Cross-Reactivity between Immunodominant Human T Lymphotropic Virus Type I \textit{tax} and Neurons: Implications for Molecular Mimicry

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Human T lymphotropic virus type I (HTLV-I)–associated myelopathy/tropical spastic paraparesis (HAM/TSP) is associated with immunoreactivity to HTLV-I \textit{tax}. Antibodies isolated from patients with HAM/TSP and monoclonal antibodies (MAbs) to HTLV-I \textit{tax} stained neurons. In neuronal extracts, HAM/TSP immunoglobulin G identified heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) as the autoantigen. Importantly, \textit{tax} MAbs reacted with hnRNP A1. To identify the epitope recognized by the \textit{tax} MAbs, the fine epitope specificity of the antibodies was determined using overlapping peptides. This analysis identified an epitope at the C-terminus (\textit{tax}^{346-353}), which overlaps a human immunodominant domain. Preincubation of this peptide with \textit{tax} MAbs inhibited antibody binding to \textit{tax}, hnRNP A1, and neurons. This indicates that a cross-reactive immune response between HTLV-I \textit{tax} and neuronal hnRNP A1 is contained within the human immunodominant epitope of \textit{tax} and suggests that molecular mimicry plays a role in the pathogenesis of HAM/TSP.

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Institutional review board approved consent (University of Tennessee and Veterans Affairs Medical Center—Memphis; protocol 6618) is on file for phlebotomy used to collect serum samples.

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Individuals infected with human T lymphotropic virus type I (HTLV-I) develop HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP), an immune-mediated disease of the central nervous system (CNS) [1]. How immune cells damage the CNS remains elusive. There is no evidence that direct infection of the CNS with HTLV-I results in cell damage. Instead, cellular and antibody-mediated immune responses have been implicated in the pathogenesis of HAM/TSP [1–3]. It is the immune response to \textit{tax}, an immunodominant and regulatory protein of HTLV-I, that differentiates patients with HAM/TSP from control populations [4, 5]. Patients with HAM/TSP develop CD8$^+$ cytotoxic T lymphocytes (CTL) specific for the N-terminus of HTLV-I \textit{tax} (\textit{tax}^{11-19}) in association with HLA-A2 [1, 4]. Patients with HAM/TSP have elevated titers of antibody to HTLV-I and a preferential IgG response to the C-terminus of \textit{tax} (\textit{tax}^{346-353}) [5]. Data also suggest that virus load is important in stimulating these responses, because patients with HAM/TSP have elevated levels of \textit{tax} [3]. Other studies suggest that elevated levels of \textit{tax} stimulate CTL that are protective, rather than pathogenic, in patients with HAM/TSP [2]. Importantly, HTLV-I–infected patients showed a marked IgG response to autoantigens [6]. These data suggest that a cross-reactive immune response between immunodominant HTLV-I \textit{tax} and a CNS autoantigen may exist and contribute to the pathogenesis of HAM/TSP.

Data from our laboratory suggest that an autoimmune mechanism involving a CNS autoantigen, specifically, molecular mimicry between HTLV-I \textit{tax} and neuronal heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), may contribute to the pathogenesis of HAM/TSP [7, 8]. IgG isolated from patients with HAM/TSP was immunoreactive with neurons. \textit{tax} monoclonal antibodies (MAbs) mimicked HAM/TSP IgG staining of neurons. HAM/TSP IgG reactivity to neurons was inhibited by HTLV-I \textit{tax} and the \textit{tax} MAbs. These data suggest that there may be a shared \textit{tax} epitope between the \textit{tax} MAbs and HAM/TSP IgG. HAM/TSP IgG was used to isolate the neuronal protein, which was identified as hnRNP A1, from brain tissue [8]. The \textit{tax} MAbs were also immunoreactive with hnRNP A1, which suggests that molecular mimicry exists between the immunodominant epitope of the \textit{tax} MAbs and neuronal hnRNP A1 [8]. The epitope specificity of these antibodies is unknown. We hypothesized that the epitope specificity of the \textit{tax} MAbs overlapped with human immunodominant epitopes for \textit{tax} and that
this immunoreactivity was related to the immune response to neurons.

Materials and Methods

MAbs were determined to be IgG2a by use of the ImmunoPure Monoclonal Antibody Isotyping kit (Pierce) and were tested for reactivity to HTLV-I gag and env and HTLV-II env with the HTLV Blot 2.4 Kit (Genelabs Diagnostics). HTLV-I tax MAbs were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, from Beatrice Langton; Hybridoma 168B17-46-34 (Tab 169), Hybridoma 168B17-46-50 (Tab 170), Hybridoma 168B17-46-70 (Tab 171), and Hybridoma 168B17-46-92 (Tab 172). HuT 102 cells, peripheral blood lymphocytes (PBLs) from HTLV-I-seronegative donors, and brain tissue were prepared for immunohistochemical analysis as described elsewhere [7, 8]. Sections (5 μm) were rehydrated and subjected to antigen retrieval (boiling for 20 min) with 10 mM citrate buffer (pH 6). MAbs (1:50–1:100) were applied overnight at 4°C and detected with biotinylated goat anti–mouse IgG (Vector Laboratories; 1:2000, for 1 h) followed by avidin-biotin-peroxidase complex (ABC; Vectastain Elite kit [Vector Laboratories]), and slides were developed in diaminobenzide HCl (1 mg/mL) and hydrogen peroxide (0.01%). Slides were counterstained with hematoxylin. A mouse MAb to neurofilament (NF-68; Vector Laboratories) was the isotype control. For adsorption studies, individual MAbs were incubated with 0.01–1000 μg of the C-terminal peptide (KHFRTEV) for 1 h and then processed as described earlier. For brain tissue, sections were incubated with biotinylated IgG from patients with HAM/TSP (1:50), biotinylated IgG from healthy individuals (1:50), or tax MAbs (1:50). IgG from patients with HAM/TSP and from healthy individuals was detected as described earlier. Mouse MAbs were determined to be IgG2a by use of the ImmunoPure Monoclonal Antibody Isotyping kit (Pierce) and were tested for reactivity to HTLV-I gag and env and HTLV-II env with the HTLV Blot 2.4 Kit (Genelabs Diagnostics). HTLV-I tax MAbs were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, from Beatrice Langton; Hybridoma 168B17-46-34 (Tab 169), Hybridoma 168B17-46-50 (Tab 170), Hybridoma 168B17-46-70 (Tab 171), and Hybridoma 168B17-46-92 (Tab 172). HuT 102 cells, peripheral blood lymphocytes (PBLs) from HTLV-I-seronegative donors, and brain tissue were prepared for immunohistochemical analysis as described elsewhere [7, 8].

Results

Each of the MAbs to HTLV-I tax stained the HTLV-I–infected cell line HuT 102 (table 1). The MAbs showed no staining of PBLs from healthy donors (table 1), and an isotype-specific MAb showed no staining of HuT 102 cells (data not shown). Western blots done using each of the MAbs showed intense immunoreactivity at 40–42 kDa, the known molecular weight of HTLV-I tax [5] (table 1). There was no reactivity when the assays for HTLV-I gag, env, and HTLV-II were used (data not shown).

Mimotope analysis of the MAbs showed strongest reactivity at the final 2 peptides of HTLV-I tax (peptides 113 and 114, corresponding to tax(337–353), table 1). Minor reactivity was found throughout the protein, but no area yielded consistent results in multiple experiments or an absorbance intensity equal to that found in the C-terminus. Alignment of peptides 113 and 114 showed a common sequence of KHFRET (tax(346–353)), because the C-terminus of tax is known to be hydrophobic [5] and to allow better antibody-antigen binding, the final 2 residues, E and V (tax(352–353)), were included in the epitope. These data suggest that the immunodominant epitope is located at tax(346–353) (KHFRETV). Preincubation of each MAb with KHFRETEV inhibited staining of HuT 102 cells in a concentration-dependent manner. Similar results were found when each of the MAbs was incubated with the peptide before Western blotting was done (table 1). These data suggest that KHFRETEV contains the immunodominant epitope recognized by the MAbs. There was no competition between the MAbs to tax when a nonspecific peptide was used (table 1). Interestingly, the epitope specificities of the tax MAbs are coincident with immunodominant

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epitopes for tax in patients infected with HTLV-I, including epitopes Rp-F (tax\(^{329-353}\)), tax 23 (tax\(^{331-350}\)), and tax 24 (tax\(^{336-353}\)) [5, 9].

In previous studies [7, 8], we showed that patients with HAM/TSP develop antibodies to human neurons (figure 1, panel 2). Importantly, the tax MAb mimicked neuronal staining (figure 1, panel 3). Recently, hnRNP A1 was identified as the neuronal protein [8]. HAM/TSP IgG reacted intensely with hnRNP A1 that was cloned and used in Western blots (figure 2, lane 2). Importantly, the MAb were also highly immunoreactive (figure 2, lane 3). This suggests that the immunodominant epitope of the tax MAb cross-reacts with hnRNP A1, a CNS autoantigen identified in patients with HAM/TSP.

Database analyses of KHFRTEEV and hnRNP A1 showed no match between the 2 proteins. There was minor sequence identity between KHFRTEEV and hnRNP A1\(^{132-133}\) (EV). These results are consistent with recent data that indicate that molecular mimicry due to immunologic cross-reactivity may have greater biological significance than does mimicry defined by primary sequence comparisons [10]. To test for this, we preincubated KHFRTEEV with the tax MAb before testing for immunoreactivity with uninfected neurons and hnRNP A1. Preincubation of the peptide abolished tax MAb immunoreactivity with neurons (figure 1, panels 4–6) and hnRNP A1 (figure 2, lanes 4–8). There was no inhibition of immunoreactivity when MAb were incubated with a control peptide (figure 2, lanes 9–14). More peptide was required to abolish the immunoreactivity signal seen on Western blot, possibly because the purified antigen (hnRNP A1) was coupled with a sensitive enhanced chemiluminescence technique.

**Discussion**

These data indicate that tax MAb (the immunodominant epitope of which overlaps with the immunodominant domain for HTLV-I tax in humans) cross-reacted with hnRNP A1, a CNS autoantigen associated with HAM/TSP. These data suggest that molecular mimicry exists between immunodominant epitopes of tax and a CNS autoantigen. Previously, we showed that patients with HAM/TSP develop antibodies to human neurons but not to systemic organs [7, 8]. This staining was mimicked using tax MAb [7, 8]. The reactivity of IgG from patients with HAM/TSP was abolished by preincubation with HTLV-I tax or tax MAb [7], which suggests that IgG from patients with HAM/TSP and tax MAb share a common tax epitope. Recently, hnRNP A1 was identified as the CNS autoantigen recognized by HAM/TSP IgG [8]. The hnRNPs are riboproteins that have been associated with autoimmune diseases such as systemic lupus erythematos [11]. In addition, hnRNP A1 plays a critical role in the functioning of neurons [12]. All patients with HAM/TSP that we tested were found to have IgG that was immunoreactive with hnRNP A1 [8]. Importantly, the tax MAb also reacted with neurons and hnRNP A1, which sug-
gests that molecular mimicry exists between the epitope of the MAb and hnRNP A1 [8].

We mapped the fine epitope specificity of these MAb, the epitope specificities of which were unknown. The MAb stained HuT 102 cells and reacted with HTLV-I tax, as was seen on Western blot. We then used the Mimotope Multipin peptide system to determine the fine epitope specificity of the MAb. To maximize specificity, the 353-aa sequence of HTLV-I tax was represented by 15-mer peptides, with 12-aa overlap and a shift of only 3 aa between peptides. Using this system, the immunodominant epitope of the MAb was mapped to tax346–353 (KHFRETEV). Adsorption studies showed that KHFRETEV inhibited binding of the MAb to tax, a result apparent on immunohistochemical analysis and Western blot, which confirms that the immunodominant epitope is tax346–353.

A concern raised by use of this system is that epitopes that rely on secondary or tertiary conformations may not be detected. Although we cannot eliminate this possibility, our data suggest that these MAb defined an epitope predicted by the primary sequence. First, MAb can define immunodominant epitopes of 6–12 aa [13]. Second, the MAb were shown to react with HTLV-I tax on Western blots under reducing conditions, in which there is minimal secondary and tertiary protein structure. Finally, the MAb detected HTLV-I tax in fixed tissue (which preserves epitopes in their native form), which suggests that the MAb detected an epitope that is available for binding in vivo.

These data are consistent with published reports of epitope binding of other tax MAb [5, 9, 14, 15]. The epitope identified in this study most closely resembles that of MAb TAXY-6 and TAXY-8, which localize to HTLV-I tax339–353 [14]. In contrast, other tax MAb showed tax epitopes that did not include the C-terminus [14, 15]. Importantly, the epitope defined by this study, tax346–353, closely aligns with human epitopes for HTLV-I tax [5, 9].

These results demonstrate molecular mimicry between immunodominant epitopes of tax and neuronal hnRNP A1, an autoantigen recently associated with the development of HAM/TSP. Mimicry occurred with minimal primary sequence alignment, which suggests that immunologic cross-reactivity without sequence identity is biologically important. The relationship of this cross-reactive antibody response (between tax and hnRNP A1) to the robust CTL response in patients with HAM/TSP is not clear. This is particularly important, because it also is not clear whether CTL to tax are protective or pathogenic in HAM/TSP [2, 4]. Future studies are needed to address where the tax MAb and IgG isolated from HTLV-I-infected patients bind hnRNP A1 and to determine whether a cross-reactive CTL epitope exists between the 2 proteins.

Acknowledgment

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References