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# Targeting p53 as a general tumor antigen

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**ABSTRACT** **A major barrier to the design of immunotherapeutics and vaccines for cancer is the idiosyncratic nature of many tumor antigens and the possibility that T cells may be tolerant of broadly distributed antigens. We have devised an experimental strategy that exploits species differences in protein sequences to circumvent tolerance of high-affinity T cells. HLA transgenic mice were used to obtain cytotoxic T lymphocytes specific for peptides from the human p53 tumor-suppressor molecule presented in association with HLA-A2.1. Although such p53-specific cytotoxic T cells did not recognize nontransformed human cells, they were able to lyse a wide variety of human tumor cell lines, thus confirming the existence of broadly distributed determinants that may serve as targets for immunotherapy.**

Peptides presented by class I major histocompatibility complex (MHC) molecules and recognized by tumor-specific cytotoxic T lymphocytes (CTLs) are most often derived from endogenously synthesized cellular proteins that are expressed uniquely by a given type of tumor (1–3). It is this tissue-specific expression of tumor-associated peptide antigens that restricts any potential immunotherapy by both peptide-based vaccines and tumor-reactive CTLs to a limited set of tumors. The most useful form of immunotherapy, however, would be one that would target a more general tumor antigen and would thus be of value for many different types of human (Hu) malignancies. As an example, peptides derived from the Hu p53 tumor-suppressor protein and presented by class I MHC molecules are candidates for such broad-spectrum, tumor-associated CTL epitopes. Expression of p53 protein is markedly upregulated in a high proportion of Hu malignancies (4, 5). This overexpression correlates with the presence of a mutated form of p53 which inactivates its normal function as a tumor suppressor (4, 5). Peptides spanning mutant sequences of Hu and murine (Mu) p53 can provide endogenously synthesized epitopes presented by Mu class I molecules and recognized by CTLs of Mu origin (6–8). Due to the diversity of mutations in p53 that can arise in tumors (4, 5), if this protein is to serve as a general tumor antigen, it will be necessary to address the immunogenicity of peptides representative of the wild-type (WT) sequence. However, low-level expression of WT p53 occurs in normal tissue including thymus and spleen (9) and may cause tolerance of p53-specific T cells either by negative selection of immature thymic T cells with high avidity for self-MHC–p53 peptide complexes or by peripheral mechanisms (10–17). Thus, derivation of CTLs capable of recognizing nonmutated p53 sequences on tumor cells may require circumvention of tolerance.

In this report, we describe a strategy that takes advantage of transgenic (Tg) mice expressing either the Hu class I molecule HLA-A2.1 or the chimeric molecule A2.1/$K^b$, which contains the $\alpha$-3 domain of the Mu H-2$K^b$ molecule (18–20), in order to generate A2.1-restricted, tumor-reactive CTLs bearing T-cell receptors (TCRs) specific for peptides derived from Hu p53. Both the antigen-processing machinery and the T-cell repertoire of humans and these Tg mice are sufficiently similar to select the same immunodominant antigens (18–22). However, due to numerous sequence differences between the Hu and Mu p53 molecules (23), tolerance to endogenous Mu p53 epitopes would not necessarily exclude recognition of peptides specific to the Hu sequence.

## METHODS

**Mice.** The derivation of the Tg lines used in these studies has been described (18, 19). A2.1/$K^b$-Tg mice were homozygous for both the H-2$^b$ gene and the A2.1/$K^b$ transgene. All A2.1-Tg mice were homozygous for the H-2$^b$ gene and heterozygous for the transgene. Mice were propagated and maintained in our vivarium at The Scripps Research Institute. C57BL/6 mice were purchased from the breeding colony of The Scripps Research Institute.

**Peptides.** Peptides were synthesized with a Gilson model AMS 422 peptide synthesizer. Purity was ascertained by reverse-phase HPLC analysis on a Vydac (Hesperia, CA) $C_{18}$ column. Some peptides were also synthesized on an Applied Biosystems model 430A synthesizer.

**Cell Lines.** Previously described transfectants utilized in these studies included EL4 A2 (EA2), EL4 A2/$K^b$ (EA2$K^b$), Jurkat A2 (JA2), and Jurkat A2/K (JA2$k^b$) (18, 24), as well as Saos-2 and Saos-2 transfected with the Hu mutant p53 gene, Saos-2/175 (25). To obtain Ramos-A2 and T2-A2/$K^b$, 10 $\mu$g of plasmid containing genomic clones of A2.1 or A2.1/$K^b$ was cotransfected with pSV2neo (2 $\mu$g) as described (24). T2 cells were obtained from Peter Cresswell (Yale University). All other Hu cell lines were obtained from the American Type Culture Collection and tested by flow cytometry for the presence of HLA-A2 (24). High levels of p53 protein as a result of functionally homozygous mutations of the p53 gene were expressed by breast cancer cell lines MDA-MB-231 and BT-549, the colorectal cancer cell line SW480, and the Burkitt lymphoma cell line Ramos, whereas the breast cancer cell line MCF7 accumulated WT p53 protein in the cytoplasm via nuclear exclusion (26–31). Both p53 alleles were deleted in the osteosarcoma cell line Saos-2 (25, 32, 33). Dendritic cells and concanavalin A (Con A)- and phytohemagglutinin (PHA)-activated lymphoblasts were prepared from peripheral blood mononuclear cells obtained from healthy, HLA-A2.1-positive volunteer donors as described (34, 35).

**Peptide Binding to HLA-A2.1.** A competition assay was used to assess binding of peptide to HLA-A2.1. EA2 cells were

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Abbreviations: CTL, cytotoxic T lymphocyte; E/T, effector/target; MHC, major histocompatibility complex; TCR, T-cell receptor; Tg, transgenic; Hu, human; Mu, murine; WT, wild type; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; Flu-NP, influenza A virus nucleoprotein; VSV-N, vesicular stomatitis virus nucleoprotein; PHA, phytohemagglutinin; IFN, interferon; TNF, tumor necrosis factor.

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pulsed with 1 μM A2-binding synthetic peptide representing residues 58–66 of the A/PR/8/34 influenza virus matrix protein M1 and 100 μM test peptide (36, 37). The A2.1-binding peptide representing residues 476–484 of the reverse transcriptase (RT) of Hu immunodeficiency virus type 1 (HIV-1) served as a positive control (38). An H-2K$^b$-binding synthetic peptide representing residues 52–59 of the vesicular stomatitis virus nucleoprotein [VSV-N-(52–59)] and an H-2D$^b$-binding synthetic peptide representing residues 366–374 of the influenza A virus (1934) nucleoprotein [Flu-NP-1934-(366–374)] served as negative controls (39–41). The A2.1-restricted, M1-specific CTL clone 12 (A clone 12) was assayed at various effector/target (E/T) ratios for lytic activity against peptide- and nonpeptide-pulsed EA2 targets in a 4-hr $^{51}$Cr-release assay (24). Percent inhibition of A clone 12-mediated lysis of M1-pulsed EA2 targets by the indicated peptides was calculated at an E/T ratio of 0.3:1.

**Peptide Priming of HLA-Tg Mice and Propagation of CTL Lines.** Mice were injected subcutaneously at the base of the tail with 100 μg of the indicated test peptide and 120 μg of the I-A$^b$-binding synthetic T helper peptide representing residues 128–140 of the hepatitis B virus core protein (21) emulsified in 100 μl of incomplete Freund's adjuvant. After 10 days, spleen cells of primed mice were cultured with irradiated A2.1/K$^b$- or A2.1-Tg lipopolysaccharide (LPS)-activated spleen cell stimulators that had been pulsed with the indicated priming peptide at 5 μg/ml and human $\beta_2$-microglobulin at 10 μg/ml (18, 19). After 6 days, the resultant effector cells were assayed in a 4-hr $^{51}$Cr-release assay at various E/T ratios for lytic activity against T2 or T2-A2K$^b$ cells that had been pulsed with either the indicated priming peptide, an unrelated A2.1-binding peptide, or no peptide. Polyclonal CTL lines specific for Hu p53-(149–157) (CTL A2/K$^b$ 149 and A2 149) and Hu p53-(264–272) (CTL A2/K$^b$ 264 and A2 264) were established by weekly restimulation of effector CTLs with irradiated JA2K$^b$ or JA2 cells that had been pulsed with 5 μg of the indicated p53 peptide, irradiated C57BL/6 spleen filler cells, and 2% (vol/vol) rat Con A supernatant (24).

## RESULTS AND DISCUSSION

Synthetic peptides representing sequences within the Hu p53 protein were selected according to the known consensus motifs for peptides bound by A2.1 (42–49). Selected WT p53 peptides were 8–11 aa long and had at their N-terminal position 2 either L, M, I, V, A, or T (single-letter code) and at their C terminus either V, L, I, A, M, T, S, or Q. A2.1 binding was determined by a competition assay that assessed the ability of each peptide to inhibit binding of a synthetic peptide representing residues 58–66 of the A/PR/8/34 (PR8) influenza virus matrix protein M1 (36, 37) to A2.1 on target cells (Table 1). Inhibition of M1 peptide binding was monitored as a decrease in target cell lysis by an M1-specific, A2.1-restricted CTL clone, clone 12. All 19 peptides with intermediate-to-high A2.1-binding activity (>23% inhibition of A2.1 binding of M1) and 3 peptides with low (10–22% inhibition) or no A2.1-binding activity (<10% inhibition) were tested for their immunogenicity in A2.1/K$^b$-Tg mice. Mice were primed with peptide and 10 days later, spleen cells from these mice were restimulated with peptide *in vitro* and tested for an A2.1/K$^b$-restricted, peptide-specific CTL response. As reported (18–21), A2.1/K$^b$-Tg mice could mount an A2.1/K$^b$-restricted CTL response specific for known A2.1-binding CTL epitopes, such as HIV-1 RT-(476–484) (Table 1). A2.1/K$^b$-restricted CTL responses specific for Hu p53-(25–35), -(65–73), -(149–157), and -(264–272) were also detectable. The peptide specificity of these responses was evidenced by the ability of CTLs to lyse cells pulsed with the immunizing peptide, but not other A2.1-binding peptides (Fig. 1 *A* and *C*; data not shown). These findings were consistent with the hypothesis that the majority of functional TCR epitopes are produced by peptides with high [as with Hu p53-(25–35), -(65–73), and -(264–272)] and intermediate affinity [as with Hu p53-(149–157)] for the presenting class I MHC molecule (21). However, the data also suggest that gaps in the functional T-cell repertoire may exist, as not all of the nonhomologous Hu p53 peptides with high A2.1-binding activity were capable of inducing a CTL response. No significant response by A2.1/K$^b$-Tg mice was detectable against Mu p53-(261–269), which is identical with Hu p53.264–272 at all but one amino acid residue, yet this Mu peptide had the highest A2.1-binding activity of all p53 peptides tested (Table 1). A

Table 1. A2.1-binding affinity and immunogenicity of WT p53 peptides

| Peptide and position | Sequence | % inhibition of A2.1 binding* | Lytic activity of peptide-specific CTLs† |
|---|---|---|---|
| Hu WT p53 | | | |
| 25–33 | **LLPENNVLS** | 42 | 3 |
| 25–35 | **LLPENNVLSPL** | 65 | 47 |
| 31–39 | VLSPLPSQA | 38 | 3 |
| 31–40 | VLSPLPSQAM | 23 | 0 |
| 42–50 | **DLMLSPDDI** | 19 | 0 |
| 43–52 | **LMLSPDDIEQ** | 25 | 3 |
| 65–73 | **RMPEAAPPV** | 62 | 85 |
| 69–76 | **AAPPVAPA** | 46 | 0 |
| 69–78 | **AAPPVAPAPA** | 41 | 0 |
| 69–79 | **AAPPVAPAPAA** | 4 | 0 |
| 73–81 | VAPAPAAPT | 12 | 0 |
| 78–86 | AAPTPAAPA | 51 | 0 |
| 110–119 | **RLGILHSGTA** | 10 | ND |
| 117–125 | **GTAKSVTCT** | 12 | ND |
| 121–129 | **SVTCTYSPA** | 8 | ND |
| 122–130 | **VTCTYSPAL** | 12 | ND |
| 129–137 | **ALNKMFCQL** | 71 | 0 |
| 136–144 | **QLAKTCPVQ** | 15 | ND |
| 146–155 | **WVDSTPPPGT** | 10 | ND |
| 149–157 | **STPPPGTRV** | 29 | 91 |
| 161–169 | **AIYKQSQHM** | 12 | ND |
| 187–195 | **GLAPPQHLI** | 62 | 1 |
| 187–197 | **GLAPPQHLIRV** | 14 | ND |
| 210–218 | **NTFRHSVVV** | 43 | 6 |
| 229–237 | **CTTIHYNYM** | 14 | ND |
| 255–264 | **ITLEDSSGNL** | 24 | 3 |
| 255–265 | **ITLEDSSGNLL** | 22 | ND |
| 263–272 | **NLLGRNSFEV** | 50 | 3 |
| 264–272 | **LLGRNSFEV** | 60 | 94 |
| 322–330 | **PLDGEYFTL** | 24 | 0 |
| 339–347 | **EMFRELNEA** | 12 | ND |
| Mu WT p53 | | | |
| -(261–269) | **LLGRDSFEV** | 75 | 10 |
| HIV-1 RT- | | | |
| (476–484) | ILKEPVHGV | 72 | 85 |
| VSV-N- | | | |
| (52–59) | RGYVYQGL | 4 | ND |
| Flu-NP-1934 | | | |
| (366–374) | ASNENMETM | 4 | ND |

Selected WT p53 peptides were synthesized; residues that are identical between Hu and Mu WT p53 are in bold type.

*Relative A2.1-binding affinity as determined by the peptide's ability to inhibit the A2.1 binding of the M1-(58–66) peptide.

†Immunogenicity of WT p53 peptides and the HIV-1 RT-(476–484) control peptide was determined by peptide priming of A2.1/K$^b$-Tg mice. Two micrograms of peptide was used to pulse T2A2/K$^b$ targets during $^{51}$Cr labeling. Lytic activity of CTLs at an E/T ratio of 60:1 was calculated as percent specific $^{51}$Cr release (24). Lysis of T2A2/K$^b$ pulsed with an unrelated A2.1-binding peptide was similar to that obtained for nonpulsed T2A2/K$^b$ and did not exceed 15%. Data represent the highest amount of lytic activity obtained after peptide priming of at least three mice. ND, not done.

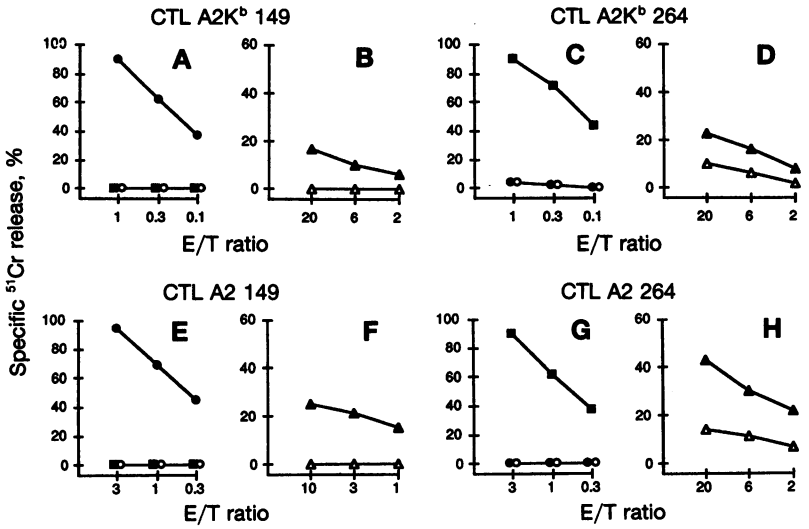


FIG. 1. A2.1-restricted recognition of endogenously synthesized p53 epitopes by p53-specific CTL lines from A2.1/K$^b$-Tg and A2.1-Tg mice. Effector CTLs were generated by peptide priming of Tg mice. CTLs were assayed for cytotoxicity in a 5-hr $^{51}$Cr-release assay against the indicated targets: (*A* and *C*) T2A2/K$^b$ (○) or T2A2/K$^b$ pulsed with p53-(149–157) (●) or p53-(264–272) (■). (*E* and *G*) T2 (○) or T2 pulsed with p53-(149–157) (●) or p53-(264–272) (■). (*B, D, F,* and *H*) Saos-2 (△) or the same cells transfected with the human p53 gene, Saos-2/175 (▲) (25, 32, 33). Both lines expressed similar levels of A2.1 as detected by flow cytometry (24).

lack of CTL responsiveness by A2.1/K$^b$-Tg mice was also observed with Hu p53 peptides that were homologous to Mu p53 sequences and had either high [Hu p53-(187–195)] or intermediate [Hu p53-(255–264) and -(322–330)] binding activity for A2.1. These results suggested that tolerance to self-p53 epitopes may indeed limit the repertoire of responsive T cells. Several peptides identified in this study had been previously shown to bind A2.1 (48–52) and also to elicit a peptide-specific response by Hu peripheral blood lymphocytes (50–52). However, the ability of such CTLs to recognize cells endogenously expressing p53 had not been reported, thereby leaving unresolved the issue of whether these or other p53 peptides are presented in association with MHC molecules on the cell surface.

To determine whether the peptides corresponding to these sequences were actually endogenously processed and presented in association with A2.1 molecules on the surface of Hu tumor cells expressing Hu p53, peptide-specific polyclonal CTL lines from A2.1/K$^b$-Tg mice were established and tested for recognition of the A2.1-expressing, p53-deficient cell line Saos-2 and this same line transfected with a Hu p53 gene, Saos-2/175 (25, 32, 33). Comparison of the levels of lysis of the transfectant relative to the p53-deficient parental line indicated that CTLs specific for Hu p53-(25–35) and -(65–73) did not lyse Saos-2/175 cells, suggesting that these peptides were not processed and presented in sufficient amount for recognition by these CTL lines (data not shown). In contrast, CTLs specific for Hu p53-(149–157) and -(264–272) were presented by cells that endogenously expressed high levels of Hu p53 (Fig. 1 *B* and *D*). However, attempts to obtain recognition by these CTL lines of A2.1-expressing tumors that naturally expressed high levels of Hu p53 were unsuccessful, even after pretreatment of target cells with both interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) (data not shown), a method that is known to augment specific cell lysis by increasing the numbers both of MHC–peptide complexes and of adhesion molecules expressed on the cell surface (53, 54). This suggested that tumor cell lines may not present p53 peptides or, more likely, that they expressed insufficient levels of the p53 peptides to be recognized by these particular CTL lines.

Due to the inability of Mu CD8 to interact with the α-3 domain of the Hu A2.1 molecule, CTLs from A2.1/K$^b$-Tg mice are at a disadvantage in recognition of cells expressing A2.1 as compared with A2.1/K$^b$ (18–20, 24). However, A2.1-restricted CTLs from A2.1-Tg mice appear to be CD8-independent in their recognition of target cells, presumably due to their selection and stimulation in the absence of the participation of Mu CD8 (18). Previous experiments indicated that CD8-independent CTL require less peptide antigen for target cell recognition (55). Therefore, if p53-specific CTLs derived from A2.1/K$^b$-Tg mice were unable to lyse Hu tumor cells due to presentation of limiting numbers of the relevant peptide–MHC complexes, it was possible that A2.1-Tg mice could provide peptide-specific CTLs capable of detecting the low amounts of p53 peptides expressed by tumor cells. To test this hypothesis, polyclonal CTL lines specific for Hu p53-(149–157) (CTL A2 149) and Hu p53-(264–272) (CTL A2 264) were established from peptide-primed A2.1-Tg mice (Fig. 1 *E* and *G*). Both CTL lines recognized endogenously synthesized p53 epitopes, as shown by their lysis of Saos-2/175 transfectants (Fig. 1 *F* and *H*). Significantly, the magnitude of lysis of Saos-2/175 targets by CTL A2 149 and 264 was higher than that obtained by CTLs from A2.1/K$^b$-Tg mice (Fig. 1 *B* vs. *F*

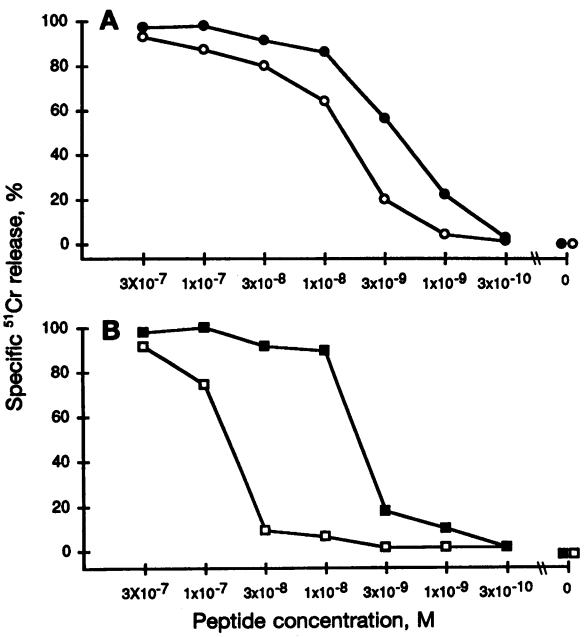


FIG. 2. Efficiency of peptide recognition by p53-specific CTL lines. CTL lines specific for Hu WT p53-(149–157) and -(264–272) were established from A2.1-Tg (CTL A2 149 and CTL A2 264) and A2.1/K$^b$-Tg (CTL A2/K$^b$ 149 and CTL A2/K$^b$ 264) mice and assayed at an E/T ratio of 10:1 for lytic activity against nonpulsed and p53-(149–157)-pulsed T2 (*A*) or nonpulsed and p53-(264–272)-pulsed T2 targets (*B*). Peptides were used at the indicated concentrations to pulse T2 targets after $^{51}$Cr labeling. Effector cells were CTL A2 149 (●), CTL A2/K$^b$ 149 (○), CTL A2 264 (■), and CTL A2/K$^b$ 264 (□). Data are from a 4-hr $^{51}$Cr-release assay.

Table 2. Hu tumor cell lines that overexpress p53 protein are lysed by A2.1-restricted, p53-specific CTL lines

| | | | Specific $^{51}Cr$ release (%) by CTL | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | A2 149 | | | A2 264 | | | | |
| | | | 10:1 E/T | | 1:1 | 10:1 E/T | | 1:1 | RT 427 | Allo A2.1/$K^b$ |
| Target cells | A2.1 | Tumor type | − α-A2 | + α-A2 | E/T | − α-A2 | + α-A2 | E/T | (10:1 E/T) | (10:1 E/T) |
| MDA-MB-231 | + | Breast | 24 | 5 | 16 | 31 | 10 | 19 | 6 | 47 |
| MCF7 | + | Breast | 38 | 13 | 28 | 79 | 38 | 67 | 8 | 52 |
| BT-549 | + | Breast | 53 | 37 | 18 | 79 | 47 | 35 | 14 | 61 |
| SW480 | + | Colorectal | 55 | 26 | 41 | 59 | 17 | 24 | 4 | 67 |
| Ramos | − | Burkitt lymphoma | 4 | 6 | 3 | 2 | 0 | 0 | 2 | 4 |
| Ramos A2.1 | + | Burkitt lymphoma | 39 | 12 | 11 | 43 | 0 | 21 | 7 | 49 |
| Saos-2 | + | Osteosarcoma | 10 | 9 | 5 | 17 | 15 | 10 | 6 | 72 |
| Dendritic cells | + | — | 0 | 3 | 0 | 2 | 0 | 1 | 0 | ND |
| Con A lymphoblasts | + | — | 7 | 4 | 3 | 8 | 4 | 7 | 3 | 55 |
| PHA lymphoblasts | + | — | 5 | 2 | 4 | 5 | 0 | 0 | 4 | 40 |

Allo A2.1/$K^b$, alloreactive, A2.1-specific effector CTLs derived from Tg mice expressing functional human CD8 α β molecules (Hu CD8-Tg mice) (18) by a 6-day primary *in vitro* culture of Hu CD8-Tg spleen cells with irradiated A2.1/$K^b$-Tg spleen cell stimulators. RT 427, an A2.1-restricted polyclonal CTL line established from peptide-primed doubly Tg (Hu CD8 and A2.1/$K^b$) mice and specific for a synthetic peptide representing residues 427–435 of HIV-1 RT. CTLs were assayed for cytotoxicity in a 6-hr $^{51}Cr$ release assay against the indicated Hu tumor cell lines, Hu dendritic cells, and Con A- or PHA-activated Hu lymphoblasts. Data are presented for noncytokine-treated Ramos and Ramos A2.1 targets, MDA-MB-231 targets that had been treated with IFN-γ (20 ng/ml for 24 hr), and the remaining targets that had been treated with both IFN-γ (20 ng/ml for 24 hr) and TNF-α (3 ng/ml for 24 hr). Anti-A2.1 inhibition was performed by exposure of $^{51}Cr$ labeled target cells to the anti-A2.1 (α-A2) monoclonal antibody PA2.1 (56) at saturating, nontoxic concentrations. ND, not done.

and *D* vs. *H*). Also, the concentrations of Hu p53-(149–157) and -(264–272) peptides required to obtain equivalent lysis of T2 targets by A2- vs. A2.1/$K^b$-derived CTLs were 3- and 10-fold less (Fig. 2 *A* and *B*), respectively. Thus, CTLs of greater sensitivity for A2.1–p53 peptide complexes could be selected in A2.1-Tg mice compared with A2.1/$K^b$-Tg mice.

Having established CTL lines with apparently higher affinity for A2.1–p53 peptide complexes, we tested the A2 149 and A2 264 CTL lines for recognition of Hu tumor cell lines that express high levels of p53 protein (MDA-MB-231, BT-549, SW480, Ramos A2.1, MCF7) (Table 2) (26–31). These tumor cell lines were lysed by both p53-specific and alloreactive, A2.1-specific control CTLs. Recognition was A2.1-restricted, as lysis was inhibited by an A2.1-specific antibody (Table 2). No response was evident when an A2.1-restricted CTL line specific for an unrelated synthetic peptide, RT 427, was used as effector. Breast and colorectal cancer cell lines had to be pretreated with either IFN-γ (MDA-MB-231) or both IFN-γ and TNF-α (MCF7, BT-549, SW480) to achieve optimal antigen-specific lysis by anti-p53 CTLs. Lysis of noncytokine-treated breast and colorectal cancer cell lines by p53-specific CTLs was low (4% to 14% specific lysis at an E/T ratio of 10:1). Since MDA-MB-231, MCF7, and SW480 are not deficient in their ability to present endogenously synthesized peptides for recognition by class I MHC-restricted CTLs (57), the observed requirement for cytokines to achieve optimal lysis suggested that p53 peptides bound by A2.1 were presented in relatively low numbers by these tumor cells as compared with Saos-2/175 and that increased expression of A2.1–peptide complexes and adhesion molecules via cytokine treatment was required to facilitate TCR-mediated recognition and target cell lysis. In contrast, Burkitt lymphoma cells that had been transfected with A2.1 (Ramos A2.1) and had high-level expression of both the transfected gene product and p53 protein (30) were efficiently lysed by p53-specific CTLs in the absence of cytokine stimulation. Again, their response was A2.1-restricted, as nontransfected Ramos targets were not lysed by p53-specific CTLs.

No significant lysis by p53-specific CTLs was evident against p53-deficient Saos-2 cells, or a variety of nontransformed targets, such as dendritic cells (34) and activated lymphoblasts that had been shown to express low amounts of p53 protein following 3–4 days of stimulation with Con A or PHA (Table 2) (35). These findings suggest that dividing and activated normal cells, even after exposure to cytokines, presented A2.1-bound p53 peptides in copy numbers too low to allow recognition by these CTLs.

In summary, these results demonstrate presentation of peptides derived from p53 by a variety of human tumors at levels sufficient for recognition by CTLs from A2-Tg mice. That normal cells were not lysed does not necessarily indicate lack of presentation of p53 peptides, but rather insufficient levels of presentation for lysis by the CTLs obtained in these studies. This may provide a window of opportunity for p53-directed immunotherapy. Whether CTLs of sufficient TCR affinity to lyse p53-overexpressing tumors could be obtained by direct priming of tumor-bearing hosts is unknown. Although the levels of p53 epitopes expressed by normal cells may not be sufficient to detect lysis, the amount of antigen required for tolerance is less than that required for effector cell recognition (58, 59). Such self-tolerance could result in deletion of T cells with receptors of sufficiently high affinity to detect p53 peptides on transformed cells, in which case it may be necessary to use Tg mice as a source of high-affinity, Hu p53-specific TCRs for immunotherapy. Finally, although this report restricts its discussion to p53, the strategy described herein could be of value for the analysis of a variety of gene products that are specifically upregulated in malignant tumors and that may represent potential targets for CTL-based immunotherapy and vaccine design.

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