Characterization of HLA-A2.1-restricted epitopes, conserved in both Hantaan and Sin Nombre viruses, in Hantaan virus-infected patients

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Nine different CTL epitopes, conserved in both Hantaan virus (HTNV) and Sin Nombre virus (SNV), were selected for study. The binding affinity of each peptide with HLA-A2.1 molecules in vitro was determined and antigen-specific responses from seven donors who had a previous field infection with HTNV were examined. Although the strength or frequency of CTL activity showed different patterns in the seven patients, five of seven patients showed significant activity against at least one or more epitope peptides. In particular, the peptide ILQDMRNTI (HTNV, aa 334–342; SNV, aa 333–341), which elicited CTL activity in five patients, was shown to be specifically HLA-A2.1-restricted in partially cloned CD8+ T cells and also induced activated and effector CD8+ T cell-producing T cytotoxic (Tc) type 1 cytokines, such as IL-2 and IFN-γ. The results suggest that this epitope would serve as a useful component for the intervention of both HTNV and SNV infection.

In Hantaan virus (HTNV) infection, there are several lines of evidence to support the role of CD8+ T cells in the pathology of disease: (i) the increase in the number of activated, circulating CD8+ cells in patients with acute haemorrhagic fever with renal syndrome (HFRS) (Huang et al., 1994); (ii) the presence of infiltrating lymphocytes in kidney biopsies from patients with acute Puumala virus infection; and (iii) an increase in the production of cytokines such as TNF-α, IFN-γ, IFN-α and IL-6 (Temonen et al., 1996; Krakauer et al., 1994; Linderholm et al., 1996). Furthermore, it has been reported recently that virus-specific and memory T cells were detected in HTNV-infected patients, as a result of the generation of CTLs to limited epitopes derived from viral proteins (Van Epps et al., 1999). It has also been reported that, during acute illness induced by Sin Nombre virus (SNV) infection, HLA-B35-restricted CD8+ cells that recognize SNV nucleocapsid protein-derived epitopes are detected in PBMCs from infected patients (Ennis et al., 1997). These results strongly suggest that, in both HTNV and SNV infection, CD8+ T cells might play a critical role in the immunopathology and clearance of the viruses in humans.

In this study, we investigated whether virus-specific CTLs, especially recognizing the epitope conserved in both HTNV and SNV, are generated in HTNV-infected patients. So far, it is believed that several MHC haplotypes, such as HLA-B8, DR3 and HLA-B35, play an important role in the cellular immune response in the pathology of both hantavirus pulmonary syndrome (HPS) and HFRS (Koster et al., 1998; Van Epps et al., 1999; Ennis et al., 1997). And yet, human HLA-A2.1-restricted CTL epitopes have not been confirmed clearly. Here, we identified HLA-A2.1-restricted epitopes conserved in both HTNV and SNV and showed that some CD8+ T cells in bulk-cultured PBMCs derived from HTNV-infected patients strongly recognized epitope peptides.

To identify conserved epitopes, we performed amino acid sequence alignments between HTNV (strains 76-118, C1-1 and C1-2) and SNV (strains NMH10 and NMR11). Amino acid sequences were obtained from the NCBI database. Using the computer program BLOSUM (NIH) (Parker et al., 1994), nine CTL epitopes, conserved in the nucleocapsid (N) and polymerase (P) proteins of both viruses, were selected based on their binding scores, as calculated by the program. The sequence of each peptide and its position in the viral genome are summarized in Table 1, along with their relative solubility in buffer.

The selected epitopes were tested for their binding affinity to HLA-A2.1 molecules using the human antigen processing-defective cell line T2 (Levy et al., 1991). Although T2 cells express a very low level of HLA-A2.1 molecules under normal culture conditions, they express the molecules at much higher levels when allowed to bind with appropriate peptides that stabilize the HLA-A2.1 molecule. Thus, up-regulation of peptide-induced HLA-A2.1 expression in T2 cells can be regarded as an indication of an HLA-A2.1-restricted epitope (Butterfield et al., 2001; Minev et al., 2000). To determine the

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Table 1. Characterization of HLA-A2-restricted epitopes derived from HTNV and SNV proteins

Anchor residues comprising HLA-A2-restricted peptide motifs are indicated in bold. The solubility of each peptide in PBS was scored as soluble (+) or insoluble (−).

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Virus (position of sequence)</th>
<th>Sequence</th>
<th>Solubility in PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>HTNV (aa 334–342)</td>
<td>ILQDMRNTE</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 333–341)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>HTNV (aa 512–520)</td>
<td>ILPSKSLEV</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 513–521)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>HTNV (aa 559–567)</td>
<td>NMSIDLNRL</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 560–568)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>HTNV (aa 625–633)</td>
<td>NLRYLIPAV</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 626–634)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>HTNV (aa 628–636)</td>
<td>YLIPAVTLS</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 629–637)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>HTNV (aa 1137–1145)</td>
<td>EMWKSMFNL</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 1138–1146)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>HTNV (aa 1188–1196)</td>
<td>ILLGSLSDL</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 1189–1197)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P8</td>
<td>HTNV (aa 1273–1281)</td>
<td>IMLATAGCI</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 1274–1282)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P9</td>
<td>HTNV (aa 1736–1744)</td>
<td>GLDCARLEI</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>SNV (aa 1737–1745)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Determination of the binding affinity of synthetic peptides derived from HTNV proteins to the HLA-A2 molecule. After incubation of different concentrations of peptide, elevation of HLA-A2 expression on the T2 cell surface was measured by flow cytometry using the antibody BB7.2. The data shown are representative of three independent experiments.

optimal concentration for binding, the amount of HLA-A2.1 molecules expressed on the T2 cell surface was quantified by staining the cells with the HLA-A2.1-specific antibody BB7.2 after the addition of peptides at different concentrations.

Among the nine different peptides tested, six peptides from HTNV (aa 334–342, 512–520, 559–567, 628–636, 1188–1196 and 1273–1281) showed significant binding affinities (Fig. 1). In this study, the hepatitis B virus S antigen (HBs) peptide, aa 271–280 (Lee et al., 1997), was used as a positive control for the class I MHC stabilization assay with T2 cells. With peptide concentrations of 10 µg/ml, surface HLA-A2 expression on T2 cells reached a plateau, indicating that the concentration is sufficient for the saturation of surface expression of HLA-A2.1 molecules in T2 cells. The P9 peptide derived from HTNV, aa 1736–1744 (SNV, aa 1737–1745), exhibited higher affinity relative to any of the other peptides tested. Peptides P1, -5, -7 and -8 were found to be moderate binders, whereas peptide P2 was found to bind relatively weakly (Fig. 1). Peptides P3-
-4 and -6 did not show significant binding under our experimental setting. These results suggested that, although the strength of HLA-A2.1 binding was variable, six of nine peptides exhibited significant binding affinities and, therefore, are expected to be immunogens for generating antigen-specific cytotoxic T cells in HTNV-infected patients.

Since the binding of a peptide to class I MHC molecules does not necessarily mean that they are able to induce and be recognized by MHC-restricted CTLs, we examined whether these peptides could generate peptide-specific CTLs in HTNV-infected patients. Initially, 13 different HTNV-infected patients were screened; seven patients were positive for the expression of HLA-A2.1 molecules (data not shown). PBMCs, isolated from HLA-A2.1-positive patients, were cultured for 2 weeks for the recovery of viable T cell repertoires. CTL assays were carried out using effector cells that had been cultured with recombinant human IL-2 and peptides and T2 target cells pre-treated with the peptides that showed significant binding affinities to HLA-A2.1 molecules. Among the various CTL epitopes tested in this study, eight peptides (P2–9) were derived from the P protein, whereas one peptide (P1) was derived from the N protein (Table 1). Our initial selection of potential CTL epitopes was, in part, guided by previous reports on other virus infections, in that several immunodominant epitopes derived from their polymerase proteins were recognized by virus-specific CD8+ T cells in both animals and humans (Tourdot et al., 2001; Belz et al., 2001; Sing et al., 2000; Mortara et al., 2000). The present study showed that CD8+ T cells recognizing P protein-derived peptides may also be generated in HTNV infections, although not as immunodominant as epitopes derived from other viral proteins, such as the glycoproteins (G1 and G2) and the N protein (Fig. 2). Interestingly, P1, which is derived from the N protein, was shown to be a very strong inducer of HLA-A2.1-restricted CTL activity (Fig. 2). This observation is consistent with and further extends previous studies on other HLA-specific epitopes from the HTNV N protein (Van Epps et
al., 1999; Ennis et al., 1997). Therefore, we investigated the P1-restricted response in HTNV-infected patients. Peptide P1 (HTNV, aa 334–342; SNV, aa 333–341) elicited the strongest CTL activity among the various peptides tested. To identify its specific CTL more closely, CD8+ T cells were purified from PBMCs using magnetic beads and characterized with regard to surface markers, profiles of secreted cytokines and MHC-restriction. Purified CD8+ cells were repeatedly stimulated with the peptide-pulsed allogenic HLA-A2.1-positive PBMCs as feeder cells in order to enrich antigen-specific lysis. To test antigen-specific lysis, a chromium-release assay was performed with CD8+ T cells. T2 cells were pre-pulsed with the HBs peptide (aa 271–280; ○) as a negative control, P1 (HTNV, aa 333–341; ●) or without peptide (▲) and then incubated with effector cells at the different effector:target cell ratios, as indicated. All assays were performed in triplicate and representative data are shown.
specific CTLs against the peptide. After 28 days, cells were harvested, stained with FITC-conjugated anti-CD8 antibody and PE-conjugated anti-CD4, -CD25, -CD45RO, -CD45RA, -CD62 or -CD69 antibodies and analysed by flow cytometry. It has been reported that, in activated or effector CD8\(^+\) T cells, CD25, CD45RO and CD69 molecules are strongly increased, whereas CD45RA and CD62L molecules are significantly down-regulated as compared to naive CD8\(^+\) T cells (Detels et al., 1996; Rutella et al., 1998). Consistent with previous reports, CD25, CD45RO and CD69 molecules were strongly increased in purified CD8\(^+\) cells, whereas no significant increases in CD45RA and CD62L molecules were observed (Fig. 3b) as compared to the pre-cultured total PBMCs derived from the same patient (Fig. 3a).

We then tested whether these CD8\(^+\) T cells were Tc type 1 or Tc type 2 cells, according to their cytokine pattern. We also examined their specific response to the HLA-A2.1-restricted peptide P1 (HTNV, aa 334–342). Cells were stained with FITC-conjugated anti-CD8 antibody and PE-conjugated anti-IL-2, -IL-5, -IL-10 or -IFN-\(\gamma\) antibodies. We used IL-2 and IFN-\(\gamma\) as markers for Tc type 1 cells and IL-5 and IL-10 for Tc type 2 cells (Mosmann & Sad, 1996; Romagnani et al., 1997). As shown in Fig. 3(c), about 33 and 87% of cells were stained with anti-IL-2 or anti-IFN-\(\gamma\) antibodies, respectively, whereas no cells were stained with anti-IL-5 or anti-IL-10 antibodies. In summary, partially cloned CD8\(^+\) cells derived from PBMCs from HTNV-infected patients are mostly CD25\(^+\)CD69\(^+\)-CD62L\(^-\)CD45RO\(^-\)CD45RA\(^-\) and are able to secrete Tc type 1 cytokines, such as IL-2 and IFN-\(\gamma\), with the known characteristics of the activated and effector CD8\(^+\) T cells.

Next, a chromium-release assay was used to test whether or not these cells show antigenic specificity against target cells pulsed with peptide P1. As shown in Fig. 3(d, ●), specific lysis of target cells pulsed with P1 was significantly high, up to about 78% at an effector:target cell ratio of 25:1, as compared to the negative controls pulsed with an irrelevant peptide (HBs, aa 271–280) (Fig. 3d, ○) or without peptide (Fig. 3d, ▲). These results confirm that the majority of CD8\(^+\) target cells are restriction by HLA-A2.1 molecules pulsed with HTNV peptide P1. Although partially cloned T cells generated in this study may not represent a homogeneous clone, their properties were very similar to that of a cloned T cell in terms of antigen-specific recognition, effector-dependence in target cell killing, phenotype and cytokine profiles.

In the present study, we predicted and selected cytotoxic T cell epitopes conserved in both HTNV and SNV proteins and examined antigen-specific responses from HTNV-infected patients. Five of seven patients exhibited significant activity against six different epitopes. Furthermore, the peptide IQD-MRNTI (HTNV, aa 334–342; SNV, aa 333–341) was shown to elicit strong CTL activity in five of seven patients tested. Following prolonged stimulation with peptide, activated and MHC-restricted CD8\(^+\) cells producing Tc type 1 cytokines could be detected in the PBMCs from HTNV-infected patients, strongly suggesting that the epitope would be potentially useful for the development of prophylactic or therapeutic vaccines for both HTNV and SNV infections.

A key issue is whether homologous peptides derived from the two related viruses are also immunogenic in SNV-infected patients. Since we did not have access to PBMCs from SNV-infected patients in our present circumstances, we opted to test cytolytic activity with PBMCs from HTNV-infected patients. Although the pathogenesis of HTNV and SNV is not well understood, it has been proposed that they might induce a similar immune response in infected patients (Lopez et al., 1996; Nichol et al., 1993; Williams et al., 1997). Since the CTL epitopes tested in this study are also present in SNV proteins as well, it is probable that specific responses in SNV infection might also be generated. In conclusion, we have identified several HLA-A2.1-restricted CTL epitopes conserved in both HTNV and SNV. These epitopes may be used as useful components for peptide-based, DNA or chimeric viral vector vaccines for the intervention of both HTNV and SNV infections.

Ki-Young Lee and Eunyoung Chun contributed equally to this work.

References


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