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CTLA-4 Blockade Enhances the CTL Responses to the p53 Self-Tumor Antigen

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Tumor-associated Ags are often derived from proteins that are also expressed on normal tissues. This fact has focused attention on self-tolerance as an important issue in the development of T cell responses capable of eliminating tumor cells. Also, during tumor growth, Ags expressed uniquely by the tumor cells can be cross-presented to T cells under conditions that result in tolerance or anergy rather than sustained effector function. Self-tolerance and tumor-induced peripheral tolerance may be responsible for the limitations of the immune system in controlling tumor growth in cancer patients. In many patients, the presence of circulating, Ag-experienced, tumor Ag-specific CD8+ T cells can be demonstrated; however, they are not able to eradicate the tumors.

In the past few years, a number of different types of approaches have been pursued in an effort to enhance tumor immunity and override tolerance. One such approach targets CTLA-4, which acts as a negative regulator of T cell responses. CTLA-4 is up-regulated on CD4+ and CD8+ T cells after activation, whereupon it can engage B7 on APCs. This engagement has been shown in vitro to prevent T cell proliferation by inhibiting IL-2 production and arresting cell cycle progression.

The p53 tumor suppressor protein represents an attractive target Ag for cancer immunotherapy. A high proportion of human tumors process and present much higher levels of p53 epitopes to T cells than are found on normal cells. The current study examines the effect of anti-CTLA-4 mAb in the development of CTL responses to p53 in A2.1/Kb-transgenic mice and found that compared with p53-deficient mice, the CD8+ T cell response was devoid of T cells with high-affinity TCRs as defined by their ability to bind HLA A2.1 tetramers that contain cognate peptide. This scenario, where only a weak response comprised of relatively low-affinity CD8+ T cells is available to respond against a tumor Ag, is likely to represent the usual situation for many tumor-associated epitopes. Accordingly, a major challenge for successful immunotherapy is to determine how to activate optimally the available repertoire to reject tumor cells.

The availability of detailed information concerning the CD8+ response to p53 makes this Ag an attractive vehicle for assessing the ability of anti-CTLA-4 mAb to override tolerance to a self-tumor Ag. The current study examines the effect of anti-CTLA-4 mAb in the development of CTL responses to p53 in A2.1/Kb-transgenic mice. The CTL response is greatly enhanced by blockade of CTLA-4 engagement, resulting from a 10-fold increase in the numbers of effector cells compared with the response in the absence of this reagent. This enhancement also persists in the number of memory cell precursors. The effect is primarily dependent on the presence of CD4+ T cell help during priming and on the augmentation of this function by the anti-CTLA-4 mAb. Despite this enhanced response, there was no increase observed in the avidity of the CD8+ T cells obtained. Therefore,
the response was altered quantitatively but not qualitatively. These results have important implications for the development of vaccination strategies in cancer immunotherapy.

Materials and Methods

Mice

A2.1/Kb-transgenic mice are on a C57BL/6 background and have been previously described (29). Mice were propagated and maintained under specific pathogen-free conditions in our vivarium at The Scripps Research Institute (La Jolla, CA). 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 that is deficient in TAP, EL4-A2.1/Kb, and T2-A2.1/Kb transfectants has already been described (29, 28). The 261 CTL clones 7 and 13 specific for p53 261–269 were previously described (29).

Reagents

The following Ab were used for in vivo treatment. Purified anti-CTLA-4 mAb UC10–4F10 was obtained from J. A. Bluestone (9). Purified hamster IgG1 was purchased from Jackson ImmunoResearch (West Grove, PA). Each animal received 100 μg of Ab injected i.p. on days −1, 0, and +1 of Ag priming.

Effector CTL generation

The procedure used to obtain peptide-specific effector CTL has been described elsewhere (28). Briefly, mice were infected s.c. at the base of the tail with 100 μg of murine p53 261–269 peptide (LLQRDSFPEV) alone or along with 120 μg of the I-Ab Th peptide 128–140 of the HBV core protein (HBVc hp)1 (TTPAYRPPNAPIL) in IFA. After 10 days, mice were sacrificed and spleen cells were stimulated in vitro with irradiated LPS-activated syngeneic spleen cells pulsed with the priming peptide at 5 μg/ml in RPMI 1640 medium containing 10% FBS, 2 mM glutamine, 5×10−3 M 2-ME, and 50 μg/ml gentamicin sulfate (complete medium). On day 6, effector cells were assayed for their lytic activity in a 5-h 51Cr release assay using T2-A2.1/Kb cells pulsed with different amounts of peptide as targets and at different E:T ratios. For the analysis of memory effector CTL, spleen cells from mice primed 1 mo earlier were maintained in culture for 12 days instead of 6 days using the same APCs as above. On day 8, medium was supplemented with 2% supernantant from Con A-stimulated rat spleen cells (28). For peptide titration analysis and analysis of tetramer binding, CTL lines were passaged in culture by weekly restimulation with irradiated ELA-A2.1/Kb cells (0.5×105 cells/well) pulsed with peptide and C57BL/6 spleen cells (6×105 cells/well) as fillers in complete medium supplemented with 2% supernantant from Con A-stimulated rat spleen cells. On day 4 after restimulation, fourth passage cells were tested in a 4-h 51Cr release assay using T2 cells pulsed with different amounts of peptide as targets at the indicated E:T ratio or stained as described below.

Cytotoxicity assays

Target cells were prepared as follows: 105 T2 or T2-A2.1/Kb cells were incubated for 1.5 h at 37°C with 200 μCi of sodium 51Cr-chromate (New England Nuclear, Boston, MA) in the presence or absence of p53 261–269 peptide at the indicated concentration in a final volume of 300 μl in complete medium. After three washings, targets were added to 96-well plates (32 wells/dilution) containing LPS-activated syngeneic spleen cells pulsed with peptide (2×105/well) in complete medium supplemented with 5% supernatant from Con A-stimulated rat spleen cells. On day 8, cultures were assayed using 261–269 peptide-loaded T2-A2.1/Kb cells as targets in an 8-h 51Cr release assay. Wells were considered positive when 3Cr release was >20% of the spontaneous release measured in replicates of wells containing medium with APCs and no spleen cells. Statistical analyses were performed as described previously (30).

Anti-CTLA-4 treatment enhances the CTL responses to p53 261–269 peptide

As demonstrated previously (28, 29), A2.1-restricted CTL specific for the p53 261–269 epitope can be retrieved after immunization of A2.1/Kb-transgenic mice with this peptide along with a class II peptide from HBVc that primes an I-Ab-restricted response (Fig. 1B). This response is dependent on such CD4+ T cell help, be

from the contents of each well was assayed. Assays were developed at 37°C in a final volume of 200 μl in complete medium for the indicated time. Percent specific lysis was calculated as [sample release − spontaneous release] / [maximum release − spontaneous release] × 100. In some cases, data are represented in LUs. An LU is defined as the number of effector CTL required to obtain 20% lysis. The number of LU contained in 106 effector CTL is calculated.

Proliferation assays

Mice were immunized with 120 μg of HBVc hp in IFA and treated with Ab as described above. Ten days later, spleen cell suspensions were prepared and enriched for CD4+ T cells by incubation with a mixture of anti-CD8 (3.155), anti-Ia-1 (11D1), and anti-MHC class II (CA4/A12) mAbs tissue culture supernatant at a ratio 2:1:1 in 8 ml for 1 h at 4°C. Rabbit complement (Low-Tox complement; Accurate Chemical, Westbury, NY) was then added at a final dilution of 10% and the cells were further incubated for 1 h at 37°C. The 2×105 enriched CD4+ spleen cells were cultured in a final volume of 200 μl of complete medium with 5×103 APCs. Irradiated syngeneic spleen cells were pulsed with the indicated amounts of peptide in 1 ml of complete medium for 2 h and then used as APCs. All cultures were set up in triplicate. Three days later, 1 μCi/well [3H]thymidine was added and cultured for an additional 8 h at 37°C. Cells were harvested using a Tomtec cell harvester and radioactivity was measured in a liquid scintillation counter (Betaplate; Wallac, Turku, Finland).

Flow cytometry

On day 4 after stimulation, cells were partially purified through a Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) cushion and then washed in HBSS. Cells (0.5×107) were incubated for 30 min at room temperature with A2.1p53 261–269 tetramers labeled with allophycocyanin (obtained from the National Institute of Allergy and Infectious Diseases Tetramer Facility and the National Institutes of Health AIDS Research and Reference Reagent Program) at 40 μg/ml and anti-murine CD8a-FITC mAb 53-6.7 (BD PharMingen, San Diego, CA) at 2 μg/ml in HBSS containing 0.1% BSA and 0.05% sodium azide. Propidium iodide was added after the final wash at 1 μg/ml to exclude dead cells in all experiments. Samples were analyzed on a BD Becton Dickinson (San Jose, CA) FACSsort apparatus at the TSRI FACS facility. Twenty thousand events were collected and analyzed using CellQuest software (BD Becton Dickinson).

Results

As demonstrated previously (28, 29), A2.1-restricted CTL specific for the p53 261–269 epitope can be retrieved after immunization of A2.1/Kb-transgenic mice with this peptide along with a class II peptide from HBVc that primes an I-Ab-restricted response (Fig. 1B). This response is dependent on such CD4+ T cell help, be

<FIGURE 1. Generation of p53 261–269-specific effector CTL requires CD4+ T cell help. Effector CTL from A2.1/Kb-transgenic mice primed with p53 261–269 peptide (A) or primed with p53 261–269 plus HBVc hp (B) were assayed for lytic activity against T2-A2.1/Kb cells (○, ○) or T2-A2.1/Kb cells pulsed with 10−6 M p53 261–269 peptide (●, ● symbols) at the indicated E:T ratio. Squares and circles represent data of CTL from two different mice in each panel. They are representative of six individually analyzed mice per group in two different experiments.

1 Abbreviation used in this paper: HBVc hp, hepatitis B virus core protein helper peptide.
cause immunization with p53\textsubscript{261–269} peptide alone is unable to elicit specific effector CTL (Fig. 1A).

We analyzed the effect of blockade of CTLA-4 engagement in vivo during the induction of a CTL response to p53\textsubscript{261–269}. First, we tested anti-CTLA-4 mAb treatment when priming with the p53\textsubscript{261–269} peptide alone. Anti-CTLA-4 mAb promotes generation of an extremely weak but specific CTL response (Fig. 2C), comparable to what is obtained with a 100-fold fewer CTL from mice that were primed with helper epitope (Fig. 2D). The specificity of this result was supported by the observation that no detectable effector CTL were observed if we used an isotype control Ab (hamster IgG) along with the 261–269 peptide (Fig. 2B). Also, treatment with anti-CTLA-4 in the absence of the p53 peptide did not induce p53\textsubscript{261–269}-specific CTL (Fig. 2A). These results suggest that a weak but specific enhancement of the CD8\textsuperscript{+} response by anti-CTLA-4 can occur in the absence of specific CD4\textsuperscript{+} help.

Next, we tested the effect of anti-CTLA-4 treatment on CTL generation by priming with p53\textsubscript{261–269} peptide in combination with the class II peptide Ag. Under such conditions, CTLA-4 blockade induced a much more vigorous CTL response (Fig. 2F) compared with that of mice treated with the isotype control Ab (Fig. 2E). The CTL responses in the presence or absence of the isotype control were essentially identical (Fig. 2, E and D, respectively), indicating the specificity of this effect. The level of lysis by CTL obtained from the anti-CTLA-4-treated mice was 10-fold enhanced over the level by CTL from nontreated or control Ab-treated mice.

Because enhancement of CTL responses was greatly dependent on the presence of CD4\textsuperscript{+} help, we analyzed the effect of anti-CTLA-4 treatment directly on CD4\textsuperscript{+} T cells. Proliferation of CD4\textsuperscript{+} T cells from mice immunized and treated with anti-CTLA-4 in response to HBVc hp was enhanced >2-fold compared with cells from mice immunized and treated with the isotype Ab (Fig. 3). No specific proliferation was observed in nonimmunized mice either treated or untreated with anti-CTLA-4 mAb (Fig. 3). Although an enhanced proliferation induced by CTLA-4 blockade was evident, the HBVc hp-specific proliferative response was rather low. These experiments were set up under conditions identical to those designed for the analysis of CTL responses. It is likely that these immunization conditions are not optimal for the analysis of class II-restricted responses. However, the data indicate that anti-CTLA-4 does enhance the CD4\textsuperscript{+} helper function that is essential for the development of p53-specific CTL responses.

We next analyzed whether the enhancement observed in the generation of p53\textsubscript{261–269}-specific CTL by the presence of anti-CTLA-4 mAb resulted in a larger pool of memory cells available for this Ag. To this end, mice were primed with p53\textsubscript{261–269} and the helper epitope in either the presence or absence of the CTLA-4 blockade and the CTL responses were analyzed 1 mo after priming (Fig. 4). The results were similar to those obtained when CTL responses were analyzed 10 days after priming in that anti-CTLA-4 treatment resulted in the induction of a much more potent CTL response (Fig. 4, B and C) compared with the controls (Fig. 4, A and C). This indicated that the boost supplied by anti-CTLA-4 mAb in the generation of a CTL response also increased the pool of memory T cells specific for p53\textsubscript{261–269}.
Anti-CTLA-4 treatment augments the frequency of the low-affinity p53261–269-specific CTL

In a previous report, we demonstrated that the CD8+ T cell repertoire available for the p53261–269 self-Ag expresses 10-fold lower affinity TCRs than do T cells from p53-deficient mice. Thus, tolerance to p53 results in the elimination from the response of T cells expressing TCRs with high affinity for this self-epitope (29). Taking this into account, two possible scenarios could explain the enhancement observed with anti-CTLA-4. First, CTLA-4 blockade may augment responsiveness by facilitating the participation in the response of T cells with TCRs that have high affinity for the p53261–269 epitope. Consistent with this possibility, it has been described that anti-CTLA-4 treatment may reverse CD8+ T cell tolerance to a tumor-expressed Ag by reversing anergy (21). Alternatively, the presence of anti-CTLA-4 may result in the enhanced expansion of the low-avidity p53-specific CTL that are usually found to respond in these mice. To distinguish between these possibilities, the CTL generated in the presence or absence of anti-CTLA-4 were compared by three criteria: 1) binding of tetramers of HLA A2.1 containing cognate peptide; 2) dose response to peptide titration in a cytolysis assay; and 3) the CTL precursor frequency.

We have shown previously that A2.1/mu p53261–269 tetramers can distinguish between CD8+ T cells expressing high- and low-affinity TCRs for this Ag. Tetratners are able to bind stably to CD8+ T cells with a relatively high-affinity TCR for this Ag; however, such high-affinity T cells could only be obtained from mice deficient in p53 (29). An example of such binding is exhibited by p53261–269-specific CTL clone 7 (Fig. 5B and Ref. 29). However, these same tetramers could not stably bind to p53261–269-specific CD8+ T cells obtained from mice that express normal levels of p53, as exemplified by CTL clone 13 (Fig. 5A and Ref. 29). It should be noted that the inability of the low-affinity T cells to stably bind tetramers containing cognate peptide is an unusual situation and most likely due to the lack of binding assistance by murine CD8, which is unable to bind human class I molecules (31), such as the HLA A2.1 present in the tetramer. Recent studies have demonstrated an important contribution by CD8 in tetramer binding (32, 33). Thus, we compared p53261–269-specific CTL lines from untreated (Fig. 5, C and D) vs anti-CTLA-4-treated mice (Fig. 5, E and F) in their ability to bind A2.1/mu p53261–269 tetramers. In agreement with our previous observations, despite being capable of specifically lysing target cells (see below), no tetramer-binding CD8+ T cells could be detected in any of the CTL lines from untreated mice. The same results were found when CTL lines from anti-CTLA-4 mAb-treated mice were analyzed, indicating that the enhancement of CTL activity observed as a result of anti-CTLA-4 treatment was not due to enhanced affinity by the resultant CTL. To further confirm this observation CTL lines from treated and untreated mice were compared in their avidity by assessing their cytolytic activity using targets pulsed with increasing concentrations of peptide (Fig. 6). Again, no difference in avidity was observed between CTL induced in the presence or absence of anti-CTLA-4. Taken together, these experiments demonstrate that high-avidity p53261–269-specific CD8+ T cells cannot be rescued from mice tolerant of p53 by the use of blocking anti-CTLA-4 mAb.

These results suggest that the enhanced response observed in mice treated with anti-CTLA-4 was due to a higher frequency of CTL. To directly assess this possibility, the p53261–269 CTL precursor frequency of treated vs untreated mice was measured by
from the response high-affinity T cells specific for the naturally Ags that are also expressed in normal tissues (4, 28, 29, 34). For that self-tolerance can shape the TCR repertoire specific for tumor importance in tumor immunity. We have demonstrated previously

\[ \frac{\text{CD8}^+ \text{T cells per spleen in anti-CTLA-4-treated animals}}{\text{CD8}^+ \text{T cells per spleen in untreated mice}} \times \frac{\text{Frequency in anti-CTLA-4 mAb-treated mice}}{\text{Frequency in untreated mice}} \]

was also observed that the total number of both CD8

1

T cells and subsequent amplification of sustained effector CTL responses is of particular importance in tumor immunity. We have demonstrated previously that self-tolerance can shape the TCR repertoire specific for tumor Ags that are also expressed in normal tissues (4, 28, 29, 34). For example, expression of p53 in the thymus and periphery eliminates from the response high-affinity T cells specific for the naturally processed epitope p53261–269 (29). The goal of the current study was to determine whether and how blocking the down-regulatory effect of signaling through CTLA-4 affects the response specific for a self/tumor Ag. This model has several advantages over previous studies that have examined the effect of anti-CTLA-4 mAb on the CD8

+ T cell response to a defined Ag. First, it uses a self/tumor Ag rather than a foreign epitope, such as OVA or viral proteins. Second, we have examined the response of the endogenous repertoire rather than that of adoptively transferred CD8

+ T cells from TCR transgenics.

Administration of blocking anti-CTLA-4 mAb has been shown to have therapeutic anti-tumor effects, and CD8

+ T cells played an important role in such tumor rejection (13, 17). McCoy et al. (19) and Ito et al. (20) have reported enhancement of effector CTL responses to a viral peptide and OVA, respectively, by anti-CTLA-4 mAb treatment. Chambers et al. (35) showed enhancement of secondary responses by CD8

+ T cells from CTLA-4

+ TCR-transgenic mice. Our results demonstrate that CTLA-4 blockade resulted in more than a 10-fold increase in the number of p53261–269-specific CTL that were obtained after immunization with a helper epitope and the p53 peptide. This increase in CTL observed early after priming was long lasting in that the pool of memory cells available to respond in vitro to p53261–269 self-Ag was comparably augmented by this same treatment. This result is consistent with the report by Whitmire et al. (36) that the number of T cells activated during the primary responses to viral epitopes determines the size of the memory pool generated.

In our experiments, anti-CTLA-4-mediated enhancement of the p53261–269-specific CTL response was found to be highly dependent on the presence of the helper epitope. The finding that the CD4

+ response to the helper epitope was augmented by anti-CTLA-4 treatment suggests that the p53261–269-specific CTL response by anti-CTLA-4 is due to enhancement of CD4 help rather than a direct effect on CD8 cells. In contrast, enhancement of effector CTL responses by anti-CTLA-4 mAb reported by McCoy et al. (19) and Ito et al. (20) were independent of CD4

+ T cells. We observed minimal enhancement by anti-CTLA-4 in the absence of the helper epitope. It is likely such conflicting results concerning the role of CD4

+ help reflect differences in the strength of the antigenic signal provided for priming in each particular case (37). The response to p53 was entirely dependent on CD4

+ help. This requirement may reflect the low affinity of the CD8

+ repertoire that is available for response to a self-Ag. It was recently demonstrated that one factor that is determinative of CD4 dependence of a CD8 response is that stability of the peptide-class I complex (38). It is likely that TCR affinity plays a similar role in determining the

Discussion

Activation of tumor-specific CD8

+ T cells and subsequent amplification of sustained effector CTL responses is of particular importance in tumor immunity. We have demonstrated previously that self-tolerance can shape the TCR repertoire specific for tumor Ags that are also expressed in normal tissues (4, 28, 29, 34). For example, expression of p53 in the thymus and periphery eliminates from the response high-affinity T cells specific for the naturally processed epitope p53261–269 (29). The goal of the current study was to determine whether and how blocking the down-regulatory effect of signaling through CTLA-4 affects the response specific for a self/tumor Ag. This model has several advantages over previous studies that have examined the effect of anti-CTLA-4 mAb on the CD8

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| Peptide dose responses of p53261–269-specific CTL lines. | CTL lines from p53261–269 plus HBVc hp-primed A2.1/Kb-transgenics, untreated (■, ○) or treated with anti-CTLA-4 (□, ○) were analyzed after 4 weeks of rounds of in vitro restimulation. Two representative CTL lines per group are shown. Four days after restimulation, effector CTL were assayed for lytic activity against T2 cells pulsed with increasing concentrations of p53261–269 peptide as indicated at an E:T ratio of 2:1 (■, ●) or 0.2:1 (□, ○). |

FIGURE 6.

<table>
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<tr>
<th>Reciprocal of CTL precursor frequency</th>
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<tr>
<td>261-269 + HBVc hp #1</td>
<td>261-269 + HBVc hp #2</td>
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<tr>
<td>261-269 + HBVc hp</td>
<td>261-269 + HBVc hp + Anti-CTLA-4 #1</td>
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<tr>
<td>261-269 + HBVc hp + Anti-CTLA-4 #2</td>
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<td>[261-269 peptide] (M)</td>
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FIGURE 7. p53261–269 peptide-specific CTL precursor frequency analysis. Anti-CTLA-4 mAb-treated or untreated, p53261–269 plus HBVc hp-primed A2.1/Kb-transgenic mice were analyzed by limiting dilution as described in Materials and Methods. Data from six mice per goup in two different experiments are presented. Dots represent data from different mice. Bars represent the average of each group. Analysis by the Mann-Whitney U test showed the two groups to be statistically significantly different (p < 0.01).
stability of the TCR-peptide-MHC complex. In CD8 responses that are not dependent on CD4+ help, the effect of anti-CTLA-4 mAb may also be less dependent on CD4+ help. Therefore, we would propose that the in vivo enhancement of CTL responses by anti-CTLA-4 treatment may occur by: 1) blockade of CTLA-4 engagement on CD8+ T cells, an effect that may be more pronounced in helper independent responses; and 2) blockade of CTLA-4 engagement on CD4+ T cells. Enhancement of CD4+ help by anti-CTLA-4 may induce a more sustained secretion of IL-2 and expression of other molecules that directly or indirectly assist clonal expansion of CD8+ cells (21, 39).

Shrikant et al. (21) reported that anti-CTLA-4 mAb could reverse tumor-induced anergy of OT-I CD8+ T cells. In their model, the interaction between tumor cells expressing OVA and OVA-specific CD8+ T cells resulted in T cell anergy. This was also reversed by priming OVA-specific CD4+ cells or by directly supplying IL-2. In previous experiments, we could not distinguish between actual deletion of high-affinity p53261–269 CD8+ T cells and their persistence in an anergic state that could not be reversed by peptide immunization. In this study, we have shown that if such anergized T cells with high affinity for p53 are present, they cannot be rescued from tolerance by anti-CTLA-4 treatment. Considering that p53 is expressed in the thymus at high levels, the most likely explanation for the absence of high-affinity T cells is their deletion in the thymus.

Due to their inherently low avidity, the repertoire of p53-specific T cells that are available appear to be of little danger for inducing autoimmunity. It will be of interest to determine whether this is also true for T cells responsive to other self-Ags. van Elsas et al. (40) demonstrated that the use of anti-CTLA-4 in conjunction with stimulation of a response to melanoma cells leads to the induction of vitiligo. This suggests that enhanced expansion of some anti-self CD8+ cells can result in autoimmune destruction of normal tissue. Presumably, the determining factor is the affinity of the CD8+ T cells that are available in the repertoire before elicitation of the response. There may be a higher affinity repertoire available for some peripheral Ags, such as melanoma-associated tumor Ags, than is there to p53. Supporting this idea is the fact that responses leading to depigmentation in that system were CD4+ independent (40). Also, It has been reported that transgenic expression of the melanoma associated Ag tyrosinase induces only partial tolerance in the repertoire, because high-affinity specific CD8+ T cells can be retrieved from these mice (41).

The results presented here represent an important advance in clarifying the role of CTLA-4/B7 interactions in the generation of CTL responses in vivo and the mechanism of anti-CTLA-4 mAb engagement of CD4+ T cell help. Therefore, we would propose that the induction of tumor antigen-specific tolerance in vivo requires CD8+ T cell help. In vivo blockade of CTLA-4 enhances the priming of responsive T cells but fails to prevent the induction of tumor antigen-specific tolerance. Proc. Natl. Acad. Sci. USA 96:11476.

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