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Adenovirus serotype 5 E1A expressing tumor cells elicit a tumor-specific CD8+ T cell response independent of NKG2D

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ABSTRACT

The expression of the Adenovirus serotype 2 or serotype 5 (Ad2/5) E1A gene in tumor cells upregulates ligands that are recognized by the NKG2D activating receptor, which is expressed on NK cells and T cells, and reduces their tumorigenicity, a process dependent on NK cells and T cells. In some model systems, the forced overexpression of NKG2D ligands on tumor cells induced antigen-specific CD8+ T cells that mediated anti-tumor immunity. We wanted to determine if the interaction of NKG2D ligands on tumor cells that express E1A with NKG2D on immune cells contributed to the ability of E1A to induce a CD8+ T cell anti-tumor response or reduce tumorigenicity. To address these questions, we used the MCA-205 tumor cell line or MCA-205 cells that expressed Ad5 E1A (MCA-205-E1A cells), a fusion protein of E1A and ovalbumin (MCA-205-E1A-OVA) or OVA (MCA-205-OVA). We found that the expression of E1A or E1A–OVA, but not OVA, upregulated the expression of the NKG2D ligand RAE-1 on the surface of MCA-205 cells. Additionally, MCA-205-E1A cells and MCA-205-E1A-OVA cells were more sensitive to NK cell lysis than MCA-205 or MCA-205-OVA cells in WT B6 mice, but not NKG2D deficient B6 mice. Next, we adoptively transferred WT or NKG2D deficient OT-1 T cells (CD8 T cells that recognize OVA residues 257–264) into WT B6 mice or B6 mice that were deficient in NKG2D respectively and measured the expansion of OT-1 cells following immunization with MCA-205-E1A-OVA or MCA-205-OVA cells. We found that the expansion of OT-1 cells following immunization of either OVA-expressing MCA-205 cell lines was not affected by the presence or absence of NKG2D in B6 mice. Finally, we found that the capacity of E1A to reduce the tumorigenicity of MCA-205 cells was not impaired in B6-NKG2D deficient mice as compared to WT B6 mice. Our results suggest that the ability of E1A to reduce the tumorigenicity of MCA-205 cells, or induce an antigen-specific CD8+ T cell response, is independent of the interaction of NKG2D ligands with the NKG2D receptor.

1. Introduction

NKG2D is an activating receptor found primarily on NK cells and CD8+ T cells in rodents and humans [1]. The NKG2D ligands are a diverse group of proteins, which are upregulated in response to cellular stress, viral infection, cellular transformation, and DNA damage [2–6]. The engagement of NKG2D on NK cells with NKG2D ligands on the cell surface of target cells, leads to target cell killing and cytokine production by NK cells [1,3]. Ectopic expression of NKG2D ligands on tumor cell surfaces has been shown to significantly reduce the tumorigenicity of tumor cells in an NK cell dependent-manner [3,6–8]. In some tumor models, it appears that the ectopic expression of NKG2D ligands also induces antigen-specific, CD8+ anti-tumor immune responses [8]. How the interaction of NKG2D on CD8 T cells and NKG2D on target cells activates naïve CD8 T cells is unclear, as NKG2D does not appear to be able to directly activate CD8 T cells, but instead provides co-stimulatory signals [4,9–11].

Adenovirus serotype 2 or serotype 5 (Ad2/5) E1A (heretofore referred to as E1A), when stably expressed in tumor cells, reduces their tumorigenicity [12,13]. Both NK cells and T cells [14] mediate the reduction of tumorigenicity by E1A. Tumor cells stably expressing E1A are sensitive to NK mediated killing *in vitro* and *in vivo*, in part through the upregulation of NKG2D ligands [7]. In

Abbreviations: E1A, early region 1 A; Ad, adenovirus; NKG2D, natural killer group 2 D; MCA, methylcholanthrene; OVA, ovalbumin; NK, natural killer; WT, wildtype; RAE-1, retinoic acid early inducible; B6, C57BL/6; TPD_{50}, tumor producing dose 50

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B6-derived MCA-205 tumor cells, E1A has been shown to only increase the expression of the NKG2D ligands, RAE-1. In B6-RAG$^{-/-}$ mice lacking adaptive immune systems, blocking the interaction of NKG2D ligands with the NKG2D receptor, using a specific mAb, inhibited the lysis of MCA-205-E1A tumor cells *in vitro* and increased their tumorigenicity *in vivo* [7].

The purpose of this study was to determine if the interaction of NKG2D ligands on MCA-205 tumor cells that express E1A with NKG2D on immune cells contributed to the ability of E1A to induce an antigen-specific CD8+ T cell response. Furthermore, we sought to determine if the upregulation of RAE-1 by E1A decreased the tumorigenicity of MCA-205-E1A cells in WT mice. We found that NKG2D–NKG2D ligand interactions were dispensable for the expansion of antigen-specific CD8+ T cells and for the anti-tumorigenic activity of E1A in WT B6 mice.

2. Results

2.1. Role of interaction of NKG2D ligands and the NKG2D receptor in the NK cell lysis of E1A-expressing MCA-205 tumor cells

We previously demonstrated that the expression of E1A in the B6-derived tumor cell line, MCA-205 cells (MCA-205-E1A), upregulated the NKG2D ligand RAE1 and sensitized MCA-205 cells to NK-mediated killing [7]. First, we generated MCA-205 cell lines that expressed a slightly truncated $OVA_{3\text{-}383}$ protein (MCA-205-OVA) or a fusion protein of E1A and $OVA_{3\text{-}383}$ (MCA-205-E1A-OVA) [16]. Next, we determined if expression of E1A–OVA fusion protein induced the NKG2D ligand, RAE1 on the MCA-205 cells as well. We found that expression of E1A–OVA and E1A, but not OVA, induced expression of RAE1 in MCA-205 cells (Fig. 1A). Next, we determined if NKG2D deficient NK cells, compared to normal B6 NK cells, were impaired in their ability to kill MCA-205-E1A or MCA-205-E1A-OVA tumor cells in a 5 h *in vitro* NK killing assay. These results demonstrated that, in comparison to normal NK cells, NKG2D deficient NK cells were markedly impaired in their capacity to lyse both MCA-205-E1A (Fig. 1B) and MC-205-E1A-OVA cells (C). In fact, the ability of E1A or E1A–OVA to sensitize MCA-205 cells to NK cell killing was nearly lost when using NKG2D deficient NK cells as effector cells (Fig. 1B and C). Collectively, these data showed that the ability of E1A or E1A–OVA to sensitize MCA-205 cells was largely dependent on the interaction between NKG2D on NK cells and RAE-1 on MCA-205 cells.

2.2. Role of the interaction of NKG2D ligands and the NKG2D receptor in the induction of antigen specific CD8+ T cells

We next asked if the interaction of NKG2D ligands on MCA-205-OVA cells or MCA-205-E1A OVA cells with NKG2D on immune cells was required for the expansion of OVA-specific CD8+ T cells. To answer this question, we adoptively transferred NKG2D sufficient OT-1 cells or NKG2D deficient OT-I CD8+ T cells into B6 WT or NKG2D deficient B6 mice, respectively. Twenty-four hours after adoptive transfer of OT-1 cells, we injected MCA-205-OVA cells or MCA-205-E1A-OVA cells into the hock of B6 WT or NKG2D deficient B6 mice. After 5 days, the draining lymph nodes were removed, and OT-1 CD8 T cells were enumerated by flow cytometry (Fig. 1A and B). We found that the expansion of OT-1 cells following immunization of E1A–OVA or OVA-expressing MCA-205 cell lines was not affected by the presence or absence of NKG2D in B6 mice. Interestingly, immunization of WT or NKG2D deficient mice with MCA-205-OVA cells, which express little RAE-1, consistently led to a more robust expansion of OT-1 T cells compared to immunization with MCA-205-E1A-OVA (Fig. 2B). The mechanism for this result is under current investigation and is outside the goals of the current manuscript.

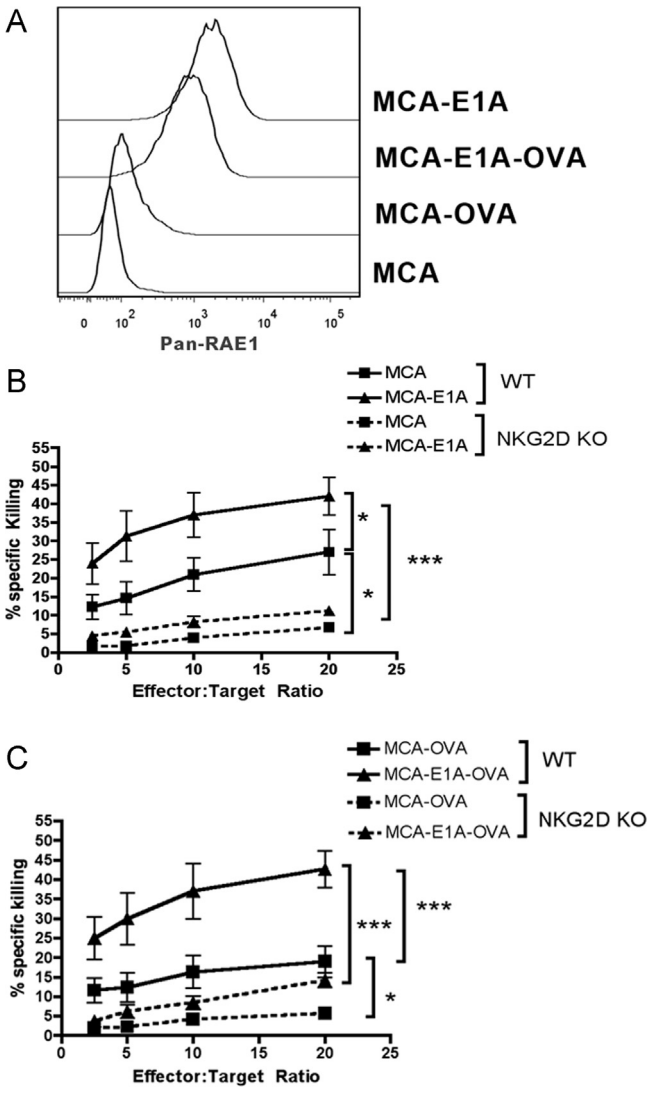

Fig. 1. Stable expression of E1A–OVA fusion protein in MCA-205 cells induced RAE1 expression and sensitized cells to NKG2D-dependent NK lysis. (A) Surface expression of RAE1 on MCA-205 cells stably transfected with E1A, OVA, or E1A–OVA was detected by flow cytometry. Representative histograms show the intensity of RAE1 staining. (B, C) MCA-205-E1A and MCA-205 cells (B) or MCA-205-E1A-OVA and MCA-205-OVA cells (C) were incubated with IL-2 activated B6 WT (solid) or NKG2D deficient (hashed) NK cells in a 5 h NK cytolysis assay and the specific lysis was determined. Data shown is the mean ± SEM from three independent experiments. Data were analyzed by ANOVA followed by Tukey's HSD *post hoc* analyses. * $p < 0.05$ and ** $p < 0.01$.

2.3. Role of interaction of NKG2D with NKG2D ligands in mediating tumor rejection of MCA-205 cells that express E1A or E1A–OVA

In previous studies using T cell deficient, B6-Rag$^{-/-}$ mice, we demonstrated that NK cells contribute to the rejection of MCA-205-E1A cells in an NKG2D–NKG2D ligand dependent manner [7]. Using this finding, we next addressed the role of NKG2D–NKG2D ligand interactions in the rejection of MCA-205 cells that express E1A in WT (T cell sufficient) mice or mice deficient in NKG2D (Fig. 3).

Tumorigenicity was measured by determining the tumor-producing dose 50 (TPD_{50}), which is the $\log_{10}$ of the number of tumor cells required to form tumors in half of the B6 mice (Material and methods). In previous studies, we demonstrated that the TPD_{50} of MCA-205-E1A cells and MCA-205-E1A-OVA cells were equivalent

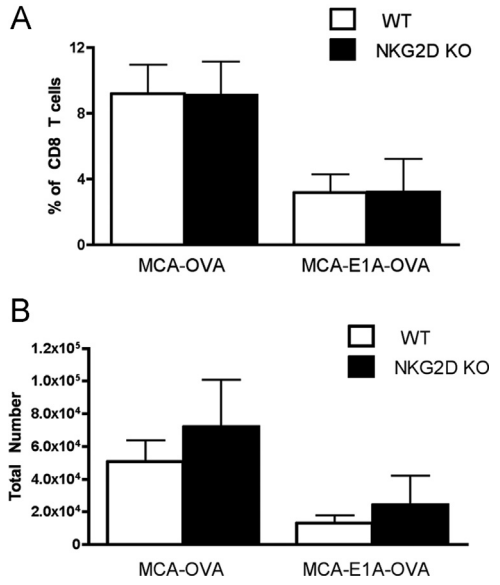

Fig. 2. NKG2D deficiency does not alter the expansion of OT-1 CD8 T cells in response to injection of MCA-205-E1A-OVA cells *in vivo*. B6 or NKG2D deficient mice were adoptively transferred with 1×10^5 CD45.1$^+$ OT-1 cells that were NKG2D sufficient or NKG2D deficient respectively and 24 h later administered MCA-205, MCA-205-OVA or MCA-205-E1A-OVA tumor cells s.c. in the flank. Five days later the draining lymph node was examined by flow cytometry for the percentage of OT-1 cells (A) and the total number of OT-1 cells (B). OT-1 cells were identified as CD45.1$^+$ CD3$^+$CD8$^+$ cells. Data shown is the mean ± SEM from two experiments, with three to six mice per group. Data were analyzed by ANOVA followed by Tukey's HSD *post hoc* analyses.

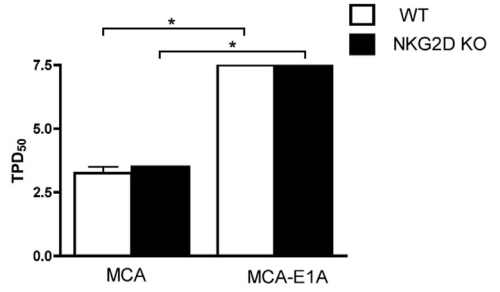

Fig. 3. NKG2D deficiency does not alter the tumorigenicity of MCA-205-E1A cells. Serial log dilutions of MCA-205 cells from 1×10^2–1×10^5 cells or MCA-205-E1A cells from 1×10^5–1×10^7 cells (3 mice/dose tumor cells) were injected s.c. into the flank of either WT B6 or NKG2D deficient mice. The TPD_{50} was calculated 12 weeks later. Data shown is the mean ± SEM from two experiments. Data were analyzed by a Student's *t*-test. * $p < 0.01$.

in B6 mice [16]. Furthermore, the ability of E1A and E1A–OVA to upregulate RAE-1 expression or sensitize MCA-205 cells to NK cell lysis was also equivalent (Fig. 1A–C). Therefore, to decrease the numbers of mice, only MCA-205 cells and MCA-205-E1A cells were used in these tumor induction studies. We found that the TPD_{50} of MCA-205-E1A cells were equivalent in WT B6 mice compared to mice that lacked NKG2D. Collectively, these studies suggest that NKG2D–NKG2D ligand interactions play a relatively minor role in the ability of E1A to reduce tumorigenicity in WT (T cell sufficient) mice.

3. Discussion

In this study, we investigated the role of the interaction of NKG2D ligands on tumor cells that express E1A and NKG2D on immune cells in elicitation of antigen-specific CD8+ T cells, and the anti-tumorigenic properties of E1A. Prior studies have shown that the reduction of tumorigenicity by E1A is dependent on NK cells and T cells [14]. Expression of E1A induces NKG2D ligands on the surface of cells, and NKG2D is expressed on NK cells and CD8+ T cells. The NK cell mediated killing of tumor cells that express E1A is dependent, at least in part, on the interaction between NKG2D–NKG2D ligands [7]. Therefore, it seemed possible that the interaction of NKG2D ligands with NKG2D could link the innate and CD8+ T cell response to E1A expressing tumor cells in mice.

We found that the expression of E1A, or a fusion protein of E1A and OVA, induced the expression of RAE-1 on MCA-205 cells (Fig. 1A). Furthermore, the lysis of MCA-205-E1A cells or MCA-205-E1A-OVA cells by NKG2D deficient NK cells was significantly decreased compared to NKG2D sufficient NK cells (Fig. 1B). These results are consistent with our prior studies and the large literature on the importance of the interaction of NKG2D ligands and NKG2D in the NK cell mediated lysis of specific target cells that express high levels of NKG2D ligands.

In contrast to NK cell mediated killing of tumor cells that express E1A, we found that the interaction of NKG2D ligands with NKG2D was dispensable for the expansion of OVA-specific CD8+ T cells following challenge with MCA-205-OVA cells or MCA-205-E1A-OVA cells (Fig. 2). Prior studies on the role of NKG2D–NKG2D interactions in the induction of CD8+ antigen-specific antitumor responses are mixed. Diefenbach et al. [8] found that the ectopic expression of NKG2D ligands on tumor cells reduced tumorigenicity and led to the induction of antigen-specific, CD8+ T cell antitumor immunity. Similar to our results, Cerwenka et al. found that the forced expression of NKG2D ligands on tumor cells reduced tumorigenicity, but did not enhance the generation of tumor-specific CD8+ T cell responses [3].

We hypothesize that MCA-205-OVA tumor cells induce a greater OVA-specific CD8+ T cell response than MCA-205-E1A-OVA cells due to increased tumor antigen load. The number of MCA-205-OVA cells used for these studies is well above the TPD_{50}, which would result in persistent and growing MCA-205-OVA tumor cells *in vivo*. Conversely, MCA-205-E1A-OVA cells are non-tumor forming and rapidly eliminated *in vivo*, which likely leads to reduced antigen load over the course of the experiment. OT-1 cell expansion similar to that of MCA-205-OVA cell immunization were observed with MCA-205-E1A-OVA cells when the number of cells inoculated was increased 10-fold, (data not shown), suggesting the amount of tumor antigen load may be responsible for the differences observed between MCA-205-OVA and MCA-205-E1A-OVA cells.

The reasons for the disparity in the role of NKG2D–NKG2D interactions in the elicitation of tumor-specific CD8+ T cell responses is unclear. It is possible that NKG2D signaling was dispensable in our model due to the presence of a strong foreign antigen, OVA. Natural tumor antigens may provide weaker activation signals than OVA, and therefore may require additional co-stimulation which could be provided by NKG2D signaling. MCA-205 is mildly immunogenic and therefore expresses an endogenous tumor antigen [23]. However, the tumorigenicity of either MCA-205 or MCA-205-E1A tumor cells was not increased in NKG2D sufficient mice compared to wild type mice. Collectively, these data suggest that in the MCA-205 model, the interaction of NKG2D with NKG2D ligands is dispensable for the anti-tumor immune response.

Finally, studies reported herein suggest that the reduction of tumorigenicity by E1A is largely independent of NKG2D–NKG2D ligand interactions in WT B6 mice. Previous work by our laboratory has shown that the rejection of tumor cells that express E1A in WT B6 mice is partially dependent on T cells. In RAG$^{-/-}$ B6 mice, blocking the interaction of NKG2D with NKG2D ligands increased the tumorigenicity of MCA-205-E1A cells [7]. Because the expansion of antigen-specific CD8+ T cells occurs in an NKG2D–

NKG2D ligand independent manner (Fig. 2), these results suggest that there is a redundant role for NK cells in WT B6 mice that is fulfilled by T cells in an NKG2D-independent manner.

Technical issues intrinsic to the MCA-205 model may contribute to the inability to detect a change in tumorigenicity in a WT B6 mice compared to B6 mice lacking NKG2D. Injection of MCA-205-E1A cells or MCA-205-E1A-OVA cells fail to cause tumor formation in a WT animal at the highest challenge dose (10^7 tumor cells), which is the highest inoculum we can give in a small volume, due to technical limitations. Thus, with a higher tumor cell burden, NKG2D dependent and independent mechanisms may be required for rejection of MCA-205 tumor cells that express E1A in WT B6 mice. This hypothesis is consistent with results demonstrating that that ectopic expression of NKG2D ligands on tumor cell lines reduces tumorigenicity [3,6–8]. Further investigations on the role of NKG2D–NKG2D ligand interactions in the reduction of tumorigenicity by E1A are needed to clarify this issue.

We found the ability of E1A to sensitize cells to NK cell mediated killing is largely NKG2D–NKG2D ligand dependent. In contrast, the interaction of NKG2D ligands with NKG2D appears to be dispensable for the ability of E1A to generate antigen specific CD8+ anti-tumor immune responses, and for the reduction of tumorigenicity in WT B6 mice. These findings are consistent with our previous work that showed that E1A may reduce tumorigenicity by modulation of tumor microenvironment through the inhibition of arginase 1 produced by tumor associated macrophages [16]. Arginase 1 produced in the tumor microenvironment results in a depletion of L-arginine, which is essential for T cell effector function [18]. Additional potential mechanisms whereby E1A may decrease tumorigenicity include sensitizing cells to immune effector molecules such as TNF-α, TRAIL, nitric oxide, perforin and fas-dependent killing [19–22].

In summary, we found that the ability of E1A to reduce the tumorigenicity of MCA-205 cells and elicit an antigen-specific CD8 T cell response is independent of the interaction of NKG2D ligands with NKG2D.

4. Materials and methods

4.1. Mice

WT C57BL6/J (B6), B6.129S7-Rag1tm1Mom/J ($RAG^{-/-}$), B6.SJL-Ptprca Pepcb/BoyJ (CD45.1) and C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-1) mice were purchased from the Jackson Laboratories. OT-1 mice express a transgene for a T cell receptor that recognizes ovalbumin (OVA) residues 257–264 in the context of H-$2K^b$. B6-$Klrk1^{tm1Dhr}$ ($NKG2D^{-/-}$) mice were kindly provided by David H. Raulet [15] (University of California, Berkeley, Berkeley, CA). $NKG2D^{-/-}$ mice were bred with $CD45.1^+$ OT-1^+ $RAG^{-/-}$ mice to generate $NKG2D^{-/-}CD45.1^+$ OT-1^+ $RAG^{-/-}$ mice. All animal work was reviewed and approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

4.2. Reagents

Roswell Park Memorial Institute (RPMI) medium with 5% Fetal Bovine Serum (FBS) (RPMI-5) or 10% FBS (RPMI-10) supplemented with Glutamax, glucose and antibiotics was used for all cell culture. FBS (Atlanta Biologicals) was heat inactivated for 45 min at 56 °C.

4.3. Flow cytometry

Flow cytometry was performed with an LSR II (BD biosciences) using BD FACSDiva software. Flow cytometry analysis was performed using FlowJo software (Tree Star). Antibodies specific to mouse CD3 (145-2C11) Alexa Fluor 488 (AF-488); Fluorescein (FITC), CD8α (5H-10) PE; Pacific Orange (PO), and CD45.1 (A20) Allophycocyanin (APC) were purchased from Biolegend. Pan-RAE1 (CX1) PE antibody was purchased from BD Biosciences.

4.4. Cell lines

MCA-205, a C57BL/6 derived fibrosarcoma cell line, was kindly provided by N. Restifo (National Cancer Institute, National Institutes of Health, Bethesda, MD). MCA-205 lines that stably expressed E1A–OVA (MCA-205-E1A-OVA), or OVA (MCA-205-OVA) were generated as previously described [16].

4.5. NK cell cytolysis assays

Mouse NK cells were enriched from either B6 or NKG2D deficient splenocytes by nylon wool enrichment as previously described [17] and used in a standard 5 h ^{51}Cr release assay [7]. NK purity was confirmed by flow cytometry, and only NK cell preparations with greater than 90% NK cells were used.

4.6. Quantification of OVA specific CD8 +T cells

OT-1 transgenic CD8+ T cells were harvested from the spleens of either $NKG2D^{+/+}CD45.1^+$ OT-1^+ $RAG^{-/-}$ mice or $NKG2D^{-/-}CD45.1^+$ OT-1^+ $RAG^{-/-}$ mice. 1×10^5 $NKG2D^{+/+}CD45.1^+$ OT-1^+ cells or $NKG2D^{-/-}CD45.1^+$ OT-1^+ cells were adoptively transferred *via* retro-orbital injection into WT or $NKG2D^{-/-}$ B6 mice respectively. The following day, mice were immunized with 1×10^5 live MCA-205-OVA or MCA-205-E1A-OVA cells subcutaneously (s.c.) in the hock (the lateral tarsal region just above the ankle). Five days after tumor cell injection, the popliteal lymph nodes were removed from WT or $NKG2D^{-/-}$ B6 mice and the $CD45.1^+$ OT-I cells were quantitated by flow cytometry by staining for $CD45.1^+$ $CD3^+$ $CD8^+$ T cells. The absolute number of CD8+ T cells was determined by multiplying the percentage of the target cell population by the total number of cells in the lymph node.

4.7. Tumor induction studies

Quantitative tumor inductions studies were performed as previously described [7]. Briefly, B6 or NKG2D deficient mice were administered serial log dilutions of MCA-205 or MCA-205-E1A tumor cells s.c. in a total volume of 0.2 cc in the flank of WT B6 or NKG2D and then monitored for tumor growth. Animals were euthanized when tumors reached 20 mm in diameter, or at the end of a 12-week monitoring period. Tumor producing dose 50 (TPD_{50}) values, which are the $\log_{10}$ of the number of cells required to form tumors, were calculated using the Spearman–Karber Formula. Due to technical limitations, 10^7 was the highest number of tumor cells that could be given in 0.2 cc volume. Therefore, if tumors did not occur at 10^7 tumor cells, the TPD_{50} was estimated 0.5 logs higher than the highest attainable dose (*e.g.* $TPD_{50}=7.5$).

4.8. Statistics

Statistical differences between groups were calculated using the ANOVA and Dunnett multiple comparison tests. Mann–Whitney tests were used to compare two sets of data. Any p values less than 0.05 were considered significant. Unless otherwise stated, all data are presented as mean ± standard error of mean (SEM). Statistical analysis was done using Prism version 5.0a software (GraphPad Software) (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

Authors' contributions

MK performed the studies and prepared the manuscript. JR conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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References

[1] Jamieson, Diefenbach, McMahon, Xiong, Carlyle, Raulet. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity 2002;17(1):19–29. http://dx.doi.org/10.1016/S1074-7613(02)00333-3. 12150888.

[2] Wu, Song, Bakker, et al. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 1999;285(5428):730–2. http://dx.doi.org/10.1126/science.285.5428.730. 10426994.

[3] Cerwenka, Bakker, McClanahan, et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. Immunity 2000;12(6):721–7. http://dx.doi.org/10.1016/S1074-7613(00)80222-8. 10894171.

[4] Groh, Rhinehart, Randolph-Habecker, Topp, Riddell, Spies. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. Nature Immunology 2001;2(3):255–60. http://dx.doi.org/10.1038/85321. 11224526.

[5] Gasser, Orsulic, Brown, Raulet. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005;436(7054):1186–90. http://dx.doi.org/10.1038/nature03884. 15995699.

[6] Diefenbach, Jamieson, Liu, Shastri, Raulet. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nature Immunology 2000;1(2):119–26. http://dx.doi.org/10.1038/77793. 11248803.

[7] Routes, Ryan, Morris, Takaki, Cerwenka, Lanier. Adenovirus serotype 5 E1A sensitizes tumor cells to NKG2D-dependent NK cell lysis and tumor rejection. Journal of Experimental Medicine 2005;202(11):1477–82. http://dx.doi.org/10.1084/jem.20050240. 16314433.

[8] Diefenbach, Jensen, Jamieson, Raulet. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001;413(6852):165–71. http://dx.doi.org/10.1038/35093109. 11557981.

[9] Bauer, Groh, Wu, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible mica. Science 1999;285(5428):727–9. http://dx.doi.org/10.1126/science.285.5428.727. 10426993.

[10] Ehrlich, Ogasawara, Hamerman, et al. Engagement of NKG2D by cognate ligand or antibody alone is insufficient to mediate costimulation of human and mouse CD8+ T cells. Journal of Immunology 2005;174(4):1922–31. http://dx.doi.org/10.4049/jimmunol.174.4.1922. 15699119.

[11] Rajasekaran, Xiong, Fong, Gorski, Malarkannan. Functional dichotomy between NKG2D and CD28-mediated co-stimulation in human CD8+ T cells. PLOS One 2010;5(9). http://dx.doi.org/10.1371/journal.pone.0012635. 20844584.

[12] Walker, Wilson, Lewis Jr. AM, Cook. E1A oncogene induction of cytolytic susceptibility eliminates sarcoma cell tumorigenicity. Proceedings of the National Academy of Sciences of the United States of America 1991;88(15):6491–5. http://dx.doi.org/10.1073/pnas.88.15.6491. 1830664.

[13] Cook, Krantz, Routes. Role of p300-family proteins in E1A oncogene induction of cytolytic susceptibility and tumor cell rejection. Proceedings of the National Academy of Sciences of the United States of America 1996;93(24):13985–90. http://dx.doi.org/10.1073/pnas.93.24.13985. 8943047.

[14] Routes, Ryan, Li, Steinke, Cook. Dissimilar immunogenicities of human papillomavirus E7 and adenovirus E1A proteins influence primary tumor development. Virology 2000;277(1):48–57. http://dx.doi.org/10.1006/viro.2000.0571. 11062035.

[15] Guerra, Tan, Joncker, et al. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity 2008;28(4):571–80. http://dx.doi.org/10.1016/j.immuni.2008.02.016. 18394936.

[16] Korrer, Routes. Possible role of arginase-1 in concomitant tumor immunity. PLOS One 2014;9(3):e91370. http://dx.doi.org/10.1371/journal.pone.0091370. 24614600.

[17] Rajasekaran, Kumar, Schuldt, et al. Signaling by Fyn-ADAP via the Carma1-Bcl-10-MAP3K7 signalosome exclusively regulates inflammatory cytokine production in NK cells. Nature Immunology 2013;14(11):1127–36. http://dx.doi.org/10.1038/ni.2708. 24036998.

[18] Rodriguez, Quiceno, Ochoa. L-arginine availability regulates T-lymphocyte cell-cycle progression. Blood 2007;109(4):1568–73. http://dx.doi.org/10.1182/blood-2006-06-031856. 17023580.

[19] Cook, Walker, Lewis Jr AM, Ruley, Graham, et al. Expression of the adenovirus E1A oncogene during cell transformation is sufficient to induce susceptibility to lysis by host inflammatory cells. Proceedings of the National Academy of Sciences of the United States of America 1986;83(18):6965–9. http://dx.doi.org/10.1073/pnas.83.18.6965. 2944117.

[20] Cook, May, Wilson, Holskin, Chen, et al. Role of tumor necrosis factor-alpha in E1A oncogene-induced susceptibility of neoplastic cells to lysis by natural killer cells and activated macrophages. Journal of Immunology 1989;142(12):4527–34. 2723438.

[21] Miura, Morris, Ryan, Cook, Routes. Adenovirus E1A, not human papillomavirus E7, sensitizes tumor cells to lysis by macrophages through nitric oxide- and TNF-alpha-dependent mechanisms despite up-regulation of 70-kDa heat shock protein. Journal of Immunology 2003;170(8):4119–26. http://dx.doi.org/10.4049/jimmunol.170.8.4119. 12682242.

[22] Routes, Ryan, Clase, Miura, Kuhl, et al. Adenovirus E1A oncogene expression in tumor cells enhances killing by TNF-related apoptosis-inducing ligand (TRAIL). Journal of Immunology 2000;165(8):4522–7. http://dx.doi.org/10.4049/jimmunol.165.8.4522. 11035092.

[23] Barth, Bock, Mulé, Rosenberg. Unique murine tumor-associated antigens identified by tumor infiltrating lymphocytes. Journal of Immunology 1990;144(4):1531–7. 2303716.