Expression of B7-1 by Highly Metastatic Mouse T Lymphomas Induces Optimal Natural Killer Cell-mediated Cytotoxicity

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Abstract

The interaction between B7-1 and CD28 provides costimulatory signals not only for T cells but also for natural killer (NK) cells. Highly metastatic mouse T lymphoma cells (BW-Li) can escape from NK cell-mediated killing by expressing H-2D\(^{a}\) molecules that negatively regulate NK lytic activity. We have analyzed whether B7-1:CD28 overrules the MHC class I-mediated inactivation of NK cells by transfecting BW-Li with the gene coding for B7-1. Expression of B7-1 rendered BW-Li cells sensitive toward NK cells. The experimental metastatic capacity of the B7-1 transfectedants was drastically reduced in both syngeneic AKR and SCID mice but could be restored in SCID-bg mice. These results provide direct evidence that B7-1 expression leads to NK-mediated elimination of metastasizing, NK-resistant tumor cells.

Introduction

B7-1 is a ligand for both CD28 and CTLA-4 and delivers a costimulatory signal for T-cell activation (1). Costimulation of T cells by B7-1 may play an important role in eliciting antitumor immunity (2). Indeed, failure to deliver a costimulatory signal may allow tumor cells to evade the immune system, even if the tumor cells express normal levels of MHC class I antigens and present tumor-specific peptides. Consequently, in a number of tumor models, de novo expression of B7-1 via transfection has proven to be an efficient means for the induction of protective antitumor T-cell responses (2-5). Recently, it has been demonstrated that besides T and B cells, most resting NK cells require also costimulatory signals for optimal proliferation (6). There is suggestive evidence that CD28:B7-1 interactions can provide such costimulatory signals to NK cells. Indeed, Nandi et al. (7) demonstrated that an optimal proliferation of SCID spleen-derived NK cells can be achieved after stimulation with anti-CD28 or L-B7@ cells. Furthermore, Azuma et al. (8) reported that a human CD28-positive NK leukemia cell line can lyse B7-1-expressing cell lines in a MHC-unrestricted fashion (8). We have shown previously that the experimental metastatic potential of BW5147-derived T lymphoma cells is determined by the level of H-2D\(^{a}\) MHC class I antigen expression, conferring NK-cell resistance to the tumor cells (9). In view of the possible role of B7-1 in NK cell activation, we have tested whether expression of B7-1 modulates the sensitivity of BW5147-derived tumor cells toward NK effector cells. In our study, we demonstrate that transfection of the B7-1 gene in NK-resistant, BW5147-derived tumor cell variants increases the tumor cell sensitivity toward NK cells and concomitantly reduces the experimental metastatic capacity.

Materials and Methods

Tumor Cell Lines. The highly malignant BW-Li is a spontaneous metastasizing variant derived from the nonmetastatic BW5147 T-cell lymphoma (AKR origin; Salk Institute, La Jolla, California) (9). Transfection of BW-Li with the gene coding for neomycin phosphotransferase alone or in combination with the mouse B7-1 gene gave rise to respectively BW-Li-neo and BW-Li(B7-1). Tumor cell lines were cultured as described earlier (9).

Mice. Virus-free, female, 6-9-week-old mice were used in all in vivo experiments. AKR mice were obtained from B&K Universal, Ltd. (England). CB-17 SCID/SCID mice and CB-17scid/scid-bg mice were purchased from Harlan CPB (Zeist, the Netherlands).

Transfection. The vector encoding the genes for mouse B7-1 and neomycin phosphotransferase, designated pcDNAneo1::MoB7-1, was obtained by cloning B7-1 cDNA, derived from the mouse B1 lymphoma cell line, in the pcDNA3 expression vector (Invitrogen BV, Leek, the Netherlands) via HindIII and XhoI restriction. For stable transfection, tumor cells were transfected by electroporation with the use of a Bio-Rad Gene Pulser apparatus as described earlier (9). A stable transfection efficiency of 20-30% resistant clones/10\(^3\) cells was achieved.

FACS Staining and Analysis. Cytofluorimetric analysis was performed as described in detail previously (9). Cells were stained indirectly with hamster anti-B7-1 (mAb 16-10A1), kindly provided by Dr. H. Reiser (10), followed by rat anti-hamster immunoglobulin coupled to FITC or with mouse anti-H-2D\(^{a}\) (mAb 15.5.55; American Type Culture Collection), followed by anti-mouse immunoglobulin coupled to FITC.

Analysis of Experimental Metastasis. Indicated mice in groups of five were given i.v. injections in the tail of 10\(^6\) tumor cells in a volume of 200 \(\mu\)l PBS. Mortality was followed up, and metastases were evaluated macroscopically at different time points as indicated.

In Vitro Cytotoxicity Assays. AKR NK cells were enriched by injecting 180 \(\mu\)g polyI:polyC (Boehringer Mannheim, Mannheim, Germany) i.p., 18—20 h before harvesting of the spleen. Subsequently, the spleen cells were subjected to a Nycoprep gradient centrifugation and used as effector cells. AKR LAK cells were obtained by incubating 10\(^6\) cells/ml of a nylon wool nonadherent fraction of spleen cells with 1000 units/ml interleukin 2 for 6-7 days. The adherent AKL cell fraction was used in the cytotoxicity tests. Percent lysis was determined in an \(^{11}I\)In release assay, which is a modification of the standard \(^{51}Cr\) release assay (11).

Statistical Analysis. The statistical differences in survival time (Mantel-Haenszel test) and organ weight (Mann-Whitney U test) were considered as significant when \(P \leq 0.05\).

Results

Expression of B7-1 on the BW-Li T Lymphoma Variant. The highly metastatic variant BW-Li was transfected with the mouse expression vector pcDNAneo1::MoB7-1 or with the pcDNA3 control construct, containing only the neomycin resistance gene. G418-resistant clones were isolated, and the membrane expression of B7-1 was determined by FACS analysis. The immunofluorescence profile of a
B7-1-induced NK Cell-Mediated Cytotoxicity

Fig. 1. Expression of B7-1 and MHC class I H-2Dk on BW-Li transfected with a control plasmid pcDNA-neo1 as compared to BW-Li transfected with pcDNA-neo1:MoB7-1. (A and C) BW-Li(neo) and (B and D) BW-Li(B7-1) were stained for B7-1 expression with hamster anti-B7-1 mAb and for H-2Dk antigen with mouse anti-H-2Dk mAb. The stained cells were detected on FACS via indirect immunofluorescence. The profiles (—) are represented as compared to the background fluorescence profiles without primary antibody (••••).

Fig. 2. In vitro sensitivity of BW-Li (○), BW-Li(neo) (▲), and BW-Li(B7-1) (●) cells toward syngeneic NK cells. Cytotoxicity was measured in an 111In release assay, incubating NK cells with their targets at different ratios. Percent lysis was determined as [(release - spontaneous release)/(maximum release - spontaneous release)] × 100. a, poly I:poly C-activated NK-mediated cytotoxicity measured after 18 h of incubation at 37°C with the effector cells. b, adherent LAK-mediated cytotoxicity toward the BW-Li transfectants measured in a 4-h assay.

B7-1 expression renders BW-Li cells more sensitive toward NK and LAK Cells. BW-Li cells are relatively resistant to lysis by poly I:poly C-activated NK cells and LAK cells, and this resistance is mediated by a high cell surface expression of class I H-2Dk molecules (9). Because BW-Li and the B7-1 transfectants express similar levels of H-2Dk, it was of interest to test the influence of B7-1 expression on in vitro NK- and LAK-mediated cytotoxicity. The results indicate that the expression of B7-1 on BW-Li renders the relatively NK-resistant tumor cell line highly sensitive toward both poly I:poly C-activated NK cells and LAK cells. A 10-fold increase in lysis was recorded with LAK cells (at an E:T ratio of 20:1; Fig. 2b) and a 3-fold increase with poly I:poly C-stimulated NK cells (at an E:T ratio of 100:1, Fig. 2a). In contrast, transfection with neo alone did not render BW-Li cells more sensitive toward NK or LAK cells (Fig. 2).

B7-1 expression reduces the experimental metastatic potential of BW-Li Cells. BW-Li cells are highly metastatic upon i.v. inoculation into syngeneic AKR mice, and this metastatic capacity relates to the NK resistance of these tumor cells (9). Because the B7-1 transfectants became highly NK sensitive, it was important to...
test their experimental metastatic capacity. To this end, 10⁶ BW-Li, BW-Li(neo), and BW-Li(B7-1) cells were injected i.v. into syngeneic AKR mice, and survival times were monitored. All parental BW-Li-inoculated mice died with an average survival time of 17.6 ± 1.3 (SD) days (Fig. 3a). The BW-Li(B7-1)-inoculated AKR mice survived much longer, with only 1 casualty 49 days after inoculation. The prolonged survival of mice injected with BW-Li(B7-1) (P < 0.01) was not due to a transfection effect and/or clonal selection because mice inoculated with 10⁶ BW-Li(neo) cells survived with an average of 18.6 ± 4.6 days, a survival time that does not significantly differ from that of the BW-Li-inoculated mice (P > 0.25).

To evaluate the pattern of organ colonization, the organs of groups of 5 mice were compared macroscopically at the time point where the BW-Li-inoculated hosts were moribund. According to the results (Table 1), BW-Li-inoculated mice die from massive tumor proliferation in liver and spleen. At this time point, no significant increase in the different target organs could be observed in BW-Li(B7-1)-inoculated animals (Table 1). Microscopic examination of different organs further revealed tumor infiltration in the brains of BW-Li-inoculated mice, whereas no or a very low number of tumor cells could be detected in the organs of the BW-Li(B7-1)-inoculated mice.

**B7-1 Expression Renders Metastasizing BW-Li Cells Susceptible to NK Cells.** To ascertain whether natural immune effector cells such as NK are involved in the elimination of metastasizing BW-Li(B7-1) cells, BW-Li and BW-Li(B7-1) cells were inoculated in SCID mice. Inoculation i.v. with 10⁶ BW-Li cells results in a fast dissemination of the tumor cells in the spleen, liver (Table 1), and brains, and all mice die within 23 days. Hence, the metastatic capacity of BW-Li cells is similar in immunocompetent and SCID mice, confirming earlier observations (9), denying a role for CD8⁺ T cells in the control of metastasizing BW-Li cells. Expression of B7-1 on BW-Li cells reduces the malignancy of the tumor cells in SCID mice because BW-Li(B7-1)-inoculated SCID mice manifest a significantly (P < 0.02) longer survival time as compared to the SCID mice receiving injections of BW-Li (Fig. 3b). Examining the organs of BW-Li(B7-1)-inoculated hosts at the time point when the BW-Li-injected mice were moribund did not reveal any visible signs of metastasis in any of the target organs (Table 1). Autopsy of the recipients injected with BW-Li(B7-1) revealed that B7-1 expression does not change the metastatic pattern of BW-Li because an identical, though less severe, metastatic pattern was observed in the BW-Li(B7-1)-inoculated animals (results not shown).

The in vivo role of NK cells in the reduced metastatic capacity of BW-Li(B7-1) as compared to BW-Li tumor cells was unequivocally confirmed by analyzing their experimental metastatic capacity in SCID-bg mice that are devoid of functional NK cells. Inoculation i.v. of both tumor cell lines in SCID-bg mice led to statistically comparable survival times (0.25 > P > 0.1 see Fig. 3c). Furthermore, the metastatic pattern of both cell lines were quite similar in SCID-bg mice (Table 1), and no statistically significant differences could be detected for the level of organ infiltration between the two experimental groups (P > 0.4 for all organs).

**Discussion**

*De novo* expression of B7-1 on tumor cells can successfully induce tumor rejection, and this B7-1-mediated antitumor effect was reported to occur for numerous (both MHC class II-positive and class II-negative) tumor cells (2–5). In all these studies T-cell costimulation via B7-1 was found to induce a T-cell-dependent tumor rejection, and until now, the B7-1-elicted antitumor response seems to be mediated primarily by CD8⁺ and/or CD4⁺ effector cells (2–5). The results, herein presented, are the first indication that an antitumor response toward B7-1-positive tumor cells can be mediated by NK cells.

The tumor cell selected for this study is a highly malignant BW tumor variant that selectively expresses high levels of H-2Dk that correlate with the metastatic potential of the tumor cells (9). Metastasis by H-2Dk-low BW tumor cells was recorded only in NK-depleted but not in CD8⁺ T cell-depleted animals, implicating that

<table>
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<tr>
<th>Recipient</th>
<th>Tumor cell</th>
<th>Δ mg increase</th>
<th>Pᵇ</th>
<th>Δ mg increase</th>
<th>Pᵇ</th>
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<tr>
<td>AKR</td>
<td>BW-Li</td>
<td>468 ± 41</td>
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<td>BW-Li(neo)</td>
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<tr>
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<td>1677 ± 224</td>
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<td>0</td>
<td>113 ± 26</td>
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<td>0</td>
<td>744 ± 207</td>
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</table>

Two the results are presented as means of increase in organ weight as compared to the organ weight of normal mice.

*b* The statistic significance of differences in organ weights of mice inoculated with BW-Li(neo) and BW-Li(B7-1) as compared to BW-Li-inoculated mice was evaluated applying the Mann-Whitney U test.

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**Table 1 Organ metastasis of indicated mice given i.v. injections of 10⁶ BW-Li, BW-Li(neo), or BW-Li(B7-1) tumor cells**

The metastatic patterns of differentially inoculated mice were compared by measuring all organ weights simultaneously when the BW-Li-inoculated mice succumbed to the tumor challenge.
NK cells but not CD8+ T cells control the metastatic potential of H-2D\(^b\)-high versus H-2D\(^b\)-low BW tumor variants. The role of NK cells in the elimination of disseminating BW tumor cells is herein corroborated because B7-1 expression in a high H-2D\(^b\)-expressing BW cell reverts the resistance of the tumor cells toward NK cells and concomitantly reduces their metastatic capacity in syngeneic and SCID recipients. In addition, the reducing effect of B7-1 expression on the metastatic potential of BW tumor cells in SCID mice was completely abrogated in SCID-bg mice, demonstrating unequivocally that NK cells eliminate efficiently B7-1-positive BW tumor cells. It should be emphasized that the metastatic potential of B7-1-positive BW tumor cells was more greatly reduced in immunocompetent mice as compared to SCID mice. At least two nonexclusive possibilities may account for this observation: (a) H-2D\(^b\)-high-expressing BW cells, albeit being weakly immunogenic, may elicit a CTL response when a potent costimulatory molecule such as B7-1 is expressed on their membrane; and (b) NK cells derived from SCID mice may either represent different subpopulations and/or reside in a different state of activation. In support of this view, at least one family of surface expressed molecules, the Ly-49 family, was shown to define separate functional NK cell subsets, which can discriminate between target cells based on their MHC class I molecule expression. Ly-49A+ NK cells are specifically unable to lyse H-2\(^d\) and H-2\(^k\) targets, whereas Ly-49C+ NK cells mediate the rejection of H-2\(^d\) but not H-2\(^k\) target cells (13). Furthermore, other studies suggested that differentially activated NK subsets can have different target specificity. Indeed, Correa et al. (14) demonstrated that freshly isolated versus interleukin 2-activated NK cells differ in their capacity to kill class I-deficient lymphoblast target cells. Additionally, others reported that, dependent on the cytokine or the cytokine concentration, different NK cell functional subsets, which can discriminate between target cells, may be defined based on their MHC class I molecule expression. Ly-49A+ NK cells mediate the rejection of H-2\(^d\) but not H-2\(^k\) target cells. In fact, stimulation of the nonadoptive arm of antitumor immune responses via B7-1 gene therapy may be of crucial importance in the elimination of disseminating tumor cells.

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References

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