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Avoidance of Self-Reactivity Results in Skewed CTL Responses to Rare Components of Synthetic Immunogens

Anthony W. Purcell, Weisan Chen, Nicholas J. Ede, Jeffrey J. Gorman, John V. Fecondo, David C. Jackson, Yuming Zhao, and James McCluskey

In studying the CTL recognition of peptide determinants derived from the nuclear Ag La (SS-B), we observed significant skewing of the response toward rare components present within the immunogen. Thus, priming of naive mouse lymphocytes in vitro with a synthetic H-2Kb-binding peptide comprising human La (hLa) residues 51–58 resulted in class I-restricted cytotoxic T cells that failed to recognize naturally presented hLa 51–58 peptide. Instead, the majority of T hybrids recognized a low abundance (≤1%) contaminant present at picomolar concentrations in the original synthesis and identified as a peptide adduct containing N,4-t-butyl asparagine at position 6 of the hLa 51–58 sequence. The preferred T cell recognition of the butyl adduct was not due to increased affinity of this peptide for the H-2Kb molecule or to the antagonism of CTL recognizing the unmodified determinant. Rather, the bias in the immune response appeared to be the result of partial self-tolerance to the homologous mouse La 51–58 determinant, which differs from its human counterpart by only a single amino acid at position 1 (T→I). Accordingly, the CTL response appeared to be focused on “non-self” ligands present within the synthesis, even though they were present at very low concentrations. These observations have significant implications for the use of synthetic peptide vaccines, especially those designed to manipulate responses to self peptides such as tumor Ags in which self-tolerance may result in unexpected reactivity.

Cytotoxic T lymphocytes recognize short peptides of 8 to 10 amino acids in length bound to class I MHC molecules expressed on the surface of most nucleated cells. These peptide Ags are generated within the cytoplasm of cells and are specifically transported into the endoplasmic reticulum to assemble with nascent class I MHC molecules (1). The peptides restricted to various class I allotypes contain specific binding motifs, and structural studies have revealed that the amino acid residues defined by these motifs make highly specific and conserved side chain contacts within pockets of the polymorphic binding cleft of the MHC molecule (2). Side chains from the solvent-exposed amino acid residues of bound peptides are thought to make contacts with the TCR expressed on the surface of specific CTL (3, 4).

The identification of these motifs (5) has allowed CTL epitopes to be predicted from the primary structure of candidate Ags and has kindled significant effort toward the development of peptide vaccines. Among the candidate vaccines are self peptides, which might serve as tumor Ags capable of eliciting protective antitumor CTL responses, and altered self peptides, which might modify T cell responses in autoimmune disease. T cells raised against synthetic peptides, however, frequently do not recognize native processed Ag (6, 7), suggesting that either unnatural CTL specificities are elicited by the synthetic immunogen or that Ag processing fails to reveal all potential epitopes of a protein. The potential for T cells to respond to contaminants within synthetic peptides is highlighted by recent studies demonstrating T cell recognition of post-translationally modified peptides that have either undergone glycosylation (8–10) or asparagine bond rearrangements (11) and nonpeptidic ligands (12–15). Despite the theoretical likelihood, the practical extent to which synthetic peptide immunogens might elicit T cells with aberrant or modified specificities is unclear.

Here, we demonstrate that a rare contaminant of a peptide derived from a H-2Kb-restricted determinant contained in the human La (SS-B) nuclear autoantigen (amino acid residues 51–58) dominates the response of T cell hybridomas resulting from T-T fusion of anti-peptide CTL. This contaminant was isolated by RP-HPLC and was structurally characterized by amino acid sequencing and mass spectroscopy. Retrospective synthesis of this species confirmed that a t-butylation Asn (ABu) residue at position 6 in the hLa 51–58 sequence determined the specificity of recognition by the majority of T hybridomas. The implications for immune responses toward impurities in synthetic peptide formulations are discussed. In addition, these findings are related to recent studies that highlight the importance of modified peptides in immune responses, particularly studies by Meadows et al. (16), who demonstrate T cell recognition of a H-Y-derived peptide with a post-translationally modified cysteine residue, and Skipper et al. (17), who demonstrate that a tumor Ag contains a deamidated Asparagine residue.

Abbreviations used in this paper: RP-HPLC, reversed phase HPLC; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; t-Boc, tertiary-butyloxycarbonyl; TFA, trifluoroacetic acid; F-moc, 9-fluorenylmethoxycarbonyl; Asu, aspartimide; ACN, acetonitrile; hLa, human La (SS-B); mLa, murine La (SS-B); ABu, N-t-butyl asparagine; m/z, mass to charge ratio; DME-10, DMEM with 10% FCS/5 × 10−5 M 2-ME/antibiotics/and 2 mM Gln; RPMI 1640 with 10% FCS/5 × 10−5 M 2-ME/antibiotics/and 2 mM Gln.
Materials and Methods

Cell culture and reagents. The thymoma cell line EL-4 (H-2b), the murine L cell line LTA-5 (H-2b), and the H-2Kb-transfected L cell I-3 (H-2b, H-2Kb) were cultured in DMEM with 10% FCS, 5 × 10⁻⁴ M 2-ME, antibiotics, and 2 mM Gln (DME-10). The H-2Kb-specific hybridomas (1F4 and 3B8) and hLa 51–58 minigene (11)-transfected I-3 cells (1-3-Mini2) were maintained in DME-10 plus 0.3 to 0.5 mg/ml G418 (Geneticin; Life Technologies, Grand Island, NY). CTL lines were primed and maintained in RPMI 1640 with 10% FCS, 5 × 10⁻⁴ M 2-ME, antibiotics, and 2 mM Gln (RP-10).

Peptide synthesis. The hLa 51–58 peptide (IMIKFNRNL) used in the initial in vitro priming experiments was synthesized using highly optimized t-Boc-based chemistry (18) with HBTU (2-benzotriazole-1,1,3,3-tetramethyluronium-hexafluorophosphate) as an activation agent. Following Boc deprotection, the peptide was cleaved from phenylacetamidomethyl (PAM) resin using the high hydrogen fluoride (HF) method (anhydrous hydrogen fluoride (HF) at 0°C for 1 h with cesol/thiocresol as scavengers) and then extracted into 10% acetic acid. All other peptides and analogues were synthesized using standard F-moc chemistry, purified by RP-HPLC, and their authenticity monitored by amino acid sequence analysis and mass spectrometry. The asparagine (Asn-6 hLa 51–58 and asparotinire (ACN-6) hLa 51–58 analogues were synthesized as described previously (19). The N-α-t-butylated Asn-6 hLa 51–58 peptide analogue (Abu-6 hLa 51–58) was synthesized using orthogonally protected F-moc-(Nα-t-buty1)-hLA 51–58) in place of Asn. The allyl-protected side chain carboxyl was deprotected with Pd(PPh₃)₄/Na₂BuSnH, and the t-buty aspartate amide was introduced by coupling 10 molar equivalents of t-buty amine/HATU/HOAt (2-1H-azabenzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluoro- phosphosphate/1-hydroxy-7-azabenzotriazole) and 20 molar equivalents of diisopropylethylamine overnight in dimethylformamide. The remainder of the peptide sequence was then completed, and the peptide was cleaved (5% thiouanisole/TFX) from the resin and purified by RP-HPLC.

Priming anti-peptide CTL in vitro and ⁵¹Cr release cytotoxicity assay

CTL were generated from spleen cells derived from 6- to 8-wk-old female C57Bl/6 mice (Animal Facility of Western Australia, Perth, Australia) using established methods. Responder cells were used in a standard 3.5-h ⁵¹Cr release assay on the fifth day after each restimulation (20).

Production of anti-peptide T cell hybridomas and assay

Peptide-specific T cell hybridomas were generated using established T-T fusion techniques (21). The hLa 51–58-primed CTL line was fused with BW5147.Lyt2.4, and stable hybridomas were assessed for further clonal specificity and dose-response experiments by measuring the [³H]thymidine incorporation of the IL-2-dependent cell line CTL1 (22) following 24 h of coculture with Ag-pulsed APC.

Amino acid sequencing, mass spectrometry, and chromatographic procedures

N-terminal automated Edman sequencing was performed on a Hewlett Packard G1000A protein sequencer (Hewlett Packard, Palo Alto, CA) using standard Edman chemistries. Electrospore ionization mass spectrometry was performed on a Perkin-Elmer-Sciex AP III triple quadrupole mass spectrometer (Perkin-Elmer-SCIEX, Ontario, Canada). MALDI-TOF MS was performed using a Bruker Reflex mass spectrometer (Bruker-Franzen Analytik, GMBH, Bremen, Germany) operated exclusively in the reflectron mode as described elsewhere (Gorman et al., manuscript in preparation). Preparative RP-HPLC was performed on a Waters (Bedford, MA) series 616 liquid chromatograph. Fractions were collected manually using a Frac 100 fraction collector (Pharmacia, Uppsala, Sweden), and purity was assessed by analytical RP-HPLC and mass spectrometry.

Results

Specific anti-hLa 51–58 CTL, which possess self-reactivity toward the mouse La 51–58 peptide, can be generated in vitro

In studies of the murine response to the human La (SS-B) molecule, a well-characterized human nuclear autoantigen (23), we raised CTL against a hLa peptide containing a conserved H-2Kb-binding motif (i.e., XXXXY/XYYXL). This peptide spans residues 51 to 58 of hLa (IMIKFNRL) and differs from the murine La analogue by only a single amino acid (T→I) at position 1. Thus, naive mouse T cells were primed in vitro by coinubcation with synthetic hLa 51–58-loaded syngeneic APC. The resulting CTL-lysed peptide pulsed target cells at concentrations <10⁻¹⁰ M of peptide, consistent with the sensitivity of many antiviral CTL. Importantly, the anti-hLa 51–58 CTL cross-reacted on the closely related murine La 51–58 peptide with approximately 20-fold less sensitivity than the hLa 51–58 peptide (Fig. 1a). Responder CD8⁺ T cells were immortalized by fusion of the anti-peptide CTL with a thymoma cell line (BW5147.Lyt2.4). Two different populations of T hybrids were obtained following this procedure (Fig. 1b). One population, represented by the T hybridoma 3B8, demonstrated sensitive recognition of the peptide immunogen (50% maximal response at 5 × 10⁻⁷ M). The other population, represented by the T hybridoma 1F4, was much less sensitive to the primary immunogen (50% maximal response at 5 × 10⁻⁷ M).

The dominant T hybridoma activity is not toward authentic hLa 51–58 peptide

T cell hybridomas 3B8 and 1F4 were tested against a cell line which constitutively presented hLa 51–58 peptide complexed to H-2Kb following transfection with a minigene encoding the hLa 51–58 determinant as a cytoplasmic peptide (11). As shown in Fig. 1c, the peptide-specific T cell hybridoma 1F4 recognized constitutively presented hLa 51–58 (I-3/Mini2). In contrast, the 3B8 T hybridoma was not activated in response to the constitutively presented hLa 51–58 peptide, a pattern that was typical of most T hybridomas recovered from the CTL line. Furthermore, 1F4 recognized I-3 cells pulsed with highly purified hLa 51–58 peptide, whereas 3B8 did not recognize these APC under the same conditions. These observations suggested that the prototypic T hybridoma 3B8 recognized another species present within the priming peptide and not the intended target peptide.

Fractionation of hLa 51–58 reveals 3B8 activity is associated with a minor synthesis by-product

To determine whether other species present within the priming hLa 51–58 peptide were responsible for stimulating the T hybrid 3B8, the priming peptide was fractionated by RP-HPLC (Fig. 2a), and individual fractions were screened for activity by the T cell hybridomas 1F4 (Fig. 2b) and 3B8 (Fig. 2c). 3B8 activity was not observed with the main hLa 51–58 peptide peak (MH⁺ = 1034 Da), as was the case for 1F4, but was associated instead with a minor species that eluted later than the parent peptide. The fraction containing the most 3B8 activity was further purified by RP-HPLC under different gradient conditions; the preparative chromatogram of this active fraction is shown in the boxed insert in Figure 2. Mass spectrometry of this fraction indicated the presence of two coeluting species with respective masses 17 Da less than (i.e., MH⁺ = 1017 Da) and 56 Da more than (i.e., MH⁺ = 1090 Da) the parent peptide. Despite further RP-HPLC of this fraction, the two species could not be resolved by changing the gradient conditions, ion-pairing agents, other mobile phase components, and the stationary phase ligand (data not shown). Since asparaginamide formation is a common side-reaction of t-Boc-based peptide synthesis (18, 24), we speculated that the species that was 17 Da less massive than the expected parental ion represented the loss of ammonia from the Asn residue yielding an asparaginamide residue at position 6 of the hLa 51–58 peptide. To test this idea, the asparaginate analogue of this peptide (Asn-6 hLa 51–58) was independently synthesized (19). While this species was chromatographically identical to the active fraction recognized by 3B8 (data not shown), it failed to stimulate this T hybridoma in an Ag presentation assay (see Fig. 3a). Similarly, the closely related ACN-6 (MH⁺ = 1018 Da) and Asp-6 hLa 51–58 (MH⁺ = 1035 Da) analogues also failed to stimulate 3B8. Thus, only crude hLa 51–58 preparations demonstrated activity. We therefore deduced that although the parent
Da species coeluting with 3B8 activity was likely to be a stable aspartimide derivative of hLa 51–58, this was not the ligand recognized by the 3B8 T hybridoma, which was likely to be the species that was 56 Da more massive than the parent peptide.

**FIGURE 1.** Specificity of CTL derived from in vitro priming with synthetic hLa 51–58 peptide. a, Anti-hLa 51–58 CTL cross-react with the mla 51–58 equivalent. 5 × 10⁷ spleen cells were pulsed with 10 μM of hLa 51–58 at 25°C for 30 min in RP-10, irradiated, and then washed. These peptide-pulsed APC were cocultured at 37°C with 5 × 10⁷ syngeneic spleen cells in an upright T25 flask in 10 ml of RP-10. Five days later, the CD4⁺ T cells were depleted, and 10⁵ in vitro primed responder cells were restimulated with 2.5 × 10⁶ Ag-pulsed syngeneic spleen cells in 24-well plates in RP-10 containing 10 U/ml of IL-2. These responder cells were used in a standard 3.5-h ⁵¹Cr release assay on the fifth day after each restimulation. Cytotoxicity of the line is shown over a range of peptide concentrations for both human and mouse La 51–58. b, Two populations of T hybrids resulted from T-T fusion of the anti-peptide CTL, one population of high responders is represented by the clone 3B8 (□), and another group of low responders by the clone 1F4 (■). T cell hybridomas were assessed for their clonal specificity and dose-responsiveness by measuring their IL-2 release following a 24-h coincubation with peptide-pulsed APC. c, The T cell hybridomas 3B8 and 1F4 were tested for recognition of a cell line that constitutively presented the hLa 51–58 peptide complexed to H-2Kb (I-3/Mini-2). T hybridomas were coincubated with either I-3, I-3/Mini-2, or I-3 cells loaded with 1 μM of highly purified hLa 51–58 peptide. IL-2 release was measured by the [³H]thyidine incorporation of the IL-2-dependent cell line CTLL. Incorporation is shown in cpm, and maximal stimulation, as assessed by Con A stimulation (10 μg/ml), is shown for each hybridoma.

3B8 recognizes hLa 51–58 peptide with a modified asparagine residue at position 6

To isolate the 56-Da adduct presumed to contain 3B8 activity from the mixed fraction, which also contained 177-Da aspartimide-modified peptide, we exploited the known base sensitivity of the latter species. Thus, the active fraction was treated with base, resulting in conversion of the aspartimide to a- and β-linked aspartate-containing species in a characteristic ratio of 3:1. These a- and β-linked aspartyl species eluted considerably earlier than the residual peptide (peak IV), which was resistant to base treatment under the same conditions and contained the activity recognized by 3B8 (Fig. 1c). The base-resistant active fractions were then evaluated by analytical RP-HPLC and mass spectroscopy and found to contain the expected homogeneous species of +56 Da relative to the predicted parent sequence. Analysis of this purified active species by Edman degradation revealed a sequencing anomaly during cycle 6 of the chemistry (data not shown). A phenylthiohydantoin (PTH) amino acid derivative was obtained in this cycle which eluted...
Asn residues with a 56-Da adduct. One potential adduct of 56 Da would represent the covalent attachment of a t-butylation group during deprotection; the formation t-butylation cations originating from the deprotection of the t-Boc group have been previously implicated in the t-butylation of peptides synthesized in this manner (24, 26). Thus, an analogue that represented an N,N4-t-butylation of Asn in hLa 51–58 was synthesized. After purification to homogeneity by RP-HPLC, the final product was shown to represent a single peptide species of MH+ = 1090 Da, which behaved identically to the active species during analytical RP-HPLC and Edman sequencing (i.e., having identical retention time and giving a false Met-6 assignment; data not shown) and was subsequently examined for H-2Kb-restricted recognition by the T cell hybridomas 3B8 and 1F4. The ABu-6 analogue of hLa 51–58 specifically activated the 3B8 hybridoma with approximately two logs greater sensitivity compared with the original hLa 51–58 peptide immunogen (Fig. 4a); this was consistent with the RP-HPLC and mass spectroscopic analysis of the crude hLa 51–58 immunogen, which indicated that a species of MH+ = 1090 Da was present at less than 1% of total peptide (data not shown). Thus, it appears that the ABu-6 hLa 51–58 analogue is potentially immunogenic and is preferentially recognized by T cells despite the presence of at least a 100-fold more of the authentic hLa 51–58 species.

The ABu-6 hLa 51–58 analogue was tested for binding to surface H-2Kb molecules by measuring its ability to stabilize conformational mAb epitopes on the surface of the mutant APC RMA-S. The modified peptide bound and stabilized H-2Kb molecules on the surface of RMA-S cells and reconstituted several mAb epitopes (data not shown), suggesting that the ABu-6 analogue specifically interacted with the Ag-binding cleft of H-2Kb with high affinity. The binding of ABu-6 hLa 51–58 to the H-2Kb molecule was compared with that of other H-2Kb-restricted peptides by assessing the relative concentrations of peptides required for stabilization of H-2Kb determinants on RMA-S cells (Fig. 4c). Our data suggest that the ABu-6 analogue of hLa 51–58 bound with essentially equivalent formation of an adduct conferring an additional 56 Da in mass upon the active species.

To confirm that the sequencing anomaly correlated directly with the mass difference of parent +56 Da, we performed postsource decay experiments using MALDI-TOF MS on the purified active material. These experiments, which are described in more detail elsewhere (Gorman et al., manuscript in preparation), revealed data consistent with N- and C-terminal fragment ion series of the peptide sequence, taking into account modification of the peptide with a moiety of 56 Da (see Table I). The N-terminal ion series contained a- and b-type ions as predicted for the unmodified hLa 51–58 sequence, which extended to the cleavage before Arg-7. In contrast, the C-terminal ion series contained y- and z-type ions, which encompassed all but the last three ion fragments. Of note was the observation that the C-terminal ions were 56 Da heavier than corresponding ions predicted for the unmodified sequence, up to and including the y3/z3 ion pair, and lost the residue mass of asparagine + 56 Da upon transition from the y3/z3 ion pair to the y2/z2 ion pair. This pattern of C-terminal fragment ions indicates that the Asn-6 residue was modified by a 56-Da adduct. The N-terminal ion b6, which suggested that Asn-6 was unmodified, may have arisen from cleavage of the adduct side chain in addition to cleavage along the peptide backbone. Further evidence for modification of Asn-6 with a 56-Da adduct was the observation of an immunoion for the modified asparagine residue (m/z = 143.2).

A N,N4-t-butylation of Asn in position 6 of hLa 51–58 binds H-2Kb and accounts for 3B8 activity

Based on this mass discrepancy at position 6 of the peptide, we retrospectively synthesized analogues of hLa 51–58 containing modified Asn residues with a 56-Da adduct. One potential adduct of 56 Da would represent the covalent attachment of a t-butylation group during deprotection; the formation t-butylation cations originating from the deprotection of the t-Boc group have been previously implicated in the t-butylation of peptides synthesized in this manner (24, 26). Thus, an analogue that represented an N,N4-t-butylation of Asn in hLa 51–58 was synthesized. After purification to homogeneity by RP-HPLC, the final product was shown to represent a single peptide species of MH+ = 1090 Da, which behaved identically to the active species during analytical RP-HPLC and Edman sequencing (i.e., having identical retention time and giving a false Met-6 assignment; data not shown) and was subsequently examined for H-2Kb-restricted recognition by the T cell hybridomas 3B8 and 1F4. The ABu-6 analogue of hLa 51–58 specifically activated the 3B8 hybridoma with approximately two logs greater sensitivity compared with the original hLa 51–58 peptide immunogen (Fig. 4a); this was consistent with the RP-HPLC and mass spectroscopic analysis of the crude hLa 51–58 immunogen, which indicated that a species of MH+ = 1090 Da was present at less than 1% of total peptide (data not shown). Thus, it appears that the ABu-6 hLa 51–58 analogue is potentially immunogenic and is preferentially recognized by T cells despite the presence of at least a 100-fold more of the authentic hLa 51–58 species.

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efficiency to the parent Asn-6 hLa 51–58 peptide and slightly less efficiently than OVA 257–264, a known high affinity ligand of the H-2K\textsubscript{b} molecule (K\textsubscript{D} \approx 1 \times 10^{-9} \text{ M}) (20). These findings suggested that the enhanced immunogenicity of the ABu-6 hLa 51–58 peptide was not due to more efficient binding of H-2K\textsubscript{b} when compared with the parental hLa 51–58 peptide.

**Discussion**

The identification of the modified ABu-6 hLa 51–58 CTL determinant explains the unusual activity of the majority of the T cell hybridomas generated by in vitro priming of naive murine T cells with synthetic hLa 51–58 peptide. The preferred recognition of this species over the parent peptide was unexpected, since the parent hLa 51–58 species represented 85% of the synthetic peptide, and CTL recognizing authentic self peptides or closely related analogues of self peptides have been generated in a number of studies (27). In attempting to understand the mechanism of skewing of the immune response toward the t-butyl-modified hLa 51–58 peptide, two possible explanations have been eliminated. First, the ABu-6 hLa 51–58 analogue does not bind the H-2K\textsubscript{b} MHC molecule with a significantly greater affinity than the parent peptide, bearing in mind that previous studies have shown a strong correlation between the affinity of peptide-MHC interaction and peptide immunogenicity (28) and the immunodominance of CTL determinants (20). Second, the activity of CTL lines raised against highly purified hLa 51–58 was not antagonized by the ABu-6 hLa 51–58 analogue (data not shown). It is therefore unlikely that the ABu-6 hLa 51–58 analogue antagonized the development of the unmodified hLa 51–58 specific CTL during the generation of the T-T hybrids. Interestingly, the CTL that could be raised against authentic hLa 51–58 were of insufficient sensitivity to lyse targets expressing intact hLa Ag (data not shown), suggesting that immune tolerance to mL 51–58 was limiting the anti-hLa 51–58 repertoire to CTL with low avidity TCR (i.e., with “1F4-like” phenotypes). In support of this contention, CTL lines raised against hLa 51–58 cross-reacted on the mL 51–58 determinant, indicating that hLa 51–58 behaved as a self Ag mimic (Fig. 1 and data not shown). Thus, it appears that the murine La equivalent comprising mL 51–58 (which only differs at the amino acid at position 1

### Table I. Post source decay of the active hLa 51–58 species reveals an Asn-6 adduct

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<td>-0.03</td>
</tr>
</tbody>
</table>

**FIGURE 4.** t-Butyl-modified Asn-6 hLa 51–58 peptide is recognized by the T hybridoma 3B8. *a.* The structure of the parental hLa 51–58 peptide (Asn-6) and the t-butyl-modified Asn-6 hLa 51–58 (ABu-6) analogue. *b.* Activation of 3B8 by highly purified hLa 51–58 ( ), the original preparation of hLa 51–58 ( ), and the ABu-6 analogue of hLa 51–58 ( ). 3B8 activation was assessed in a standard IL-2 production assay. Each point represents duplicate assays, and IL-2 release was determined as cpm. *c.* The ABu-6 hLa 51–58 analogue binds to H-2K\textsubscript{b} with equivalent efficiency to parental hLa 51–58. RMA-S cells (3 × 10\textsuperscript{5}) were incubated in 24-well plates in 0.4 ml of DME-10 for 12 to 14 h at 25°C; graded amounts of the ABu-6 hLa 51–58 analogue ( ), hLa 51–58 ( ), or OVA 257–264 ( ) were then added for 1 h before the cells were shifted to a 37°C incubator for two h to allow “empty” Kb molecules to be lost from the surface. The cells were then harvested, stained at 4°C by the Y-3 mAb, and analyzed by flow cytometry. Peptide stabilization of H-2K\textsubscript{b} is proportional to the observed increase in relative fluorescence.
Ile → Thr) is naturally presented in vivo and effectively eliminates from the T cell repertoire many of the high affinity CTL clones that may recognize hLa 51–58. This hypothesis would explain why T cells with unnatural or abiotic specificities may be preferentially expanded during in vitro priming or immunization, when the immunogen is closely related to a self peptide.

The T cell recognition of an exotic modification of the hLa 51–58 asparagine residue is consistent with the growing information on the plasticity of MHC binding and T cell recognition. Recent studies have revealed that CTL may recognize a variety of chemical functionalities and peptide adducts (10, 11, 14, 15). Consistent with this hypothesis are the recent observations by Meadows et al. (16) who demonstrate that peptides derived from the H-Y-specific Ag SMCY and recognized by HLA A*0201-restricted T cells contain modified cysteine residues, one peptide incorporating a dicysteinyl structure at position 7. This peptide was identified in fractionated peptides eluted from purified HLA A*0201 complexes and presumably represents a naturally processed and presented form of this Ag. Similarly, Skipper et al. (17) have identified a post-translationally modified, naturally processed and presented HLA A2-restricted peptide derived from a tyrosinase Ag and purified from a melanoma cell line. Interestingly, this peptide contained an aspartate residue that resulted from the amidation of a genetically encoded asparagine residue.

This study has revealed the potential of rare contaminants, originating from the synthesis or spontaneous degradation of peptides, to create novel ligands. The use of synthetic peptide vaccines to alter or enhance anti-self responses is currently receiving much attention because of its relevance to the development of antitumor immunity as well as to potential treatments for autoimmune disease. Here, we show that when synthetic peptides are used in the context of generating T cell responses toward self peptides or peptides very closely related to self, tolerance mechanisms may skew the response away from the intended ligand toward unwanted specificities. This study not only highlights the potential of impurities to act as surrogate immune targets, but previous studies of ours (11, 19) and others (16, 17) suggest that other spontaneously occurring modifications may occur in peptide ligands during processing, storage, or administration, which may lead to unwanted and potentially hazardous immune responses. The findings do not just signal a need for caution in using synthetic peptides to induce tumor autoimmunity. They also suggest that the identification of tumor-specific modifications to self Ag might provide opportunities for more effective tumor immunotherapy.

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References


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