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A novel dendritic cell subset involved in tumor immunosurveillance

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The interferon (IFN)-γ–induced TRAIL effector mechanism is a vital component of cancer immunosurveillance by natural killer (NK) cells in mice[1,2]. Here we show that the main source of IFN-γ is not the conventional NK cell but a subset of B220$^+$Ly6C$^-$ dendritic cells, which are atypical insofar as they express NK cell-surface molecules. Upon contact with a variety of tumor cells that are poorly recognized by NK cells, B220$^+$NK1.1$^+$ dendritic cells secrete high levels of IFN-γ and mediate TRAIL-dependent lysis of tumor cells. Adoptive transfer of these IFN-producing killer dendritic cells (IKDCs) into tumor-bearing *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice prevented tumor outgrowth, whereas transfer of conventional NK cells did not. In conclusion, we identified IKDCs as pivotal sensors and effectors of the innate antitumor immune response.

Immune surveillance against tumors is mediated by both innate and adaptive components of cellular immunity[3,4]. IFN-γ and lymphocytes are crucial effectors against methylcholanthrene-induced and spontaneous tumors in mice, setting the stage for the concept of 'tumor immunoediting'[3]. IFN-γ is instrumental for enhancing antigen processing and presentation for optimal recognition of tumor cells by T cells[3,4]. But the source of IFN-γ at the onset of tumor progression is unclear. A 2001 study showing a higher incidence of primary tumors in *Rag2*$^{-/-}$*Stat1*$^{-/-}$ mice compared with *Rag2*$^{-/-}$ littermates suggested that IFN-γ was not produced only by activated T cells[3]. The innate component of IFN-γ production has been largely attributed to NK cells, which can lyse tumor cells[1,2,5–7]. NK cells exert their lytic functions through perforin, CD95 ligand (FasL) or tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathways, depending on the cytokines to which NK cells respond and on the expression patterns of NKG2D ligands on tumor cells[8].

We recently reported that the specific tyrosine kinase inhibitor imatinib mesylate (also called STI571 or Gleevec, which inhibits c-Kit, Abl and PDGFR-α) promotes antitumor effects mediated by NK1.1$^+$ cells *in vivo*[9]. Notably, regressing tumors were infiltrated by a novel subset of dendritic cells (DCs) that secreted high levels of IFN-γ when in contact with tumor cells and killed tumor cells in a TRAIL-dependent fashion. These IFN-γ–producing killer DCs (IKDCs) are unique in their capacity to recognize a broad array of transformed cells and to participate in tumor surveillance *in vivo*.

To augment the NK cell–mediated antitumor effect induced by imatinib mesylate, we combined imatinib mesylate with interleukin (IL)-2. We observed enhanced antitumor effects against B16F10 melanoma lung metastases when we combined imatinib mesylate and IL-2 *in vivo*, as compared with either agent alone (data not shown). Administration of depleting NK1.1-specific monoclonal antibody completely abrogated the tumoricidal activity induced by the combination of imatinib mesylate and IL-2, suggesting a role for NK1.1-expressing cells in the antitumor effects. Immunohistochemistry of residual lung metastases showed prominent infiltrates of CD11c$^+$ in tumor beds and in the surrounding parenchyma in imatinib mesylate + IL-2–treated mice but not in controls (532 ± 92 and 60 ± 13 CD11c$^+$ cells/field, respectively; **Supplementary Fig. 1** online). Tumor-infiltrating CD11c$^+$ cells were rare in the groups treated with IL-2 or imatinib mesylate alone (136 ± 50 and 22 ± 9 CD11c$^+$ cells/field, respectively), suggesting that DCs and/or NK cells are crucial effectors of the antimetastatic effects achieved with the combination of imatinib mesylate and IL-2. To reconcile these data with the previously identified role of NK1.1$^+$ cells, we performed cytofluorometric analyses of single-cell suspensions obtained from lung metastases, searching for CD11c$^+$NK1.1$^+$ cells. Classical NK cells, defined as CD3$^-$B220$^-$NK1.1$^+$ cells, represented about 4 ± 2% of viable cells and were not significantly expanded by the combination therapy. In contrast, the number of CD11c$^+$ cells increased two- to threefold in response to imatinib mesylate + IL-2 (**Supplementary Fig. 1** online). The vast majority (72 ± 5%) of tumor-infiltrating

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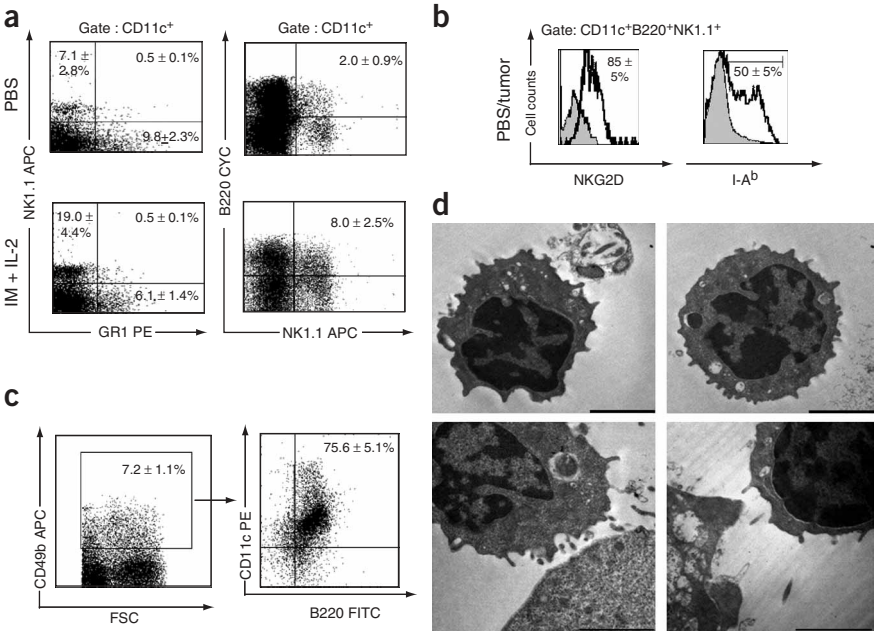


Figure 1 A unique DC subset expands in spleen. (**a**) Three-color flow cytometry studies using FITC-conjugated CD11c-specific, APC-conjugated NK1.1-specific, PE-conjugated Gr1-specific monoclonal antibodies were performed on splenocytes harvested from PBS- or imatinib mesylate (IM) + IL-2–treated C57BL/6 mice. The characterization of CD11c$^+$NK1.1$^+$Gr1$^-$ was performed by four-color staining in spleens from mice treated with PBS or IM+IL-2. (**b**) The CD11c$^+$NK1.1$^+$ subset was characterized by four-color staining using CyC-conjugated B220-specific, PE-conjugated I-Ab–specific or PE-conjugated NKG2D-specific monoclonal antibodies in lung metastases from naive mice. The gray area represents the isotype control antibody and the white area represents the NKG2D-specific or I-Ab–specific antibody. (**c**) Identification of CD11c$^+$B220$^+$CD49b$^+$ cells performed by flow cytometry on splenocytes of *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. The two panels depict one representative of three mice. (**d**) Transmission electron microscopy of CD49b$^+$B220$^+$NK1.1$^+$ cells obtained by magnetic separation and FACS. Cells were either freshly obtained (upper panels) or cultured with B16F10 tumor cells for 20 min (lower left) or overnight (lower right). Scale bars, 2 μm.

CD11c$^+$ cells expressed B220 but lacked Ly6C and Gr1 (**Supplementary Fig. 1** online). All tumor-infiltrating CD11c$^+$ cells that expressed NK1.1 molecules were Gr1$^-$. Up to 19 ± 4% of splenic CD11c$^+$ cells from mice treated with imatinib mesylate + IL-2 expressed NK1.1 (**Fig. 1a**). Classical plasmacytoid DCs expressing CD11c, B220 and Gr1 represented about 3% of tumor-infiltrating CD11c$^+$ cells (**Supplementary Fig. 1** online). Therefore, we focused our attention on the subset of CD11c$^+$ cells that expresses B220 and NK1.1. The number of B220$^+$NK1.1$^+$ cells increased fourfold during treatment with imatinib mesylate + IL-2, representing up to 8 ± 2% of splenic CD11c$^+$ cells (versus 2 ± 0.9% in PBS-treated controls; **Fig. 1a**). In contrast, either imatinib mesylate or IL-2 alone did not boost the expansion of B220$^+$NK1.1$^+$ cells (data not shown). The B220$^+$Gr1$^-$NK1.1$^+$ population expressed other NK cell markers such as the integrin

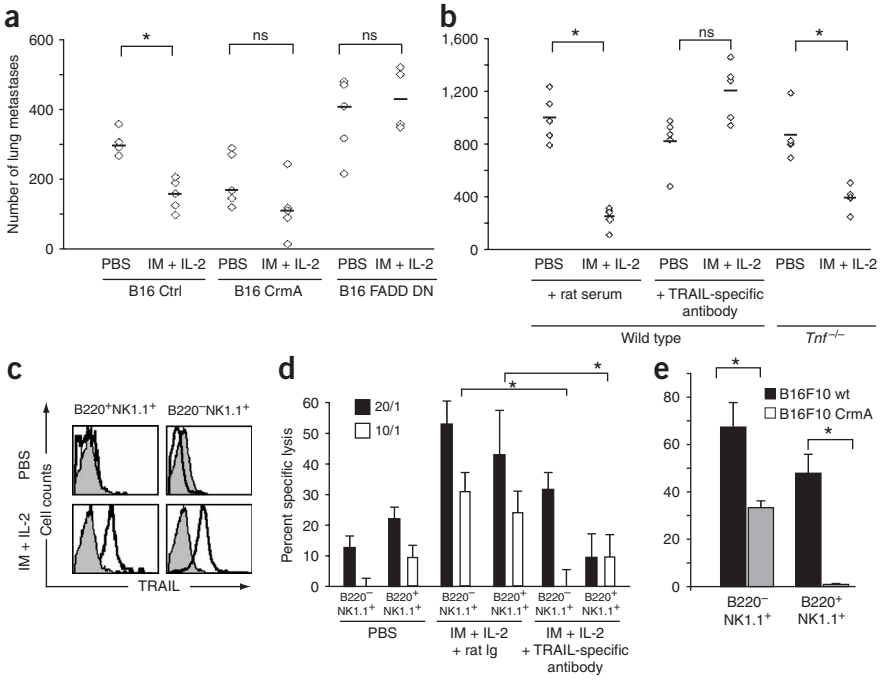
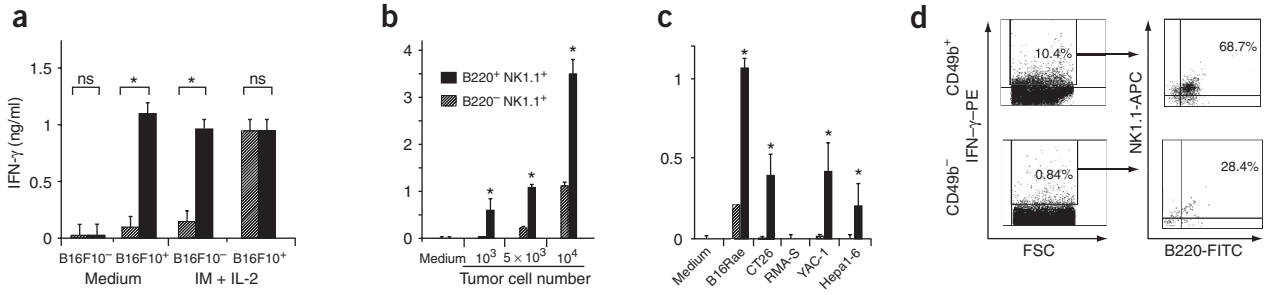


Figure 2 TRAIL-dependent death of tumor cells by B220$^+$NK1.1$^+$ DCs. (**a**,**b**). C57BL/6 mice were subjected to oral feeding with imatinib mesylate (IM; 150 mg/kg) twice a day for 11 d along with intraperitoneal administration of recombinant human IL-2 (Roussel Uclaf) or PBS at 10^5 IU/mouse, twice a day for the last 4 d. B16F10 or tumor variants expressing DISC blockers (CrmA or FADD DN) were injected into the tail vein (**a**) at day 0. Mice were killed at day 11 and B16F10 lung metastases were counted. (**b**) As in **a** but wild-type or *Tnf*$^{-/-}$ C57BL/6 mice were injected intravenously with B16F10. Neutralization of TRAIL *in vivo* using N2B2 monoclonal antibody was also performed in wild-type mice in parallel. One representative experiment of two is shown and analyzed according to Fisher exact method at 95% confidence interval. (**c**). B220$^+$NK1.1$^+$ and NK cells (B220$^-$NK1.1$^+$) were harvested from splenocytes of mice treated with PBS or IM+IL-2. Splenocytes enriched in CD49b$^+$ cells were further separated by flow cytometry after staining with FITC-conjugated B220-specific and PE-conjugated NK1.1-specific antibodies. Membrane expression of TRAIL was assessed using biotinylated TRAIL-specific (N2B2) monoclonal antibody. The gray area represents the isotype control antibody and the white area represents the relevant antibody. (**d**,**e**) Cells were separated as in **c** from spleens of IM+IL-2–treated mice. After separation, 3×10^4 B220$^+$NK1.1$^+$ or NK cells were incubated with B16F10 (**d**,**e**) or CrmA-transfected B16F10 at an effector/target ratio of 20:1 (**e**) in the presence or absence of TRAIL-specific neutralizing antibody for 72 h (**d**), and cell viability was assessed using a crystal violet bioassay[26] (**d**,**e**). All experiments were performed three to four times with identical results. Representative data are shown. ns, not significant. $^*P < 0.05$.



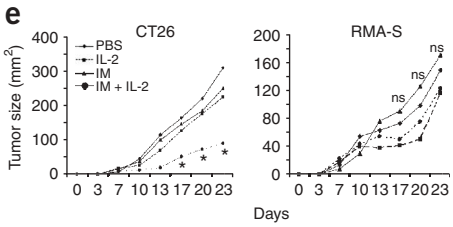


Figure 3 Secretion of IFN-γ is the specialized function of B220⁺NK1.1⁺ DCs. (**a–d**) Secretion of type II IFN by B220⁺NK1.1⁺ cells in contact with tumor cells or imatinib mesylate (IM) + IL-2. B220⁺NK1.1⁺ or NK cells were purified from naive mice according to the procedure described in **Figure 2** and stimulated with either B16F10 tumor cells at an effector/target (E:T) ratio of 10:1 or with IM+IL-2 for 24 h (**a**). A dose effect by augmenting the E:T ratio is shown in **b**. (**c**) As in **b**, but replacing B16F10 with various tumor cell lines at 10:1 E:T ratio. IFN-γ levels were measured in the supernatants using ELISA kits and statistical analyses were performed using Fisher exact method at 95% confidence interval. (**d**) CD49b⁺ cells were purified from bone marrow and incubated with B16F10 tumor cells at an E:T of 10:1 for 40 h before intracellular staining and flow cytometric analyses using IFN-γ–specific or isotype control monoclonal antibody. (**e**) Results of the combination therapy in RMA-S and CT26 tumor models. We subcutaneously inoculated 3×10^5 CT26 tumor cells (left panel) and 5×10^5 RMA-S (right panel) into BALB/c and C56BL/6 mice, respectively, and mice were treated as specified in **Figure 2a**. Tumor growth was monitored twice a week. One representative out of three to five experiments including six mice per group is shown. ns, not significant.* $P < 0.05$, 95% confidence interval.

VLA-2 (recognized by the CD49b-specific monoclonal antibody), CD122 (**Supplementary Fig. 2** online), NKG2D (**Fig. 1b**), CD11b (**Supplementary Fig. 2** online), but did not express CD3, CD4, CD8α, CD25, c-KIT, PDCA-1 (**Supplementary Fig. 2** online) or costimulatory molecules CD40, CD80 (data not shown) and CD86 (**Supplementary Fig. 2** online). Because up to 50% of the CD11c⁺B220⁺NK1.1⁺ cells expressed I-A^b in tumors (**Fig. 1b**), these CD11c⁺ cells might belong to the DC lineage. To further confirm that this B220⁺Gr1⁻NK1.1⁺ DC subset is distinct from the classical NK lineage, we examined splenocytes from *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice for the presence of such cells. We found that most CD49b⁺ splenocytes coexpressed B220 and CD11c (**Fig. 1c**) and that 60% among them carried I-A^b (major histocompatibility complex (MHC) class II) molecules at the surface (data not shown). We detected CD11c⁺B220⁺NK1.1⁺ cells in naive mice in all primary and secondary lymphoid organs including liver and lungs, representing from 0.5% (liver) to 2–3% (spleen) of all CD11c⁺ cells (**Supplementary Fig. 3** online). Their phenotype was similar in all locations, with the exception of bone marrow, in which CD11c⁺B220⁺NK1.1⁺ cells do not express NKG2D (data not shown). Morphological analyses using transmission electron microscopy highlighted that freshly isolated B220⁺Gr1⁻NK1.1⁺ DCs have a typical diameter of 5–6 μm and hence are smaller than NK cells, have a smooth plasma membrane with small pseudopodia, a high nucleocytoplasmic ratio and a dense cytoplasm containing few mitochondria but numerous multivesicular structures (**Fig. 1d**). Altogether, we identified a subset of CD11c⁺B220⁺ DCs with a unique morphology that coexpresses the NK markers CD49b, NK1.1 and NKG2D, expands during therapy with imatinib mesylate + IL-2 and infiltrates tumor beds.

We evaluated the role of death receptors in the combination therapy using several approaches. First, B16F10 tumor cells manipulated to express proteins that block the proapoptotic signal transduction complex initiated through death receptors (such as dominant-negative mutant FADD or the cowpox virus caspase-8 inhibitor CrmA) were resistant to the combination therapy with imatinib mesylate + IL-2 *in vivo* (**Fig. 2a**). This suggested that death receptors and their ligands such as tumor necrosis factor (TNF) or TRAIL have a crucial role in the antitumor effects of imatinib mesylate + IL-2. The combination therapy maintained a significant antitumor activity in *Tnf*$^{-/-}$ mice (**Fig. 2b**). In contrast, the efficacy of imatinib mesylate + IL-2 was completely abolished in mice treated with a neutralizing antibody to mouse TRAIL (**Fig. 2b**), establishing the pivotal role of TRAIL in the tumoricidal activity. Membrane expression of TRAIL was detectable only on splenic CD11c⁺B220⁺NK1.1⁺ DCs and conventional NK cells (CD11c⁺B220⁻NK1.1⁺) after therapy with imatinib mesylate + IL-2 (**Fig. 2c**). To further elucidate the relevance of TRAIL-dependent death pathways in the tumoricidal activity of NK1.1⁺ cells, we determined whether splenic B220⁺NK1.1⁺ DCs and conventional NK cells (B220⁻NK1.1⁺) could kill wild-type B16F10 cells or CrmA-expressing tumor variants in a TRAIL-dependent manner *ex vivo*. We first carried out magnetic separation of splenic cells using CD49b-specific monoclonal antibody and then carried out fluorescence-activated cell sorting (FACS) using NK1.1- and B220-specific monoclonal antibodies. Both subsets (that is, CD49b⁺NK1.1⁺B220⁻ (conventional NK cells) and CD49b⁺NK1.1⁺B220⁺ DCs) were isolated from PBS-treated or imatinib mesylate + IL-2–treated mice and evaluated for their capacity to lyse B16F10 tumor cells in the absence or presence of TRAIL-specific neutralizing monoclonal antibody. Both innate effector cell subsets exerted lytic activity with a similar efficacy *ex vivo*, in a manner that was partially or completely dependent on TRAIL, respectively (**Fig. 2d**). CrmA-expressing tumor cells were not significantly lysed by CD49b⁺NK1.1⁺B220⁺ DCs, underscoring the importance of death-receptor signaling in tumor-cell lysis (**Fig. 2e**). In conclusion, the novel subset of DCs coexpressing B220 and NK cell markers killed tumor cells through a predominantly TRAIL-dependent pathway. Transmission electron microcopy showed close contacts between CD49b⁺NK1.1⁺B220⁺ DCs and tumor cells that may lead to tumor cell death (**Fig. 1d**).

Because expression of TRAIL is controlled by IFN type II[1,2,10,11], we investigated the requirement for IFN type II receptors in the efficacy of

the combination therapy. Indeed, IFN type II receptor signaling was crucial for the antitumor effects *in vivo*, as indicated by the failure of imatinib mesylate + IL-2 to mediate antitumor effects in mice expressing a dominant-negative IFN type II receptor transgene (**Supplementary Fig. 4** online). These data prompted us to examine the regulation of IFN-γ secretion by splenic B220$^+$NK1.1$^+$ DCs and conventional (B220$^-$NK1.1$^+$) NK cells. Notably, splenic B220$^+$NK1.1$^+$ DCs stimulated *in vitro* with tumor cells alone (that is, in the absence of exogenous cytokines) produced copious amounts of IFN-γ (up to 40 ng per 10^6 cells per day; **Fig. 3a**). This secretion of IFN-γ was proportional to the number of stimulating tumor cells and remained significant even at effector/target ratios as low as 50:1 (**Fig. 3b**). Stimulation of the cells with imatinib mesylate + IL-2 (but not with either stimulus alone) also resulted in the secretion of IFN-γ (**Fig. 3a**). B220$^+$NK1.1$^+$ DCs could produce IFN-γ in response to a broad array of allogeneic or syngeneic transformed cells except the transporter associated with antigen processing (TAP) deficient RMA-S lymphoma tumor cells, whereas NK cells did not produce IFN-γ in the absence of exogenous stimulation (**Fig. 3c**), despite showing lytic activity against RMA-S. Of note, B220$^+$NK1.1$^+$ DCs did not secrete IFN-γ in contact with healthy tissues such as primary hepatocyte or thymocyte cultures (data not shown). Both spleen- and bone marrow–derived B220$^+$NK1.1$^+$ DCs could produce IFN-γ when cocultured with B16F10 tumor cells and constituted the vast majority of IFN-γ–producing cells within the subset of CD49b$^+$ bone marrow cells (**Fig. 3d**). Similarly, B220$^+$NK1.1$^+$ DCs from *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice, which constitute the entire CD49b$^+$ subset (**Fig. 1c**), are efficient producers of IFN-γ (**Supplementary Fig. 5** online). B220$^+$NK1.1$^+$ DCs could also produce IFN-α after stimulation with influenza virus (data not shown). Therefore, the B220$^+$NK1.1$^+$ DCs were termed interferon-producing killer dendritic cells or IKDCs. Supporting the primary role of IKDCs in the antitumor effects mediated by imatinib mesylate + IL-2, imatinib mesylate + IL-2 was not effective against RMA-S tumor cells *in vivo* but was synergistic against established CT26 tumors (**Fig. 3e**), as expected (**Fig. 3c**). Together, these data suggest that IKDCs represent a novel class of innate effectors that produce IFN-γ in response to tumor cells.

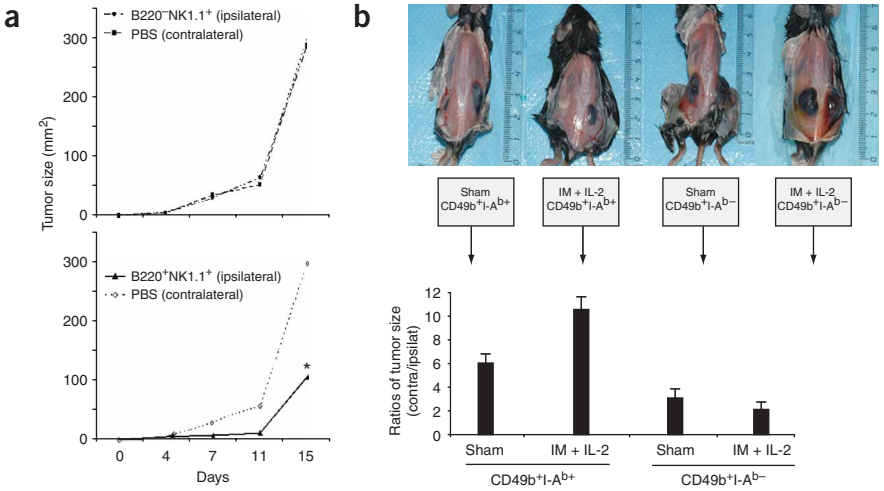


Figure 4 Adoptive transfer of B220$^+$NK1.1$^+$ or NK cells into tumors carried by immunodeficient mice. (**a**) Splenocytes enriched in CD49b$^+$ cells by magnetic separation were sorted from imatinib mesylate (IM) + IL-2–treated mice using a flow cytofluorometer into B220$^+$NK1.1$^+$ DCs or B220$^-$ NK cells and inoculated into day 3 established B16F10 melanomas in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. The contralateral flank also bearing a B16F10 tumor was inoculated with PBS in parallel, as an internal standard of uncontrolled tumor growth. (**b**) Splenocytes from C57BL/6 mice treated with sham or IM+IL-2 were enriched in CD49b$^+$ cells and then further subjected to a magnetic separation using I-A^b–specific monoclonal antibody. The CD49b$^+$I-A^{b+} DCs or CD49b$^+$I-A^{b-} NK cells were then inoculated into established B16F10 melanomas implanted in the left flank of isogenic *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ carriers. As a control, PBS was injected into the contralateral right B16F10 tumor. The ratios ($x \pm$ s.e.m., $n = 5$) of tumor growth between contralateral and ipsilateral flanks were determined 15 d later. Statistical analyses using Kruskall-Wallis test indicated significant differences at 95% confidence interval. One representative mouse was selected for macroscopic examination in each group. This experiment was repeated twice, yielding similar results.

We determined whether IKDCs and/or NK cells would be sufficient to prevent tumor outgrowth in effector-deprived hosts such as B16F10-bearing *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. We used two different methods to isolate IKDCs and NK cells. First, splenocytes derived from imatinib mesylate + IL-2–treated mice were enriched in CD49b$^+$ cells and then sorted using a flow cytometer into B220$^+$NK1.1$^+$ and B220$^-$NK1.1$^+$ cells and directly inoculated into established B16F10 tumors. As an internal control, the contralateral flank also bearing a B16F10 tumor was injected with PBS. The B220$^+$NK1.1$^+$ DCs were the only effectors capable of impairing tumor outgrowth *in vivo* (**Fig. 4a**). To further establish that MHC class II$^+$ DCs are tumoridical, splenocytes enriched in CD49b$^+$ cells were further subjected to a magnetic separation using I-A^b–specific monoclonal antibody. The CD49b$^+$I-A^{b+} DCs or CD49b$^+$I-A^{b-} NK cells were inoculated into established B16F10 melanomas. CD49b$^+$I-A^{b+} DCs (which are mostly IKDCs) mediated potent tumoricidal effects *in vivo*, irrespective of whether they were purified from imatinib mesylate + IL-2–treated or sham-treated mice. Moreover, CD49b$^+$I-A^{b-} NK cells did not show significant antitumor activity (**Fig. 4b**). Thus, adoptive transfer experiments indicated that IKDCs are unique in their ability to mediate tumor rejection *in vivo*.

This study describes a novel DC subset called IKDCs that shows unique phenotypic and functional properties. These CD11c$^+$MHC class II$^+$ DCs coexpress markers related to the plasmacytoid DC and NK cell lineages such as B220 and NK1.1, NKG2D and CD49b, respectively. This peculiar DC category exerts specialized functions and may have the role of 'tumor scavenger', capable of producing large amounts of IFN-γ and of TRAIL-dependent killing in contact with transformed cells. Although IKDCs possess NK markers and exert NK functions, they diverge from canonical NK cells in their developmental origin. Thus, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice lack NK cells but do possess functional B220$^+$NK1.1$^+$CD11c$^+$ splenocytes (**Fig. 1c** and **Supplementary Fig. 5** online). Moreover, IKDCs expressed MHC class II molecules, which are normally lacking on mouse NK cells (**Fig. 1b**), and could recognize tumor cells that are ignored by conventional NK cells (**Fig. 3c**) in the absence of any exogenous stimuli. As implied by another study[12], B220$^+$NK1.1$^+$ cells thus share the ontogeny of conventional DCs, which develop in mice lacking Rag-1, Rag-2, IL-7 receptor or Pax5. More importantly, IKDCs have the cardinal feature of DCs, that is, the capacity of antigen presentation after activation with Toll-like receptor (TLR)4 and TLR9 ligands (data not shown), as

corroborated by others[13]. IFN-γ has long been considered as the exclusive product of T, NK and NKT cells. But after appropriate stimulation (IL-12, IL-18, *Staphylococcus aureus*, Cowan strain I), B cells[14], macrophages[15] and DCs[16,17] can produce IFN-γ as well. It is not clear whether a small subset of IKDCs could account for the reported IFN-γ secretion. Some DC subsets were shown to exhibit killing activity[18]. One report has suggested a regulatory role for mouse $CD11c^+CD49b^+$ DCs, which were found to kill RMA-S and YAC-1 tumor cell targets and to produce IFN-γ after stimulation with cytokines. These regulatory DCs were identified in virally infected hosts treated with blocking CD40L-specific antibody and could protect diabetes-prone hosts after their adoptive transfer *in vivo*[19]. But these $CD11c^+CD49b^+$ DCs were obtained after negative selection, after elimination of $B220^+$ cells, ruling out similarities with IKDCs. Others have described TRAIL-dependent killing activity by *ex vivo* propagated DCs, but the pathophysiological relevance of this observation has not been documented[20,21]. Imatinib mesylate has been described to license myeloid $CD11c^+CD11b^+$ c-kit$^+$ DCs to activate resting NK cells *in vitro* and *in vivo*[9], to synergize with lipopolysaccharide for the upregulation of inflammatory cytokines and chemokines[22] and to upregulate FLT3 on bone marrow precursors, facilitating the differentiation of plasmacytoid DCs in individuals with chronic myeloid leukemia[23]. Because IKDCs did not express c-Kit, a potential signaling effect of the tyrosine kinase inhibitor on Abl or PDGFR-α transduction pathways remains to be elucidated.

IKDCs may constitute an important link between the innate and cognate arms of antitumor immunity. Conceivably, IKDCs could trigger tumor-cell apoptosis through the TRAIL pathway substituting for therapy targeting TRAIL receptors[1,2], followed by the cross-presentation of tumor-cell antigens to specific T cells. Future work in the human system might corroborate the cardinal role of IKDCs in antitumor surveillance and immunotherapy.

METHODS

Reagents and assays. We used imatinib mesylate (synthesized by C. Auclair, Ecole Normal Superior de Cachan) for *in vitro* experiments at 10^{-6} M and administered it orally twice a day *in vivo*. We intraperitoneally administered the N2B2 TRAIL-specific monoclonal antibody at 250 μg/mouse three times a week for *in vivo* neutralizing experiments and at 2 μg/ml for *in vitro* experiments[1,2]. We intraperitoneally administered depleting NK1.1-specific monoclonal antibody (300 μg of PK136 monoclonal antibody/mouse) or normal mouse serum at days –4, –2, 0 and 4 to C57BL/6 mice. We used commercial ELISA kits measuring mouse IFN-γ (Genzyme) and mouse IFN type 1 (R&D Systems) according to the manufacturer's instructions.

Mice and cell lines. We obtained female C57BL/6 (H-2^b) or BALB/c (H-2^d) wild-type mice and Swiss *nu/nu* mice from the Centre d' Elevage Janvier or the Centre d' Elevage Iffa Credo and used them at 6–10 weeks of age. We maintained mice according to the Animal Experimental Ethics Committee Guidelines (Val de Marne, France). *Tnf*$^{-/-}$, *Ifnar2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ backcrossed on a C57BL/6 background were provided by Centre d'Elevage d'Orléans and Centre d'Immunologie de Marseille. B16F10 and Hepa 16 are a melanoma and a hepatocarcinoma cell line syngeneic to C57BL/6, respectively (provided by M.T. Lotze, University of Pittsburgh). We stably transfected B16F10 cells with the cDNA encoding CrmA[24] (provided by G. Salvesen, Burnham Institute) or dominant-negative FADD[25] (provided by J. Tschopp, University of Lausanne). We injected 3×10^5 cells of these transfected cells into the tail vein for the induction of lung metastases. CT26 is colon cancer cell line syngeneic to BALB/c mice. YAC-1, B16-Rae1 and RMA-S were provided by the Centre d'Immunologie de Marseille.

Antibodies and flow cytometry analyses. We purchased all antibodies from Pharmingen, except the PE–TRAIL-specific antibody, which was purchased from e-Bioscience. We incubated cells with the antibodies and then analyzed them by FACScan (Becton Dickinson) using CellQuestPro Software.

Preparation of NK and $B220^+NK1.1^+$ cells. Splenocytes from C57BL/6 mice were enriched in $CD49b^+$ cells by a magnetic separation using CD49b-specific (Dx5) monoclonal antibody (Miltenyi Biotech) according to the manufacturer's protocol. We then stained $CD49b^+$ cells with FITC- or Cychrome-conjugated B220-specific monoclonal antibody and FITC- or PE-conjugated NK1.1-specific monoclonal antibody for cell sorting on a FACS Vantage instrument (BD Biosciences). Purified NK cells ($CD49b^+NK1.1^+B220^-$ cells) and $CD49b^+NK1.1^+B220^+$ cells were then used for functional experiments. The purity of cell separation exceeded 70%.

Electron microscopy. IKDCs were either allowed to adhere to poly-L-Lysin–coated cover slips for 30 min or cocultured for 30 min or 15 h with B16 melanoma cells first grown on cover slips overnight. We fixed cells with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 h or overnight. After several washes with cacodylate buffer, we post-fixed cells with 2% OsO_4 for 45 min on ice, dehydrated them in increasing concentrations of ethanol and embedded them in Epon while they were on cover slips. We prepared ultrathin sections with a Reichert UltracutS ultramicrotome (Leica) and viewed them with a TEM CM120 Philips electron microscope (FEI) after counterstaining them with uranyl acetate.

Statistical analyses. We compared groups using analysis of variance (ANOVA) followed by multiple comparison of means. When the variables studied were not normally distributed, we used nonparametric statistical methods. We used the Wilcoxon two-sample rank sum test to compare the values of continuous variables between two groups. When three or more groups were compared, we used the Kruskall-Wallis test. Paired comparisons were made using Wilcoxon paired test. P values of <0.05 were considered significant.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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