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The Use of HLA A2.1/p53 Peptide Tetramers to Visualize the Impact of Self Tolerance on the TCR Repertoire

Javier Hernández,* Peter P. Lee,† Mark M. Davis,‡ and Linda A. Sherman2*

p53 is an attractive target for cancer immunotherapy since it is overexpressed in half of all tumors. However, it is also expressed in normal lymphoid tissue, and self tolerance leaves a p53-specific repertoire purged of high avidity CTL. To better understand the mechanism of tolerance and the basis for such low avidity interaction, p53-specific CTL from p53 deficient (p531) and sufficient (p53*) A2.1/Kb transgenic mice were compared with respect to their ability to bind HLA-A2.1 tetramers containing cognate murine p53 peptide Ag, p53 261–269. Since the murine CD8 molecule cannot interact with human HLA-A2.1, this tests the ability of the TCR to bind the A2.1/peptide complex tetramer. CTL from p531 mice demonstrated strong binding of such A2.1/p53 tetramers; however, the CTL from tolerant p53* mice were devoid of tetramer-binding CD8+ T cells. Examination of TCR expression at the clonal level revealed that CTL from p531 and p53* mice each expressed comparable levels of the p53-specific TCR. These results indicate that normal expression of p53 promotes elimination of T cells expressing TCRs with sufficient affinity to achieve stable binding of the A2.1/p53 tetramers. The Journal of Immunology, 2000, 164: 596–602.

To avoid autoimmunity, the immune system has developed multiple strategies by which T cells can achieve tolerance to self Ags. The issue of self tolerance has increasingly become of concern in tumor immunology, since many promising candidates for tumor Ags are derived from proteins that are also expressed on normal tissue (1–5). However, not much information is available concerning the extent to which self tolerance has shaped the repertoire specific for these tumor Ags.

The first and arguably most effective form of self tolerance is thymic deletion, by which thymocytes recognizing self peptides undergo apoptosis (6–8). This process, however, is highly dependent on the avidity of T cell recognition and the amount of epitope presented in the thymus (9–12). As a consequence, many T cells that recognize self epitopes with an avidity below a certain threshold are permitted access to the periphery (13–15). In addition to thymic tolerance, antigenic encounter in the periphery, in the absence of the appropriate immunostimulatory environment, can lead to deletion or anergy (16–21). However, the presence of the Ag in the periphery does not always affect T cells, and some may remain ignorant of Ag (22). Again, this is likely to be determined by T cell avidity and the amount of Ag presented in the periphery.

We and others have been studying the feasibility of targeting p53 as a tumor Ag since it is overexpressed in more than half of all human tumors (23). However, p53 is expressed in normal tissues, including thymus, spleen, and lymphohemopoietic cells (24–26), and may act as a tolerogen during thymic development and in the periphery (5, 27). Previously, we compared the HLA-A2.1-restricted response to p53 epitopes in p531 deficient (p531) and sufficient (p53*) HLA-A2.1/Kb transgenic mice (27). The effect of tolerance varied for different peptides. CTL specific for the epitope spanning residues 187–197 were completely eliminated in p531 A2.1/Kb mice (27). In contrast, a CTL response specific for the 261–269 epitope was detected in p531 mice; however, it was of low avidity as compared with CTL from p53* mice and required more than 10-fold more peptide to achieve the same levels of lysis (27). This low avidity repertoire is likely to represent the only CTL precursors available for activation by a putative vaccine or immunotherapeutic agent targeting p53 and, as such, merits further examination.

In this report we have used A2.1/peptide tetramers for further characterization of the residual low avidity A2.1/Kb-restricted p53 261–269-specific CTL by comparing the ability of CTL from p531 and p53* A2.1/Kb mice to bind A2.1/p53 tetramers (28, 29). The results demonstrate that this self tumor Ag promotes functional deletion of the CD8+ T cells carrying TCRs with the highest affinity for p53, resulting in CTL incapable of stable binding to the tetramer complexes.

Materials and Methods

Mice

All mice used in these studies are on a C57BL/6 background. p531 A2.1/Kb and p53* A2.1/Kb transgenic mice have already been described (27, 30). p531 mice were obtained from Tyler Jacks (Massachusetts Institute of Technology, Cambridge, MA) and mated with A2.1/Kb transgenics. Progeny were interbred, and offspring were screened for mice that were p531−/− and expressed A2.1/Kb. C57BL/6 mice were purchased from the breeding colony of The Scripps Research Institute. Mice were propagated and maintained under specific pathogen-free conditions in our vivarium at The Scripps Research Institute. All experimental procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cell lines

The T2 cell line, which is deficient in TAP, was propagated in RPMI 1640 media containing 10% FBS, 2 mM glutamine, 5 × 10−3 M 2-ME and 50 μg/ml gentamicin sulfate (complete media). EL4-A2.1/Kb and Jurkat-A2.1 transfectants have already been described (27, 30). They were propagated in complete media supplemented with 0.4 mg/ml G418 (Genetic Bio-Products, Calabasas, CA).
Derivation of p53<sup>+</sup> A2.1/K<sup>b</sup> and p53<sup>−</sup> A2.1/K<sup>b</sup> effector CTL lines

The procedure used to obtain peptide-specific CTL lines has been described (27). Briefly, mice were injected s.c. at the base of the tail with 100 μg of either murine p53 261–269 peptide or human p53 149–157 peptide along with 120 μg of the I-A<sup>B</sup> T helper peptide 128–140 of the hepatitis B virus core protein in IFA. After 10 days, mice were sacrificed, and spleen cells were stimulated in vitro (24-well plates) with irradiated LPS-activated syngeneic spleen cells pulsed with the priming peptide at 5 μg/ml in complete media. On day 6, effector cells were assayed for their lytic activity. The resultant effector CTL lines were restimulated weekly with irradiated EL4-A2.1/K<sup>b</sup> cells (5 × 10<sup>5</sup> cells/well) pulsed with peptide and C57BL/6 spleen cells (6 × 10<sup>5</sup> cells/well) as fillers in complete media supplemented with 2% supernatant from Con A-stimulated rat spleen cells (27). On day 4 after restimulation, cells were tested in a standard 4-h<sup>51</sup>Cr release assay using T2 cells pulsed with different amounts of peptide as targets at the indicated E:T ratio.

CTL clones

p53<sup>+</sup> A2.1/K<sup>b</sup> 261 clone 12 and 13 were derived from p53<sup>+</sup> A2.1/K<sup>b</sup> 261 CTL line 3 by limiting dilution methods previously described (31). p53<sup>−</sup> A2.1/K<sup>b</sup> 261 clone 6 and 7 were derived by limiting dilution from the p53<sup>−</sup> A2.1/K<sup>b</sup> CTL line 2, previously sorted in sterile conditions into a CD8<sup>+</sup> subpopulation. They were propagated for the parental clone at 5 μg/ml, with 5% instead of 2% rat Con A supernatant. A2.1 149 clone 5 and A2.1 261 clone 45 were derived by limiting dilution from human p53 149–157 and murine p53 261–269, respectively, specific A2.1 CTL lines described previously (27). They were propagated as mentioned above using peptidelpulsed Jurkat-A2.1 cells as APCs instead of EL4-A2.1/K<sup>b</sup>.

Tetramer construction and specificity

Production of MHC/peptide tetramers was described in detail elsewhere (28). Briefly, a 15-amino acid substrate peptide for BirA-dependent biotinylation (BSP) has been engineered onto the COOH terminus of HLA-A2.1. The A2.1-BSP fusion protein and human β<sub>2</sub>-microglobulin were expressed in Escherichia coli and were folded in vitro with the specific peptide ligand. The properly folded MHC-peptide complexes were extensively purified using fast performance liquid chromatography and anion exchange, and biotinylated on a single lysine within the BSP using the BirA enzyme (Avidity, Denver, CO). Tetramers were produced by mixing the biotinylated MHC-peptide complexes with PE-conjugated avidin (PharMingen, San Diego, CA) at a molar ratio of 4:1.

HLA A2.1/peptide tetramers used in this study contained either murine p53 261–269 (A2.1/p53 261–269 tetramers), or human p53 149–157 (A2.1/p53 149–157 tetramers). To test their specificity of binding, CTL clones specific for each of these A2.1 binding peptides (27) were stained with both A2.1/p53 261–269 tetramers-PE and A2.1/p53 149–157 tetramers-PE. A2.1 261 clone 45 demonstrated binding of A2.1 tetramers containing its nominal Ag, the murine 261–269 peptide, but not A2.1 tetramers containing the human p53 149–157. Similarly, the A2.1 149 clone 5 can be stained specifically by A2.1/p53 149–157 tetramers, but not by A2.1/261–269 tetramers.

Flow cytometry

On day 5 after stimulation, cells were partially purified through a Ficoll-Paque (Pharmacia Biotechnology, Uppsala, Sweden) cushion and then washed in HBSS. Cells (0.5 × 10<sup>6</sup>) were incubated with different combinations of the following staining reagents for 30 min at room temperature in HBSS containing 0.1% BSA and 0.05% sodium azide: A2.1 tetramers described above at 40 μg/ml; anti-murine CD8α-PE (BD Biosciences, San Diego, CA) at 2 μg/ml; anti-murine pan-TCR<sup>b</sup>-PE (PharMingen, San Diego, CA) at 2 μg/ml; anti-murine pan-CD4-PE (PharMingen, San Diego, CA) at 2 μg/ml; anti-murine pan-CD8-PE (PharMingen, San Diego, CA) at 2 μg/ml; anti-murine panto-T8-PE MAb H57-597, at 2 μg/ml; anti-murine CD11a-biotin MAb 2D7, at 1 μg/ml; and streptavidin-FITC, at 5 μg/ml. All reagents were supplied by PharMingen. Propidium iodide was added after the final wash at 1 μg/ml to exclude dead cells in all experiments. Samples were analyzed on a Becton Dickinson (San Jose, CA) FACSort apparatus at The Scripps Research Institute FACS facility. Twenty thousand events were collected and analyzed using CellQuest software (Becton Dickinson).

TCR Vα usage and sequencing of V-J-C junctions region

Poly(A)<sup>+</sup> RNA from 4 × 10<sup>6</sup> cells of the 261–269 peptide-specific CTL clones was extracted using MicroFastTrack Kit (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. cDNA was synthesized using SuperScript II Reverse Transcriptase (Life Technologies, Gaithersburg, MD) and oligo(dT)<sub>12–18</sub> primers (Life Technologies). cDNA (1/100) was used as a template for PCR amplification with 1 unit Taq polymerase (Life Technologies) in a Hybrid thermal cycler (Hybaid, Middlesex, UK). Methods and primer sequences for PCR-based screening of the TCR V<sub>α</sub> usage have been previously described (32). PCR fragments obtained from CTL clones 7 and 13 were cloned into pCR2.1 vector using TA Cloning Kit (Invitrogen) and following the manufacturer’s instructions. M13 forward and reverse primers were used to determine sequences of three different molecular clones for each PCR fragment by the Nucleic Acids Core Facility at Scripps Research Institute using Applied Biosystems (Foster City, CA) automated sequencers.

Results

Binding of A2.1/I<p>53</p> 261–269 tetramers by CTL lines from p53<sup>+</sup> and p53<sup>−</sup> mice

Previous experiments suggested a lower avidity A2.1-restricted p53 261–269-specific CTL response in A2.1/K<sup>b</sup> transgenics that express p53 than in p53<sup>−</sup> A2.1/K<sup>b</sup> mice, since the first needed a 10-fold higher concentration of peptide to achieve similar levels of lysis (27). As defined, avidity is a multifactorial property in T cell recognition, in which TCR and CD8 are main contributors to variability. Although murine CD8 can interact with the chimeric A2.1/K<sup>b</sup> molecule that contains the α3 domain from the K<sup>d</sup> molecule, it does not effectively interact with the intact HLA-A2.1 molecule (30). Accordingly, it is possible to evaluate the contribution of TCR affinity to the overall T cell avidity (in the absence of participation of CD8) by examining its interaction with the intact A2.1 molecule, rather than A2.1/K<sup>b</sup>. To do so, we used two different approaches: 1) analysis of the lytic activity of targets expressing A2.1; and 2) analysis of the binding to A2.1/peptide tetramers.

We analyzed the lytic activity of 261–269-specific CTL lines from p53<sup>+</sup> and p53<sup>−</sup> A2.1/K<sup>b</sup> using T2 cells loaded with peptide as targets (Fig. 1A). The results obtained in the absence of CD8 contribution were similar to those obtained previously when the coreceptor was operative. CTL lines from p53<sup>+</sup> mice required far less peptide than those from p53<sup>−</sup> mice to achieve similar levels of lysis. There is approximately a 10-fold difference between lines 2 and 3 in the amount of peptide required to obtain 50% lysis (Fig. 1A). The very same 261–269-specific CTL lines were tested for their ability to bind A2.1/p53 261–269 tetramers (Fig. 1B). The p53<sup>−</sup> CTL lines contained a subset of CD8<sup>+</sup> cells capable of binding these tetramers, whereas p53<sup>+</sup> lines did not (Fig. 1B). An average of 21% of the CD8<sup>+</sup> T cells from six different high avidity p53<sup>−</sup> A2.1/K<sup>b</sup> CTL lines analyzed could bind tetramers. In contrast, only an average of 1.2% of the CD8<sup>+</sup> T cells from six different low avidity CTL lines from p53<sup>+</sup> A2.1/K<sup>b</sup> mice could bind tetramer. The level of staining observed using allospecific CTL, an average of 1% of the CD8<sup>+</sup> T cells (data not shown), suggested this was the background level in our conditions. Also, no evidence for specific binding could be found among freshly isolated splenocytes from p53 261–269-immunized mice.

Despite their inability to bind A2.1/p53 261–269 tetramers, these CTL specifically lysed A2.1-expressing cells loaded with the 261–269 peptide (Fig. 1A). Taken together with the results of the cytolytic assay using A2.1 targets, there is good correlation between high avidity, as defined by the lytic assay, and tetramer binding.

Tetramer binding of cloned CTL from p53<sup>−</sup> and p53<sup>+</sup> mice

The poor binding and lysis exhibited by p53<sup>−</sup> A2.1/K<sup>b</sup> lines could be explained by the presence in these CTL populations of a very few T cells that are actually specific for this complex and capable of binding and cytolysis. To determine whether cells that did not
demonstrate tetramer binding could actually be responsible for specific lysis, CTL clones were derived from p53\(^{A2.1/Kb}\) CTL line 3 by limiting dilution (Fig. 2A). As a control, clones were also isolated from the tetramer binding, CTL line 2 derived from a p53\(^{2}\) mouse (Fig. 2A). Resulting clones were tested for both tetramer binding (Fig. 2B) and lytic activity (Fig. 2C). Clones 12 and 13 were unable to bind stably A2.1/p53 261–269 tetramers, whereas clones 6 and 7 demonstrated staining, although at different levels (Fig. 2B). Both tetramer\(^{+}\) and tetramer\(^{-}\) clones were able to kill specifically p53 261–269 peptide-loaded T2 targets; however, they displayed different lytic capabilities (Fig. 2C). A 10-fold difference in avidity in the dose-response curve translated into either strong tetramer binding as exhibited by clone 7 or no binding at all, as observed for clone 13 (Fig. 2, B and C). Clones displaying p53 261–269-specific lytic activity yet unable to bind tetramer were also isolated from a CD8\(^{a}\) tetramer\(^{2}\) subpopulation from p53\(^{2}\) CTL line 2 (data not shown), indicating the presence of both high and low avidity CTL in this population.

It has been shown previously that one way to achieve tolerance and a low avidity phenotype is by decreasing the density of the specific TCR on the cell surface (33–35). We therefore examined the levels of total TCR on the surface of both tetramer\(^{2}\) high avidity and tetramer\(^{4}\) low avidity clones, and no significant differences were observed (Fig. 3). Surface TCR levels were also equivalent in the CTL lines described above (data not shown). Even when total levels of TCR were similar, the expression of a second \(\alpha\)-chain, which can compete for the same \(\beta\)-chain to produce another TCR with a different specificity, may reduce the actual density of the TCR specific for nominal Ag (36–38). To test this possibility, a fragment containing the V-J-C junctions region of the TCR \(\alpha\)-chain mRNA from clones 7 and 13 was amplified and sequenced (Fig. 4). Two different \(\alpha\)-chain mRNA were found in each clone, but only one was productively rearranged: V\(\alpha\)3-J\(\alpha\)17-C\(\alpha\), in clone 7; and V\(\alpha\)1-J\(\alpha\)8-C\(\alpha\), in clone 13 (Fig. 4). Analysis of the TCR V\(\alpha\) usage by PCR for clones 6 and 12 revealed identical results to that obtained for clones 7 and 13, respectively (data not shown). This suggested that they could be clonally related.

Finally, the expression of CD8 and LFA-1 was analyzed, since interaction with its ligands plays an important role in T cell avidity (30, 39–42). Again, no significant differences were observed (Fig. 3). Taken together, these results suggest a difference in TCR affinity is responsible for the difference in lytic ability and tetramer binding observed between CTL from p53\(^{2}\) and p53\(^{1}\) mice. Thus, A2.1 tetramers can discriminate the distribution of affinities present in a CTL population. Only the highest affinity TCRs can be

![FIGURE 2. Isolation and characterization of tetramer\(^{+}\) and tetramer\(^{-}\) murine p53 261–269 peptide-specific CTL clones. A, Schematic representation of the origin of p53\(^{A2.1/Kb}\) clones 6 and 7 and p53\(^{A2.1/Kb}\) clones 12 and 13. B, p53 261–269 peptide-specific clones were stained with A2.1/p53 261–269 tetramers-PE on day 5 after restimulation. Shaded profiles correspond to unstained controls. C, The lytic activity of clones 6, 7, 12, and 13 was assessed on day 4 after restimulation using T2 cells pulsed with the indicated concentrations of p53 261–269 peptide as targets at an E:T ratio of 2:1.](http://www.jimmunol.org/)

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**FIGURE 1.** Lytic activity and tetramer binding ability of murine p53 261–269 peptide-specific CTL lines. CTL lines obtained from p53 261–269 peptide-primed p53\(^{1}\) and p53\(^{2}\) A2.1/Kb transgenics were analyzed after four weekly rounds of in vitro restimulation. Data from two of six independently established lines from p53\(^{1}\) A2.1/Kb mice (lines 1 and 2) and p53\(^{2}\) A2.1/Kb mice (lines 3 and 4) is provided. A, Effector CTL were assayed for lytic activity against T2 cells pulsed with different concentrations of p53 261–269 peptide as indicated at an E:T ratio of 2:1 4 days after restimulation. B, The same CTL lines 1, 2, 3 and 4 were stained on day 5 after restimulation with A2.1/p53 261–269 tetramers-PE and anti CD8\(\alpha\)-FITC.
detected by FACS. It is also likely that the difference in intensity of tetramer staining between clones 6 and 7 (mean fluorescence intensity of 52 for clone 6, and 202 for clone 7) reflects differences in their TCR affinities (Fig. 2). However, since cell surface TCR expression of clone 6 is 50% lower than that of clone 7 (Fig. 3), the lower level of tetramer binding may also reflect a difference in TCR density.

**p53**+ A2.1/Kb CTL are not tolerant of human p53149–157 foreign peptide

To ascertain that the differences between the CTL obtained from p53 expressing and p53 deficient mice were actually related to the fact the epitope under examination induced tolerance in the p53-expressing mice, we examined the CTL responses by these same mice containing a peptide epitope that is not expressed in either strain of mouse. The human p53 149–157 epitope is also A2.1 restricted, mice against a peptide epitope that is not expressed in either strain of mouse. The human p53 149–157 epitope is also A2.1 restricted, and biotinylated anti-CD11a plus streptavidin-FITC. Shaded profiles correspond to unstained controls.

**FIGURE 3.** Expression of surface receptors in tetramer+ and tetramer− murine p53 261–269 peptide-specific CTL clones. Analysis of total TCR, CD8, and LFA-1 surface expression of clones described in Fig. 3 on day 5 after restimulation with an anti-murine pan-TCRβ-PE, anti CD8α-FITC, and biotinylated anti-CD11a plus streptavidin-FITC. Shaded profiles correspond to unstained controls.

p53+ A2.1/Kb CTL lines from both p53 expressing mice were actually related to the relatively high concentration of peptide used for their propagation in vitro. It has been shown that high concentrations of peptide may induce apoptosis of high avidity CTL (44). However, since identical concentrations of peptide were used to propagate 261-specific CTL from p53− mice, which were capable of strong tetramer binding, it is unlikely this can explain the lack of tetramer binding among 261-specific CTL from p53+ mice. Also, 149-specific CTL from both p53+ and p53− mice stimulated under the same conditions contained high avidity CD8+ T cells capable of tetramer binding.

Our studies found no evidence for down-regulation of TCR levels or expression of a second TCR as a mechanism for decreasing T cell avidity in p53+ mice. The later was somewhat surprising since it is well documented that more than one TCR can normally be expressed by T cells, thereby decreasing the actual concentration of the TCR specific for the first epitope (36–38, 45, 46). It has been shown in TCR transgenic models how down-regulation of the total levels of TCR may allow T cells to escape tolerance (33). Also, it was shown using TCR transgenics that cells expressing an autoreactive TCR can escape thymic deletion if they express a second TCR (34, 35). Perhaps more extensive analysis of T cell clones from p53-expressing mice will reveal CD8+ cells that have escaped tolerance by expression of a second TCR. Alternatively, it is possible that, for this particular Ag, it was not possible to achieve sufficiently low avidity to avoid deletion by these alternative mechanisms.

p53 261–269-specific effector CTL from p53+ and p53149−157-specific CTL from either p53- or p53+ mice contain a mixture of both specific tetramer binding and nonbinding CD8+ T cells. These results indicate that, in response to a foreign peptide Ag, a heterogeneous set of TCR affinities may arise, including CTL with a relative high affinity TCR, as well as very low avidity CTL. Furthermore, the use of MHC tetramers represents an important tool to separate CTL by avidity. Several reports have correlated differences in the intensity of tetramer staining with differences in TCR affinity for the Ag for both class II and class I MHC.
tetramers (47–50). Interestingly, in two of these reports it has been demonstrated that a higher intensity of tetramer staining reflects a lower tetramer dissociation rate (49, 50). Probably, the dissociation rate of the A2.1/p53 peptide tetramers is too high to detect binding to the low affinity p53-specific TCR by FACS. However, the difference in avidity, as measured in a cytolysis assay, between the CTL that bind tetramer and those that cannot is quite narrow, no higher than 10-fold.

The existence of CD8\(^+\) T cells within p53 261–269 peptide-specific CTL lines, which are not able to bind tetramers yet display specific lytic activity, contrasts with results from several other studies using HLA A2.1, Mamu-A0.1, or Kb tetramers. It was reported that the tetramer\(^+\) but not tetramer\(^-\) cells from human, monkey, or mouse effector CTL were able to lyse peptide-pulsed targets (28, 51–53). An explanation for these different findings is that human CD8\(^+\) T cells bind A2.1 tetramers better than murine CTL do. In previous studies, some contribution to the avidity of tetramer binding may have come from CD8 (M. M. Davis, unpublished observation). In the current study, since murine CD8 does not bind the human A2.1 molecule, this interaction was not possible and therefore could not increase the avidity of tetramer binding to the CTL. This suggests that, in the absence of CD8 contribution, cytotoxicity assays are more sensitive than tetramer binding in detecting murine A2.1-restricted 261–269-specific CTL.

The experiments presented here suggest the occurrence of functional elimination of the CD8\(^+\) T cells carrying TCRs with relatively high affinity for the Ag, when it is expressed as a normal self Ag. Since there is expression of p53 in the thymus (24–26), it is likely that high avidity interaction with physiological amounts of the 261–269 peptide on thymic APCs results in negative selection. On the other hand, thymocytes expressing TCRs that have low affinity for this peptide may be positively selected in the same environment. Using TCR transgenic mice, several studies have demonstrated that weak agonist peptides are capable of positive activation of T cells.

**FIGURE 4.** Expression of TCR \(\alpha\)-chain in murine p53 261–269 peptide-specific CTL clones. Expression of two different TCR \(\alpha\)-chain mRNA was found in each of the tetramer\(^+\) and tetramer\(^-\) clones analyzed using the method described by Zisman et al. (32). Partial sequences containing the V-J-C junctions region of TCR \(\alpha\)-chains found in clones 7 and 13 are presented. Each clone expressed a productive and a nonproductively rearranged sequence. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers AF151729, AF151730, AF15131, and AF151732.
Possible to activate CTL specific for self epitopes, such as p53, and rejection. Promisingly, two recent studies have demonstrated it is effector CTL could provide a window of opportunity for tumor threshold. The existence of a residual p53-specific, low avidity tolerational tolerance to a natural self epitope has been achieved. TCR 4 weekly rounds of in vitro restimulation. One representative p53
selection (10, 54–57). It is interesting to speculate that the 261–269 endogenous peptide may have contributed to the selection of some of the peptide-specific CTL obtained from p53+ mice.

In summary, this study shows the mechanism by which functional tolerance to a natural self epitope has been achieved. TCR affinity for a given MHC class I/peptide complex appears to be the main factor determining the overall avidity in CTL recognition for p53. In order for a potentially auto-reactive T cell to be maintained in the repertoire, its TCR affinity must be kept under a certain threshold. Resolving, two recent studies have demonstrated it is possible to activate CTL specific for self epitopes, such as p53, and get tumor rejection by the use of viral vectors or dendritic cells as vaccines (58, 59). Future studies will compare the ability of CTL-expressing high and low affinity TCRs to eliminate tumors in vivo.

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References