Lassa Fever Virus Peptides Predicted by Computational Analysis Induce Epitope-Specific Cytotoxic-T-Lymphocyte Responses in HLA-A2.1 Transgenic Mice

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Received 30 March 2005/Returned for modification 17 May 2005/Accepted 16 June 2005

Lassa fever is a hemorrhagic disease caused by Lassa fever virus (LV). Although the precise host defense mechanism(s) that affords protection against LV is not completely understood, cellular immunity mediated by cytotoxic T lymphocytes (CTLs) plays a pivotal role in controlling viral replication and LV infection. To date, there have been no reports mapping major histocompatibility complex (MHC) class I-binding CTL epitopes for LV. Using computer-assisted algorithms, we identified five HLA-A2.1-binding peptides of LV glycoprotein (GP) and two peptides from LV nucleoprotein (NP). Synthesized peptides were examined for their ability to bind to MHC class I molecules using a flow cytometric assay that measures peptide stabilization of class I. Three of the LV-GP peptides tested (LLGTFTWT, SLYKGVYEL, and YLISIFLHL) stabilized HLA-A2. The LV-NP peptides tested failed to stabilize this HLA-A2. We then investigated the ability of the HLA-A2-binding LV-GP peptides to generate peptide-specific CTLs in HLA-A2.1 transgenic mice. Functional assays used to confirm CTL activation included gamma interferon enzyme-linked immunospot (ELISPOT) assays and intracellular cytokine staining of CD8+ T cells from peptide-primed mice. CTL assays were also performed to verify the cytolytic activity of peptide-pulsed target cells. Each of the LV-GP peptides induced CTL responses in HLA-A2-transgenic mice. MHC class I tetramers prepared using one LV-GP peptide that showed the highest cytolytic index (LLGTFTWT) confirmed that peptide-binding CD8+ T cells were present in pooled lymphocytes harvested from peptide-primed mice. These findings provide direct evidence for the existence of LV-derived GP epitopes that may be useful in the development of protective immunogens for this hemorrhagic virus.

Among the emerging infectious diseases, viral hemorrhagic fever (VHF) represents a serious public health problem with recurrent outbreaks worldwide (16). In addition to infections occurring in areas of VHF endemicity and those imported from such areas to other parts of the world, a new concern has recently emerged due to weaponization of these pathogens for use as bioweapons. Four distinct families of viruses are known to have members that cause VHF, including Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. Among the Arenaviridae family of viruses, Lassa fever virus (LV) is a pathogen that causes high mortality rates in infected individuals. It is known to have been weaponized, and it is thus designated a Category A agent by the Centers for Disease Control and Prevention (9). LV is endemic in rural Africa and has been estimated to cause ~300,000 infections/year and several thousand deaths due to hemorrhagic fever (15). The fatality rate of hospitalized patients is about 17%, but in certain groups of patients, such as pregnant women, there is a >30% mortality rate, and fetal and neonatal deaths approach 88% (7, 20). The principal vector for LV transmission in humans is a highly commensal rodent host. LV infection in rodents is persistent and asymptomatic. However, once infected with this virus, animals exhibit profuse urinary excretion of LV. The virus is transmitted from rodents to humans by contact with animal urine or feces or even blood that contains high virus titers. Transmission between humans has been reported as a result of exposure to blood, sexual contact, and breast-feeding (15). The incubation period of Lassa fever may last up to three weeks. Therefore, the virus may be imported to other regions of the world while infected individuals are asymptomatic or show early nonspecific signs of LV infection. So far, about 20 cases of imported Lassa fever have been reported worldwide (4).

The molecular mechanisms underlying the pathogenicity of LV are not clearly understood. Currently, there are no vaccines available for LV and the only effective therapy available for the treatment of VHF caused by LV is the antiviral drug ribavirin (9). However, ribavirin treatment has been shown to be effective only if it is administered early after infection (14). Although the mechanism of protection against LV is not completely understood, cell-mediated immunity is believed to play a pivotal role in controlling LV infection and replication (7, 28, 29). Neutralizing antibodies specific for LV are of low titers or nonexistent in infected individuals (23). Moreover, treatment of LV-infected individuals using pooled immunoglobulin containing high titers of anti-LV antibodies is not always effective (14). This observation suggests that humoral immunity plays a minor role in controlling infections caused by LV. Fisher-Hoch et al. (7) have demonstrated the critical role of T-cell response in controlling virus replication and prevent-
ing the cascade of events that leads to death. Their data strongly implicate the primary role of cytotoxic T lymphocytes (CTLs) in conferring protection from infection with LV. To date, there have been no reports identifying candidate protective CTL epitopes for this hemorrhagic virus.

In the current study, we identified three HLA-A2.1-restricted CTL epitopes present within the LV glycoprotein (GP). These peptides were initially predicted as candidate epitopes using computer-assisted algorithms. The immunogenic properties of these LV epitopes were then confirmed using HLA-A2.1 transgenic mice together with functional assays to measure specific immune parameters associated with CTL responses.

MATERIALS AND METHODS

**Computational prediction of candidate CTL epitopes for LV.** Two matrix-based prediction algorithms, BIMAS (http://bimas.dct.nih.gov/molbio/hla_bind/) and SYFPEITHI (http://www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm) were used for the prediction of LV peptides binding to HLA-A2.1 (19, 21). The amino acid sequences of LV (Josiah strain) nucleoprotein (NP; Swiss-Prot accession no. P56969; NCBI accession no. NP_694869.1) and glycoprotein (GP; Swiss-Prot accession no. P53314; NCBI accession no. NP_694870.1), each encoded by the S segment of LV RNA, were individually tested using these algorithms. The total NP and GP sequences were parsed into peptides of nine amino acids in length, each overlapping by eight amino acids. The selected peptides were then screened for similarity to the human genome using the National Institutes of Health (NIH) BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/). Peptides showing homology with regard to the human proteome were eliminated from further study.

**Cell line and peptides.** TAP-deficient T2 cells expressing HLA-A2 were obtained from American Type Culture Collection (Manassas, VA) and maintained in Iscove's modified Dulbecco medium (Invitrogen, Grand Island, NY) supplemented with 20% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) at 37°C with 5% CO2. All peptides used in this study were synthesized commercially (Sigma-Genosys, Woodlands, TX) and were of >95% purity. Peptides were dissolved either in water or dimethyl sulfoxide (DMSO) and diluted to desired concentrations in phosphate-buffered saline (PBS).

**Mice.** Six- to eight-week-old C57BL/6J transgenic mice expressing HLA-A2.1 (C57BL/6-Tnp (HLA-A2.1) T [Engel]) were obtained from The Jackson Laboratory (Bar Harbor, ME). The animals were maintained in our animal facility under specific-pathogen-free conditions.

**MHC stabilization assay.** The ability of peptides to bind to HLA-A2.1 molecules was evaluated using a major histocompatibility complex (MHC) class I stabilization assay (18). Briefly, T2 cells were grown overnight at 37°C in Iscove's modified Dulbecco medium supplemented with 20% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) at 37°C with 5% CO2. All peptides used in this study were synthesized commercially (Sigma-Genosys, Woodlands, TX) and were of >95% purity. Peptides were dissolved either in water or dimethyl sulfoxide (DMSO) and diluted to desired concentrations in phosphate-buffered saline (PBS).

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**RESULTS**

**Computational prediction of CTL epitopes.** The total protein sequences of LV-NP or LV-GP were parsed into peptides of nine amino acids in length, each overlapping by eight amino acids, as described in Materials and Methods. The ten highest-ranking peptides for GP and NP analyzed by BIMAS and SYFPEITHI are shown in Table 1.

Based on the ranking scores on both computational predic-
tion methods, five peptides from the GP and two peptides from the NP of LV were selected for further study. GP peptides with a score of >150 in BIMAS and >24 in SYFPEITHI were selected for further analysis. In the case of LV-NP, peptides with BIMAS scores of >50 and SYFPEITHI scores of >24 were selected (Table 1). Based on these selection criteria, five GP peptides and two NP peptides were chosen as candidate CTL epitopes and evaluated in vitro and in vivo. The peptide WLV with a SYFPEITHI score of 20 was not among the ten highest-scoring peptides but was among the top ten ranking peptides using BIMAS. This peptide was the only one tested in further analysis that did not fulfill our criteria for candidate CTL epitopes.

**Evaluation of synthetic peptides as candidate CTL epitopes.**

When evaluated for MHC class I binding, three out of the five selected GP-derived peptides (LLG, YLI, and SLY) stabilized the expression of HLA-A2 on T2 cells, as evidenced by their MFI profiles when compared with the no-peptide control (Fig. 1). An increase in MFI values of >300% was noted with the LLG and SLY peptides. This increase was similar to that observed using the positive peptide GIL. The YLI peptide caused a 175% increase in MFI over the no-peptide control (Table 2). The other two GP peptides, GLV and WLV, induced 60% and 0% increases in MFI, respectively. Thus, based on our selection criterion for the identification of peptides as high binders, nanomers LLG, SLY, and YLI were selected as candidates among the LV-GP peptides for further analysis. Neither of the computationally selected NP-derived LV peptides (CML and GILK) was able to stabilize HLA-A2.1 expression on T2 cells (Fig. 1; Table 2). Thus, these peptides were excluded as candidates for further study.

**In vivo investigation of immunogenicity of LV-GP and -NP peptides.**

In order to gain insight into the functional properties of the high binding peptides, we tested them for their ability to elicit CTL responses in vivo. HLA-A2.1 transgenic mice were immunized with the following selected single peptides: LLG, SLY, YLI, and WLV. Control mice were immunized with the positive control peptide, GIL. Immune responses were assessed using several functional assays, including IFN-γ ELISPOT assay, intracellular IFN-γ staining, CTL assay, and MHC class I tetramer staining.

**IFN-γ ELISPOT assay.** Peptide-specific T cells were enumerated by measuring IFN-γ-producing cells by ELISPOT assay. As shown in Fig. 2, a substantial number of T cells har-

### TABLE 1. Candidate Lassa fever virus CTL epitopes from GP and NP predicted by computational methods

| Epitope          | BIMAS | SYFPEITHI |
|------------------|-------|-----------|
| **LV-GP (Protein ID: NP_694870.1 - 491 AA)** |       |           |
| 1 258 LLGTFTWTLL | 1.035.01 | 1 60 SLYKGVYEL | 32 |
| 2 434 FVFSTSFYLL | 1.014.87 | 2 23 LIALSVLAVL | 28 |
| 3 386 WLVSNGSYLL | 540.47 | 3 42 GLVGLVTLFL | 28 |
| 4 257 RLLGTFTWTLL | 288.13 | 4 258 LLGTFTWTLL | 27 |
| 5 314 RLDFVTAQL | 286.39 | 5 336 LINKAVNAL | 27 |
| 6 42 GLVGLVTLFL | 270.23 | 6 444 SIFLHLV | 26 |
| 7 441 YLISIFLHL | 186.71 | 7 442 LISIFLHLV | 26 |
| 8 60 SLYKGGYEL | 157.23 | 8 18 VMNIVLIAL | 25 |
| 9 442 LISIFLHLV | 142.09 | 9 441 YLISIFLHL | 25 |
| 10 212 CMTSYQYLY | 89.66 | 10 24 IALSVLAVL | 24 |

| Epitope          | BIMAS | SYFPEITHI |
|------------------|-------|-----------|
| **LV-NP (Protein ID: NP_694869.1 - 569 AA)** |       |           |
| 1 257 CMLDGGNML | 234.05 | 1 101 ILAADLEK | 30 |
| 2 497 AVWDQYKDL | 95.41 | 2 277 GILKSILKLY | 28 |
| 3 277 GILKSILKLY | 81.39 | 3 194 DLNDAVQAL | 26 |
| 4 288 ALGMFISDST | 65.13 | 4 69 DLNOAVNVL | 25 |
| 5 101 ILAADLEK | 40.93 | 5 98 DLLILAADL | 24 |
| 6 531 LMDCIMFAD | 32.55 | 6 257 CMLDGGNML | 24 |
| 7 355 SLOQAGFCTA | 28.81 | 7 66 RLRDLNQAV | 23 |
| 8 403 YOPSSGCVY | 26.76 | 8 240 NISGYNFSL | 23 |
| 9 370 LMIRKDAML | 26.23 | 9 274 TMDGILKSI | 23 |
| 10 66 RLRDLNQAV | 21.67 | 10 365 LTTVMLMTL | 23 |

* The peptides in boldface type were selected for further analysis based on the criteria discussed in Materials and Methods.
vested from mice immunized with peptides responded by producing IFN-γ. We observed a dose-dependent relationship between the concentration of peptide used for stimulation and the IFN-γ-producing T cells (Fig. 2). Two of the LV-GP peptides, LLG and SLY, were found to be more effective in generating peptide-specific T cells by virtue of their ability to produce IFN-γ than the other high-binder peptide, YLI. The frequency of IFN-γ-producing cells in the LLG- and SLY-primed splenocyte population was found to be 225 and 235 per one million cells, respectively, when cells were stimulated with 100 μM peptide (Fig. 2).

These results were similar to those observed using the positive control GIL peptide (255 per million cells). The frequency of IFN-γ-producing cells generated in response to YLI (100 μM) was 155/10^6 cells. Similar to results observed with each of the other peptides tested, this response decreased with decreasing peptide concentrations, showing a dose-dependent profile. Interestingly, the nonbinding peptide, WLV, failed to either prime or to stimulate splenocytes from WLV-primed mice for IFN-γ production (Fig. 2).

**Intracellular IFN-γ staining.** Antigen-specific T cells were also enumerated using intracellular cytokine staining (ICCS) to measure IFN-γ. In contrast to the ELISPOT assays, the YLI peptide stimulated the highest level of intracellular IFN-γ production in comparison with the other HLA-A2.1 high-binding LV peptides tested. The frequency of intracellular IFN-γ-containing cells among the LLG-stimulated CD8-positive cells was 0.084% (Fig. 3B). This frequency was similar in cells stimulated with the positive control GIL peptide (0.1%; Fig. 3A). In contrast with results using ELISPOT assays, 0.11% of CD8-positive cells were IFN-γ positive when primed and stimulated

**FIG. 2.** Enumeration of IFN-γ-producing cells by ELISPOT. The IFN-γ ELISPOT assay was performed as described in Materials and Methods. LV-GP-derived peptides tested included LLG, WLV, SLY, and YLI (abbreviations for nanomer peptides are given in the legend of Fig. 1). A peptide (GIL) derived from influenza matrix antigen was used as a positive control. Peptide-specific ELISPOT assays reflect total number of spots minus backgrounds observed in the absence of peptide. The experiment was performed at least three times and the results shown represent the mean IFN-γ ELISPOTs/10^6 ± the standard deviation from a representative experiment.

**TABLE 2. MHC stabilization by selected Lassa fever virus peptides**

| Peptides | Start position | Peptide | BIMAS score | SYFPEITHI score | % Increase in MFI^a |
|----------|----------------|---------|-------------|-----------------|---------------------|
| From LV-GP | 258 | LLG | 1,035.01 | 27 | 325 ± 32.78 |
| 386 | WLV | 540.47 | 20 | 0 |
| 42 | GLVGLVTL | 270.23 | 28 | 60 ± 18.03 |
| 441 | YLI | 186.71 | 25 | 175 ± 5 |
| 60 | SLY | 157.23 | 32 | 305 ± 10 |
| From LV-NP | 257 | CML | 234.05 | 24 | 75 ± 8.89 |
| 277 | GIL | 81.39 | 28 | 0 |
| Control | NA | GIL | NA | NA | 310 ± 8.66 |
| | NA | IAG | NA | NA | 0 |

^a Percent increase in MFI was calculated over the no-peptide control using the following formula: [(MFI of test peptide – MFI of no peptide)/MFI of no peptide] × 100.

^b These peptides were selected for further evaluation. The letters in boldface type indicate the three- or four-letter abbreviations of the peptides used in the text.

^c NA, not applicable.
with SLY peptide (Fig. 3C) and 0.43% of the cells produced IFN-γ/H9253 when stimulated with YLI peptide (Fig. 3D). We speculate that the discrepancy between IFN-γ/H9253 measurements obtained using the ELISPOT and ICCS assays may be attributable to the threshold chosen for flow cytometric measurements, which may have overestimated the percentage of IFN-γ/H9253-positive CD8 T cells observed in response to the YLI peptide.

CTL assay. Pooled splenocytes and lymph node cells from peptide-primed HLA-A2.1 transgenic mice were tested for their cytolytic activity by measuring their ability to serve as effector cells that kill peptide-pulsed T2 target cells expressing the HLA-A2.1 molecule. As shown in Fig. 4, effector cells generated after 5 days of culture with specific peptides killed peptide-pulsed target cells in a dose-dependent manner. Cells stimulated with the LLG peptide exhibited the highest level of specific lysis (30%) at an effector/target cell (E:T) ratio of 40:1. This value is comparable to that of the positive control GIL peptide (37%) at the same E:T ratio. This was followed by the SLY and YLI peptides, showing specific lysis of 17% and 9%, respectively, at the 40:1 E:T ratio (Fig. 4).

Tetramer staining. The frequency of LLGTFTWTL-specific CTLs in peptide-immunized mice was analyzed by tetramer staining using PE-conjugated HLA-A2.1-LLG tetramers. Tetramer staining of pooled spleen and lymph node cells revealed a small but significant population (0.32%) of tetramer-positive cells in the LLG-primed mice, as compared with nonimmunized mice (Fig. 5).

DISCUSSION

Cellular immunity is essential for host defense against pathogenic viral infections such as those caused by LV, although humoral immunity also plays an important role in eliminating these pathogens. Previous studies have indicated a major role for the cellular arm of the immune system in generating protective immune responses to LV. A greater understanding of the nature of protective immune responses is necessary to facilitate the development of future vaccines against this hemorrhagic fever virus (2, 7, 28). To date, there have been no reports showing CTL responses induced by LV-derived peptides. The LV structural protein, GP, has been previously shown to elicit T-cell responses in nonhuman primates (7). The present study was undertaken to identify candidate CTL epitopes from the two structural proteins of LV, namely, GP and NP. Synthetic peptides derived from the GP and NP of LV with known MHC class I-binding motifs predicted by computational algorithms were studied for their ability to up-regulate and stabilize HLA-A2.1 molecules expressed by T2 cells. Peptides with confirmed HLA-A2.1 binding poten-
tial were then used to immunize the HLA-A2.1 transgenic mice in order to investigate their ability to induce LV peptide-specific CTL responses. Using this experimental strategy, we have identified LV HLA-A2.1-binding motifs that may be candidate immunogenic epitopes capable of inducing protective immune responses to this virus.

Experimental strategies that utilize computational methods combined with in vitro/in vivo studies have proven to be very useful in the identification of immunogenic T-cell epitopes from defined antigens (10, 30). Using two matrix-based algorithms, BIMAS and SYFPEITHI, we were able to predict epitopes within the GP and NP structural proteins of LV. Using quantitative flow cytometric parameters as criteria to define candidate epitopes, we selected five peptides from LV-GP and two peptides from LV-NP as predicted MHC class I binders (Table 1). The utility of the BIMAS and SYFPEITHI algorithms in predicting viral and tumor antigen-specific CTL epitopes has been demonstrated by many other groups (10, 12, 27, 30, 31). Previous studies have also validated the use of computational algorithms by mapping CTL epitopes previously identified using conventional methods to explore immunogenic motifs of pathogens (25). Based on our computational prediction data, the following GP-derived peptides were selected for testing of MHC class I binding ability: LLGTFTWTL (LLG), WLVSNGSYL (WLV), GLVGLVTFL (GLV), and YLISIFLHL (YLI), SLYKGVYEL (SLY). We also investigated two selected NP-derived LV peptides: CMLDGGNML (CML) and GILKSIILKV (GILK).

The glycoprotein precursor of LV-GP is posttranslationally cleaved into two proteins, GP1 and GP2. The epitopes SLY and YLI lie on GP1 and GP2, respectively. It is interesting to note that the peptide LLG lies right at the cleavage site that is between L259 and G260 (11). Since the cleavage is a posttranslational modification, some of the precursor proteins produced may escape cleavage and be subjected to proteosomal digestion. Thus, we can’t rule out the possibility of LLG presented as a CTL epitope. It would be interesting to see whether this is the case in human LV infections.

To validate the ability of these predicted class I binders to ligate HLA-A2.1, we employed an MHC class I stabilization assay. Three out of the five selected GP-derived peptides (LLG, YLI, and SLY) stabilized the expression of HLA-A2 molecules expressed on T2 cells, as evidenced by a positive shift in MFI relative to the no-peptide control. T2 cells treated with the other two GP-derived peptides (WLV and GLV) did not show any appreciable increase in MFI when stained with FITC-conjugated anti-HLA-A2-specific monoclonal antibody. Indeed, the peptide WLV caused a negative shift in MFI relative to the no-peptide control. T2 cells treated with the other two GP-derived peptides (WLV and GLV) did not show any appreciable increase in MFI when stained with FITC-conjugated anti-HLA-A2-specific monoclonal antibody. Indeed, the peptide WLV caused a negative shift in MFI (Fig. 1), suggesting destabilization of HLA-A2 expressed by T2 cells. Neither of the computationally selected NP-derived peptides (CML and GILK) stabilized HLA-A2 expression on these cells (Fig. 1; Table 2). Despite the fact that each of these nonstabi-
lizing peptides contained the required anchoring amino acid residues (L, M, or I at the second position; V at the ninth position), they failed to bind HLA-A2 when evaluated using this class I stabilization assay. It has been shown that the success rate of these algorithms in predicting MHC class I binding ranges broadly from 30 to 70% depending on the class I allele and peptide motif under study (6, 24). Other factors that contribute to the success or failure of these prediction algorithms to map MHC class I-binding epitopes include peptide length, spacing between required anchoring residues, and the presence of other amino acids which may act as secondary anchor residues that may play a role in binding (5, 22, 32). Combinations of these factors may also influence the ability of peptides to bind MHC class I molecules. Thus, it is difficult to accurately explain the failure of some of the LV-derived peptides identified computationally as candidate epitopes to stabilize HLA-A2.1 molecules.

Peptide-induced stabilization of MHC class I molecules is directly correlated with their ability to induce CTL response in vivo (24). Based on the MHC class I binding profiles of the LV peptides tested, LLG, YLI, and SLY were selected for in vivo studies. As an LV peptide-negative control, the HLA-A2.1 nonbinding peptide WLV was included in initial ELISPOT studies to measure the ability of peptides to induce IFN-γ-secreting cells. As shown in Results, the high-binding peptide LLG was found to induce stronger T-cell responses than the other LV-GP peptides tested. When we measured intracellular cytokine responses induced by these peptides, however, YLI was found to consistently induce higher levels of IFN-γ than LLG and SLY (Fig. 3). As expected, the HLA-A2.1 nonbinding (WLV) failed to induce T-cell responses as measured by IFN-γ ELISPOT assay.

ELISPOT quantitation of IFN-γ-secreting cells yielded results that correlated well with CTL responses. T cells from LV peptide-primed HLA-A2.1 transgenic mice efficiently lysed corresponding LV peptide-loaded target cells. Among the LV peptides tested for their ability to generate peptide-specific CTLs, LLG induced the highest responses at the 40:1 E:T ratio, followed by SLY and YLI, respectively.

In light of the efficiency of LLG in generating CTL responses, we prepared LLG-HLA-A2.1 tetramers to allow us to perform more quantitative and confirmatory studies to enumerate LLG-specific cells flow cytometrically (1). Tetramer staining of pooled splenocytes and lymph node cells from LLG-primed mice confirmed the presence of a small but significant population (0.32%) of LLG-specific CD8+ T cells.

Given the biosafety issues associated with LV and the paucity of individuals previously exposed to this virus, our knowledge of protective immunity to this hemorrhagic virus is far from complete. Recently it has been shown that cellular immunity is clearly associated with recovery from a natural course of LV infection (28, 29). ter Meulen et al. (28) have examined CD4+ T cells with regard to their LV epitope specificity and identified six T helper epitopes (NP specific) using overlapping epitopes expressed within the NP structural protein. In a more recent study by these investigators (29), overlapping peptide pools as well as single peptides derived from LV-GP were screened to map CD4+ T-cell epitopes. Using T-cell clones generated from peripheral blood mononuclear cells (PBMC) obtained from LV-seropositive individuals, they identified three class II LV epitopes which were capable of inducing proliferative responses of PBMC. These epitopes were later confirmed using the prediction software, ProPred, indicating a good correlation between experimentally observed and computationally predicted HLA-DR-restricted peptides.

Heretofore, no other LV epitope mapping studies have been

![FIG. 5. Enumeration of LV LLG-specific CTLs by tetramer staining. Tetramer-positive antigen-specific T lymphocytes were analyzed in spleen and draining lymph nodes from LLG peptide-immunized and nonimmunized control HLA-A2.1 transgenic mice. Prior to staining, LLG-primed cells were stimulated in vitro overnight with 10 μM of LLG peptide; control cells were cultured in the absence of peptide. Lymphocytes were stained with the LLG/HLA-A2.1-PE-conjugated tetramers (1:800), FITC-conjugated anti-CD3, and PerCP-conjugated anti-CD8 monoclonal antibodies.](http://cvi.asm.org/Downloaded from http://cvi.asm.org/)

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reported other than these two reports aimed at mapping MHC class II-binding motifs. Using nonhuman primates, another study demonstrated protection against live-virus challenge in animals vaccinated with glycoproteins GP1 and GP2 despite minimal LV-specific antibody responses, suggesting a greater role for T cell-mediated immunity (7).

A full understanding of the protective contribution of T-helper and CTL responses to LV together with broader knowledge of the immunogenic viral motifs that effectively induce such responses is not only of theoretical significance, but also of practical significance in designing appropriate vaccination strategies. Our approach to mapping and testing selected human MHC class I allele-specific LV binding motifs has confirmed the existence of three epitopes capable of generating peptide-specific CTL responses. The present study indicates that LV-GP peptides LLG and SLY are highly immunogenic and that YLI is moderately immunogenic in HLA-A2 transgenic mice. This is the first report describing MHC class I-binding CTL epitopes for LV. Future studies using nonhuman primates to examine these candidate immunogenic epitopes should be undertaken to confirm their ability to stimulate LV-specific effector T cells capable of protecting animals from challenge with live virus. Future studies to explore the utility of these mapped LV epitopes as diagnostic tools in monitoring and assessing T-cell immunity to this virus following infection or vaccination will also be useful.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Viera Lima and Igor Toporovsky. We are also grateful to Jeffery Walker for his help with the flow cytometric analyses and Stephen O’Neill of SEPPIC Inc. for providing the adjuvant used in this work.

REFERENCES

1. Altman, J. D., P. A. Moss, P. J.oulder, D. H. Barouch, M. G. McHeyzer-Williams, J. I. Bell, J. A. McMichael, and M. M. Davis. 1996. Phenotypic analysis of antigen-specific T lymphocytes. Science 274:94–96.
2. Baize, S., J. Kaplon, C. Faure, D. Pannetier, M.-C. Georges-Courbot, and V. Deubel. 2004. Lassa virus infection of human dendritic cells and macrophages is productive but fails to activate cells. J. Immunol. 172:2861–2869.
3. Bhasin, M., H. Singh, and G. P. S. Raghava. 2003. MHCBN: a comprehensive database of MHC binding and non-binding peptides. Bioinformatics 19:665–666.
4. Drosten, C., B. M. Kummerer, H. Schmitz, and S. Gunther. 2003. Molecular diagnostics of viral hemorrhagic fevers. Antivir. Res. 57:51–87.
5. Eberl, G., A. Sabbatini, C. Servis, P. Romero, J. L. Maryanski, and G. Corradin. 1993. MHC class I-H-2d-restricted antigenic peptides: additional constraints for the binding motif. Int. Immunol. 5:1489–1492.
6. Feldkamp, M. C., M. P. M. Vierboom, W. M. Kast, and C. J. Melief. 1994. Efficient MHC class I-peptide binding is required but does not ensure MHC class I-restricted immunogenicity. Mol. Immunol. 31:1391–1401.
7. Fischer-Hoch, S. P., L. Hutmager, B. Brown, and J. B. McCormick. 2000. Effective vaccine for Lassa fever. J. Virol. 74:6777–6783.
8. Foster, B., and C. Prussin. 2002. Detection of intracellular cytokines by flow cytometry, p. 6.21.1–6.24.15. In J. Coligan et al. (ed.). Current protocols in immunology. John Wiley & Sons Inc., Hoboken, N.J.
9. Hilleman, M. R. 2002. Overview: cause and prevention in biowarfare and bioterrorism. Vaccine 20:3085–3087.
10. Huang, Y. H., M. H. Tao, C. P. Hu, W. J. Sys, and J. C. Wu. 2004. Identification of novel HLA-A*0201-restricted CD8+ T-cell epitopes on hepatitis delta virus. J. Gen. Virol. 85:3089–3098.
11. Lenz, O., J. ter Meulen, H. Feldmann, H. D. Klenk, and W. Garten. 2000. Identification of a novel consensuses sequence at the cleavage site of the Lassa virus glycoprotein. J. Virol. 74:11419–11421.
12. Lu, J., and E. Celis. 2000. Use of two predictive algorithms of the world wide web for the identification of tumor-reactive T-cell epitopes. Cancer Res. 60:5223–5227.
13. Lycke, N. V., and R. F. Coico. 1996. ELISPOT assay for measurement of antigen-specific and polyclonal antibody responses, p. 7.141–7.147. In J. Coligan et al. (ed.). Current protocols in immunology. John Wiley & Sons Inc., Hoboken, N.J.
14. McCormick, J. B., J. I. King, P. A. Webb, C. L. Scribner, R. B. Craven, K. M. Johnson, L. H. Elliott, and J. R. Monogram-Williams. 1986. Lassa fever. Effective therapy with ribavirin. N. Engl. J. Med. 314:20–26.
15. McCormick, J. B., P. A. Webb, K. M. Krebs, K. M. Johnson, and E. S. Smith. 1987. A prospective study of the epidemiology and ecology of Lassa fever. J. Infect. Dis. 155:437–444.
16. McCormick, J. B., J. I. King, P. A. Webb, K. M. Johnson, R. O’Sullivan, E. S. Smith, S. Trippel, and T. C. Tong. 1987. A case-control study of the clinical diagnosis and course of Lassa fever. J. Infect. Dis. 155:445–455.
17. Feltkamp, M. C., M. P. M. Vierboom, W. M. Kast, and C. J. Melief. 2000. Phenotypic analysis of antigen-specific T lymphocytes. Science 274:94–96.
18. Price, M. E., S. P. Fischer-Hoch, R. B. Craven, and J. B. McCormick. 1988. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. Br. Med. J. 297:548–555.
19. Corradin, G., O. A. Bachor, and S. Stevanovic. 1999. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50:213–219.
20. Ruppert, J., J. Sidney, E. Celis, R. T. Kubo, H. M. Grey, and A. Sette. 1993. Prominent role of secondary anchor residues in peptide binding to HLA-A2 molecules. Cell 74:929–937.
21. Schmitz, H. B. Kohler, T. Laue, C. Drosten, P. J. Veldkamp, S. Gunther, P. Emmerich, H. P. Geisen, K. Fleischer, M. F. Beersma, and A. Hoerauf. 2002. Monitoring of clinical and laboratory data in two cases of imported Lassa fever. Microbes Infect. 4:43–50.
22. Sette, A., A. Vitelli, B. Reherman, P. Fowler, R. Nayersina, M. Kast, C. J. M. Melief, C. Overhoff, L. Yuan, J. Ruppert, J. Sidney, M. D. Guercio, S. Southwood, R. T. Kubo, R. W. Betcht, H. M. Grey, and P. S. Raghava. 1994. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. J. Immunol. 153:586–592.
23. Simmons, G., A. Lee, A. J. Rennecke, X. Fan, P. Bates, and H. Shen. 2004. Identification of murine T-cell epitopes in Ebola virus nucleoprotein. Virology 318:224–230.
24. Singh, R. A., L. W. Ma, A. Barry. 2002. Generation of genome-wide CD8 T cell responses in HLA-A*0201 transgenic mice by an HIV-1 ubiquitin expression library immunization vaccine. J. Immunol. 166:379–391.
25. Sun, Y., M. Song, S. Stevanovic, C. Jankowiak, A. Paschen, H. G. Rammensee, and D. Schadendorf. 2000. Identification of a new HLA-A*(0201)-restricted T-cell epitope from the tyrosine-related protein 2 (TRP2) melanoma antigen. Int. J. Cancer 87:930–940.
26. ter Meulen, J., M. Badusche, K. Kuhn, A. Doebe, J. Satoguitina, T. Marti, C. Loechler, K. Kouleoum, L. Koivogui, H. Schmitz, B. Fleischer, and A. Hoerauf. 2000. Characterization of human CD4+ T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J. Virol. 74:2186–2192.
27. ter Meulen, J., M. Badusche, J. Satoguitina, T. Strecker, O. Lenz, C. Loechler, M. Sako, K. Kouleoum, L. Koivogui, H. Schmitz, B. Fleischer, and A. Hoerauf. 2000. New and Old World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology 321:134–143.
28. Turcanova, V., and P. Hollberg. 2004. Sustained CD8+ T-cell immune response to a novel immunodominant HLA-B*0702-associated epitope derived from an Epstein-Barr virus helicase-prime-associated protein. J. Med. Virol. 72:635–645.
29. Vonderheide, R. H., W. C. Hahn, J. L. Schultz, and L. M. Nadler. 1999. The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. Immunity 10:673–679.
30. Zhou, X., U. M. Abdel Motal, L. Berg, and M. Jondal. 1992. In vivo priming of cytotoxic T lymphocyte responses in relation to in vitro up-regulation of major histocompatibility complex class I molecules by short synthetic peptides. Eur. J. Immunol. 22:3085–3090.