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Expression of B7 co-stimulatory molecules by B16 melanoma results in a natural killer cell-dependent local anti-tumour response, but induces T-cell-dependent systemic immunity only against B7-expressing tumours

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Summary In an attempt to enhance the anti-tumour immune response, the co-stimulatory molecules B7-1 or B7-2 were expressed on the surface of B16 melanoma cells. B7-expressing tumours grew more slowly in both syngeneic immunocompetent mice and athymic T cell-immunodeficient nude mice. The delay in growth of B7-expressing tumours was dependent on natural killer (NK) cells, as reductions in tumour growth rates were minimized in mice depleted of NK cells. Systemic immunity to B16 melanoma was examined by vaccination with irradiated tumour cells. Inoculation with irradiated B16 B7-1 cells failed to protect against a subsequent challenge with live parental B16 cells, but conferred partial protection against challenge with live B16 B7-1 cells. In contrast to the local anti-tumour reaction, this protective response was dependent on T cells. The results presented here reveal some of the mechanisms involved in the in vivo response to a poorly immunogenic tumour modified to express co-stimulatory molecules.

Keywords: melanoma; B7-1; B7-2; co-stimulation; natural killer cell

Attempts to modulate the immune response against tumour cells as a potential therapeutic modality have centred mainly on T cells, as these represent the immune cell population with antigen specificity and memory (Tepper and Mule, 1994; Colombo and Forni, 1996). An effective lytic response against tumour cells requires activation of precytotoxic $CD8^-$ T cells by cytokines secreted from $CD4^-$ T helper cells which, in turn, have been activated by professional antigen-presenting cells (APCs) that have taken up antigens derived from tumour cells (Pardoll, 1993). In addition, professional APCs may also activate $CD8^+$ cytotoxic T cells directly (Huang et al, 1994). Efficient activation of T cells requires antigen-non-specific signals, as well as the antigen-specific signal received by the T-cell receptor/CD3 complex. An important co-stimulatory signal is provided by the CD28 receptor on T cells (Linsley and Ledbetter, 1993), the ligands for which belong to the B7 family, including B7-1 (CD80) (Freeman et al, 1991) and B7-2 (B70/CD86) (Azuma et al, 1993; Freeman et al, 1993). These co-stimulatory molecules are expressed by professional APCs, such as dendritic cells and macrophages, enabling them to present antigens effectively to T cells. Co-stimulatory signals give rise to increased expression of a variety of cytokines that have autocrine and paracrine effects on the proliferation, activation and maturation of T cells (Gimmi et al, 1991).

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In recent years, it has become evident that many human and experimental tumours possess specific antigens that may be recognized by T cells and that may act as targets for an immune rejection response (Boon et al, 1994). Such specific antigens have been identified in the B16 murine melanoma (Naftzger et al, 1996; Bloom et al, 1997). However, as most tumour cells do not express co-stimulatory molecules, tumour-specific antigens would fail to be presented to T cells efficiently. Indeed, this may represent one mechanism by which tumour cells evade recognition by the immune system (Vile et al, 1996).

In vitro, activation of cytotoxic T cells may be achieved by stimulation via the TCR and CD28, without requirement for exogenous cytokines provided by T helper cells (Azuma et al, 1992*a*; Harding and Allison, 1993). In attempts to enhance immune recognition and augment anti-tumour responses, co-stimulatory molecules have been expressed on the surface of tumour cells. The engineered tumour cell would then be capable of presenting the tumour-associated antigens together with the co-stimulatory signal directly to T cells, thereby bypassing the requirement for helper T cells and APCs. B7-expressing tumours in vivo have elicited an effective local anti-tumour response that is mediated by $CD8^+$ cells independent of $CD4^+$ cells, in support of this model, at least in a primary response against the tumour (Chen et al, 1992; Townsend and Allison, 1993). Recently, it has been shown that the in vivo response against some B7-1-expressing tumours is mediated primarily by natural killer (NK) cells, instead of (Geldhof et al, 1995; Yeh et al, 1995), or in addition to, T cells (Cavallo et al, 1995; Wu et al, 1995). Here, we report for the first time that the response against a poorly immunogenic tumour

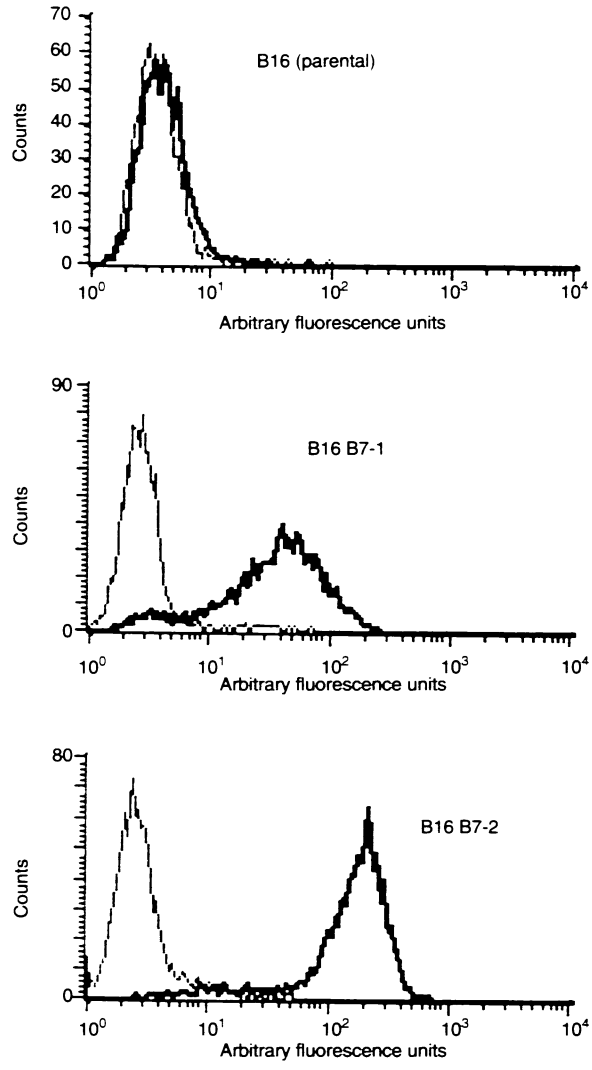

Figure 1 Expression of B7-1 or B7-2 in parental and gene-modified B16 cells. **A** Parental B16. **B** B16 B7-1. **C** B16 B7-2. Cells were incubated with mCTLA4-Hγ1, indicated by the bold lines, followed by FITC-conjugated rabbit anti-human IgG as described in Materials and methods. The faint lines represent control samples in which cells were incubated with normal medium instead of the fusion protein. The *x*-axis shows fluorescence on a $\log_{10}$ scale and the *y*-axis represents relative cell number

expressing B7-2 is also NK-cell dependent, as well as confirming the involvement of NK cells in the response against a B7-1-expressing tumour.

Generation of a response against metastatic tumour cells represents a primary aim of immunotherapy. Therefore, besides attempting to elicit a local anti-tumour response, it is also of importance to investigate whether the B7-expressing tumour cells give rise to systemic protective immunity. Recently, B7-1 tumours have been shown to elicit a systemic immune response by cross-priming of host professional APCs, in addition to direct antigen presentation to T cells by the tumour cells (Huang et al, 1996; Cayeux et al, 1997). Indeed, the former mechanism may be more effective than direct presentation (Huang et al, 1996).

We examined the ability of irradiated, B7-expressing B16 cells to evoke systemic protective immunity against a subsequent live tumour cell challenge. Although B7-1-expressing B16 cells were ineffective in eliciting a protective response against parental B16 cells, they afforded partial protection against B7-1-expressing B16 cells. Unlike the local reaction against the tumour, this response was T cell dependent. These data raise questions concerning the role of exogenously transferred B7 molecules in the generation of anti-tumour immune responses in vivo.

MATERIALS AND METHODS

Cell culture

B16 is a long-established murine melanoma cell line (Fidler, 1970). The B16.F1 subline was used in this study. CMT93 is a murine colorectal tumour cell line (Franks and Hemmings, 1978). All cell lines were monitored routinely and found to be free of *Mycoplasma* infection. The cells were cultured in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% (vol/vol) fetal calf serum and 4 nM L-glutamine.

Expression plasmids and transfection of tumour cells

Subcloning was performed using standard recombinant techniques (Sambrook et al, 1989). Plasmid pTyr-B7-1 is a tissue-specific expression vector where murine B7-1 is driven by the 5′ promoter of the murine tyrosinase gene (Vile and Hart, 1993; Chong et al, 1996). The expression vector BCMGSNeo-mB70 was kindly provided by Professor M Azuma (Tokyo) (Azuma et al, 1993). Adherent B16 melanoma cells (10^6) were transfected with 10 μg of plasmid DNA by calcium phosphate precipitation using the Profection method (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cells were selected in 1.25 μg ml^{-1} puromycin (Sigma, Poole, UK) or in 5 mg ml^{-1} G418 sulphate (Gibco, Paisley, UK). After incubating in selection medium for 3 weeks, surviving colonies were expanded and assayed for transgene expression. B7-1 was expressed in CMT93 cells using a retroviral vector, as described previously (Chong et al, 1996).

Flow cytometry

Expression of B7-1 and B7-2 was determined by staining with the fusion protein mCTLA4-Hγ1 (Lane et al, 1993) (kindly provided by Dr P Lane, Basle) or the specific monoclonal antibodies 16-10-A1, hamster IgG (anti-B7-1) (Razi-Wolf et al, 1992) (generously given by Dr H Reiser, Boston) or GL1, rat IgG_{2a} (anti-B7-2) (Pharmingen, Cambridge, UK). An appropriate fluoroisothiocyanate-conjugated secondary antibody was used (all obtained from Dako, Bucks, UK). The samples (5000 cells) were analysed using a Becton Dickinson FACScan.

In vivo injection of tumour cells

C57BL/6 mice and BALB/c nude (*nu/nu*) mice were obtained from colonies bred at the Imperial Cancer Research Fund (Herts, UK). C57 beige mice (C57BL/6OlaHsd-*bg*) were purchased from Harlan (Oxfordshire, United Kingdom). The NK cell activity of the nude mice was higher than that of C57BL/6 mice (data not shown), whereas C57 beige mice were NK cell deficient. Mice were age and sex matched for individual experiments. Institutional guidelines for care and welfare of animals were adhered to strictly. To establish subcutaneous (s.c.) tumours, 1×10^5 cells (experiments involving

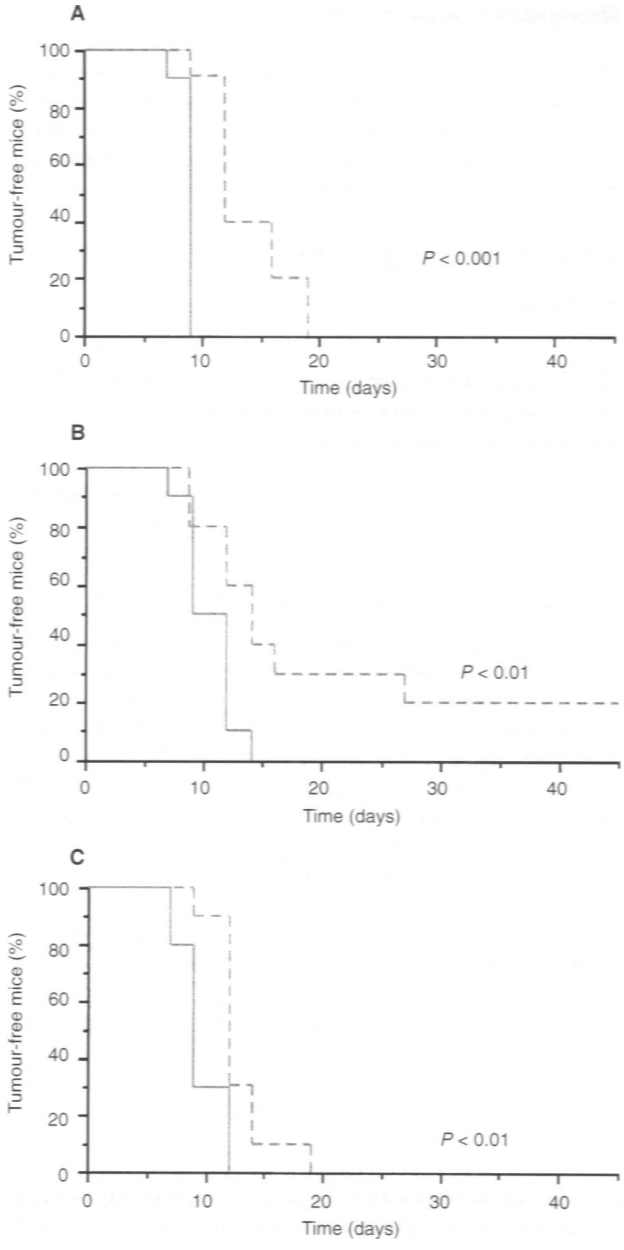

Figure 2 Growth of parental and B7-1-expressing B16 cells in vivo. Parental B16 and B16 B7-1 cells (5×10^5 cells per mouse) were injected s.c. into **A** immunocompetent C57BL/6 mice, **B** athymic nude BALB/c mice and **C** C57 beige mice (ten mice per group). This experiment was performed in parallel using the three types of mice and all mice received the same preparation of B16 of B16 B7-1 cells. The result with C57BL/6 mice is representative of nine independent experiments, that with nude mice is representative of seven experiments and that with beige mice is representative of two experiments. —, B16; - - -, B16 B7-1

NK cell depletion with anti-asialo GM1) or 5×10^5 cells (all other experiments) were suspended in 0.1 ml of phosphate-buffered saline (PBS) and injected s.c. into the flank region. Animals were examined daily until the tumour became palpable, whereafter the diameter, in two dimensions, was measured thrice weekly. Animals were killed when tumour size reached 1.0×1.0 cm. The minimal tumorigenic dose for parental B16 cells is 1×10^5 (s.c.).

Depletion of natural killer cells

In vivo depletion of NK cells was performed by using 25 µl of rabbit polyclonal anti-asialo GM1 (Wako, Neuss, Germany), made up to a volume of 0.2 ml with PBS and injected intravenously (Habu et al, 1981). The animals were challenged with tumour cells 1 day later. Each mouse received a second dose of antibody 1 week later. Control mice were injected with normal rabbit serum.

In vitro assay for natural killer cell activity

The assay was based on lysis of ^{51}Cr-labelled tumour cells by freshly isolated splenocytes (Brunner et al, 1976). Target cells (2×10^6) were incubated with [^{51}Cr]sodium chromate (3.4 MBq) for 1 h, washed and suspended at a concentration of 1×10^5 cells ml^{-1}. Single cell suspensions of spleen cells were prepared. An aliquot of 0.1 ml of the effector and target cell suspensions was mixed at various effector–target ratios, in replicates of at least four, and incubated for 4 h. An aliquot of 0.1 ml of supernatant was aspirated to determine radioactivity. As a positive control in the assays, splenocytes were obtained from mice that had been injected with 100 mg of poly [I]:poly [C] (polyinosinicpolycytidylic acid) 24 h previously. This agent is a potent inducer of interferons and rapidly activates NK cell activity (Djeu et al, 1979).

Vaccination of mice with irradiated tumour cells

Tumour cells were suspended in PBS and irradiated (50 Gy). Mice were injected s.c. with 5×10^5 irradiated cells (0.1 ml) in the flank region and two further doses were administered at weekly intervals. The mice received a live cell challenge (5×10^5 B16 or B16 B7-1 cells, 5×10^6 CMT93 B7-1 cells) in the opposite flank 1 week later.

Statistical analyses

Data from the animal experiments were analysed by plotting Kaplan–Meier curves using the 'occurring event' as the time at which a tumour appeared. A tumour was considered to be present when a palpable mass >0.2 cm was noted. Different groups of mice were compared using the log-rank test (Altman, 1991).

RESULTS

Transfection of B7-1 or B7-2 or cDNA into B16 melanoma cells

Parental B16 melanoma cells did not express detectable levels of B7-1 or B7-2 (Figure 1). Stable expression of murine B7-1 was achieved by co-transfection of plasmid pTyr-B7-1 with a plasmid bearing the puromycin-resistance gene (Figure 1). Murine B7-2/B70 was expressed using plasmid BCMGSNeo-mB70 (Figure 1). Expression of these molecules was stable in in vitro culture, up to approximately ten subcultures. The in vitro growth rates of the B7-expressing sublines were similar to the parental line (data not shown).

Expression of the co-stimulatory molecule B7-1 or B7-2 retards the growth rate of B16 melanoma cells in immunocompetent mice

Subcutaneous injection of B16 B7-1 cells into syngeneic immunocompetent C57/BL6 mice resulted in the development of tumours

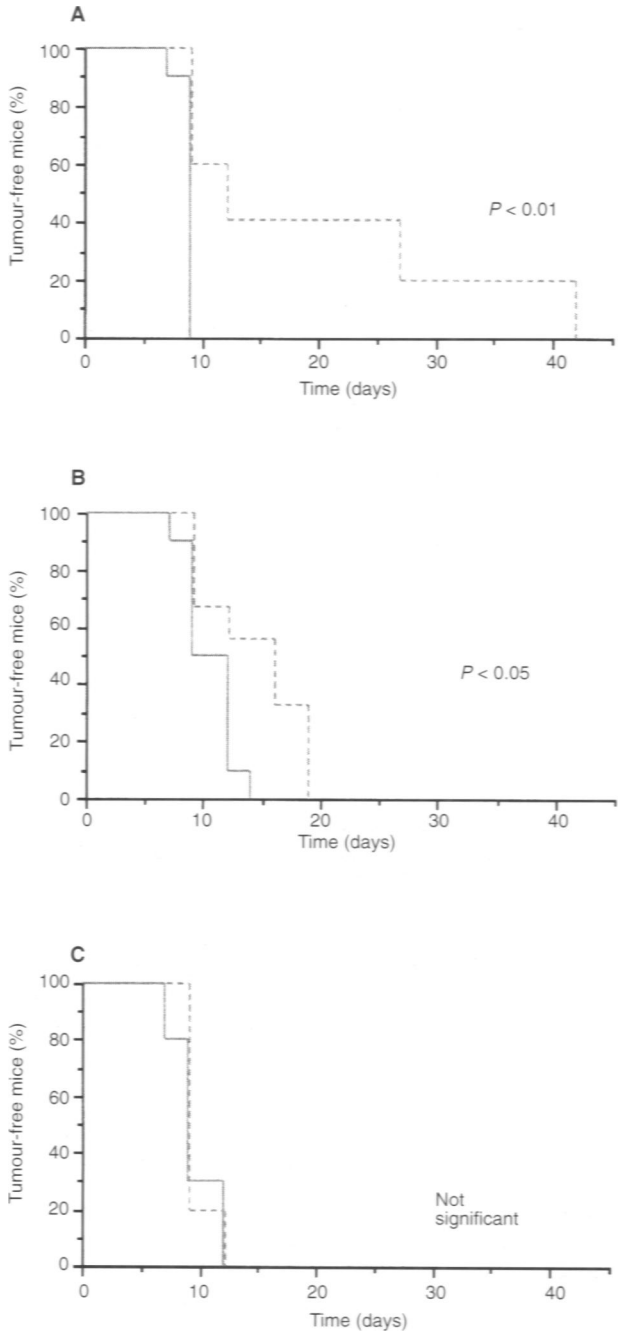

Figure 3 Growth of parental and B7-2-expressing B16 cells in vivo. Parental B16 and B16 B7-2 cells (5×10^5 cells per mouse) were injected s.c. into **A** immunocompetent C57BL/6 mice, **B** athymic nude BALB/c mice and **C** C57 beige mice (ten mice per group). This experiment was performed in parallel using the three types of mice and all mice received the same preparation of B16 or B16 B7-2 cells. The results with C57BL/6 mice and nude mice are representative of three independent experiments and that with beige mice is representative of two experiments. —, B16; - - -, B16 B7-2

in most cases. However, there was a delay in the appearance of the B7-1 tumours, compared with tumours resulting from inoculation of parental B16 cells ($P<0.001$, log-rank test) (Figure 2A), which was seen consistently in nine independently repeated experiments (data not shown). In the experiment depicted in Figure 2A, 10/10 mice inoculated with parental B16 cells had developed tumours by day 9, whereas only 1/10 of those given B7-1 cells had formed a tumour. Similarly, it was found that expression of B7-2 decreased the growth rate of B16 melanoma cells in vivo ($P<0.01$, log-rank test). In the experiment represented in Figure 3A, tumours had appeared in all ten mice given parental B16 cells by day 9, compared with only 4/10 of mice injected with B16 B7-2 cells. Control B16 cells transfected with the puromycin or neomycin selection marker, without B7, grew at the same rate as parental B16 cells in vivo (data not shown).

Expression of B7-1 or B7-2 also retards the growth rate of B16 melanoma cells in athymic T-cell immunodeficient nude mice

A delay in emergence of tumours was also observed when B16 B7-1 melanoma cells were inoculated into athymic T-cell immunodeficient nude mice ($P<0.01$, log-rank test) (Figure 2B). Moreover, a number of these mice completely rejected the B7-expressing tumour cells. For instance, 2/10 nude mice injected with B16 B7-1 cells remained tumour free, whereas all the mice given parental B16 cells developed tumours (Figure 2B). The decreased tumorigenicity of B16 B7-1 cells in nude mice was observed in seven other independently repeated experiments (data not shown), where it was also apparent that the retardation of growth of B16 B7-1 cells in nude mice was more marked than that seen in immunocompetent mice. Expression of B7-2 also decreased the growth rate of B16 tumours in nude mice consistently ($P<0.05$, log-rank test) (Figure 3B).

The growth rates of B7-2 and parental tumours were similar in beige mice

No difference was seen in the growth rates of B7-2 and parental tumours in C57 beige mice (Figure 3C). However, a delay in appearance of B7-1 tumours was seen compared with parental tumours ($P<0.01$, log-rank test) (Figure 2C).

The difference in growth rates between B7-expressing and parental B16 tumours in nude mice was minimized by treatment with anti-asialo GM1 antibody

Nude mice that were treated with 25 µl of rabbit polyclonal anti-asialo GM1 antibody showed decreased NK cell activity compared with control mice given normal rabbit serum (NRS), as demonstrated by an NK cell lysis assay performed using splenocytes from these mice against ^{51}Cr-labelled NK-sensitive YAC cells (data not shown).

When control NRS-treated nude mice were inoculated with parental B16 and B16 B7-1 cells, there was a delay in the emergence of B7-1 tumours (Figure 4A), in keeping with previous results. In contrast, when the same cells were inoculated into anti-asialo GM1-treated nude mice, there was no delay in the appearance of the B7-1 tumours (Figure 4B).

Similar results were seen when B7-2 and parental B16 cells were compared. The growth rate of B16 B7-2 cells was retarded in control NRS-treated nude mice (Figure 4C) but the difference in the B7-2 and parental lines was markedly diminished when compared in anti-asialo GM1-treated mice (Figure 4D).

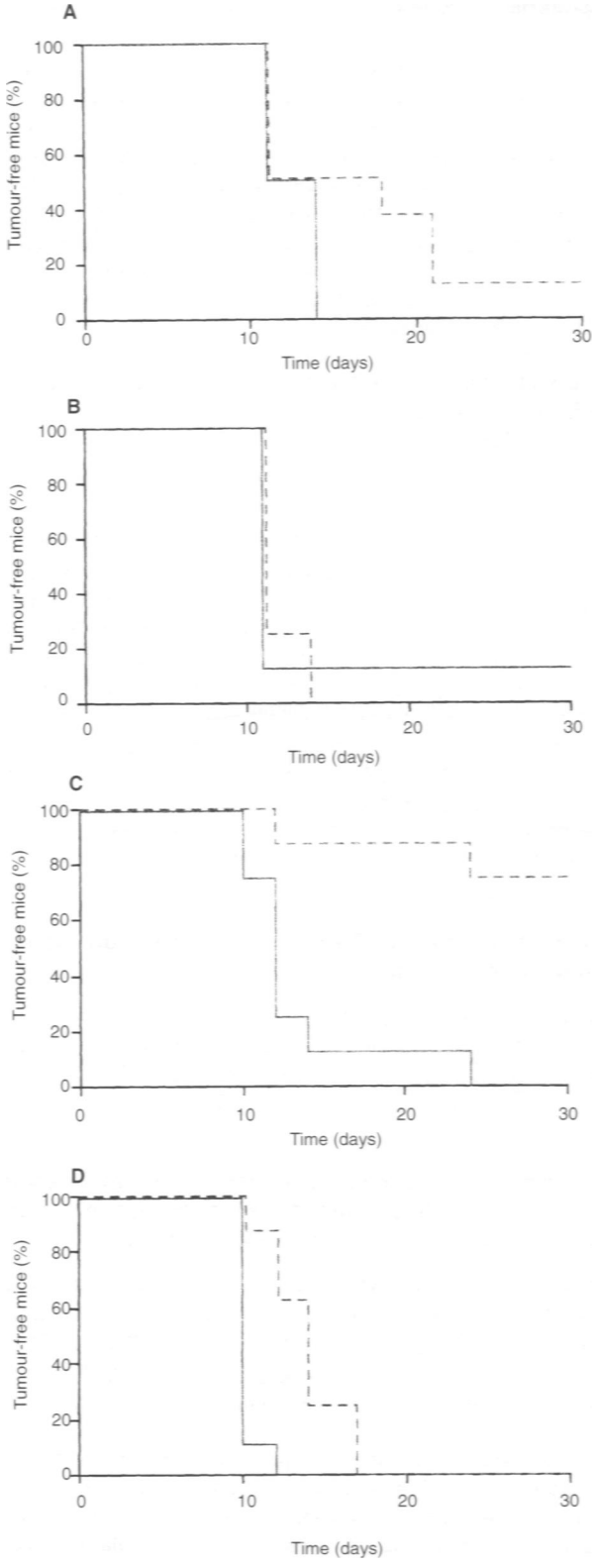

Figure 4 Growth of parental, B7-1-expressing cells and B7-2-expressing B16 cells in nude mice rendered NK-deficient by treatment with anti-asialo GM1 antibody. BALB/c nude mice were injected i.v. with (**A** and **C**) 25 μl of normal rabbit serum (NRS) (control mice) or (**B** and **D**) 25 μl of anti-asialo GM1 antibody (eight mice per group). On the following day, the mice were challenged with 1×10^5 parental B16 cells and (**A** and **B**) B16 B7-1 cells or (**C** and **D**) B16 B7-2 cells. A second injection of antibody was given 1 week after the first dose. This result is representative of two independent experiments. —, B16; - - -, B16 B7-1 (**A** and **B**) and B16 B7-2 (**C** and **D**)

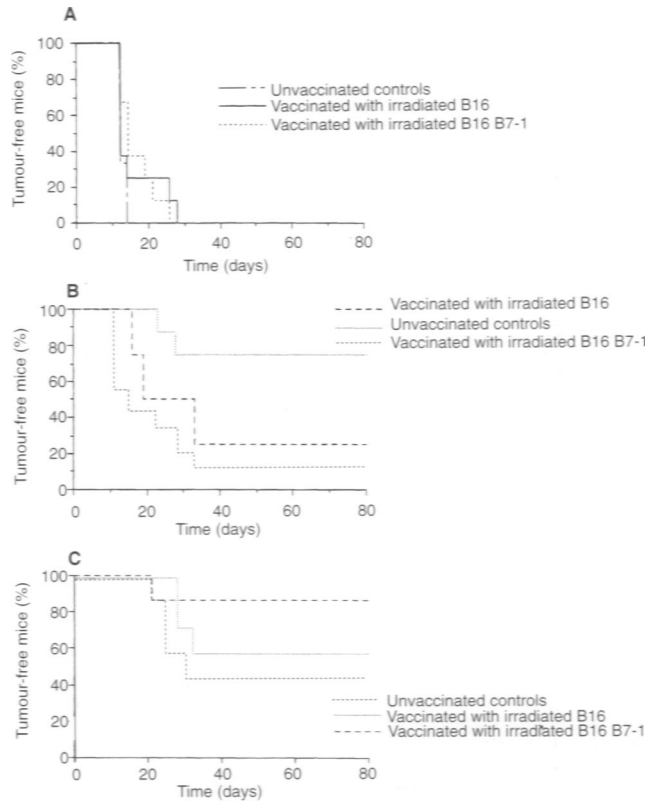

Figure 5 Generation of systemic protective immunity in immunocompetent mice by vaccination with irradiated parental B16 and B16 B7-1 cells. C57BL/6 mice were vaccinated with 5×10^5 irradiated parental or B7-1 cells, as indicated (eight or nine mice per group) (three vaccinations at weekly intervals). One week later, the mice were challenged with 5×10^5 live (**A**) parental B16 cells or (**B**) B16 B7-1 cells. The figure shows the growth of tumours arising from the live cell challenge. These results are representative of three independent sets of experiments. In **C**, C57BL/6 mice that received the same vaccination schedule were challenged with 5×10^6 live CMT93 B7-1 cells (seven mice per group). The figure shows the growth of tumours arising from the live cell challenge

Expression of B7-1 gave rise to systemic protective immunity against B7-1-expressing tumour cells

The ability of tumour cells to elicit systemic protective immunity against parental cells was investigated by vaccination with irradiated tumour cells followed by challenge with live B16 cells. In immunocompetent mice, vaccination with irradiated parental B16 cells did not result in any protection against challenge with live parental B16 cells (Figure 5A). Therefore, under these conditions, B16 tumour cells were poorly immunogenic. Vaccination with irradiated B7-1 cells did not induce any protective immunity against live parental B16 challenge either (Figure 5A). In contrast, vaccination with irradiated B7-1 cells, but not parental B16 cells, consistently elicited partial protection against challenge with live B16 B7-1 cells ($P<0.02$, log-rank test) (Figure 5B). The majority of mice was completely protected against live cell challenge. These vaccination experiments were also performed in parallel in T-cell-immunodeficient athymic nude mice. In these, however, vaccination with B7-1 cells did not protect against challenge with live B7-1 cells (Figure 6).

Immunocompetent mice were also vaccinated with irradiated B16 B7-1 cells and subsequently challenged with live B7-1-expressing

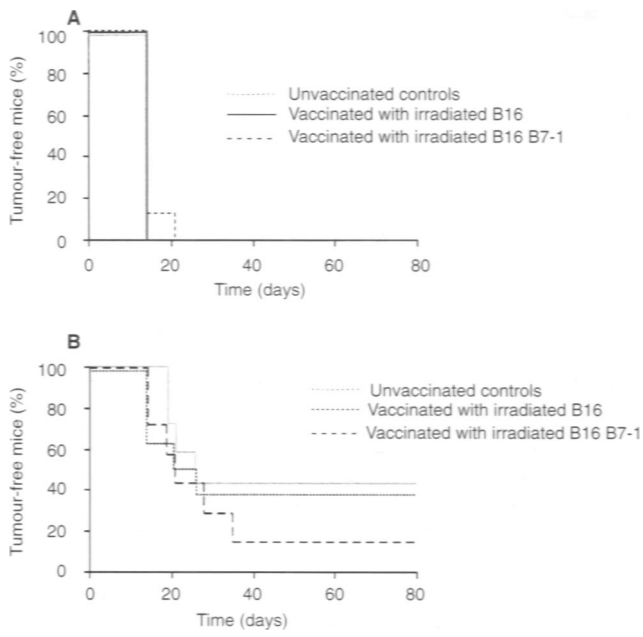

Figure 6 Lack of systemic protective immunity in T-cell immunodeficient athymic nude mice following vaccination with irradiated parental B16 and B16 B7-1 cells. BALB/c nude mice were vaccinated with 5×10^5 irradiated parental or B7-1 cells, as indicated (seven or eight mice per group) (three vaccinations at weekly intervals). One week later, the mice were challenged with 5×10^5 live (**A**) parental cells or (**B**) B7-1 cells. The figure shows the growth of tumours arising from the live cell challenge

CMT93 cells, an unrelated syngeneic tumour cell line (Chong et al, 1996). Most of these mice (six of seven) did not develop tumours following challenge with CMT93 B7-1 cells and remained tumour free (Figure 5C). In comparison, mice vaccinated with irradiated parental B16 cells and unvaccinated mice developed tumours more readily, although the difference compared with the group vaccinated with B16 B7-1 cells did not reach statistical significance.

DISCUSSION

We have shown here that B16 melanoma cells that express B7-1 or B7-2 grew more slowly than parental B16 cells in immunocompetent syngeneic animals. This retardation in growth was dependent on NK cells, as the B7 tumours also grew more slowly in athymic nude mice, which possess NK cell activity but which are largely deficient in T cells. Also, in nude mice depleted of NK cells, the difference in growth rates between the parental and B7 tumours was minimal. Similarly, in NK cell-deficient beige mice, at least in the case of the B7-2 tumours, the growth rates between parental and gene-modified B16 cells did not differ. These results show clearly the involvement of NK cells in the response against B7-expressing tumours, although they do not exclude the participation of other effector cell types, as is suggested by the delayed growth of B7-1 tumours in beige mice.

Natural killer cells represent a heterogeneous population of lymphocytes that possess spontaneous cytolytic activity against some tumour cells and, unlike T cells, they are unrestricted by antigen specificity (Whiteside and Herberman, 1995). NK cells also secrete a variety of cytokines that contribute to the activation of other cells, including T cells. It has been presumed that NK cells play a role in the response against tumours in vivo as they can eliminate circulating tumour cells (Riccardi et al, 1980; Barlozzari et al, 1983). Recently, a subpopulation of NK cells was identified that is able to extravasate, migrate into solid tumour tissue and function as cytolytic cells therein (Vujanovic et al, 1995). Also, of late, there has been considerable progress in the definition of receptors on NK cells that regulate their activity (Lanier, 1997). A family of receptors recognizes MHC class I molecules and generates an inhibitory signal to NK cells. In the mouse these belong to the Ly49 family of proteins, whereas in humans this function is provided by the family of killer cell inhibitory receptors (KIR) (Raulet, 1996). Low expression of MHC class I by tumours such as B16 melanoma leads to poor recognition by T cells, but may favour NK cell activation as MHC class I interacts with these inhibitory molecules.

The stimulatory receptors on NK cells are not as well understood, but a variety of different molecules are assumed to be responsible for this activity. One family of receptors with stimulatory activity is NKR-P1, which recognizes oligosaccharide moieties on tumour cells (Bezouska et al, 1994). CD28, which is expressed on murine NK cells, also provides a stimulatory signal on binding with B7, promoting NK cell proliferation and increased cytokine secretion (Nandi et al, 1994). Recently, B7-1 was reported to bind to an unidentified receptor on murine NK cells and trigger cytolytic activity (Chambers et al, 1996). Moreover, this signal was able to override the inhibitory signals imparted by MHC class I molecules. Human NK cell lines derived from the YT line also express CD28, and the cytolytic activity of these cell lines is enhanced by the interaction of CD28 with B7 (Azuma et al, 1992*b*; Montel et al, 1995). Moreover, fresh human NK cells have been found to lyse B7-1-expressing tumour cells preferentially (Dessureault and Gallinger, 1996).

Other groups have described varied effects of B7-1 expression on in vivo growth of B16 melanoma. In some cases, B7-1 did not affect the growth pattern (Chen et al, 1994; Townsend et al, 1994), whereas in others it resulted in complete rejection of B16 tumours, a response that was mediated by NK cells and $CD8^-$ cells (Wu et al, 1995). These apparently conflicting results probably reflect differing levels of B7-1 expression, as it has been reported that only clones expressing high levels of B7-1 were rejected completely, whereas low-expressing clones grew more slowly but were not rejected (Wu et al, 1995). The results we present here are compatible with this possibility. The clone used in our experiments expressed a comparable level of B7-1 to the low-expressing clone in the latter study. Under in vitro conditions, B7-expressing tumour cells were no more susceptible to lysis by fresh splenocytes than were the parental cells (data not shown). This does not correlate with the in vivo results, but may simply reflect the less than ideal nature of the in vitro assay.

As well as the effects on local tumour growth, it is also of importance to determine the effects of B7 co-stimulatory molecules on systemic immunity. In some studies, B7-expressing tumours have generated protective immunity against a subsequent challenge with parental tumour cells (Cavallo et al, 1995; Gajewski et al, 1996; Dunussi-Joannopoulos et al, 1996) although in other studies such tumours did not elicit any protective immunity (Ramarathinam et al, 1994; Katsanis et al, 1995). Previously, in the moderately immunogenic tumour model (K1735 murine melanoma) we demonstrated that B7-expressing tumours may even reduce the degree of systemic immunity relative to that elicited by the parental tumour (Chong et al, 1996). In contrast, B16 melanoma is a tumour of low intrinsic immunogenicity

(Dranoff et al, 1993; Vile et al, 1994). The ability of B16 B7-1 cells to elicit systemic protective immunity was examined by inoculation of irradiated tumour cells followed by challenge with live tumour cells. Irradiated B7-1 cells failed to evoke any protection against rechallenge with parental B16 cells, in keeping with reports by other groups (Cavallo et al, 1995; Wu et al, 1995) and consistent with the observation that B7 is generally weak at eliciting systemic immunity against tumours of low intrinsic immunogenicity (Chen et al, 1994).

In contrast, irradiated B16 B7-1 cells consistently protected the majority of mice against challenge with live B16 B7-1 cells. Unlike the local response against B7-expressing tumours, protective immunity was dependent on T cells, as no protection was evident in athymic nude mice. A similar phenomenon was noted in a murine mammary adenocarcinoma model (Zitvogel et al, 1996). Similarly, tumour-infiltrating lymphocytes from a B7-expressing plasmacytoma were able to lyse the B7-expressing tumour cells, but the parental tumour cells were lysed inefficiently (Ramarathinam et al, 1994). It is possible, therefore, that an epitope derived from the B7-1 molecule may act as an antigenic target against which a memory T-cell response is directed. In support of this was the tendency for vaccination with irradiated B16 B7-1 cells to confer a degree of protection against an unrelated syngeneic tumour, CMT93, engineered to express B7-1. Although B7-1 is a 'self' molecule, it may be that in gene-modified tumour cells, where B7-1 is expressed out of its normal context, novel epitopes arise. Alternatively, the requirement for the live challenge tumour cells to express B7-1 may be a reflection of the low degree of protective immunity afforded by inoculation with irradiated B7-1 cells, such that an additional trigger is needed at the time of live challenge.

In this report, we confirm reports by others that the local response against B7-1-expressing B16 melanoma is mediated, at least in part, by NK cells (Wu et al, 1995). We have also shown that the reaction to the B7-2-expressing tumour is similar, and this constitutes the first report describing NK cells as the main effector cells responsible for an in vivo response against a B7-2-expressing tumour. The involvement of NK cells in the local response may help to explain the recent reports concerning the role of host APCs in production of systemic immunity, as elicited by B7-expressing tumour cells (Huang et al, 1996; Cayeux et al, 1997). It is possible that NK cells cause local tumour cell lysis, thus making tumour antigens available in abundance for uptake by professional APCs attracted to the local environment, and these in turn would then present the antigens to T cells efficiently. This situation may be comparable with the systemic immune response associated with tumour cell lysis resulting from activation of a 'suicide gene' such as herpes simplex virus thymidine kinase with ganciclovir treatment (Vile et al, 1997). Therefore, although our results suggest that B7 molecules, by themselves, have limited therapeutic use, it may be useful to attempt co-expression of B7 with a cytokine, such as granulocyte–macrophage colony-stimulating factor (GM-CSF), which promotes maturation of professional APCs. This might then enhance systemic immunity against poorly immunogenic tumours. Experiments to explore such approaches are currently under way.

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