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Effective TRAIL-based immunotherapy requires both plasmacytoid and CD8 α dendritic cells

Britnie R. James^{*,†}, Erik L. Brincks^{*}, Tamara A. Kucaba^{*}, Louis Boon[†], and Thomas S. Griffith^{*,†,§,¶}

^{*}Department of Urology, University of Minnesota, Minneapolis, MN 55455, USA [†]Department of Microbiology, Immunology, and Cancer Biology Graduate Program, University of Minnesota, Minneapolis, MN 55455, USA [§]Center for Immunology, University of Minnesota, Minneapolis, MN 55455, USA [¶]Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA [†]Bioceros Holding BV, Utrecht, The Netherlands

Abstract

It is now appreciated there are distinct subsets of dendritic cells (DC) with specialized functions. Plasmacytoid DC (pDC) and CD8 α DC can contribute to the priming, activation, and function of antitumor CD8 T cells; however, their specific roles and necessity in stimulating antitumor immunity are not clearly understood. We examined the importance of pDC and CD8 α DC during immunotherapy of an orthotopic model of metastatic renal cell carcinoma (RCC). Immunotherapy that utilizes a recombinant adenovirus encoding TNF-related apoptosis-inducing ligand (Ad5-TRAIL) in combination with an immunostimulatory CpG-containing oligodeoxynucleotide (CpG) resulted in the clearance of primary and metastatic tumors in wild-type (WT) replete BALB/c mice and prolonged survival. In comparison, mice deficient in either pDC (accomplished by using a depleting mAb specific for PDCA1) or CD8 α DC (through utilization of CD8 DC-deficient *Batf3*^{-/-} BALB/c mice) had uncontrolled tumor growth and high mortality after Ad5-TRAIL/CpG administration. The ineffectiveness of Ad5-TRAIL/CpG therapy in the anti-PDCA1-treated and *Batf3*^{-/-} BALB/c mice was marked by an altered activation phenotype of the DC, as well as significantly reduced expression of type I IFN-stimulated genes and IL-15/IL-15R complex production. In addition, pDC-depleted and *Batf3*^{-/-} BALB/c mice had significantly decreased effector CD8 T cell infiltration in the primary tumor site compared to WT mice after therapy. These data collectively suggest that pDC and CD8 α DC carry out independent, but complementary, roles that are necessary to initiate an efficacious antitumor immune response after Ad5-TRAIL/CpG therapy.

Keywords

TRAIL; dendritic cells; immunotherapy; RCC

Address correspondence to Dr. Thomas S. Griffith, Department of Urology, University of Minnesota, 3-125 CCRB, 2231 6th St. SE, Minneapolis, MN 55455, USA. tgriffit@umn.edu.

DISCLOSURES

B. James, E. Brincks, T. Kucaba, and T. Griffith declare that they have no conflict of interest. L. Boon is Chief Scientific Officer at Bioceros Holding BV.

INTRODUCTION

Immune-based therapy for solid tumors provides the potential for long-lived protection against cancers of various stages—including metastatic cancer [1]. The development of successful cancer immunotherapeutic protocols depends on identifying the mechanisms that efficiently prime and activate effector cells within the immune system. One goal of many immunotherapeutic protocols is the generation of a systemic antitumor T cell response, which largely depends on the proper function of antigen presenting cells (APC). Dendritic cells (DC) are the most potent APC population that capture, process, and present tumor-derived Ag to T cells via MHC I or II [2,3]. When presented with tumor-derived Ag in combination with co-stimulation and immunostimulatory cytokines, tumor-specific T cells differentiate into effectors that can directly kill tumor cells [2,4,5]. Because DC are essential for initiating antitumor T cell responses, understanding the roles played by specific DC subsets becomes paramount for optimizing immunotherapeutic protocols. Plasmacytoid DC (pDC) and CD8 α DC are two DC subsets crucial in the generation of robust antitumor responses [4,6]. pDC are recognized as the primary producers of type I interferon (IFN) after stimulation with various proteins or molecules containing pathogen- or damage-associated molecular patterns [6,7], while CD8 α DC cross-present Ag from exogenous sources (e.g., apoptotic tumor cells) in MHC I to CD8 T cells [8,9].

The tumor necrosis factor (TNF) superfamily member TNF-related apoptosis-inducing ligand (TRAIL) is best known for its ability to selectively induce apoptosis in tumor cells [10,11]. Moreover, systemically-administered recombinant TRAIL protein and TRAIL death receptor-specific agonistic mAb have been evaluated preclinically and in clinical trials for direct tumoricidal potential [12]. Our laboratory was the first to describe the utility of a recombinant replication-defective adenovirus encoding TRAIL (Ad5-TRAIL) delivered locally into a tumor [13,14]. When combined with immunostimulatory CpG-containing oligodeoxynucleotides (CpG), Ad5-TRAIL induces a systemic antitumor T cell response in mice bearing localized subcutaneous Renca tumors and in a mouse model of advanced renal cell carcinoma where an orthotopic primary Renca tumor gives rise to lung metastases [15,16].

CD8 T cells are required for the complete eradication of the tumors in these models, suggesting the need for DC to cross-present tumor-derived Ag. In addition, one expected function of the CpG is to stimulate DC maturation for enhanced MHC/costimulatory molecule expression and inflammatory cytokine production. To address the specific necessity and function of pDC in the efficacy of the Ad5-TRAIL/CpG therapy, mice orthotopically challenged with Renca tumors were depleted of pDC using a mAb against PDCA1 [17]. We also examined the necessity and function of CD8 α DC by examining the efficacy of Ad5-TRAIL/CpG immunotherapy in Renca-bearing *Batf3*^{-/-} BALB/c mice [18]. We hypothesized that both pDC and CD8 α DC would be required for the induction of antitumor immunity and the subsequent clearance of tumor. Data is presented to support this hypothesis, and the implications of these findings in regard to the development of current and future immunotherapeutic protocols are discussed.

Materials and Methods

Animals

Female wild-type BALB/c mice (7–8 wk old) and *Batf3*^{-/-} BALB/c breeder mice were purchased from the National Cancer Institute (Frederick, MD) and Jackson Laboratories (Bar Harbor, ME), respectively. *Batf3*^{-/-} BALB/c mice were housed and bred under pathogen-free conditions at the AALAC-accredited University of Minnesota Animal Care Facility. All animal procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Cell lines

The murine renal adenocarcinoma cell line, Renca [19], was obtained from Dr. Robert Wiltout (National Cancer Institute, Frederick, MD). Renca cells were maintained in RPMI supplemented with 10% FBS, penicillin, streptomycin, sodium pyruvate, non-essential amino acids, 2-mercaptoethanol, and HEPES (a.k.a. complete RPMI) [15,16]. Renca-GLE stably expresses firefly Luciferase and green fluorescent protein (GFP), and was obtained from Dr. Andrew Wilber (Southern Illinois University School of Medicine, Springfield, IL). Renca-GLE cells were maintained in complete RPMI supplemented with 0.3 µg/ml puromycin and 300 µg/ml zeosin.

Tumor challenge

For intrarenal (IR) tumor challenge, mice were anesthetized, a skin incision was made on the left flank, and 2×10^5 Renca or Renca-GLE cells were injected through the intact peritoneum into the left kidney in a 100 µl volume of HBSS [16,20]. pDC depletion was accomplished with 2 consecutive doses of anti-PDCA1 mAb (clone 120G8 [21]) at 500 µg/mouse [17] in 100 µl PBS i.p. on d 5 and 6 after tumor challenge. Mice treated with rat IgG1 anti-βGAL mAb (clone GL113) served as controls for pDC-depleted mice. On d 7, mice were re-injected in the same kidney with sterile PBS, Ad5-TRAIL (10^9 pfu), and/or the nonmethylated CpG ODN 1826 (5'-tccatgacgttcctgacgtt-3', 100 µg; IDT, Coralville, IA) in a 100 µl volume. Renal and metastatic tumor burden was measured via bioluminescence imaging using a Xenogen IVIS Spectrum [16,20]. Renca-GLE generated radiance (photons/second/cm²) was calculated within a defined region of interest using Living Image software (Version 2.5). For end time-point experiments, renal tumor burden was measured via weight (g) between d 18–21 when mice became moribund.

Flow cytometry

Spleens, tumor-bearing kidneys, and contralateral kidneys were harvested, homogenized into a single cell suspension via gentleMACS Dissociator (Miltenyi BioTeck Inc., Auburn, CA), and then digested for 15–30 min in HBSS containing 0.56 Wünsch units/ml Liberase Blendzyme 3 (Roche, Branford, CT) and 0.15 mg/ml DNase I (Sigma, St. Louis, MO). Cells were then blocked with anti-CD16/32 and normal mouse serum prior to staining with the following mAb (eBioscience (San Diego, CA) or BioLegend (San Diego, CA)) and analyzed on a BD LSR II (BD Biosciences, San Diego, CA) and FlowJo software (TreeStar Inc., Ashland, OR): DC – anti-CD11c-APC/Cy7 or -PE/Cy7, I-A^d-PacBlue or -PE, CD45R/

B220-APC/Cy7 or -PacBlue, CD317-APC or -FITC, CD8-PE-Cy7, CD40-PE, CD86-PE/Cy5, CD80-BV605; T cells – anti-CD3-PerCP/Cy5.5, CD4-PE/Cy7, CD8-PacBlue, CD44-PE or -AF700, KI-67-AF647, CD62L-PE, CD45.2-PE and -BV650. Intracellular staining for Foxp3 was done, as a dump gate, using a Foxp3 staining kit (eBioscience).

To specifically identify leukocytes within the tumor-bearing kidney tissue or kidney vasculature, intravascular (i.v.) staining was done [22]. Briefly, mice were injected i.v. with 3 µg of PE-conjugated anti-CD45.2 mAb 3 min before death and prepping the organs for flow cytometric analysis as described above. Additionally, cells were stained *ex vivo* with BV650-conjugated anti-CD45.2 mAb. Dual staining of CD45.2 discriminates cells within the vasculature (CD45.2 PE⁺ and BV650⁺) or the tissue (CD45.2 PE⁻ and BV650⁺) at the time of harvest. Blood and inguinal lymph nodes served as positive and negative controls for i.v. staining, respectively.

Quantitative real-time PCR (qPCR)

Tumor-bearing kidneys were harvested 4 h after treatment, and homogenized via gentleMACS Dissociator. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), and 1 µg was reverse-transcribed using Superscript III. Resulting cDNA was used as a template for qPCR using TaqMan primer/probe sets for *Cxcl10*, *Ifit*, *Irf7*, *Isg15*, *Mda5*, *Mx1*, and 18s rRNA (PE Applied Biosystems, Foster City, CA).

IL-15/IL-15R complex quantitation by ELISA

Tumor-bearing kidneys were harvested 24 h after treatment and homogenized in Cell Extraction Buffer (Invitrogen, Grand Island NY) supplemented with Complete Mini protease inhibitor (Roche Applied Science, Indianapolis, IN) and 1 mM PMSF (Cell Signaling Technologies, Danvers, MA). Samples were incubated on ice for 30 min, spun at 3000g at 4°C for 10 min, and resulting supernatants were frozen at -80°C until use. The amount of IL-15/IL-15R complex was determined via ELISA (eBioscience).

Statistical Analysis

Statistical analysis between groups was determined by 1-way ANOVA with a Tukey post hoc test or unpaired Student's *t*-test, where appropriate. Statistical analyses of tumor growth kinetics and survival between groups were performed via 2-way ANOVA, and Log-rank tests, respectively. Data was analyzed with Prism4 GraphPad software and statistical significance is indicated in figure legends (*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$; not significant (n. s.)).

Results

DC-depleted mice succumb to tumor despite receiving Ad5-TRAIL/CpG therapy

Different DC populations play synergistic and mutually exclusive roles during the initiation of an antitumor immune response [2,17,23,24]. To determine the *in vivo* significance of pDC and CD8α DC in the efficacy of Ad5-TRAIL/CpG therapy in a model of advanced RCC, we treated WT BALB/c mice with anti-PDCA1 mAb (clone 120G8 [17]), which depletes pDC, and utilized *Batf3*^{-/-} BALB/c mice, which are deficient in CD8α DC [18]. Renca is derived

from a spontaneously arising renal adenocarcinoma in BALB/c mice[25], and is widely used to model RCC. The H-2K/D^d restriction of this model consequently limited us to using *Batf3*^{-/-} BALB/c mice; to our knowledge there are no mice on the BALB/c background genetically engineered to be deficient in pDC. Tumor-free WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice showed normal development of splenic conventional DC (CD11c⁺ MHC II⁺) and CD8⁺ T cells, while having the expected reduction in frequency and number of pDC and CD8 α DC (Fig. 1). Both pDC-depleted mice and *Batf3*^{-/-} mice also showed normal development and maintenance of the converse DC subset, and we did not detect any major shifts in the major immune populations (i.e., T cells or B cells [26–28]) shown to upregulate PDCA-1 (CD317) in certain settings in the anti-PDCA1-treated mice (data not shown), although smaller effects on such subsets cannot be completely excluded. The degree of pDC and CD8 α DC depletion observed in tumor-free mice was also obtained in tumor-bearing mice (data not shown). The use of *Batf3*^{-/-} BALB/c mice proved to be superior to administering a depleting anti-CD8 mAb, as mice given this mAb had significant reductions in both CD8 α DC and CD8 T cells. Together, these mice and reagents gave us the ability to examine the therapeutic efficacy of Ad5-TRAIL/CpG in Renca-bearing mice missing either pDC or CD8 α DC, while maintaining an intact CD8 T cell compartment.

Ad5-TRAIL/CpG therapy induces a systemic antitumor T cell response in RCC-bearing mice capable of clearing local and metastatic tumors and prolonging survival [15,16]. Use of Ad5-TRAIL or CpG individually can slightly decrease tumor burden, but only the Ad5-TRAIL/CpG combination significantly reduces tumor burden and enhances survival [15,16]. These data suggested that Ad5-TRAIL-induced death creates the necessary tumor Ag debris that is taken up by DC and presented to tumor-specific T cells, while CpG serves to activate the DC for optimal Ag presentation and T cell stimulation [15]. As our previous study did not specifically investigate the importance of pDC and CD8 DC in the Ad5-TRAIL/CpG-induced tumor eradication, we compared tumor outgrowth and survival of WT replete, pDC-depleted, or *Batf3*^{-/-} BALB/c mice following treatment with PBS or Ad5-TRAIL/CpG therapy. (Note – we did not treat mice with Ad5-TRAIL or CpG alone as they proved to be suboptimal therapy in our previous studies.) As before, WT replete mice given Ad5-TRAIL/CpG displayed significantly suppressed tumor outgrowth, decreased frequency of mice with lung metastases, and prolonged survival compared to mice given PBS (Fig. 2A–D). In contrast, mice devoid of either pDC or CD8 α DC displayed significant primary and metastatic tumor outgrowth and these groups had no survival benefit after Ad5-TRAIL/CpG therapy. Together, these data demonstrate the necessity of both pDC and CD8 α DC in the efficacy of Ad5-TRAIL/CpG therapy in this model of advanced RCC.

Phenotype of pDC and CD8 DC in *Batf3*^{-/-} and anti-PDCA1-treated mice following Ad5-TRAIL/CpG therapy

Given the lack of efficacy of Ad5-TRAIL/CpG therapy in the anti-PDCA1-treated and *Batf3*^{-/-} mice, we initially investigated the phenotype of pDC from *Batf3*^{-/-} BALB/c and CD8 DC from anti-PDCA1-treated mice after Ad5-TRAIL/CpG therapy. A number of reports have described the importance for pDC/CD8 DC crosstalk during an immune response, suggesting that one DC subset can “license” another DC subset [5,23,24]. Most notably, pDC can specifically license myeloid DC and CD8 α DC to process and present Ag

to CD8 T cells. With this in mind, we hypothesized that the absence of pDC would lead to decreased activation status in the CD8 α DC compartment. However, there is little-to-no published data describing alterations in pDC activation as a result of a lack in CD8 α DC. Splenocytes from tumor-bearing WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice were analyzed via flow cytometry 24 h after PBS or Ad5-TRAIL/CpG treatment to examine the activation status of the remaining endogenous DC populations. Interestingly, pDC from *Batf3*^{-/-} Balb/c mice exhibited a blunted activation phenotype 24 h after Ad5-TRAIL/CpG administration, as seen by significantly decreased CD40, CD80, and CD86 expression (Fig. 3A). Expression of these same proteins on CD8 α DC in pDC-depleted mice was unchanged compared to WT replete mice (Fig. 3B). These results suggest that pDC maturation after Ad5-TRAIL/CpG therapy is dependent on cellular and/or soluble cues from CD8 α DC, but the phenotypic maturation of CD8 α DC does not require the presence of pDC.

pDC-depleted and *Batf3*^{-/-} BALB/c mice have an altered cytokine signature after Ad5-TRAIL/CpG therapy

An early hallmark of both adenovirus type 5 (Ad5) infection and CpG stimulation is the induction of type I IFN cytokines, and both pDC and CD8 α DC can make type I IFN after Ad5 and CpG stimulation [29–31]. Quantitation of type I IFN (be it IFN or IFN) can be difficult from tissue or serum samples in some settings because the cytokines are quickly sequestered by the interferon receptor (IFNAR), reducing the amount a free cytokine below the level of detection of commonly used assays (such as ELISA). To overcome this potential obstacle, we utilized quantitative real-time PCR (qPCR) to measure mRNA expression of a panel of type I IFN-stimulated genes (*Cxcl10*, *Ifit*, *Irf7*, *Isg15*, *Mda5*, and *Mx1*) within the tumor-bearing kidney of WT replete mice 4 h after injection of PBS, Ad5-TRAIL, and/or CpG. Mice treated with both Ad5-TRAIL/CpG had the greatest increase in *cxcl10* and *irf7* mRNA expression compared to mice injected with PBS, Ad5-TRAIL alone, or CpG alone (Fig. 4A–B). A similar trend was seen for *ifit*, *isg15*, *mda5*, and *mx1* (data not shown). These results support our previous data demonstrating the superior therapeutic efficacy of Ad5-TRAIL/CpG over either component alone. We then examined the expression of the 6 type I IFN-stimulated genes in WT replete, pDC-depleted, and *Batf3*^{-/-} BALB/c mice 4 h after PBS or Ad5-TRAIL/CpG injection. Tumor-bearing WT replete mice that received Ad5-TRAIL/CpG had substantial mRNA upregulation of the 6 type I IFN-stimulated genes when normalized to tumor-free controls (Fig. 4C–H). By comparison, Ad5-TRAIL/CpG-treated *Batf3*^{-/-} BALB/c mice had significantly reduced induction of mRNA for these genes, and there was a decreased trend in mRNA expression in similarly treated pDC-depleted mice. The data in Figure 4 suggest alterations in the type I IFN response after Ad5-TRAIL/CpG injection contribute to the reduced efficacy of therapy.

Type I IFNs can induce DC production of IL-15, and DC production of proinflammatory cytokines (including IFN) after CpG-stimulation is dependent on IL-15 [24,32]. Further, the presence of endogenous or exogenous IL-15 during a tumor challenge promotes an antitumor CD8 T cell response capable of eradicating established tumors [33]. When we measured the amount of IL-15/IL-15R complex within tumor-bearing kidneys of WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice after Ad5-TRAIL/CpG

administration, we found a significant increase in the amount of IL-15/IL-15R complex within the kidneys of WT replete mice over PBS-treated mice and when compared to anti-PDCA1-treated or *Batf3*^{-/-} BALB/c mice (Fig. 5). Collectively, the data in Figures 4 and 5 suggest both pDC and CD8 DC are required for the Ad5-TRAIL/CpG-induced production of proinflammatory cytokines important for stimulating antitumor T cell responses.

DC-depleted mice have a blunted CD8 T cell response after Ad5-TRAIL/CpG therapy

pDC and CD8 α DC have been implicated in the activation and expansion of tumor-specific CD8 T cells, and a CD8 T cell response is induced with Ad5-TRAIL/CpG therapy [15,16]. The inability of Ad5-TRAIL/CpG therapy to control tumor outgrowth in pDC- and CD8 α DC-deficient mice along with the decreased production of proinflammatory cytokines and DC maturation prompted us to evaluate CD8 T cell infiltration into tumor-bearing kidneys following therapy. As no Renca-specific tumor Ag have been identified to our knowledge, we determined the frequency of “effector phenotype” CD8 T cells (CD3⁺CD8⁺CD44^{hi}) in the tumor-bearing kidneys of WT replete, anti-PDCA1-treated, or *Batf3*^{-/-} BALB/c mice 5 d after receiving Ad5-TRAIL/CpG therapy. There was a significant increase in the frequency of CD3⁺CD8⁺CD44^{hi} cells in kidneys from Ad5-TRAIL/CpG-treated WT replete tumor-bearing mice compared to PBS-treated mice when calculated from total live cells (Fig. 6A) or from total CD45.2⁺ leukocytes (Fig. 6B). Similar increases in frequency in CD3⁺CD8⁺CD44^{hi} cells were not seen within the tumor-bearing kidneys from Ad5-TRAIL/CpG-treated pDC-depleted or *Batf3*^{-/-} BALB/c mice. It is important to note that the CD8 T cell expansion seen in the WT replete mice was not in response to adenovirus, as mice treated with Ad5-TRAIL alone did not display this increase in CD8 T cell infiltration (data not shown). The diminished infiltration of CD8 T cells into the kidneys of tumor-bearing anti-PDCA1-treated mice after Ad5-TRAIL/CpG therapy was also not due to anti-PDCA1-mediated depletion of activated (IFN-stimulated) CD8 T cell (data not shown), as suggested by fact that this mAb can deplete stromal cells and IFN-stimulated hematopoietic cells [26].

Our orthotopic RCC model is based on injection of Renca cells into the kidney to form the primary tumor. Kidneys are highly vascular organs, making it likely that evaluation of leukocytes from tumor-bearing kidneys would include T cells “localized” within the kidney tissue and present in the kidney vasculature (i.e., in the circulating blood) at the time of organ harvest. Thus, we used an intravascular staining technique [22], in which PE-conjugated anti-CD45.2 mAb was injected i.v. immediately prior to tumor-bearing kidney harvest to label cells within the vasculature of the kidney at the time of harvest. The cells were then stained *ex vivo* with a BV650-conjugated anti-CD45.2 mAb, allowing for the clear distinction between tissue (BV650⁺PE⁻) vs. blood (BV650⁺PE⁺) T cells. Using this method, it was determined that ~90% or more of the CD45.2⁺CD3⁺CD8⁺CD44^{hi} T cells were localized within the kidney tissue at the time of harvest – regardless of the experimental group (Fig. 6C & D).

Subsequent analysis of the CD45.2⁺CD3⁺CD8⁺CD44^{hi} T cells localized within the kidney at the time of harvest revealed a significant reduction in the frequency of Ki-67⁺ cells from Ad5-TRAIL/CpG-treated pDC-depleted or *Batf3*^{-/-} BALB/c mice (Fig. 6E). CD11a is upregulated on Ag-experienced CD8 T cells [34] and is also crucial for CD8 T cell-mediated

killing of Renca cells [35]. There was a significant reduction in CD11a expression on the tumor-infiltrating CD45.2⁺CD3⁺CD8⁺CD44^{hi} T cells from Ad5-TRAIL/CpG-treated pDC-depleted and *Batf3*^{-/-} BALB/c mice compared to WT replete mice (Fig. 6F). Collectively, these data demonstrate that the absence of pDC or CD8 α DC results in the abrogation of the Ad5-TRAIL/CpG therapy-induced CD8 T cell immune response essential for the eradication of tumor cells in this model of advanced RCC.

Discussion

The ability to stimulate antitumor immunity is the primary goal of many active clinical trials [1]. The generation of T cell-mediated antitumor immunity should be (in theory) relatively straightforward, since a variety of Ag expressed by tumors (including overexpressed tissue-differentiation Ag, mutant proteins, and/or viral Ag) can be recognized by the immune system. However, there are many aspects unique to T cells and other components of the immune system that inhibit optimal T cell activation. Among the cells needed to generate antitumor T cell immunity, the importance of DC cannot be underemphasized. DC engulf fragments of dying tumor cells, and process the numerous proteins into antigenic peptides for presentation via MHC I/II to T cells. A variety of methods can be employed to kill tumor cells *in vivo* (e.g., chemotherapy, radiation, or cryotherapy), but these methods frequently lack the specificity to safely and selectively target tumor cells and not harm normal, healthy cells. The immune system also has a wide arsenal of molecules that can induce cell death, some of which possess discrete tumoricidal activity. TRAIL and agonistic mAb against TRAIL death receptors kill tumor cells, but have little-to-no toxicity to normal cells [36]. Our laboratory developed an immunotherapy that utilizes TRAIL-mediated tumor cell death *in vivo* that induces a robust systemic antitumor immune response mediated by tumor-specific CD8 T cells [13,15,16]. To further understand the mechanism by which this immunotherapeutic approach is successful, we performed a series of experiments that demonstrated the importance of two distinct DC populations, pDC and CD8, in the generation of the antitumor CD8 T cell response.

The development of immunotherapies that specifically use or target DC is a major area of interest in the treatment of cancer [37]. While the potential for DC-mediated tumoricidal activity has been reported [38–40], the intended role of DC in tumor immunotherapy is to generate an antitumor T cell response with the potential to deal with disseminated and/or recurrent disease. While the data presented suggest the key role of pDC and CD8 α DC in the success of Ad5-TRAIL/CpG therapy in a model of advanced RCC, it is also important to note that the roles played by these DC populations are partially dependent on the successful induction of TRAIL-mediated apoptotic tumor cell death. It is reasonable to think that any agent that induces tumor cell apoptosis would achieve similar effects, but there are many factors that can influence whether apoptotic cell death leads to an immunogenic or tolerogenic outcome [41]. TRAIL is unique in the sense that it selectively induces apoptosis in tumor cells [10], but there is still much to learn in regard to how tumor cells killed by TRAIL are perceived by DC and other cells of the immune system.

We focused our investigation to pDC and CD8 α DC, as these DC subsets are classically defined by being able to respond to CpG (and producing proinflammatory cytokines) and

cross-present tumor Ag from the apoptotic tumor cells. The CpG ODN used in our studies is thought to function as an adjuvant to activate the immune system to more efficiently respond to the tumor [42]. Unmethylated CpG-containing motifs are specifically recognized by TLR9, and murine pDC, M, B cells, and all myeloid DC subsets identified to date express TLR9 and respond to CpG [43,44]. Thus, the inability of pDC-depleted mice to eliminate tumor after Ad5-TRAIL/CpG therapy cannot be explained simply by a failure to respond to CpG. TLR-activated pDC have increased MHC and costimulatory molecule expression giving them the ability to stimulate T cells [45]. Activated pDC also undergo a differentiation process that features production of proinflammatory cytokines and other molecules that act on other cells of the immune system. In our hands, anti-PDCA1-treated tumor-bearing mice had reduced proinflammatory cytokine production (based on IFN-stimulated gene expression and IL-15/IL-15R production) and effector CD8 T cell infiltration into the tumor-bearing kidney after Ad5-TRAIL/CpG therapy compared to WT replete mice, which together likely contributed to the increased tumor growth and decreased survival.

In vivo depletion of CD8 DC can be achieved a number of ways. For us *Batf3*^{-/-} BALB/c mice proved to be ideal, as these mice are devoid of CD8α DC while still having CD8 T cells [18]. *Batf3* encodes a transcription factor highly expressed in conventional CD11c⁺ DC, and splenic CD11c⁺ conventional DC from *Batf3*^{-/-} mice lack the ability to cross-present Ag to CD8 T cells [18]. Thus, the lack of significant effector CD8 T cell infiltration into the tumor-bearing kidneys of *Batf3*^{-/-} BALB/c mice and absence of a therapeutic benefit after Ad5-TRAIL/CpG administration was expected. It was unexpected to see, however, the minimal response by the *Batf3*^{-/-} BALB/c mice in regard to IFN-responsive gene expression and IL-15/IL-15R production after Ad5-TRAIL/CpG therapy – especially since the *Batf3*^{-/-} BALB/c mice still have pDC (Fig 1). In addition, there was minimal upregulation of costimulatory molecules on the pDC from *Batf3*^{-/-} BALB/c mice after Ad5-TRAIL/CpG therapy. These data suggest that of the two DC populations investigated, CD8α DC may play a more important role in the successful treatment of advanced RCC with Ad5-TRAIL/CpG than pDC.

There is increasing evidence suggesting the necessity for DC subset interaction for the development of optimal T cell responses. Lou *et al.* showed that a mixture of CpG-stimulated pDC plus CD11c^{hi}CD11b⁺ B220⁻ myeloid DC synergized to induce increased Ag-specific CD8 T cell responses and antitumor immunity compared to either DC subset alone [23]. Similarly, Kuwajima *et al.* found pDC were required for the production of IL-12 by CpG-stimulated conventional DC [24]. The role for pDC being essential to license CD8α DC function has been shown in multiple models [23,46], and data presented herein suggests the possibility for pDC/CD8α DC crosstalk is important in the success of Ad5-TRAIL/CpG immunotherapy. Both cell-to-cell contact and soluble factors have been suggested to be the molecular mechanism behind DC subset communication [5,47,23,24,46], but our experiments were not designed to define which of these previously defined (or perhaps some other) mechanisms were being used by the pDC and CD8α DC to communicate with one another, as this would have significantly broadened the scope of the study beyond its initial intention. Future studies are planned to specifically address this aspect, as it will be

important to understand the signaling methods that allow pDC and CD8 α DC to talk with one another for the generation of a successful antitumor immune response after Ad5-TRAIL/CpG therapy and apply this knowledge to scenarios where the therapy is ineffective.

For years (and still today), metastatic or unresectable cancers of many types have been treated by systemic administration of a drug that directly or indirectly (via modification of the tumor microenvironment) leads to tumor cell death. This approach required careful dosing to demonstrate a reduction in tumor growth without inducing toxic side effects. The refinement of powerful imaging and minimally invasive techniques have improved the identification and access to tumors *in situ*, as well as increasing the types of therapies that can be performed to treat cancer in patients that may have previously had limited therapeutic options [48]. Thus, it seems logical to envision a setting where cancer immunotherapies designed to activate DC would benefit from local injection of the therapeutic molecules directly into the tumor microenvironment. A number of CpG oligonucleotides have been tested in clinical trials, mostly in combination with tumor vaccines and given subcutaneously (perhaps for convenience), and have shown evidence of antitumor activity [49]. However, it is not unreasonable to consider a protocol consisting of CpG delivered directly into the tumor – alone or in combination with another drug with tumoricidal activity. For example, we have examined the tolerability of direct intratumoral injection of Ad5-TRAIL in a phase I clinical trial in men with locally-confined prostate cancer [50], or the systemic administration of TRAIL death receptor agonists [51], which could easily be combined with local CpG therapy. Other drug regimens are feasible, but the potential benefit of intralesional administration of cancer therapies, like CpG, in human remains to be determined.

The findings presented herein are important to the basic biological understanding of how Ad5-TRAIL/CpG therapy for cancer works, as well as being essential for understanding how to modify the use of Ad5-TRAIL/CpG when immunomodulatory co-morbidities are present. For example, Renca-bearing diet-induced obese (DIO) mice do not respond to Ad5-TRAIL/CpG therapy and ultimately succumb to tumor burden, unlike lean controls [20]. I-A^{d+}CD11c^{hi} CD11b⁺ splenic DC from the DIO mice had decreased T cell stimulatory capacity compared to the same population of cells from lean mice. However, these markers identify a unique “immunosuppressive” DC population [20] that is mutually exclusive from pDC and CD8 α DC (based on the markers used in this study). It is tempting to speculate that in addition to the immunosuppressive I-A^{d+}CD11c^{hi} CD11b⁺ DC in DIO mice, altered function of pDC and/or CD8 α DC (be it cytokine production, Ag uptake/processing/presentation, or communication needed for DC licensing) also contribute to the failure of Ad5-TRAIL/CpG therapy in tumor-bearing obese mice. Regardless of the different DC populations (pDC, CD8 α DC, or immunosuppressive CD11b⁺ DC) examined, knowing which specific DC populations and their functional characteristics are essential for the success of Ad5-TRAIL/CpG therapy.

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Abbreviations

Ad5-TRAIL	recombinant adenovirus encoding TRAIL
Ag	antigen
APC	antigen presenting cell
Batf3	Basic Leucine Zipper Transcription Factor, ATF-Like 3
BV650	Brilliant Violet 650
CpG	CpG-containing oligodeoxynucleotide
CXCL10	C-X-C motif chemokine 10
DC	dendritic cell
GFP	green fluorescent protein
HBSS	Hank's buffered salt solution
IFIT	Interferon Induced proteins with Tetratricopeptide repeats
IFN	interferon
IFNAR	interferon $\alpha\beta$ receptor
IL	interleukin
IR	intrarenal
i.v	intravascular
mAb	monoclonal antibody
IRF7	interferon regulatory factor 7
ISG15	interferon-stimulated gene 15
MDA5	melanoma differentiation-associated protein 5
MHC	major histocompatibility complex
PBS	phosphate-buffered saline
Mx1 (IFI78)	interferon inducible protein 78
pDC	plasmacytoid dendritic cell
PDCA	plasmacytoid dendritic cell antigen
PE	phycoerytherin
RCC	renal cell carcinoma

Rx	combination Ad5-TRAIL/CpG therapy
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
WT	wild-type

References

1. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: Harnessing the T cell response. *Nature Rev Immunol.* 2012; 12(4):269–281.10.1038/nri3191 [PubMed: 22437939]
2. van Mierlo GJ, Boonman ZF, Dumortier HM, den Boer AT, Fransen MF, Nouta J, van der Voort EI, Offringa R, Toes RE, Melief CJ. Activation of dendritic cells that cross-present tumor-derived antigen licenses CD8+ CTL to cause tumor eradication. *J Immunol.* 2004; 173(11):6753–6759. [PubMed: 15557168]
3. de Brito C, Tomkowiak M, Ghittoni R, Caux C, Leverrier Y, Marvel J. CpG promotes cross-presentation of dead cell-associated antigens by pre-CD8alpha+ dendritic cells [corrected]. *J Immunol.* 2011; 186(3):1503–1511.10.4049/jimmunol.1001022 [PubMed: 21187449]
4. Shirota H, Klinman DM. CpG-conjugated apoptotic tumor cells elicit potent tumor-specific immunity. *Cancer Immunol Immunother.* 2011; 60(5):659–669.10.1007/s00262-011-0973-y [PubMed: 21318638]
5. Liu C, Lou Y, Lizée G, Qin H, Liu S, Rabinovich B, Kim GJ, Wang YH, Ye Y, Sikora AG, Overwijk WW, Liu YJ, Wang G, Hwu P. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. *J Clin Invest.* 2008; 118(3):1165–1175.10.1172/JCI33583 [PubMed: 18259609]
6. Nierkens S, den Brok MH, Suttmüller RP, Grauer OM, Bennink E, Morgan ME, Figdor CG, Ruers TJ, Adema GJ. In vivo colocalization of antigen and CpG within dendritic cells is associated with the efficacy of cancer immunotherapy. *Cancer Res.* 2008; 68(13):5390–5396.10.1158/0008-5472.CAN-07-6023 [PubMed: 18593941]
7. Drobits B, Holcmann M, Amberg N, Swiecki M, Grundtner R, Hammer M, Colonna M, Sibilio M. Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. *J Clin Invest.* 2012; 122(2):575–585.10.1172/JCI61034 [PubMed: 22251703]
8. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med.* 2000; 192(12):1685–1696. [PubMed: 11120766]
9. Schulz O, Reis e Sousa C. Cross-presentation of cell-associated antigens by CD8alpha+ dendritic cells is attributable to their ability to internalize dead cells. *Immunology.* 2002; 107(2):183–189. [PubMed: 12383197]
10. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, Sutherland GR, Smith TD, Rauch C, Smith CA, Goodwin RG. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity.* 1995; 3(6):673–682. [PubMed: 8777713]
11. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, Smith C, Smolak P, Goodwin RG, Rauch CT, Schuh JC, Lynch DH. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med.* 1999; 5(2):157–163. [PubMed: 9930862]
12. den Hollander MW, Gietema JA, de Jong S, Walenkamp AM, Reyners AK, Oldenhuis CN, de Vries EG. Translating TRAIL-receptor targeting agents to the clinic. *Cancer Lett.* 2013; 332(2):194–201.10.1016/j.canlet.2012.04.007 [PubMed: 22531313]
13. Griffith TS, Anderson RD, Davidson BL, Williams RD, Ratliff TL. Adenoviral-mediated transfer of the TNF-related apoptosis-inducing ligand/Apo-2 ligand gene induces tumor cell apoptosis. *J Immunol.* 2000; 165(5):2886–2894. [PubMed: 10946322]
14. Griffith TS, Broghammer EL. Suppression of tumor growth following intralesional therapy with TRAIL recombinant adenovirus. *Mol Ther.* 2001; 4(3):257–266. [PubMed: 11545617]

15. VanOosten RL, Griffith TS. Activation of tumor-specific CD8⁺ T Cells after intratumoral Ad5-TRAIL/CpG oligodeoxynucleotide combination therapy. *Cancer Res.* 2007; 67(24):11980–11990. [PubMed: 18089829]
16. Norian LA, Kresowik TP, Rosevear HM, James BR, Rosean TR, Lightfoot AJ, Kucaba TA, Schwarz C, Weydert CJ, Henry MD, Griffith TS. Eradication of metastatic renal cell carcinoma after adenovirus-encoded TNF-related apoptosis-inducing ligand (TRAIL)/CpG immunotherapy. *PLoS One.* 2012; 7(2):e31085.10.1371/journal.pone.0031085 [PubMed: 22312440]
17. Nierkens S, den Brok MH, Garcia Z, Togher S, Wagenaars J, Wassink M, Boon L, Ruers TJ, Figdor CG, Schoenberger SP, Adema GJ, Janssen EM. Immune adjuvant efficacy of CpG oligonucleotide in cancer treatment is founded specifically upon TLR9 function in plasmacytoid dendritic cells. *Cancer Res.* 2011; 71(20):6428–6437.10.1158/0008-5472.CAN-11-2154 [PubMed: 21788345]
18. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, Calderon B, Schraml BU, Unanue ER, Diamond MS, Schreiber RD, Murphy TL, Murphy KM. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science.* 2008; 322(5904):1097–1100.10.1126/science.1164206 [PubMed: 19008445]
19. Hrushesky WJ, Murphy GP. Investigation of a new renal tumor model. *J Surg Res.* 1973; 15(5): 327–336. [PubMed: 4759685]
20. James BR, Tomanek-Chalkley A, Askeland EJ, Kucaba T, Griffith TS, Norian LA. Diet-induced obesity alters dendritic cell function in the presence and absence of tumor growth. *J Immunol.* 2012; 189(3):1311–1321.10.4049/jimmunol.1100587 [PubMed: 22745381]
21. Asselin-Paturel C, Brizard G, Pin JJ, Briere F, Trinchieri G. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. *J Immunol.* 2003; 171(12):6466–6477. [PubMed: 14662846]
22. Anderson KG, Mayer-Barber K, Sung H, Beura L, James BR, Taylor JJ, Qunaj L, Griffith TS, Vezys V, Barber DL, Masopust D. Intravascular staining for discrimination of vascular and tissue leukocytes. *Nat Protocols.* 2014; 9(1):209–222.10.1038/nprot.2014.005
23. Lou Y, Liu C, Kim GJ, Liu YJ, Hwu P, Wang G. Plasmacytoid dendritic cells synergize with myeloid dendritic cells in the induction of antigen-specific antitumor immune responses. *J Immunol.* 2007; 178(3):1534–1541. [PubMed: 17237402]
24. Kuwajima S, Sato T, Ishida K, Tada H, Tezuka H, Ohteki T. Interleukin 15-dependent crosstalk between conventional and plasmacytoid dendritic cells is essential for CpG-induced immune activation. *Nat Immunol.* 2006; 7(7):740–746.10.1038/ni1348 [PubMed: 16715101]
25. Salup RR, Wiltout RH. Adjuvant immunotherapy of established murine renal cancer by interleukin 2-stimulated cytotoxic lymphocytes. *Cancer Res.* 1986; 46(7):3358–3363. [PubMed: 3486710]
26. Blasius AL, Giurisato E, Cella M, Schreiber RD, Shaw AS, Colonna M. Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. *J Immunol.* 2006; 177(5):3260–3265. [PubMed: 16920966]
27. Vinay DS, Lee SJ, Kim CH, Oh HS, Kwon BS. Exposure of a distinct PDCA-1+ (CD317) B cell population to agonistic anti-4-1BB (CD137) inhibits T and B cell responses both in vitro and in vivo. *PLoS One.* 2012; 7(11):e50272.10.1371/journal.pone.0050272 [PubMed: 23185591]
28. Bao Y, Han Y, Chen Z, Xu S, Cao X. IFN-alpha-producing PDCA-1+ Siglec-H- B cells mediate innate immune defense by activating NK cells. *Eur J Immunol.* 2011; 41(3):657–668.10.1002/eji.201040840 [PubMed: 21287550]
29. Asselin-Paturel C, Boonstra A, Dalod M, Durand I, Yessaad N, Dezutter-Dambuyant C, Vicari A, O'Garra A, Biron C, Briere F, Trinchieri G. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol.* 2001; 2(12):1144–1150.10.1038/ni736 [PubMed: 11713464]
30. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, Gajewski TF. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. *J Exp Med.* 2011; 208(10):2005–2016.10.1084/jem.20101159 [PubMed: 21930765]

31. Basner-Tschakarjan E, Gaffal E, O'Keeffe M, Tormo D, Limmer A, Wagner H, Hochrein H, Tuting T. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN- α production. *J Gene Med.* 2006; 8(11):1300–1306.10.1002/jgm.964 [PubMed: 16952196]
32. Mattei F, Schiavoni G, Belardelli F, Tough DF. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. *J Immunol.* 2001; 167(3):1179–1187. [PubMed: 11466332]
33. Epardaud M, Elpek KG, Rubinstein MP, Yonekura AR, Bellemare-Pelletier A, Bronson R, Hamerman JA, Goldrath AW, Turley SJ. Interleukin-15/interleukin-15R α complexes promote destruction of established tumors by reviving tumor-resident CD8 $^{+}$ T cells. *Cancer Res.* 2008; 68(8):2972–2983.10.1158/0008-5472.CAN-08-0045 [PubMed: 18413767]
34. Rai D, Pham NL, Harty JT, Badovinac VP. Tracking the total CD8 T cell response to infection reveals substantial discordance in magnitude and kinetics between inbred and outbred hosts. *J Immunol.* 2009; 183(12):7672–7681. [PubMed: 19933864]
35. Seki N, Brooks AD, Carter CR, Back TC, Parsonneault EM, Smyth MJ, Wiltout RH, Sayers TJ. Tumor-specific CTL kill murine renal cancer cells using both perforin and Fas ligand-mediated lysis in vitro, but cause tumor regression in vivo in the absence of perforin. *J Immunol.* 2002; 168(7):3484–3492. [PubMed: 11907109]
36. Gonzalez F, Ashkenazi A. New insights into apoptosis signaling by Apo2L/TRAIL. *Oncogene.* 2010; 29(34):4752–4765.10.1038/onc.2010.221 [PubMed: 20531300]
37. Le DT, Pardoll DM, Jaffee EM. Cellular vaccine approaches. *Cancer J.* 2010; 16(4):304–310.10.1097/PPO.0b013e3181eb33d7 [PubMed: 20693840]
38. Fanger NA, Maliszewski CR, Schooley K, Griffith TS. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J Exp Med.* 1999; 190(8):1155–1164. [PubMed: 10523613]
39. Anguille S, Lion E, Tel J, de Vries IJ, Coudere K, Fromm PD, Van Tendeloo VF, Smits EL, Berneman ZN. Interleukin-15-induced CD56(+) myeloid dendritic cells combine potent tumor antigen presentation with direct tumoricidal potential. *PLoS One.* 2012; 7(12):e51851.10.1371/journal.pone.0051851 [PubMed: 23284789]
40. Tel J, Smits EL, Anguille S, Joshi RN, Figdor CG, de Vries IJ. Human plasmacytoid dendritic cells are equipped with antigen-presenting and tumoricidal capacities. *Blood.* 2012; 120(19):3936–3944.10.1182/blood-2012-06-435941 [PubMed: 22966165]
41. Griffith TS, Ferguson TA. Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells. *Immunity.* 2011; 35(4):456–466.10.1016/j.immuni.2011.08.011 [PubMed: 22035838]
42. Krieg A, Kline J. Immune effects and therapeutic applications of CpG motifs in bacterial DNA. *Immunopharmacology.* 2000; 48(3):303–305. [PubMed: 10960672]
43. Gilliet M, Boonstra A, Paturel C, Antonenko S, Xu XL, Trinchieri G, O'Garra A, Liu YJ. The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J Exp Med.* 2002; 195(7):953–958. [PubMed: 11927638]
44. Suzuki K, Suda T, Naito T, Ide K, Chida K, Nakamura H. Impaired toll-like receptor 9 expression in alveolar macrophages with no sensitivity to CpG DNA. *Am J Respir Crit Care Med.* 2005; 171(7):707–713.10.1164/rccm.200408-1078OC [PubMed: 15640365]
45. Salio M, Palmowski MJ, Atzberger A, Hermans IF, Cerundolo V. CpG-matured murine plasmacytoid dendritic cells are capable of in vivo priming of functional CD8 T cell responses to endogenous but not exogenous antigens. *J Exp Med.* 2004; 199(4):567–579.10.1084/jem.20031059 [PubMed: 14970182]
46. Yoneyama H, Matsuno K, Toda E, Nishiwaki T, Matsuo N, Nakano A, Narumi S, Lu B, Gerard C, Ishikawa S, Matsushima K. Plasmacytoid DCs help lymph node DCs to induce anti-HSV CTLs. *J Exp Med.* 2005; 202(3):425–435.10.1084/jem.20041961 [PubMed: 16061729]
47. Lorenzi S, Mattei F, Sistigu A, Bracci L, Spadaro F, Sanchez M, Spada M, Belardelli F, Gabriele L, Schiavoni G. Type I IFNs control antigen retention and survival of CD8 α (+) dendritic cells

- after uptake of tumor apoptotic cells leading to cross-priming. *J Immunol.* 2011; 186(9):5142–5150.10.4049/jimmunol.1004163 [PubMed: 21441457]
48. Chu KF, Dupuy DE. Thermal ablation of tumours: biological mechanisms and advances in therapy. *Nat Rev Cancer.* 2014; 14(3):199–208.10.1038/nrc3672 [PubMed: 24561446]
49. Krieg AM. CpG still rocks! Update on an accidental drug. *Nucleic Acid Ther.* 2012; 22(2):77–89.10.1089/nat.2012.0340 [PubMed: 22352814]
50. Holloch PA, Griffith TS. TNF-related apoptosis-inducing ligand (TRAIL): a new path to anti-cancer therapies. *Eur J Pharmacol.* 2009; 625(1–3):63–72.10.1016/j.ejphar.2009.06.066 [PubMed: 19836385]
51. Micheau O, Shirley S, Dufour F. Death receptors as targets in cancer. *Br J Pharmacol.* 2013; 169(8):1723–1744.10.1111/bph.12238 [PubMed: 23638798]

Figure 1.

Validation of DC depletion models. Spleens were harvested from WT replete, anti-CD8 α -treated, anti-PDCA1-treated, or *Batf3*^{-/-} BALB/c mice. Anti-CD8 α or -PDCA1-treated mice were given 250 μ g and 500 μ g mAb i.p., respectively, on 2 consecutive days, after which spleens were harvested and processed for flow cytometry. (A) Representative flow plots showing the gating for conventional DC (cDC, MHCII⁺CD11c⁺), CD8 α DC (gated from MHCII⁺CD11c⁺), pDC (gated from MHCII^{lo}CD11c^{lo}B220⁺) and CD8 T cells (gated from CD3⁺). Samples from *Batf3*^{-/-} mice were run independently of the other groups, resulting in slight changes in the gating. (B) Mean (+ SEM) frequency of DC and T cell populations.

(C) Mean (+ SEM) total number of DC and T cell populations. Cumulative data from two independent experiments, n=3–4 mice/group/experiment. ns = not significant, * $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$ using 1-way ANOVA with Tukey post hoc test.

Figure 2.

Absence of pDC or CD8 α DC abrogates the therapeutic benefit of Ad5-TRAIL/CpG. WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice were implanted IR with 2×10^5 Renca or Renca-GLE cells, and then treated with either PBS or Ad5-TRAIL/CpG (Rx) on d 7. (A) Mean $\pm$ SEM tumor-bearing kidney mass (g) for mice harvested when they became moribund (18–21 days, cumulative data from 3 independent experiments, n for each group: tumor-free ctl= 5, WT PBS= 9, WT Rx= 18, ctl Ab= 8, anti-PDCA1= 8, *Batf3*^{-/-} = 7). (B) Mean $\pm$ SEM orthotopic tumor burden in mice monitored via bioluminescence. Excised lungs were imaged on d 17 via bioluminescence, for the presence of metastatic tumor burden (cumulative data of multiple independent experiments, n=3–5 mice/group/experiment). (C–D) Overall survival for WT replete vs. (C) anti-PDCA1-treated mice and (D) *Batf3*^{-/-} BALB/c mice that received PBS or Ad5-TRAIL/CpG therapy. All test groups were compared to Ad5-TRAIL/CpG-treated WT replete mice (cumulative data from 3 independent experiments; n= 3–5 mice/group/experiment). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ using 1-way ANOVA with Tukey post hoc test in A and 2-way ANOVA in B. Log-rank tests were performed in C–D.

Figure 3.

Absence of CD8α DC results in decreased pDC activation following Ad5-TRAIL/CpG therapy. WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice were implanted IR with 2×10^5 Renca cells, and then treated with either PBS or Ad5-TRAIL/CpG (Rx) on d 7. Spleens were harvested 24 h later, made into single cell suspensions, and stained for flow cytometric analysis. Mean + SEM geometric MFI for CD40, CD80, and CD86 expression on (A) pDC in *Batf3*^{-/-} mice and (B) CD8α DC in anti-PDCA1-treated mice. Data are representative of two independent experiments, n= 4–5 mice/group/experiment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ using 1-way ANOVA with Tukey post hoc test.

Figure 4.

The absence of CD8α DC results in a decreased type I IFN signature within the tumor-bearing kidney after Ad5-TRAIL/CpG therapy. (A–B) WT replete BALB/c mice were implanted IR with 2×10^5 Renca cells, treated with PBS, Ad5-TRAIL alone, CpG alone, or Ad5-TRAIL/CpG on d 7, and tumor-bearing kidneys were harvested 4 h later. Total RNA was isolated from tumor-bearing kidneys, reverse transcribed into cDNA, and analyzed by qPCR for the abundance of the type I IFN-stimulated genes *cxcl10* and *irf7*. Mean fold change of type I IFN-stimulated genes was determined relative to tumor-free kidneys. (C–H) WT replete, pDC-depleted, and *Batf3*^{-/-} BALB/c mice were implanted IR with Renca cells, treated with either PBS or Ad5-TRAIL/CpG (Rx), and harvested as in A. Mean fold change of type I IFN-stimulated genes was determined relative to tumor-free kidneys. Data are representative of three independent experiments, n = 3–5 mice/group/experiment. ns = not significant, * p < 0.05, ** p < 0.01, *** p < 0.001 using 1-way ANOVA with Tukey post hoc test.

Figure 5.

Mice deficient in pDC or CD8 α DC have decreased IL-15/IL-15R expression in tumor-bearing kidneys after therapy. WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice were implanted IR with 2×10^5 Renca cells, and then treated with PBS or Ad5-TRAIL/CpG (Rx) on d 7. Tumor-bearing kidneys were harvested 24 h later, homogenized, and supernatants were analyzed in duplicate by ELISA for IL-15/IL-15R complexes. $n = 4$ mice/group/experiment. * $p < 0.05$, ** $p < 0.01$ using 1-way ANOVA with Tukey post hoc test.

Figure 6.

Absence of pDC or CD8 α DC results in a blunted effector CD8 T cell infiltration into the tumor after Ad5-TRAIL/CpG. WT replete, anti-PDCA1-treated, and *Batf3*^{-/-} BALB/c mice were implanted IR with 2 $\times$ 10⁵ Renca cells, treated with either PBS or Ad5-TRAIL/CpG (Rx) on d 7, and kidneys were harvested on d 12. Prior to harvest, all mice were injected i.v. with PE-conjugated anti-CD45.2 mAb (3 μ g) in PBS and sacrificed 3 min later. Single cell suspensions of tumor-bearing kidneys were prepared, and stained for flow cytometric analyses to assess immune cell infiltration. (A–B) Mean + SEM frequency of CD3⁺CD8⁺CD44^{hi} T cells among (A) total live cells or (B) CD45.2⁺ cells within tumor-bearing kidneys. (C–D) Intravascular staining reveals that the majority of CD3⁺CD8⁺CD44^{hi} T cells within tumor-bearing kidneys, regardless of receiving PBS or Ad5-TRAIL/CpG, are located within the tissue. Representative gating from each group are presented in (C), and mean + SEM data are in (D). (E) Mean + SEM frequency of Ki67⁺ cells within the CD3⁺CD8⁺CD44^{hi} effector phenotype CD8 T cell gate. (F) Mean + SEM geometric MFI for CD11a expression on the CD3⁺CD8⁺CD44^{hi} effector phenotype CD8 T cells. Cumulative data from two independent experiments shown, n= 5 mice/group/experiment. * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 using 1-way ANOVA with Tukey post hoc test.