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Primary Human Tumor Cells Expressing CD155 Impair Tumor Targeting by Down-Regulating DNAM-1 on NK Cells

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The activating NK cell receptor DNAX accessory molecule-1 (DNAM-1) contributes to tumor immune surveillance and plays a crucial role in NK cell-mediated recognition of several types of human tumors, including ovarian carcinoma. Here, we have analyzed the receptor repertoire and functional integrity of NK cells in peritoneal effusions from patients with ovarian carcinoma. Relative to autologous peripheral blood NK cells, tumor-associated NK cells expressed reduced levels of the DNAM-1, 2B4, and CD16 receptors and were hyporesponsive to HLA class I-deficient K562 cells and to coactivation via DNAM-1 and 2B4. Moreover, tumor-associated NK cells were also refractory to CD16 receptor stimulation, resulting in diminished Ab-dependent cellular cytotoxicity against autologous tumor cells. Coincubation of NK cells with ovarian carcinoma cells expressing the DNAM-1 ligand CD155 led to reduction of DNAM-1 expression. Therefore, NK cell-mediated rejection of ovarian carcinoma may be limited by perturbed DNAM-1 expression on tumor-associated NK cells induced by chronic ligand exposure. Thus, these data support the notion that tumor-induced alterations of activating NK cell receptor expression may hamper immune surveillance and promote tumor progression. 

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Natural killer cells are lymphocytes of the innate immune system that recognize and kill tumor cells without prior sensitization (1). Human NK cells can be divided into two main functional subsets based on the intensity of CD56 expression (2). Although there is a degree of functional overlap, CD56dim NK cells have a potent cytotoxic function, whereas perforinlowCD56bright NK cells are thought to have immunoregulatory properties (2–5). Recent data support the notion that CD56bright and CD56dim NK cells represent different stages of maturation, with CD56dim NK cells being the more differentiated cell type (6). 

NK cells are regulated by a balance of activating and inhibitory signals from cell surface receptors (7). The inhibitory signals are mediated mainly by HLA class I-binding receptors, including killer cell Ig-like receptors (KIRs),3 CD94/NKG2A, and leukocyte Ig-like receptor B1 (LILR-B1) (8). Activating signals are encoded by a wide array of receptors, including NKG2D, DNAX accessory molecule-1 (DNAM-1), natural cytotoxicity receptors (NKp30, NKp44, NKp46), CD94/NKG2C, and KIR with activating intra-cellular domains (7). Additionally, the low-affinity FcyRIIIA receptor CD16 mediates Ab-dependent cellular cytotoxicity (ADCC). Costimulatory receptors and adhesion molecules such as 2B4 and LFA-1 are also involved in the regulation of NK cell activity (7). Engagement of specific combinations of activating receptors on NK cells dictates qualitatively distinct responses and can lead to synergistic effects for activation of effector cell function (9, 10). 

The ability of NK cells to kill tumors in vitro has been taken as indirect evidence for their participation in tumor immune surveillance. However, more direct evidence for a role of NK cells in tumor immune surveillance is limited. In murine models, however, two groups have independently reported an increased risk of tumor development in mice lacking either the NKG2D or DNAM-1 receptor (11, 12). DNAM-1-deficient mice developed significantly more DNAM-1 ligand-expressing fibrosarcoma and papilloma tumors compared with wild-type mice in response to the chemical carcinogens methylcholanthrene and 7,12-dimethylbenz[a]anthracene (12). These results substantiate the notion of DNAM-1 playing an important role in immune surveillance of tumor development. 

The DNAM-1 receptor is involved in the induction phase of both T and NK cell activation (13). Poliovirus receptor (CD155) and Nectin-2 (CD112) have been identified as ligands for DNAM-1, with CD155 appearing to have a predominant role in inducing DNAM-1-dependent responses (14). CD155 is widely expressed on normal cells and overexpressed on many tumor types (14–21). We recently demonstrated that the DNAM-1/CD155 interaction is crucial for recognition and killing of freshly isolated human ovarian carcinoma cells by resting allogeneic NK cells (15). Since the ovarian carcinoma cells constitutively express CD155 in combination with reduced levels of HLA class I molecules (15, 22, 23), autologous NK cells could theoretically target...
CD155-MEDIATED LOSS OF DNAM-1 IMPAIRS TUMOR TARGETING

Flow cytometry

All cells were thawed, washed, and preincubated with 10 μg of human IgG per 10^6 cells for 30 min on ice to block Fc receptors before phenotyping. Ethidium monoazide bromide was added at a final concentration of 1 μg/ml, followed by incubation for 10 min on ice in the dark. Cells were then exposed to light on ice for 10 min followed by extensive washing. Where appropriate, cells were stained with biotin-conjugated primary anti-NKG2A mAb and washed intensively before streptavidin-Pacific Blue was added together with primary labeled mAbs followed by incubation for 15 min on ice. Cells were washed twice and immediately analyzed on a CyAn ADP LX 9 color flow cytometer (Dako). Isotype-matched control Abs were used at similar concentrations. The data were analyzed with FlowJo software (Tree Star).

Analysis of NK cell activation

NK cells were coincubated with target cells at a ratio of 1:1 in a final volume of 200 μl in 96-well plates at 37°C and 5% CO₂. After 1 h of coincubation, GolgiPlug was added. Before analysis, cells were stained with a dead cell marker and a combination of anti-CD3, anti-CD56, and anti-CD107a mAbs for 15 min on ice, followed by washing and permeabilization (BD Biosciences). Intracellular staining was performed with anti-IFN-γ and anti-TNF-α Abs before acquisition. P815 cells used in the reverse lysis assays were preincubated with agonistic Abs at a final concentration of 2.5 μg/ml. Abs used for ADCC were present in the assay at 10 μg/ml. Experiments involving redirected lysis or ADCC were run for 6 h. Experiments involving stimulation by PMA (200 μM) and ionomycin (1000 μg/ml) were stopped after 2 h of incubation.

Flow cytometry-based cytotoxicity assays

Fresh ovarian carcinoma cells were isolated and HLA class I was blocked by the anti-HLA class I mAbs A6-136 and 12B4 at the dilution 1/1 and 1/100, respectively (both mAbs were provided by Dr. D. Pende, Genoa, Italy). Purified overnight IL-2-activated NK cells were coincubated with target cells at a ratio of 10:1 in a final volume of 200 μl for 3 h at 37°C and 5% CO₂. At the end of the assay, tumor cells were stained with the anti-EP-CAM mAb on ice following Fc receptor blockade with IgG (1 μg/10^6 cells). Cells were shielded from light and incubated 10 min with 7-aminoactinomycin D (BD Biosciences) at room temperature before immediate acquisition on the CyAn instrument.

Analysis of DNAM-1 expression

Healthy donor-derived PBMCs were coincubated with target cells at a ratio of 10:1 as described above. Before analysis, cells were stained with a dead cell marker and a combination of anti-CD3, anti-CD14, anti-CD56, and anti-DNAM-1 mAbs for 15 min on ice. GolgiPlug and an anti-CD107a mAb were used for the analysis of DNAM-1 expression on activated cells as described for the CD107a assay above. The relative expression (MFI) was calculated as the mean fluorescence intensity of DNAM-1 on NK cells coincubated with target cells divided by the mean fluorescence intensity of DNAM-1 on NK cells incubated without target cells.

Statistics

Statistical analyses were performed with GraphPad Prism (GraphPad Software) using the Wilcoxon and the Mann-Whitney t tests for paired and unpaired groups, respectively, and one-way ANOVA with Dunn’s multiple comparison for multiple comparison analyses. Correlation analysis was performed using Spearman’s correlation test.

Results

Perturbed NK cell receptor repertoires and altered subset frequencies in peritoneal effusions of patients with ovarian carcinoma

We performed a high-resolution phenotypic analysis of NK cells from peritoneal effusions and peripheral blood of patients with ovarian carcinoma. Tumor-associated NK cells differed substantially from autologous NK cells in peripheral blood and NK cells from sex- and age-matched healthy controls. First, we observed an increased proportion of CD56bright NK cells constituting on average 32% of all NK cells in the peritoneal effusions, compared with ~10% in peripheral blood (Fig. 1, A and B) (28). Containing with CD16, NKG2A, KIRs, CD57, and LIRLR-B1 confirmed a classical phenotype of the CD56bright NK cells (Fig. 1A) (3). Second,
FIGURE 1. Increased proportion of CD56bright NK cells and perturbed expression of NK cell receptors on CD56dim NK cells in the tumor environment. A, Representative dot plot diagrams of PBLs from a healthy donor (HD; left), as well as of PBLs (middle) and autologous tumor-associated lymphocytes (TAL) in peritoneal effusion (right) from an ovarian carcinoma patient. The histograms show expression of markers used to define the two NK cell subsets. B, Proportion of CD56bright NK cells in total NK cells in PBLs from HDs (■; n = 6), PBL (▲; n = 11) and TAL in patients (▼; n = 11). C, Representative histograms for the expression of activating and costimulatory NK cell receptors on CD56dim NK cells in PBLs (dashed line) and peritoneal effusions (line) compared with isotype-matched control mAb (filled). D, Expression of activating and costimulatory NK cell receptors on CD56dim NK cells in PBLs from HDs (■; n = 6), PBL (▲; n = 11) and TAL (▼; n = 11) from patients. Lines indicate mean fluorescence intensity for each receptor. * p < 0.05; ** p < 0.01; *** p < 0.001.
tumor-associated NK cells displayed multiple alterations in the receptor repertoires of both the CD56<sup>dim</sup> and CD56<sup>bright</sup> NK cell subsets (Fig. 1, C and D, and supplemental Fig. 1, respectively). Analysis of the CD56<sup>dim</sup> NK cell subset revealed that expression of the activating receptor DNAM-1 was significantly lower on tumor-associated NK cells compared with NK cells isolated from peripheral blood of patients and healthy donors (Fig. 1D). Similarly, the coactivating receptor 2B4 (CD244) was relatively lower on tumor-associated NK cells (Fig. 1D). Furthermore, the CD16 receptor, primarily expressed on CD56<sup>dim</sup> NK cells, was significantly decreased on the tumor-associated CD56<sup>dim</sup> NK cells (Fig. 1D). In contrast, the expression of NKp46 and NKG2D was slightly higher on NK cells within the tumor environment, whereas the expression of NKp30 was unaltered compared with NK cells in peripheral blood (Fig. 1D). Analysis of the CD56<sup>bright</sup> NK cell subset revealed similar receptor alterations as for CD56<sup>dim</sup> NK cells (supplemental Fig. 1).

These results demonstrate that NK cells in the tumor environment display altered proportions of the CD56<sup>bright</sup> and CD56<sup>dim</sup> NK cell subsets compared with peripheral blood and perturbed expression of activating NK cell receptors, including significant down-modulation of DNAM-1, 2B4, and CD16.

Changes in 2B4 and NKG2D expression on CD56<sup>dim</sup> NK cells correlate with increased proportion of CD56<sup>bright</sup> NK cells in the peritoneal effusion

The variable degrees of receptor modification among patients prompted us to examine whether there was any correlation between the increased proportion of CD56<sup>bright</sup> NK cells and the observed down-regulation of DNAM-1, 2B4, and CD16. Interestingly, the increased relative proportion of CD56<sup>bright</sup> NK cells in the peritoneal effusion correlated to the loss of expression of the 2B4 receptor (p < 0.05). An opposite tendency was observed for the expression of NKG2D (p = 0.07), whereas no correlation was observed for none of the other receptors studied, including DNAM-1, NKp30, NKp46, and CD16 (Fig. 2). This observation indicates that the coordinated increase in CD56<sup>bright</sup> NK cells and changes in 2B4 and NKG2D are related and may depend on environmental factors. However, despite the fact that several cytokines, including IL-2, IL-10, and IFN-γ, were increased in peritoneal effusions compared with autologous plasma, exposure of peripheral blood-derived NK cells to peritoneal effusion from patients with high proportions of CD56<sup>bright</sup> NK cells did not mimic the observed changes in receptor expression (data not shown).

Importantly, neither of these observations could explain the reduced expression of the DNAM-1 receptor. Since this receptor is of fundamental importance for the recognition and killing of several human tumors, including ovarian carcinoma (15–17, 29), we next investigated the mechanism behind the modulation of DNAM-1 receptor expression in the tumor environment.

Loss of DNAM-1 expression upon interaction with CD155-expressing freshly isolated ovarian carcinoma cells

Receptor-ligand interactions have been shown to down-modulate surface expression of several NK cell receptors, including NKG2D (30, 31). Therefore, we studied if there was a correlation between the reduced levels of DNAM-1 expression on NK cells in peritoneal effusions with the expression of CD155 on autologous ovarian carcinoma cells. This analysis revealed a significant inverse correlation between the relative reduction of DNAM-1 expression on tumor-associated CD56<sup>dim</sup> NK cells and the expression of CD155 on carcinoma cells (Fig. 3A). To more directly assess the role of
CD155, we used a recently described system in which S2 insect cells are transfected with ligands of human NK cell receptors ligands (27). Coincubation of peripheral blood NK cells from healthy donors with S2 cells expressing CD155 induced specific down-modulation of DNAM-1 (Fig. 3B). Importantly, no reduction of DNAM-1 expression was observed upon interaction with S2 cells expressing the NKG2D ligand ULBP1 (Fig. 3B).

To investigate the consequences of receptor ligation in a more physiological setting, we used a panel of freshly isolated ovarian carcinoma cells and tumor cell lines, all expressing CD155 (supplemental Fig. 2). In agreement with our previous report, all tested tumor samples were negative for Nectin-2 (data not shown and Ref. 15). Coincubation of NK cells with the ovarian carcinoma cell lines CaOV-4 and Skov3 as well as the freshly isolated ovarian carcinoma cells resulted in a reduction of DNAM-1 expression (Fig. 3C). Similarly, DNAM-1 was significantly down-modulated upon coincubation with K562 cells that also express CD155. In contrast, coincubation with the NK cell-resistant P815 cell line and the NK-sensitive 721.221 cell line, both lacking CD155 expression, did not induce DNAM-1 down-modulation, regardless of the degree of NK cell activation (Fig. 3C and data not shown). Kinetic experiments demonstrated a gradual reduction of DNAM-1 expression, occurring over 12 h during coincubation with fresh tumor cells (Fig. 3D).

To assess the role of soluble factors in the modulation of DNAM-1 receptor expression, we next coincubated healthy donor-derived NK cells with target cells in the presence of a transwell membrane abrogating physical interactions between DNAM-1 and CD155. DNAM-1 expression remained intact when physical interactions were prevented, whereas reduced DNAM-1 expression was observed when target cells expressing CD155 were allowed to interact with the NK cells (Fig. 4A). Furthermore, maintaining NK cells in peritoneal effusions for 36 h did not affect surface expression of DNAM-1 ligands and/or cytokines in the down-modulation of this receptor within this time frame (Fig. 4B).

Hence, these data suggests that a physical interaction between CD155 and DNAM-1 is required to induce a loss of DNAM-1 receptor expression.

**Impaired activation of tumor-associated NK cells upon specific stimulation via the DNAM-1 receptor**

To explore the functional responsiveness of CD56dim tumor-associated NK cells, we monitored the cell surface expression of CD107a as a surrogate marker for degranulation (32). The overall responsiveness of patient-derived CD56dim NK cells following
expression on tumor-associated NK cells (Figs. 1D and 5, C and D). Costimulation via the NKG2D and NKP46 receptors, which trigger Ca\(^{2+}\) flux synergistically (10) but are consistently less efficient in triggering degranulation (our unpublished observation), was not significantly different between tumor-associated NK cells and peripheral blood NK cells, despite a slight up-regulation of these receptors on tumor-associated NK cells (Figs. 1D and 5, C and D) (10). Similar response patterns were observed for the CD56\(^{dim}\) NK cells when the production of IFN-\(\gamma\) and TNF-\(\alpha\) was assessed (supplemental Fig. 4).

Finally, we used a previously described flow cytometry-based cytotoxicity assay (15) to examine how dysregulation of NK receptors affected recognition of autologous freshly isolated ovarian carcinoma cells. To allow a direct comparison of allogeneic vs autologous NK cells and to assess the net signaling through activating receptors, the cytotoxicity experiments were performed in the presence of complete blockade of HLA class I. Tumor-associated NK cells displayed impaired killing of autologous carcinoma cells compared with both autologous and allogeneic peripheral blood-derived NK cells (Fig. 5E).

Taken together, these data demonstrate that tumor-associated NK cells were fully functional upon receptor-independent stimulation, but hypofunctional upon stimulation via specific or multiple receptors mediating natural cytotoxicity, reflecting the net sum of alterations in the receptor expression.

**Discussion**

DNAM-1 is an activating NK cell receptor recently demonstrated to play an important role in tumor immune surveillance. Here we describe that receptor engagement attenuates DNAM-1 expression on NK cells, leading to hyporesponsiveness. These results suggest that chronic receptor-ligand interactions may cause loss of DNAM-1 expression on NK cells in the tumor environment, thereby contributing to poor NK cell-mediated elimination of ovarian carcinoma cells and possibly also other tumors expressing DNAM-1 ligands.

The importance of DNAM-1 on both T cells and NK cells was recently highlighted in a DNAM-1 knockout mouse model (12, 34). Data revealed a central role for DNAM-1 in costimulation of CD8 T cells upon recognition of nonprofessional APCs (34). DNAM-1 was also shown to be critical in the recognition of tumor cell lines expressing DNAM-1 ligands. Additional evidence for
DNAM-1-dependent rejection of tumors was provided by increased formation of ligand-expressing fibrosarcomas and papillomas after treatment of mice with the carcinogens methylcholanthrene and 7,12-dimethylbenz[a]anthracene, respectively (12). These studies provide the first direct evidence for a central role for DNAM-1 in tumor immune surveillance.

DNAM-1 is important for NK cell-mediated recognition of several human tumors, including neuroblastoma, myeloma, and Ewing sarcoma (16, 17, 29, 35). We have previously shown that the DNAM-1/CD155 interaction is crucial for the recognition of freshly isolated ovarian carcinoma by allogeneic NK cells (15). Thus, loss of DNAM-1 expression on NK cells may explain the inability of patient-derived NK cells to kill autologous tumors (24).

Suppression of NK cell function in cancer patients has been associated with reduced expression of activating NK cell receptors, but the mechanisms for receptor modulations are not fully understood (36–38). Mechanisms such as shedding of ligands, chronic ligand exposure, and trogocytosis have been described (30, 31, 39, 40). Interestingly, CD96, a NK cell receptor that like DNAM-1 binds CD155, was shown to be down-regulated upon ligand engagement (41). Since ovarian carcinoma cells constitutively express CD155 (15), we speculated that similar mechanisms could be responsible for the down-modulation of DNAM-1 on tumor-associated NK cells. Indeed, we were able to demonstrate that peripheral blood NK cells lost DNAM-1 expression within hours of exposure to CD155-expressing targets. Down-modulation of
DNAM-1 was dependent on physical contact with target cells expressing CD155 since no change in DNAM-1 expression was observed when effectors and targets were separated in transwell experiments or when NK cells were exposed to peritoneal effusions. Interestingly, we found an inverse correlation between the expression of CD155 on ovarian carcinoma cells and the expression of DNAM-1 on autologous tumor-associated NK cells, supporting the notion that these events take place in vivo.

Receptor-ligand interactions occurring during target recognition could not explain the loss of 2B4 expression since the ligand for 2B4, CD48, is not expressed by ovarian carcinoma cells. Furthermore, such interactions could not explain the subtle but statistically significant increase in NKG2D and NKP46 on TA-NK cells. We observed here a significant overrepresentation of the CD56bright NK cell subset among NK cells in peritoneal effusions compared with peripheral blood. This finding is in agreement with a study by Belisle et al. (28), and has also been described in several other tissues, including secondary lymphoid organs and the uterus of pregnant women, as well as in some human diseases (42–45). Intriguingly, we found a clear correlation between the proportion of CD56bright cells in peritoneal effusions and the loss of 2B4 in this compartment. Furthermore, there was a tendency for higher NKG2D expression in patients with high relative frequencies of CD56bright cells. Importantly, DNAM-1 expression, here shown to be modulated by ligand engagement, was independent on the frequencies of CD56bright NK cells. Although the exact chain of events leading to altered proportions of CD56bright cells and the associated receptor modifications remain elusive, it seems likely that environmental factors play a role. Whether changes in CD56bright frequencies are caused by factors that simultaneously act on receptor expression or in fact are responsible for the modification of 2B4 and NKG2D expression should be important to assess in future studies. Selective recruitment of CD56bright NK cells and apoptosis of CD56dim NK cells are mechanisms that may yield an enriched population of CD56bright NK cells (46, 47). However, these mechanisms have not been shown to have a central role in ovarian carcinoma (28). Preferential proliferation of CD56bright NK cells due to expression of high-affinity cytokine receptors and increased sensitivity of CD56dim NK cells to oxidative stress are two possible mechanisms that may explain the altered CD56bright/CD56dim ratios in ovarian carcinoma (48–51). A relative contraction of the more cytotoxic CD56dim NK cell subset may limit the tumor killing capacity, facilitating immune evasion.

There is abundant evidence for a role of soluble factors as mediators of NK cell receptor regulation. MUC-16 (CA125), known to inhibit the cytotoxicity of NK cells, was recently shown to down-modulate the expression of CD16 on NK cells from ovarian carcinoma patients, although the role in regulating DNAM-1 expression was not investigated (28, 52). Furthermore, several reports have noted that cytokines can modulate the expression of natural cytotoxicity receptors and NKG2D (38, 53, 54). IL-2 and TNF-α have been shown to increase the expression of DNAM-1 in T cells, whereas TGF-β can counteract this effect (55). A recent publication also demonstrated a MIF-mediated transcriptional down-regulation of NKG2D in ovarian carcinoma (37). Although soluble factors may be involved in changing the relative proportions of CD56bright cells and in modulating expression of 2B4, NKG2D, and NKP46, it does not seem to play a role for the observed loss of DNAM-1. Indeed, culturing NK cells in the presence of patient-derived peritoneal effusions had no effect on DNAM-1 expression, and transwell experiments revealed a requirement for physical contact between NK cells and target cells for the loss of DNAM-1 expression.

Increasing knowledge of the molecular specificities in NK cell-mediated tumor recognition provides new possibilities for developing more effective immunotherapeutic interventions (56). For efficient tumor rejection, strategies to circumvent the immunomodulatory effects of tumor environments are likely needed in combination with adoptive transfer of NK cells. A concern raised by the present study is that sequential killing of multiple target cells (57) may be hampered through the loss of DNAM-1 expression following initial target cell contact. Repetitive adoptive transfer of DNAM-1-expressing NK cells may help to override the continuous down-regulation of DNAM-1 upon interaction with CD155-expressing tumor targets. Chimeric NKG2D receptors

**FIGURE 6.** Abolished ADCC by tumor-associated NK cells upon specific stimulation via the CD16 receptor. The expression of CD107a was measured on CD56dim NK cells isolated from PBL of healthy donors (PB-NK (HDs); n = 4; dashed bars) and ovarian carcinoma patients (PB-NK (Patients); n = 7; open bars) as well as from peritoneal effusions of patients (TA-NK (Patients); n = 10; filled bars). A, Representative FACS plots showing the CD107a expression after stimulation with P815 cells coated with agonistic CD16 mAb. B, CD107a expression after stimulation with P815 cells coated with agonistic CD16 mAb. IgG1 isotype control mAbs were used as negative control stimuli. C, CD107a expression on patient derived CD56dim NK cells (n = 6) after coincubation with trastuzumab-coated freshly isolated autologous ovarian carcinoma cells. The same target cells, coated with rituximab Ab targeting CD20 Ags, were used as negative control stimuli. Columns show mean of CD107a expression (%); bars, SD. *, p < 0.05; **, p < 0.01.
been shown to enhance tumor targeting by CTLs (58). Similar approaches based on effector cells that stably express chimeric DNAM-1 receptors could theoretically also enable effective tumor rejection by NK cells.

The efficacy of mAbs for the treatment of malignancies such as lymphoma and breast cancer is well established (59). Since metastatic ovarian carcinoma cells uniformly express the tumor Ag Her2/ neu, they could serve as targets for the humanized mAb trastuzumab (33). Apart from changes in DNAM-1 expression, our phenotypic analysis demonstrated a dramatic loss of CD16 expression, which severely impaired tumor-associated NK cell ADCC toward trastuzumab-coated fresh ovarian carcinoma cells. In contrast, autologous peripheral blood NK cells displayed robust activation upon cocultivation with trastuzumab-coated targets. It has previously been reported that the loss of the signal transducing molecules FcεRIα and CD3-ζ in tumor-associated lymphocytes of patients with ovarian carcinoma led to reduced expression of CD16 and depressed the proliferative response to CD16 stimulation (60). Moreover, monocyte-derived macrophages from both peripheral blood and peritoneal effusions of ovarian cancer patients had less ADCC activity than did the corresponding cells from normal donors (61). These results indicate that targeting of metastatic ovarian carcinoma with mAbs, via NK cell-mediated ADCC, may be less effective than anticipated.

In conclusion, we have demonstrated that the loss of DNAM-1 expression on tumor-associated NK cells results in impaired NK cell activation and that loss of CD16 abrogates the killing of trastuzumab-coated autologous ovarian carcinoma cells. Moreover, we provide evidence for the contribution of DNAM-1 to ADCC interactions to the reduction of DNAM-1 expression, suggesting that chronic receptor-ligand interactions in the tumor environment may induce loss of DNAM-1 on tumor-associated NK cells. These results may have implications for the design of future protocols of adoptive NK cell- and Ab-based immunotherapies for ovarian carcinoma and possibly other human tumors.

Disclosures

The authors have no financial conflicts of interest.

References