A Nonamer Peptide Derived from *Listeria monocytogenes* Metalloprotease Is Presented to Cytolytic T Lymphocytes

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*Listeria monocytogenes* is an intracellular bacterium that secretes proteins into the cytosol of infected macrophages. Major histocompatibility complex (MHC) class I molecules bind peptides that are generated by the degradation of bacterial proteins and present them to cytolytic T lymphocytes (CTL). In this study we have investigated CTL responses in *Listeria monocytogenes*-immunized mice to peptides that (i) derive from the *L. monocytogenes* protein phosphatidylinositol-specific phospholipase C, lecinthinase (most active on phosphatidylcholine), metalloprotease (Mpl), PrfA, and the ORF-A product and (ii) conform to the binding motif of the H2-K\(^d\) MHC class I molecule. We identified a nonamer peptide, Mpl 84–92, that is presented to *L. monocytogenes*-specific CTL by H2-K\(^d\) MHC class I molecules. Unlike other motif-conforming peptides derived from the secreted Mpl of *L. monocytogenes*, Mpl 84–92 is bound with high affinity by H2-K\(^d\). Mpl 84–92 is the fourth *L. monocytogenes*-derived peptide found to be presented to CTL by the H2-K\(^d\) molecule during infection and demonstrates the importance of high-affinity interactions between antigenic peptides and MHC class I molecules for CTL priming.

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**TABLE 1. Sequences of H2-K\(^d\) motif-conforming *L. monocytogenes* peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrfA 61–69</td>
<td>Gln-Tyr-Tyr-Lys-Gly-Ala-Phe-Val-Ile</td>
</tr>
<tr>
<td>PrfA 94–102</td>
<td>Ala-Tyr-Val-Ile-Lys-Ile-Asn-Glu-Leu</td>
</tr>
<tr>
<td>PrfA 206–214</td>
<td>Phe-Tyr-Val-Gln-Asn-Leu-Asp-Tyr-Leu</td>
</tr>
<tr>
<td>OrfA 34–42</td>
<td>Leu-Tyr-Gly-Lys-Ile-Glu-Phe-Ile</td>
</tr>
<tr>
<td>OrfA 137–145</td>
<td>Ile-Tyr-Ser-Glu-His-Ile-Asn-Asn-Leu</td>
</tr>
<tr>
<td>OrfA 167–175</td>
<td>Arg-Tyr-Ser-Met-Asn-Gly-Phe-Ile</td>
</tr>
<tr>
<td>PlcA 4–12</td>
<td>Asn-Tyr-Leu-Gln-Arg-Thr-Leu-Val-Leu</td>
</tr>
<tr>
<td>PlcA 92–100</td>
<td>Leu-Tyr-Gln-Gln-Leu- Ala-Gly-Ile</td>
</tr>
<tr>
<td>PlcB 110–118</td>
<td>Pro-Tyr-Tyr-Asp-Thr-Ser-Thr-Phe-Leu</td>
</tr>
<tr>
<td>PlcB 195–203</td>
<td>Ala-Tyr-Glu-Asn-Tyr-Val-Asp-Thr-Ile</td>
</tr>
<tr>
<td>Mpl 84–92</td>
<td>Gly-Tyr-Leu-Thr-Asp-Asn-Glu-Ile</td>
</tr>
<tr>
<td>Mpl 289–297</td>
<td>Glu-Tyr-Lys-Asn-Val-His-Glu-Leu</td>
</tr>
<tr>
<td>Mpl 363–371</td>
<td>Glu-Tyr-Gly-Gln-Ser-Gly-Ala-Leu</td>
</tr>
</tbody>
</table>

* These peptide sequences are derived from the deduced amino acid sequences of PrfA (17), OrfA and PlcB (31), PlcA (3), and Mpl (5).
we decided to scan several other known L. monocytogenes proteins for nonamer peptide sequences that conform with the H2-Kd peptide binding motif. Since H2-Kd binds peptides that contain tyrosine in the second position from the amino terminus and either leucine or isoleucine in the ninth, carboxy-terminal position (7), peptides that conform to this sequence were synthesized. Because PrfA-regulated proteins are associated with virulence, we reasoned that these proteins might be targets for L. monocytogenes-specific CTLs. Thirteen peptides derived from PrfA, PlcA, PlcB, the Orf-A product, and Mpl were identified (Table 1) and tested for the ability to stimulate L. monocytogenes-immune murine splenocytes. C57BL/6 × BALB/c F1 (H2b × H2d) (CB6) mice were infected intravenously with a sublethal dose (5,000 organisms) of L. monocytogenes ATCC 43251, and 7 days later immune splenocytes were isolated. Immune splenocytes were restimulated in vitro with peptide-coated (10^{-6} M), irradiated (3,000 rads), naive CB6 splenocytes as previously described (20, 27). Five days later, restimulated splenocytes were assayed for peptide-specific cytolytic activity, using 51Cr-labeled P815 (H2d) target cells in the presence of the respective stimulating peptide. A known target of L. monocytogenes-specific CTLs, p60 217–225, was included as a positive control (20). Indeed, immune splenocytes restimulated with p60 217–225 lysed peptide-coated target cells (Fig. 1). Immune splenocytes did not respond to any of the other peptides except one (Fig. 1), Mpl 84–92, corresponding to amino acids 84 to 92 of the secreted L. monocytogenes Mpl. The response to Mpl 84–92 5 days after in vitro peptide restimulation is quantitatively similar to the response to p60 217–225. Of note, splenocytes from unimmunized mice did not respond to in vitro restimulation with either p60 217–225 or Mpl 84–92 (results not shown). Staining with anti-CD8 and anti-CD4 antibodies revealed that Mpl 84–92-specific CTLs are exclusively CD8^+ CD4^- (results not shown). Thus, Mpl 84–92 is the fourth identified peptide that is presented to CTLs by the H2-Kd MHC class I molecule during L. monocytogenes infection. In common with LLO 91–99, p60 217–225, and p60 449–457, Mpl 84–92 derives from a secreted protein. Interestingly, Mpl 84–92 derives from the predicted pro-region of Mpl (6), suggesting that entry of Mpl into the
MHC class I antigen processing pathway may be linked to activation of the proenzyme.

Many of the H2-Kd motif-conforming peptides were not detected by immune splenocytes (Fig. 1). Similarly, several H2-Kd motif-conforming peptides derived from LLO and p60 were also not detected by immune splenocytes (27, 32). In the case of p60- and most LLO-derived peptides, the nonantigenic peptides were bound by H2-Kd with significantly lower affinity than the antigenic peptides (27, 32). To determine if the non-antigenic peptides described in this report might similarly be of lower affinity, we performed a peptide competition experiment (32). 51Cr-labeled P815 target cells were incubated in the presence of $5 \times 10^{-11}$ M p60 217–225 and various concentrations of each of the H2-Kd motif-conforming peptides. CTL clone L9.6 (specific for p60 217–225) was determined and plotted.

**FIG. 2.** Mpl 84–92 binds to H2-Kd with high affinity. 51Cr-labeled P815 target cells were incubated in the presence of $5 \times 10^{-11}$ M p60 217–225 and a range of concentrations of each of the H2-Kd motif-conforming peptides. The percent specific lysis of target cells obtained with CTL clone L9.6 (specific for p60 217–225) was determined and plotted.
H2-Kd binding. Since Mpl 84–92 competes for H2-Kd binding as effectively as the other three known peptides presented by H2-Kd, our findings confirm the importance of MHC/pptide affinity in determining antigenicity. Why some peptides, such as PrfA 206–214, do not prime specific CTLs following L. monocytogenes infection remains unknown, but the reason may relate to a lack of PrfA access to the MHC class I antigen processing pathway. Alternatively, peptides such as PrfA 206–214 may not be generated by host cell proteasomes or they may not be transported by the transporter associated with antigen processing. Yet another possibility is that these peptides are not be transported by the transporter associated with antigen presentation. Further experiments.

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REFERENCES


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