during primary infection (47), a decline in CTL activity associated with the development of AIDS (11, 24), and the ability of CD8⁺ lymphocytes to inhibit viral replication *in vitro* (53, 57).

Efforts to develop a vaccine against HIV-1 have been hindered by the remarkable genomic variability of the virus, particularly in gp160. Sequence variation in gp160 among

HIV-1 isolates obtained from different geographic regions may range as high as 30% (37). Consequently, a candidate vaccine that induces only type-specific immunity may produce little or no protection against challenge with a heterologous strain of HIV-1. Neutralizing antibodies found in HIV-1-infected individuals are often type specific (3, 8), and neutralizing antibodies induced by candidate HIV-1 vaccines derived from the LAI/IIIB strain of HIV-1 have also been predominantly type specific (19). In contrast, gp160-specific CTL obtained from both HIV-1-seropositive subjects and HIV-1-seronegative vaccine recipients have been shown to recognize a number of relatively conserved epitopes (15, 18, 23, 28, 29, 49). Vaccines that induce gp160-specific CTL may therefore offer relatively broad protection against heterologous virus strains.

Recently, a novel prime-boost vaccination approach has been described (13, 19) that is able to induce both CD4⁺ and CD8⁺ HIV-1-specific CTL in seronegative humans (13, 21). Volunteers were immunized with a live recombinant vaccinia virus carrying the HIV-1 envelope gene (*vac-env*) and then boosted with purified recombinant gp160. This regimen resulted in the elicitation of neutralizing antibodies, significant proliferative responses, and CD4⁺ and CD8⁺ CTL specific for gp160 (13, 19, 21). In this report, we demonstrate that these CTL recognize multiple epitopes in relatively conserved regions of gp120 and gp41. In particular, an immunodominant CD8⁺ CTL response is directed against a highly conserved region in gp120.

* Corresponding author. Mailing address: Infectious Disease Unit, Massachusetts General Hospital, 149 13th St., Charlestown, MA 02129. Phone: (617) 726-5772. Fax: (617) 726-5411. Electronic mail address: johnson@helix.mgh.harvard.edu.

MATERIALS AND METHODS

Vaccine trial. Vaccinia virus-naïve HIV-1-seronegative volunteers were immunized with candidate HIV-1 vaccines in a phase I vaccine trial organized by the NIAID AIDS Vaccine Clinical Trials Network. This vaccine regimen involved an initial immunization with a live vac-env vector (HIVAC-le; Oncogen/Bristol-Myers Squibb, Seattle, Wash.), which carried the *env* gene of the LAI isolate of HIV (26). These volunteers were subsequently boosted with 640 µg of recombinant HIV-1 (LAI) gp160 (VaxSyn; MicroGeneSys, Inc., Meriden, Conn.) as described in detail elsewhere (13, 19). Peripheral blood mononuclear cells (PBMC) used for CTL analysis in this report were obtained from two volunteers (A42 and A52).

Cell lines. Epstein-Barr virus-transformed B-lymphoblastoid cell lines (B-LCL) were established and maintained as described previously (54) in RPMI 1640 medium (Sigma, St. Louis, Mo.) containing 10 or 20% (vol/vol) heat-inactivated fetal bovine serum (Sigma). RPMI 1640 medium used for all cell lines was supplemented with L-glutamine (2 mM), penicillin (50 U/ml), streptomycin (50 µg/ml), and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; 10 mM). HLA-typed reference B-LCL were also obtained from the American Society for Histocompatibility and Immunogenetics B cell line repository. Mutant B-LCL consisting of the C1R cell line (HLA-A⁻ B*3503^{low} Cw*401⁺) (59) transfected with HLA-A2.1 and HLA-A3.1 were provided by William E. Bidson (National Institutes of Health).

Isolation of gp160-specific CTL. Isolation and initial characterization of the Env-specific CTL clones has been described in detail previously (21). Briefly, PBMC isolated from two vaccinees were stimulated in vitro with an autologous gp160-transfected B-LCL. Following in vitro stimulation, lymphocytes were cloned at limiting dilution in the presence of phytohemagglutinin and irradiated allogeneic PBMC. These clones were subsequently screened for Env-specific CTL activity in ⁵¹Cr release assays and maintained by periodic restimulation with phytohemagglutinin and feeder cells in the presence of interleukin-2.

Recombinant vaccinia viruses. Preparation of recombinant vaccinia viruses expressing serial truncations of the HIV-1 envelope glycoprotein has been previously described (18). In brief, a series of truncated envelope genes was prepared from the BH8 molecular clone of HIV-1 (42) by digestion at unique restriction enzyme sites, and recombinant vaccinia viruses were generated by using homologous recombination. A recombinant vaccinia virus, vSC8, expressing the *lacZ* gene was used as a control (12).

Synthetic HIV-1 peptides. Synthetic peptides corresponding to the HIV-1 envelope LAI/PV22 sequence (36) were synthesized as previously described (25, 28). All amino acids are numbered as indicated for the HXB2 sequence (37), and references to other peptides and epitopes have been modified accordingly. Additional smaller peptides were synthesized for fine mapping by using a technique similar to that described above (2) or were obtained commercially (Research Genetics, Huntsville, Ala.; Bio-Synthesis, Lewisville, Tex.; Quality Controlled Biochemicals, Hopkinton, Mass.). Lyophilized peptides were reconstituted at 2 mg/ml in sterile distilled water with 10% dimethyl sulfoxide (Sigma) with or without 1 mM dithiothreitol (Sigma).

Cytotoxicity assay. Target cells consisted of B-LCL infected with recombinant vaccinia viruses or preincubated with synthetic HIV-1 peptides. Vaccinia virus-infected targets were prepared by incubating 2.5×10^6 to 5×10^6 B-LCL in log-phase growth with recombinant vaccinia virus at 1 to 2 PFU

per cell for 16 h at 37°C. Cells were then labeled with 100 µCi of Na₂(⁵¹CrO₄) (New England Nuclear, North Billerica, Mass.) for 45 to 60 min and washed three times with RPMI supplemented with 10% fetal calf serum. Peptide-coated targets used for analysis of CD8⁺ CTL were obtained by incubating 2×10^6 to 3×10^6 B-LCL with peptide at 100 µg/ml for 60 min during ⁵¹Cr labeling or by adding peptides at the indicated concentrations to ⁵¹Cr-labeled targets for 50 to 60 min in 96-well plates prior to the addition of effector cells. Peptide-sensitized targets used for analysis of CD4⁺ CTL were prepared by incubating 10^6 B-LCL with 100 µg of peptide per ml overnight prior to the ⁵¹Cr labeling. Cytolytic activity was determined in a standard ⁵¹Cr release assay (54) using U-bottom microtiter plates containing 10^4 targets per well. Plates were incubated in a humidified incubator at 37°C or for 4 to 5 h (CD8⁺ CTL clones) or 5 to 6 h (CD4⁺ CTL clones). All assays were performed in duplicate. Supernatants were then harvested and counted on a Cobra Gamma Counter (Packard Instrument Co., Meriden, Conn.), and percent lysis was determined from the following formula: $100 \times [(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})]$. Maximum release was determined by lysis of targets in detergent (1% Triton X-100; Sigma). Spontaneous release was less than 30% of maximal release for all reported assays.

RESULTS

Vaccine-induced envelope-specific CTL clones are HLA restricted. As described previously, following in vitro stimulation with a gp160-expressing autologous B-LCL, multiple CTL clones were obtained from two seronegative volunteers who had been immunized with the vac-env/gp160 regimen (21). In contrast to previous observations that only CD4⁺ CTL clones were obtained following immunization with purified recombinant gp160 (41, 48), both CD4⁺ and CD8⁺ CTL clones were obtained from volunteers who were immunized with vac-env and then boosted with recombinant gp160. To characterize the HLA molecules responsible for restricting recognition of specific epitopes in gp160, clones were tested for CTL activity against antigen-sensitized allogeneic B-LCL. For all CD8⁺ CTL clones tested, HLA class I restriction elements were readily identified. Five CD8⁺ CTL clones obtained from volunteer A42 (A42.6, A42.9, A42.29, A42.34, and A42.46) were found to be HLA-A3.1 restricted (Fig. 1 and data not shown). An additional CD8⁺ CTL clone, A52.161, obtained from a second volunteer, was also found to be HLA-A3.1 restricted (data not shown). Two other CD8⁺ CTL clones from subject A42 were each restricted by different class I alleles. Clone A42.52 was restricted by HLA-B35, although not all HLA-B35-matched target cells were lysed (Fig. 1), probably because of the existence of several molecular variants of HLA-B35 (7). Similarly, clone A42.53 was HLA-B18 restricted, although variable recognition of HLA-B18-expressing target cells was observed (Fig. 1), most likely attributable to HLA-B18 subtypes. For HLA-A3.1- and HLA-B35-restricted clones, further confirmation of major histocompatibility complex (MHC) restriction was provided by the antigen-specific lysis of cell lines transfected with the relevant restriction elements HLA-A3*01 (Fig. 1) and HLA-B35*01 (23a).

Attempts to define the restricting HLA elements for the CD4⁺ CTL clones revealed reproducible antigen-specific lysis of a small subset of a panel of over 15 allogeneic target cells matched at DR or DQ alleles (data not shown). However, no consistent HLA DR or DQ allele was shared by the lines susceptible to lysis by a given CD4⁺ CTL clone. These data

FIG. 1. CD8⁺ CTL clones are HLA class I restricted. Clone A42.46 is restricted by HLA-A3.1. Similar data were obtained for clones A42.6, A42.9, A42.29, A42.34, and A52.161 (data not shown). Clone A42.52 is HLA-B35 restricted and clone A42.53 is HLA-B18 restricted. Target cells consisted of autologous (Auto) or allogeneic B-LCL matched at indicated HLA alleles, with shared class I alleles as indicated. Mutant B-LCL C1R, which is HLA-A* B*3503^{low} Cw*401⁺ (59), transfected with either HLA-A2.1 or HLA-A3.1, was also used for selected assays. Target cells used for clones A42.46 and A42.53 were incubated with either the appropriate envelope peptide (env/96 and env/129, respectively; see Fig. 4) or a control peptide consisting of an adjacent envelope peptide (env/97 and env/129, respectively). B-LCL used for assays with clone A42.53 were either peptide-sensitized targets (as for clone A42.46) or B-LCL infected with recombinant vaccinia virus containing either the HIV-1 envelope or *lacZ* control. Effector-to-target ratios were 3:1 for clone A42.46, 10:1 for clone A42.52, and 1:1 for clone A42.53. Envelope-specific lysis was calculated for each target tested by subtracting the specific lysis of targets incubated with the control peptide from the specific lysis of envelope peptide-sensitized targets. Lysis of control targets ranged from 1 to 17%. The complete HLA type of subject A42 is A3,11; B18,35; Bw4,6; Cw4,5; DR3,6(13); DQ1,2. The complete HLA type of subject A52 is A1,3; B8,35; Bw6; Cw4; DR1,DR5(DRw12); DQw1, DQw3.

suggest that lysis of infected target cells by the CD4⁺ clones is, in fact, HLA restricted but may be restricted either by an HLA DP allele, a class which is not routinely defined with use of serologic reagents, or by a variant of the DQ or DR alleles.

Envelope-specific CTL clones recognize multiple regions of gp160. CTL recognize processed antigen consisting of peptides 8 to 11 amino acids (aa) in length for class I-restricted epitopes and 13 to 18 aa in length for class II-restricted epitopes (20, 27, 46, 58). Definition of the precise region of a protein recognized by CTL may be accomplished by using overlapping synthetic peptides (51). Mapping of CTL epitopes to conserved or variable regions of gp160 can therefore allow a determination as to whether a CTL response directed at a particular epitope is likely to be largely group specific or type specific. To localize epitopes recognized by these vaccine-induced CTL, we first used a series of recombinant vaccinia viruses expressing serial truncations of gp160 (18). Testing of CD8⁺ CTL against a panel of autologous B-LCL infected with these vectors revealed two different patterns (Fig. 2 and data not shown). The HLA-A3.1-restricted CTL clones A42.6, A42.9, A42.29, A42.34, A42.46, and A52.161 all recognized the smallest construct expressing the NH₂-terminal 204 aa of gp160. In addition, the HLA-B18-restricted clone also recognized this NH₂-terminal 204-aa portion of gp160. The HLA-B35-restricted clone A42.52, in contrast, recognized an epitope located between aa 508 and 635, in the extracellular domain of gp41.

Using the same panel of recombinant vaccinia viruses expressing serial truncations of gp160, we identified two different classes of epitope specificity for the CD4⁺ CTL clones (Fig. 3). A42.5 and A42.45 were both found to recognize an epitope located between aa 287 and 393. The epitope for A42.32 was localized to between aa 204 and 287.

To identify the precise regions recognized by these CTL clones, all clones were tested for the ability to lyse autologous target cells incubated with synthetic peptides spanning the regions identified by the truncated envelope vectors (Fig. 4). The HLA A3.1-restricted CD8⁺ CTL clone A42.46 recognized the 25-aa peptide env/96 (aa 31 to 55), which lies in the NH₂-terminal region of gp160 that immediately follows the signal sequence. All other HLA-A3.1-restricted clones (A42.6, A42.9, A42.29, A42.34, and A52.161) were also found to recognize this peptide (data not shown). Remarkably the CD8⁺ CTL clone A42.53, although restricted by a different HLA class I molecule (HLA-B18), also recognized peptide env/96. The HLA-B35-restricted gp41-specific clone A42.52 recognized env/129 (aa 592 to 616).

The CD4⁺ CTL clones were found to recognize two different Env peptides in gp120 (Fig. 4). Clones A42.5 and A42.45 lysed target cells sensitized with env/114 (aa 337 to 361), and clone A42.32 was specific for peptide env/106 (aa 201 to 225).

Epitopes recognized by both CD4⁺ and CD8⁺ CTL were further characterized by using smaller peptides. Detailed characterization of the HLA-A3.1-restricted CTL response is described below. Initial fine mapping of the fine specificity of the HLA-B35-restricted clone A42.52 demonstrated recognition of the peptide TTAVPWNASWS (aa 605 to 615) at concentrations down to 100 ng/ml (data not shown). Further fine mapping using 9-aa peptides spanning aa 605 to 615 defined the peptide TAVPWNASW (aa 606 to 614) as the optimal epitope for CTL clone A42.52 (Fig. 5). This 9-aa region includes codon 607 in which an alanine-to-threonine substitution is observed in the HIV-1 MN and RF strains, a finding which presumably accounts for the inability of this clone to recognize gp160 derived from these strains (21). The fine specificity of the HLA-B18-restricted clone A42.53 could not

FIG. 2. Epitope mapping of CD8⁺ envelope-specific CTL clones, using recombinant vaccinia viruses expressing serial truncations of the HIV-1 envelope gene. Autologous B-LCL were infected with recombinant vaccinia viruses expressing serial truncations of the HIV-1 envelope or the *lacZ* control. Clones were incubated with target cells at several effector/target ratios, and data are shown for clones A42.46 and A42.52 at an effector/target ratio of 3:1 and for clone A42.53 at an effector/target ratio of 2:1. Envelope-specific lysis was calculated for each target by subtracting the specific lysis of targets infected with the control vaccinia virus from the specific lysis of envelope-expressing targets. Lysis of *lacZ*-expressing targets was less than 2% for all assays. Clone A42.53 was not tested against vaccinia virus constructs containing portions of gp41 (vPE16, vPE17, and vPE18), as only limited quantities of this clone were available and it was previously shown to recognize gp120 (21). nd, not determined.

be determined because of the failure of this clone to recognize overlapping 15-aa peptides and the limited ability to propagate this clone. The epitope specificity of the CD4⁺ clone A42.5 was further localized to the peptide NNTLKQIDSKLREQFG (data not shown).

Recognition of a highly conserved region of gp160 by HLA-A3.1-restricted CTL. The presence of an immunodominant HLA-A3.1-restricted response to a conserved region of gp120, as judged by the isolation from two different donors of several CD8⁺ CTL clones that recognized this epitope, prompted detailed characterization of the fine specificity of these CTL. Clone A42.46 was tested against a series of synthetic peptides spanning aa 31 to 55 (Table 1). Initial mapping of this epitope localized CTL activity to a 9-aa peptide, VYYGVPVWK (aa 38 to 46). The other HLA-A3.1-restricted CTL clones (A42.6, A42.9, A42.29, A42.34, and A52.161) also recognized this nonamer. However, titrations performed with peptide gp120/38-46 revealed a mean sensitizing dose of approximately 50 ng/ml (Fig. 6), a value that is

significantly higher than has been noted for other optimal MHC class I-restricted CTL epitopes (4, 35, 52) or naturally processed peptides (44). On the basis of homology with other identified HLA-A3-restricted CTL epitopes (33, 49) and the recently described HLA-A3.1 binding motif (17), an additional peptide corresponding to the decamer TVYYGVPVWK (aa 37 to 46) was synthesized. Serial dilutions of this decamer produced sensitization of target cells at peptide concentrations as low as to 500 pg/ml (Fig. 6).

Given the extent of sequence variation in gp160, we examined the ability of CTL clones specific for the HLA-A3.1-restricted epitope gp120/37-46 to recognize variant envelope sequences. Analysis of sequenced HIV-1 strains revealed no variation in epitope gp120/38-47 in the majority of virus strains (Table 2). No variation in this region was observed in the HIV-1 consensus B subtype, which represents the North American and European HIV-1 isolates. Two HIV-1 isolates (HIV-1 NDK and HIV-1 TN243) showed a single conservative amino acid substitution. More divergence was noted for the

FIG. 3. Epitope mapping of CD4⁺ envelope-specific CTL clones, using recombinant vaccinia viruses expressing serial truncations of the HIV-1 envelope gene. Envelope-specific lysis was calculated as in Fig. 2. Representative CTL data are shown for clones A42.5 and A42.45 at an effector/target ratio of 10:1 and for clone A42.32 at an effector/target ratio of 3:1. Lysis of *lacZ*-expressing targets ranged from 3 to 11%.

FIG. 4. Epitope mapping of gp160-specific CTL clones. Autologous B-LCL were incubated with overlapping synthetic peptides corresponding to the regions of gp160 identified by experiments using the envelope truncations in Fig. 2 and 3. Targets for CD4⁺ CTL clones were incubated with peptides at 100 μ g/ml for 60 min during ⁵¹Cr labeling. Targets for CD8⁺ CTL clones were incubated with peptides at 100 μ g/ml for 12 to 16 h prior to ⁵¹Cr labeling. Effector-to-target ratios were 10:1 for clones A42.5, A42.45, A42.46, and A42.52, 3:1 for A42.32, and 1:1 for A42.53. Sequences of peptides containing CTL epitopes are as follows: env/96, TEKLWVTVYVGVPVWKEATTTLFCA; env/106, ITQACPKVSFEPIPIHYCAPAGFAI; env/114, KWNNTLKQIDSKLREQFGNNKTIIF; and env/129, LLGIWGCSCGLICTTAVPWNASWSN.

HIV-2 ROD sequence, which exhibited three substitutions in the 10-aa epitope. Analysis of CTL recognition of peptides corresponding to these variant sequences showed equivalent recognition of the HIV-1 NDK strain over a wide range of peptide concentrations and a lesser degree of recognition of the HIV-1 TN243 and HIV-2 ROD sequences (Fig. 7). Comparable data regarding recognition of variant peptides were obtained with clones A42.9 and A42.46 (data not shown). Thus, given the published sequence data and recognition of variant peptides, the HLA-A3.1-restricted CTL response induced by this vaccine appears likely to recognize the vast majority of HIV-1 isolates.

DISCUSSION

Sequence variation in HIV-1 represents a considerable barrier to AIDS vaccine development. Given the extensive variation in gp160, one of the prime target antigens for an HIV-1 vaccine, a major concern has been that immunity induced by immunization with any one viral strain will be largely type specific. In this report, we identify the epitopes recognized by CD4⁺ and CD8⁺ CTL clones derived from two HIV-1-seronegative volunteers who were immunized with vac/env and gp160. CTL induced by this vaccine regimen were heterogeneous, recognizing multiple epitopes and restricted by multiple class I and class II HLA types (Fig. 8). The immunodominant MHC class I-restricted CTL response induced in these vaccinees is directed against a highly conserved region of

FIG. 5. Definition of the optimal epitope for the HLA-B35-restricted CTL clone A42.52. ⁵¹Cr-labeled B-LCL were incubated with peptides at the indicated concentrations for 60 min, and then effector cells were added at an effector/target ratio of 3:1. Sequences of peptides are as follows: GG06-17, ICTTAVPWNASWSNKS(GC) (■); env/129D, TTAVPWNAS (●); env/129E, TAVPWNASW (▲); and env/129F, AVPWNASWS (◆).

TABLE 1. Fine mapping of the epitope specificity of the gp120-specific CTL clone A42.46

Peptide	Sequence	% Specific lysis ^a	
		10:1	3:1
env/96	TEKLWVTVYYGVPVWKEATTTLFCA	66	53
KAO2-1	TEKLWVTVYYGVPVW	4	3
KAO2-2	VTVYYGVPVWKEATT	75	72
KAO2-3	GVPVWKEATTTLFCA	4	2
KAO2-4	KEATTTLFCASDAKA	4	4
env/96A	VYYGVPVWK	67	36
env/96B	YVGVPVWKE	16	8
env/96C	YGVVPVWKEA	2	1
env/96D	TVYYGVPVWK	92	84

^a The HLA-A3.1-restricted CTL clone A42.46 was incubated at the indicated effector/target ratios with autologous B-LCL pulsed with 100 µg of peptide per ml during ⁵¹Cr labeling.

gp120 that is conserved among all sequenced North American isolates. Notably, no CTL were found to recognize the variable regions of gp160, V1 to V5.

In the volunteers analyzed, CD4⁺ and CD8⁺ CTL clones induced by the vac-env/gp160 vaccination protocol were found to recognize a total of five different novel epitopes in gp120 and gp41. In light of the need to induce immunity against a wide number of HIV-1 strains, the breadth of this response is highly encouraging. These results add a new perspective to prior studies of class I-restricted CTL responses in mice in which only one or two dominant epitopes were identified (9) and in which the presence or absence of a particular MHC haplotype determined the ability of animals to mount a CTL response to given virus (5). The diversity of responses induced by this vaccine is similar to that observed by HIV-1-infected humans, in that multiple epitopes were recognized by CTL and that multiple HLA types served as restricting elements (10, 14, 28, 30, 55). In the limited number of HIV-1-seropositive subjects who have been studied in detail so far, up to five different epitopes in a single HIV-1 protein have been shown

to be recognized by CTL from a single individual (reference 30 and unpublished data), and up to 14 different CTL epitopes have been shown to be recognized by an infected person (31). Similar results have been reported for human CTL responses to hepatitis B virus (38). Induction of CTL responses to multiple epitopes presented by diverse HLA molecules is therefore likely to provide a broadly directed cell-mediated immunity effective against heterologous strains of HIV-1.

The five novel CTL epitopes described in this report add to the increasing number of epitopes that have now been defined in gp160 (reviewed in reference 31). Most of the CD8⁺ CTL epitopes that have been previously defined by using CTL clones lie in gp41 (16, 23, 28, 49), with the exception of a Cw4-restricted epitope in gp120 (29). None of the epitopes characterized in this report have been previously described in either HIV-1-infected humans or seronegative vaccine recipients, although we have recently identified an HLA-B35-restricted CTL response to peptide env/96 (aa 31 to 55) in an HIV-1-seropositive subject (57a). It is not clear whether the failure to detect these CTL epitopes in HIV-1-seropositive subjects is due to some difference in the immune response induced by this immunization regimen in HIV-1-seronegative subjects or to our incomplete knowledge of CTL epitopes recognized by HIV-1-seropositive individuals.

The induction of a dominant CTL response to a highly conserved region of gp120 is a particularly desirable feature of a candidate HIV-1 vaccine. No variation in the epitope gp120/38-47 has been reported in the majority of HIV-1 isolates

FIG. 6. Delineation of the optimal epitope for the representative HLA-A3.1-restricted CTL clone A42.29. ⁵¹Cr-labeled B-LCL were incubated with peptides at the indicated concentrations for 60 min, and then effector cells were added at an effector/target ratio of 5:1. Sequences of peptides are as follows: env/96, TEKLWVTVYYGVPVWKEATTTLFCA (■); env/96A, VYYGVPVWK (●); env/96D, TVYYGVPVWK (▲); and env/96J, VYYGVPVWKE (◆). Peptide p24/110 (VHQAISPTLNWVKVVEEKAF) (×), an unrelated HIV-1 Gag peptide, served as a negative control peptide.

TABLE 2. Natural sequence variation in the HLA-A3-restricted epitope gp120/38-47

Virus	Sequence ^a	SD ₅₀ (µg/ml) ^b
HXB2/LAI	TVYYGVPVWK	0.0005
Consensus A	-----	0.0005
Consensus B	-----	0.0005
Consensus C	-----	0.0005
Consensus D	-----	0.0005
HIV-1 NDK	-----I--	0.0005
Consensus E (TN243)	-----R	0.05
HIV-2 ROD	--F----A-R	0.05

^a Sequence data were obtained from Myers et al. (37). Consensus sequences refer to the subtypes of HIV-1 that have been identified to date. North American and European HIV-1 isolates are represented in the consensus B sequence, representing over 27 sequenced isolates, in which no variation is observed in the epitope gp120/38-47. The HIV-1 NDK sequence is the only variant in consensus D sequences in which variation in aa 38 to 47 is observed.

^b SD₅₀ indicates sensitizing dose of peptide required for 50% maximal lysis.

FIG. 7. Recognition of variant envelope sequences by the representative HLA-A3.1-restricted CTL clone A42.29. ^{51}Cr -labeled B-LCL were incubated with peptides at the indicated concentrations for 60 min, and then effector cells were added at effector/target ratio of 10:1. Sequences of peptides are as follows: env/96D, TVYYGVPVWK (■); env/96D NDK, TVYYGVPIWK (●); env/96D, TN243 TVYYGVPVWR (▲); and env/96D/HIV-2ROD, TVEYGVPAWR (◆) (variant positions are underlined). Peptide p24/110, an unrelated HIV-1 Gag peptide, served as a negative control.

sequenced to date (37), and the only HIV-1 and HIV-2 variants reported were also recognized. Similar cross-reactivity between HIV-1 and HIV-2 CTL epitopes in Gag has been previously reported (39). However, since the epitope specificity of CTL is largely determined by the HLA type of the donor, the ability of this vaccination regimen to induce responses to this conserved region of gp120 is likely to vary significantly from one subject to another. Analysis of additional vaccinated subjects will be necessary in order to better define the breadth of specificity of the CTL response induced by this vaccination regimen. Nonetheless, since HLA-A3 is a relatively common

allele (present in approximately 23% of Caucasians and 14% of African-Americans [50]), and in light of the demonstration of HLA-B18- and HLA-B7-restricted CTL responses to this region of gp120, it appears likely that CTL responses to this region may be induced in a significant proportion of vaccinees and in the context of a variety of HLA types. The immunogenicity of this NH_2 -terminal region of gp120 is further demonstrated by the identification of CD8^+ CTL responses to two distinct epitopes in this region (aa 31 to 39 and 42 to 50) in baboons immunized with HIV-1 gp120 formulated in an oil emulsion adjuvant (56a). Other regions that contain overlapping or adjacent class I-restricted CTL epitopes has been reported in Nef (14), Gag (10, 30), and gp41 (16, 28). However, the proximity of the region of gp120 containing HLA-A3, HLA-B18, and HLA-B35-restricted CTL epitopes (aa 31 to 55) to the envelope signal peptide (aa 1 to 30) raises an intriguing possibility regarding the immunogenicity of this region. Association of antigenic peptides with class I HLA molecules normally requires translocation of these peptides into the endoplasmic reticulum by the TAP-1/TAP-2 transporter complex (58). It is possible that the proximity of amino acids in the NH_2 region of gp120 to the signal peptide allows MHC class I processing by an alternative pathway. This possibility is favored by the demonstration that the TAP-1/TAP-2-defective cell line T2, when infected with vac/env, can be lysed by the Env-specific HLA-A3.1-restricted clone A42.46 (22).

The ability of the vac-env/gp160 vaccination regimen to induce an immunodominant class I-restricted CTL response to a highly conserved region of gp120 adds to the existing evidence favoring this regimen as a particularly attractive vaccine candidate. Previous studies have documented that priming of individuals by infection with vac-env followed by boosting with purified recombinant gp160 results in a dramatic proliferative response and heightened humoral response, including the induction of neutralizing antibodies (13, 19). The broadly directed specificity of the cellular immune response induced by this vaccination regimen in these subjects is a

FIG. 8. Summary of epitopes recognized by CD8^+ and CD4^+ CTL clones isolated from vac-env/gp160 recipients. Epitopes recognized by CD4^+ CTL clones are listed in the top half, and those recognized by CD8^+ CTL are shown in the bottom half. The HLA-restricting alleles for the CD8^+ CTL clones are listed in parentheses. The five variable regions of gp120 (V1 to V5) are indicated. sig, signal sequence; TM, transmembrane region of gp41.

particularly desirable feature of a candidate AIDS vaccine and supports the further development and testing of vaccine approaches that are able to induce heterogeneous CTL responses.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-33327, AI-28568, AI-28108, AI-30194, AI 32871, and AI-26503 from the National Institute of Allergy and Infectious Disease and by a Burroughs Wellcome Foundation/Infectious Disease Society of America Young Investigator Award to R.P.J. S.A.H. was supported by NIH training grant T32 AI-07247. The vaccine trial was supported by NIH contract N01-05051. R.P.J. is a Scholar of the American Foundation for AIDS Research.

We thank Mary Lou Clements for organizing the vaccine trial at Johns Hopkins, Pat Earl and Bernard Moss for the donation of recombinant vaccinia viruses, William Biddison for donation of the HMy.CIR.A2 and HMy.CIR.A3 cell lines, and Thomas Buchanan for donation of envelope peptides.

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