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HER-2/neu and hTERT Cryptic Epitopes as Novel Targets for Broad Spectrum Tumor Immunotherapy

Antonio Scardino,²* David-Alexandre Gross,²* Pedro Alves,*, Joachim L. Schultzze,† Stéphanie Graff-Dubois,*, Olivier Faure,*, Sophie Tourdot,*, Salem Chouaib,† Lee M. Nadler,‡ François A. Lemonnier,‡ Robert H. Vonderheide,³† Angelo A. Cardoso,³† and Kostas Kosmatopoulos⁴*

Tolerance to tumor-nonautomated self proteins represents a major obstacle for successful cancer immunotherapy. Since this tolerance primarily concerns dominant epitopes, we hypothesized that targeting cryptic epitopes that have a low affinity for HLA could be an efficient strategy to breach the tolerance to tumor Ags. Using the P1Y heteroclitic peptide approach, we identified low affinity cryptic HLA-A*0201-restricted epitopes derived from two widely expressed tumor Ags, HER-2/neu and hTERT. The P1Y variants of four HER-2/neu (neu391, neu402, neu466, neu659) and two hTERT (hTERT727 and hTERT986)-derived low affinity peptides exhibited strong affinity for HLA-A*0201 and stimulated specific CTL from healthy donor PBMCs. These CTL specifically recognized HER-2/neu- and hTERT-expressing tumor cells of various histological origins. In vivo studies showed that HLA-A*0201 transgenic HHD mice vaccinated with the P1Y variant peptides generated CTL that specifically lysed Ag-expressing tumor cells, thus recognizing the cognate endogenous Ags. These results suggest that heteroclitic variants of low affinity, cryptic epitopes of widely expressed tumor Ags may serve as valid tools for tumor immunotherapy. The Journal of Immunology, 2002, 168: 5900–5906.

*Tumor-specific CTL recognize tumor Ag (TuAg) derived peptides complexed with MHC class I molecules. The large majority of the known human TuAgs are nonmutated self proteins that either are overexpressed by tumors or are developmentally regulated (1, 2). HER-2/neu and hTERT are two Ags that are particularly appealing for a broad spectrum immunotherapy because of their overexpression in a variety of tumors of different origins (3, 4). HER-2/neu is a 185-kDa transmembrane glycoprotein with tyrosine kinase activity and homology to the epidermal growth factor receptor (5). The HER-2/neu gene is present in normal cells as a single copy. In contrast, the HER-2/neu gene is amplified and the associated protein is overexpressed in breast, ovary, uterus, lung, renal, stomach, and pancreas cancers (6–8). TERT is a reverse transcriptase that maintains telomere length in rapidly dividing cells, a crucial mechanism for the unrestricted survival of cancer cells (9). Recently, we and others identified the hTERT as a widely expressed TuAg found in greater than 85% of all human cancers (10, 11). To date, multiple high affinity, dominant epitopes derived from these two Ags have been identified and used as targets of specific CTL responses (10–19).

However, like the majority of TuAgs, HER-2/neu and hTERT are also expressed on normal tissues, including the thymus (20). This raises the problem of the tolerance of their specific CTL repertoire, and consequently their inability to trigger a strong and efficient antitumor response, as it has been demonstrated for other TuAgs (21–25). Breaking tolerance to TuAgs is, indeed, one of the major goals of tumor vaccination (26). Tolerance to self Ags involves dominant epitopes and much lesser cryptic epitopes (26–28). Hence, we can hypothesize that the recruitment of the CTL repertoire specific for cryptic determinants should circumvent immunologic tolerance. Results obtained in the murine p53 and more recently in the transgenic adenocarcinoma mouse prostate mouse model fit in favor of this hypothesis (21, 24, 25). Cryptic tumor epitopes might, therefore, be considered good candidates for tumor immunotherapy provided that they are efficiently presented by tumor cells.

In this study, we used the heteroclitic peptide approach (29) to identify six low affinity cryptic HER-2/neu and hTERT epitopes presented by HLA-A*0201. These epitopes, in their P1Y heteroclitic form, stimulated CTL that specifically lysed HER-2/neu- or hTERT-expressing tumor cells of various histological origins.

Materials and Methods

Animals

HHD mice have been previously described (30). They are β2-microglobulin (β2m)−/−, D8−/− and express an HLA-A*0201 monochain composed of a chimeric heavy chain (α1 and α2 domains of HLA-A*0201 and the α3 and intracellular domains of D8) linked by its N terminus to the C terminus of the human β2m by a 15-aa peptidic arm.
Cell lines
Murine RMA/HHD and RMSA/HHD cells were obtained by transfection of RMA and RMS cells with the HHD construct (30). Human tumor cells used as targets for HER-2/neu-specific CTL were the HLA-A*0201 HER-2/neu MC1F-7 (breast cancer), HCT-116 (colon cancer), PUB/N (lung cancer) kindly provided by M. Chouaib (Institut Gustave Roussy, Villejuif, France), LAW (renal cell carcinoma) kindly provided by F. Triebe (Institut Gustave Roussy), and the HLA-A*0201 HER-2/neu ZR75.1 (breast cancer) and SUP/M2 (anaplastic lymphoma) kindly provided by C. Gambacorti (Istituto di Tumori, Milan, Italy). The level of HER-2/neu expression by these lines has been reported previously (18). Human tumor cells used as targets for hTERT-specific CTL were the HLA-A*0201 hTERT U266 (melanoma), 36 M (ovarian carcinoma), K029 (myeloma), and SKW6.4 (EBV-transformed lymphoblasts), and the HLA-A*0201 hTERT U2OS (osteosarcoma) and the HLA-A*0201 hTERT HSS (melanoma) and Calu-1 (lung cancer). The hTERT expression by these lines has been described previously (10). Unless indicated otherwise, the human cell lines were obtained from American Type Culture Collection (Manassas, VA) or as previously reported (10). The TAPI/2 mutant T2 cells and the NK-sensitive K562 cells were also used. All cells were grown in RPMI 1640 medium supplemented with 10% FCS.

Peptides
Peptides were synthesized by Syntem S.A. (Nimes, France).

Viruses
T39 (vac-neu HER-2/neu-expressing recombinant and TBC-Wy wild-type (vac-wt) vaccinia viruses were kindly provided by G. Mazarra (Therion Biologics, Cambridge, MA).

Generation of CTL in HHD mice
HHD mice were injected s.c. at the base of the tail with 100 μg peptide emulsified inIFA in the presence of 140 μg of the I-A<sup>a</sup>-restricted hepatitis B virus core-derived Th epitope (126–140: sequence TPOPYRFPNAIL). After 14 days, greater than 85% of the cells were CD19/CD80/CD86/FITC emulsified in IFA in the presence of 140 μg peptide (dissociation complex, DC) was determined as: percentage of specific lysis was determined as: percentage of specific lysis = (experimental release – spontaneous release)/maximal release – spontaneous release) × 100. Spontaneous release was always <20% of maximal release induced by 3 N HCl.

Measurement of peptide relative affinity to HLA-A*0201
T2 cells (3 × 10<sup>5</sup> cells/ml) were incubated with various concentrations of peptides ranging from 100 to 0.1 μM in serum-free RPMI 1640 medium supplemented with 100 ng/ml human β2M at 37°C for 16 h. Cells were then washed twice and stained with the BB7.2 mAb, followed by FITC-conjugated goat anti-mouse Ig mAb to quantify the expression of HLA-A*0201. For each peptide concentration, the HLA-A*0201-specific staining was calculated as the percentage of the staining obtained with 100 μM of the reference peptide HIPv1039 (IgGAEFFVY). The relative affinity (RA) is determined as: RA = concentration of each peptide that induces 20% of HLA-A*0201-expression/concentration of the reference peptide that induces 20% of HLA-A*0201 expression. The lower the RA value, the stronger is the peptide binding to HLA-A*0201. The definitive RA value for each peptide was determined from at least three independent experiments. In all experiments, 20% of HLA-A*0201 expression using the reference peptide was obtained at 1–3 μM.

Assessment of peptide/HLA-A*0201 complex stability
T2 cells (10<sup>4</sup>ml) were incubated overnight with 100 μM of each peptide in serum-free RPMI 1640 medium supplemented with 100 ng/ml β2M at 37°C. Cells were then washed four times to remove free peptides, incubated with brefeldin A (10 μg/ml) for 1 h to block cell surface expression of newly synthesized HLA-A*0201 molecules, washed, and incubated at 37°C for 0, 2, 4, 6, or 8 h. Subsequently, cells were stained with the BB7.2 mAb, followed by FITC-conjugated goat anti-mouse Ig mAb. For each time point, peptide-induced HLA-A*0201 expression was calculated as: mean fluorescence of peptide-preincubated T2 cells – mean fluorescence of T2 cells treated in similar conditions in the absence of peptide. DC<sub>50</sub> (dissociation complex, DC) was defined as the time required for the loss of 50% of the HLA-A*0201/peptide complexes stabilized at t = 0. The definitive DC<sub>50</sub> value for each peptide was determined from at least three independent experiments.

Results
Low affinity HER-2/neu and hTERT peptides
Four previously described HER-2/neu peptides with low affinity for HLA-A*0201 (neu<sub>466</sub>, neu<sub>391</sub>, neu<sub>402</sub>, neu<sub>660</sub>, and neu<sub>580</sub>) (18, 29) were used in this study. Two hTERT peptides predicted by the Bioinformatics and Molecular Analysis Section algorithm (32) to have low HLA-binding affinity were also included. Their capacity to bind and stabilize HLA-A*0201 molecules was assessed using the T2-binding assay. As shown in Table I, all these peptides form unstable complexes with HLA-A*0201 (DC<sub>50</sub> < 2 h). Analysis of the relative binding affinity showed that neu<sub>466</sub>, neu<sub>391</sub>, and hTERT<sub>727</sub> and hTERT<sub>988</sub> were weak binders to HLA-A*0201 (RA > 5).

### Table I. HLA A2.1 affinity of HER-2/neu and hTERT peptides

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Sequence</th>
<th>RA&lt;sub&gt;50&lt;/sub&gt;</th>
<th>DC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>KIFGSLAF</td>
<td>2.3</td>
<td>6</td>
</tr>
<tr>
<td>neu&lt;sub&gt;466&lt;/sub&gt;</td>
<td>TLEIEGTYL</td>
<td>19.0</td>
<td>&lt;2</td>
</tr>
<tr>
<td>neu&lt;sub&gt;391&lt;/sub&gt;</td>
<td>PLQPEQLOV</td>
<td>&gt;70</td>
<td>2</td>
</tr>
<tr>
<td>neu&lt;sub&gt;402&lt;/sub&gt;</td>
<td>PLTSIISAV</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>neu&lt;sub&gt;580&lt;/sub&gt;</td>
<td>ALIHHTNL</td>
<td>4.8</td>
<td>2</td>
</tr>
<tr>
<td>hTERT&lt;sub&gt;740&lt;/sub&gt;</td>
<td>ILAKFLHWL</td>
<td>0.5</td>
<td>6</td>
</tr>
<tr>
<td>hTERT&lt;sub&gt;727&lt;/sub&gt;</td>
<td>RLFFYRKSV</td>
<td>25.3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>hTERT&lt;sub&gt;988&lt;/sub&gt;</td>
<td>DLQVNSLQT</td>
<td>28.6</td>
<td>2</td>
</tr>
<tr>
<td>Heteroclitic</td>
<td>TLEIEGTYL</td>
<td>3.6</td>
<td>4</td>
</tr>
<tr>
<td>LQPEQLOV</td>
<td>1.3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>YLTSIISAV</td>
<td>0.2</td>
<td>4–6</td>
<td></td>
</tr>
<tr>
<td>YLHIHTNL</td>
<td>1.4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>YLFYRKSV</td>
<td>2.2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>DLQVNSLQT</td>
<td>2.1</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The RA is determined as described in Materials and Methods.
<sup>b</sup> DC<sub>50</sub> was defined as the time required for the loss of 50% of the HLA-A*0201/peptide complexes stabilized at t = 0.
<sup>c</sup> Results of RA and DC<sub>50</sub> have been published previously (see Refs. 18 and 29).
whereas the peptides neu_{466} and neu_{650} exhibited a strong binding affinity (RA < 5).

**Generation of CTL against low affinity peptides**

The generation of specific CTL is a prerequisite for the identification of CTL epitopes. However, low affinity peptides are generally nonimmunogenic in either humans or HLA-A*0201 transgenic mouse models (33).

To generate CTL against low affinity peptides, we used the P1Y heteroclitic peptide approach we described previously (29). It consists of substituting the amino acid at position 1 with a tyrosine. Indeed, P1Y variants of neu_{391}, neu_{402}, neu_{466}, neu_{650}, hTERT_{572}, and hTERT_{988} exhibited a high affinity (RA < 5 and DC_{50} ≥ 4 h), fulfilling the criteria of peptides predicted to be immunogenic (Table I). We then used the heteroclitic P1Y variants of the low affinity HER-2/neu and hTERT peptides to induce CTL from the PBMC of healthy donors. Four to five donors were tested for each peptide. The generation of CTL was evaluated on cognate peptide-pulsed T2 cell targets after the fourth in vitro restimulation. Peptide-specific CTL were generated from PBMC stimulated with all these heteroclitic peptides. CTL killed T2 cells pulsed with the cognate peptide, but not T2 cells pulsed with an irrelevant peptide (HIVgag_{76} or MAGE3_{271}) (Fig. 1).

**Low affinity HER-2/neu and hTERT peptides are naturally processed by tumor cells expressing the relevant TtAg**

To evaluate whether these low affinity HER-2/neu and hTERT peptides are naturally processed and presented by tumor cells, peptide-specific CTL were tested for their ability to lyse HER-2/neu- or hTERT-expressing HLA-A*0201− tumor cells. For the HER-2/neu-derived peptides, CTL generated were tested as effector cells in cytotoxic assays using MCF-7 (HLA-A*0201, HER-2/neu), ZR75.1 (HLA-A*0201, HER-2/neu), and K562 (NK-sensitive) cells as targets. As shown in Fig. 2A for a representative case, CTL specific for neu_{391Y}, neu_{402Y}, neu_{466Y}, and neu_{650Y} lysed the MCF-7 cells, but not the ZR75.1 nor the K562 cells. Similarly, for the hTERT-derived peptides, CTL generated by stimulation with hTERT_{572Y} and hTERT_{988Y} lysed the SKW6.4 cells (HLA-A*0201, hTERT+), but failed to lyse the U2OS (HLA-A*0201+hTERT+) and the K562 cells (Fig. 2B). We further demonstrated that the MCF-7 lysis by HER-2/neu-reactive CTL involves the specific recognition of the HLA-A*0201/neu peptide complexes in cold target inhibition experiments. MCF-7 killing was inhibited by T2 cells loaded with the corresponding native HER-2/neu peptide, but not by T2 cells loaded with the irrelevant HIVgag_{76} peptide (Fig. 3). Inhibition was dependent on the cold-hot target ratio.
These studies demonstrate that the low affinity, cryptic epitopes neu\textsubscript{369}, neu\textsubscript{466}, neu\textsubscript{650} and hTERT\textsubscript{572} are naturally processed and presented by human tumor cells expressing the relevant TuAg.

**Low affinity HER-2/neu and hTERT epitopes are presented by tumor cells of various histological origins**

HER-2/neu and hTERT are appealing TuAgs because of their wide expression on multiple cancers. To determine the potential of low affinity, cryptic HER-2/neu and hTERT epitopes for broad spectrum immunotherapy, we tested the capacity of CTL induced by the PY1 variants of these epitopes to lyse malignant cells derived from tumors of different histological origins.

For the HER-2/neu epitopes, CTL specific for the four cryptic epitopes were used as effectors, as well as CTL generated using the dominant peptide neu\textsubscript{650} (12). The CTL were tested for their capacity to lyse the HLA-A*0201\textsuperscript{+} HER-2/neu\textsuperscript{+} MCF-7, HCT116, LAW, and PUB/N cells. These cell lines express variable amounts of HER-2/neu (18). The HLA-A*0201\textsuperscript{+} HER-2/neu\textsuperscript{+} cell lines ZR75.1 and SUP/M2 were used as controls. As shown in Fig. 4, CTL generated using the PY1 variants of the cryptic epitopes lysed all HER-2/neu\textsuperscript{+} tumor cells (black symbols) irrespective of their tissue of origin, including those with low expression of HER-2/neu (LAW) (18). Importantly, these CTL showed a cytotoxic efficacy equivalent to that mediated by neu\textsubscript{650} specific CTL (Fig. 4, donors 1 and 2), thus demonstrating that presentation of low affinity epitopes does not require high expression of the endogenous Ag.

A similar analysis was performed for the hTERT-derived epitopes (Fig. 5). Four HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} tumor cells (U266, 36 M, KO29, and SKW6.4) were used as targets for CTL specific for the two hTERT cryptic epitopes or the dominant peptide hTERT\textsubscript{540} (10, 11). The HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cell line U265 and two HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cells (HSS and Calu-1) were used as negative controls. CTL lysed all the HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cells (filled symbols) irrespective of their histological origin. In contrast, they did not lyse targets that exclusively express hTERT or HLA-A*0201 (open symbols).

**FIGURE 3.** Tumor cell killing by HER-2/neu-specific CTL involves specific HLA-A*0201/neu peptide recognition. CTL were generated by in vitro stimulation of CD8\textsuperscript{+} PBMC with P1Y heteroclitic low affinity HER-2/neu peptides. They were tested for cytotoxicity against MCF-7 cells in the presence of cold T2 cells loaded with the corresponding native HER-2/neu peptide (○) or with the irrelevant HIVgag\textsubscript{26} peptide (△). Effector-hot target ratio: 40:1.

**FIGURE 4.** Recognition by HER-2/neu peptide-specific CTL of tumor cells of various origins. CTL were generated by in vitro stimulation of CD8\textsuperscript{+} PBMC from four healthy donors with P1Y variants of HER-2/neu peptides and tested for cytotoxicity against the HLA-A*0201\textsuperscript{+} HER-2/neu\textsuperscript{+} ZR75.1 (○), SUP/M2 (□), and the HLA-A*0201\textsuperscript{+} HER-2/neu\textsuperscript{+} MCF-7 (○), HCT-116 (■), LAW (▲), and PUB/N (▼) targets.

The capacity of the cryptic HER-2/neu and hTERT epitopes to induce in vivo tumor-specific CTL was assessed using the HLA-A*0201-transgenic HHD mouse model. Mice were vaccinated with the native peptides and their P1Y heteroclitic variants. After 11 days, their spleens were removed and the splenic cells were restimulated in vitro with the corresponding native peptide. Results in Fig. 6 show that native peptide-vaccinated mice did not develop CTL, confirming previous results showing that low affinity peptides are not immunogenic in HHD mice (29). In contrast, CTL were generated in mice vaccinated with HER-2/neu (Fig. 6A) and hTERT (Fig. 6B) heteroclitic peptides. These CTL lysed RMAS/HHD targets pulsed with the cognate and the P1Y heteroclitic, but not with vac-wt vaccinia virus. These studies indicate that low affinity, cryptic HER-2/neu and hTERT epitopes are presented by tumor cells of different types and may serve as targets for broad spectrum antitumor immunotherapeutic strategies.

**P1Y heteroclitic low affinity HER-2/neu and hTERT peptides induce in vivo tumor-specific CTL**

CTL specific for the cryptic epitopes and those specific for the dominant epitope hTERT\textsubscript{540} recognized hTERT\textsuperscript{+} tumor cells with equivalent efficacy (Fig. 5, donors 1 and 2).

These studies indicate that low affinity, cryptic HER-2/neu and hTERT epitopes are presented by tumor cells of different types and may serve as targets for broad spectrum antitumor immunotherapeutic strategies.

**FIGURE 5.** Four HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} tumor cells (U266, 36 M, KO29, and SKW6.4) were used as targets for CTL specific for the two hTERT cryptic epitopes or the dominant peptide hTERT\textsubscript{540} (10, 11). The HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cell line U265 and two HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cells (HSS and Calu-1) were used as negative controls. CTL lysed all the HLA-A*0201\textsuperscript{+} hTERT\textsuperscript{+} cells (filled symbols) irrespective of their histological origin. In contrast, they did not lyse targets that exclusively express hTERT or HLA-A*0201 (open symbols).
endogenous HER-2/\(\text{neu}\) recognition. To evaluate the endogenous TERT recognition by TERT-specific CTL, we used the HLA-A*0201/H11001 mTERT/H11001 RMA/HHD cells as targets, since the hTERT 572 and hTERT 988 epitopes are identical in mouse and human TERT sequences. CTL induced in mice vaccinated with hTERT 572Y and hTERT 988Y lysed RMA/HHD cells, but failed to lyse TERT/H11001 HLA-A*0201-negative RMA cells (Fig. 7B).

These studies show that the P1Y heteroclitic variants of low affinity HER-2/\(\text{neu}\) and TERT epitopes are immunogenic, and induce, in vivo, CTL capable of specifically lysing tumor cells expressing the relevant TuAg.

**FIGURE 5.** Recognition by hTERT peptide-specific CTL of tumor cells of various origins. CTL were generated by in vitro stimulation of CD8\(^+\) PBMC from three healthy donors with P1Y variants of hTERT peptides and tested for cytotoxicity against the HLA-A*0201\(^+\) hTERT\(^+\) U2OS (\(\square\)), the HLA-A*0201\(^+\) hTERT\(^+\) HSS (\(\bigcirc\)), Calu-1 (\(\triangle\)), and the HLA-A*0201\(^+\) hTERT\(^+\) U266 (\(\bullet\)), 36 M (\(\blacksquare\)), SKM6.4 (\(\blacktriangle\)), and KO29 (\(\blacktriangledown\)) targets.

**FIGURE 6.** In vivo generation of peptide-specific CTL in HHD mice. HHD mice were vaccinated with the native and the P1Y heteroclitic low affinity HER-2/\(\text{neu}\) (A) and hTERT (B) peptides, and their spleen cells were in vitro stimulated with the cognate native peptides, as described in Materials and Methods. CTL were tested for cytotoxicity against RMA/HHD targets coated with an irrelevant HIV-gag\(\text{gag}_{76}\) (\(\bigcirc\)), the cognate native (\(\bullet\)), and the P1Y heteroclitic (\(\blacksquare\)) peptide.

**Discussion**

This work aimed at determining whether cryptic epitopes derived from widely expressed TuAgs can serve as targets of antitumor CTL and whether they have potential for use in tumor immunotherapy. We identified six low affinity HLA-A*0201-restricted epitopes of the ubiquitous HER-2/\(\text{neu}\) and hTERT TuAgs and showed that they are targets of antitumor CTL. These epitopes were presented by various tumor cells irrespective of their histological origin and the level of Ag expression. Moreover, we showed that these epitopes used as heteroclitic P1Y variants induce a tumor-specific CTL response in vivo in HLA-A*0201 transgenic HHD mice.

This observation, if extended to low affinity epitopes of other widely expressed TuAgs, is of great importance for a broad spectrum tumor immunotherapy. In fact, the majority of TuAgs, including HER-2/\(\text{neu}\) and hTERT, corresponds to nonmutated self proteins overexpressed in tumors, and the CTL repertoire against their high affinity immunodominant epitopes may be tolerized. Such tolerance mainly concerns high avidity CTL. This has been demonstrated in HLA-A*0201/K\(^b\) mice for HLA-A*0201-restricted p53-specific and tyrosinase-specific CTL (21–23), and more recently in HHD mice for the HLA-A*0201-restricted CTL specific for the mTERT (D.-A. Gross, unpublished observations).

Low affinity cryptic epitopes are weakly or not at all involved in tolerance induction. For instance, B6 mice possess a fully functional CTL repertoire against low affinity D\(^b\)-restricted mgp100 and K\(^b\)-restricted gp75 epitopes (25, 34). Moreover, in the transgenic adenocarcinoma mouse prostate model, specific CTL can be generated against a subdominant/cryptic SV40T epitope, but not against a dominant SV40T epitope (24). Given that the efficacy of tumor immunotherapy depends on the avidity of recruited CTL (35, 36), these results point out the potential of using low affinity tumor epitopes, provided that they are able to mobilize their specific CTL repertoire and that they are presented by tumor cells efficiently enough to be recognized by CTL.

An effective strategy to mobilize CTL targeting low affinity epitopes is the use of heteroclitic variants that have a higher affinity and more stable binding to HLA molecules (25, 34, 37). We have
previously described two heteroclitic variant approaches that enabled the generation of CTL directed to low affinity \( D^b \)-restricted and HLA-A*0201-restricted epitopes (29, 37). Heteroclitic variants of low affinity \( D^b \) epitopes from flu nucleoprotein induced a potent protective immunity against lethal challenge of mice with flu virus (37). In this study, we demonstrate in HHD mice that this strategy can be used to mobilize antitumor CTL specific for cryptic HER-2/neu and hTERT epitopes. We are presently evaluating the potential of \( D^b \) heteroclitic peptides as tumor vaccines in HHD mouse tumor models.

The low affinity peptides we described are presented by tumor cells at a level sufficient to be targets of antitumor CTL, an observation that raises several issues. First, low affinity HER-2/neu and hTERT epitopes are presented by tumor cells of different origins; they can, therefore, be used alone or in association with other previously described HER-2/neu and hTERT peptides for a broad spectrum immunotherapy (38–40). The vaccine potential of a polyepitopic vector containing the four HER-2/neu \( D^b \) variant along with intermediate or high affinity HER-2/neu epitopes described by us and by others is currently being studied in HHD mice. Second, low affinity epitopes, even those with the lowest affinity, such as neu403 and neu405, are efficiently presented by tumor cells expressing low amounts of HER-2/neu such as LAW. The level of HER-2/neu expression is not, therefore, a barrier to the use of low affinity epitopes for tumor immunotherapy. This is most likely explained by the fact that CTL effectors require a small number of peptide/HLA complexes on the target surface to be activated (41). Third, six of six low affinity peptides we have studied in this work are naturally processed by tumor cells. Although the small number of peptides does not permit any conclusion about the diversity of the epitopic repertoire of an Ag, these results suggest that the epitopic repertoire contains a very large number of cryptic epitopes. This is strengthened by our previous observation that three of five flu nucleoprotein-derived peptides with low or intermediate \( D^b \) affinity tested are processed by flu-infected murine cells (42) and by recent results showing that low HLA-A*0201 affinity peptides from MAGE are presented by tumor cells. It is noteworthy that all these peptides had the appropriate HLA primary anchor motifs (PSN and P9 M for \( D^b \) molecule; P2L and P9/10V/L for HLA-A*0201 molecule). This raises the question of whether the presence of anchor motifs is by itself sufficient to ensure a natural presentation independently of the overall HLA affinity. Investigation of a large panel of randomly selected peptides having the anchor motifs or not and exhibiting variable affinities is necessary before drawing any definitive conclusion.

In summary, we provide results demonstrating that low affinity tumor epitopes for HER-2/neu and hTERT are efficiently presented by tumor cells and can, therefore, be used for tumor immunotherapy.

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References


