Chimeric NKG2D Receptor–Bearing T Cells as Immunotherapy for Ovarian Cancer

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Abstract

Despite advancements in the treatment of ovarian cancer, this disease continues to be a leading cause of cancer death in women. Adoptive transfer of tumor-reactive T cells is a promising antitumor therapy for many cancers. We designed a chimeric receptor linking NKG2D, a natural killer (NK) cell–activating receptor, to the CD3ζ chain of the T-cell receptor to target ovarian tumor cells. Engagement of chimeric NKG2D receptors (chNKG2D) with ligands for NKG2D, which are commonly expressed on tumor cells, leads to T-cell secretion of proinflammatory cytokines and tumor cytotoxicity. In this study, we show that >80% of primary human ovarian cancer samples expressed ligands for NKG2D on the cell surface. The tumor samples expressed MHC class I–related protein A, MICB, and UL-16 binding proteins 1 and 3. ChNKG2D-expressing T cells lysed ovarian cancer cell lines. We show that T cells from ovarian cancer patients that express chNKG2D secreted proinflammatory cytokines when cultured with autologous tumor cells. In addition, we show that chNKG2D T cells can be used therapeutically in a murine model of ovarian cancer. These data indicate that treatment with chNKG2D-expressing T cells is a potential immunotherapy for ovarian cancer. [Cancer Res 2007;67(10):5003–8]

Introduction

Ovarian cancer is the fifth leading cause of cancer death in women, and this carcinoma has the highest mortality rate of gynecologic malignancies. Current immunotherapy strategies for ovarian cancer include using tumor antigen vaccines, targeting regulatory T cells, and cytokine therapy (1). Clinical trials in which recombinant human IFNγ was given i.p. showed minimal toxicity and an increased survival in some ovarian cancer patients (2, 3). Treatment with tumor-specific T cells has been shown to be a promising antitumor therapy for melanoma (4). In addition, adoptive T-cell therapies include infusing patients with tumor antigen–specific T cells, such as HER-2/neu–specific T cells (5).

Natural killer (NK) cells recognize tumors without MHC restriction, and one of the NK cell–activating receptors is NKG2D (6). In humans, the NKG2D receptor is expressed by $CD8^+$ T cells, NK cells, NKT cells, and γδ T cells (7, 8). Although NKG2D provides a primary activation signal in NK cells, NKG2D in T cells associates with DAP10 and only provides costimulation for the T-cell receptor (9–11). The ligands for the human NKG2D receptor are MHC class I–related protein A (MICA), MICB, UL-16 binding proteins (ULBP) 1 to 3, and lymphocyte effector cell toxicity-activating ligand (Letal/ ULBP4/Raet1E; refs. 7, 12, 13). Mice also express the NKG2D receptor on NK cells, activated and memory $CD8^+$ T cells, and a subset of γδ T cells. Murine NKG2D ligands include retinoic acid early transcript-1 proteins (Rae1α-ε), a minor histocompatibility antigen (H-60), and mouse ULBP-like transcript 1 (Mult-1; refs. 7, 8). In humans, the ligands for NKG2D are commonly expressed on tumor cells, including ovarian carcinomas (14–18). NKG2D ligand mRNA is found in some normal tissues, but cell-surface protein expression is absent or limited (7, 13, 19). Thus, NKG2D ligands can potentially be used as tumor-specific targets with minimal cross-reaction with normal tissues.

In previous studies, we have described a chimeric NKG2D receptor (chNKG2D) that consists of a full-length NKG2D sequence fused to the cytoplasmic region of the CD3ζ chain (20, 21). When chNKG2D is expressed in T cells, this receptor activates T cells on engagement with NKG2D ligand–positive tumor cells. In this study, we investigated the expression of NKG2D ligands in human ovarian cancer and the function of chNKG2D T cells against human and murine ovarian cancers. These results indicate that chNKG2D-expressing T cells are able to generate potent antitumor responses to ovarian tumors and represent a promising therapeutic approach against ovarian cancer.

Materials and Methods

Mice. Six- to eight-week-old female C57BL/6 and C57BL/6 $Ly5.2^+$ mice were purchased from the National Cancer Institute (Frederick, MD). All animal work was done in the Dartmouth Medical School Animal Facility in accordance with institutional guidelines.

Tissues and cell lines. Patient ovarian ascites samples were obtained from Dartmouth Medical Center after surgery with informed patient consent for all samples obtained. Human ovarian samples were isolated from newly diagnosed stage III ovarian cancer patients. RBC in the ascites samples were lysed with ACK lysis buffer [0.15 mol/L NH_4Cl, 1 mmol/L $KHCO_3$, 0.1 mmol/L EDTA (pH 7.3)] before cell culture. Human ovarian cancer cell lines A2008, A2780, and SKOV3 were cultured in RPMI supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 1 mmol/L pyruvate, 10 mmol/L HEPES, 0.1 mmol/L non-essential amino acids, and 50 μmol/L 2-mercaptoethanol. ID8 and ID8-GFP cells were grown in DMEM supplemented with 4% heat-inactivated fetal bovine serum, 1× insulin, transferrin, and selenium (Sigma, I3146), and the same supplements as in RPMI.

Isolation and transduction of $CD8^+$ T cells from ascites. Human *wtNKG2D* and *chNKG2D* vectors were made as previously described (21).

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Human peripheral blood mononuclear cells (PBMC) were isolated from healthy donors and transduced as previously described (21). All procedures were approved by the Internal Review Board at Dartmouth College. $CD8^+$ cells were isolated from ascites samples by a magnetic cell sorting kit (Miltenyi Biotec, Inc.) according to the manufacturer's instruction. The $CD8^+$ T cells were then stimulated with phytohemagglutinin (1 μg/mL) for 3 days. Retroviral transduction of PBMCs was done as previously described (21). Two days after infection, transduced primary T cells were selected in complete RPMI medium containing G418 (0.5 mg/mL) plus 100 units/mL recombinant human interleukin (IL)-2 for an additional 3 days. Live cells were isolated using lymphocyte separation medium (density, 1.077; Mediatech) and were cultured for an additional 2 days in 100 units/mL recombinant human IL-2 before use.

Reverse transcription-PCR. Total RNA from mouse and human tumor cell lines and total human ascites samples was extracted as described (21). The primers used for amplification of the human sequences are shown in Supplementary Table S1 and primers used for amplification of mouse NKG2D ligands are shown in Supplementary Table S2. The PCR products were run on agarose gels and visualized by staining with ethidium bromide or SYBR Safe (Invitrogen).

Flow cytometry. For fluorescence-activated cell sorting analysis of NKG2D ligand expression, human tumor cells and ascites samples were incubated with human FcR blocking reagent (Cohn's fraction; Sigma), then stained with human NKG2D-mouse immunoglobulin G1 fusion protein, followed by staining with phycoerythrin-labeled rat anti-mouse IgG1 (A85-1, BD PharMingen). Similarly, ID8 cells were stained with mouse NKG2D-human IgG1 fusion protein (R&D Systems) with a FITC-labeled goat anti-human IgG secondary (Jackson ImmunoResearch). Allophycocyanin-anti-Rae-1 (186107, rat IgG2a) was obtained from R&D Systems. FITC-anti-CD45 (HI30, mouse IgG1) was purchased from Caltag. FITC-anti-CD8 (RPA-T8) and all isotype controls were obtained from eBiosciences. Cell fluorescence was monitored using a FACSCalibur cytometer (Becton Dickinson).

Cytokine production and cytotoxicity by gene-modified T cells. Culture of ovarian tumor cells (2.5×10^4) with gene-modified primary human or mouse T cells (10^5) was done in flat-bottomed 96-well plates. Tumor cells were irradiated (120 Gy) before use. Cell-free supernatants were collected after 24 and 72 h. Twenty-four-hour supernatants were assayed for IFNγ by ELISA using mouse and human Duoset ELISA kits (R&D Systems). Seventy-two-hour supernatants were used for detection of other cytokines and chemokines using Bio-Plex kits (Bio-Rad) based on the manufacturer's protocol. Bio-Plex analysis was done by the Immune Monitoring Laboratory of the Norris Cotton Cancer Center (Lebanon, NH). Lysis of target cells was determined by a 4-h ^{51}Cr release assay as previously described (20, 21). To block NKG2D receptors, T cells were preincubated at 37°C for 2 h with the anti-NKG2D antibodies (clone 1D11; 20 μg/mL, sodium azide–free) or isotype control antibodies before addition of target cells.

Treatment of mice with ID8-GFP cells and genetically modified T cells. To detect ID8 tumor cells, the green fluorescent protein (*gfp*) gene (Clontech) was inserted into a retroviral pFB-neo vector (Stratagene), and ID8-GFP cells were made by retroviral transduction of *gfp* into ID8 cells. Generation of retrovirus using packing cell line PT67 and retroviral transduction was done (20, 21). Mouse spleen cells were isolated and transduced as previously described (20). Female C57BL/6 mice were injected i.p. with 10^6 ID8-GFP cells in 500 μL of PBS. After 2 or 7 days, mice were treated i.p. with PBS, 5×10^6 *wtNKG2D*, or 5×10^6 *chNKG2D*-modified T cells in a volume of 500 μL. Eight weeks after the mice received the tumor injection, the mice were sacrificed and a peritoneal wash was done using 10-mL PBS. Solid tumors on the peritoneal lining were also counted. RBC in the peritoneal washes were lysed with ACK lysis buffer, the number of cells was counted, and percent GFP^+ cells was determined by flow cytometry. Absolute number of tumor cells in the peritoneal washes was determined by multiplying percent GFP^+ cells by the number of cells in the peritoneal wash.

Statistical analysis. Differences between groups were analyzed using Student's t test. $P < 0.05$ was considered significant.

Results

Human ovarian cancer cell lines and primary ovarian cancer samples express ligands for the NKG2D receptor. To determine whether human ovarian cancer cells express ligands for NKG2D, ovarian cancer cell lines A2780 and A2008 were stained with a soluble human NKG2D receptor-mouse IgG fusion protein (sNKG2D-Ig; Fig. 1*A*). All of the ovarian cancer cell lines tested expressed cell-surface NKG2D ligands. Human ovarian cancer ascites samples were also stained with sNKG2D-Ig to determine the extent that fresh ovarian cancer samples express NKG2D ligands on $CD45^-$ cells. We observed that amounts of ligand expression varied between samples as represented by four different patient samples (Fig. 1*B*). More than 80% of the patient samples (9 of 11) had cell-surface expression of NKG2D ligands. We observed no NKG2D ligand expression on the $CD45^+$ cells from the tumor samples (data not shown). In addition, reverse transcription-PCR (RT-PCR) was done to determine which NKG2D ligands were expressed by ovarian cancer cells. We analyzed two ovarian cell lines and five primary samples for expression of each NKG2D ligand (Table 1). All samples tested were positive for mRNA expression of at least one NKG2D ligand. Whereas a majority of ovarian cancer samples expressed MICA and MICB, the expression of the ULBPs varied, with only A2008 expressing ULBP2 and all ascites samples expressing low levels of ULBP3. We did not observe expression of Letal/ULBP4 in any of the primary tumor samples.

T cells expressing chNKG2D lyse ovarian cancer cell lines in an NKG2D-dependent manner. Because human ovarian cancer cells express the ligands for NKG2D, we investigated whether chNKG2D-expressing T cells lysed ovarian tumor cells. T cells from

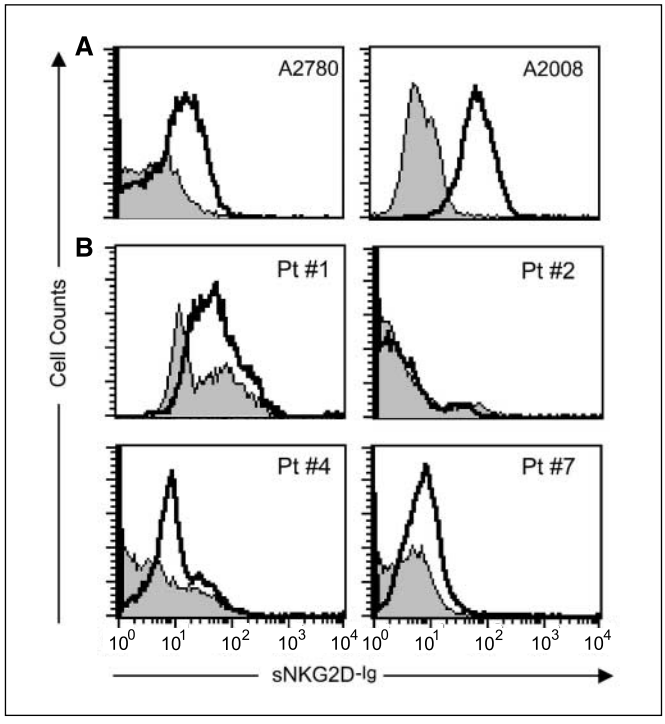

Figure 1. Surface expression of NKG2D ligands on human ovarian cancer cells. *A,* A2780 and A2008 cells were stained with a soluble human NKG2D-mouse IgG1 fusion protein (*open histogram*) or mouse IgG1 isotype control (*filled histogram*) and a phycoerythrin-antimouse IgG1 antisera. *B,* ovarian cancer ascites Pt #1, Pt #2, Pt #4, and Pt #7 were stained in a similar manner and gated on $CD45^-$ cells.

Table 1. NKG2D ligand expression in human ovarian cancer

Samples*	MICA	MICB	ULBP1	ULBP2	ULBP3	Letal/ ULBP4
A2008	+ +	+	+	+ +	+ +	+ +
A2780	+ + +	+	+ + +	−	−	+
Pt #2	+ +	−	−	−	+	−
Pt #3	+	+	−	−	+	−
Pt #4	+ + +	+ + +	+	−	+	−
Pt #5	+ +	+ +	−	−	+	−
Pt #6	+ + +	+	+	−	+	−

NOTE: Levels of expression were classified as follows: −, no expression; +, low expression; + +, medium expression; + + +, high expression.
*Patient samples are represented as Pt #. A2008 and A2780 are human ovarian cell lines.

healthy donors transduced with vector alone, wild-type NKG2D (*wtNKG2D*), or *chNKG2D* were used as effector cells in cytotoxicity assays with A2008 or A2780 cells as target cells (Fig. 2). For both ovarian cell lines, chNKG2D-expressing T cells had a greater cytotoxic activity than wtNKG2D- or vector-expressing T cells. We used primary T cells from three different donors and found specific lysis by chNKG2D T cells in all cases. To show that cytotoxicity was dependent on NKG2D, we tested whether blocking NKG2D reduced cytotoxic activity. As shown in Fig. 2*C* and *D*, when incubated with blocking NKG2D antibodies, chNKG2D T-cell cytotoxicity was reduced to background amounts against A2008 and A2780 tumor cells. These data are similar to data with other tumors in which cytotoxicity by chNKG2D T cells was dependent on NKG2D ligand expression and not due to MHC-peptide recognition by the T-cell receptor (21). Thus, T cells expressing chNKG2D kill ovarian cancer cells.

ChNKG2D T cells secrete proinflammatory cytokines when cultured with autologous tumor cells. In addition to killing NKG2D ligand–expressing tumor cells, we wanted to determine whether T cells isolated from ovarian cancer patients recognized autologous tumor cells when their T cells expressed chNKG2D receptors. $CD8^+$ T cells isolated from patient ascites samples were transduced to express wtNKG2D or chNKG2D receptor and cocultured with their primary ovarian cancer cells. ChNKG2D T cells originating from autologous tumor ascites or healthy donors produced IFNγ, granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNFα), and chemokines CCL3 and CCL5 (Fig. 3), but they did not secrete the anti-inflammatory cytokine IL-10. In contrast, T cells expressing wtNKG2D did not produce significant amounts of proinflammatory cytokines and chemokines when cultured with the ovarian tumor cells. T cells cultured alone did not spontaneously produce these cytokines (data not shown; ref. 21).

The murine ovarian cancer cell line ID8 expresses Rae-1, a murine NKG2D ligand. The murine ovarian cell line ID8 was originally derived from spontaneously transformed mouse ovarian surface epithelial cells (22). Injection of ID8 tumor cells i.p. is a robust mouse model of ovarian cancer and can form ascites and solid tumors in immunocompetent mice in a way similar to human ovarian cancer. ID8 cells expressed cell-surface NKG2D ligands, as determined by staining with a soluble murine NKG2D receptor-human IgG fusion protein (Fig. 4*A*). RT-PCR was done for each of the mouse NKG2D ligands, and we determined that ID8 cells expressed the NKG2D ligand Rae-1 but not other murine NKG2D ligands (Fig. 4*B*). Cell-surface protein expression of Rae-1 was confirmed by specific staining (Supplementary Fig. S1). Thus, like human ovarian tumor cells, ID8 cells express NKG2D ligands.

ChNKG2D-expressing T cells secrete IFNγ and lyse ID8 ovarian cancer cells. Because ID8 cells express the NKG2D ligand Rae-1, we predicted that mouse T cells expressing a murine version of the chNKG2D receptor would lyse ID8 cells. As shown in Fig. 4*C*, the chNKG2D-expressing T cells killed ID8 cells, whereas wtNKG2D-expressing T cells did not. In addition, when T cells were cocultured with irradiated ID8 cells for 24 h, the chNKG2D T cells secreted a large amount of IFNγ (>30 ng/mL) compared with wtNKG2D T cells (Fig. 4*D*).

Treatment with chNKG2D T cells reduces ID8 tumor growth *in vivo*. Having shown that chNKG2D T cells lyse ID8 cells and secrete high levels of IFNγ when cocultured with this tumor, we investigated the ability of chNKG2D T cells to therapeutically reduce tumor burden *in vivo*. ID8 cells (10^6) expressing GFP (ID8-GFP) were injected i.p. into mice.

Seven days later, the mice were treated with 5×10^6 wtNKG2D T cells or chNKG2D T cells. Eight weeks after tumor injection, the mice were sacrificed and the tumor burden was determined by quantitation of the GFP^+ cells in the peritoneal wash and the number of solid tumors in the peritoneum. ChNKG2D T cells were able to significantly reduce ID8 tumor burden, as shown by a

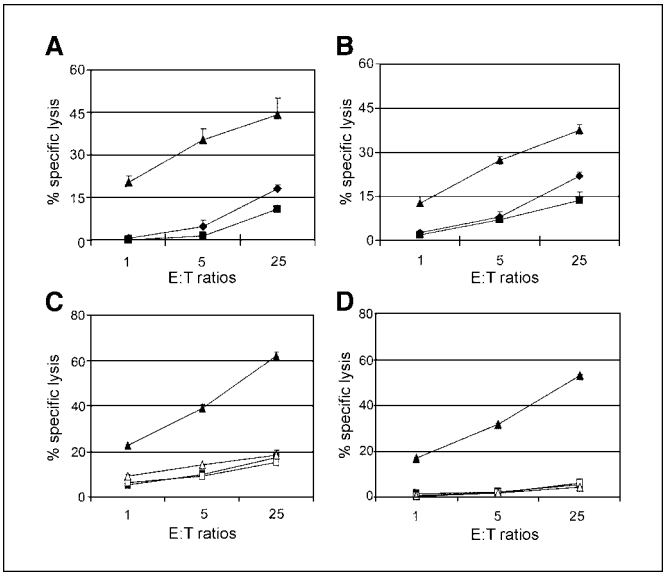

Figure 2. ChNKG2D-expressing T cells kill human ovarian cell lines in an NKG2D-dependent manner. Primary human T cells were transduced with vector only (*diamonds*), *wtNKG2D* (*squares*), or *chNKG2D* (*triangles*). These T cells were used as effector cells in a ^{51}Cr release assay against A2008 (*A* and *C*) or A2780 (*B* and *D*) cells. The ^{51}Cr-labeled tumor cells were cocultured with the effector cells at the indicated effector/target (*E:T*) ratios (1:1, 5:1, or 25:1). *ChNKG2D*-transduced T cells had higher specific lysis at all effector/target ratios compared with wtNKG2D- or vector-transduced T cells ($P < 0.05$). To show NKG2D dependence, wtNKG2D (*squares*) or chNKG2D (*triangles*) T cells were incubated with anti-NKG2D antibodies (*open symbols*) or with isotype control IgG antibodies (*closed symbols*) before incubation with tumor cells. Blocking NKG2D significantly reduced the cytotoxicity of *chNKG2D*-transduced T cells against tumor cells at all ratios compared with control ($P < 0.05$). *Points,* mean; *bars,* SD.

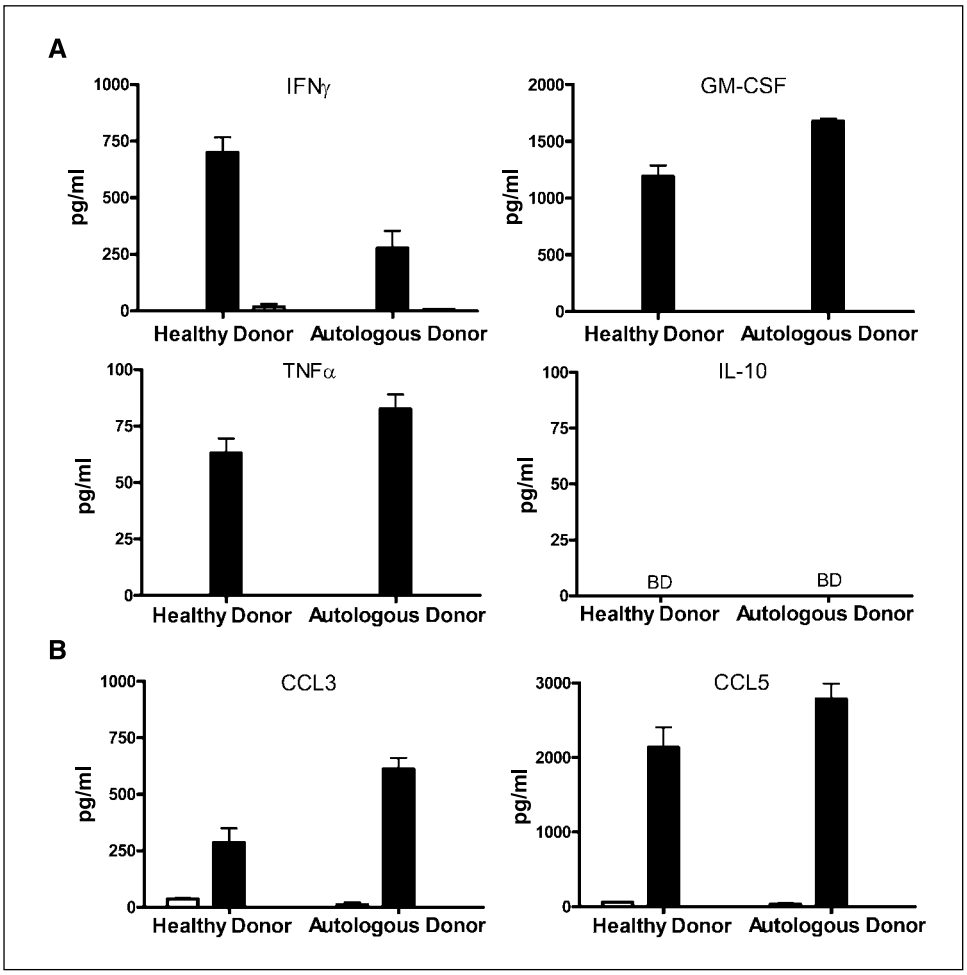

Figure 3. Nonautologous and autologous chNKG2D-modified T cells secrete proinflammatory cytokines during culture with primary ovarian cancer samples. T cells from a healthy donor or isolated from Pt #7 ovarian cancer ascites (*autologous donor*) were transduced with wtNKG2D (*open columns*) or chNKG2D (*filled columns*). These T cells were cultured with irradiated Pt #7 ovarian cancer cells. *Hashed columns,* tumor cells cultured alone. Cell-free supernatants were analyzed for cytokines (IFNγ, GM-CSF, TNFα, and IL-10; *A*) and chemokines (CCL5 and CCL3; *B*). *Columns,* mean; *bars,* SD. Representative of three different primary tumor samples cultured with T cells from a healthy or autologous donor. When cultured with tumor cells, chNKG2D T cells produced more cytokines than wtNKG2D T cells or tumor cells cultured alone ($P < 0.001$). *BD,* below detection level (4 pg/mL).

decrease in the percent of GFP^+ cells and number of solid tumors in the peritoneum (Fig. 5*A* and *B*). The difference in the absolute numbers of tumor cells in the peritoneal wash was similar: 17.29×10^4 ID8-GFP cells from wtNKG2D T-cell–treated mice and 0.52×10^4 ID8-GFP cells from chNKG2D T-cell–treated mice (data not shown). Therefore, treatment with chNKG2D T cells can be used therapeutically to reduce an established ovarian tumor burden. Mice treated with chNKG2D T cells at earlier time points (e.g., day +2) also showed tumor growth prevention (Supplementary Fig. S2). We have not observed a significant difference in tumor burdens between PBS-treated and wtNKG2D T-cell–treated mice.

As a model for T cells from ovarian cancer patients, we used T cells from tumor-bearing mice as a source of chNKG2D-bearing T cells. $Ly5.2^+$ mice were injected with ID8-GFP cells and treated with $Ly5.1^+$ wtNKG2D T cells. Eight weeks later, the spleens from the tumor-bearing mice were removed and the splenocytes were transduced with *chNKG2D*. The spleens from these mice did not contain $Ly5.1^+$ wtNKG2D T cells or tumor cells (data not shown). ID8-GFP cells were injected into naïve B6 mice, and the mice were given chNKG2D T cells generated from naïve mice or from tumor-bearing mice 1 week later (Fig. 5*C* and *D*). After 8 weeks, the chNKG2D T cells generated from a tumor-bearing host had similar efficiency at reducing the ID8-GFP tumor burden as chNKG2D T cells prepared from a naïve mouse, as shown by a similar reduction in the percent GFP^+ cells in the peritoneal wash and the number of solid tumors on the peritoneum. Mice treated with chNKG2D T cells did not seem to have any clinical signs of unwanted side effects due to chNKG2D T-cell treatment. Thus, it is possible to use autologous T cells from tumor-bearing mice as chNKG2D effector cells against ovarian cancer.

Discussion

This study shows a novel immunotherapy for ovarian cancer. More than 80% of human ovarian cancer ascites samples tested expressed NKG2D ligands on the cell surface. Previous studies have shown expression of various NKG2D ligands in human ovarian cell lines (12, 14, 16, 18). This study investigated the expression of NKG2D ligands in ovarian cancer samples, looking both at the cell-surface expression and at mRNA expression for individual NKG2D ligands. Our data show that all samples express mRNA for NKG2D ligands, but not all express NKG2D ligands on the cell surface. It is important to verify cell-surface expression because functional activity of chNKG2D T cells required expression on the cell surface. None of the primary ascites samples tested in this study expressed mRNA for Letal/ULBP4, as a previous study showed that >50% of stage III ovarian carcinomas express Letal (18). One possible reason for this discrepancy may be a difference in Letal expression in tumor cells from ascites examined in this study compared with solid tumors.

$CD8^+$ T cells isolated from human ovarian cancer ascites samples and transduced with the chNKG2D receptor secreted

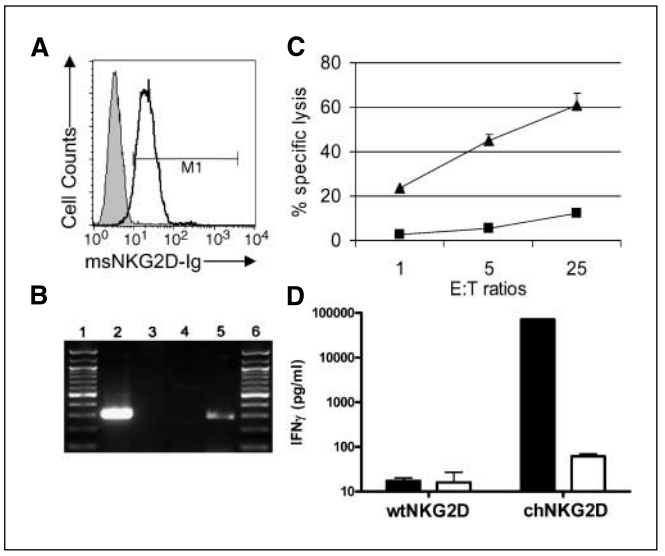

Figure 4. ID8 cells express Rae-1, and culture of ID8 cells with mouse chNKG2D T cells results in production of IFNγ and ID8 cell lysis. *A,* ID8 cells were stained with a soluble mouse NKG2D-human IgG1 fusion protein (*open histogram*) or human IgG1 isotype control (*filled histogram*). *B,* RT-PCR was done on cDNA from ID8 cells. Primers were used for β-actin (*lane 2*) or mouse NKG2D ligand H-60 (*lane 3*), Mult-1 (*lane 4*), or Rae-1 (*lane 5*). *Lanes 1* and *6* contain a 100-bp marker. *C,* mouse T cells transduced with wtNKG2D (■) or chNKG2D (▲) were used as effector cells with ^{51}Cr-labeled ID8 cells at the indicated effector/target ratios (1:1, 5:1, or 25:1). ChNKG2D-expressing T cells lysed ID8 cells better than wtNKG2D T cells ($P < 0.05$). *D,* wtNKG2D- or chNKG2D-expressing T cells were cultured with irradiated ID8 cells (*closed columns*) or in media alone (*open columns*). Data are shown on a log scale. After 24 h, chNKG2D T cells produced more IFNγ than wtNKG2D T cells when cultured with ID8 cells ($P < 0.001$). *Columns,* mean; *bars,* SD. Representative of five experiments.

proinflammatory cytokines when cultured with autologous tumor cells. Yet, the same CD8$^+$ T cells transduced with wtNKG2D did not produce any cytokines when cultured with autologous tumor cells. This may be due to immunosuppression at the tumor site inducing T-cell anergy, low frequency of antigen-specific T cells, or some other mechanism through which the tumor-associated T cells do not respond to autologous tumor cells. Similarly, T cells isolated from tumor-bearing mice were able to reduce the ID8 tumor burden at efficiency similar to effector cells taken from naïve mice. Thus, although tumor-specific T cells in a patient may be functionally suppressed, it is feasible that expression of chNKG2D receptors will provide a means for activation of the patient's T cells and recognition of the autologous tumor.

There are many advantages to using chNKG2D T cells as a therapy for ovarian cancer. Our previous studies have shown that T cells expressing chNKG2D receptor are able to both lyse and produce proinflammatory cytokines when cultured with NKG2D ligand–expressing tumors, and this killing is independent of MHC class I expression and T-cell receptor recognition but dependent on NKG2D ligand expression (20, 21). Because chNKG2D T cells are MHC class I independent, they will be able to recognize tumor cells even if tumors down-regulate expression of MHC class I, as has been shown in many cancers including primary ovarian cancer samples (23). It is difficult to obtain sufficient numbers of antigen-specific T cells to treat patients because the frequency of tumor-specific T cells can be low, and those T cells that are present in patients often have low affinities for tumor antigens. Treatment with chNKG2D T cells does not require the isolation and expansion of tumor-specific T cells from the patient because the chNKG2D receptor provides T cells a way to become activated to the tumor without requiring MHC-peptide antigen recognition on the tumor cell.

In addition, it has been shown that the CD3ζ chain of the T-cell receptor is often down-regulated in ovarian cancer, thus contributing to the lack of a host T-cell response to the tumor (24, 25). The chNKG2D receptor consists of the NKG2D receptor fused to the CD3ζ chain and, thus, tumor-induced down-regulation of CD3ζ in T cells may not effect chNKG2D expression and signaling. Tumor cells can shed a soluble form of MICA (sMICA), and this soluble ligand can lead to the down-regulation of NKG2D expression and the impairment of NK cell and CD8$^+$ T-cell functions (26–28).

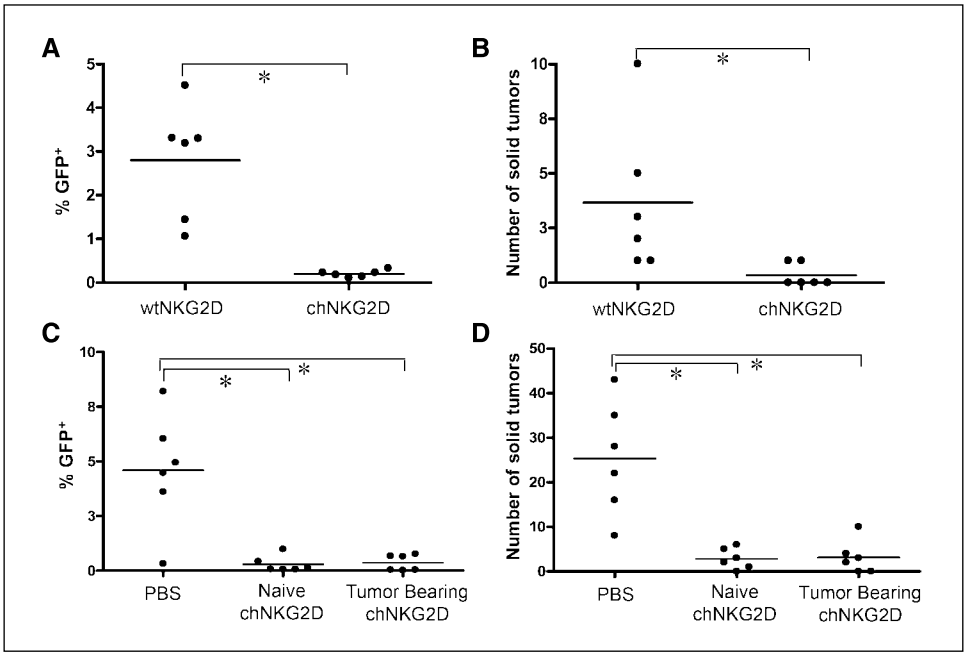

Figure 5. ChNKG2D-expressing T cells reduce ID8 tumor growth *in vivo*. Female C57BL/6 mice were injected with 10^6 ID8-GFP cells i.p. After 7 d, mice received a treatment of 5×10^6 wtNKG2D T cells or 5×10^6 chNKG2D T cells i.p. as indicated. Tumor burden was determined after 8 wk by measuring the percent GFP$^+$ cells (*A*) or the number of solid tumors on the peritoneum (*B*). *C,* seven days after tumor injection, mice were treated with chNKG2D T cells derived from naïve or ID8 tumor–bearing mice or with PBS alone. After 8 wk, percent GFP$^+$ cells in a peritoneal wash and number of solid tumors in the peritoneum (*D*) were counted. Each data point represents an individual, and the average of each group is shown. Representative of two or three separate experiments. $P < 0.05$, Student's *t* test.

However, we have previously shown that chNKG2D T cells are not inhibited when cultured with soluble MICA at concentrations reported in the sera of cancer patients (0.2–10 ng/mL). ChNKG2D T-cell activity was only inhibited at an amount greater than 1,500 ng/mL, an amount that is much higher than found in cancer patients (29, 30). Thus, treatment with chNKG2D T cells may be able to overcome many of the immune evasion strategies of ovarian cancer.

We have previously shown that mice treated with chNKG2D T cells that cleared a tumor challenge with RMA/Rae-1, an NKG2D ligand-positive lymphoma cell line, were subsequently resistant to a challenge with the wild-type RMA tumor cells. These data are consistent with the generation of host immunity to other tumor antigens (8, 20). IFNγ and GM-CSF may activate antigen-presenting cells and induce tumor antigen presentation, and CCL3 and CCL5 may increase inflammation at the tumor site. It has been shown that CCL5 levels correlate with the number of $CD3^+$ and $CD8^+$ T cells infiltrating ovarian cancer (31, 32). Therefore, chNKG2D T cells may not only directly kill the tumor cells but also secrete proinflammatory cytokines that induce a host antitumor immune response.

Patients with stage III and stage IV ovarian cancer have formation of ascites and solid tumors on the peritoneum. In the mouse ovarian cancer model, treatment with chNKG2D T cells was able to reduce the number of tumor cells in the ascites and solid tumors on the peritoneum. However, this could be due to chNKG2D T cells attacking the solid tumors or due to the ability of chNKG2D T cells to prevent solid tumor formation. Because ovarian cancer is confined within the peritoneal cavity, administering the T cells locally may not require extensive T-cell trafficking and reduced potential toxicity. It has been shown that activation of DNA damage repair pathways induces the expression of NKG2D ligands (33). Many chemotherapy drugs used to treat ovarian cancer induce DNA damage, such as carboplatin, and thus it is possible that chNKG2D T-cell therapy may synergize with current chemotherapy protocols in ovarian cancer.

In summary, this study shows that treatment with chNKG2D T cells is a novel, potential immunotherapy for ovarian cancer. The majority of ovarian cancer samples express the ligands for NKG2D, and treatment of ovarian tumor–bearing mice with chNKG2D T cells was able to significantly reduce the tumor burden. Finally, we show that T cells can be isolated from a tumor-bearing individual and these T cells can generate antitumor responses after transduction with the chNKG2D receptor. Thus, administration of chNKG2D T cells to ovarian cancer patients is a promising new immunotherapy.

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