Role of B7-1 in Mediating an Immune Response to Myeloid Leukemia Cells

By Ursula A. Matulonis, Chrysoula Dosiou, Clare Lamont, Gordon J. Freeman, Peter Mauch, Lee M. Nadler, and James D. Griffin

A costimulatory signal from B7-1 (CD80) to its counter-receptor CD28 is required for T-cell activation. Many tumors, including most human leukemias, lack expression of B7-1, and this has been suggested to contribute to the failure of immune recognition of these diseases. A murine leukemia model system was developed to assess the potential role of B7-1 in the induction immunity to leukemia cells. The nonleukemic 32Dc13 myeloid cell line was transformed by transfection of the BCR/ABL gene, generating a subline (32Dp210/clone 26) that was leukemic and rapidly lethal to syngeneic, immunocompetent C3H/HeJ mice or T-cell-deficient nude mice. B7-1-modified leukemic cells remained lethal in nude mice, but caused only a transient, nonlethal leukemia in C3H/HeJ mice. After a single exposure to live, nonirradiated B7-1-modified leukemic cells, C3H/HeJ mice developed protective immunity against subsequent challenge with B7-1(-) leukemic cells. Further, hyperimmunization with B7-1(+) leukemic cells prolonged the survival of mice previously injected with a lethal number of B7-1(-) leukemic cells. These results indicate that myeloid leukemic cells may be attractive candidates for B7-1 gene transfer.

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From the Division of Hematologic Malignancies, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

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Address reprint requests to Ursula Matulonis, MD, Division of Hematologic Malignancies, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

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**MATERIALS AND METHODS**

**Animals.** Eight- to 12-week-old C3H/HeJ mice (H-2^k^) (Jackson Laboratories, Bar Harbor, ME) were maintained in standard conditions. Swiss nu/nu mice (Tacolicn Laboratories, Germantown, NY) were obtained at 6 to 8 weeks, and studies were performed at 8 to 10 weeks. All experiments included 5 to 10 animals in each group, and all experiments were repeated at least once. All experiments involving mice were reviewed and approved by the New England Deaconess Hospital and Dana-Farber Cancer Institute Animal Use Committees. In some experiments, blood was obtained from anesthetized animals by retro-orbital sinus puncture.

**Cell lines.** The 32Dc13 (32D) (H-2^k^) cell line is an IL-3-dependent murine myeloidoid line obtained from Dr Joel Greenberger (University of Pittsburgh). It was cultured at 37°C, 5% CO_2 in RPMI 1640 media containing 10% fetal calf serum (FCS), 2% glutamine, 1% penicillin-streptomycin, and 1% WEHI-conditioned media as a source of IL-3. Generation of a subline transformed by the BCR/ABL oncogene has been described.\(^{3,6}\) One subclone (clone 26, hereafter referred to as 32Dp210/clone 26) was then cotransfected with the full-length murine B7-1 expression construct and a hygromycin resistance vector pPGK-hyg.\(^{3,6}\) The transfection, performed by electroporation (BioRad [Hercules, CA] gene pulsar, using 0.25 V at 10^3 cells/800 μL of serum-free RPMI 1640 media) used DNA at a ratio of 10:1 with 20 μg of B7-1 and 2 μg of pPGK-hyg. Cells were selected in RPMI 1640 media with 10% FCS, 2% glutamine, 1% penicillin-streptomycin, and 1.5 mg/mL of hygromycin (Calbiochem-Novabiochem Corp, La Jolla, CA). Hygromycin-resistant cells were subcloned in soft agar, and seven subclones were studied for p210BCR/ABL expression by antiphosphotyrosine immunoblotting and B7-1 surface expression by surface immunophenotyping. All experiments were also performed with a second subclone, 32Dp210/clone 2, which was selected for further study and is designated 32Dp210/B7-1/clone 2 cell line. Briefly, cells were lysed by 1% NP-40, 150 mmol/L NaCl, 20 mmol/L TRIS pH 7.4, 10% glycerol containing 1 mmol/L phenylmethylsulfonylfluoride, 20 μg/mL aprotinin, and 1 mmol/L Na orthovanadate at 10^6 cells/mL, and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 7.5% polyacrylamide. Immunoblots using antiphosphotyrosine monoclonal antibody (MoAb) 4G10 (gift of Dr Brian Drucker, Oregon Health Sciences University, Portland, OR) and antialb MoAb 24-214\(^{4}\) a gift from Dr Naomi Rosenberg, Tufts University School of Medicine, Boston, MA; diluted 1:200 in TRIS-buffered saline [TBS] with 0.05% Tween 20 were performed as described.\(^{3,6}\)

**Injection of cells into mice.** 32Dc13, 32Dp210/clone 26, and 32Dp210/B7-1/clone 2 cells were injected in small volumes (100 to 200 μL) in the tail vein or subcutaneously in the flank. In some experiments, cells were first irradiated at a dose of 100 Gy at a rate of 12.8 Gy/min using a Gamacell 1000 gamma radiator (Nordion International Inc, Kanata, Ontario, Canada). This dose of radiation was selected based on pilot studies which showed that 100 Gy was the minimum dose required to stop proliferation and prevent leukemic cell outgrowth in culture. In other experiments, cells were first fixed in paraformaldehyde as follows: cells were washed once in 1X PBS to remove serum, resuspended in freshly prepared 2% paraformaldehyde (Sigma Co, St Louis, MO) in 1X PBS pH 7.4 at a concentration of 10^6 cells/mL at 4°C for 20 minutes, washed three times in cold 1X PBS, and recounted before using in experiments. CD8^+^ T-cell depletion studies. The hybridomas producing rat-antimouse CD8 (lyt-2.1) MoAb 116-13.1 and rat-antimouse CD4 (L3T4) MoAb GK1.5, obtained from the American Type Culture Collection (ATCC; Rockville, MD), were injected into either pristine-treated Balb/c mice or Swiss nu/nu mice, respectively. Ascites containing anti-CD8 MoAb was purified by passing the fluid over protein A coupled to sepharose CL-4B (Pharmacia, Piscataway, NJ). Ascites obtained from nude mice containing anti-CD4 MoAb was purified by ammonium sulfate precipitation as follows: after ascsites were centrifuged at 3,000 rpm for 30 minutes, an equal volume of saturated ammonium sulfate was added and the supernatant kept at 4°C overnight. The supernatant was then resuspended in 0.5 vol of 1X PBS and was dialyzed in 1X PBS for 24 hours. For the CD8^+^ depletion experiments, mice received between 0.5 mg and 1.0 mg per mouse of anti-CD8 antibody IV 1 week before and on the day of tumor injection. Control mice undergoing CD4^+^ depletion received 1.0 mg of MoAb 1 week before, on the day of tumor injection, and 3 days and 6 days after tumor cell injection. Mice receiving antibody alone were killed 1 week after tumor cell injection, and lymph nodes and spleens were prepared by gentle dissection on a frosted glass slide and examined by immunostaining and flow cytometry using rat-antimouse Ly2 (anti-CD8^+^) or rat-antimouse L3T4 (anti-CD4^+^) conjugated to FITC (BioSource International, Cama-rillo, CA).

**RESULTS**

**Generation of a subline of 32Dp210/clone 26 that expresses murine B7-1.** The parental cell line used in these studies is 32Dc13, an IL-3-dependent, nonleukemic, myeloid cell line derived from a long-term BM culture.\(^{31}\) A transformed, factor-independent, subline of 32Dc13, termed 32Dp210/clone 26, was derived by transfection with a p210BCR/ABL retrovirus, and has been previously described.\(^{32}\) 32Dp210/clone 26 was transfected with a murine B7-1 plasmid as described in Materials and Methods, and sublines resistant to hygromycin were grown out. The 32Dp210/B7-1/clone 2 cell line selected for further study was strongly reactive with murine B7-1 antibody and with CTLA4Ig fusion protein, indicating surface expression of...
Both 32Dp210/cIone 26 and 32Dp210/B7-1/cIone 2 cell lines express equivalent amounts of p210BCR/ABL protein. p210BCR/ABL expression, determined by antiphosphotyrosine (Fig 1B, left panel) and anti-ab1 immunoblotting (Fig 1B, right panel), was present at equivalent levels in the 32Dp210/cIone 26 and the 32Dp210/B7-1/cIone 2 cell line and was absent in the 32Dc13 cell line. Both the 32Dp210/cIone 26 and 32Dp210/B7-1/cIone 2 cell lines were factor-independent in vitro whereas the 32Dc13 cell line was factor-dependent. The in vitro growth rate of the 32Dp210/cIone 26 cell line was indistinguishable from the 32Dp210/B7-1/cIone 2 cell line (Fig 1C).

Immunocompetent syngeneic mice survived injections with B7-1—transduced 32Dp210/cIone 26 cells, but not 32Dp210/cIone 26 cells. Groups of 10 mice were administered 10⁷ to 10⁸ cells intravenously (IV) (Fig 2A), and survival was measured. The parental cell line, 32Dc13, was not lethal in C3H/HeJ mice (up to 10⁷ cells) and no morphologic evidence of leukemia was detected in blood on days 7, 14, 28, or 60 after injection or BM on sacrifice at 60 days (data not shown). In contrast, 10⁴ 32Dp210/cIone 26 cells killed all mice in less than 18 days, apparently because of rapidly progressive leukemia. In animals killed at 12 days, 25% to
90% of PB, BM, and spleen cells were blasts, and spleens were massively enlarged (data not shown). Although 32Dp210/clone 26 and 32Dp210/B7-1/clone 2 cell lines were indistinguishable in their in vitro growth characteristics, 32Dp210/B7-1/clone 2 cells were not lethal at 60 days (up to 10^5 cells; Fig 2B). Mice administered 10^3, 10^4, or 10^5 32Dp210/B7-1/clone 2 cells all showed 100% survival after IV injection (data not shown). Mice injected with 10^6 32Dp210/B7-1/clone 2 cells were bled on days 7, 14, and 28 and found to reproducibly have a transient leukemia manifested as abundant blasts in the PB on day 7 that were absent by day 14 (data not shown). However, IV administration of 10^3 32Dp210/B7-1/clone 2 cells did produce persistently leukemic mice and was uniformly lethal (Fig 2B). Thus, the dose of 32Dp210/B7-1/clone 2 cells sufficient to cause a lethal leukemia was about 3 logs higher than that of 32Dp210/clone 26 cells.

32Dp210/clone 26 and 32Dp210/B7-1/clone 2 are both lethal in Swiss nu/nu mice. 32Dc13, 32Dp210/clone 26 and 32Dp210/B7-1/clone 2 cells were injected IV into Swiss nu/nu mice (10^5 cells each; Fig 2C). Mice receiving 32Dc13 cells were not observed to be sick and all survived at greater than 60 days of follow-up. 32Dp210/clone 26 cells or 32Dp210/B7-1/clone 2 cells caused the death of all animals within 13 days after injection.

32Dp210/B7-1/clone 2 cells, but not 32Dc13 cells, protect against rechallenge with 32Dp210/clone 26 cells. The observation that mice receiving 32Dp210/B7-1/clone 2 cells appeared to be only transiently leukemic suggested the development of an immune response to the tumor cells. This is further supported by the fact that both 32Dp210/B7-1/clone 2 and 32Dp210/clone 26 cells are lethal to nude mice. Therefore, we looked for evidence of immunity by challenging mice surviving 36 days after a single injection of either 32Dc13 or 32Dp210/B7-1/clone 2 cells with 10^5 32Dp210/clone 26 cells. Mice that had received 32Dc13 cells rapidly became leukemic and died (Fig 3A). However, five of five mice that had received prior 32Dp210/B7-1/clone 2 cells survived (minimum follow up was >60 days). Similar results were seen in six different experiments and were repeated with another clone of 32Dp210/B7-1/clone 2 cells, 32Dp210/B7-1/clone 10 (data not shown). This protective effect was dependent on the number of 32Dp210/B7-1/clone 2 cells injected; a single injection of 10^9, 10^8, or 10^7 cells conferred protection to challenge, whereas 10^6 cells did not (Fig 3B). This protective effect was long-lasting, but not permanent. In studies not shown, a group of five mice that received 10^6 32Dp210/B7-1/clone 2 cells were challenged with 10^6 32Dp210/clone 26 cells 150 days later. All mice died, although survival was prolonged compared with naive controls (median survival 30 days v 14 days).

To determine the length of time necessary for the genera-
Fig 3. (A) Mice receiving prior injections of 32Dp210/B7-1/clone 2 cells are protected from further rechallenges with 32Dp210/clone 26 cells; mice receiving 32Dc13 cells are not. Thirty-six days after receiving either a single IV injection of 10^6 32Dc13 cells (-----) or 32Dp210/B7-1/clone 2 cells (●), 10 mice per group were rechallenged with 10^6 32Dp210/clone 26 cells. The experiment was repeated five times. (B) 10^5 32Dp210/ B7-1/clone 2 cells must be given for effective protection against 32Dp210/clone 26 cells; 10^4 32Dp210/B7-1/clone 2 cells will not confer protection. Mice administered either 10^5 (○) or 10^6 (●) 32Dp210/B7-1/clone 2 cells on day 0 were rechallenged with 10^5 32Dp210/clone 26 cells 36 days later. Five mice were present in each group, and this experiment was repeated four times. (C) Ten days is necessary for the generation of protection against leukemia after exposure to 32Dp210/B7-1/clone 2 cells. The time interval between initial injection with 32Dp210/B7-1/clone 2 cells and subsequent rechallenge with IV 10^6 32Dp210/clone 26 cells was varied. Intervals chosen were 0 (simultaneous administration) (-----), 4 (○), and 10 (●) days. Each group contained five mice, and the experiment was repeated twice. (D) 32Dp210/B7-1/clone 2 is lethal in mice depleted of CD8⁺ T cells. Mice were depleted of either CD8⁺ (●) or CD4⁺ (□) T cells before injection IV of 10^6 32Dp210/B7-1/clone 2 cells. A non-T-cell-depleted group was also given 10^6 32Dp210/B7-1/clone 2 cells IV (○). Five mice were included in each group, and the experiment was repeated twice.

Abrogation of leukemogenicity observed after 32Dp210/B7-1/clone 2 injection is mediated by CD8⁺ cells. Mice were administered between 0.5 mg and 1.0 mg of anti-CD8 antibody or 1.0 mg of anti-CD4 antibody as described in Materials and Methods. The 32Dp210/B7-1/clone 2 cells were administered simultaneously with the second injection of either anti-CD8 or anti-CD4 antibody. Mice receiving only anti-CD8 or anti-CD4 antibody injections were killed 1 week after the second antibody injection and were found to have greater than 95% depletion of either CD8⁺ or CD4⁺ T-cell subsets via MoAb staining of either lymph nodes or whole-spleen suspensions as described in Materials and Methods (data not shown). Mice undergoing CD8⁺ depletion all died of leukemia by day 15 after injection of 10⁶ cells of 32Dp210/B7-1/clone 2 (Fig 3D). However, 100% of mice...
that received the anti-CD4 MoAb injections with subsequent 32Dp210/B7-1/clone 2 injections survived, as did non-T cell-depleted mice receiving 32Dp210/B7-1/clone 2 injections alone (Fig 3D).

*Immunization with irradiated cells.* The results above suggested that a single exposure of mice to 32Dp210/B7-1/clone 2 cells, but not to 32Dc13 cells, protected against subsequent challenge against B7-1(-) leukemic cells, possibly by enhancing an antileukemia immune response. Mice previously exposed to live 32Dp210/clone 26 cells could not be evaluated because all of these mice died of leukemia. To assess the immunogenicity of 32Dp210/clone 26 cells and to more directly examine the role of B7-1 in inducing protection against leukemia challenge, we attempted to immunize mice using irradiated cells. Mice were administered two doses (10⁷ cells) of irradiated 32Dp210/clone 26 or 32Dp210/B7-1/clone 2 cells IV, 2 weeks apart. Two weeks after the second immunization, mice were challenged with 10⁶ IV 32Dp210/clone 26 cells. As shown in Fig 4, none of the groups were protected against challenge with 32Dp210/clone 26 cells compared with the unimmunized control. In studies not shown, mice were given higher doses (10⁸) of irradiated 32Dc13, 32Dp210/clone 26, or 32Dp210/B7-1/clone 2 cells administered weekly subcutaneously for four doses and were rechallenged with 10⁶ 32Dp210/clone 26 cells 2 weeks after the last injection. No protective immunity was observed against the 32Dp210/clone 26 cells.

Because fixed cells can also temporarily present antigen and because B7-1 costimulation is resistant to paraformaldehyde fixation,⁴⁵ we also attempted to induce protection against challenge using paraformaldehyde-fixed 32Dc13, 32Dp210/clone 26, or 32Dp210/B7-1/clone 2 cells. Groups of five mice were administered two IV injections of 10⁶ fixed cells 2 weeks apart, and challenged with 10⁴ IV 32Dp210/clone 26 cells 2 weeks later. Again, no protection against leukemic death was observed in any group (data not shown). These results suggested that the protective effect of exposure to 32Dp210/B7-1/clone 2 cells required exposure to live cells.

*Hyperimmunization of leukemic mice with 32Dp210/B7-1/clone 2 cells prolongs survival.* Because results from other investigators⁴⁶ have shown that established B7-1(-) metastases could sometimes be rejected after treatment with B7-1(+) tumor injections, we examined the effect of hyperimmunization with 32Dp210/B7-1/clone 2 cells in mice that had previously received 32Dp210/clone 26 cells. Mice received 10⁴ 32Dp210/clone 26 cells on day 1. Twenty-four hours later, one group of mice received 10⁶ 32Dp210/B7-1/clone 2 cells IV every day for 5 days, a second group received 10⁴ 32Dp210/B7-1/clone 2 cells SQ every day for 5 days, and the control group received no further therapy. As shown in Fig 5, the control group died within 12 days. The group receiving 32Dp210/B7-1/clone 2 cells SQ all died of acute leukemia by day 15. The group receiving IV 32Dp210/ B7-1/clone 2 cells were found to have prolonged survival (60%) with follow-up > 60 days. This protective effect was no longer observed when administration of 32Dp210/B7-1/clone 2 cells was delayed until 3 days after establishment of leukemia (data not shown), possibly because of the extremely rapid growth of this tumor.

**DISCUSSION**

In this study, we have examined the in vivo effects of introducing the B7-1 (CD80) gene into a rapidly lethal, non-immunogenic, leukemic cell line. B7-1 expression was associated with loss of tumorigenicity and induction of protective immunity against subsequent challenges with the parental B7-1(-) leukemia line in immunocompetent, syngeneic mice. Remarkably, a single IV injection of 32Dp210/B7-1/clone 2 cells was sufficient to protect mice against subsequent challenge with B7-1(-) 32Dp210/clone 26 cells for up to 5 months. Further, survival of mice with established leukemia could be prolonged if live 32Dp210/B7-1/clone 2 leukemia cells were repeatedly administered IV shortly after the administration of 32Dp210/clone 26 leukemic cells.

Several possible explanations exist for the reduced leukemogenicity of the 32Dp210/clone 26 cell line after introduction of B7-1. One explanation would be that the cell line is no longer transformed. The parent cell line, 32Dc13, is factor-dependent and nonleukemic in immunocompetent mice. However, after introduction of the p210 BCR/ABL gene, the cells become transformed, factor-independent, and, as shown here, cause leukemia in C3H/HeJ mice. After B7-1 transfection into the 32Dp210/clone 26 cell line, the resulting subclones were indistinguishable from 32Dp210/clone 26 cells in vitro. Specifically, the subclones remained factor-independent and had an identical doubling time, expression of p210 BCR/ABL, and pattern of other cellular tyrosine phosphoproteins. Additional evidence suggesting that the 32Dp210/B7-1/clone 2 cell line remained transformed was the observed lethality of this cell line in nude mice and in syngeneic mice depleted of CD8+ T-cell subsets. Thus, B7-
There are several observations suggesting that 32Dp210/B7-1/clone 2 cells induce an antileukemia immune response. First, mice injected with 32Dp210/B7-1/clone 2 cells appear to be transiently leukemic, with easily demonstrable leukemic blasts in blood, BM, and spleen on day 7 that are reduced or absent by day 14. The time course and outcome suggest immunologic rejection of the leukemic cells. Further, mice exposed to 32Dp210/B7-1/clone 2 cells are protected against subsequent challenge by 32Dp210/clone 26 cells, whereas mice receiving live 32Dc13 cells or irradiated 32Dp210/clone 26 cells are not protected. We have shown that CD8+ T cells mediate the abrogation of leukemogenicity after the initial injection of 32Dp210/B7-1/clone 2 cells, whereas CD4+ depletion before 32Dp210/B7-1/clone 2 cells resulted in 100% survival of the mice. Future studies will examine depletion of CD4+ and/or CD8+ subsets after immunization but before rechallenge with 32Dp210/clone 26. 32Dp210/B7-1/clone 2 cells also can cure established leukemia, suggesting that T cells have not yet been anergized in vivo to the leukemia cells.

Overall, our studies support the concept that expression of B7-1 is important in the immune response against neoplastic cells as other groups have shown. Introduction of the B7-1 gene into B7-1(-) tumors has now been shown to enhance antitumor immune responses in several murine tumors.17,18,27,28,45 However, an effect has not been observed in other tumors25 (G. Dranoff, personal communication, December 1993), and the inherent immunogenicity of the tumor has been suggested to be an important variable.26 The leukemia cell lines reported here indicate that B7-1 can also alter the immune response to a nonimmunogenic, hematopoietic neoplasm. We were unable to immunize mice with even repeated injections of irradiated or paraformaldehyde-fixed B7-1(-) 32Dp210/clone 26 leukemia cells, yet transfer of B7-1 had dramatic effects.

The antigen(s) in these 32Dp210 cell lines are unknown. Unless transformed by BCR/ABL, the parent cell line, 32Dc13, is not leukemic. Because p210BCR/ABL is a human protein, it could potentially be an effective tumor antigen. In fact, a peptide representing the junction between BCR and ABL sequences has been shown to induce a T-cell response in BALB/c mice,47 even though the sequences of murine BCR and ABL are otherwise identical to human BCR and ABL in this region. It is possible that this cell line has only weak tumor antigens or low levels of tumor antigens, but is capable of particularly efficient and effective antigen presentation in the presence of B7-1. This may be of particular relevance to human leukemias, where tumor antigens may also be present in low abundance. It is also possible that B7-1 is only one of several costimulatory molecules that is important as a group, and that 32Dc13 cells may already express other important surface molecules. Recent identification of B7-2 (CD86) as a second ligand for CD28 and CTLA-4 suggests that costimulation in tumor immunity may be complex. 32Dc13 does not express B7-2 because there is no CTLA4lg binding. Indeed, in preliminary studies, expression of B7-2 in 32Dp210 cells also reduces leukemogenicity and induces tumor immunity (U.A.M., J.D.G., unpublished results, August 1994).

Our experiments suggest that the degree of immunization with 32Dp210/B7-1/clone 2 cells is highly dependent on dose, schedule, and route, which are critical variables not appreciated in previous studies with B7-1. Effective protective immunity against 32Dp210/clone 26 challenge was obtained with a single dose of 10⁶ cells, but not with 10⁴. Live cells may initially proliferate after injection, thereby increasing the potential for presentation of antigen to T cells. Immunization with paraformaldehyde-fixed, dead cells or nonproliferating irradiated cells was not effective. Irradiation of cells may abrogate the immunization potential of the B7-1-expressing cells by shortening their in vivo life span, impairing antigen presentation, reducing antigen production, altering homing in vivo, or other mechanisms. Irradiated cells remained alive in culture for several days, but viability in vivo could not be assessed. Unexpectedly, the route of immunization was critical in our model. Subcutaneous immunization was markedly inferior to IV administration of the immunization. IV administered cells may accumulate preferentially in the spleen, thereby facilitating direct T-cell interaction. However, in a subcutaneous site, access to potentially reactive T cells may be limited, requiring the tumor cells to interact with an intermediary APC that will process and subsequently present the antigen. Introduction of cytokine genes, such as IL-2 and GM-CSF, may augment the APC migration to the site of SQ immunization making this route more effective in eliciting an immune response. Because cytokine expression in hematopoietic cells has led to transformation in normal cells and leukemic proliferation in
neoplastic cells, the use of cytokines in live leukemia cells might be problematic clinically. However, cytokine expression in leukemia cells may have a role when the vaccine is administered killed and subcutaneously, and these experiments are currently underway.

Of potential relevance for the design of future human leukemia therapies, we have shown that 32Dp210/B7-1/clone 2 cells can produce therapeutic immunity, i.e., some mice with leukemia can be cured with aggressive administration of 32Dp210/B7-1/clone 2 cells. IV administration of live 32Dp210/B7-1/clone 2 cells starting 24 hours after injection of 32Dp210/clone 26 cells resulted in long-term survival of greater than 40% to 60% of the mice. The rapid course of this leukemia in mice (12- to 14-day survival) dictates that any therapeutic immune response must be developed immediately. Delaying administration of 32Dp210/B7-1/clone 2 cells even to day 3 was ineffective, but we do not know if this was due to the development of clonal anergy or simply to the possibility that the immune response could not catch up with the proliferation of leukemia at this point. We are currently evaluating the effects of B7-1 expression in hematopoietic neoplasms with longer courses in vivo. Overall, however, our results suggest that expression of B7-1 can dramatically alter the virulence of a very aggressive leukemia and enhance protective and therapeutic immunity in this system. We conclude from these results that B7-1 gene transduction could be particularly useful in the development of live cellular vaccines for the treatment of human leukemias.

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