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p53, a potential target for tumor-directed T cells

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1. Summary

Cell lineage-specific cellular proteins, oncogenes from viral or cellular origin and tumor suppressor genes encode tumor-specific/associated antigens. Such antigens can elicit an major compatibility complex (MHC) class I-restricted cytotoxic T lymphocyte (CTL) response, either naturally in cancer patients or following appropriate immunostimulation (in vitro or in vivo). The reported immune responses in humans to the melanoma-associated MAGE gene products, GP100 and tyrosinase, all self-proteins, support the idea to use wild-type p53 products as targets for T cells. An important step towards this goal is identification of potential p53 CTL epitopes. We identified the wild-type p53 peptides with the highest affinity to the HLA-A*0201 molecule using two assays: the previously described MHC peptide-binding assay and the peptide competition assay. We obtained CTL against four p53 peptides with a high affinity for the HLA-A*0201 molecule. These findings are discussed next to a short review concerning the p53 literature.

2. Introduction

The therapeutic role of CTL and CTL epitopes has recently been reviewed [1–4]. In mice viral oncogenes of simian virus 40, polyoma virus, adenovirus and human papilloma virus (HPV) type 16 have been shown to code for major histocompatibility complex (MHC) class I-binding immunogenic peptides [3,5]. Immunization of mice with peptides derived from the E7 oncoprotein of HPV-16 protected those mice against outgrowth of HPV-16 transformed tumors [6]. Eradication of tumors using adoptive transfer with adenovirus-specific CTL is achieved in mice [7]. CTL epitopes of Epstein-Barr virus (EBV) antigens restricted to different human MHC class I molecules have been mapped during the last few years [4]. The HLA-A*0101, A*0201, A*0301, A*1101 and A*2401 binding HPV-16 E6 and E7 derived peptides were recently described [8,9]. Peptides derived from mutated proteins, e.g., mutated p21 ras protein, and abnormal chromosome translocation can also code for tumor-specific CTL epitopes [1–3]. A mutant p53 peptide was shown to give rise to K^d-restricted CTL responses recognizing the synthetic and processed mutant p53 peptide [10]. The well-documented immune responses in humans to the melanoma-associated MAGE gene products [11–14], GP100 [15] and tyrosinase [16], all self-proteins, with the latter two also expressed in normal melanocytes support the idea to use wild-type p53 products as targets for T cells.

The nuclear phosphoprotein p53 is composed of 393 amino acids [17]. The normal p53 protein acts as a suppressor of cell division. Although it is not completely clear how the p53 protein inhibits cell growth, recent work provided some clues. The p53 protein promotes the transcription of a 21 kDa protein by binding to a specific DNA binding site [18]. This 21 kDa protein inhibits cyclin-dependent kinases (CDK), which are needed to drive cells through the cell cycle. The 21 kDa protein is called CIP1 (CDK-

Key words: p53; Peptide; Cytotoxic T lymphocyte; Peptide major histocompatibility complex affinity; Cancer; (Human)

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interacting protein 1) [19] or WAF1 (wild-type p53-activated fragment 1) [20]. Cell cycle arrest is needed to prevent the replication of damaged DNA. p53 protein levels increase when cells are exposed to DNA-damaging agents; in other words, there are physiologically relevant situations in which p53 is over-expressed [21,22]. p53 can send a cell into a programmed spiral of cell death, apoptosis, in response to DNA damage [24–26]. This linkage between p53 and apoptosis is of direct importance for chemotherapy and radiation treatments based on inducing apoptosis [27] because drug-resistant tumor cells might be the cells exhibiting loss of the p53 protein [22,25]. Mutant p53 confers resistance to radiation and chemotherapy [22]. Otherwise the existence of p53 null cells shows that p53 is not an absolute requirement for cell division [28,29].

Inactivation of the p53 gene contributes to the development of 50–60% of all human cancers [30,31]. An inactivated p53 protein is correlated with more aggressive tumors, metastasis and lower 5-year survival rates in different forms of cancer [30–33]. Whether p53 alteration is an early or late event in carcinogenesis may depend on tumor type. Inactivation of the p53 gene can be caused by different mechanisms. A mutation of one of the p53 alleles and deletion of the other allele is the most frequent cause of p53 inactivation [30,33]. The mutations are mostly missense mutations [30,33]. Mutational hot-spots are described depending on the particular type of cancer [30,33,34]. Germline p53 mutations as in Li-Fraumeni patients provide a high risk for development of cancer in many tissues including colon, ovarian and breast carcinomas, soft tissue sarcomas, osteosarcomas, brain tumors and leukemias [35]. Some viruses are capable of inactivating the p53 protein. The SV-40 T antigen blocks the ability of the p53 protein to bind to DNA by direct binding to the middle part of the p53 protein [36]. The E6 oncoproteins of HPV-16 and -18 interact with the carboxyl-terminal region of p53, promoting the degradation of p53 via the ubiquitin-dependent proteolysis pathway [37–40]. Recently a gene has been described that encodes an E6-associated protein (E6AP) which mediates the interaction between E6 and p53 and induces p53 degradation [41]. The E1B protein of the adenovirus type 5 is also able to complex and inactivate wild-type p53 [42,43]. In soft tissue sarcomas the 90 kDa mdm-2 oncogene product is capable of binding to the p53 protein, thereby inhibiting p53 function [44,45]. Another mechanism of p53 inactivation is found in breast cancer, where the p53 protein was found sequestered in the cytoplasm instead of being transported into the nucleus [46]. The mechanism of such abnormal sequestration has not yet been determined.

The half-life of wild-type p53 is less than 30 min; therefore the protein level in the cell is very low [33,47]. Mutations of p53 are associated with a dramatically prolonged protein stability resulting in much higher intracellular concentrations of the mutant p53 protein [33,48]. Viral oncoproteins influence the half-life of p53 differently. The SV40 T antigen and the Ad5 E1B protein stabilize the p53 protein in the cell in contrast to protein level and half-life of p53 in E6 immortalized cells in which degradation of p53 is enhanced by the ubiquitin-dependent proteolytic system [38].

Antibodies against human p53 are demonstrable in an appreciable proportion of patients with breast cancer [49–51], childhood cancers [52], lung cancer [53] and prostate, bladder and thyroid cancer next to lymphoma and leukemia [54]. The presence of p53 antibodies is associated with poor prognosis [51]. Over-expression of the p53 protein is important for the formation of antibodies [53]. The antibodies present in sera of cancer patients are directed against wild-type p53 epitopes [54]. By a peptide mapping procedure the wild-type epitopes recognized by p53 antibodies were identified [54]. The immunodominant regions are located at the carboxyl and amino terminal ends of the protein [51,54]. The existence of anti-p53 IgG antibodies indicates that a p53-specific CD4 response occurs. Proliferative responses of $CD4^+$ lymphocytes from cancer patients with over-expressed p53 have indeed been observed and are directed against wild-type p53 HLA class II-binding peptides (Soussi, personal communication).

Our work is based on the hypothesis that p53-specific and therefore self-reactive T cells might evade clonal deletion during thymic development because the homologous p53 epitopes are not presented effectively through endogenous processing. These p53 epitopes could become immunogenic in tumor cells displaying an altered quantity and/or quality of p53 protein expression, and subsequently activate T-cell responses. T cells directed against p53 might recognize lower levels of p53 epitopes once the T cells have been activated. In other words self-epitopes, such as p53 epitopes, presented in a density not sufficient for T-cell activation can be sufficient for target cell recognition by already activated T cells. Stimulation with p53 CTL epitopes in vitro could break tolerance and activate an autoreactive tumor-specific T-cell response. We will present and discuss our latest data concerning p53 peptide-specific CTL.

3. Material and Methods

3.1. MHC peptide binding assay

The selection of p53 peptides was done by a computer scoring program based on an HLA-A*0201 peptide motif [55] which has been previously described [56]. The HLA-A*0201 motif was adjusted by adding a threonine at position 2 and the C-terminal end as anchor residue [8]. Peptides were synthesized by solid-phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) using Fmoc chemistry. The purity of the peptides was determined by reversed-phase HPLC and was found to be routinely over 90% pure. Peptides were dissolved in DMSO (final DMSO concentration 0.25%) and diluted in 0.9% NaCl to a peptide concentration of 2 mg/ml and stored at −20°C. The T2 cell line, a gift from Dr. P. Cresswell (Dept. of Immunology, Yale University, New Haven, CT), was cultured in Iscove's modified Dulbecco's medium (IMDM) (Biochrom, Seromed Berlin) with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml kanamycin and 10% fetal calf serum (FCS, Hyclone Laboratories, Logan, UT). The T2 binding assay was performed as previously described [56]. In short, washed T2 cells were incubated overnight with peptide or 0.9% NaCl. Peptides binding to the HLA-A*0201 molecule will stabilize this molecule at the cell surface of the T2 cell line and therefore increase the HLA-A*0201 cell surface expression. Cells were stained by indirect immunofluorescence with the mouse anti-HLA-A2 monoclonal antibody BB7.2 as a first antibody and goat anti-mouse FITC-labeled $F(ab')_2$ fragments as a second antibody and measured on a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ). The fluorescence index (FI) was calculated by the formula:

$$\frac{\text{(mean fluorescence experimental sample} - \text{mean fluorescence background)}}{\text{mean fluorescence background}}$$

Subsequent serial dilutions of peptide concentrations were used to determine the concentration needed to upregulate HLA-A*0201 to half maximum ($^1/_2$ max) fluorescence on the T2 cell line.

3.2. Peptide competition assay

The influenza matrix specific HLA-A*0201 restricted CTL clone Q66.9 was a generous gift from Dr. H. Spits (NKI, Amsterdam, The Netherlands). The CTL clone was grown on HLA-A*0201-positive EBV-transformed B-cell line irradiated with 30 Gy in RPMI (Gibco, Paisley, Scotland) with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml kanamycin and 10% FCS (Hyclone Laboratories, Logan, UT). In the competition cytotoxicity assay 2000 europium ($Eu3^+$) labeled T2 cells (40 μl complete IMDM medium per well) were incubated with 10 μl of the influenza CTL epitope (500 pg/ml, final concentration at which plateau level of lysis by Q66.9 is just reached) in the presence or absence of 10 μl of one of the competitor peptides in a 96-well U-bottomed plate. The controls, background (no peptide) and maximum lysis (influenza CTL epitope only), were included on every plate. The target cells were labeled with $Eu3^+$ as described elsewhere [57]. After 30-min incubation the influenza-specific CTL clone was added in 40 μl of complete IMDM medium at an effector-to-target ratio of 5:1 for an additional 4 h of incubation. After 4 h 20 μl of each culture supernatant was collected and mixed with 200 μl Enhancer Solution (Wallac, Turku, Finland). Measurement of the samples took place in a 1234 Delfia fluorometer (Wallac). The percentage $Eu3^+$ release was calculated by the formula:

$$\frac{(Eu3^+ \text{ release experimental well} - \text{background } Eu3^+ \text{ release})}{(\text{maximum } Eu3^+ \text{ release} - \text{background } Eu3^+ \text{ release})} \times 100$$

Serial dilutions of the concentration of competitor peptide were used to determine the concentration needed to block the lysis to $^1/_2$ max.

3.3. Induction of CTL responses

The in vitro CTL response induction method recently published [58] was slightly modified. Peptide loaded and mitomycin-C-treated T2 cells were used as antigen-presenting cells (APC) and co-cultivated with HLA-A*0201-positive PBMC of a healthy donor at a ratio of 1 T2 per 4 PBMC. Cells were cultured for 10 days in 2 ml of standard medium (RPMI-1640 Dutch modification (Gibco, Paisley) containing L-glutamine, antibiotics, 15% pooled human serum and 40 μg/ml peptide) in 24-well Costar plates at a density of 2 million cells/well. Responder cells were harvested on a Ficoll layer (Lymphoprep, Nycomed-pharma, Oslo, Norway) and depleted for $CD4^+$ T cells using CD4-coated magnetic beads (Dynal, Oslo, Norway) according to the manufacturer's instructions. Two million CD4-depleted responder cells were re-stimulated with a feeder mix

consisting of 1×10^6 irradiated (3000 rad) autologous PBMC and 2×10^5 irradiated (10 000 rad) autologous B-LCL. Feeder cells were sensitized with 50 μg/ml peptide in serum-free IMDM for 2 h at 37°C washed and added to the responders. The cells were cultured in standard medium, however, without free peptide and supplemented with 60 IU/ml human recombinant interleukin-2 (rIL-2) (Eurocetus, Amsterdam, The Netherlands). At day 17, responder cells were harvested on Ficoll and cloned by limiting dilution: 10 or less responder cells were co-cultured with 1×10^5 irradiated (3000 rad) PBMC and 5000 irradiated (10 000 rad), peptide sensitized, washed B-LCL (from at least 2 different HLA-A*0201-positive donors), in 100 μl of standard medium containing 60 IU rIL-2/ml and 1% leucoagglutinin (Sigma, St. Louis, MO). Growing clones were expanded but stimulated with peptide-sensitized washed B-LCL at a 2-week interval only. Cytotoxic specificity of responding cells against peptide-sensitized target cells was tested in standard 4 h ^{51}Cr-release assays. The percentage ^{51}Cr release was calculated by the formula:

$$\frac{(^{51}\text{Cr release experimental well} - \text{background } ^{51}\text{Cr release})}{(\text{maximum } ^{51}\text{Cr release} - \text{background } ^{51}\text{Cr release})} \times 100$$

4. Results

4.1. T2 binding assay

Based on selection by a computer scoring program (see Material and Methods) we synthesized 63 HLA-A*0201 motif bearing p53 peptides with a cut-off of more than 72 points or more (25 peptides with a length of 9 amino acids, 22 decamers and 16 elevenmers). Among them 11 HLA-A*0201 binding p53 peptides (6 nonamer, 2 decamer and 3 elevenmer) were found using the T2 binding assay (see Materials and Methods) with a FI cut-off of 1.0 (final peptide concentration 100 μg/ml, Table 1 and 2A). In addition to the already published HLA-A*0201-binding p53 peptides [58] we add 3 new HLA-A*0201-binding peptide. Serial dilutions of peptide concentrations were used to determine peptide-binding affinity (Table 2A). The concentration needed for up-regulation of the HLA-A*0201 molecule to $^1/_2$ max was calculated from the results in Table 2A and is shown in Table 3, second column. The ranking of the peptides in Tables 2A and 3 is almost the same. Four relatively strong binding peptides (p53(25–35), p53(187–197), p53(65–73), and p53(65–74)) are identified next to two moderately well-binding peptides (p53(139–147) and p53(264–272)) and five poorly binding peptides (p53(24–32), p53(193–203), p53(122–130), p53(256–265) and p53(149–157)).

4.2. Peptide competition assay

Peptides selected by the MHC peptide-binding assay were tested in a peptide competition experiment. First to demonstrate that the p53 peptides actually bind to the groove of the HLA-A*0201 molecule in a functional assay such as peptide competition for CTL recognition. Second to compare the binding assay data and the competition assay data with respect to the affinity of peptides for the HLA-A*0201 molecule. The first issue was settled by the observation that all eleven p53 peptides detected in the binding assay as binding peptides could, at the highest peptide concentrations, inhibit the lysis of CTL clone Q66.9 which recognizes a HLA-A*0201-restricted matrix influenza-derived peptide (Table 2B). Peptides which did not bind to the HLA-A*0201 molecule were not able to block lysis by clone Q66.9 (data not shown). Comparison of affinity for HLA-A*0201 in both tests, showed strong binding peptides are strong/moderate competitors, that moderately binding peptides are relatively strong-to-poor competitors, and that three out of four poorly binding peptides are moderate/poor competitors (Tables 2B and

TABLE 1
p53 PEPTIDES IDENTIFIED AS PEPTIDES BINDING TO HLA-A*0201 (cut-off level FI ≥ 1.0 at a final peptide concentration of 100 μg/ml)

The sequence numbers (seq. nr.) of the first and last amino acids are shown. The peptides are ranked according to first seq. nr. Peptides were selected using the sequence analysis and in vitro assay for identifying peptides capable of binding to HLA-A*0201 [56]. *The new HLA-A*0201-binding p53 peptides in addition to the published HLA-A*0201-binding p53 peptides [58].

Seq. nr.	Peptide
24–32	K L L P E N N V L
25–35	L L P E N N V L SPL
65–73	R M P E A A P P V
65–74	R M P E A A P P VA
122–130	V T C T Y S P A L*
139–147	K T C P V Q L W V*
149–157	S T P P P G T R V*
187–197	G L A P P Q H L IRV
193–203	H L I R V E G N LRV
256–265	T L E D S S G N LL
264–272	L L G R N S F E V

TABLE 2
A: TITRATION OF THE p53 PEPTIDES IN THE BINDING ASSAY (avg of 3 exp)

Binding of p53 peptides at concentrations of 100, 50, 25, 12.5, 6.25, 3.1, and 1.6 μg/ml (final concentration in the test). Indicated is the Fluorescence Index (FI). –, FI ≤ 0.20. The peptides are ranked in order of binding affinity to HLA-A*0201.

p53 peptides (seq. nr.)	100	50	25	12.5	6.25	3.1	1.6
LLPENNVLSPL (25–35)	2.4	2.2	1.9	1.9	1.1	0.7	0.4
GLAPPQHLIRV (187–197)	2.2	1.8	1.5	1.3	0.8	0.5	0.3
RMPEAAPPV (65–73)	1.9	1.8	1.2	1.0	0.7	0.4	0.3
RMPEAAPPVA (65–74)	2.1	1.7	1.1	1.0	0.6	0.4	–
KTCPVQLWV (139–147)	1.5	1.3	0.9	0.5	0.3	–	–
LLGRNSFEV (264–272)	1.7	1.3	0.8	0.4	–	–	–
KLLPENNVL (24–32)	1.6	1.2	0.7	0.4	–	–	–
VTCTYSPAL (122–130)	1.7	1.0	0.6	0.3	–	–	–
STPPPGTRV (149–157)	1.5	0.9	0.5	0.3	–	–	–
TLEDSSGNLL (256–265)	1.2	0.7	0.5	0.3	–	–	–
HLIRVEGNLRV (193–203)	1.2	0.8	0.6	0.3	–	–	–

B: TITRATION OF THE p53 PEPTIDES IN THE COMPETITION ASSAY (avg of 3 exp)

Competition of p53 peptides at concentrations of 100, 50, 25, 12.5, 6.25, 3.1, 1.6 and 0.8 μg/ml (final concentration in the test). Indicated is the percentage specific lysis. The peptides are ranked in order of competition ability.

p53 peptides (seq. nr.)	0.8	1.6	3.1	6.25	12.5	25	50	100
HLIRVEGNLRV (193–203)	44	25	26	13	16	10	7	3
RMPEAAPPV (65–73)	71	69	50	41	22	5	1	0
LLPENNVLSPL (25–35)	71	59	51	26	22	11	6	4
RMPEAAPPVA (65–74)	73	67	62	33	25	6	3	3
LLGRNSFEV (264–272)	67	64	51	39	27	23	2	0
TLEDSSGNLL (256–265)	75	68	52	43	32	11	4	3
KLLPENNVL (24–32)	84	73	65	49	38	17	5	2
GLAPPQHLIRV (187–197)	83	74	67	55	36	38	8	7
STPPPGTRV (149–157)	81	85	76	64	58	37	18	5
VTCTYSPAL (122–130)	71	65	68	47	56	36	30	16
TCPVQLWV (139–147)	74	75	69	51	47	34	26	20

3). Peptide p53(193–203) was a relatively poorly binding peptide. In the competition assay, however, this peptide was one of the best competitors (Tables 2B and 3). The explanation for this difference is at present not clear. Different circumstances during the two tests might influence the stability of the peptides or the interaction of the peptides with the HLA-A*0201 molecule.

4.3. Induction of CTL responses

We have previously reported the succesful generation of stable, peptide-specific CTL clones against the p53(264–272) peptide [58]. With the same CTL response induction protocol we obtained only weakly specific responses against other wild-type p53 peptides: p53(25–33), p53(65–73) and p53(187–197) [59]. After modification of the CTL response induction protocol (CD4 depletion at day 10, and limiting dilution for cloning at day 17) we again tried to obtain peptide-specific CTL clones against the three best binding p53 peptides (p53(25–35), p53(187–197), and p53(65–73), Table 2A). We succeeded in inducing peptide specific clones against all three p53 peptides (Table 4): one clone specific for peptide p53(25–35), five against p53(187–197) and five specific for p53(65–73). The newly obtained clones are as specific as the clones obtained with the former T-cel induction protocol against peptide p53(264–272) (Table 4).

5. Discussion

The HLA-A*0201 molecule is the most frequently occurring class I allele of caucasians (45%). Inactiva-

TABLE 3
COMPARISON BETWEEN THE $^1/_2$ max OF BINDING AND THE $^1/_2$ max OF COMPETITION FOR THE HLA-A*0201 *BINDING* p53 *PEPTIDES*

The peptides are ranked in order of $^1/_2$ max of binding. The $^1/_2$ max of binding is derived from Table 2A. The $^1/_2$ max of competition is derived from Table 2B. A summary for binding and competition ability is shown in the last column using an arbitrary score. +, relative strong binding ($\leqslant$13 μg/ml) or competitor ($\leqslant$8 μg/ml) peptide; $\pm$, a relative moderate binding (14–26 μg/ml) or competitor (9–16 μg/ml) peptide; −, a relative poor binding (27–40 μg/ml) or competitor (17–24 μg/ml) peptide.

p53 peptides (seq. nr.)	$^1/_2$ max binding	$^1/_2$ max comp	Bind/ comp
LLPENNVLSPL (25–35)	6	6	+/+
GLAPPQHLIRV (187–197)	8	12	+/±
RMPEAAPPV (65–73)	12	8	+/+
RMPEAAPPVA (65–74)	12	6	+/+
KTCPVQLWV (139–147)	20	20	±/−
LLGRNSFEV (264–272)	26	8	±/+
KLLPENNVL (24–32)	32	12	−/±
HLIRVEGNLRV (193–203)	34	2	−/+
VTCTYSPAL (122–130)	40	22	−/−
TLEDSSGNLL (256–265)	40	10	−/±
STPPPGTRV (149–157)	40	24	−/−

tion of the p53 protein is reported in approximately 50–60% of all malignancies [30–33]. HLA-A*0201 binding wild-type p53 peptides or HLA*0201-restricted CTL recognizing wild-type p53 epitopes may therefore have therapeutic potential for a large number of patients. We identified the p53 peptides with the highest affinity to the HLA-A*0201 molecule using two assays: the peptide binding assay and the peptide competition assay. The results of both tests were mostly complementary. One peptide p53(193–203) showed a striking difference in the two tests. In the peptide-binding assay it turned out to be a weakly binding peptide but in the competition assay it was one of the best competitors. It is unclear to us how to interpret this finding. The immunological relevance will be clarified by testing whether this peptide or a length variant of it is processed and presented in context of HLA-A*0201 and recognized by peptide-specific CTL. We tried to induce CTL clones against three p53 peptides with a high affinity for the HLA-A*0201 molecule. Through modification of the CTL induction protocol we succeeded in culturing peptide-specific clones against all three p53 peptides in addition to previous reports [58,59]. It remains to be demonstrated which if any of these HLA-A*0201-binding p53 peptides are processed selectively in tumor cells versus normal cells.

In the near future we will compare cancer patients and healthy controls for the frequencies of CTL-p against HLA-A*0201-binding p53 peptides with a limiting dilution analysis. Such an assay will help us estimating the importance of p53 peptides as possible T-cell targets in cancer cells [60]. Results of such an assay will also inform us how to optimize vaccines, if CTL-p will be measured before and after therapeutic vaccinations in cancer patients.

Using p53-knock-out mice, one can obtain more insight into tolerance for p53 peptides. We are testing if K^b and D^b binding mouse p53 peptides which are non-immunogenic in normal mice can give rise to p53 peptide-specific T-cell responses in p53-knock-out mice in the absence of immunologic tolerance for p53.

It is probably possible to overcome tolerance espe-

TABLE 4
LYTIC ACTIVITY OF CTL CLONES AGAINST p53 PEPTIDES

The code for the clones: e.g., 25–19 = p53 peptide (25–35) clone 19. An HLA-A*0201 positive EBV was used as the target cell line in the ^{51}Cr release assay. Spec: % specific lysis in ^{51}Cr release assay (target cell loaded with the specific peptide). Aspec: % aspecific lysis in ^{51}Cr release assay (target cell not loaded with a peptide). The effector/target ratio was 10:1, 5:1, $2^1/_2$:1 and $1^1/_4$:1.

Clone	25 19	187 2	187 3	187 12	187 17	187 27	65 4	65 8	65 11	65 15	65 16	264* 1A5	264* 3C5
Spec. target	37	59	43	28	67	71	37	53	67	46	64	60	57
	30	63	30	23	73	46	40	45	65	40	65	72	46
	27	36	27	42	57	49	51	48	49	13	45	55	46
	15	26	18	38	40	48	48	32	39	14	47	35	46
Aspec. target	6	5	10	15	0	9	10	5	0	4	3	3	4
	6	5	7	10	−5	4	5	0	0	4	5	2	4
	7	3	0	12	6	6	−4	5	4	5	5	2	1
	9	5	−1	4	6	2	−1	2	0	5	−2	3	1

*These two previously described clones [58] are shown for comparison with the present data.

cially if the self-proteins are not expressed at sufficient levels at the time and place of tolerance induction. The results of serological analysis detecting p53 antibodies recognizing wild-type p53 means that B- and T-cell responses against p53 do occur [54]. The detection of CTL responses specific for wild-type p53 cross-reactive on processed peptides expressed at the surface of tumor cells will hopefully only be a question of time.

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