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NK Cell TRAIL Eliminates Immature Dendritic Cells In Vivo and Limits Dendritic Cell Vaccination Efficacy

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Recent studies have implicated a possible role for NK cells in regulating dendritic cells (DC) in vitro. In the present study, we demonstrate that immature DC are rapidly eliminated by NK cells in vivo via a pathway dependent on the TNF-related apoptosis-inducing ligand (TRAIL). Elimination of NK cells and/or neutralization of TRAIL function during immunization with immature DC loaded with nonself or tumor Ags significantly enhanced T cell responses to these Ags and Ag-specific tumor immunity. These data suggested that NK cell TRAIL might regulate responses to vaccination by controlling the survival of Ag-loaded DC. The Journal of Immunology, 2004, 172: 123–129.

Dendritic cells (DC) have been referred to as nature’s adjuvant for their ability to stimulate and activate a broad spectrum of immune cell types, including B and T cells (1, 2). The expression of innate receptors on the surface of DC allows them to respond to infectious agents by taking up, processing, and then presenting Ags in complex with MHC molecules while concomitantly increasing their expression of costimulatory molecules and synthesis of cytokines (2). All of these combined factors ensure that mature DC are potent stimulators of T cells. In this way, DC link the primary innate control of infectious agents to the development of long-term adaptive immunity to infectious agents (2).

The in vitro generation of DC from precursors in the blood or bone marrow has led to the development of DC-based vaccines (3, 4). The type of Ag, activation state of the DC, and route of immunization can all affect the development of subsequent T cell immune responses (4), but productive T cell-DC interactions are key to a good vaccine. In general, devising strategies in which the frequency of T cell-DC interactions is increased should strengthen the efficacy of immunization to Ag.

We and others have found that NK cells efficiently lyse immature DC in vitro (5–13). However, whether and how NK cells might kill injected DC have not been assessed in vivo. Nor do we appreciate the impact NK cell-mediated killing may have on the development of adaptive immune responses following DC-based immunization. In the present study, we have evaluated the survival of transferred bone marrow-derived DC in the absence of NK cells and/or specific NK cell effector mechanisms. We demonstrate that NK cells eliminate immature DC in vivo using TRAIL, and thereby restrict the induction of T cell immunity to Ag-loaded DC vaccination.

Materials and Methods

Mice

C57BL/6 (B6), B6 recombination-activating gene (RAG)-1−/− mice, B6 common γ-chain (γc)−/− RAG-2−/− mice, B6 TAP-1−/− mice (14), B6 perforin (pfp)−/− mice (15), B6 gld mice (Fas ligand (FasL) mutant), B6 TRAIL−/− mice (16), B6 pfp−/− TRAIL−/− mice (bred at the Peter MacCallum Cancer Centre), and B6 gld TRAIL−/− mice (bred at the Peter MacCallum Cancer Centre) (6–10 wk old) were housed under standard conditions at the Microbiology and Tumor Biology Center, Karolinska Institutet and/or the Peter MacCallum Cancer Centre. All procedures were performed under both institutional and national guidelines.

Antibodies

Anti-NK1.1 mAb (PK136) and anti-TRAIL mAb (N2B2) (17) were purified from cell culture medium. For NK cell depletion, mice were injected i.p. with 200 μg/mouse. Anti-TRAIL mAb was injected i.p. at 500 μg/mouse 24 h before immunization. Anti-TRAIL-R2 (DR5) mAb (MD5-1) was generated by immunizing an Armenian hamster with a mouse DR5-human IgG1 fusion protein purchased from DAKO (Glostrup, Denmark). Reactivity to DR5 was confirmed by flow cytometry with DR5 transfectants and immunoprecipitation. All other Abs were bought commercially from BD PharMingen (San Diego, CA), except anti-TRAIL-PE mAb (eBioscience, San Diego, CA).

Generation of bone marrow-derived DC

DC were generated, as described previously (18). Bone marrow cells were cultured in DMEM (Invitrogen, Paisley, U.K.) containing 10% supernatant from a GM-CSF-secreting X63 cell line or 10 ng/ml rGM-CSF (PeproTech, London, U.K.). The cells were harvested after 6 days and replated overnight. When DC were further purified using anti-CD11c mAb-coated beads (Miltenyi Biotec, Bergisch Gladbach, Germany), these cells were cultured for a further 48 h, after which time no Ab was detected on the cell surface by FACS. DC were matured with 1 μg/ml LPS overnight. OVA257–264 peptide was obtained from Interactiva (Ulm, Germany), while E719–26 was purchased from Auspep (Melbourne, Australia). zVAD-fmk and zFA-fmk (Enzyme System Products, Dublin, CA) were incubated with DC overnight at a concentration of 100 μM.
Rapid elimination assay
DC were incubated for 1 h in the presence of Na232CrO4 and then washed thoroughly in PBS. Groups of four to six mice were injected with 5 × 10^6 DC in 200 µl of PBS i.v. via the tail vein, and the organs were removed after 8 h and run on a gamma radiation counter (Wallac Oy, Turku, Finland). The percentage of remaining radioactivity was determined as follows: (organ − background/dose of cells − background) × 100. For experiments using CFSE (Molecular Probes, Eugene, OR), DC were labeled with 1 µM of CFSE for 15 min and then thoroughly washed. Cells were injected i.v., and organs were removed 12–16 h later. Single cell suspensions were prepared from the organs. The cells were then stained with CD11c-PE. The number of DC was calculated as the percentage of CFSE^+ DC/organ.

NK cell cytotoxicity
NK cells were purified from the livers of RAG-1^−/− and pfp^−/− RAG-1^−/− mice, as previously described (16). These effectors were examined in an 8-h ^51Cr release assay using immature bone marrow-derived DC as targets. In some wells, TRAIL was neutralized (20 µg/ml anti-TRAIL) and/or pfp-mediated granule exocytosis was inhibited (5 mM EGTA). Specific lysis was calculated as below, and spontaneous lysis was always <20%.

CTL generation
Groups of mice were treated with both anti-NK1.1 and/or anti-TRAIL mAb 1 wk (day −7) and 2 days (day −2) before bone marrow-derived DC injection. Bone marrow-derived DC were loaded with either 30 µM of K^b-binding OVA257-264 (SIINFEKL) or D^b-binding E749-57 (RAHYNVITF) peptide for 1 h and washed thoroughly, and 4 × 10^5 DC were injected i.v. via the tail vein, i.p., or s.c. in the footpad of mice. After 10–14 days, the spleens were removed. To assess CTL activity, spleen cells were set up in culture in the presence of 0.1 µM OVA257-264 or E749-57 peptide in complete medium (αMEM, 10 mM HEPES, 2 × 10^{-5} M 2-ME, 10% FCS, 100 U/ml penicillin, 100 U/ml streptomycin) (Sigma-Aldrich, St. Louis, MO). In some experiments, 20 µg/ml anti-TRAIL mAb was added to neutralize TRAIL in the cultures for 5 days. Five days later, these cells were used as effectors in ^51Cr release assay using OVA257-264-labeled, E749-57-labeled, and unlabeled RMA-S cells as targets (19). After 4-h incubation, the specific lysis was calculated according to the formula: percentage of specific lysis = (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100. Killing of RMA-S target cells alone was always <3%, and no mice inoculated with unpulsed DC displayed significant CTL activity against RMA-S target cells pulsed with SIINFEKL or RAHYNVITF (<3%). To assess Ag-specific proliferation, spleen cells (harvested from i.v. immunized mice, 1–8 × 10^6/well) were set up in culture in the presence of irradiated RMA-S cells (5 × 10^5/well, 20,000 rad) with or without SIINFEKL peptide (2 µM) at various responder to stimulator ratios (16:1 to 2:1) for 3–6 days. IL-2 (2000 U/ml) was added in the culture as a positive control for the proliferation assay. Proliferative responses were assessed by pulsing cultures with [3H]thymidine (0.5 µCi) for the last 16 h.

Tumor challenge in vivo
Groups of mice were immunized with bone marrow-derived DC loaded with or without the 30 µM E749-57 peptide for 1 h and washed thoroughly, and 4 × 10^5 DC were injected s.c. in the footpads of mice. After 14 days, mice were given another immunization with E749-57 peptide-loaded DC. Mice were treated with anti-NK1.1 or anti-TRAIL mAb 1 wk (day −7) before first and 2 days (day −2) before first and second bone marrow-derived DC immunization. Fourteen days after the second immunization, mice were inoculated s.c. (right hind leg) with RMA or RMA-E7 cells (5 × 10^5, 5 × 10^6) (20). All mice were observed every other day, and tumor growth was measured with a caliper square as a product of two diameters.

Results
NK cells target DC in vivo
To determine whether NK cells can eliminate DC in vivo, we used a classical rapid elimination assay (21, 22) to compare the recovery of i.v. injected ^51Cr-labeled bone marrow-derived DC in control and NK cell-depleted B6 mice. In this assay, the radioactivity recovered is an accurate determinant of intact viable cells remaining in the lung (22). After 8 h, the amount of remaining radioactivity recovered in the lungs of NK cell-depleted mice was 3-fold greater than the levels observed in the lungs of the control mice, indicating that NK cells rapidly eliminated the transferred DC in vivo (Fig. 1A). Although at 16 h the amount of radioactivity was reduced further, there was still at least a 3-fold increase in the amount of remaining radioactivity in NK cell-depleted mice (data not shown). This level of NK cell activity against DC in vivo was comparable to that previously reported for NK cells against tumor cells using the same assay (22).

To strengthen the evidence that NK cells were the effector cells involved in the elimination of DC, similar experiments were performed with DC injected into RAG-1^−/− mice that lack B and T cells. These mice eliminated the DC as efficiently as B6 mice, and recovery was increased between 3- and 4-fold when mice were treated with anti-NK1.1 mAb (Fig. 1B). Elimination of DC was also similarly decreased in γc^−/− RAG-2^−/− mice, which lack B, T, and NK cells (Fig. 1C). Notably, by using the RAG-1^−/− mice, we ruled out the role of NK1.1^+ T cells in the elimination of DC.

Because the rapid elimination assay was only useful for detecting DC retention in the lungs, we confirmed the increased number of surviving DC in the lungs of NK cell-deficient mice by flow cytometric analysis using CFSE-labeled DC (Fig. 2, A and B). Similarly, we observed increased numbers of injected DC in the lung (Fig. 2, A and B), spleen (Fig. 2C), and liver (data not shown) of NK cell-deficient γc^−/− RAG-2^−/− mice compared with RAG-1^−/− mice. Thus, using two distinct methods and assessing several organ sites, these data indicated that NK cells were eliminating transferred DC in vivo.

FIGURE 1. DC are eliminated by NK cells in the lungs of mice. ^51Cr-labeled bone marrow-derived DC were injected i.v., and lungs were removed after 8 h to measure the remaining radioactivity. A, B6 mice untreated or treated with anti-NK1.1 mAb. B, RAG-1^−/− mice untreated or treated with anti-NK1.1 mAb. C, RAG-1^−/− and γc^−/− RAG-2^−/− mice. All data accumulated from at least three independent experiments. *, p < 0.001 as determined by ANOVA.
FIGURE 2. Elimination of CFSE-labeled DC in vivo. CFSE-labeled DC were injected i.v., and organs were removed after 12–16 h. The number of CFSE+ cells in the different organs was determined by flow cytometry. A, CFSE+ CD11c+ DC in the lungs. Upper panel, Control; middle panel, RAG-1−/− mouse; lower panel, γc−/− RAG-2−/− mouse. B, Comparison of the percentage of CFSE+ CD11c+ DC found in the lungs of RAG-1−/− and γc−/− RAG-2−/− mice (n = 9). C, Comparison of the percentage of CFSE+ CD11c+ DC found in the spleens of RAG-1−/− and γc−/− RAG-2−/− mice (n = 7). *, p < 0.01 as determined by ANOVA; **, p < 0.001 as determined by paired t test.

Mechanism of NK cell elimination of DC

To examine the effector mechanism responsible for NK cell-dependent elimination of DC in vivo, we injected radiolabeled DC into mice deficient in either pfp (pfp−/−), Fasl (gld), or TRAIL (TRAIL−/−). DC were efficiently eliminated in pfp−/− or gld mice, but not in TRAIL−/− mice, in which, similar to mice depleted of NK cells, a 3- to 4-fold increase in remaining radioactivity was observed (Fig. 3A). TRAIL−/− mice treated with anti-NK1.1 mAb displayed no additional effect on DC elimination above that observed in TRAIL−/− mice or anti-NK1.1-treated wild-type mice, consistent with TRAIL being functional on NK cells (23), rather than other effector cell types (Fig. 3B). Blocking of the TRAIL pathway by anti-TRAIL mAb treatment in B6 mice demonstrated similar results to TRAIL−/− mice (data not shown), indicating that TRAIL played a predominant role in the elimination of DC in vivo. Confirming these observations, the number of surviving DC was also increased in syngeneic mice deficient in both TRAIL and pfp (pfp−/−/TRAIL−/−) or deficient in TRAIL and Fasl (gld/ TRAIL−/−) (Fig. 3, C and D). Most importantly, in the absence of T or B cells, RAG-1−/− mice pretreated with a neutralizing anti-TRAIL mAb before DC inoculation displayed a 3-fold greater recovery of DC than RAG-1−/− mice alone (Fig. 3E). Taken together, these data showed that the TRAIL was the major effector mechanism by which immature DC were eliminated by NK cells in the lungs.

TRAIL receptor 2 (DR5) was detected on the surface of both untreated immature and LPS-treated (matured) CD11c+ bone marrow-derived DC (Fig. 4A). To further illustrate that elimination of DC occurred via a TRAIL- and therefore caspase-dependent apoptotic pathway, DC were preincubated with the pan-caspase inhibitor zVAD-fmk. DC preincubated with zVAD-fmk, but not zFA-fmk, were protected from NK-mediated elimination in B6 mice (Fig. 4B). Furthermore, zVAD-fmk was without effect in wild-type mice depleted of NK cells or TRAIL−/− mice, attributing the caspase dependence to the TRAIL pathway. LPS-matured DC were protected from NK cell-mediated elimination in vivo despite their high expression of DR5 (Fig. 4C). This protection did not appear to be related to increased expression of FLIP or other death inhibitors (24, 25), because LPS-matured DC from TAP1−/− β2-microglobulin−/− (class I−) mice were eliminated equivalently to immature DC in B6 mice (data not shown). These data were consistent with our previous observations that LPS maturation elevated DC MHC class I expression and reduced their sensitivity to NK cells (10).

To directly demonstrate that NK cells were capable of killing DC through a TRAIL-mediated mechanism, purified NK cells from RAG-1−/− or RAG-1−/− pfp−/− mice were incubated with immature DC in vitro. NK cells from each of these strains of these mice effectively lysed the DC. Although pfp was responsible for part of the cytotoxic activity in vitro, significant levels of NK cell-mediated lysis were measured in the absence of pfp, and this was blocked by the addition of anti-TRAIL mAb (Fig. 4, D and E). Neutralization of both pfp and TRAIL pathways completely blocked NK cell-mediated killing of DC. These data were consistent with the ability of NK cells to eliminate immature DC in vivo by a TRAIL-dependent mechanism.

T cell responses to DC immunization are enhanced in the absence of NK cells or TRAIL

Because the absence of NK cells or TRAIL led to an increased recovery of DC, the functional consequences of increased DC survival on CTL induction were next examined. CTL activity from spleens of mice immunized with the OVA257–264 (SIINFEKL) peptide-loaded DC was enhanced >6-fold in mice depleted of NK
cells compared with CTL generated in control mice (comparing E:T ratios, Fig. 5A). Furthermore, CTL responses to peptide-loaded DC in TRAIL−/− mice were similar to those observed in NK cell-depleted mice (Fig. 5A). Similar results were also obtained when DC were injected i.p. or s.c. (Fig. 5B and data not shown), indicating that NK cell TRAIL eliminated DC and restricted CTL responses generated, regardless of the route of DC immunization. Lytic unit calculation supported the conclusion that mice lacking NK cells or TRAIL exhibited a 6-fold increase in their cytotoxicity compared with control mice (data not shown). To ensure that the observed increased CTL activity was not related to any inherent defect in the T cells from TRAIL−/− mice (26, 27), wild-type mice were pretreated with anti-TRAIL mAb 24 h before the day of DC immunization. In this experiment, at least a 10-fold enhancement in CTL response was detected in the anti-TRAIL mAb-treated mice, indicating that TRAIL neutralization during the first few days after DC immunization was sufficient (Fig. 5C). CTL generated in mice transiently depleted of NK cells and neutralized for TRAIL displayed an equivalent CTL activity to those from mice depleted of NK cells or anti-TRAIL mAb treated alone. The addition of anti-TRAIL mAb to the spleen cell culture upon restimulation with OVA257–264 had no effect on the CTL activity generated. These experiments provide no evidence for a role for TRAIL on T cells in influencing DC immunization of T cell responses. Rather, all the data indicated that NK cell TRAIL suppressed T cell responses to peptide-loaded immature DC.

To assess any potential role for pfp in regulating such Ag-loaded DC immunization, we concomitantly assessed Ag-specific T cell proliferation. Improved T cell proliferation to Ag was observed in immunized NK cell-depleted wild-type mice and TRAIL−/− mice compared with untreated wild-type mice (Fig. 5D). However, in line with the data from the DC elimination assay, no significant increase in Ag-specific T cell proliferation was observed in the spleen T cells restimulated from immunized pfp−/− mice compared with wild-type mice. Taken together, effective immunization for CTL was enhanced by inhibiting TRAIL-dependent apoptosis.

**Improved DC immunization enhances tumor rejection**

To explore the biological effect of inhibiting the NK cell TRAIL pathway during DC immunization, we examined tumor challenge subsequent to s.c. vaccination with DC loaded with a high affinity H-2Db-binding peptide of the human papillomavirus type 16 protein E7, E749–57. Similar to the enhanced CTL responses observed in response to DC pulsed with OVA257–264 (Fig. 5), neutralization of TRAIL or depletion of NK cells during immunization enhanced E7-specific CTL responses detected in the spleen by >6-fold above those observed in wild-type B6 mice (data not shown). Similarly, immunized mice were challenged with two different lethal doses of RMA lymphoma cells expressing the E7 protein, 2 wk after immunization. Although wild-type B6 mice twice immunized with E749–57-loaded DC were somewhat able to resist RMA-E7
tumor growth, B6 mice that were depleted of NK cells or neutralized for TRAIL at the time of immunization completely rejected s.c. challenge with a dose of \(5 \times 10^5\) RMA-E7 tumor cells (Fig. 6A). Although B6 mice that were immunized with Ag-loaded immature DC only weakly resisted challenge with a higher dose of tumor rejection was Ag specific because no immunized mice resisted challenge with parental RMA tumor cells. Furthermore, mice immunized with unloaded DC (control) were unable to suppress RMA-E7 tumor growth (Fig. 6). No statistical improvement in immunization with mature DC was obtained by NK cell depletion or TRAIL neutralization (data not shown). These results highlight the potential benefit of temporarily depleting NK cells and/or neutralizing TRAIL during vaccination protocols that use immature DC and confirm the increased effectiveness of mature DC for vaccination.

**Discussion**

In our study, the elimination of transferred DC was examined in the presence and absence of NK cells. Significantly increased survival of bone marrow-derived immature DC was obtained in mice lacking NK cells, indicating that NK cells could restrict DC survival in vivo. Immature, but not mature DC were efficiently eliminated by NK cells, and the primary effector mechanism used was TRAIL. The ability of NK cells to suppress immature DC survival after adoptive transfer highlighted a potentially novel role for NK cell-mediated killing in vitro, some mice were also immunized twice with mature DC loaded with E7\(_{49-57}\). Clearly, immunization with mature DC afforded greater protection from higher tumor doses than immunization with immature DC, and the level of protection was comparable or slightly superior to that observed for B6 mice immunized with immature DC and additionally depleted of NK cells or neutralized for TRAIL (Fig. 6B). Tumor rejection was Ag specific because no immunized mice resisted challenge with parental RMA tumor cells. Furthermore, mice immunized with unloaded DC (control) were unable to suppress RMA-E7 tumor growth (Fig. 6). No statistical improvement in immunization with mature DC was obtained by NK cell depletion or TRAIL neutralization (data not shown). These results highlight the potential benefit of temporarily depleting NK cells and/or neutralizing TRAIL during vaccination protocols that use immature DC and confirm the increased effectiveness of mature DC for vaccination.
that inhibition of TRAIL-mediated apoptosis or TRAIL responsiveness of immature DC at the time of DC immunization may specifically improve DC survival upon transfer and thereby enhance ensuing adaptive immune responses.

NK cells are armed with many different pathways by which they can kill target cells. Although pfp may appear to be an important mechanism in NK cell-mediated cytotoxicity ex vivo and in vivo (15, 28–32), FasL (32–36) and TRAIL (16, 17, 37, 38) death ligand mechanisms have begun to receive more attention. Previous studies have suggested that neither pfp nor FasL pathways were involved in the death of DC in vivo (39, 40). The data generated in our study support these previous studies, and for the first time identify TRAIL as a key pathway that NK cells can use to eliminate DC in vivo. In particular, the fact that DC were similarly protected from elimination in TRAIL−/−, pfp−/−TRAIL−/−, and gld/TRAIl−/− mice indicated that TRAIL was the dominant mode for elimination of transferred DC in vivo. DC maturation in vivo has been shown to be dependent on FasL-Fas interactions (39), and hence a lack of maturation of DC in gld mice may explain why transferred DC were eliminated slightly more effectively in gld mice. Thus, following immunization, those DC that undergo maturation in vivo may survive NK cell elimination and subsequently stimulate T cells. By contrast, pfp appeared to play little role in the elimination of transferred immature DC. Although these data are similar to that of Ludewig et al. (40), clearly pfp is critical in the control of tumor metastases (17, 30) and during infections (41), and may in these and other situations make an important contribution to the development of adaptive immunity or immunopathology associated with infection (42, 43). Although NK cells can lyse DC in vitro in a TRAIL- or pfp-dependent manner, it remains unclear why the pfp pathway is not critical in vivo. Indeed, no study has demonstrated whether pfp-mediated cytotoxicity directly regulates DC function in vivo. Nevertheless, there may be many potential interactions between cytotoxic lymphocytes and DC, in which cell death mechanisms may positively or negatively regulate the immune response.

Mature DC have been found to be more effective in vaccines than immature DC (3, 4). These findings have been attributed to the up-regulation of costimulatory molecules on the DC and the release of cytokines by the DC (1, 2). Cytokines such as IL-12 released by activated DC would drive Th1-type immune responses and enhance CTL responses (44). Mature DC have been found to stimulate IFN-γ production by NK cells in vitro (11–13, 45), which could also help to drive Th1 responses. Mature DC were comparably resistant to NK cell-mediated killing in vitro, and this resistance was related to the increase of MHC class I expression on the DC (10, 13). Therefore, the efficacy of the mature DC as adjuvants may be due not only to their surface expression of costimulatory molecules and release of Th1-inducing cytokines, but also to their increased resistance to NK cell-mediated lysis. Our studies raise the likelihood that DR5 expression and/or TRAIL sensitivity may also impact upon the sensitivity of DC to NK cell-mediated apoptosis and their effectiveness as adjuvants. Future studies will address the mechanisms by which some DR5-positive DC (e.g., LPS-matured DC) remain refractory to TRAIL-mediated killing in vivo.

A recent study has shown that there is a 1:1 ratio between T cells and DC in the lymph nodes of DC-immunized mice (46). Because we observe at least a 3-fold increase in the number of DC surviving in the absence of NK cells or TRAIL, it may not be surprising that T cell proliferation and CTL responses to immunization increased in these mice. When NK cells were present, the immunizing immature DC remained capable of stimulating T cell proliferation and CTL activity. It may be that only the TRAIL-resistant DC survived, but these were sufficiently matured in vivo to stimulate a T cell response to Ag. Clearly, following DC immunization, a potentially complex network is created in which the DC interact with other cells of the innate immune system. Dissecting this in vivo will not be trivial, but it will now be important to consider the potential impact TRAIL may have on shaping the DC when they capture and respond to Ag.

With the evolution of the adaptive immune system to cope with persistent and recurring infections, NK cells not only provide innate protection from infection, but may also serve to control adaptive immune responses by eliminating DC (47–49). Under conditions in which a high DC:NK cell ratio exists in vitro, NK cell activation and maturation of DC were induced (11–13, 45). Thus, a cycle may be envisaged in which mature DC can stimulate NK cell activation that in turn can limit the available immature pool of DC that could be potentially harmful to the host, e.g., by inducing tolerance during an infection (50). Although our data support the concept that innate cells might play a role in the development of the adaptive immune response (47, 49, 51), the ability of NK cells to kill endogenous DC remains unproven and must be examined. Indirect evidence for NK cell interactions with endogenous DC can be gleaned from the studies of autoimmune diseases (52). In most autoimmune models, NK cells have been associated with reduced severity of disease. However, in some models, increased severity of disease has been linked to NK cells. It is tempting to speculate that the latter cases of autoimmunity may be enhanced by NK cells eliminating tolerogenic DC, while in the former cases NK cells may have eliminated immunizing DC. This disease induction is often complex, and many of these studies have involved a general depletion or assessment of NK cells, rather than considering the possibility that NK cell subsets exist and that NK cells and DC may encounter one another in the tissue or the lymph node. We need a better understanding of what types of NK cells can kill DC and where this may be occurring in vivo, before any sense of data from disease models can be easily interpreted. It has recently been shown that NK cells can migrate to the epidermis and physically interact with DC in an atopic eczema/dermatitis syndrome (53). Therefore, NK cells may traffic to sites of inflammation and interact with DC in the periphery. Further extensive studies are now required to examine where and how NK cells interact with endogenous DC in vivo and to establish whether the numbers and functions of specific subsets of DC are altered in the absence of NK cells.

DC-based vaccines have had mixed results in clinical trials (3). The data presented in this work suggest that responses to some DC vaccines may be enhanced in the temporary absence of TRAIL. Obviously, chronically neutralizing TRAIL may not be a prudent course of action because TRAIL has been shown to be important in tumor immune surveillance (23, 54). Temporary inhibition of TRAIL was sufficient to considerably enhance the tumor Ag-specific activity of a weakly immunogenic antitumor DC vaccine. Although further analysis will be required to determine whether TRAIL has any impact on the strength of long-term T cell memory responses and other arms of the adaptive immune system, our study highlights the potential importance of innate effector mechanisms in determining the success or failure of DC-based vaccination.

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