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Rae1 and H60 ligands of the NKG2D receptor stimulate tumor immunity

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Abstract

Natural killer cells attack many tumor cell lines and have long been thought to play a critical role in anti-tumor immunity[1-7], but the interaction between NK cells and tumor targets is poorly understood. The stimulatory lectin-like NKG2D receptor[8-13] is expressed by NK cells, activated $CD8^+$ T cells and activated macrophages in mice[11]. Several distinct cell surface ligands related to class I MHC molecules have been identified[11-14], some of which are expressed at high levels by tumor cells but not by normal cells in adults[11,13,15,16]. However, no direct evidence links the expression of these “induced self” ligands and tumor cell rejection. Here we demonstrate that ectopic expression of the murine NKG2D ligands Rae1β or H60 in several tumor cell lines results in dramatic rejection of the tumor cells by syngeneic mice. Rejection is mediated by NK cells and/or $CD8^+$ T cells. The ligand-expressing tumor cells induce potent priming of cytotoxic T cells and sensitization of NK cells in vivo. Strikingly, mice exposed to live or irradiated tumor cells expressing Rae1 or H60 are specifically immune to subsequent challenge with tumor cells lacking NKG2D ligands, suggesting application of the ligands in the design of tumor vaccines.

As demonstrated by staining with a tetramerized derivative of the extracellular portion of NKG2D, NKG2D ligands are expressed by the majority of tumor cells tested, including various lymphoid, myeloid, and carcinoma cell lines (ref. 12 and unpublished data). Northern blot analysis revealed that many of the positive cell lines express Rae1 transcripts, while H60 transcripts were limited to only one or two of the cell lines tested (data not shown). Rae1 transcripts have not been detected in normal cells from adult mice[15], suggesting that these genes are specifically upregulated in tumor cell lines.

To investigate whether tumor cells expressing NKG2D ligands stimulate anti-tumor immune responses, we used a retrovirus expression system to ectopically express high levels of Rae1β or H60 in EL4, a thymoma, RMA, a T cell lymphoma and B16-BL6, a melanoma. These cell lines are all from C57BL/6 (hereafter B6) mice and do not normally express NKG2D ligands[11]. Ligand-transduced cells were selected based on staining with NKG2D tetramers. To serve as controls, tumor cells transduced with empty retrovirus vector (designated as EL4/−, B16/− and RMA/−) were selected by genomic PCR (see Methods).

For analysis of the response to EL4 and B16-BL6 tumor cells, groups of five B6 mice were inoculated subcutaneously with syngeneic tumor cell transductants. Control-transduced EL4 or B16-BL6 cells grew progressively at a rate similar to untransduced cells (Fig. 1a, c, d, data not shown), leading to uniform terminal morbidity by approximately 28 days. Strikingly, Rae1β- or H60-transduced tumor cells of both types were rejected rapidly and completely, as they failed to yield detectable tumors at any time point (Fig. 1a, c, d). A ten-fold increase in the dose of Rae1β- or H60-transduced EL4 cells (to 5×10^7 cells) did not

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change the outcome, while a higher dose (1×10^5) of Rae1β- or H60-transduced B16-BL6 cells resulted in progressive, though substantially delayed, tumor growth in all the mice compared to the control-transduced tumor cells (data not shown). Ligand-transduced tumor cells of both types also failed to grow in B6 mice that had been depleted of $CD8^+$ T cells or in B6-$Rag1^{-/-}$ mice, which lack all T and B cells, but grew progressively in normal and B6-$Rag1^{-/-}$ hosts that had been depleted of $NK1.1^+$ cells (Fig. 1a-e). Thus, these doses of Rae1β- or H60-transduced EL4 cells and B16-BL6 cells are rejected rapidly by conventional NK cells without a requirement for T and B cells, including $NK1.1^+$ T cells or γδ T cells. Interestingly, Rae1β- or H60-transduced B16-BL6 cells reproducibly exhibited retarded growth in NK-depleted B6-$Rag1^{-/-}$ mice (Fig. 1e). It is possible that a residual response against these cells is mediated by non-lymphoid cells such as macrophages, or by small numbers of NK cells that survive antibody treatment.

Rae1β- or H60-expression by B16-BL6 cells also reduced the frequency of lung metastases by over 10-fold after i.v. injection (Fig. 1f). In another experiment where mice were examined at a later time point, control-transduced B16-BL6 cells formed massive contiguous lung metastases, but ligand-transduced B16-BL6 cells were almost completely rejected (Fig. 1g). NK1.1-depletion before tumor cell inoculation dramatically depressed the rejection of the metastases.

Rae1β- or H60-transduced RMA tumor cells were also rejected by B6 mice (Fig. 2). Unlike the responses to the other tumor cells, however, the primary rejection of ligand-transduced RMA cells was mediated by both $CD8^+$ T cells and NK cells, though the specific outcome depended on the dose of tumor cells. Depletion of both $NK1.1^+$ cells and $CD8^+$ T cells was necessary to abrogate rejection of the smallest inoculum of 10^4 ligand-transduced tumor cells, while depletion of either population allowed tumor growth in at least some animals injected with the largest dose of 10^6 tumor cells (Fig. 2a). With the intermediate dose of 10^5 tumor cells, depletion of CD8 cells allowed tumor cell growth, but NK cell depletion did not (Fig. 2a). Thus, either subset is sufficient for tumor rejection at the lowest tumor cell dose, CD8 cells (but not NK cells) are sufficient at the intermediate dose, and the two cell types must cooperate to achieve consistent rejection at the highest tumor cell dose. In B6-$Rag1^{-/-}$ mice, NK cells were sufficient to reject the Rae1β- or H60-transduced RMA cells at the two lower tumor doses (Fig. 2b), suggesting that NK cells are more active or effective in these B6-$Rag1^{-/-}$ mice than in B6 mice. Parallel analysis of mice inoculated with the RMA/S cell line, an MHC class I^{low} version of RMA cells, confirmed previous reports that these cells are rejected by NK cells and not $CD8^+$ T cells[2], and demonstrated the efficacy of our NK cell depletion procedure (Fig. 2a, b).

The question arises why NKG2D ligand expression does not cause the rejection of the majority of tumor cell lines, which naturally express these ligands[11]. In some cases, the host response to the ligands may be overwhelmed by an excess tumor load. Furthermore, the response may critically depend on the levels of NKG2D ligands expressed by tumor cells, especially if the NK cells are also subject to inhibitory signaling via MHC-specific receptors. Notably, the levels of Rae1β and H60 expression in our transductants were substantially higher than the levels of endogenous NKG2D ligands on most of the naturally expressing tumor cell lines we tested (Fig. 2c). Indeed, a comparison showed that RMA-transductants that had intermediate ligand levels, comparable to most tumor cells[11] (Fig. 2c, data not shown), were less efficiently rejected than were transductants with higher ligand levels (Fig. 2d). Therefore, the anti-tumor response to naturally arising tumor cells is likely to vary depending on the level of NKG2D ligands that are expressed.

To address whether prior immunization with tumor cells expressing NKG2D ligands induces protective immunity to ligand-negative tumor cells, mice that had previously rejected

Rae1β- or H60-transduced tumor cells (EL4, B16-BL6 or RMA cells) were rechallenged with corresponding ligand-negative tumor cells 8-12 weeks after the first exposure. The ligand-negative tumor cells grew progressively in naïve B6 mice, but were rejected by the mice that had been previously exposed to the corresponding Rae1β- or H60-transduced tumor cells (Fig. 3a). The immunity was specific, since mice that had previously rejected ligand-transduced tumor cells of each type were immune only to untransduced tumor cells of the same type (Fig. 3b). Some delay was observed in the rejection of RMA cells by mice primed with ligand-transduced EL4 cells (Fig. 3b), and this may be in line with evidence indicating a common origin of EL4 and RMA cells in the distant past[17,18]. However, the cross-protection was only partial, and was absent or very weak in the reciprocal situation, suggesting that our lines have an independent origin or that most of the response is focused on antigens that are unique to each cell line. We conclude that ligand-expressing tumor cells specifically vaccinated the mice against ligand-negative tumor cells of the same type.

Primary rejection of ligand-transduced EL4 and B16-BL6 cells by naïve mice was mediated by NK cells (Fig. 1), but it was not expected that NK cells could provide a specific "memory" immune response. Indeed, no immunity to any of the tumor cells developed in $Rag1^{-/-}$ mice (Supplementary information Fig. 1). Furthermore, normal mice pretreated with anti-CD8 antibody before the initial exposure to all three types of ligand-transduced tumor cells were unable to reject ligand-negative tumor cells upon rechallenge (Fig. 3c), despite the fact that the ligand-transduced tumor cells had been rejected in each case (Fig. 1, 2). Pretreatment of mice with control Ig did not alter the memory response to ligand negative tumor cells. Thus, although $CD8^+$ T cells were not required to reject the primary inoculum of ligand-transduced EL4 and B16-BL6 cells, they were essential for a protective immune response against untransduced tumor cells.

It was possible that immunity arose in this system because NK cell rejection of the transduced tumor cells resulted in excess tumor cell debris, enhancing "cross priming" of tumor specific $CD8^+$ T cells[19]. Indeed, irradiated untransduced RMA cells, which are expected to die in vivo yielding considerable cell debris, are reportedly effective in vaccinating mice[20]. However, this was not the case with irradiated ligand negative B16-BL6 tumor cells[21], since no immunity resulted when mice were vaccinated once or three times in succession with irradiated control-transduced B16-BL6 tumor cells (Fig. 3d). Mice vaccinated in parallel with irradiated ligand-transduced B16-BL6 cells did develop immunity to untransduced tumor cells, suggesting that ligand expression results in priming of tumor-specific T cells independent of the generation of cell debris by NK cells. Consistent with this conclusion, irradiated ligand-transduced B16-BL6 cells were effective at priming a tumor-specific CTL response, even in mice that had been previously depleted of NK cells, whereas control-transduced B16-BL6 cells failed to prime $CD8^+$ T cell responses (see below and Supplementary information Fig. 2). Similar results were obtained in the RMA system (Fig. 4).

Consistent with the role of $CD8^+$ T cells in responses to ligand-transduced tumor cells, irradiated Rae1β- or H60-transduced tumor cells were more effective than ligand-negative tumor cells in priming tumor-specific CTLs in vivo, as tested by restimulating with ligand-transduced cells and testing effector function in vitro. Priming with irradiated ligand-transduced RMA cells, for example, increased RMA target cell lysis substantially and augmented the percentage of RMA-specific IFN-γ producing $CD8^+$ T cells several-fold (Fig. 4a, e). The effector cells were considerably more active against ligand-transduced RMA cells, and this enhancement could be completely blocked with anti-NKG2D antiserum (compare Fig. 4e, g with f, h), indicating that the NKG2D-ligand interaction enhances not only CTL induction in vivo, but also the effector activity of the CTL. The enhanced induction of CTL, which were conventional $CD8^+$ $NK1.1^-$ cells (Fig. 4c-f), occurred even in

mice that had been previously depleted of NK1.1$^+$ cells (Fig. 4i), demonstrating that ligand-dependent augmentation of the CTL response does not depend directly or indirectly on NK cell activity.

Importantly, the CTL were tumor cell specific, as they did not lyse any of three other tumor cells (EL4, B16-BL6 or MC38), nor did they lyse Rae1β-transduced B16-BL6 cells (Fig. 4j). Furthermore, the CTL did not lyse syngeneic T cell blasts, suggesting that priming with ligand-transduced tumor cells does not break self-tolerance of CTL (Fig. 4j). Highly comparable results were obtained in the B16-BL6 tumor cell system, though the responses were generally weaker, probably because of the low expression of class I MHC molecules by these cells (Supplementary information Fig. 2). Similar results were also obtained with CTL derived from mice that had previously rejected live ligand-transduced tumor cells (data not shown). Thus, tumor cell expression of NKG2D ligands results in a significant enhancement in the priming of tumor specific CTL in vivo, as well as in the activation of preformed CTL. These data are in line with evidence that NKG2D engagement costimulates human CTL responses in vitro[22], and extend these findings to in vivo responses to tumor cells.

Induction of NK cell activity in vivo by tumor cells expressing Rae1β or H60 was also demonstrable. When inoculated intraperitoneally in naïve mice, irradiated Rae1β- or H60-transduced RMA tumor cells increased the number of peritoneal NK cells 2-4-fold within two days (Fig. 5a), and substantially enhanced the cytotoxic activity of the cells versus YAC-1 target cells (Fig. 5b, left panel), whereas control-transduced RMA cells had no effect. A substantially higher fraction of these NK cells also produced IFN-γ after in vitro stimulation with YAC-1 tumor cells (Fig. 5c). As previously reported, class I-deficient RMA/S cells also induced the local accumulation of NK cells and enhanced their functional activity[23] (Fig. 5a-c). In either case, the cytotoxic activity was nearly abolished by pretreatment of effector cells with anti-NK1.1 plus complement or by in vivo depletion of NK1.1$^+$ cells before tumor cell inoculation (Fig. 5b, d). In contrast, depletion of CD8$^+$ cells before tumor cell inoculation or immediately before the cytotoxicity assay had no effect (Fig. 5b, d), and similar cytotoxic activity was induced in Rag1$^{-/-}$ and wildtype mice (Supplementary information Fig. 3). Thus, the effector cells are conventional NK cells and do not depend on CD8$^+$ cells for their induction. Similar cytotoxicity and cytokine data were obtained with ligand-expressing B16-BL6 and EL4 cells (Supplementary information Fig. 4). Thus, expression of NKG2D ligands, like MHC-deficiency, provokes NK cell recruitment and sensitization in vivo.

A nondepleting anti-NKG2D antiserum injected in vivo almost completely blocked the ligand-dependent induction of NK cytotoxicity and cytokine production against YAC-1 cells (Fig. 5e). The effect of the antibody was specific, since NK cell induction by class I-deficient RMA/S cells, which do not express NKG2D ligands, was unaffected by the anti-NKG2D antibody. Therefore, the induction process with ligand-transduced cells required interactions with the NKG2D receptor in vivo.

Our results demonstrate that NKG2D ligand expression and consequent activation of NK cells and CD8$^+$ T cells can impose a substantial barrier to the establishment of tumors in vivo. The ligands activate NK cells and T cells via NKG2D, which associates in the membrane with KAP/DAP10, an adapter signaling protein that is thought to deliver costimulatory signals[10]. It is unknown whether NKG2D engagement in NK cells results in direct stimulation or supplies a costimulatory signal that acts in conjunction with signals from other stimulatory receptors[24,25]. Regardless, NKG2D receptor engagement clearly dramatically enhances the effective NK cell response against the three tumor cell lines tested. Furthermore, ligand expression by tumor cells also strongly enhanced the generation

of tumor specific CD8 T cells. Since this enhancement also occurred in mice devoid of NK cells (Fig. 2a, Fig. 4), and activated $CD8^+$ T cells express NKG2D, we propose that $CD8^+$ T cell activation is enhanced as a consequence of direct interactions with ligand-expressing tumor cells. It remains possible that the priming of $CD8^+$ T cells is accomplished indirectly as a result of ligand-expressing tumor cells interacting with NKG2D-expressing macrophages[11]. In either case, the results suggest that NKG2D ligands provide an example of innate immune stimuli that function to enhance the adaptive immune response[26]. The effectiveness of ligand transduction of tumor cells in stimulating an anti-tumor response and protective immunity to tumor rechallenge suggests that ligand-expressing cells may have applications in tumor therapy and the development of tumor vaccines. The strong response against B16-BL6 cells is particularly notable in this regard, given that the BL6 variant was selected for high invasiveness and is poorly immunogenic[27]. Indeed, other manipulations such as ectopic B7 expression or CTLA4-blockade do not, by themselves, result in rejection of B16-BL6 cells[28]. The finding that typical levels of NKG2D ligands naturally found on most tumor cell lines are suboptimal in inducing anti-tumor immunity (Fig. 2c, d) raises the possibility that immunity to such tumors can be boosted by engineering cells with higher levels of ligands. The effectiveness of this approach against preestablished tumors and naturally arising tumors remains to be established.

Methods

Ectopic expression of NKG2D ligands

Three NKG2D ligand negative cell lines (EL4, a B6 thymoma; RMA, a B6 T lymphoma derived from the Rauscher virus-induced RBL-5 cell line[2] and B16-BL6, a B6 melanoma derived from the B16-F0 cell line[27]) were retrovirally transduced as described[11]. The retroviral vectors containing the H60 or Rae1β cDNAs used for these experiments did not direct synthesis of GFP or any other selection marker. Transduced cells expressing equivalent high levels of the NKG2D ligands were sorted after staining with a tetrameric soluble version of NKG2D[11]. Control staining was performed with an irrelevant tetramer of the T22 class Ib molecule. Control tumor cells were infected with “empty” retrovirus, and transduced clones were identified by PCR with primers corresponding to the MSCV 5′ and 3′ LTR (5′ primer: GTCCTCCGATAGACTGCGTCGCCCGGG; 3′ primer: GCTTGCCAAACCTACAGGTGGGG). Approximately 100-150 clones with integrated provirus were pooled and used as control tumor cells (designated as EL4/−, B16-BL6/− and RMA/−).

Mice, antibody depletion, tumor inoculation and rechallenge

All tumor rejection experiments employed five mice per group. C57BL/6J (B6) and B6-$Rag1^{-/-}$ mice were purchased from Jackson Laboratories and the latter mice were bred in our animal facilities under specific pathogen free conditions. All mice were used between 8 and 18 weeks of age. NK cells and $CD8^+$ T cells were depleted[28] by i.p. injection of 200 μg of monoclonal antibody (PK136 against NK1.1 and 2.43 against CD8) at day −1, 1, 8, 15, and 22. Control mice received the equivalent amounts of normal mouse IgG. Depletions were confirmed in lymph node and spleen cells 3 weeks after tumor challenge by flow cytometry using non-competing antibodies. In general, less than 1.5% of the depleted cell population could be detected in spleen and lymph nodes. Tumor cells were injected subcutaneously in 100μl of PBS in the right flank. Tumor development was monitored by measuring the tumor size twice weekly with a metric caliper. For the metastasis assay, 3×10^5 B16-BL6 cells were injected intravenously via the tail vein in groups of five mice, and lung metastases were examined 14-21 days later. For the rechallenge experiments, groups of five mice that had completely rejected the initial tumor (8-12 weeks after initial

tumor challenge) were injected in the opposite flank with the respective untransduced or control transduced tumor cells (i.e., lacking NKG2D ligands).

Tumor cell vaccination and and ex vivo analysis of CTL activation

Transduced B16-BL6 tumor cells were irradiated (16,000 rad) and 5×10^6 cells were injected in groups of five mice in 100μl PBS subcutaneously in the left flank either once at day −10 or three times at day −10, −7 and −4 as indicated. Some mice received injections of unirradiated tumor cells at day −10. At day 0, mice were challenged with 10^4 untransduced B16-BL6 cells in 100μl PBS in the opposite flank. For the ex vivo analysis of CTL activity, groups of five mice received two vaccinations (day 0 and 3) with irradiated (16,000 rad) RMA or B16-BL6 tumor cell transductants. 2-3 weeks after vaccination, mice were sacrificed and pooled splenocytes were restimulated for 5 days with the respective Rae1β-transduced tumor cells. The cells were expanded for another three days before testing cytotoxicity and IFN-γ production. In some experiments $NK1.1^+$ or $CD8^+$ T cells were depleted prior to the cytotoxicity assay by complement-mediated lysis as described[29]. In vitro blocking of NKG2D was as described previously, using a rat anti-NKG2D antiserum and a control rat serum[11].

Ex vivo analysis of NK cell activation

Tumor cells were irradiated (12,000 rad) and injected intraperitoneally as described[23]. After 72h peritoneal cells were harvested[23]. The percentages (± s.d.) of NK cells in the peritoneal cavity of individual mice were quantified by flow cytometry and electronic gating on lymphocytes (FSC^{lo}/SSC^{lo}). Peritoneal wash cells were pooled for cytotoxicity and IFN-γ assays. In some experiments $NK1.1^+$ or $CD8^+$ T cells were depleted prior to the cytotoxicity assay by complement-mediated lysis as described[29]. For the in vivo blocking experiments 100μl of a non-depleting antiserum specific to NKG2D or a control antiserum[11] were injected i.p. As determined by flow cytometry, the antibody did not deplete $NK1.1^+$ cells from the peritoneal cavity (data not shown).

Assays

Cytotoxicity was tested in a standard 4h ^{51}Cr release assay[11]. Data are given as the mean of triplicate measurements. Standard deviations were generally less than 5% and are omitted from the figures for reasons of clarity. IFN-γ production was assayed by stimulating CTLs or NK cells with an equal number of target cells for 5-7 hours and staining intracellular IFN-γ as described[30], with gating on $CD8^+$ $CD3^+$ cells or $NK1.1^+$ $CD3^-$ cells. Data are presented as the mean (±s.d.) of triplicate measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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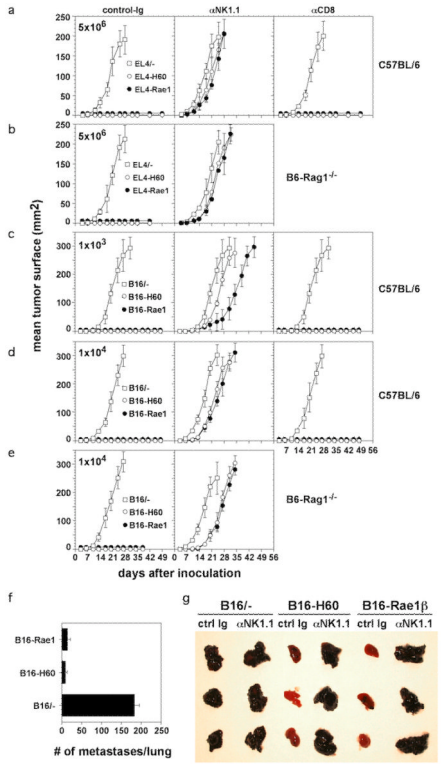

Figure 1.

EL4 and B16-BL6 tumor cells expressing NKG2D ligands are rejected by NK cells in syngeneic mice. C57BL/6 or B6-Rag1$^{-/-}$ mice were treated with the indicated antibodies before inoculation with the indicated number of EL4 (**a**, **b**) or B16-BL6 (**c-e**) tumor cell transductants. Tumor growth was monitored thereafter. Data are representative of at least three experiments. **f**, **g**, B6 mice were injected i.v. with 3×10^5 B16-BL6 transductants. Lung metastases were counted two weeks after the tumor challenge (**f**) or the lung lobes from representative mice in a separate experiment were examined three weeks after tumor challenge (**g**).

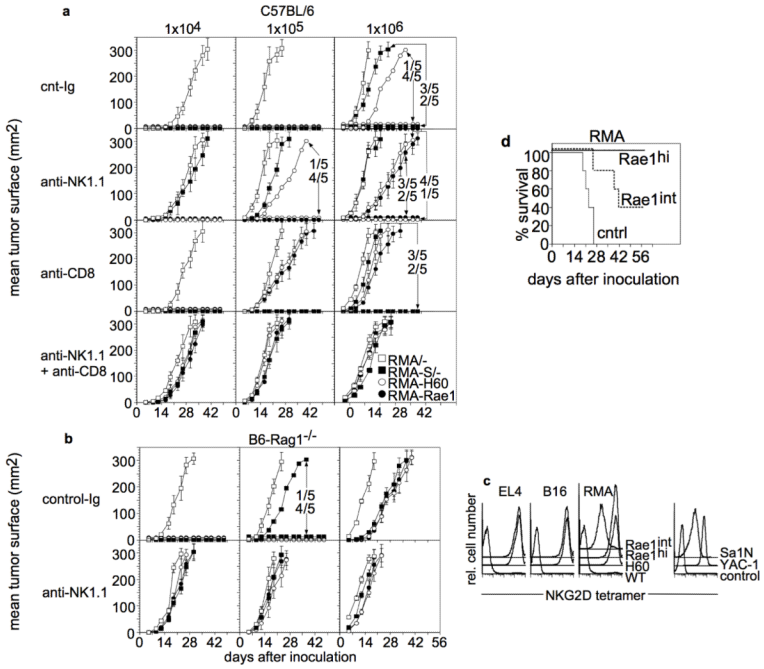

Figure 2.

RMA cells expressing NKG2D ligands are rejected by NK cells and CD8 T cells in syngeneic mice. B6 (**a**) or B6-Rag1$^{-/-}$ (**b**) mice were pretreated with the indicated antibodies and inoculated subcutaneously with the indicated numbers of RMA transductants or RMA/S cells. In instances where tumors grew in some but not all mice, the fractions are indicated in the panels. Data are representative of four experiments. **c**, Levels of NKG2D ligands determined by staining with fluorescently labeled tetrameric NKG2D. Tumor cell transductants, the YAC-1 lymphoma and the Sa1N fibrosarcoma are compared. **d**, The anti tumor response depends on Rae1β levels. Survival of B6 mice that had been inoculated with 1×10^5 RMA cells, or RMA cells expressing high (Rae1^{hi}) or intermediate (Rae1^{int}) levels of Rae1β. Terminally moribund mice were sacrificed. Data are representative of two experiments.

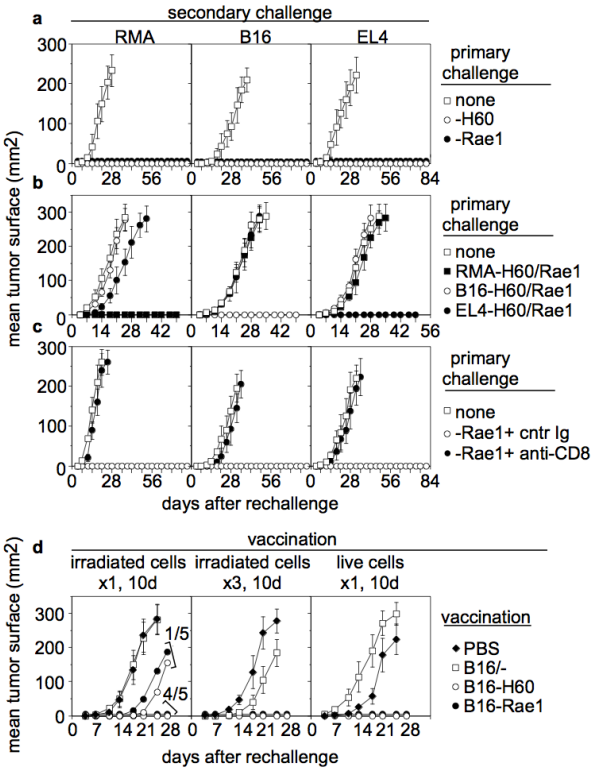

Figure 3.

Vaccination with ligand-expressing tumor cells confers specific immunity to the corresponding ligand-negative tumor cells. **a**, B6 mice that had previously rejected ligand-transduced tumor cells (5×10^6 EL4, 1×10^4 B16-BL6 or 1×10^4 RMA transductants) were inoculated subcutaneously with control-transduced tumor cells of the same type (EL4: 5×10^6 ; B16-BL6: 1×10^4 ; RMA: 1×10^5). Primary exposure occurred 8-12 weeks before challenge. **b**, B6 mice that had been vaccinated 12 weeks earlier with each ligand-transduced tumor cell type were injected with the same or different ligand-negative cell lines. The primary and secondary tumor doses were as in (**a**). **c**, Depletion of $CD8^+$ cells during the primary challenge prevents development of immunity. Rechallenge with control-transduced tumor cells occurred 8-12 weeks after vaccination. Naïve B6 mice were challenged in parallel. **d**, B6 mice were injected with PBS or were vaccinated with 5×10^6 irradiated or 1×10^4 living transduced or untransduced B16-BL6 tumor cells. Mice were challenged 10 days later with 10^4 untransduced B16-BL6 cells in the opposite flank. Data are representative of two experiments.

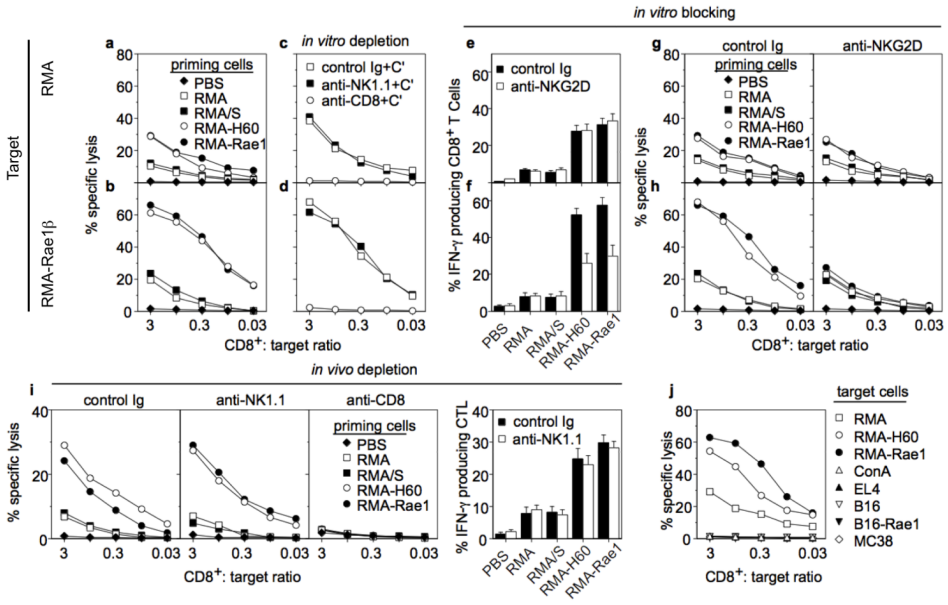

Figure 4.

CTL responses primed by irradiated ligand-transduced RMA cells. Mice were vaccinated with 5×10^6 irradiated RMA transductants or PBS. Two weeks later splenocytes were restimulated with irradiated RMA-Rae1β cells. **a**, Lysis of RMA cells. **b**, Target cell expression of Rae1 resulted in enhanced CTL lysis. **c**, **d**, Complement mediated-depletion of CD8 cells but not NK cells abrogates activity of effector cells from RMA-Rae1β vaccinated mice. **e**, Elevated percentage of RMA-specific IFN-γ producing effector $CD8^+$ $CD3^+$ T cells in mice primed with ligand-transduced RMA cells. Priming cells indicated at bottom. **f**, Expression of Rae1 by target cells enhances IFN-γ response. The enhancement was completely blocked by anti-NKG2D antibody. **g, h,** Enhanced lysis of target cells expressing Rae1β is blocked by anti-NKG2D antibody. **i**, CTL priming occurs in the absence of NK cells. Mice were depleted of $NK1.1^+$ or $CD8^+$ cells prior to and during vaccination with tumor cell transductants. Effector cells were tested for lytic activity and IFN-γ production versus RMA target cells. **j**, CTL from RMA-Rae1β vaccinated mice specifically recognize RMA cells and remain tolerant of syngeneic T cell blasts. Effector cells from RMA-Rae1β vaccinated mice were tested for lysis of the indicated tumor cells as well as Con A blasts from syngeneic mice. Data are representative of three experiments.

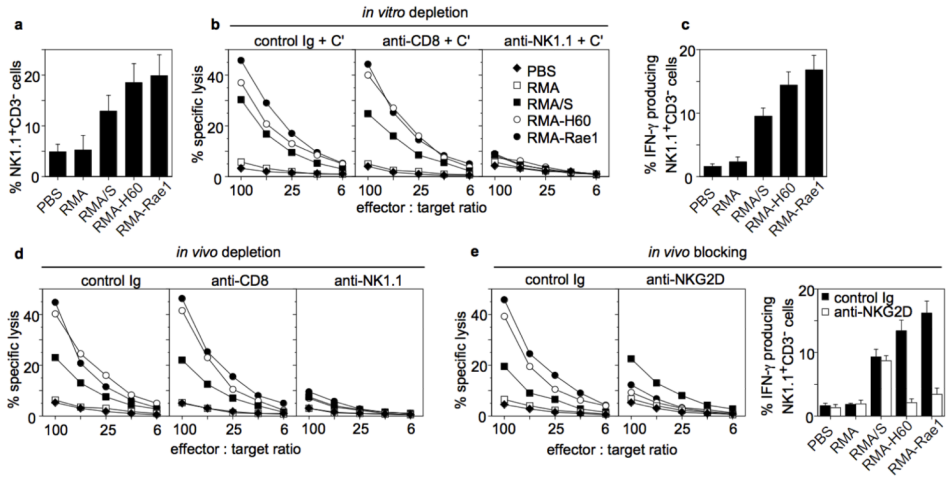

Figure 5.

RMA cells expressing NKG2D ligands stimulate NK cell recruitment and activation in vivo. Groups of five B6 mice were injected intraperitoneally with 5×10^6 irradiated RMA transductants, RMA/S cells or PBS. Peritoneal wash cells were recovered two days later. Compared to RMA cells, ligand-transduced RMA cells stimulated elevated percentages of NK ($NK1.1^+$ $CD3^-$) cells (**a**), which exhibited enhanced capacity to lyse YAC-1 target cells (**b**), and more of which produced IFN-γ when stimulated with YAC-1 target cells (**c**). Effector cells were destroyed by complement lysis with anti-NK1.1 but not anti-CD8 antibody (**b**), and pretreatment of mice with anti-NK1.1 antibody but not with anti-CD8 antibody prevented induction of cytotoxic activity (**d**). **e**, NK cell induction by ligand-transduced cells was blocked by injection of a non-depleting anti-NKG2D antiserum, but not by a control antiserum, just prior to tumor cell inoculation. The response to RMA/S cells was unaffected. Lysis of YAC-1 tumor cells and production of IFN-γ after stimulation with YAC-1 target cells were assayed. Data are representative of two experiments.