Does B7-1 Expression Confer Antigen-presenting Cell Capacity to Tumors In Vivo?

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Summary

Tumors engineered to express the costimulatory molecule B7-1 can elicit $CD8^+$ cytotoxic T lymphocyte (CTL)–dependent antitumor responses in immunocompetent mice. It has been postulated that this result reflects direct priming of CTL by the modified tumor in vivo. Previous studies of the immune response to a $B7-1^-$ murine colon carcinoma expressing influenza nucleoprotein (NP) as a model tumor antigen have demonstrated the crucial role of bone marrow–derived antigen-presenting cells (APCs) in the priming of NP-specific CTL in vivo. In this system, no evidence of direct CTL priming by tumor was detected. We have performed a similar analysis to determine if a B7-1 transfectant of this tumor results in the direct priming of CTL, and to compare this response to that primed by host APCs. When $H-2^b \rightarrow H-2^{bxd}$ bone marrow chimeras were immunized with a single injection of CT26/NP/B7-1 ($H-2^d$), NP-specific CTL were detected that were restricted to the bone marrow haplotype ($H-2^b$), but not to the tumor haplotype. In contrast, CTL recognizing the NP antigenic epitope in the context of the tumor's major histocompatibility complex were detectable only after multiple immunizations. These results suggest that whereas $B7-1^+$ tumor vaccines result in some degree of direct presentation to $CD8^+$ T cells, the dominant mechanism of CTL priming is through the uptake and presentation of tumor antigens by bone marrow–derived APCs. However, repeated immunization with $B7-1^+$ tumor cells can efficiently expand the directly primed $CD8^+$ CTL population.

T cell activation requires two distinct signaling events (1–3). The first signal originates from the binding of the TCR to its antigen–MHC ligand, and provides the specificity of the interaction. The second signal is either provided by soluble factors such as IL-2 or the interaction of cell surface molecules on the T cell with their ligands on APCs. This second signal is thought to provide the necessary constimulation to the TCR-mediated signaling event. Binding of the TCR with peptide–MHC complexes in the absence of costimulation can result in T cell inactivation or anergy, which is associated with a block in the IL-2 gene transcription (4–7).

Among the known costimulatory molecules, the B7 family of molecules appears to be the most potent. Both B7-1 and B7-2 can interact with their counterreceptor, CD28, and CTLA-4 on T cells. Interaction with CD28 contributes to activation of helper T cell subsets and is important for an intact in vivo immune response (8). The B7–CD28 interaction has also been shown to be necessary and sufficient costimulation for $CD8^+$ CTL generation in the absence of exogenous help (4, 9). Recently, a number of experimental systems have provided evidence suggesting the importance of both B7-1 and B7-2 as costimulators involved in generating antitumor immune responses (10–12). In some studies, B7-1–expressing tumor cells are rejected in syngeneic hosts, whereas unmodified $B7-1^-$ parental tumors are not. These observations appear to vary depending on the tumor system studied, with the greatest effect of B7-1 transfection occurring with more inherently immunogenic tumors (13). In some of these models, mice that successfully reject the B7-1 tumor transfectants as a live vaccine generate T cell–mediated systemic immunity to the parental tumor. These studies suggest that tumors are capable of delivering antigen-specific signals to T cells, but may escape immunologic rejection by failure to deliver the costimulatory signals necessary for the full activation of these tumor-specific T cells. The current working hypothesis is that $B7-1^+$ tumor cells can provide both signals one and two directly to activate naive CTL in the absence of other bystander help. However, this hypothesis has not been formally tested, and in particular, the role of host APCs in the priming of antigen-specific CTL by the $B7-1^+$ tumor cells

has not been examined. Indeed, the finding that B7-1 expression by tumor cells confers enhanced lysis by NK cells suggests the alternate mechanism of increased antigen release and availability to host APCs in vivo.

We have demonstrated that for certain tumors that originated from "nonprofessional APCs" such as epithelial cancers, the priming of naive, tumor-specific CTL in vivo is mediated exclusively by the host's bone marrow–derived APCs (14). In a series of in vivo and in vitro experiments with parent-into-F_1 bone marrow chimeras, we have shown that a tumor lacking the appropriate costimulatory molecules could not directly activate CTL, but did so indirectly by shedding tumor antigens for uptake, processing, and presentation by professional APCs, a phenomenon termed "cross-priming" (15, 16). In the present study, we employ the same bone marrow chimera system to assess the role of B7-1$^+$ tumor cells relative to the host APC in mediating the priming of tumor-specific, MHC class I–restricted, CD8$^+$ CTL. We have found that whereas B7-1$^+$ tumor cells are capable of directly priming naive CTL in vivo, they do so much less efficiently than host APCs. The data demonstrate that whereas some direct presentation capacity is indeed conferred by B7-1 expression, most of the CTL priming is related to other mechanisms, such as increased lysis of B7-1$^+$ tumors in vivo, which facilitates subsequent antigen uptake and processing by professional APCs.

Materials and Methods

Mice. 6–8-wk-old female C57BL/6 (H-2^b), BALB/c (H-2^d), and their F_1 progeny CB6F$_1$ (H-2^{bxd}) were obtained from the National Institutes of Health (Frederick, MD). Parent-into-F_1 bone marrow chimera mice were created as described previously (14) and are summarized below.

Cell Lines and Clones. CT26 is a moderately immunogenic colonic epithelial tumor derived from the BALB/c mice. CT26NP was derived by infecting CT26 with a Moloney-based defective recombinant retrovirus encoding both the nucleoprotein (NP)[1] gene of the PR8 influenza strain and a neomycin-resistance gene (*Neo*) (14, 17). Cotransfection of CT26NP with plasmids encoding the hB7-1 and a hygromycin-selectable marker resulted in CT26NPB7 lines. The transduced and transfected cell lines were cloned by limiting dilution cloning in selecting media. The resulting clones of CT26NP and CT26NPB7 were stained for the level of NP (mAb HB65) and B7 (CTLA4-Ig), and tested as targets for lysis by a NP-specific, H-2^d–restricted CTL line. Intracellular NP staining was performed first by permeabilizing cells with 70% ice-cold ethanol, followed by incubation with NP-specific mAb HB65 at 4°C for 30 min. FITC-labeled secondary goat anti–mouse IgG (Southern Biotechnology Associates, Birmingham, AL) was then added at 4°C for 30 min. Surface B7-1 staining was performed by first adding CTLA4-Ig at 4°C for 18 min, followed by FITC-labeled secondary goat anti–human IgG (Southern Biotechnology Associates) at 4°C for 18 min. A CT26NP clone and a CT26NPB7 clone positive for both antibody staining and CTL lysis were chosen for further experiments. CT26NP and CT26NPB7 clones exhibited similar patterns of

growth kinetics compared to that of CT26WT both in vitro as well as in SCID mice. Whereas CT26 and CT26NP showed similar growth kinetics in intact BALB/c and CB6F$_1$ mice (100% tumor incidence in 3 wk when injected with 10^6 live tumors), mice injected with live CT26NPB7 clone showed slowing of tumor growth with complete rejection in 20% of animals at 10^6 cell dose and 80% of animals at 2.5 × 10^5 cell dose).

Synthetic NP Nanomeric Peptides. The immunodominant nanomeric H-2K^d–restricted NP(147-155) peptide (TYQRTRALV) and H-2D^b–restricted NP(366-374) peptide (ASNENMETM) were synthesized by the Protein/Peptide/DNA Facility at the Johns Hopkins University School of Medicine. The sequences were confirmed by amino acid analysis and mass spectrometry.

Construction of Bone Marrow Chimera Mice. The parent-into-F_1 bone marrow chimeria mice were constructed as described previously (14). Briefly, recipient female CB6F$_1$ mice were depleted of NK1.1$^+$ cells with mAb, PK136 (American Type Culture Collection, Rockville, MD), 1 d before and after the marrow transplant to avoid the Hh rejection phenomenon. On the day of the transplant, donor bone marrows were harvested from both the femur and the tibia of the C57BL/6, BALB/c, and CB6F$_1$ mice. The resultant bone marrow cells were depleted of mature T cells by treatment with mAb RL172 (anti-CD4), 3-155 (anti-CD8), J1J (anti-Thy1), C3PO (anti-CD5; American Type Culture Collection), and rabbit complement (Low-Tox M; Cedarlane, Accurate Chemical and Scientific Corp., Westbury, NY). 2–5 × 10^6 of the T cell–depleted bone marrow cells were injected intravenously into lethally irradiated (10 Gy) CB6F$_1$ recipients. The control mice received injection of saline alone and expired between days 12 and 21 after transplant. 5 mo after the transplant, representative chimera mice were killed. Splenocytes from the chimeras were stained with haplotype-specific, anti-MHC class II mAb Y3P (anti-I-A^b) and 14.4.4 (anti-I-E^d) followed by goat anti–mouse IgG2a-FITC (Southern Biotechnology Associates), and analyzed by FACScan® (Becton Dickinson & Co., Braintree, MA) to confirm complete bone marrow reconstitution.

In Vivo Immunization. CT26, CT26NP, and CT26NPB7 were harvested and washed in HBSS three times. Before immunization, samples of the different cell lines were analyzed on the FACScan® for the level of NP and B7-1 expression. Two to five mice from each of the chimera groups (H-2^b→H-2^{bxd}; H-2^d→H-2^{bxd}; and H-2^{bxd}→H-2^{bxd}) received 10^6 cells of live or irradiated (50 Gy) CT26, CT26NP, or CT26NPB7 subcutaneously in the left flank. 2 wk later, mice were either reimmunized with a second dose of tumor cells, or were killed for subsequent secondary in vitro stimulation and chromium release assays.

Secondary In Vitro Stimulation and Chromium Release Assay. Splenocytes were harvested from immunized chimeric mice 2 wk after either the first or the second dose of immunization. A fraction of the splenocytes from each chimera was analyzed on the FACScan® to test for the presence of any contaminating residual H-2^{bxd} APC using haplotype-specific anti-MHC class II antibodies. In all cases, the level of detectable staining with antibody recognizing the opposite haplotype as compared to the bone marrow was the same as that of the background staining with secondary FITC-labeled antibody alone. A portion of the splenocytes from each mouse was incubated either with NP(147-155) or NP(366-374) peptides in the presence of live CB6F$_1$ (H-2^{bxd}) splenocytes and IL-2. After 7 d, responder cells were harvested, washed, and incubated with chromium-51 (30 mCi/ml; Amersham Corp., Arlington Heights, IL)–labeled surrogate target cells, P815 (H-2^d) or MC57G (H-2^b), pulsed with either NP(147-155) or NP(366-374) peptides. After a 4–5-h incubation at 37°C and 5% CO$_2$, su-

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[1]*Abbreviation used in this paper:* NP, influenza nucleoprotein.

pernatants were assayed for radioactivity on a gamma counter. The percent specific lysis = 100% × (sample cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm). The percent peptide specific lysis was the difference between the percent specific lysis of the target cells pulsed with the MHC-matched peptide and that with the MHC-mismatched peptide.

Results

Characterization of Tumor Constructs. We have developed clones of CT26 that express the viral protein, NP, with or without the expression of the costimulatory molecule, B7-1. (Fig. 1 *A*). Both CT26NP and CT26NPB7 clones exhibited similar in vitro as well as in vivo growth kinetics in SCID mice compared to that of the parental tumor, CT26 (data not shown). The NP-expressing tumor clones were also recognized as targets in an in vitro chromium release assay by $H-2K^d$-restricted, NP-specific CTL lines generated from infection of BALB/c mice with a sublethal dose of the PR8 strain of influenza (Fig. 1 *B*). Mice immunized with irradiated CT26NP or CT26NPB7 were able to elicit a CTL response recognizing a surrogate target pulsed with the NP nanomeric peptide in vitro. Furthermore, mice infected with a sublethal dose of PR8 strain of influenza were able to reject a live CT26NP challenge, whereas mice challenged with parental CT26 failed to re-

ject the inoculation (data not shown). Taken together, NP represents a good model tumor antigen since (*a*) the presence of the viral antigen did not alter the tumorigenicity and immunogenicity of CT26; (*b*) the NP antigen is presented by the two different tumor clones at levels recognizable by NP-specific, MHC class I–restricted CTL; and (*c*) CT26NP was able to be rejected in a NP-specific manner in mice previously immunized against the antigen. Similar to other investigators' observations, CT26NPB7 injected subcutaneously can be rejected in a CD8-dependent manner (20–80% rejection depending on the challenge dose). Tumor explants from mice that failed to reject the primary CT26NPB7 challenge showed a decreased level of B7 expression, but retained NP expression as determined by their ability to be lysed by NP-specific CTL in vitro.

Comparison of In Vivo CTL Priming by Tumor Cells versus Host APCs. Analysis of the specificity of anti-NP CTL in parent→F_1 chimeras immunized with NP-transfected tumors allows the direct assessment of presentation by the tumor versus host bone marrow–derived cells. This is because the bone marrow–derived cells and tumor cells can express different MHC class I alleles and thus will present different epitopes of NP. When $H-2^b$→$H-2^{bxd}$ bone marrow chimeric mice were initially immunized with irradiated CT26NPB7 ($H-2^d$), no detectable CTL activity was observed against $H-2^d$ targets pulsed with the NP(147-155)
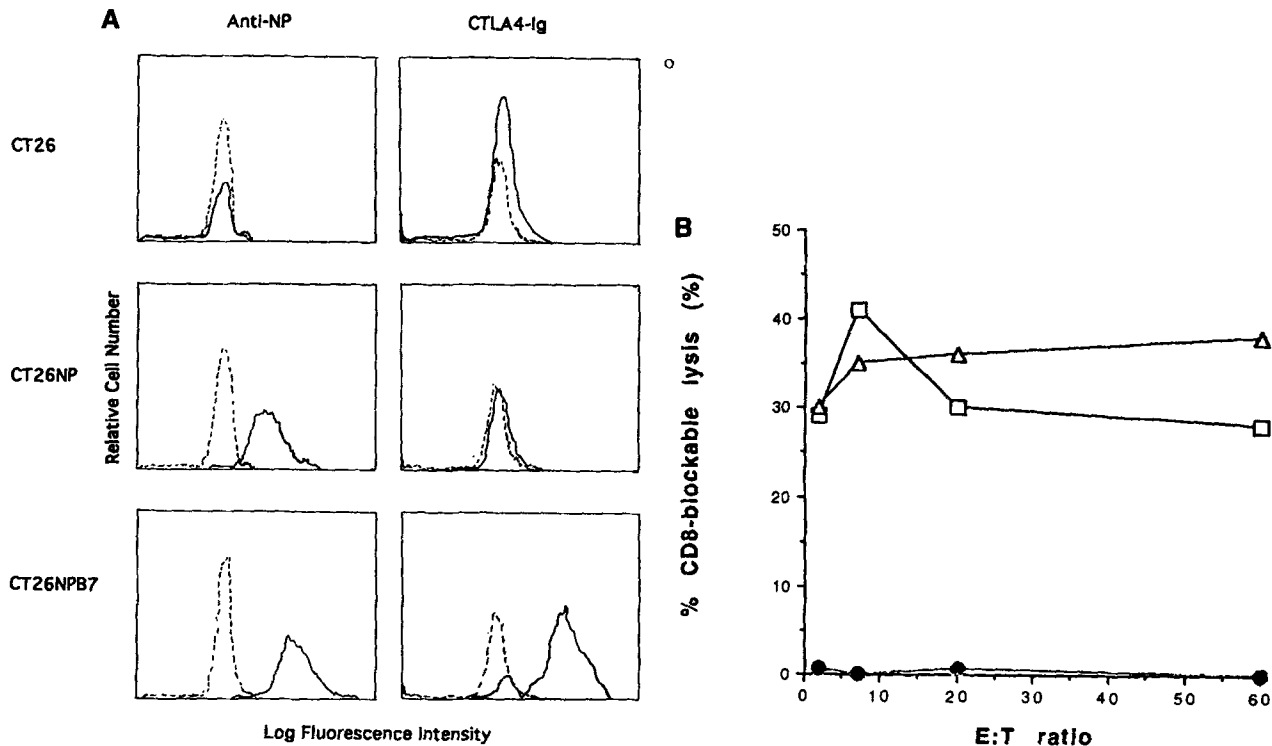

Figure 1. Expression of NP and B7-1 in various CT26 constructs. (*A*) parental CT26, CT26NP, and CT26NPB7 are stained with either mAb HB6.5 (*Anti-NP*, intracellular) or CTLA4-Ig (anti-B7, surface), followed by FITC-labeled goat anti–mouse IgG (HB6.5) or goat anti–human IgG (*CTLA4-Ig*) (*solid lines*). As controls, each cell construct was stained with FITC-labeled secondary antibody alone (*broken lines*). (*B*) CT26 (*closed circles*), CT26NP (*open squares*), and CT26NPB7 (*open triangles*) were labeled with ^{51}Cr and used in an in vitro chromium release assay as targets against CTL lines generated from mice infected with the PR8 strain of influenza. CD8-blockable lysis (%) was the difference between percent specific lysis with and without the presence of mAb 2.43 (anti-*CD8*). Non-CD8–blockade lysis never exceeded 5% at a 100:1 E/T ratio.

peptide, indicating either that no direct priming of CTL by the tumor cell had occurred, or that the frequency of $H-2^d$-restricted CTL was below the sensitivity of the in vitro assay (Fig. 2 *A*). As described previously, mice immunized with CT26NP or CT26 also failed to generate CTL against NP in the context of the tumor's MHC. In contrast, $H-2^d$-restricted CTL were generated when $H-2^d \rightarrow H-2^{bxd}$ or $H-2^{bxd} \rightarrow H-2^{bxd}$ chimeras were immunized with either CT26NP or CT2NPB7 (Table 1). CTL which recognize the $H-2^b$ targets pulsed with the NP(366-374) peptide were observed when $H-2^b \rightarrow H-2^{bxd}$ chimeric mice were immunized with either CT26NP or CT26NPB7, indicating that anti-NP CTL were primed in vivo by the bone marrow–derived APCs (Fig. 2 *B* and Table 1). These data suggest that whereas CT26NPB7 fails to directly prime CTL in vivo to levels above the assay's detection limit, the cross-priming pathway results in readily measurable CTL activity.

To increase the ability to detect low levels of direct priming by CT26NPB7, mice were immunized and then boosted 2 wk later. Reimmunization of the $H-2^b \rightarrow H-2^{bxd}$ chimera with irradiated CT26NPB7 resulted in NP-specific CTL restricted by $H-2^b$, as was seen after a single vaccination (Fig. 3 *B* and Table 1). However, with boosting, $H-2^d$-restricted, anti-NP CTL were also observed (Fig. 3 *A*). This is in sharp contrast to similar chimeras immunized with CT26NP alone, in which the $H-2^d$-restrictd, anti-NP CTL were never detected, even after boosting (Fig. 3 *A* and Table 1). Table 1 summarizes the results of experiments using different chimeras constructs, cell clones, and immunization dosing schedules.

The lower efficiency of CTL priming by CT26NPB7 compared to that by host APC may be due to the effects of irradiation on the vaccinating cells. Although the mechanism is not well understood, it has been shown that irradiating B7-1–transfected tumor cells may diminish the ability to generate systemic antitumor immunity (18). This may be the reason why direct tumor priming was observed only after multiple vaccinations with irradiated CT26NPB7, whereas cross-priming was detected after a single immunization. To test this hypothesis, $H-2^b \rightarrow H-2^{bxd}$ chimeras were immunized with either irradiated or live CT26NPB7, and the splenocytes were analyzed 2 wk later. As stated above, 20–80% of mice injected with live CT26NPB7 can reject the tumor. To eliminate the confounding issue of tumor burden on the immune response, only the mice that rejected the primary tumor challenge were used for this assay. Fig. 5 shows the result of such experiments. The $H-2^b \rightarrow H-2^{bxd}$ chimera immunized with live CT26NPB7 failed to generate detectable levels of $H-2^d$-restricted (Fig. 5), NP-specific CTL, even though CTL were generated that recognize NP in the $H-2^b$ haplotype (data not shown). These data suggest that the cross-priming pathway is dominant over the direct-priming pathway, regardless of whether immunizing tumor cells were irradiated or live.

Lack of Residual Radio-resistant $H-2^{bxd}$ APCs in the $H-2^b \rightarrow H-2^{bxd}$ Chimeras. Whereas the appearance of the $H-2^d$-restricted, anti-NP CTL in the $H-2^b \rightarrow H-2^{bxd}$ chimeras
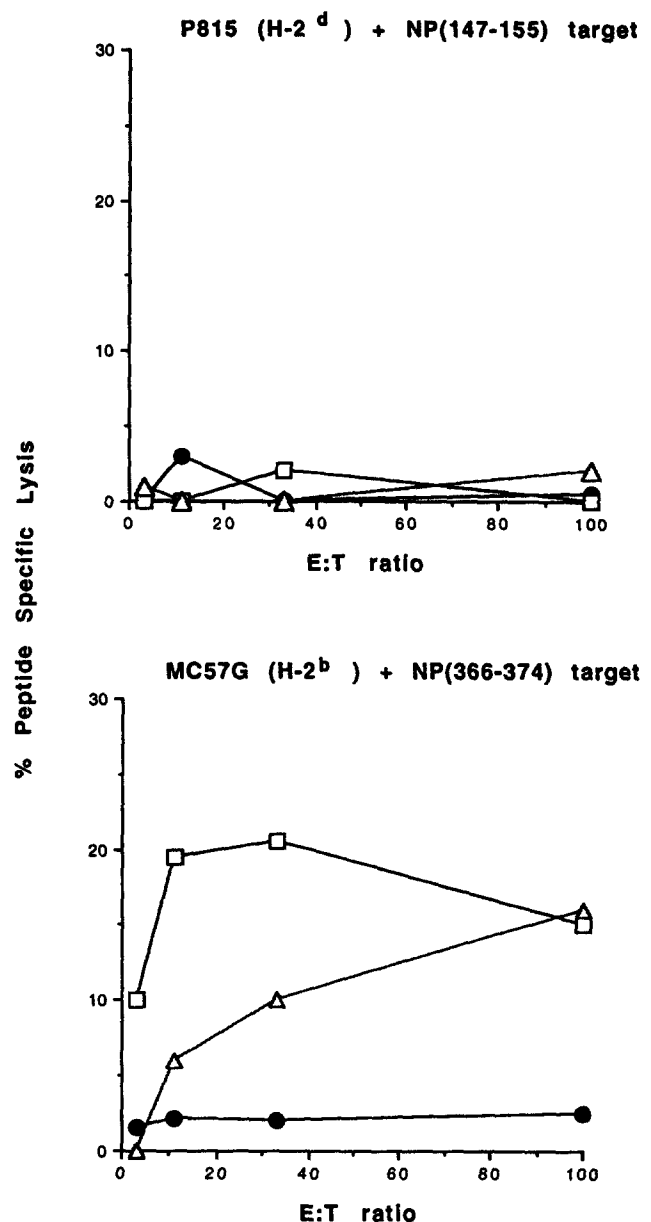

Figure 2. Epitope specificity of T cells from $H-2^b \rightarrow H-2^{bxd}$ chimera mice immunized once with various tumor constructs. Mice were immunized once with either CT26 (*closed circles*), CT26NP (*open squares*), or CT26NPB7 (*open triangle*). 2 wk later, splenocytes from these mice were then stimulated with either NP(147-155) (*top*) or NP(366-374) (*bottom*) + $H-2^{bxd}$ splenocytes. Cells were then mixed with ^{51}Cr-labeled P815 targets and NP(147-155) ($H-2^d$, *top*) or MC57G targets and NP(366-374) ($H-2^b$, *bottom*). Peptide-specific lysis represents the difference between the percent specific lysis of the target cells with the MHC-matched NP peptide and the percent specific lysis of the target cells with the MHC-mismatched peptide. In all cases, lysis of targets in the presence of the MHC-mismatched NP peptide was <5% at all E/T ratios. Data are representative of three separate experiments.

immunized twice with CT26NPB7 may reflect direct priming of naive CTL in vivo, it is possible that CT26NPB7 cannot directly initiate the priming of CTL, per se, but can amplify, in the subsequent immunization, the activated CTL precursors that were initially primed by residual radio-resistant $H-2^{bxd}$ APCs. The precursor frequency of such

Table 1. *Summary of In Vivo Cross-priming Experiments*

Chimera		(MHC)	CT26		(MHC)	Vaccine no.	Lysis against*	
			NP	B7			P815+NP (H-2^d)	MC57G+NP(H-2^b)
a	CB6F$_1$→CB6F$_1$	b/d→b/d	−	−	d	1	−	−
b	CB6F$_1$→CB6F$_1$	b/d→b/d	+	−	d	1	+	++
c	CB6F$_1$→CB6F$_1$	b/d→b/d	+	+	d	1	+++	+++
d	CB6F$_1$→CB6F$_1$	b/d→b/d	−	−	d	2	−	−
e	CB6F$_1$→CB6F$_1$	b/d→b/d	+	−	d	2	+++	+++
f	CB6F$_1$→CB6F$_1$	b/d→b/d	+	+	d	2	+	++
g	C57BL/6→CB6F$_1$	b→b/d	−	−	d	1	−	−
h	C57BL/6→CB6F$_1$	b→b/d	+	−	d	1	−	++
i	C57BL/6→CB6F$_1$	b→b/d	+	+	d	1	−	++
j	C57BL/6→CB6F$_1$	b→b/d	−	−	d	2	−	−
k	C57BL/6→CB6F$_1$	b→b/d	+	−	d	2	−	+++
l	C57BL/6→CB6F$_1$	b→b/d	+	+	d	2	+++	++
m	BALB/c→CB6F$_1$	d→b/d	−	−	d	1	−	−
n	BALB/c→CB6F$_1$	d→b/d	+	−	d	1	+	−
o	BALB/c→CB6F$_1$	d→b/d	+	+	d	1	++	−
p	BALB/c→CB6F$_1$	d→b/d	−	−	d	2	−	−
q	BALB/c→CB6F$_1$	d→b/d	+	−	d	2	++	−
r	BALB/c→CB6F$_1$	d→b/d	+	+	d	2	+++	−

Different bone marrow chimeras and tumor constructs were used. H-2^{bxd}→H-2^{bxd} chimera (*a–f*), H-2^b→H-2^{bxd} (*g–l*) chimera, or H-2^d→H-2^{bxd} (*m–r*) chimera were immunized with different irradiated tumor constructs. The magnitudes of the epitope-specific CTL against P815+NP(147-155) target or MC57G+NP(366-374) target are indicated by the plus (+) signs.

*At a 100:1 E/T ratio: − (5%), + (10%), ++ (20%), +++ (<30%), ++++ (<40%), +++++ (>40%).

CTL may have been too low after the initial immunization to be detected in the in vitro CTL assays. To address this latter possibility, we first sought to detect residual H-2^{bxd} APCs in the chimeras by FACScan® (Becton Dickinson & Co.) as well as in two functional assays. Splenocytes from each experimental chimera were stained with haplotype-specific antibody and analyzed on a FACScan®. In no case was there any detectable level of contaminating H-2^{bxd} APC detected (sensitivity to <2%) 5 mo after the bone marrow reconstitution. Detection of residual H-2^{bxd} APCs in the H-2^b→H-2^{bxd} chimeras was further sought in an allogeneic mixed lymphocyte reaction (data not shown). Stimulation of C57BL/6 splenocytes with APCs from the H-2^b→H-2^{bxd} chimeras gave levels of proliferation no greater than the autologous mixed lymphocyte reaction. In contrast, C57BL/6 splenocytes proliferated vigorously in the presence of splenocytes from either H-2^{bxd}→H-2^{bxd} chimeras, BALB/c, or C57BL/6 mixed with 50, 25, 10, and 0.5% of H-2^{bxd} splenocytes.

To further exclude the possibility that CT26NPB7 acts to amplify H-2^d–restricted, anti-NP CTL previously activated by residual host H-2^{bxd} APCs, a double immunization experiment was performed. H-2^b→H-2^{bxd} chimera mice were first immunized with irradiated CT26NP, and reimmunized 2 wk later with irradiated CT26NPB7. If the primary induction was mediated by the host's residual APCs, then it should not matter whether mice were initially immunized with CT26NP or CT26NPB7, as long as CT26NPB7 was used in the subsequent immunization. Fig. 4 demonstrates that anti-NP, H-2^d-restricted CTL were observed only in the case where the chimeras were immunized sequentially with two doses of irradiated CT26NPB7. Mice immunized either with two doses of irradiated CT26NP or one dose of irradiated CT26NP followed by a dose of CT26NPB7 failed to generate appreciable levels of H-2^d–restricted, NP-specific CTL activity.

Discussion

Several studies have explored the use of B7-1–transfected tumor cells as a strategy for generating systemic antitumor immunity. Using a melanoma cell line, Townsend and Allison (11) observed that mice successfully rejected an innoculum of live B7-1$^+$ melanoma. In a related study, Chen et al. (10) reported that rejection of a murine melanoma line required the expression of B7-1 as well as a foreign antigen, HPV-16 E7. In both of these studies, the primary rejection of the tumor cells resulted in long-term protection against a secondary challenge with the B7$^-$ negative parental tumor, as well as eradication of preestablished
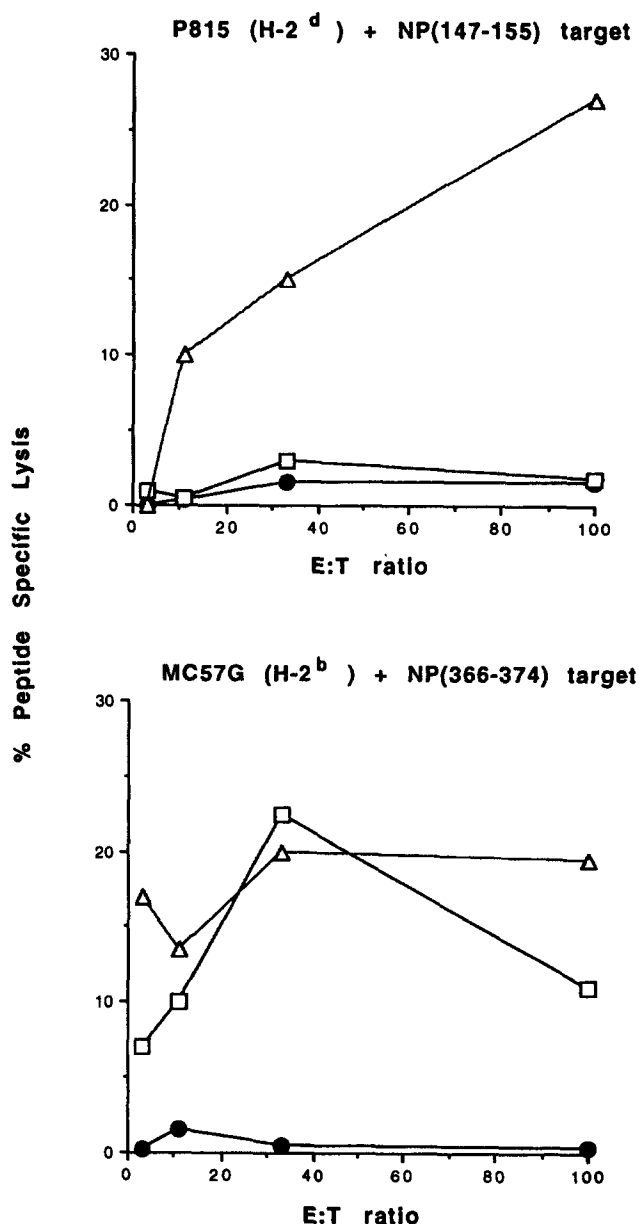
P815 (H-2^d) + NP(147-155) target

P815 (H-2^d) + NP(147-155) target

Figure 4. Direct priming of NP-specific CTL by B7$^+$ tumor and the role of residual H-2^{bxd} APCs in priming such CTL. H-2^b→H-2^{bxd} chimera mice were immunized either twice with CT26 (*closed circles*), CT26NP (*open circles*), or CT26NPB7 (*open triangles*), or once with CT26NP followed by one injection of CT26NPB7 (*closed squares*). 2 wk after the last injection, splenocytes were analyzed as described in the legend to Fig. 2. Only the H-2^d epitope specificity is shown. Closed circles and closed squares are superimposed.

MC57G (H-2^b) + NP(366-374) target

Figure 3. Epitope specificity of T cells from H-2^b→H-2^{bxd} chimera mice immunized twice with various tumor constructs. Mice were immunized twice 2 wk apart with either CT26 (*closed circles*), CT26NP (*open squares*), or CT26NPB7 (*open triangles*). 2 wk after the second immunization, splenocytes were removed and analyzed under the same condition as described in the legend to Fig. 2. The results are representative of three separate experiments.

micrometastases. Tumor rejection was shown to require CD8$^+$ but not CD4$^+$ T cells, since depletion of the former rendered mice tumor prone. The failure to demonstrate a requirement for CD4$^+$ T cells in this system is supported by earlier studies (4) demonstrating CD4-independent priming of CTL when TCR engagement is accompanied by B7-1–mediated cross-linking of CD28. Baskar et al. (19) have subsequently reported that CD4$^+$ T cells play a critical role in the rejection of tumors cotransfected with the genes encoding MHC class II antigen as well as B7-1,
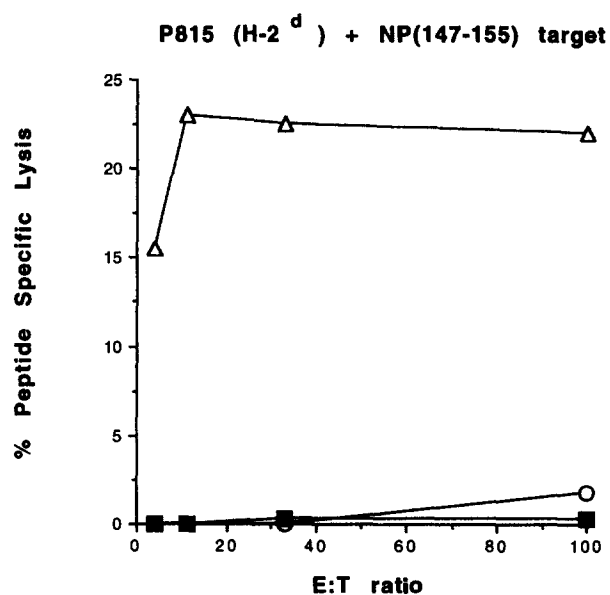
which also resulted in generation of potent systemic immunity against the nontransfected parental tumor.

It has been postulated that tumor-specific CD8$^+$ CTL are primed directly by the B7-1–transfected tumor which provides both signals 1 and 2 to the CTL precursor (6). In contrast to the tumor cells acting as APCs, we have previously reported that host bone marrow–derived professional APCs are the dominant cell population mediating tumor-specific CTL priming. Using parent-into-F$_1$ bone marrow chimeras in which the haplotype of the reconstituted APC population is distinct from that of the tumor, we have consistently found that B7-1$^-$, MHC class I$-$ tumors cannot prime tumor-specific, CD8$^+$, MHC class I–restricted CTL in vivo. Rather, the host bone marrow–derived professional APCs are the exclusive APCs in priming the naive CTL through a MHC class I–processing and –presenting pathway for exogenous tumor antigens.

The current study extends these observations to examine the role of B7-1$^+$ tumor cells in generating an antitumor immune response. Mice immunized with irradiated CT26NPB7 were found to have detectable NP-specific CTL restricted to the haplotype of the tumor, but only after multiple immunizations. In contrast, NP-specific CTL restricted to the haplotype of the bone marrow were easily detected after a single immunization. These results indicate that B7-1–transfected tumor can directly serve as APC for the priming of naive CTL in vivo, but the efficiency of this process is far less than that of CTL priming by the host's professional APCs. If the cross-priming pathway is so potent and B7-1$^+$
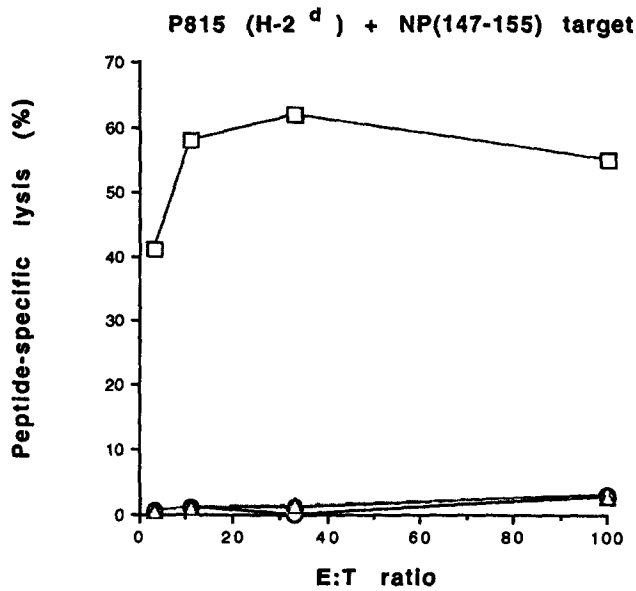
P815 (H-2^d) + NP(147-155) target

Figure 5. Epitope specificity of T cells from H-2^b→H-2^{bxd} immunized with either live (*open circles*) or irradiated (*open triangles*) CT26NPB7. As a control, H-2^{bxd}→H-2^{bxd} chimeras were injected with live CT26NPB7 (*open squares*). Mice were killed 2 wk after the immunization, and their splenocytes were analyzed as described.

tumor cells do not efficiently prime naive CTL in vivo, why then should there be an enhanced immunogenicity seen with B7-1$^+$ tumors? One possible explanation is that B7-1 expression on the tumor cell makes the cell more susceptible to lysis in vivo. Several studies (20–22) suggest that B7-1 may enhance the susceptibility of the tumor to CTL killing. In one model system, the in vivo killing of B7-1$^+$ tumors was shown to be dependent on both NK1.1$^+$ cells and CD8$^+$ T cells (22). The enhanced immunogenicity of the B7-1$^+$ tumor may be caused by the increased lysis of the tumor cells, thus releasing more antigens available for the cross-priming pathway. Our cross-priming experiment with live CT26NPB7 tumor cells supports this hypothesis (Fig. 5). The injected live CT26NPB7 tumor cells were rejected, even though antigen-specific CTL restricted to the tumor's MHC class I cannot be demonstrated in vitro. On the other hand, NP-specific CTL primed by the host's APCs can be readily detected. This may explain earlier observations indicating that the ability of B7-1–transfected tumor vaccines to generate effective systemic immunity is dependent on the inherent immunogenicity of the parental tumor (13). Immunogenic tumors transfected with B7-1

that are lysed in vivo by CTL or NK cells would release antigens in a form that is efficiently processed by host APCs; this would lead to systemic immunity to the parental tumor. B7-1 transfection of nonimmunogenic tumors may result in the elimination of the transfected population, but generate little systemic immunity. This issue cannot be precisely addressed in the present study, and awaits further analysis.

It has been recently shown that the microenvironment greatly influences the ability to prime MHC class I–restricted CTL to tumor cell antigens. Using a fibrosarcoma cell line and different model tumor antigens, Kundig et al. (23) have shown that this tumor, which fails to express known costimulatory molecules or cytokines, is capable of directly priming and activating tumor-specific CTL in vivo, but only in the environment of a lymphoid organ. Intraperitoneal injection was found to result in lymphoid metastases and direct CTL priming, whereas subcutaneous injection, as was the case in our current report, did not result in direct CTL priming. Given the well-demonstrated ability of paracrine cytokine stimulation, such as by IL-2, to provide costimulation for CTL generation, this result is not inconsistent with the two-signal model for T cell activation, and generally supports our findings of the inability of nonhematopoietic tumors to directly prime naive CTL in situ at their site of origin, or at a subcutaneous vaccination site. It does raise the following question, however: why are so many solid tumors present clinically with early stage disease, with lymph node invasion as the only site of micrometastases, if in fact this microenvironment efficiently promotes tumor-specific CTL generation? In contrast to our observations, these authors (23) failed to observe any cross-priming by host APCs. The reason for this difference is unclear, but it is likely to involve features of the tumor models that affect the efficiency of exogenous antigen uptake and processing by APCs. Given the incompletely understood pathways of antigen trafficking for cross-priming, the explanation of this discrepancy awaits further study.

In conclusion, we have examined the ability of a B7-1$^+$, MHC class I$^+$, MHC class II$^-$ colonic carcinoma to directly mediate the induction and activation of tumor-specific CTL in vivo. Contrary to the current working model of immune activation by B7-1$^+$ tumor, the primary route of antitumor induction does not appear to be through the tumor cells, but via the host's hematopoietic-derived professional APCs. The in vivo pathways for exogenous antigen uptake, processing, and presentation on MHC class I molecules are under active investigation.

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