T Cells Gene-engineered with DAP12 Mediate Effector Function in an NKG2D-dependent and Major Histocompatibility Complex-independent Manner*

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NKG2D is an important activating/co-stimulatory receptor harnessed by NK and T cells in immune surveillance. In contrast to NK cells, T cells fail to express the activation-signaling molecule DAP12 even when activated and, therefore, ligation of NKG2D alone is insufficient to induce T cell cytolytic function. To test whether we could endow T cells with NK cell-like effector function, we have engineered DAP12 into T cells by retroviral transduction (T-DAP12). T-DAP12 cells were demonstrated to specifically secrete interferon-γ following receptor ligation and to mediate potent and specific lysis of the NKG2D ligand (NKG2D-L) (Rae-1β) expressing MHC class I-deficient and class I-sufficient tumors. To circumvent the inability of T-DAP12 cells to proliferate following NKG2D ligation by Rae-1β expressing tumors, DAP12 was engineered into OT-1 cells with an endogenous T cell receptor specific for chicken ovalbumin peptide (amino acids 257–264). Importantly, following a period of proliferation through endogenous T cell receptor ligation, OT-1-DAP12 cells retained specificity against NKG2D-L expressing major histocompatibility complex class I-deficient tumor. In adoptive transfer experiments, T-DAP12 cells enhanced the survival of NK cell-depleted RAG-1-deficient mice inoculated with RMA-S-Rae-1β but not parental RMA-S tumors. Overall, this study demonstrated the significant potential of suppressing tumors and other cellular targets expressing NKG2D-L by endowing T cells with innate NK cell-like function.

NKG2D is an activating cell surface receptor expressed on a wide range of effector cells including NK, NKT, γδT, and CD8+ αβ T cells (1, 2). NKG2D recognizes several families of ligands expressed on interacting stressed, transformed, or pathogen-infected cells. These NKG2D ligands (NKG2D-Ls) are distantly related to MHC class I molecules, including the MHC class I chain-related proteins A and B (MICa and MICb) encoded in the human MHC, and a diverse family of proteins present in both mice and humans, including mouse Rae1 (retinoic acid early transcript 1), H60, Multi1 (murine UL16-binding protein-like transcript 1), and the human UL16-binding proteins or RAET1 proteins. The NKG2D ligands are generally expressed poorly by normal adult cells but are abundant in transformed epithelial cells and thus represent a potentially effective tumor-associated antigen (3, 4). Like many leukocyte activating receptors, NKG2D only signals when co-expressed and associated non-covalently with membrane-bound signaling adaptor proteins. Two adaptor proteins known to associate with NKG2D are DAP10 and DAP12 (5–7).

NKG2D is known to confer either co-stimulatory or activatory signals in different lymphocyte subsets depending upon the expression of two NKG2D protein species encoded by distinct mRNA splice isoforms and the differential expression of the DAP10 and DAP12 adaptor proteins (2). The long isoform of NKG2D, NKG2D-L, associates only with DAP10, and ligation of this receptor complex is thought to provide co-stimulatory function because of the presence of an YXXM motif, which delivers signals by activation of a lipid kinase pathway (5, 8). By contrast, the short isoform of NKG2D, NKG2D-S, may associate with either DAP10 or DAP12. Ligation of the NKG2D-S/DAP12 complex directly activates cytokine production and cytotoxicity, because DAP12 contains intracytoplasmic immunoreceptor tyrosine-based activation motifs that activate the Syk-family protein tyrosine kinases (5). Both isoforms of NKG2D are present in activated NK cells and CD8+ T cells, but T cells do not express DAP12 (2). Hence, in conventional TCRαβ CD8+ T cells NKG2D-DAP10 provides a co-stimulatory signal in combination with TCR triggering, whereas NKG2D ligation may result in the direct activation of NK cells. Notably, NKG2D only provides a co-stimulatory signal to CD8+ T cells under restricted conditions (9).

CD8+ αβ T cells are activated and proliferate through interaction of their endogenously rearranged TCR with the variety of MHC-I peptide complexes displayed on target cells (host, infected, or tumor cells). T cells possess a powerful ability to proliferate strongly in response to antigens when compared with NK cells whose proliferation is mostly cytokine-driven (10). T cells are optimally activated by the provision of signal 1 from the TCR and signal 2 from co-stimulatory molecules (11). Tumors rarely express co-stimulatory molecules such as CD80/CD86, and few conventional T cells specific for tumor antigen can generally be found infiltrating the tumor microenvironment. Furthermore, although tumors may often express NKG2D ligands, most T cells even when

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6 The abbreviations used are: NKG2D-L, NKG2D ligand; IFN-γ, interferon γ; IL, interleukin; mAb, monoclonal antibody; MHC, major histocompatibility complex; OT-1-DAP12, OT-1 cells expressing DAP12; OT-1α-MOV, OT-1 cells expressing scFv-anti-MOV-γ; PE, phosphatidylethanolamine; pfp, perforin; scFv, single chain fragment of variable regions; TCR, T cell receptor; T-DAP12, T lymphocytes transduced with DAP12; Tα-erbB2, T lymphocytes transduced with scFv-anti-erbB2-CD28-ζ; WT, wild-type.
engaged fail to express DAP12 and, therefore, ligation of NKG2D alone is insufficient to stimulate T cell proliferation or effector function in response to tumor.

We rationalized that such activated T cells might be able to respond directly to tumors by NKG2D ligation if they additionally expressed the DAP12 adaptor protein. Herein, we have demonstrated that genetic engineering of T cells with DAP12 enabled NKG2D-dependent recognition and effector function against tumor cells independently of the TCR or tumor MHC class I expression. The bi-potential of such DAP12 engineered T cells was evidenced by their ability to retain NKG2D ligand specificity following TCR-mediated expansion. Furthermore, these DAP12-expressing T cells mediated significant enhanced survival of mice inoculated with MHC class I-deficient tumors expressing NKG2D-L.

EXPERIMENTAL PROCEDURES

Mice—Inbred wild-type (WT) C57BL/6 (B6) and B6 OT-I mice were purchased from the Walter and Eliza Hall Institute of Medical Research (Melbourne, Australia). B6 RAG-1−/− mice were purchased from the Animal Resources Centre (Perth, Australia). B6 perforin-deficient (B6 pfp−/−) and B6 gld (Fasl mutant) mice were bred at the Peter MacCallum Cancer Centre (East Melbourne, Australia). Mice that were 8–12 weeks of age were used in all experiments, which were performed according to Animal Experimental Ethics Committee guidelines.

Cell Culture—RMA and RMA-S are T cell lymphomas derived from the Rauscher murine leukemia virus-induced RBL-5 cell line (from B6 mice) (12). RMA-S-Rae1β and RMA-Rae1β tumor cells were prepared as described elsewhere (4) and analyzed by flow cytometry using PE-labeled mNKG2D tetramer as described previously (13).

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Amplification and Cloning of Mouse DAP12 and Retroviral Transduction of Primary Mouse T Lymphocytes—DAP12 was amplified from cDNA of activated spleens from B6 mice. A c-Myc tag epitope (for detection purposes) was inserted between its leader and the extracellular domain using splice overlap extension PCR and the primers 5′-GACTACGTCACACTGAGGGGCTTGAGGCC-3′ (sense) and 5′-ATTCCAGTTCTTCCTTGATGAGTTTCTGCTGTGCCTGTACGGGACTTAATC-3′ (antisense) and 5′-GAAGGGCAGACAAACACTACCTCAGAACAGGACTCGATCAGATGCACACTTACCAAG-3′ (sense) and 5′-GACCTGAAGTCATCCTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG
cells were also demonstrated to specifically kill the highly metastatic B16-Rae-1β tumor, but not B16 parental tumor cells (data not shown).

To investigate whether the mechanism of cytotoxicity employed by our engineered T-DAP12 cells was mediated via perforin, cytotoxicity assays were carried out using either perforin-deficient pfp−/− T-DAP12 effector cells or WT T-DAP12 cells in the presence of EGTA. The use of pfp−/− T-DAP12 effector cells or the blockade of Ca2+ by EGTA resulted in complete abrogation of RMA-Rae-1β and RMA-S-Rae-1β tumor cell cytotoxicity, suggesting that T-DAP12 cells were rapidly killing targets in a perforin-dependent manner (Fig. 2, C and D). To investigate whether the FasL/Fas pathway was also playing a role, we performed another 16-h cytotoxicity assay using FasL-sensitive, MHC class I-sufficient Lewis lung carcinoma 3LL cells as a tumor target. Wild-type T-DAP12 cells specifically killed 3LL tumor cells, but this tumor target was insensitive to death mediated by pfp−/− T-DAP12 cells, suggesting that the FasL/Fas pathway was not involved (Fig. 2E). These data were further supported by the WT levels of cytotoxicity of FasL mutant gld-derived T-DAP-12 cells against 3LL tumor targets (data not shown). As an additional control for NKG2D specificity, an anti-NKG2D blocking antibody (C7), but not a control hamster Ig (data not shown), abolished T-DAP12-specific killing of RMA-Rae-1β and RMA-S-Rae-1β tumor targets (Fig. 2, C–E). Collectively these results demonstrated that primary mouse T cells genetically engineered with DAP12 were able to mediate specific perforin-dependent killing of tumor cells through an NKG2D-NKG2D-L interaction. The complete absence of cytotoxicity in the absence of the NKG2D-NKG2D-L pathway suggested that DAP12 association with any other receptors (e.g. Ig-like) was of no functional consequence in this assay.

Given that T-DAP12 cells mediated specific lysis of NKG2D-L-expressing tumor cells, we were further interested in determining what other effector functions DAP12 might trigger in mouse T cells after NKG2D ligation alone. NK cells are reported to secrete IFN-γ after NKG2D ligation (13). Hence, we examined the ability of T-DAP12 cells to secrete cytokines upon NKG2D-L interaction or cross-linking with a plate-bound c-Myc tag mAb. T-DAP12 cells demonstrated a specific low level production of IFN-γ following co-culture with RMA-S-Rae-1β tumor cells, but not parental RMA-S cells (Fig. 3A). By contrast, control T-α-erbB2 cells did not produce IFN-γ when incubated with RMA-S-Rae-1β tumor cells. Interestingly, cross-linking by plate-bound c-Myc tag mAb triggered optimal T cell IFN-γ production from both T-α-erbB2 and T-DAP12 cells that expressed surface receptors containing c-Myc tag epitope (Fig. 3A). This result is consistent with other reports that have demonstrated greater cytokine release by gene-engineered T cells following stimulation with an immobilized antibody as compared with antigen-specific activation by tumor cells (25).

The ability of T-DAP12 cells to proliferate was examined in a 3-day [3H]thymidine assay following stimulation with immobilized c-Myc tag mAb or irradiated RMA-S-Rae-1β tumor cells. T-DAP12 cells demonstrated strong proliferation following cross-linking by plate-bound c-Myc tag mAb but, interestingly, not against RMA-S-Rae-1β or RMA-S parental tumor cells (Fig. 3B). Similarly, T-α-erbB2 control T cells demonstrated comparatively strong proliferation following stimulation through plate-bound c-Myc tag mAb, but not following co-culture with RMA-S-Rae-1β or RMA-S parental cells. The effect of c-Myc tag ligation in stimulating CD8+ T cell IFN-γ production and proliferation was in concert with an earlier report of DAP12 transgenic mouse CD8+ T cells producing IFN-γ and proliferating in response to ligation with plate-bound anti-NKG2D mAb (2). Thus DAP12 can endow CD8+ T cells with the ability to kill, produce IFN-γ, and proliferate directly in response to NKG2D ligation. However our data extend these findings by

**RESULTS**

The expression of DAP12 in primary mouse T cells was achieved by retroviral transduction. Using a mAb specific for the c-Myc tag that was incorporated into the transgene, high level expression of both DAP12 (T-DAP12) (Fig. 1A) and the control scFv receptor T-α-erbB2 (Fig. 1B) were reproducibly detected in T cells. The transduced T cell populations consisted of a high proportion of CD8+ T cells (80–85%) and a low number of CD4+ T cells (10–15%) as reported previously (14, 15). Importantly, equivalent expression of the NKG2D receptor was detected on both T-DAP12 (Fig. 1C) and T-α-erbB2 cells (Fig. 1D) following retroviral transduction.

It has been previously reported (13, 22–24) that specific ligation of the NKG2D-DAP12 complex in NK cells results in their ability to kill NKG2D ligand-expressing tumor cells. To determine whether ligation of NKG2D-DAP12 conferred a similar cytotoxic function on our retrovirus-transduced T cells, we assessed the ability of T-DAP12 cells to specifically kill an MHC class I-sufficient RMA-Rae1β tumor (Fig. 2A) and a MHC class I-deficient RMA-S-Rae1β tumor (Fig. 2B) targets in a 16 h 51Cr assay. T-DAP12 cells demonstrated significant and specific cytotoxicity of both RMA-Rae-1β and RMA-S-Rae-1β tumor cells, but not parental NKG2D-L negative RMA or RMA-S tumor cells (Fig. 2, A and B). In contrast, similarly activated and transduced control T-α-erbB2 cells mediated only background cytotoxicity of all tumor targets (Fig. 2, A and B). Specificity was maintained even at much higher effector/target ratios (e.g. 40:1). Similar experiments using a 4-h assay yielded weaker levels of specific cytotoxicity by T-DAP12 cells (data not shown). Because T-DAP12 effector cells required a long incubation period to mediate optimal cytotoxicity, we first pre-activated these cells using plate-bound c-Myc tag mAb in an overnight culture before examining their cytotoxic function in a 4-h 51Cr assay. Under these conditions, pre-activated T-DAP12 cells mediated even greater and more rapid cytotoxicity of both RMA-Rae-1β and RMA-S-Rae-1β tumors, but not RMA or RMA-S parental tumors (Fig. 2, C and D). As demonstrated previously, pre-activated T-α-erbB2 cells exhibited no cytotoxicity of Rae-1β expressing tumor targets (data not shown). T-DAP12
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illustrating that when the NKG2D-L is expressed in the context of a tumor cell, IFN-γ production and, even more strikingly, the proliferation mediated via NKG2D are abrogated. We have observed that RMA/S-Rae-1β and other tumor cell lines secrete factors that inhibit T cell proliferation, and, indeed, supernatants from RMA-S-Rae-1β cells were capable of inhibiting proliferation following direct DAP12 activation via a c-Myc tag (data not shown). Collectively, it appeared that Rae-1β expressed by MHC class I-deficient tumor cells was more effective in triggering cytotoxicity than either IFN-γ production or proliferation.

Because T-DAP12 cells did not proliferate effectively against RMA/S-Rae-1β tumor cells, it was important to establish whether such tumor-specific effector cells could be expanded via conventional TCR ligation and, perhaps, distant from the tumor site. To test this approach, we engineered T cells from OT-1 TCR (reactive with the ovalbumin peptide, amino acids 257–264) transgenic mice with DAP12 using the same transduction procedure demonstrated earlier for T cells from B6 mice. Importantly, the expression of both DAP12 and NKG2D in OT-1 T cells was comparable with that in similarly engineered WT B6 T cells (data not shown). Following retroviral transduction, the cytotoxic function of OT-1 cells expressing DAP12 (OT-1-DAP12) or control scFv-α-

![Figure 2](image-url)

**FIGURE 2.** Specific lysis of Rae-1β expressing tumors by primary mouse T cells expressing DAP12. A and B, cytolytic function of transduced T cells was evaluated in a 16-h 51Cr release assay. T-DAP12 cells (closed squares) specifically lysed RMA-Rae-1β (A) and RMA-S-Rae-1β (B), but not parental tumor cells RMA or RMA-S. C–E, control T cells (expressing the scFv-α-erbB2-CD28-ζ chimeric receptor) mediated background killing of all tumors. Engineered T-DAP12 cells that were stimulated overnight on plate-bound c-Myc tag mAb (1 µg/ml) were used in a 4-h (C and D) or a 16-h 51Cr release assay (E). The addition of EGTA (5 mM) or the use of perforin-deficient T-DAP12 (pfp−/−) cells abrogated specific killing of RMA-Rae-1β (C) and RMA-S-Rae-1β (D) tumors, indicating that the cytotoxic effector function employed by T-DAP12 cells was perforin-dependent. This finding was further confirmed by the lack of killing of 3LL cells by T-DAP12 (pfp−/−) cells in the absence or presence of the NKG2D blocking antibody C7 (D). Lysis of RMA-Rae-1β and RMA-S-Rae-1β was mediated through NKG2D-ligand interaction as the addition of a blocking antibody C7 (100 µg/ml) abrogated tumor lysis (C–E). Results are expressed as means ± S.E. of triplicate samples and are representative of at least two experiments.
MOV-γ chimeric receptor (OT-1-α-MOV) was tested in a 16-h 51Cr assay. Similar to B6 WT T-DAP12 cells, OT-I-DAP12 cells specifically killed RMA-S-Rae-1β, but not RMA-S, tumor cells (Fig. 4A). Only background cytotoxicity was observed with control OT-1-α-MOV cells against RMA-S or RMA-S-Rae-1β tumor targets (Fig. 4A). We next assessed whether OT-1-DAP12 cells could maintain DAP12 expression (through the detection of c-Myc tag epitope) as well as the specific killing of NKG2D-L expressing tumor cells following a 6-day expansion period with irradiated ovalbumin (257SIINFEKL264)-pulsed splenocytes. Transduced OT-1-DAP12 or OT-1-α-MOV cells labeled with carboxyfluorescein succinimidyl ester were found to have undergone at least five rounds of cell division (data not shown). After a period of expansion, stimulated OT-1-DAP12 cells retained their ability to specifically kill RMA-S-Rae-1β targets and SIINFEKL-pulsed RMA-S targets, but not unpulsed RMA-S target cells (Fig. 4B). However, stimulated OT-1-α-MOV cells demonstrated only specific lysis of SIINFEKL-pulsed RMA-S targets, but not RMA-S or RMA-S-Rae-1β tumor cells (Fig. 4C). These results demonstrated that DAP12-engineered OT-1-T cells maintained DAP12 expression and specific lysis of NKGR2D-L-expressing tumor cells even following expansion via their specific TCR.

The strong effector function exhibited by T-DAP12 cells against NKGR2D-L-expressing tumors in vitro encouraged us to examine their anti-tumor potential in vivo. We have previously shown that RMA-S-Rae-1β tumor cells are exclusively rejected by NK cells and that growth of this tumor is equivalent between B6 WT and B6 RAG-1−/− mice (26). Therefore we examined the intraperitoneal ascites growth of RMA-S and RMA-S-Rae-1β tumors in B6-RAG−1−/− mice that were additionally depleted of NK cells. Mice were given a lethal dose of RMA-S-Rae-1β tumor (2 × 10^5 cells) and then received either T-DAP12 or
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T-DAP12 effectors could be expanded through their specific TCR without loss of DAP12 expression or NKG2D-L specificity. Furthermore, T cell- and NK cell-deficient mice challenged with NKG2D-L-expressing tumors demonstrated enhanced survival following adoptive transfer of T-DAP12 cells. Collectively, these experiments demonstrate the principle that DAP12-engineered T cells, like naturally occurring NK cells, can respond to a single signal mediated by NKG2D ligation. Their tight specificity of action and ability to respond concomitantly via their TCR offer the possibility of expanding T cells capable of innate anti-tumor activity in vivo.

Our data are in concert with a previous report (2) that demonstrated that DAP12 transgenic CD8+ T cells could respond directly via NKG2D to produce IFN-γ production and proliferate. However, we also demonstrated that tumors expressing NKG2D-L could suppress the proliferation, but not cytotoxicity, of responding T-DAP12 cells. The lack of proliferation of T-DAP12 cells following NKG2D-L interaction could explain the failure of T-DAP12 cells to cure all mice (Fig. 5) and the only minor prolongation in survival when T-DAP12 effector cells were administered 3 days after tumor inoculation. However, T-DAP12 cells were able to significantly enhance the survival of RAG1-/- mice challenged with RMA-S-Rae-1B tumors as compared with mice treated with control T cells or those that had received no T cell transfer. We consider this effect impressive, given that it was as protective as the natural host NK cell response and considering that the host contains many more than 2 million NK cells that can potentially respond to the tumor. To improve the anti-tumor efficacy of T-DAP12 cells and circumvent the inability of these cells to proliferate following interaction with NKG2D-L on tumor cells, we tested the idea of engineering DAP12 into T cells with a known TCR specificity. Encouragingly, DAP12 expression could still be detected (using c-Myc mAb) on transgenic OT-1-DAP12 T cells expanded for 6 days via their endogenous TCR (data not shown) while being able to specifically kill NKG2D-L-expressing tumor targets. An analogous situation in humans might be the genetic engineering and expansion of allogeneic, Epstein-Barr virus-specific, or human cytomegalovirus-specific T cells by immunization. Thus, dual specific T-DAP12 cells could potentially prove to be efficient in the eradication of NKG2D-L tumors if they can be expanded in vivo by immunization that triggers their TCR at a distance from the tumor sites. Future experiments will now involve extensively testing the anti-tumor efficacy of OT-1-DAP12 T cells in RMA-S-Rae-1B-challenged mice following immunization with the ovalbumin peptide. It will also be important to test this approach in mice with an intact immune system where the natural host response to tumors expressing NKG2D-L is ineffective.

Although we and others have genetically engineered DAP12 in mouse T cells, it is quite possible that some subsets of mouse T cells with “innate” activity may naturally exist or that DAP12 may under certain microenvironmental conditions be induced in T cell subpopulations. Good examples are two recent reports by Dhanji et al. (27, 28) describing the existence of a subset of CD8+ T cells that are characterized by high expression levels of CD44 in normal C57BL/6 mice (27, 28). These CD8+CD44hi cells could be directly activated following a period of culture in high dose IL-2 compared with conventional CD8+CD44lo T cells. Activated CD8+CD44hi cells demonstrated a preference in the killing of syngeneic tumor cells, and this killing of syngeneic tumor cells was greatly enhanced by the expression of NKG2D ligands on the target cell. Interestingly, the authors found that activated CD8+CD44hi cells expressed both DAP12 and DAP10 mRNA transcripts unlike the activated CD8+CD44lo T cells that only expressed DAP10, making CD8+CD44hi cells similar to NK cells in this regard. In concert, we...
showed that the DAP12 gene-transduced CD8⁺ T cells were able to mediate specific killing of NKG2D-L-expressing tumors and respond additively to signals via the TCR and NKG2D in IFN-γ production assays (data not shown). However, our experiments illustrate that conventional CD8⁺ T cells can also acquire similar functions as those of NK cells when transduced with DAP12, further suggesting that DAP12 was the critical adaptor molecule for conferring innate effector function. The existence of CD8⁺/CD44hi cells in normal mice has also been documented by others (29), and these cells may be an early source of IFN-γ in innate immune responses (30). The potential role of DAP12 in γδ⁺ T cells and additional populations of T cells with non-MHC related activities deserves further attention.

A recent elegant report (31) described the inability of human DAP12 to interact with human NKG2D and showed that human DAP10 was sufficient for human NKG2D signal transduction. Structural differences in the transmembrane of mouse and human NKG2D account for the species-specific difference for this immune receptor. Nevertheless, genetic engineering strategies that enable human NKG2D to associate with a chimeric form of human DAP12 should allow our approach of endowing human T cells with NK cell-like function. For example, the chimeric human DAP12 may consist of the extracellular and transmembrane domain of human DAP10 linked to the cytoplasmic domain of human DAP12. CD4⁺/CD28null T cells express the killer inhibitory receptor KIR2DS2 that may associate with DAP12, and the expression of DAP12 is sufficient to convert a co-stimulatory KIR into a stimulatory molecule (32). Thus, a model where DAP12 endows T cells with innate activities may also be a common mode of action for specialized T cell subsets in humans. In summary, our ability to confer NK cell function in T cells by expression of the activation adaptor molecule DAP12 may potentially broaden the applicability of gene-engineered T cells as an immunotherapy for cancer and infectious diseases where transformed or stressed cells express NKG2D ligands.

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