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Definition of an HLA-DPw2-Restricted Epitope on NS3, Recognized by a Dengue Virus Serotype-Cross-Reactive Human CD4+ CD8- Cytotoxic T-Cell Clone

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We previously reported that the clone JK34 was cross-reactive for dengue virus types 1, 2, 3, and 4 and recognized NS3 (I. Kurane, M. A. Brinton, A. L. Samson, and F. A. Ennis, J. Virol. 65:1823–1828, 1991). In the present experiments, we defined the epitope at the amino acid level, with 93 15-mer overlapping peptides which cover the entire NS3. A peptide 4 which contains amino acids 251 to 265 of NS3 sensitized the autologous B lymphoblastoid cell line (LCL) to the lysis by JK34. The smallest peptide recognized by JK34 was a 10-mer peptide which contains amino acids 255 to 264 (EIVDLNCHAT). A monoclonal antibody to HLA-DP inhibited the lysis of epitope peptide-pulsed autologous LCL by JK34. Genotypic typing revealed that the HLA-DP of this donor is DPA1*01, DPB1*0201, which is serologically defined as HLA-DPw2. JK34 lysed peptide 4-pulsed allogeneic LCL which carried HLA-DPw2. These results indicate that HLA-DPw2 is the restriction allele for recognition of this epitope by JK34.

Dengue virus infections are a serious cause of morbidity and mortality in many areas of the world: southeast and south Asia, Central and South America, and the Caribbean (9, 11). Dengue virus infection can be asymptomatic or cause two forms of disease (8). Dengue fever is a self-limited febrile disease. In some situations, patients infected with dengue virus leak plasma into interstitial spaces, resulting in hypovolemia and sometimes circulatory collapse. This severe life-threatening syndrome is termed dengue hemorrhagic fever (DHF).

Development of dengue virus vaccines is a potential method for preventing dengue virus infections; however, the epidemiological observations have shown that DHF is observed much more commonly in secondary dengue virus infections than in primary infections (1, 9). These observations raise the possibility that dengue virus vaccine may induce immune responses which could lead to the immunopathology of DHF. Dengue virus vaccines should induce protective immune responses but should not induce immunity which may increase the risk of DHF during future dengue virus infections. Although protective immune mechanisms against dengue viruses are not understood, it is believed that serotype-specific neutralizing antibodies can prevent dengue virus infections and that dengue virus-specific cytotoxic T lymphocytes (CTL) contribute to recovery from infection. Therefore, experimental subunit vaccines should include neutralizing B-cell epitopes and dominant CTL and helper T-cell epitopes.

We have reported that the NS3 protein was recognized by the majority of CD4+ CTL clones established from a dengue virus-infected individual (14). Identification and characterization at the amino acid levels of these CD4+ CTL epitopes will provide useful information for the future development of dengue vaccines. In this paper, we define an epitope on NS3 recognized by a dengue virus serotype-cross-reactive CD4+ CD8- CTL clone, JK34, with overlapping synthetic peptides.

Dengue virus type 1, Hawaii strain; type 2, New Guinea C strain; type 3, CH53489 strain; and type 4, 814669 strain; yellow fever virus (17D strain); and West Nile virus (E101 strain) were used. Dengue virus, yellow fever virus, and West Nile virus antigens were prepared by using dengue virus-infected Vero cells as previously reported (16). Synthetic peptides of the NS3 protein of dengue virus type 4, 814669 strain (17), were synthesized with the RaMPS system (DuPont, Boston, Mass.) as previously reported (6, 23). The peptides consist of 15 amino acids (aa) which overlapped each other by 7 to 10 residues.

Cytotoxic assays were done as previously reported (14). In cytotoxic assays using synthetic peptides, 105 cells in 0.1 ml were incubated with peptide in 0.05 ml for 30 min, and effector cells in 0.05 ml were then added to each well. After incubation at 37°C for 6 h, the supernatant fluid was collected from each well and counted in an automatic gamma counter. The percent specific 51Cr release was calculated by the following formula: 100 × (counts per minute of experimental release – counts per minute of spontaneous release)/(counts per minute of maximal release – counts per minute of spontaneous release). Concentrations of the peptide which induce 50% maximum lysis were calculated on the basis of the dose-response curves with peptide at concentrations from 25 μM to 2.5 × 10-6 μM, and the percent specific lysis of dengue virus type 3 antigen-cultured target cells was considered as the maximum lysis in the experiment. The 10th International Histocompatibility Workshop lymphoblastoid cell lines (LCL) (10w9023, -9011, -9029, -9038, -9022, -9052, and -9077) (American Society for Histocompatibility and Immunogenetics, Lenexa, Kans.) were used in HLA-DP restriction experiments.

The HLA-DPA1 and DPB1 genotype of the donor was established by polymerase chain reaction amplification of

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the first domain of DPAl and DPB1 genes and hybridization with sequence-specific oligonucleotide probes as previously described (19, 20).

The establishment and partial characterization of the JK34 clone have been already reported (14). Briefly, JK34 was established from the peripheral blood mononuclear cells of a donor who had been infected with dengue type 3 virus (CH53489) 1 year earlier, with a limiting dilution technique. JK34 has a CD3+ CD4+ CD8- phenotype and has dengue virus-specific cytotoxic activity. JK34 is cross-reactive for dengue virus types 1, 2, 3, and 4 but not for yellow fever virus or West Nile virus.

After demonstrating that clone JK34 recognized the NS3 protein (14), we attempted to determine the epitope recognized by JK34, with 93 15-mer overlapping peptides which covered the entire NS3 protein. Only peptide 4 which contains aa residues 251 to 265 (HTGREIVDLMCHATF) sensitized autologous LCL to the lysis by JK34 (Fig. 1). None of the other peptides sensitized autologous LCL to the lysis by JK34 (data not shown). To further delineate the smallest peptide recognized by this clone, we synthesized N- and C-terminal truncations of peptide 4 as shown in Table 1. Peptides 4a, 4b, 4c, 4d, 4f, and 4k sensitized autologous LCL to the lysis by JK34. These results indicate that the smallest peptide recognized by JK34 is located on aa 255 to 264, which have the amino acid sequence of EIVDLMCHAT.

HLA restriction in the recognition of the epitope by JK34 was first examined with monoclonal antibodies to HLA molecules (Table 2). A monoclonal antibody to HLA-DR inhibited the lysis of peptide 4-pulsed target cells and dengue virus type 3 antigen-cultured target cells by JK34, but monoclonal antibodies to HLA-DQ, HLA-DR, and HLA class I did not. This result confirmed that recognition of the epitope by JK34 is HLA-DR restricted as previously reported (14). In order to determine HLA-DR allelic restrict-

![Table 2. HLA-DP-restricted lysis of the target cells by JK34](image)

![Table 1. Determination of the core epitope recognized by JK34 with truncated synthetic peptides](image)

**FIG. 1.** Recognition of a peptide 4 (aa 251 to 265) by a dengue serotype-cross-reactive CD4+ CTL clone, JK34. A total of 10^9 autologous LCL were incubated with 8 x 10^5 JK34 cells (effector/target ratio = 8:1) for 6 h in the presence of peptides at 20 μM. Dengue 4 Ag, dengue virus type 4 antigen.
TABLE 3. HLA-DPw2-restricted lysis of peptide 4-pulsed autologous LCL by JK34

<table>
<thead>
<tr>
<th>Target</th>
<th>HLA-DP</th>
<th>Genotype</th>
<th>% Specific  ( ^{31} )Cr release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>w2</td>
<td>01 0201</td>
<td>89</td>
</tr>
<tr>
<td>9023</td>
<td>w1</td>
<td>02 0101</td>
<td>4</td>
</tr>
<tr>
<td>9011</td>
<td>w2, w4</td>
<td>01 0201, 0401</td>
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<td>w2</td>
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<td>40</td>
</tr>
<tr>
<td>9022</td>
<td>w3</td>
<td>01 0301</td>
<td>4</td>
</tr>
<tr>
<td>9052</td>
<td>w4</td>
<td>01 0401</td>
<td>5</td>
</tr>
<tr>
<td>9077</td>
<td>w5</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

* A total of 10⁶ autologous or allogeneic LCL were incubated with 1.2 x 10⁶ JK34 cells (effector/target ratio = 12:1) for 6 h in the presence of peptide 4 at 25 μM. 

The role of dengue virus-specific CD4+ CTL in dengue virus infections is poorly understood. It is likely that CD4+ CTL contribute to prevention of infection and recovery from dengue virus infection by helping the generation of neutralizing antibodies and CD8+ CTL and by lysing dengue virus-infected HLA class II-bearing cells. It is also possible that these CD4+ CTL contribute to the pathogenesis of DHF by lymphokine production and cytotoxic activities (15). If CD4+ CTL do contribute prevention and recovery, the epitopes recognized by these dengue serotype-cross-reactive clones should be included in the subunit vaccines. It is therefore important to map the epitopes recognized by other dengue virus-specific T-cell clones and determine the HLA restriction of these clones. This information will be useful in the development of safe and effective dengue virus vaccines in the future.

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