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J Immunol 1999; 162:3388-3394; http://www.jimmunol.org/content/162/6/3388

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Fine Specificity and MHC Restriction of Trinitrophenyl-Specific CTL

Alessandra Franco,* Takashi Yokoyama,† Dung Huynh,* Cole Thomson,‡ Stanley G. Nathenson,‡ and Howard M. Grey2* 

In this study, the fine specificity and MHC restriction of a CTL response specific to the trinitrophenyl (TNP) hapten was analyzed. Based on the structure of peptide/Kb complexes and ternary TCR/Ag/MHC complexes, four TNP peptides, two octamers, and two nonamers were chosen for eliciting anti-TNP CTL responses. Hapten was conjugated at position 4 in the octamers and at position 5 in the nonamers, positions which should allow engagement of the hapten by TCRs. Potent CTL activity for each of the TNP peptides was obtained that was highly hapten-specific; however, there were considerable differences in the extent of cross-reactivity with other TNP peptides, with the octamers generating more cross-reactive CTL than the nonamers. MHC restriction analysis suggested that anti-hapten responses were less dependent on MHC recognition than anti-peptide responses. This was evidenced by the relative ease of detecting cross-reactivity to haptenated peptides presented by allo-MHC and by the relative insensitivity of anti-hapten vs anti-peptide CTL to mutations in the Kb molecule at potential TCR interaction sites. One potential explanation for this insensitivity to MHC mutation was the finding that the anti-hapten response appeared to be of higher avidity, since a >100-fold difference in the amount of Ag required to sensitize target cells was found between these two types of Ags. The Journal of Immunology, 1999, 162: 3388–3394.

It is widely accepted that hapten-specific T lymphocytes can play an important role in immunopathology. In several allergic diseases, not only hapten-specific B cells but also T cells are pivotal. Nitrobenzene derivatives such as trinitrocholorobenzene are well known for their capacity to induce a contact hypersensitivity that is mediated by hapten-specific CD4+ and CD8+ T cells (1–3). CD4+ T cells specific for the β-lactam ring of penicillin have been generated from peripheral blood of allergic patients (4), and CD4+ and CD8+ T cells specific for the hapten urushiol play an important role in the hypersensitivity caused by contact with poison ivy or oak (5). Antireactive hapten-specific T cells may also play a role in certain autoimmune diseases. For instance, in a murine collagen arthritis model, the immunodominant epitope is a glycopeptide in which an oligosaccharide O linked to hydroxylsine is critical for T cell recognition (6).

Because of the unique structure of haptenated peptides that results in longer, bulkier side chains extending from the peptide backbone than are normally found, and because many haptenants represent unique chemical structures that are distinctively different from the natural endogenous peptides involved in thymic selection, we have, in this study, investigated several parameters of the T cell response to the extensively studied TNP hapten, including the role of the peptide backbone in defining the specificity, MHC restriction of the response, and relative avidity of the CTL for Ag/MHC complexes.

Materials and Methods

Mice
Female C57BL/6 mice (8–12 wk old) purchased from The Jackson Laboratory (Bar Harbor, ME) were used in all the experiments.

Peptide synthesis
The peptides used in this study were synthesized by Fmoc chemistry using a multiple peptide synthesizer (Symphony/Multiplex; Protein Technologies, Tucson, AZ). Peptides were cleaved automatically on the synthesizer using trifluoroacetic acid as a cleavage reagent. Peptides were >90% pure as assessed by C18 reverse phase HPLC, and the identity of the peptides was verified by mass spectrometry. TNP modifications were introduced by using e-N-TNP lysine derivatives for peptide synthesis (Bachem Bioscience, King of Prussia, PA).

MHC purification and MHC peptide binding assay
EL-4 cells were used as a source of Kb and Dd molecules, and RDM4 as a source of Kk molecules. Nonidet P-40 cell lysates from large-scale (1010 to 1011) cell cultures were filtered through 0.45-μm filters and purified by affinity chromatography as described (7). To measure peptide binding to MHC molecules, previously identified MHC binding peptides were 125I-radiolabeled (sequences vesicular stomatitis virus (VSV) NP 52–59 for Kb, Y240 substituted adenovirus, E1a 234–243 for Dd, and Y240 substituted Flu-HA 240–248 for Kk) and incubated with 5–10 nM of purified MHC molecules for 48 h in PBS containing 5% DMSO, 0.05% Nonidet P-40, and protease inhibitors. The Kb, Dd, and Kk complexes were subsequently separated from free peptide by gel filtration TSK columns (7). The binding of peptide to these MHC molecules was measured by their capacity to inhibit binding of the radiolabeled ligand. The affinity of the binding was estimated by determining the quantity of peptide required to inhibit by 50% (IC50) the binding of the radiolabeled peptide.

Generation and characterization of T cell lines and clones
To generate hapten-specific CTL, C57BL/6 mice were immunized s.c. at the base of the tail with 50 μg of TNP-conjugated peptide in IFA, together with 140 μg of a known Th epitope (hepatitis B virus surface Ag 128–140) (8). Two weeks after priming, mice were sacrificed, and splenocytes were...
The Journal of Immunology

3389

Table I. Kb binding peptides used for hapten conjugation

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Kb Binding Capacity (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEV</td>
<td>12</td>
</tr>
<tr>
<td>SEV*</td>
<td>13</td>
</tr>
<tr>
<td>VSV</td>
<td>14</td>
</tr>
<tr>
<td>VSV*</td>
<td>17</td>
</tr>
<tr>
<td>Poly-(A)(9)</td>
<td>4</td>
</tr>
<tr>
<td>Poly-(A)(9)*</td>
<td>4</td>
</tr>
<tr>
<td>Poly-(A)(8)</td>
<td>9</td>
</tr>
<tr>
<td>Poly-(A)(8)*</td>
<td>3</td>
</tr>
</tbody>
</table>

*Kb binding capacity was measured as the quantity of peptide required to inhibit by 50% the binding of a well-characterized radioiodinated Kb binding peptide.

**TNP*-lysine.

sentative data from a CTL line derived from mice primed with TNP-poly(A)9. Good CTL activity to the haptenated immunogen was observed, and no activity above the control was observed against the nonhaptenated peptide. These initial data clearly demonstrated that it was possible to generate a hapten-specific T cell response using as immunogen a high-affinity MHC binding peptide that was haptenated at a central position and that the predominant CTL population elicited was hapten-specific.

T cell lines generated against each of the four TNP peptides tested are shown in Fig. 2. Each line was assayed for its reactivity with the haptenated immunogen as well as the other three TNP peptides, along with a fourth haptenated Kb-restricted peptide, OVA 257–264. In general, cross-reactive recognition of all TNP peptides was observed; however, the different immunogens varied with respect to the extent of cross-reactivity elicited. T cell lines derived from mice immunized with the poly(A)8 (Fig. 2b) and the VSV peptide (also an octapeptide) (Fig. 2c) were more highly cross-reactive with the other TNP peptides than T cell lines generated against the two nonapeptides, poly(A)9 (Fig. 2a) and SEV (Fig. 2d). These data are quantitatively summarized in Table II, in which LUs obtained with each of the TNP peptides were calculated and compared with that of the immunogen.

One LU is defined as the number of effector cells required to obtain 30% lysis of target cells. In cases where 30% lysis was not reached, values were obtained by extrapolation. The number of LU contained in 10⁶ effectors cells can then be calculated by dividing the number of effectors required for 30% lysis into 10⁶. The number of effector cells required for 30% lysis can be derived from the data in Fig. 2, which plots E:T ratios, by multiplying the E:T ratio by 100.

FIGURE 1. TNP-specificity of CTL culture derived from TNP peptide-immunized mice. EL-4 cells (H-2b) were ⁵¹Cr-labeled and incubated with different numbers of T cells in the presence or absence of 1 μg/ml of TNP-poly(A)9 peptide (O), the nonhaptenated peptide (.), or no peptide (I). Cell culture supernatants were harvested 4 h later and ⁵¹Cr release calculated as [(sample-spontaneous release)/(maximum release-spontaneous release)] × 100.
Cross-reactive recognition by TNP-specific CTL of TNP-conjugated peptides

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>VSVa</th>
<th>Poly(A)b</th>
<th>SEVa</th>
<th>Poly(A)b</th>
<th>OVAa</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSVb</td>
<td>0.56</td>
<td>0.25</td>
<td>0.57</td>
<td>0.10</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Poly(A)b</td>
<td>0.14</td>
<td>—</td>
<td>0.06</td>
<td>0.78</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>SEVa</td>
<td>0.03c</td>
<td>0.24c</td>
<td>0.27</td>
<td>0.00c</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Poly(A)b</td>
<td>0.01c</td>
<td>0.26c</td>
<td>0.05c</td>
<td>0.00</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.06</td>
<td>0.35</td>
<td>0.12</td>
<td>0.54</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

LU ratios were calculated for the cross-reacting TNP-conjugated peptides. One LU is defined as the number of effector cells required to obtain 30% lysis of target cells. The number of lytic units contained in 10⁶ effector cells was then calculated and used as a measure of CTL activity in culture. The LU contained within each of the haptenated peptides were divided by the LU obtained with the immunogen to obtain a cross-reactivity index.

a TNP peptide.
b Cross-Reactivity to TNP Peptides

c Indicates LU values extrapolated.

MHC restriction

Haptenated peptides present a unique topography compared with conventional peptides bound in the Ag binding groove (the hapten is predicted to protrude further away from the α helices that make up the walls of the Ag binding groove compared with the side chains of the natural amino acids). Thus, it was of interest to determine the extent of MHC restriction demonstrated by the cytotoxic T cells specific for the TNP hapten. To study this, two kinds of experiments were performed: 1) The capacity of CTL to recognize K⁺ mutant molecules presenting TNP peptides; and 2) The capacity of hapten to be recognized by CTL when presented by MHC alleles other than the original MHC.

Ag presentation by mutant K⁺ molecules

A series of transfected cells containing mutant K⁺ molecules (and wild-type K⁺) were previously generated (10, 11). A subset of these mutants, which were predicted to have a mutation at a position in the α1 or the α2 helix that pointed up toward the TCR rather than into the peptide binding groove, were selected to assess the relative importance of MHC recognition by anti-TNP-specific CTL. Table IV lists the mutants studied and the position of the mutation in the K⁺ α-chain. These cells were used as targets for killing by TNP-specific CTL in the experiments described below.

Twelve T cell clones generated from mice that had been immunized with TNP-VSV, 7 T cell clones generated from mice that had the other TNP peptides tested, as exemplified by the data in Fig. 3c. Most of the clones exhibited intermediate patterns of cross-reactivity, reacting with 1 (2 of 12 clones), 2 (4 of 12 clones) or 3 (2 of 12 clones) of the 4 heterologous TNP peptides tested. One such intermediate pattern of cross-reactivity is shown in Fig. 3b. It is to be noted that in no instance was there any significant cross-reactivity with the corresponding nonhaptenated peptides.

FIGURE 2. Cross-reactive recognition by TNP-specific CTL of TNP conjugated to different peptide backbones. T cell lines were derived from mice immunized with the following TNP conjugated peptides: a, poly(A)b; b, poly(A)b; c, VSV; and d, SEV. The T cell lines were tested for their capacity to lyse the immunizing peptide and four other haptenated peptides: poly(A)b, poly(A)b, VSV, SEV, and OVA. The number of target cells in the assay (5×10⁶). For example, in Fig. 2b, the E:T ratio to achieve 30% lysis with the immunogen poly(A)b was 1.72. Thus, the number of LU is provided by the equation: [10⁶/1.72 × 5 × 10⁶] = 116.

Cross-reactivity between the immunogen and the other haptenated peptides was calculated as a ratio by dividing the LU obtained with the immunogen into the LU obtained with the other peptides tested. For instance, lysis of VSV targets by anti-poly(A)b CTL resulted in 15.9 LU of activity, giving a LU ratio relative to the anti-VSV activity of 0.14 (15.9/116). The data represented in this manner in Table II suggest the following conclusions.

Whereas the two octapeptides, VSV and poly(A)b, generated relatively highly cross-reactive CTL responses, with average cross-reactivity indices of 0.37 and 0.33, the indices of the two nonapeptides, SEV and poly(A)b, were only 0.13 and 0.08. Furthermore, when the data were analyzed with respect to which of the peptides tested for their capacity to cross-react, it was evident that the two poly(A)b peptides were the most cross-reactive, with average cross-reactive indices of 0.35 for the poly(A)b and 0.54 for the poly(A)b. In contrast, VSV had an average index of 0.06, SEV of 0.12, and OVA of 0.11.

The fine specificity of the hapten-specific response was further analyzed at the clonal level with a panel of T cell clones derived from three T cell lines specific for TNP-VSV, TNP-SEV, and TNP-poly(A)b. When analyzed collectively, the data obtained were consistent with the data described above for the T cell lines. As shown in Table III, T cell clones generated against TNP-VSV were more highly cross-reactive than the clones generated against TNP-SEV or TNP-poly(A)b. Also, TNP-poly(A)b and TNP-poly(A)b were more often recognized as cross-reactive Ags than TNP-SEV, TNP-VSV, or TNP-OVA. However, when individual clones specific for a given TNP peptide were analyzed, a great deal of variation in their cross-reactivity patterns was observed. Examples of this heterogeneity are shown in Fig. 3, which illustrates the cross-reactivity pattern of three TNP-VSV-specific clones. Of the 12 anti-TNP-VSV clones analyzed, 2 gave a pattern, as shown in Fig. 3a, that indicated good CTL activity with the immunogen and no cross-reactivity with any of the other TNP peptides. At the other extreme, 2 of 12 clones showed extensive cross-reactivity with all
been immunized with TNP-poly(A)9, and 10 T cell clones generated from mice that had been immunized with TNP-SEV were analyzed for their ability to recognize the haptenated peptide presented by the 10 K\textsuperscript{b} mutants in a 51 Cr release assay. For comparative purposes, 10 T cell clones generated from mice primed with the nonhaptenated SEV peptide were also analyzed. The results obtained with this series of clones are summarized in Table V.

When the anti-TNP clones were analyzed as a group, almost 50% of the clones studied (14 of 29) recognized the appropriate TNP peptide presented by all 10 of the K\textsuperscript{b} mutants. In contrast, none of the 10 anti-SEV peptide-specific clones recognized SEV presented by all mutants. Whereas the anti-TNP clones failed to recognize, on average, 0.6–2.5 mutants per clone, the anti-peptide clones failed to respond to Ag presented by 4.6 mutants per clone. When the three sets of anti-TNP clones were analyzed separately, there were considerable differences in their reactivity with the K\textsuperscript{b} mutants. Anti-TNP-SEV clones were most efficient at recognizing Ag presented by the K\textsuperscript{b} mutants, with 8 of 10 clones reacting with all K\textsuperscript{b} mutants. Anti-poly(A)9 clones were somewhat less capable of recognizing Ag in the context of the K\textsuperscript{b} mutants, and anti-TNP-VSV clones were the least promiscuous in their recognition. However, in comparison to the anti-peptide clones, each of these sets of anti-TNP clones were more cross-reactive, with the most striking difference being the comparison between the anti-TNP-SEV clones and the anti-SEV clones. It is to be noted that the data obtained with the anti-SEV peptide-specific clones are consistent with a previous study of two anti-SEV clones using the same panel of K\textsuperscript{b} mutants (11).

### Hapten recognition in the context of other MHC specificities

To further evaluate the role of MHC in the recognition of TNP, we investigated the possibility that CTL could be generated that recognized haptenated peptides presented by MHC molecules other than the MHC molecules used to present the immunizing peptide. The first set of experiments determined whether a TNP peptide capable of binding D\textsuperscript{b} (but not K\textsuperscript{b}) could be recognized by CTL generated against a TNP-conjugated K\textsuperscript{b} binding peptide. D\textsuperscript{b} was chosen for these experiments because it, like K\textsuperscript{b}, binds peptides with primary anchor residues at position 5 and the C terminus (although K\textsuperscript{b} preferentially binds octamers, whereas D\textsuperscript{b} binds nonamers) (13). To generate a TNP-D\textsuperscript{b} binding peptide, the poly(A) sequence AAAK*NAAAM was selected. Binding experiments indicated high-affinity binding to D\textsuperscript{b} (IC\textsubscript{50} = 1 nM) and no appreciable binding to K\textsuperscript{b} molecules (IC\textsubscript{50} > 50,000 nM). After an initial immunization with the TNP-K\textsuperscript{b} binding peptide poly(A)9\textsuperscript{*} (IC\textsubscript{50} K\textsuperscript{b} = 4 nM; IC\textsubscript{50} D\textsuperscript{b} > 50,000 nM), a very weak but detectable response was observed when target cells were incubated with the TNP-D\textsuperscript{b} binding peptide (Fig. 4a). However, following limiting dilution cloning with the TNP-D\textsuperscript{b} binding peptide, T cell clones could be generated that responded equivalently to the 10 K\textsuperscript{b} mutants in a 51Cr release assay. For comparative purposes, 10 T cell clones generated from mice primed with the nonhaptenated SEV peptide were also analyzed. The results obtained with this series of clones are summarized in Table V.

When the anti-TNP clones were analyzed as a group, almost 50% of the clones studied (14 of 29) recognized the appropriate TNP peptide presented by all 10 of the K\textsuperscript{b} mutants. In contrast, none of the 10 anti-SEV peptide-specific clones recognized SEV presented by all mutants. Whereas the anti-TNP clones failed to recognize, on average, 0.6–2.5 mutants per clone, the anti-peptide clones failed to respond to Ag presented by 4.6 mutants per clone. When the three sets of anti-TNP clones were analyzed separately, there were considerable differences in their reactivity with the K\textsuperscript{b} mutants. Anti-TNP-SEV clones were most efficient at recognizing Ag presented by the K\textsuperscript{b} mutants, with 8 of 10 clones reacting with all K\textsuperscript{b} mutants. Anti-poly(A)9 clones were somewhat less capable of recognizing Ag in the context of the K\textsuperscript{b} mutants, and anti-TNP-VSV clones were the least promiscuous in their recognition. However, in comparison to the anti-peptide clones, each of these sets of anti-TNP clones were more cross-reactive, with the most striking difference being the comparison between the anti-TNP-SEV clones and the anti-SEV clones. It is to be noted that the data obtained with the anti-SEV peptide-specific clones are consistent with a previous study of two anti-SEV clones using the same panel of K\textsuperscript{b} mutants (11).

### Table IV. K\textsuperscript{b} mutants used for MHC-restriction studies

<table>
<thead>
<tr>
<th>K\textsuperscript{b} Mutant</th>
<th>α-Chain Residue</th>
<th>Wild Type</th>
<th>Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>R8 347</td>
<td>166</td>
<td>E</td>
<td>K</td>
</tr>
<tr>
<td>R8 353</td>
<td>174</td>
<td>N</td>
<td>K</td>
</tr>
<tr>
<td>R8 10</td>
<td>167</td>
<td>W</td>
<td>R</td>
</tr>
<tr>
<td>R8 110.2</td>
<td>158</td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td>R8 341</td>
<td>162</td>
<td>G</td>
<td>D</td>
</tr>
<tr>
<td>R8 24</td>
<td>80</td>
<td>T</td>
<td>E</td>
</tr>
<tr>
<td>R8 208</td>
<td>82</td>
<td>L</td>
<td>F</td>
</tr>
<tr>
<td>R8 110.43</td>
<td>75</td>
<td>R</td>
<td>Q</td>
</tr>
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<td>K</td>
</tr>
<tr>
<td>R8 14</td>
<td>141</td>
<td>L</td>
<td>R</td>
</tr>
</tbody>
</table>

### Table V. Ability of TNP-specific T cell clones to recognize Ag presented by K\textsuperscript{b} mutants

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>n</th>
<th>0–1</th>
<th>1–2</th>
<th>2–3</th>
<th>3–4</th>
<th>4–5</th>
<th>5–6</th>
<th>6–7</th>
<th>7–8</th>
<th>Average\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV\textsuperscript{c}</td>
<td>12</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Poly(A)9\textsuperscript{c}</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>SEV\textsuperscript{c}</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0.6</td>
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<tr>
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<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>4.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} n = number of T cell clones analyzed.

\textsuperscript{b} Average number of mutations/clone that affect T cell recognition. LU were calculated for all mutant MHC and normalized to the LU generated with wild-type (wt) MHC. The numbers of T cell clones unable to recognize the hapten when presented by the K\textsuperscript{b} mutant (ratio to wt $<0.1$) are indicated.

\textsuperscript{c} TNP peptide.
TNP-D<sup>b</sup> binding peptide and the TNP-K<sup>b</sup>-binding peptide (Fig. 4b).

As a second system to study the recognition of TNP peptides presented by allo-MHC, we synthesized a K<sup>a</sup> binding TNP peptide (13) (AEAK<sup>a</sup>AAAI) and studied the capacity of anti-TNP/K<sup>a</sup>-restricted CTL to kill K<sup>a</sup>-expressing target cells (RDM4) sensitized with the TNP-K<sup>a</sup> binding peptide (Fig. 4c). In contrast to the very weak K<sup>d</sup>/D<sup>b</sup> cross-recognition by an early T cell clone derived from the bulk T cell line shown in a to kill EL-4 target cells in the presence of haptenated peptides either presented by K<sup>b</sup> molecules (○) or by D<sup>b</sup> molecules (△). The response to nonhaptenated poly(A)<sup>9</sup> (▲) is also shown. c, Bulk CTL culture of C57BL/6 (H-2<sup>b</sup>) spleen cells from mice immunized with the TNP-K<sup>b</sup> binding poly(A)<sup>8</sup> and cultured 7 days with the same peptide. The ability of the T cell line to kill H-2<sup>b</sup> (RDM4) targets presenting a TNP-K<sup>b</sup> binding peptide (□) was compared with its ability to present the TNP-K<sup>b</sup> binding poly(A)<sup>8</sup> (○).

Comparison of TCR avidity between TNP-specific and peptide-specific T cell clones

The data obtained comparing the capacity of anti-TNP and anti-peptide-specific clones to recognize Ag presented by the K<sup>b</sup> mutants suggested that the anti-TNP clones were less dependent on the integrity of the potential TCR contact residues in the K<sup>b</sup> mol-ecule than the anti-peptide clones. One possible explanation for this observation is that TNP peptide/MHC has a higher binding affinity for their respective TCRs than does peptide/MHC for their TCRs. If this were the case, then anti-TNP CTL would be expected to tolerate MHC mutations that affect the affinity of binding to TCR to a greater extent than anti-peptide CTL. To obtain information concerning this possibility, several anti-TNP-SEV clones and anti-SEV peptide clones were studied to determine the amount of Ag required to sensitize target cells for an equivalent degree of lysis. The data shown in Fig. 5 indicate a striking difference between these two sets of CTL clones, exemplified by the fact that the anti-TNP-specific clones required an average Ag concentration of 0.0263 ± 0.0145 nM to achieve 20% lysis, whereas the anti-SEV peptide-specific clones required 3.2898 ± 3.205 nM, a 125-fold difference (p < 0.027).

Discussion

In the present study, we examined several aspects of the immune response to haptenated peptides that bind with high affinity to class I MHC. We were particularly interested in characterizing the fine specificity of the CTL generated with respect to their capacity to recognize the hapten in the context of different peptide backbones, as well as determining the relative role of MHC recognition by the anti-hapten-specific CTL. TNP was chosen as the hapten because a considerable amount of data have been published on the CTL response to this particular hapten. For instance, Weltzien and colleagues (14, 15) demonstrated that it is possible to generate MHC class I- as well as MHC class II-restricted T cells either by priming in vitro with trinitrobenzene sulfonic acid-treated APCs or by immunizing with haptenated peptides. They also showed that clones could be isolated that were promiscuous for MHC recognition as well as cross-reactive on other TNP peptides (16–18).

The strategy we used for the positioning of the hapten and the choice of the peptide backbone was influenced by immunochemical studies showing that P4 and P6 of an octamer VSV peptide were immunodominant for TCR recognition (19). For instance, Weltzien and colleagues (14, 15) demonstrated that it is possible to generate MHC class I- as well as MHC class II-restricted T cells either by priming in vitro with trinitrobenzene sulfonic acid-treated APCs or by immunizing with haptenated peptides. They also showed that clones could be isolated that were promiscuous for MHC recognition as well as cross-reactive on other TNP peptides (16–18).

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backbones derived from previously described dominant T cell epitopes containing effective TCR-contact residues. Our a priori prediction was that the TNP polyalanine nonapeptide would be the best Ag for eliciting a hapten-specific CTL response, due to the lack of potent TCR-contact residues other than the hapten itself, as well as having the TNP moiety in a position that could bulge out to optimally interact with a TCR. The experimental data we obtained, however, did not confirm this prediction. All four of the TNP peptides tested elicited similar levels of CTL activity as measured by LU in bulk T cell cultures (data not shown). The degree to which the anti-TNP CTL cross-reacted with other TNP-binding peptides varied in the following order: VSV<sub>poly(A)8</sub>, SEV<sub>poly(A)9</sub>. This variation in cross-reactive recognition occurred despite the fact that in all instances there was little or no detectable recognition of the peptides’ backbones per se. Two possible explanations exist for the lack of more extensive cross-reactivity. First, detrimental residues in the peptide backbone of some of the potentially cross-reactive TNP peptides interfered with the effective engagement of the TNP peptide/MHC complexes with some TCRs. This explanation is supported by the finding that the two TNP poly(A) peptides were, in general, more cross-reactive than the two TNP peptides derived from known viral epitopes, since the alanine backbone would be unlikely to contain detrimental residues (22). Another explanation is that although the peptide backbone is not recognized in the absence of the hapten, amino acid side chains do contact the TCR and contribute to the overall binding energy. Regardless of the mechanism, the failure of some TNP peptides to elicit a broadly cross-reactive CTL response is an important consideration if an anti-hapten response is to be considered as part of a prophylactic or therapeutic vaccine strategy (for instance, in the generation of a glycopeptide-specific CTL response).

Two lines of evidence were obtained that suggested that the anti-TNP CTL response may be less dependent than conventional anti-peptide CTL on self-MHC recognition. First, it was relatively easy to demonstrate the presence of T cells capable of lysing cells sensitized with TNP peptides bound to allo-MHC molecules. Especially striking was the recognition of TNP peptides bound to K<sub>K</sub> where the CTL analysis suggested that 50% or more of the anti-K<sub>K</sub>-specific CTLs in a bulk culture could recognize TNP presented by K<sub>K</sub>. The second approach, using K<sub>β</sub> mutants, allowed for a detailed comparison between anti-TNP CTL and anti-peptide CTL for the requirement of self-MHC recognition. When CTL specific for an immunodominant SEV peptide were compared with TNP-specific CTL that used the same SEV peptide as a backbone for TNP conjugation, a striking difference in the sensitivity to K<sub>β</sub> mutations at potential TCR-contact sites was observed. Eighty percent (8 of 10) of the anti-TNP T cell clones analyzed were capable of lysing all 10 K<sub>β</sub> mutants, and the average effect of the K<sub>β</sub> mutants on the TNP-SEV-specific clones indicated that <1 mutation (0.6) per clone had a deleterious effect on CTL recognition. In contrast, none of the 10 SEV peptide-specific clones analyzed recognized all 10 K<sub>β</sub> mutants, and on average, almost 50% (4.6) of the 10 mutants had deleterious effects on CTL recognition. Although the reason for this striking difference has not been elucidated, one
potential mechanism is related to the apparent differences in affinity for Ag/MHC complexes detected between TNP-specific and SEV-specific clones. As estimated by the amount of Ag required for sensitization of target cells, there was a strong indication that the TNP-specific clones had a significantly higher affinity than the SEV-specific clones (Fig. 5). We postulate that the reason for this is the potential for the TNP hapten to interact extensively with side chains in the pocket formed by the CDR3α and the CDR3β segments, especially if multiple hydrogen bonds are established with the three NO2-groups of the TNP moiety. This high-affinity interaction could compensate for any potential decrease in affinity when the TNP peptide is presented by the mutant Kb molecules. That this mechanism may be involved is supported by the observation that Ag dose-response analysis indicates that 10- to 100-fold more TNP peptide was required to sensitize the Kb mutants for lysis than was needed for cells expressing the wild-type Kb (data not shown).

Acknowledgments
We thank Joyce Joseph for assistance in preparation of the manuscript.

References