Recognition of Adult T-Cell Leukemia/Lymphoma Cells by CD4⁺ Helper T Lymphocytes Specific for Human T-Cell Leukemia Virus Type I Envelope Protein

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ABSTRACT

Purpose: Human T-cell leukemia virus type I (HTLV-I) can cause an adult T-cell leukemia/lymphoma (ATLL). Because ATLL is a life-threatening lymphoproliferative disorder and is resistant to chemotherapy, the establishment and enhancement of T-cell immunity to HTLV-I through the development of therapeutic vaccines could be of value. Thus, the identification of HTLV-I epitopes for both CD8⁺ and CD4⁺ T cells should facilitate the development of effective vaccines. Although numerous HTLV-I epitopes for CTLs have been identified, few epitopes recognized by CD4⁺ helper T cells against this virus have been described.

Experimental Design: Synthetic peptides prepared from several regions of the HTLV-I envelope (Env) sequence that were predicted to serve as helper T-cell epitopes were prepared with use of computer-based algorithms and tested for their capacity to trigger in vitro helper T-cell responses using lymphocytes from normal volunteers.

Results: The results show that the HTLV-I–Env173–331, and HTLV-I–Env198–210 reactive helper T lymphocytes restricted by HLA-DQw6 and HLA-DR15, respectively, could recognize intact HTLV-I⁺ T-cell lymphoma cells and, as a consequence, secrete lymphokines. In addition, HTLV-I Env184–395 reactive helper T lymphocytes restricted by HLA-DR9 were able to directly kill HTLV-I⁺ lymphoma cells and recognize naturally processed antigen derived from killed HTLV-I⁺ lymphoma cells, which was presented to the helper T cells by autologous antigen-presenting cells.

Conclusions: The present findings hold relevance for the design and optimization of T-cell epitope-based immunotherapy against HTLV-I-induced diseases such as ATLL.

INTRODUCTION

Human T-cell leukemia virus type I (HTLV-I) was the first human retrovirus identified (1). This virus has a tropism for CD4⁺ T lymphocytes and is responsible for inducing an uncontrolled proliferation and transformation, progressing to fatal adult T-cell leukemia/lymphoma (ATLL; ref. 2). HTLV-I infections may also develop into a chronic inflammatory neurologic disorder known as HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP; ref. 2). However, HTLV-I infections are not a cause of significant pathology in the most infected subjects (asymptomatic carriers), possibly because of the role that CD8⁺ CTLs and CD4⁺ helper T lymphocytes play in the control of the infection and disease progression (3–8). HTLV-I–specific T lymphocytes will recognize viral-derived antigens as molecular complexes formed by the association of processed peptide epitopes with MHC molecules, which are expressed on the surface of the infected/transformed CD4⁺ T lymphocytes. As a consequence of recognition of the peptide/MHC complexes by the T-cell receptor for antigen, the T cells will exert their effector function via cytolysis or through the production of lymphokines, which ultimately should help prevent viral/tumor spread.

Although being an asymptomatic virus carrier is usually not a life-threatening situation, aggressive ATLL is difficult to treat and is usually fatal. Thus far, current prophylactic and therapeutic approaches for ATLL are far from optimal. Because of the limitations in the standard treatment of ATLL, much focus has been placed in the development of vaccine-based immunotherapy, but unfortunately, vaccines to induce HTLV-I–specific T-cell responses are not yet available.

It is known that both antibody and CTL responses can play a role in protection from viral infection and in eradicating viral-infected cells (9, 10). An attractive and relatively expedient approach to develop vaccines that are intended to elicit antigen-specific T-cell responses is the use of synthetic peptides representing CTL and helper T lymphocyte epitopes. This strategy has been explored for various viral and malignant diseases (11–14). A large number of T-cell epitopes derived from HTLV-I Tax protein have been identified, and most of these efforts have focused on MHC class I-restricted peptide epitopes to induce CD8⁺ CTL responses to HTLV-I because these cells are considered to be the prime effector cells that will presumably annihilate the virus-infected and -transformed cells (4, 5, 15–17).

Recent experiments by a number of groups have demonstrated that CD4⁺ helper T lymphocytes play an important role in potentiating antiviral and antitumor cellular and humoral protection against HTLV-I–induced diseases such as ATLL.
Immune responses (18, 19). Moreover, in some instances, helper T lymphocytes can exhibit a direct effector function by directly recognizing and killing MHC class II+ virally infected or tumor cells that present helper T lymphocyte epitopes on their surface (20–22). Because helper T lymphocytes play an important role both in the induction and maintenance of CTL responses, vaccines that activate both CTLs and helper T lymphocytes should be more effective than vaccines that only target CTL responses. However, there is limited information available regarding MHC class II-restricted T-cell responses against HTLV-I and the existence of the corresponding helper T lymphocyte peptide epitopes.

In the present study, we report the identification of MHC class II epitopes from the HTLV-I envelope (Env) glycoprotein that are capable of stimulating CD4+ T-cell responses from HTLV-I–naive individuals. These peptides, Env196–210, Env317–331, and Env364–378 induced HTLV-I–specific responses restricted by the HLA-DR9, HLA-DQw6, and HLA-DR15 alleles, respectively. Most importantly, some of the peptide-generated helper T lymphocytes were also capable of directly recognizing HTLV-I–infected, MHC class II+ T-cell lymphomas and naturally processed antigen in the form of cell lysates or apoptotic cells prepared from HTLV-I+ T cell lymphomas, which were indirectly presented by autologous antigen presenting cells (APCs). The present findings should hold some value for the development of epitope-based vaccines designed to elicit CTL and helper T lymphocyte responses for the treatment/prevention of ATLL and other diseases mediated by HTLV-I.

**MATERIALS AND METHODS**

**Cell Lines.** EBV-transformed lymphoblastoid cells (EBV-LCLs) were produced from peripheral blood mononuclear cells (PBMCs) of HLA-typed volunteers using culture supernatant from the EBV-producing B95–8 cell line (American Type Culture Collection, Manassas, VA). Mouse fibroblast cell lines (L cells) transfected and expressing individual human MHC class II molecules were kindly provided by Dr. Robert W. Karr (Pfizer Global R&D, New London CT) and by Dr. Takehiko Sasazuki (Tokyo, Japan). The HTLV-I–transformed T-cell lymphoma cell lines, TL-Su, TCL-Kan, MAT, and HUT102, were supplied by the Cell Resource Center for Biomedical Research Institute of Development, Aging, and Cancer (Tohoku University, Sendai, Japan). The Jurkat T-cell lymphoma cell line (HTLV-I negative) was purchased from American Type Culture Collection. MT2 is a HTLV-I–transformed T-cell line that was kindly provided by Dr. Yorio Hinuma (Kyoto, Japan; ref. 23).

**Synthetic Peptides.** Potential HLA-DR–restricted CD4+ T-cell epitopes were selected from the amino acid sequence of the HTLV-I–Env protein using the algorithm tables for three HLA-DR alleles (DRB1*0101, DRB1*0401, and DRB1*0701) described by Southwood et al. (24). The predicted peptide epitopes were synthesized by solid phase organic chemistry and purified by high-performance liquid chromatography. The purity (>80%) and identity of peptides were assessed by high-performance liquid chromatography and mass spectrometry, respectively.

**In vitro Induction of Antigen-Specific Helper T Lymphocytes Lines with Synthetic Peptides.** The procedure selected for the generation of HTLV-I–Env-reactive helper T lymphocyte lines using peptide-stimulated PBMCs has been described in detail (25, 26). Briefly, dendritic cells were produced in tissue culture from purified CD14+ monocytes (using antibody-coated magnetic microbeads from Miltenyi Biotech, Auburn CA) that were cultured for 7 days at 37°C in a humidified CO2 (5%) incubator in the presence of 50 ng/mL granulocyte macrophage colony-stimulating factor and 1000 IU/mL interleukin 4. Peptide-pulsed dendritic cells (3 µg/mL for 2 hours at room temperature) were irradiated (4200 rad) and cocultured with autologous-purified CD4+ T cells (Miltenyi Biotech) in 96 round-bottomed well culture plates. One week later, the CD4+ T cells were restimulated with peptide-pulsed irradiated autologous PBMCs, and 2 days later, human recombinant interleukin 2 was added at a final concentration of 10 IU/mL. One week later, the T cells were tested for antigen reactivity using a cytokine release assay as described below. Those cultures exhibiting a significant response of cytokine release to peptide (at least 2.5-fold over background) were expanded in 24- or 48-well plates by weekly restimulation with peptides and irradiated autologous PBMCs. Complete culture medium for all procedures consisted of AIM-V medium (Life Technologies, Inc., Rockville, MD) supplemented with 3% human male antibody serum. All blood samples were obtained after the appropriate informed consent.

**Measurement of Antigen-Specific Responses with Helper T Lymphocytes.** CD4+ T cells (3 x 10^5/well) were mixed with irradiated APCs in the presence of various concentrations of antigen (peptides, tumor lysates, and apoptotic cells) in 96-well culture plates. APCs consisted of either autologous PBMCs (1 x 10^5/well), HLA-DR–expressing L cells (3 x 10^5/well), MHC-typed EBV-LCLs (3 x 10^5/well), T-cell lymphoma cell lines (3 x 10^5/well), or autologous dendritic cells (5 x 10^5/well). Tumor cell lysates were prepared by three freeze-thaw cycles of 1 x 10^5 tumor cells and resuspended in 1 mL of serum-free RPMI 1640. Lysates were used as a source of antigen at 5 x 10^5 cell equivalents per mL. Culture supernatants were collected after 48 hours for measuring antigen-induced lymphokine (granulocyte macrophage colony-stimulating factor or IFN-γ) production by the helper T cells using commercially available ELISA kits (PharMingen, San Diego, CA). To demonstrate antigen specificity and MHC restriction, blocking of the antigen-induced proliferative response was assessed by adding anti-HLA-DR monoclonal antibody L243 (IgG2a, prepared from supernatants of the hybridoma HB-55 obtained from the American Type Culture Collection), anti-HLA-DQ monoclonal antibody SPV-L3 (IgG2a; Beckman Coulter, Inc., Fullerton, CA) or anti-HLA-A, anti-HLA-B, and anti-HLA-C monoclonal antibody W6/32 (IgG2a: American Type Culture Collection). All antibodies were used at a final concentration of 10 µg/mL throughout the 48-hour incubation period. All assessments of ELISA were carried out at least in triplicate, and results correspond to the mean values with the SD of the mean.

**Cell-Mediated Cytotoxicity Assays.** Cytotoxic activity of CD4+ T cells was determined in a colorimetric CytoTox 96 assay (Promega, Madison WI) for measuring the release of lactate dehydrogenase (LDH) from target cells. Targets were
prepared by incubating T-cell lymphoma cells or EBV-LCLs with or without 10 μg/mL peptides and tumor cell lysates at 37°C overnight. T Cells were mixed with 2 × 10⁴ targets at different E:T ratios in 96 round-bottomed well plates. After 6 to 9 hours of incubation at 37°C, 50 μL of supernatant were collected from each well to measure LDH content. To correct for spontaneous LDH release from effector cells, LDH levels were measured for each individual effector cell concentration used in the experimental set up (effector spontaneous). All measured values were assayed in triplicate and corrected for the culture medium LDH background. The percentage of specific LDH release was determined as percentage of cytotoxicity = [(experimental − effector spontaneous − target spontaneous)/(target maximum − target spontaneous)] × 100.

RESULTS

Selection of Potential Helper T-Cell Epitopes for HTLV-I–Env Glycoprotein. As stated above, the main goal of the present studies was to identify and select MHC class II CD4⁺ T-cell epitopes that could be used to facilitate in the development of a T-cell–based vaccine against HTLV-I. Thus, we first examined the amino acid sequence of HTLV-I Env for the presence of short peptides (15 mer) containing predicted binding motifs for HLA-DRB1*0101, DRB1*0401, and DRB1*0701, using the algorithms described by Southwood et al. (24). This strategy has been successful in our hands, allowing the identification several helper T-cell epitopes for diverse tumor associated antigens such as HER2/neu, gp100, MAGE-A3, PSMA, EBNA2, and CEA (20, 21, 25–28). We have previously observed that when peptide sequences that score high to all three MHC class II alleles are selected, some of the T-cell responses induced by these peptides can be restricted by several MHC class II alleles, which include others than HLA-DR1, HLA-DR4, and HLA-DR7 such as HLA-DR9, HLA-DR16, HLA-DR52, HLA-DR53, HLA-DQ2, and HLA-DQ6. For the present study, only one peptide sequence, HTLV-I–Env196–210 (AVWLVSALAMGAGVA) from the entire HTLV-I–Env protein (containing a total 488 residues) was predicted as a probable binder to HLA-DR1, HLA-DR4, and HLA-DR7, thus being a potential promiscuous MHC class II-restricted T-cell epitope. In addition to peptide Env₃₁₇₋₃₃₁, which is positioned in the gp21 portion of HTLV-I Env, we decided to also include for further study peptides HTLV-I–Env₁₉₆₋₂₁₀ (LDHILEPSIPWKSKL) and HTLV-I–Env₃₈₄₋₃₉₈ (LLFWEQGLCKALQE), which lie within HTLV-I–Env gp46 and gp21, respectively. Although these epitopes did not score as promising epitopes in the Southwood algorithms (24), they were previously reported to function as T-cell epitopes restricted by HLA-DR9 (Env₁₉₆₋₂₁₀) and HLA-DR15 (Env₃₈₄₋₃₉₈). However, the data presented in these reports did not demonstrate that the peptide-reactive T cells were capable of directly recognizing antigen-derived from HTLV-I–transformed cells, failing final validation that these peptides truly represent naturally processed T-cell epitopes (29).

Induction of T-Cell Responses to Peptide Epitopes from HTLV-I–Env Protein. Peptides representing the three sequences derived from HTLV-I Env described above were synthesized and tested for their ability to stimulate T-cell responses using PBMCs obtained from four MHC class II-typed, healthy individuals (HLA-DR1/15, HLA-DR4/15, HLA-DR4/9, and HLA-DR8/9). Purified CD4⁺ T cells were stimulated in primary cultures using peptide-pulsed autologous dendritic cells as APCs as described in Materials and Methods. After three to four cycles of peptide restimulation using autologous irradiated PBMCs as APCs, the lymphocyte cultures were tested for their capacity to respond to the peptide presented by autologous PBMCs as APCs using cytokine release assays. Those cultures that exhibited at least 2.5-fold increase of cytokine release to peptide over background were selected and expanded for additional analysis. Interestingly, peptide-reactive helper T lymphocyte lines were obtained from all four individuals, and after expansion, these were analyzed for their antigen specificity and MHC restriction pattern. In the case of the DR4/DR9 individual, the CD4⁺ T-cell line that was successfully isolated responded to peptide HTLV-I–Env₁₉₆₋₂₁₀ when either autologous PBMCs or dendritic cells were used as APCs (Fig. 1A). Furthermore, these responses were inhibited to a great extent by anti-HLA-DR antibodies but not by anti-MHC class I antibodies (Fig. 1A). These T cells did not respond significantly to an irrelevant control peptide (data not shown). When mouse fibroblasts (L cells) transfected with HLA-DR4, HLA-DR9, or HLA-DR53 were used as APCs, it became evident that the helper T lymphocytes from this individual recognized peptide HTLV-I–Env₁₉₆₋₂₁₀ in the context of HLA-DR9 (Fig. 1B). To evaluate the overall avidity (avidity) of the HLA-DR9–restricted helper T lymphocytes for its ligand, peptide titration curves were performed using various types of autologous APCs (dendritic cells, PBMCs, CD14⁺ monocytes, and EBV-LCLs). These results show that the helper T lymphocyte line displayed high avidity for peptide HTLV-I–Env₁₉₆₋₂₁₀ because <0.1 μg/mL peptide was required to attain 50% of maximal response, regardless of the type of APCs used in these assays (Fig. 1C).

Two separate T-cell lines reactive with peptide HTLV-I–Env₃₁₇₋₃₃₁, the one predicted to serve as a promiscuous T-cell epitope by the Southwood algorithm (24), were isolated from two individuals whose MHC class II alleles were HLA-DR1/15, HLA-DQ5/6 and HLA-DR8/9, HLA-DQ6/9, respectively. Studies performed to determine the HLA restriction elements for these T cells indicated that both responses to peptide HTLV-I–Env₃₁₇₋₃₃₁ were restricted by HLA-DQw6 (Fig. 2, A and B). In both cases, the T cells responded to peptide presented by autologous PBMCs in a dose-dependent manner, and the avidity of T-cell responses derived from the DQ6/9 donor appeared to be slightly higher than the response derived from the DQ5/6 donor, as determined by the amount of peptide required to achieve 50% maximal cytokine release (Fig. 2, C and D).

Peptide HTLV-I–Env₃₈₄₋₃₉₈ was able to stimulate a T-cell response in the HLA-DR4/15 individual, which was restricted by HLA-DR15 (Fig. 3A). In addition, as shown in Fig. 3B, the dose-response curve to peptide HTLV-I–Env₃₈₄₋₃₉₈ indicated that the affinity of this cell for antigen seemed to be high, requiring ~0.1 μg/mL peptide to achieve 50% of the maximal response.

Recognition of Processed HTLV-I Viral Antigen by Peptide-Responsive Helper T Lymphocyte. One of the most critical attributes that peptide-induced anti-HTLV-I CD4⁺ T cells must exhibit to assess their therapeutic potential is their capacity to recognize the naturally processed viral antigen,
which would be expressed by the HTLV-I–infected or transformed T-cell lymphoma cells. Thus, we proceeded to determine whether HTLV-I–transformed T-cell lines, which naturally process HTLV-I Env, would be capable of stimulating the peptide-reactive helper T lymphocytes. As shown in Fig. 4A, the HLA-DR9–restricted HTLV-I-Env[196–210]–specific CD4+ T-cell line was very effective in directly recognizing DR9/HLA-DR9 HTLV-I–infected T-cell lymphoma cell lines (Kan and Su) but did not react with the DR9-negative HTLV-I–infected T-cell lymphoma cell line MT2 or autologous lymphoblastoid B cells that do not express HTLV-I viral antigens. Moreover, the recognition of HTLV-I+ T-cell lymphoma cells by the helper T lymphocyte was inhibited by anti-HLA-DR antibodies (Fig. 4B), confirming that this interaction requires the presentation of peptide by MHC class II molecules.

We also wished to evaluate whether APCs would be able to take up viral antigens derived from dead HTLV-I+ tumor cells and process these antigens appropriately to stimulate helper T lymphocytes (indirect presentation). Thus, we proceeded to determine whether the DR9-restricted HTLV-I–Env[196–210]–reactive helper T lymphocytes were capable of recognizing the naturally processed viral antigen using various type of autologous APCs (dendritic cells, CD14+ monocytes, and EBV-LCLs), which were fed with freeze/thaw cell lysates or apoptotic/dead cells prepared from HTLV-I+ tumors. The data presented in Fig. 5 demonstrate that the DR9-restricted HTLV-I–Env[196–210]–specific T-cell line was able to respond in a dose-dependent manner to APCs presenting cell lysates (Fig. 5A) or apoptotic/dead tumor cells (Fig. 5C) from the HTLV-I+ T-cell lymphoma MT2 but not with APCs presenting a lysate or apoptotic/dead cell from the HTLV-I–negative Jurkat T-cell lymphoma. In addition, these results also indicate that dendritic cells and EBV-LCLs were the most potent types of APCs and that were able to capture and process the cell lysates but not the apoptotic/dead tumor cells (Fig. 5A and C). The capacity of the helper T lymphocytes to recognize naturally processed HTLV-I Env antigen in the form of tumor lysates (MAT, Kan, Su, and MT2) or apoptotic/dead tumor cells (MT2) presented by dendritic cells was effectively blocked by anti-HLA-DR antibodies but not by anti-HLA class I antibodies (Fig. 5B and D), confirming that the epitopes are presented via MHC class II molecules. In summary, the overall results indicate that the T-cell epitope represented by peptide HTLV-I–Env[196–210] can be effectively recognized by helper T lymphocytes, indirectly by APCs that process cell lysates or apoptotic/dead cells or directly by MHC class II+ HTLV-I–transformed T-cell lymphoma cells.

Next, we proceeded to determine whether the two DQw6-restricted helper T lymphocyte lines reactive with peptide
HTLV-I–Env317–331 could also recognize naturally processed antigen. Using a similar set of experiments as described previously, both the DQw6-restricted helper T lymphocytes derived from DQ5/6 or DQ6/9 donors were not effective in recognizing cell lysates or apoptotic cells prepared from HTLV-I/H11001 T-cell lymphoma cells, which were presented by autologous dendritic cells (data not shown). On the other hand, both helper T lymphocytes were able to recognize antigen directly on DQw6/H11001 HTLV-I–infected T-cell lymphoma cells (HUT102 or Su). Moreover, these helper T lymphocytes were not effective in responding to the DQw6-negative, HTLV-I/H11001 T-cell lymphoma (Kan), the HTLV-I–negative Jurkat T-cell lymphoma, or DQw6+ autologous EBV-LCLs, which is not infected with HTLV-I (Fig. 6, A and B). Most importantly, the recognition of HTLV-I–T-cell lymphomas by these helper T lymphocytes was inhibited by anti-HLA-DQ antibodies (Fig. 6), indicating that the T-cell epitopes are also expressed on MHC class II molecules on HTLV-I–infected T-cell lymphoma cell lines.

In similar experiments, the HLA-DR15-restricted, specific for peptide HTLV-I-Env384–398 helper T lymphocytes, showed the capacity to produce lymphokine when directly stimulated with the HTLV-I+, HLA-DR15+ T-cell lymphoma cell (Su) but not with the Jurkat (HTLV-I negative) or the Kan (HLA-DR15 negative) T-cell lymphomas. The response of this helper T lymphocyte line to the Su T-cell lymphoma was inhibited by anti-HLA-DR antibodies but not by anti-DQ antibodies (Fig. 7). Because this helper T lymphocyte line ceased to continuously grow in tissue culture, we were not able to assess whether autologous dendritic cells fed with tumor lysates or apoptotic cells would be able to function as APCs. Nevertheless, these results indicate that the HTLV-I–Env384–398 peptide epitope can be presented to helper T lymphocytes in the context of HLA-DR15 directly on the HTLV-I+ T-cell lymphoma cells.

Cytotoxic Activity of HTLV-I–Env-specific CD4+ T Cells. It has been reported that MHC class II-restricted T lymphocytes can behave as effector cells being capable of killing their APCs, including viral-infected and tumor cells. First, the cytotoxic activity of the DR9-restricted HTLV-I–Env196–210-specific helper T lymphocytes was evaluated against various target cells. As shown in Fig. 8A, this helper T lympho-
cyte was very efficient in killing the DR9–/H11001 HTLV-I–/H11001 T-cell lymphoma cells (Su and Kan) but was unable to kill the DR9–negative HTLV-I–/H11001 MT2 T-cell lymphoma and autologous EBV-LCLs. In addition, the HTLV-I–Env196–210-reactive T-cell line was not only able to kill the autologous EBV-LCLs pulsed with synthetic peptide, but also the autologous EBV-LCLs that were pulsed with the cell lysate from HTLV-I–/H11001 T-cell leukemia MT2 but not the EBV-LCLs pulsed with Jurkat lymphoma (HTLV-I negative) lysates (Fig. 8B). These results demonstrate that lysis by this helper T lymphocyte is MHC restricted and requires the presence of the specific MHC peptide complexes on the target cells. With respect to the other HTLV-I–Env peptide-reactive helper T lymphocyte lines, although low levels of cytotoxicity were observed, these did not appear to be statistically significant (data not shown).

**DISCUSSION**

In the past, most of the research pertaining to T-cell immunity to HTLV-I has focused on CD8+ HLA class I-restricted CTL responses, and little attention has been given to the study of role of CD4+ HLA class II-restricted T cells in the immune...
resistance to this virus. Although antiviral CD8\(^+\) CTLs are probably the most effective immune elements for eradicating the virus-infected or virus-transformed cells, the importance of CD4\(^+\) T-cell responses to HTLV-I should not be underestimated. For example, antiviral antigen-specific CD4\(^+\) T cells are likely to play a role both in the induction and maintenance of CTLs and in humoral immune responses in the production of IgG antibodies against this virus. Because the HTLV-I Env glycoprotein is a candidate to target for vaccination to induce both CD8\(^+\) CTLs and antibodies, it would be useful to identify the immunodominant helper T lymphocyte epitopes.

Our original goal was to identify epitopes of HTLV-I that elicit strong MHC class II-restricted CD4\(^+\) T-cell responses, which could be used as components of immunotherapeutic vaccines against ATLL. Here, we have described three potent MHC class II-binding CD4\(^+\) helper T lymphocyte epitopes represented by sequences HTLV-I–Env\(_{196-210}\), HTLV-I–Env\(_{317-331}\), and HTLV-I–Env\(_{384-398}\). To our knowledge, this is the first description of peptide epitopes capable of stimulating MHC class II-restricted helper T lymphocyte responses that represent true T-cell epitopes because these perform as naturally processed antigenic peptides. One of the most important functions of CD4\(^+\) helper T lymphocytes is related to their helper activity for B cells, which is necessary for IgG class switching and antibody affinity maturation. Interestingly, two of the described peptide sequences described here (HTLV-I–Env\(_{196-210}\) and HTLV-I–Env\(_{384-398}\)) overlap or lie proximal to previously described humoral B-cell epitopes for the HTLV-I envelope protein (9, 30–32). It has been reported that peptide HTLV-I–Env\(_{190-209}\) frequently binds antibodies in samples from HTLV-I\(^+\) individuals (9). Thus, these regions of HTLV-I Env glycoprotein seem to be highly immunogenic and should be considered as important targets for inducing both for T-cell and the B-cell immune responses (T-B vaccine). Thus, the two newly identified HTLV-I–Env helper T lymphocyte peptide epitopes containing both T-helper epitopes and humoral B-cell epitopes of HTLV-I–Env\(_{196-210}\) and HTLV-I–Env\(_{384-398}\).
epitope should offer significant therapeutic benefits for the development of immunotherapy against HTLV-I–mediated lymphoproliferative disorders.

In addition to the two newly described helper T lymphocyte epitopes, we present data demonstrating that peptide HTLV-I–Env196–210 was able to induce helper T lymphocyte responses to naturally processed epitope, which were restricted by HLA-DR9. The same peptide was previously described to function as a cytotoxic helper T lymphocyte epitope restricted by the HLA-DQ5 and HLA-DR16 (33). In other studies, it was reported that peptides HTLV-I–Env382–403 and HTLV-I–Env381–395 could elicit T-cell responses restricted by HLA-DR1 and HLA-DR15, respectively (29, 34). Thus, peptides represented by sequences HTLV-I–Env196–210 and HTLV-I–Env384–398 should be categorized as promiscuous MHC class II epitopes because they are capable of being presented to CD4+ T cells by multiple HLA-DR (and HLA-DQ) alleles and therefore are attractive candidates for vaccine development because they would offer expanded population coverage. Additional studies are warranted to determine whether these peptides can elicit helper T lymphocyte responses to additional MHC class II alleles.

An interesting finding that deserves additional consideration was the observation that some of the peptide-induced helper T lymphocyte lines were capable of recognizing antigen directly on HTLV-I+ lymphoma cells but not indirectly on APCs that were fed with dead tumor cells. It is generally thought that MHC class II-restricted responses are directed against exogenous antigens that are captured by APCs via phagocytosis/endocytosis and are processed in the endosomal compartments. However, it should be noted that the majority of the peptides (>85%) that are isolated from cell surface MHC class II molecules are derived from endogenous cell components, which predominantly are membrane proteins (35). Thus, it should not be all that surprising that the HTLV-I+ lymphoma cells are capable of effectively producing helper T lymphocyte epitopes from the endogenous envelope membrane protein. The inability of APCs to present some of the HTLV-I Env epitopes to helper T lymphocytes via the exogenous pathway could be due to the presence of the large number of other proteins/peptides present in the tumor lysates/apoptotic cells, which would compete for binding to MHC class II. This type of competition with exog-
CD4+ helper T lymphocytes can exhibit high levels of cytotoxicity via either the perforin or Fas/Fas ligand pathway against CD4+ inhibiting viral/tumor spread. It has been widely reported that production but also as potent effectors capable of directly in- eradicating HTLV-I/H11001 cells or HTLV-I/H11001 epitopes, it is clear that direct recognition of HTLV-I–infected T cells and other professional APCs (e.g., dendritic cells) may differ. If this was the case, some helper T lymphocyte epitopes could prove to be effective against tumors or viral infections affecting MHC class II-expressing tissues. Nevertheless, vaccination strategies involving both helper T lymphocyte and CTL epitopes would most likely offer better results than vaccines that focus in only one of the two cell types. Interestingly, three potent CD8+ CTL epitopes restricted by HLA-A2 (one of the most common MHC class I alleles) represented by peptides Env175–183, Env182–190, and Env395–403, lie closely to the Env196–210 and Env384–398 helper T lymphocyte epitopes (38). One attractive strategy for the selection of peptides to be used as vaccines is the possibility of using a single peptide of relatively small size (<25–30 residues) that contains epitopes for both CTLs and helper T lymphocytes. Thus, a synthetic peptide of 29 residues (HTLV-I–Env182–210) or 1 of 20 residues (HTLV-I–Env384–403) could be capable of eliciting antivirus CTL and helper T lymphocyte responses in patients expressing HLA-A2 and one of the MHC class II allele that restricts the corresponding HTL response.

In conclusion, we present here experimental evidence that the HTLV-I–Env196–210, HTLV-I–Env317–331, and HTLV-I–Env384–398 helper T lymphocyte epitopes are effective at eliciting potent anti-HTLV-I CD4+ T-cell responses capable of recognizing directly the HTLV-I+ T-cell lymphoma cells. Two of these epitopes (HTLV-I–Env196–210 and HTLV-I–Env384–398) lie proximal to or overlap with previously characterized antibody epitopes and HLA-A2–restricted CTL epitopes found in the HTLV-I envelope glycoprotein. The present findings advocate that some synthetic peptides derived from the HTLV-I–Env glycoprotein could be used as prophylactic/therapeutic vaccines to effectively and simultaneously stimulate CTLs, helper T lymphocytes, and antibody responses against ATLL and other diseases mediated by HTLV-I.

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