BCR/ABL Promotes Dendritic Cell–Mediated Natural Killer Cell Activation

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Abstract

***BCR/ABL* fusion gene, encoding a paradigmatic tyrosine kinase involved in chronic myelogenous leukemia (CML), can modulate the expression of genes involved in natural killer (NK) cell target recognition. Recent reports outline the role of allogeneic antileukemic NK effectors in the graft-versus-leukemia effect but the regulation of NK cell activation in the setting of graft-versus-leukemia effect remains unknown. Here we show that dendritic cells derived from monocytes of CML patients are selectively endowed with NK cell stimulatory capacity *in vitro*. We further show, using a gene transfer approach in mouse bone marrow progenitors, that BCR/ABL is necessary to promote dendritic cell–mediated NK cell activation. The dendritic cell/NK cell cross-talk in BCR/ABL-induced CML seems unique because JunB or IFN consensus sequence binding protein loss of functions, associated with other myeloproliferative disorders, do not promote dendritic cell–mediated NK cell activation. NK cell activation by leukemic dendritic cells involves NKG2D activating receptors and is blocked by imatinib mesylate. Indeed, BCR/ABL translocation enhances the expression levels of the NKG2D ligands on dendritic cells, which is counteracted by imatinib mesylate. Altogether, the clonal BCR/ABL dendritic cells display the unique and selective ability to activate NK cells and may participate in the NK cell control of CML. This study also highlights the deleterious role of imatinib mesylate at the dendritic cell level for NK cell activation.** (Cancer Res 2005; 65(14): 6409-17)

Introduction

Chronic myelogenous leukemia (CML) is a clonal malignancy of the hematopoietic stem cell harboring a 9;22 translocation which fuses the *ABL* proto-oncogene to the *BCR* gene leading to constitutive tyrosine kinase activity necessary and sufficient for massive overproduction of granulocytic cells (1). Natural killer (NK) cells may be involved in the control of the malignant CML clone (2). The efficacy of graft-versus-leukemia effect following allogeneic stem cell bone marrow transplantation in CML prompted the search for immune effectors. The role of NK cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation has been recently unraveled (3, 4). In addition, Cervantes et al. (5) previously showed that interleukin (IL)-2–activated NK cells from patients with CML suppress autologous primitive CML progenitors in long-term culture. Moreover, BCR/ABL confers to target cell susceptibility to NK cell lysis (6).

However, NK cells from CML patients (CML-NK) gradually decrease in number during disease progression from chronic phase to blast crisis (7). The BCR/ABL transgene in $CD34^{+}DR^{+}$ cells causes abnormal NK cell differentiation (8, 9). CML-NK cells proliferate less in response to IL-2 stimulation (7). Interestingly, significant numbers of NK cells from advanced-phase CML patients are BCR/ABL^{+} whereas T cells remain negative regardless of the disease stage. Chiorean et al. (10) have recently shown that BCR/ABL directly alters the function of NK cells (i.e., induces partial IL-2 independent growth and increases killer immunoglobulin-like receptor expression in primary $CD56^{bright}$ NK cell subsets).

We described in 1999 that dendritic cells have unique capacities to trigger NK cell effector functions *in vitro* (11). Dendritic cell–mediated NK cell activation can lead to the control of (*a*) viral replication (12) and (*b*) the growth of NK cell–sensitive tumors (11, 13). Human studies have clearly shown that mostly mature dendritic cells [i.e., cells activated by lipopolysaccharide (LPS) or type I IFN or mycobacteria] are endowed with NK cell stimulatory capacities *in vitro* (14–18). In CML patients, between 73% and 100% of monocyte-derived dendritic cells (CML dendritic cells) are positive for the chimeric BCR/ABL gene (19, 20). Many reports showed that dendritic cells derived from both normal volunteers (normal dendritic cells) and CML patients differentiated and matured in culture (19, 21–24) but there are conflicting data regarding the ability of CML dendritic cells compared with normal dendritic cells to stimulate T cells (19, 20, 25–31). However, the effects of BCR/ABL translocation on the capacity of dendritic cells to activate NK cells have never been studied. Here we show that the BCR/ABL translocation specifically confers to dendritic cells a selective NK cell stimulatory function by up-regulating the expression of NKG2D ligands in both mouse and human models.

Materials and Methods

Patients

Blood samples were collected from CML patients and normal volunteers concurrently after informed consent. Clinical status and current treatment are described in Table 1. Most patients presented with CML in chronic

Note: The first authors M. Terme and C. Borg, and the last authors A. Caignard and L. Zitvogel have equally contributed to the work.

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phase at diagnosis whereas other patients were in accelerated-phase CML despite IFNα or hydroxyurea therapy.

Mice

Female C57Bl/6 wild-type (H-2b), Rag2$^{-/-}$ (H-2b), and SCID (H-2d) mice were obtained from the Centre d'Elevage Janvier (Le Genest St Isle, France), and maintained in our animal facility according to the Animal Experimental Ethics Committee Guidelines. JunB knockout mice were generated as previously described (32) and bred in the animal facility of the Institute of Molecular Pathology, Vienna. ICSBP knockout mice were generated as described (33). All mice were used at 6 to 25 weeks of age.

Generation of Dendritic Cells

Monocyte-derived dendritic cells from chronic myelogenous leukemia patients and normal volunteers. Mononuclear cells (peripheral blood mononuclear cells) were isolated from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Adherent cells were propagated in AIM V complete medium containing 1,000 IU/mL of both recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) and recombinant human IL-4 (Novartis-Schering Plough, Kenilsworth, NJ). For *in vitro* testing, dendritic cells were used from day 8 to day 12. Maturation was induced by LPS (1 μg/mL, Sigma, St. Quentin Fallavier, France) or tumor necrosis factor α (TNFα; 50 ng/mL, Boehringer, Germany) for 24 hours. Dendritic cells were assessed by flow cytometry using the anti-DC-SIGN (R&D Systems, Inc., Minneapolis, MN), CD14, CD1a, CD83, HLA-DR and CD80, CD86, CD40 monoclonal antibodies (mAb; PharMingen, San Diego, CA) and human NKG2DFc (R&D Systems) coupled with a FITC anti-human immunoglobulin G (IgG; PharMingen).

Murine dendritic cells. *Bone marrow–derived dendritic cells.* Bone marrow–derived dendritic cells were used from day 7 to day 12 and propagated as previously described (34). *Spleen-derived dendritic cells.* Spleen-derived dendritic cells were propagated from RBC-deprived splenocytes as previously described (35). For phenotypic analyses, bone marrow–derived dendritic cells and spleen-derived dendritic cells were incubated with cychrome-conjugated anti-I-A^b (AF6-120.1) and phycoerythrin-conjugated anti-CD11c (HL-3), phycoerythrin-conjugated anti-CD80 (16-10A1), CD86 (GL1), CD40 (3/23), H-2Kb (AF6-885), and H-2Db (KH95). For Rae-1 staining, purified anti-mouse Rae-1 (Pan-specific) antibody (186107) coupled with a phycoerythrin-conjugated goat anti-rat IgG1 antibody as a secondary developing reagent was used. All antibodies were purchased from PharMingen, except for purified anti-Rae-1 antibody (R&D Systems).

Genetic Analyses of Chronic Myelogenous Leukemia–Derived Dendritic Cells by Fluorescence *In situ* Hybridization

Dual-color BCR/ABL translocation DNA probes (Oncor, Irvine, CA) were used to analyze interphase nuclei of CML dendritic cells. Fluorescence *in situ* hybridization (FISH) procedure was done according to the instructions of the manufacturer. Whereas normal cells were expected to display randomly distributed two red (*BCR* gene) and two green (*ABL* gene) hybridization signals, leukemic cells were expected to exhibit a merged yellow signal that resulted from the fusion of the BCR and the ABL genes. One-hundred cells were examined in each sample to quantify the percentage of cells bearing the BCR/ABL translocation.

BCR/ABL Constructs and Retroviruses

We used two different *BCR/ABL* oncogene constructs with their appropriate negative controls. The first vector, MSCV BCR/ABL p210, was constructed by subcloning 7 kb BCR/ABL cDNA into the MSCV Neo retroviral vector (MSCV neo p210) as previously described (36); the control vector did not contain the BCR/ABL cDNA cassette. The second vector, MIGRp210 BCR/ABL, was kindly provided by Dr. W. Pear (University of Pennsylvania, Philadelphia, PA) and contained both the BCR/ABL- and

Table 1. CML dendritic cells are selectively endowed with NK cell stimulatory capacities

Patients	Treatment	Disease phase	CD83* (%)	Mixed lymphocyte reactions† (CML dendritic cells/normal dendritic cells)	Lysis of K562† (CML dendritic cells + NK)/ (normal dendritic cells + NK)	Lysis of DAUDI† (CML dendritic cells + NK)/ (normal dendritic cells + NK)
1	IFNα	Chronic	6	ND	ND	20
2	Hydroxyurea	Accelerated	22	ND	ND	10
3	0	Chronic	30	0.7	2.18	ND
4	0	Chronic	3	0.73	1.89	ND
5	Hydroxyurea	Chronic	5	0.72	1.63	ND
6	0	Chronic	1	0.79	1.15	ND
7	Hydroxyurea	Chronic	0.8	0.73	1.25	ND
8	0	Chronic	2	0.72	5.12	ND
9	Pipobroman	Chronic	2.5	0.83	1.18	ND
10	0	Chronic	4.5	0.92	4.72	ND
11	0	Accelerated	0.7	1	1.88	ND
12	0	Chronic	26.6	1.1	4.30	ND
13	0	Chronic	6.4	1.15	0.79	ND
14	0	Chronic	19	1.15	1.32	ND
15	Hydroxyurea	Chronic	50	1.52	1.52	ND
16	Hydroxyurea	Chronic	10.3	1.42	1.07	ND
17	0	Chronic	2.7	2.4	2.30	ND

Abbreviation: ND, not determined.

*CD83 expression was determined on CML dendritic cells in flow cytometry.

†The CML dendritic cell and normal dendritic cell capacities to activate allogeneic T cells and NK cells were compared. The ratio of proliferation between allogeneic T cells exposed to normal dendritic cells or CML dendritic cells at a stimulator/effector ratio of 1:25 is shown (mixed lymphocyte reaction column). The ratio of specific lysis against K562 or DAUDI of allogeneic NK cells stimulated with normal dendritic cells or CML dendritic cells at 20:1 effector/target ratio is depicted. CML dendritic cell and normal dendritic cell capacities to activate allogeneic T cells were comparable ($P > 0.05$, Student's t test), but CML dendritic cell capacity to activate allogeneic NK cells was superior to normal dendritic cells ($P < 0.05$).

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green fluorescent protein–encoding cDNAs driven by the 5′ long terminal repeat (LTR) promoter and linked by internal ribosome entry site. In the control vector, green fluorescent protein alone was driven by the 5′ LTR. To generate defective BCR/ABL retroviruses, 5 to 10 μg of DNA were transfected into the ecotropic Phoenix or Bosc23 cell lines using standard $CaPO_4$ precipitation techniques. Transient retroviral supernatants were collected at days 2, 3, and 4 after the transfection, filtered and frozen or directly used to transduce post-5-fluorouracil (5-FU) bone marrow. The titers obtained using this protocol varied between 5×10^5 and 1×10^6 CFU/mL of supernatant.

BCR/ABL Gene Transfer in Murine Bone Marrow

C57Bl/6 mice were injected with 150 mg/kg of 5-FU. Bone marrow cells were harvested 5 days after 5-FU injection. RBC-deprived marrow cells were cultured for 48 hours in the presence of stem cell factor (50 ng/mL), Flt3 ligand (100 ng/mL), WEHI-conditioned medium (10%), recombinant murine IL-6 (IL-6; 50 ng/mL), and thrombopoietin (100 ng/mL). At day +3, marrow cells were infected by the retroviral supernatant in the presence of polybrene and growth factors. Three rounds of infection with 8 to 16 hours of incubation were done over the following 2 days. Cells were then washed, counted, and injected retro-orbitally into lethally irradiated (9.5 Gy) syngeneic mice in the presence of appropriate irradiation controls. Mice were followed by performing blood counts starting at day 14. In these conditions, fatal leukemia developed in mice over the next 3 to 4 weeks with appearance of hyperleukocytosis, splenomegaly, cachexia, and death.

BCR-ABL–Expressing Ba/F3 Cells

BCR/ABL gene transfer into Ba/F3 cells was done using MSCV p210 retrovirus as previously described (36).

Preparation of Natural Killer Cells

Human natural killer cells. NK cells were negatively selected from healthy volunteer's peripheral blood lymphocytes (PBL) using NK cell isolation kit II (Miltenyi Biotech, Paris, France). The purity of $CD56^+/CD3^-$ NK cells was assessed by flow cytometry (PharMingen) and ranged between 90% and 98%.

Mouse natural killer cells. Splenocytes were harvested from BALB/c SCID or C57Bl/6 $Rag2^{-/-}$ mice. Splenic nonadherent cells were generated by subjecting RBC-deprived splenocytes to 3-hour adherence at 37°C. Nonadherent cells were analyzed in FACScan using anti-CD3-FITC, NK1.1-phycoerythrin, or DX5-phycoerythrin before coculture with bone marrow–derived dendritic cells or spleen-derived dendritic cells. Up to 40% of such splenocytes were $CD3^-/DX5^+$ or $CD3^-/NK1.1^+$.

Dendritic Cell/Natural Killer Cell Cocultures

Human setting. Procedures have been previously described (14). In blocking experiments, the CML dendritic cell/NK cell cocultures were incubated with STI571 (10^{-6} mol/L) or with anti-NKG2D monoclonal antibody (R&D Systems) or mouse IgG1 as an isotype control.

Mouse setting. Procedures have been previously described (11). In blocking experiments, anti-mouse NKG2D (CX5) antibody (eBioscience, San Diego, CA) was added at 10 μg/mL in the bone marrow–derived dendritic cell/NK cell coculture. Rat IgG1 was used at the same dosage as an isotype control.

Assessment of Natural Killer Cell Cytolytic Activity

Viable trypan blue–excluded NK cells of the dendritic cell/NK cocultures were counted and used as effector cells. Cytotoxicity of NK cells was measured in a standard 4-hour ^{51}Cr-release assay using $Na_2^{51}CrO_4$-labeled K562 (human settings) or YAC-1 and BAF3p210 targets (mouse settings). Experiments were conducted in triplicate at various effector/target ratios.

Mixed Lymphocyte Reactions

Mixed lymphocyte reaction assays were done using allogeneic human PBLs as responder cells (PBL cells and NK cells from several individuals were used in Table 1). CML dendritic cells or normal dendritic cells were used as stimulators at various ratios. Lymphocyte proliferation was measured at day 5 after pulsing with [^{3}H]thymidine for the last 16 to 18 hours of culture.

Results

Human BCR/ABL monocyte-derived dendritic cells (chronic myelogenous leukemia–derived dendritic cells) promote natural killer cell activation. Monocyte-derived dendritic cells were propagated from 20 normal volunteers and 17 CML patients at diagnosis or during the course of their disease after optimal therapy for 8 to 12 days. CML dendritic cells failed to differentiate in 20% cases (versus 10% in normal dendritic cells). These cases were excluded from the study. From 30 mL of peripheral blood, the yield of dendritic cell recovery was 5.2 ± 3.1 ($\times 10^6$) from CML specimen versus 6.4 ± 3.4 ($\times 10^6$) from normal samples as described (30). The immunophenotypic pattern of immature CML dendritic cells is characterized by the loss of CD14 and the expression of DC-SIGN, CD86, HLA-DR (Fig. 1*A*) and CD1a, CD11c, CD80 (not shown). Both immature CML dendritic cells and normal dendritic cells were capable of significantly up-regulating the expression of cell surface markers such as CD83, CD86 (Fig. 1*A*), and CD40 (not shown) after 24 hours of incubation with recombinant human TNFα. Interphase FISH analyses revealed that >80% of dendritic cells derived from CML patients carried the *BCR/ABL* fusion gene (Fig. 1*B*). In these cohorts of normal volunteers and CML patients, no statistical differences in MHC class I and II and in costimulatory molecules could be detected at days 8 to 12 in the presence or absence of TNFα (Fig. 1 and data not shown). Therefore, we investigated the relative capacity of CML dendritic cells, as compared with normal dendritic cells, to promote allogeneic NK cell recognition and killing of K562 or DAUDI cells. In parallel, the alloreactivity of NK cells to CML dendritic cells was compared with that of PBLs (mixed lymphocyte reactions) of similar donors. Figure 1*C* to E depicts a typical case and Table 1 delineates the data for the 17 patients tested. Table 1 shows the index of proliferation of T lymphocytes stimulated by CML dendritic cells versus normal dendritic cells and the index of lytic activity of NK cells stimulated by CML dendritic cells or normal dendritic cells. Proliferation of allogeneic lymphocytes in coculture with CML dendritic cells was comparable (Fig. 1*C*) or somewhat inferior (8 of 15 patients—ratio ≤1 in Table 1) to that achieved with normal dendritic cells (as previously reported; ref. 21). However, both enhanced killing activity (Fig. 1*D*) and up-regulation of the activation marker CD69 on $CD3^-/CD56^+$ NK cells (Fig. 1*E*) were promoted only after incubation of NK cells with immature CML dendritic cells (and not normal dendritic cells). As previously reported (15), immature normal dendritic cells cannot boost resting NK cell functions in 40-hour coculture assays (Fig. 1*D* and *E*). For 14 of 17 CML patients, immature CML dendritic cells were more effective than normal dendritic cells to induce NK cell–specific lysis of K562 or DAUDI (ratio >1 in Table 1). The CML dendritic cell–mediated NK cell activation was not correlated with CD83 expression levels (Table 1), suggesting that the results cannot be accounted for by a minority of spontaneously mature cells contaminating the whole CML dendritic cell population. However, even after a maturation signal, CML dendritic cells were more effective than normal dendritic cells in triggering NK cell activation *in vitro* (TNFα or LPS; data not shown). It is noteworthy that CML dendritic cells were not more effective than normal dendritic cells to trigger NK cell IFNγ production in similar settings (data not shown).

These data show for the first time that immature CML dendritic cells display a NK cell stimulatory advantage over wild-type (WT) dendritic cells, electively promoting NK cell cytolytic functions *in vitro*.

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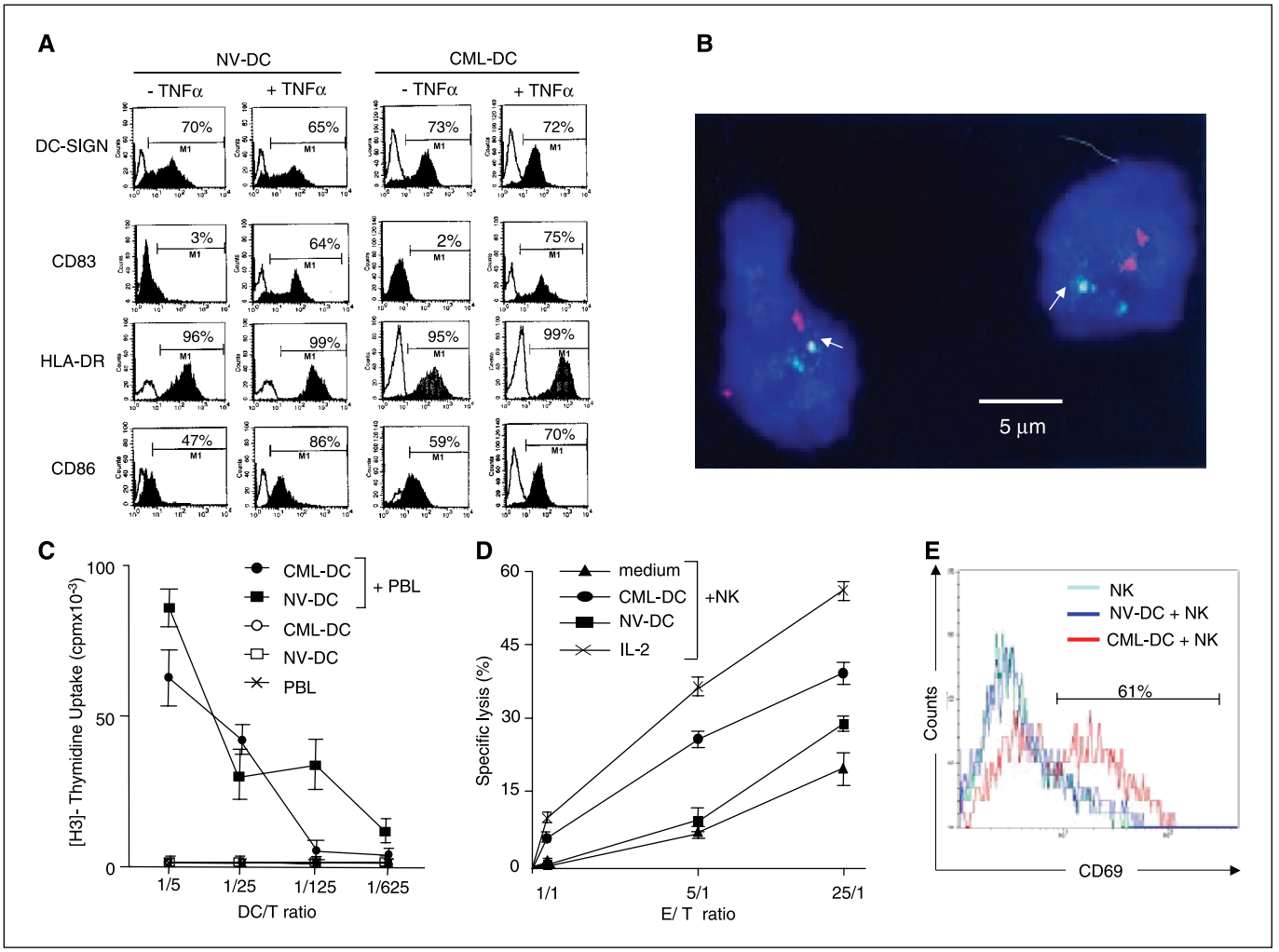

Figure 1. Immature CML dendritic cells induce the activation of resting allogeneic NK cells. *A,* CML dendritic cells (*CML-DC*) and normal dendritic cells (*NV-DC*) exhibit a similar phenotype before and after stimulation with TNFα. Cell surface expression of dendritic cell lineage (DC-SIGN)– and maturation status (CD83, CD86, HLA-DR)–related markers for monocyte-derived dendritic cells from normal volunteers (*NV-DC, left*) or from leukemic donors (*CML-DC, right*) is shown as representative overlay histograms (of 20 tested) where control mAbs are displayed using empty curves. *B,* CML dendritic cells bear the BCR/ABL translocation in FISH. One-hundred dendritic cells from CML (or normal) patients were examined by FISH to quantify the percentage of nuclei that display a fusion of the BCR and the ABL genes using specific fluorescently labeled DNA probes. *C,* mixed lymphocyte reaction comparing the allostimulatory capacity of CML dendritic cells versus normal dendritic cells for PBL proliferation. Allogeneic PBLs (1×10^5) were stimulated with titrated numbers of GM-CSF– and IL-4–propagated normal dendritic cells or CML dendritic cells during 4 days before pulsing with [^{3}H]thymidine. One representative experiment (of 15 in Table 1) is shown. *D* and *E,* CML dendritic cells promote NK cell lytic functions. The differential ability of normal dendritic cells versus CML dendritic cells to trigger allogeneic is shown NK cell functions was assessed in a cytotoxicity assay against K562 (*D*). Representative experiment (of 17 in Table 1). Results represent the mean of K562 lysis using chromium release assay done in triplicate wells ($P < 0.05$, Student's *t* test). The expression of the activation marker CD69 on the surface of the purified resting NK cells cultured alone (*light blue line*) or with normal (*dark blue line*) or CML dendritic cells (*red line*) was assessed by flow cytometry. This experiment has been done on three different patients. A representative experiment out of three is shown (*E*).

BCR/ABL gene transfer endows mouse bone marrow–derived dendritic cells with natural killer cell stimulatory capacities. We hypothesized that the BCR/ABL translocation was necessary and sufficient to endow dendritic cells with NK cell stimulatory function. Therefore, we transduced bone marrow stem cells with BCR/ABL-encoding retroviruses and transplanted these bone marrows in lethally irradiated syngeneic BL6 mice to generate a CML-like disease (37, 38). Transduction efficiency of bone marrow with the ecotropic retroviral vector encoding MIGRp210 was about 5% to 10% as assessed in flow cytometry, allowing the induction of a CML-like disorder within 25 days in 70% of mice. After leukemia development, marrow cells were cultured in the presence of recombinant murine GM-CSF (1,000 units/mL) and recombinant murine IL-4 (1,000 units/mL) for 7 to 12 days to propagate bone marrow–derived dendritic cells. Up to 67.7 ± 6.7% $CD11c^+$/$I\text{-}Ab^+$ bone marrow–derived dendritic cells expressed enhanced green fluorescent protein in flow cytometry analyses (Fig. 2). When bone marrow–derived BCR/ABL dendritic cells were cocultured for 20 hours with allogeneic NK cells, NK cells acquired enhanced killing activity against YAC-1 and BAF3p210 leukemic cell lines (Fig. 3*A*). In contrast, the control bone marrow–derived dendritic cells [nontransduced bone marrow–derived dendritic cell counterparts or mock bone marrow–derived dendritic cell recombinant for the mock retroviral constructs (Neo- or green fluorescent protein–encoding cDNA alone)] could not boost NK cell lytic activity in similar coculture conditions. Identical results were achieved with syngeneic NK cells (Fig. 3*B*). Importantly, not only bone marrow–derived BCR/ABL dendritic cells but also

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spleen-derived BCR/ABL dendritic cells could boost NK cell lytic activity against YAC-1 cells *in vitro* (Fig. 3*C*), whereas the controls could not. In addition, autologous BCR/ABL$^+$ NK cells which did not respond to short-term IL-2 stimulation for IFNγ production could be induced by autologous bone marrow–derived BCR/ABL$^+$ dendritic cells (Fig. 3*D*).

Altogether, these data suggest that BCR/ABL gene product was necessary to convert mouse dendritic cells into NK cell stimulators, confirming the results achieved with the CML dendritic cells.

The unique role of BCR/ABL dendritic cells in the dendritic cell–mediated natural killer cell activation. To confirm the role of the paradigmatic BCR/ABL translocation in the bone marrow–derived dendritic cell–mediated NK cell activation, we investigated whether the repression of the tyrosine kinase activity by imatinib mesylate (STI571) would interfere with NK cell cytolytic functions. Incubation with STI571 blocked the capacity of bone marrow–derived dendritic cells to activate mouse NK cells, suggesting that BCR/ABL translocation promotes the NK stimulatory capacity of the dendritic cells (Fig. 4*A*).

We next addressed whether BCR/ABL leukemic cell lines such as the BAF3p210 could substitute for bone marrow–derived BCR/ABL dendritic cells in activating mouse NK cells in a similar experimental setting. Figure 4*B* shows that BAF3p210 cells cannot enhance NK cell killing activity against YAC-1 cells *in vitro*, in contrast to WT bone marrow–derived dendritic cells propagated in GM/IL-4 as previously reported (11) or bone marrow–derived BCR/ABL dendritic cells. However, as shown in Fig. 3, Baf3p210 are targets susceptible to NK cell lysis.

We next asked whether genetic defects leading to myeloproliferative disorders other than BCR/ABL translocation could promote NK cell activation. Bone marrow–derived dendritic cells were derived from mice bearing a null mutation of IFN consensus sequence binding protein (ICSBP) developing a systemic CML-like syndrome leading to blast crisis with clonal expansion of undifferentiated cells (33). The phenotypic analyses of such ICSBP$^{-/-}$ bone marrow–derived dendritic cells did not reveal atypical features but all bone marrow–derived dendritic cells were CD8α$^-$ (data not shown). As shown in Fig. 4*C*, the ICSBP$^{-/-}$ bone marrow–derived dendritic cells were not able to promote NK cell activation either. Transgenic mice specifically lacking JunB expression in the myeloid lineage develop a myeloproliferative disease eventually progressing to blast crisis, which resembles human CML (32). Although the differentiation of bone marrow–derived dendritic cells from JunB$^{-/-}$ marrow precursors was less productive than with WT marrow, we derived bone marrow–derived dendritic cells from JunB$^{-/-}$ mice and phenotypic analyses of JunB$^{-/-}$ bone marrow–derived dendritic cells and WT bone marrow–derived dendritic cells did not reveal major differences. However, JunB$^{-/-}$ bone marrow–derived dendritic cells lost their capacity to enhance NK cell lytic activity compared with their WT counterparts (Fig. 4*D*).

Therefore, BCR/ABL confers to dendritic cells unique capacities to activate NK cells and STI571 specifically interferes in the BCR/ABL dendritic cell–mediated NK cell activation.

NKG2D receptors are involved in BCR/ABL dendritic cell–mediated natural killer cell activation. We next investigated the mechanism involved in BCR/ABL dendritic cell–mediated NK cell activation. In mouse setting, when bone marrow–derived BCR/ABL dendritic cells and NK cells were physically separated by a porous membrane (transwell), bone marrow–derived BCR/ABL dendritic cells lost their capacity to activate NK cells (Fig. 5*A*), indicating a cell contact-dependent mechanism. Because ligands of the NK cell activating receptor NKG2D are induced by stress or neoplastic transformation and are present on leukemic cells (39, 40), we investigated the role of NKG2D in the NK cell activity mediated by bone marrow–derived BCR/ABL dendritic cells. Addition of anti-NKG2D blocking antibody in the coculture significantly hampered

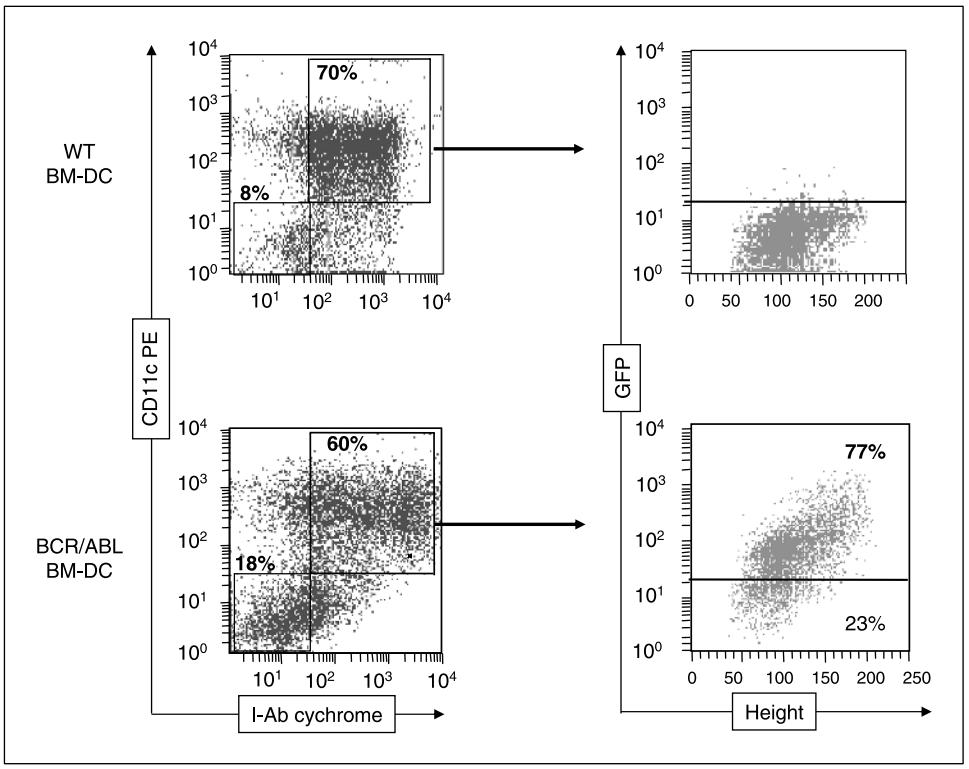

Figure 2. BCR/ABL expression in mouse bone marrow–derived dendritic cells (*BM-DC*). Bone marrow–derived dendritic cells propagated from bone marrow precursors of mice developing CML-like disease in GM-CSF and IL-4 were analyzed in flow cytometry using a two-color staining (I-A^b-cychrome, CD11c-phycoerythrin). Green fluorescent protein expression (and consequently BCR/ABL expression) was assessed on gated I-A^{b+}/CD11c$^+$ cells. Nontransduced bone marrow-derived dendritic cell or bone marrow dendritic cells transduced with Neo retroviral constructs (not shown) were used as a negative control for green fluorescent protein expression. Representative of two experiments.

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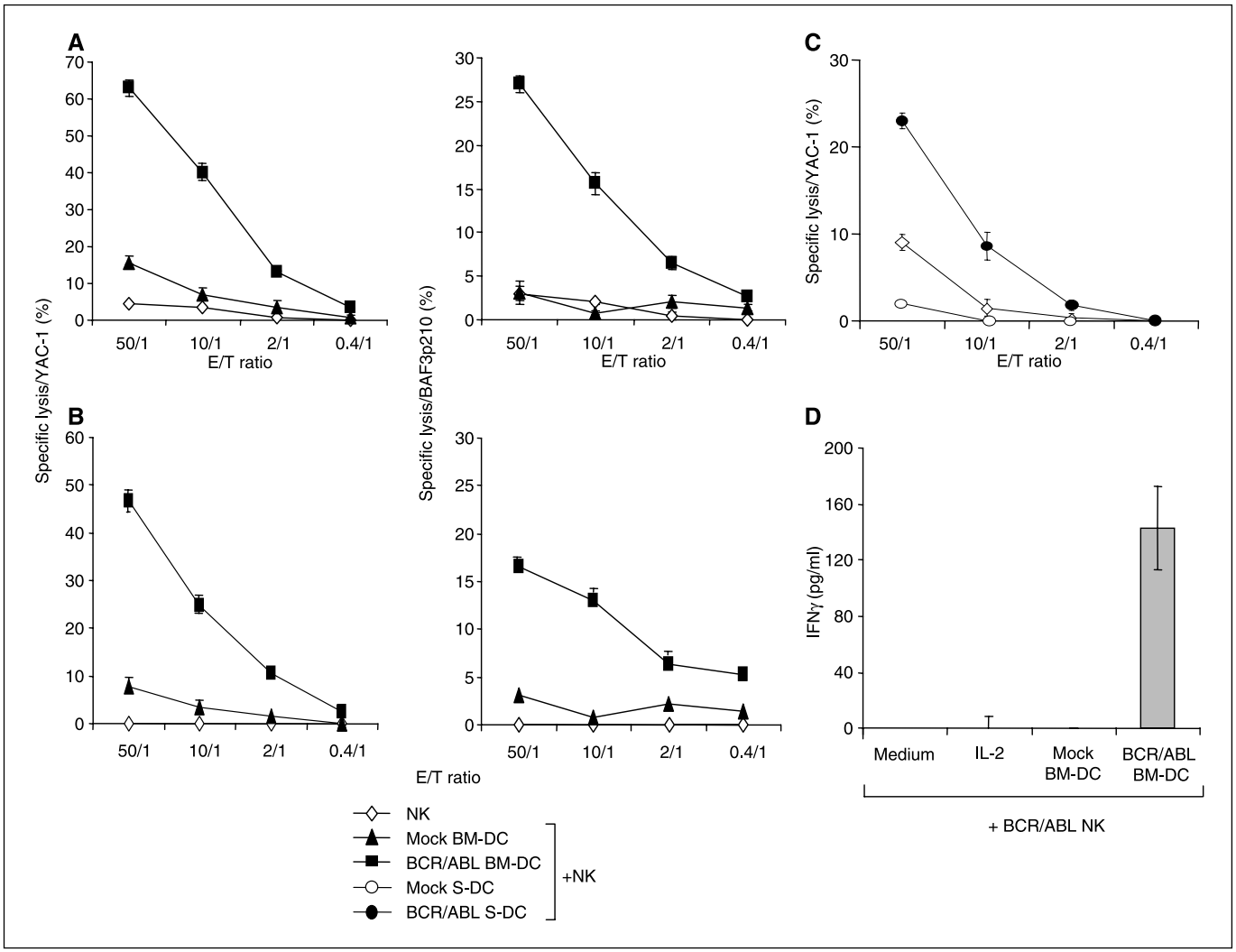

Figure 3. BCR/ABL confers NK cell stimulatory capacities to spleen- and bone marrow–derived dendritic cells. *A,* bone marrow–derived dendritic cells derived from leukemic mice (70% are bearing the BCR/ABL translocation) or from mice reconstituted with bone marrow infected with mock retroviral vectors (*Mock BM-DC*) were used to stimulate allogeneic BALB/c SCID–derived splenocytes at 1 NK:0.5 dendritic cells for 20 hours. Cocultures were then subjected to a ^{51}Cr-release assay against YAC-1 (*left*) or BAF3p210 (*right*) target cells at various effector/target (*E/T*) ratios. *B,* similar experimental settings as in (*A*), but using NK cells derived from syngeneic C57Bl/6 Rag2$^{-/-}$. *C,* similar experimental settings as in (*A*), but using day 20 spleen-derived dendritic cells (*S-DC*) propagated from nonleukemic or leukemic mice (*BCR/ABL S-DC*). *D,* autologous NK cells derived from leukemic mice and bearing BCR/ABL translocation (not shown) were incubated with recombinant human IL-2 (100,000 IU/mL) or with autologous bone marrow–derived BCR/ABL dendritic cells derived from leukemic mice or with syngeneic mock vector–transduced dendritic cells (*Mock BM-DC*). IFNγ levels were monitored after 20 hours in ELISA. Each depicted experiment has been reproduced three times with similar results. Dendritic cells alone did not lyse YAC-1 or BAF3p210 (data not shown).

NK cell activation (Fig. 5*B*). Furthermore, we showed in flow cytometry analyses that BCR/ABL translocation did up-regulate Rae-1 molecules on bone marrow–derived dendritic cells compared with WT dendritic cells (Fig. 5*C*). Whereas Rae-1 were not expressed on control bone marrow–derived dendritic cells (WT bone marrow-derived dendritic cells or bone marrow–derived dendritic cells transduced with a mock retroviral vector), dendritic cells propagated from CML developing mice expressed Rae-1 (Fig. 5*C*). Interestingly, incubation with STI571 reduced Rae-1 expression on bone marrow–derived BCR/ABL dendritic cells (Fig. 5*C*).

We confirmed these data achieved in the mouse setting in human CML because CML dendritic cells did express low levels of NKG2D ligands whereas normal dendritic cells did not (Fig. 5*D*). Moreover, the killing activity of NK cells promoted by CML dendritic cells was partially inhibited by STI571 (Fig. 5*E*) or by anti-NKG2D mAb but not by irrelevant isotype control antibody (Fig. 5*F*).

Altogether, these data suggest that activation of NK cells by BCR/ABL dendritic cells involves the NKG2D activating receptors, and that CML translocation confers to dendritic cells a NK cell stimulatory capacity by up-regulating NKG2D ligands, a process inhibited by imatinib mesylate.

Discussion

BCR/ABL is both a critical oncogene and a tumor-specific antigen in CML. The consequences of the BCR/ABL translocation on dendritic cell functions are the subject of major clinical and scientific interest because such antigen-presenting cells could represent ideal T-cell–based vaccines. CML dendritic cells have a reduced capacity to capture exogenous antigen via macropinocytosis or via mannose receptors when compared with healthy individuals (30). Chemokine-induced migration of CML dendritic

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cells *in vitro* was significantly reduced. Such defects may be related to underlying cytoskeletal changes induced by the $p210^{BCR/ABL}$ fusion protein. However, we report here the first observation stating that BCR/ABL selectively confers to dendritic cells NK cell stimulatory capacities. Indeed, we show that CML dendritic cells at an immature stage trigger NK cell activation in 14 of 17 patients. We formally confirmed that BCR/ABL expression is necessary and sufficient to endow dendritic cells with NK cell stimulatory functions in mouse *in vitro* model systems.

This observation is surprising because immature normal dendritic cells cannot promote NK cell activation in 40-hour coculture assays *in vitro* (15, 18). However, in contrast to mature LPS-stimulated normal dendritic cells, CML dendritic cells only enhance NK cell cytolytic functions and not IFNγ secretion. As previously reported in mouse setting using WT dendritic cells, BCR/ABL dendritic cell–mediated NK cell activation is dependent on cell-to-cell contact (Fig. 5*A*). We could not find down-regulation of MHC class I expression levels on BCR/ABL dendritic cells (as confirmed by others; ref. 21). Delivery of positive signals through NK cell receptors does contribute to antitumor defense. Ectopic expression of ligands for NK cell activating receptors on tumor cells allowed NK cell–mediated tumor rejection in various mouse tumor models *in vivo* (41). Delivery of positive signals through NK cell receptors (Ly49H) was also relevant in anti–murine cytomegalovirus viral defense (12). Our data outline a major role of NKG2D activating receptors in BCR/ABL dendritic cell– and CML dendritic cell–mediated NK cell activation in both mouse and human experimental settings. Moreover, BCR/ABL translocation up-regulates expression levels of NKG2D ligands on BCR/ABL dendritic cell and CML dendritic cell surfaces (Fig. 5*C* and *D*). In accordance with this finding, STI571 inhibited Rae-1 expression on BCR/ABL dendritic cells *in vitro* (Fig. 5*C*). Importantly, neither CML dendritic cells nor bone marrow–derived BCR/ABL dendritic cells were susceptible targets for resting NK cell lysis (our data not shown). Because imatinib mesylate or anti-NKG2D antibody blocked only partially NK cell activation, we cannot exclude a role for another molecular interaction. A putative role of IL-15 in the CML dendritic cell–mediated NK cell activation could be discussed because a recent report establishes that CML dendritic

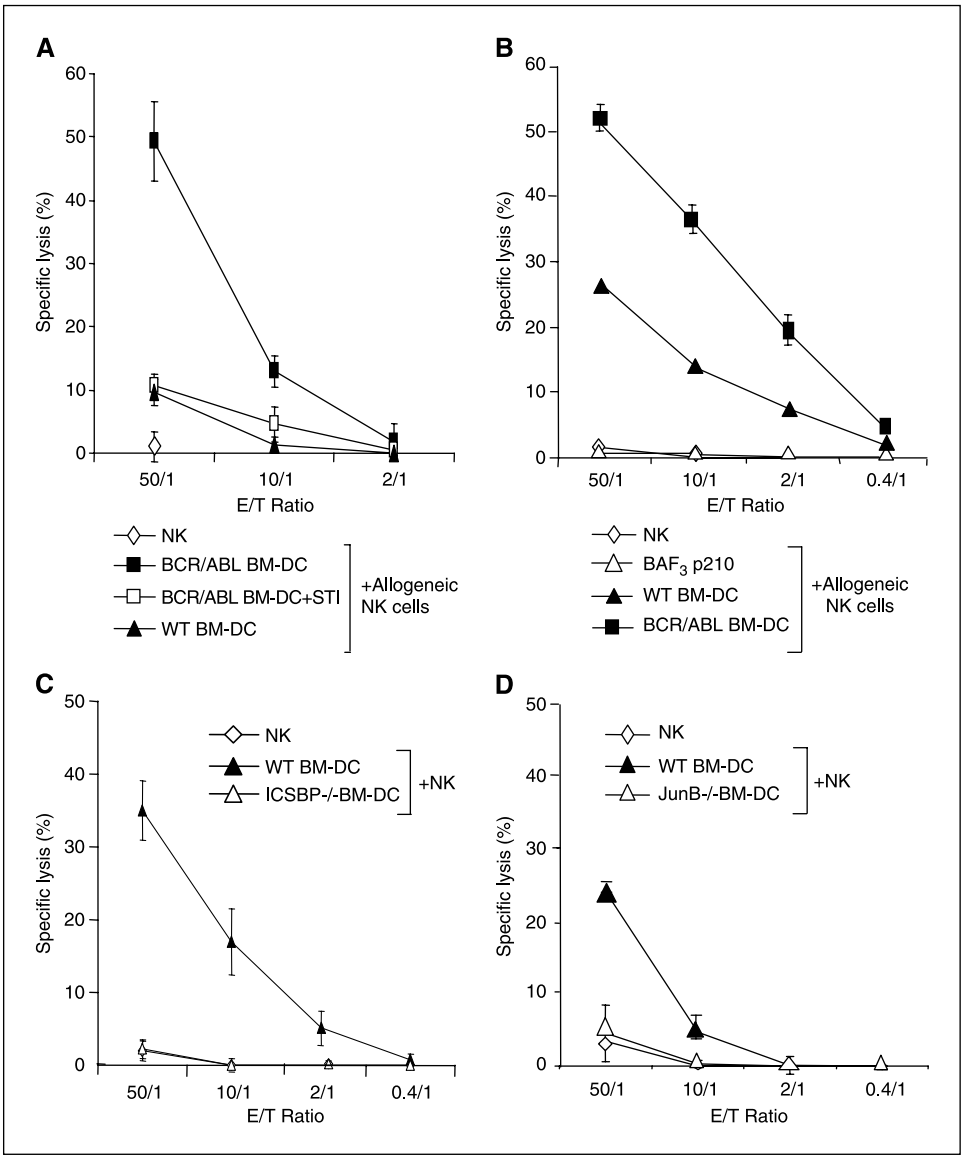

Figure 4. The BCR/ABL translocation has a unique role in promoting dendritic cell–mediated NK cell activation. *A,* similar experimental setting as in Fig. 3*A*, but comparing bone marrow–derived BCR/ABL dendritic cells (*BCR/ABL BM-DC*) with STI571-pretreated bone marrow–derived BCR/ABL dendritic cells and WT bone marrow–derived dendritic cells. This experiment has been repeated twice with similar results. *B,* similar experimental settings as in Fig. 3*A*, but using BAF3p210 cells or WT bone marrow–derived dendritic cells or bone marrow–derived BCR/ABL dendritic cells. *C* and *D,* similar setting as in (*A*), but comparing WT dendritic cells with either $ICSBP^{-/-}$ bone marrow–derived dendritic cells (*C*) or $JunB^{-/-}$ bone marrow–derived dendritic cells (*D*). Bone marrow–derived dendritic cells alone, either WT or mutated, did not directly lyse YAC-1 cells (not shown).

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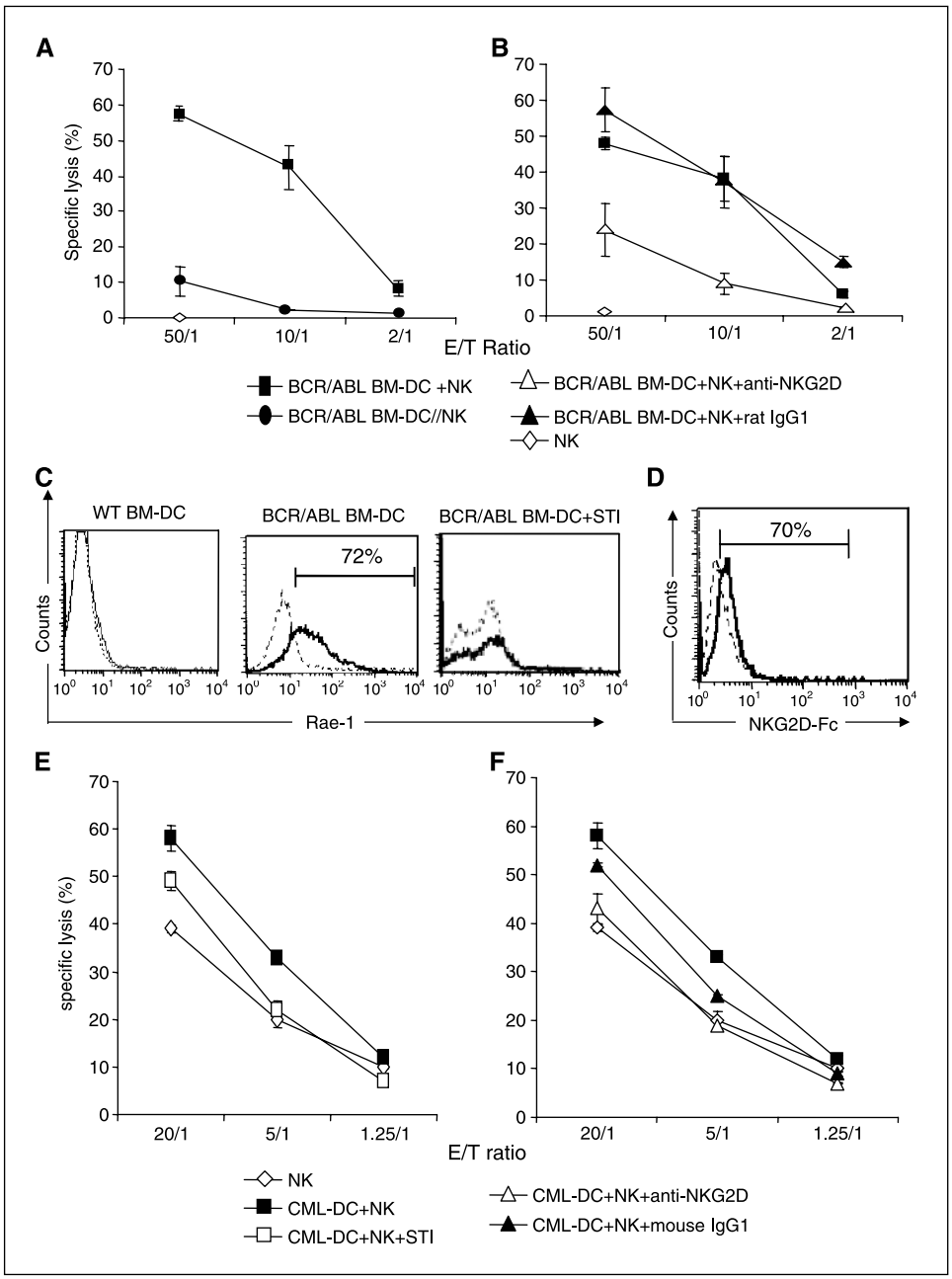

Figure 5. NKG2D receptors are involved in BCR/ABL dendritic cell–mediated NK cell activation (*A*). Similar experimental setting as in Fig. 3*A*, but bone marrow dendritic cells and NK cells were physically separated by a transwell (//) in the coculture. *B,* similar experimental setting as in Fig. 3*A*, but adding 10 μg/mL of anti-NKG2D blocking antibody or isotype control (rat IgG1) in the coculture. *C,* Rae-1 expression was assessed using flow cytometry analyses on bone marrow–derived BCR/ABL dendritic cells, pretreated or not with STI571, and on WT bone marrow-derived dendritic cells using anti-pan-Rae-1 antibody revealed by a second goat anti-rat IgG phycoerythrin. *Datched lines,* staining with isotype control. *D,* NKG2D ligand expression on CML dendritic cells was assessed in flow cytometry using human NKG2DFc revealed by a FITC mouse anti-human IgG antibody. *Datched lines,* staining with isotype control. A representative culture is shown but another CML dendritic cell culture derived from a second patient also stained with NKG2DFc reagents (*E*). Same experimental setting as in Fig. 1*D*, but using STI571-pretreated CML dendritic cells (*F*). Same experiment as in Fig. 1*D*, but in the presence of 4 μg/mL of blocking anti-NKG2D antibody or mouse IgG1 as isotype control. Each depicted experiment has been reproduced two to three times with similar results.

cells differentiated in the presence of IFNα + GM-CSF express IL-15 (42). Moreover, Koka et al. (43) just showed that IL-15 receptor α expressed on dendritic cells is required to prime NK cells.

To our knowledge, it is the first time that BCR/ABL is described to promote the expression of NKG2D ligands on the cell surface of the cell it is transforming. Not only the BCR/ABL translocation could induce the expression of Rae-1 on bone marrow–derived dendritic cells (Fig. 5) but it also triggered MIC (MHC-Class I related protein) expression on UT7.9, a monocytic cell line transformed with the BCR/ABL transgene.[9] The expression of NKG2D ligands on bone marrow–derived dendritic cells and UT7.9 was down-regulated when cells were subjected to STI571 *in vitro*, suggesting that aberrant transduction signaling through BCR/ABL is responsible for the up-regulation of NKG2D ligand expression. It is likely that the induction of NKG2D ligand expression on bone marrow–derived dendritic cells accounts for NK cell triggering because NK cell activation was hampered in the presence of bone marrow–derived BCR/ABL dendritic cells and anti-NKG2D antibody (Fig. 5). However, if therapy with Gleevec might, on one hand, directly destroy BCR/ABL leukemic targets by a cell autonomous mechanism, it might, on the other hand, also interfere with the NK cell activation promoted by bone marrow–derived BCR/ABL dendritic cells and with the NKG2D-dependent NK cell recognition of BCR/ABL leukemic targets. Whether T-cell costimulatory function via NKG2D receptors might also be hindered in this tumor model remains to be determined.

The relevance of our finding is questionable. It is conceivable that residual bone marrow–derived BCR/ABL dendritic cells could

[9] A. Caignard, unpublished observation.

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participate in donor NK cell activation after allogeneic bone marrow transplantation and/or donor lymphocyte infusion and, therefore, control/switch relapse and/or disease-free status. In the model system previously reported by Ruggeri et al. (3, 4), it is unclear how allogeneic donor NK cells undergo activation to operate graft-versus-leukemia effects. It is possible that recognition of HLA-mismatched acute myelogenous leukemia or CML targets is a necessary and sufficient signal to promote NK cell activation. It is also likely that the conditioning regimen used provide a "cytokine storm" beneficial for NK cell priming. Likewise, the malignant bone marrow–derived BCR/ABL dendritic cells might provide the necessary activating signals to trigger donor NK cells.

Experimental and clinical studies already suggest that imatinib mesylate might not be sufficient to eradicate Philadelphia chromosome–positive stem cells. Thus, data reported in this work could be of major interest for the design of novel cell therapies, especially in patients with chronic as well as more advanced phases of CML developing resistance to imatinib mesylate.

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References

1. Sawyers CL. Chronic myeloid leukemia. N Engl J Med 1999;340:1330–40.
2. Pierson BA, Miller JS. The role of autologous natural killer cells in chronic myelogenous leukemia. Leuk Lymphoma 1997;27:387–99.
3. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 1999;94:333–9.
4. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002;295:2097–100.
5. Cervantes F, Pierson BA, McGlave PB, Verfaillie CM, Miller JS. Autologous activated natural killer cells suppress primitive chronic myelogenous leukemia progenitors in long-term culture. Blood 1996;87:2476–85.
6. Baron F, Turhan AG, Giron-Michel J, et al. Leukemic target susceptibility to natural killer cytotoxicity: relationship with BCR-ABL expression. Blood 2002;99:2107–13.
7. Verfaillie C, Kay N, Miller W, McGlave P. Diminished A-LAK cytotoxicity and proliferation accompany disease progression in chronic myelogenous leukemia. Blood 1990;76:401–8.
8. Carayol G, Giron-Michel J, Azzarone B, et al. Altered natural killer cell differentiation in CD34+ progenitors from chronic myeloid leukemia patients. Oncogene 2000;19:2758–66.
9. Nakajima H, Zhao R, Lund TC, et al. The BCR/ABL transgene causes abnormal NK cell differentiation and can be found in circulating NK cells of advanced phase chronic myelogenous leukemia patients. J Immunol 2002;168:643–50.
10. Chiorean EG, Dylla SJ, Olsen K, Lenvik T, Soignier Y, Miller JS. BCR/ABL alters the function of NK cells and the acquisition of killer immunoglobulin-like receptors (KIRs). Blood 2003;101:3527–33.
11. Fernandez NC, Lozier A, Flament C, et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses *in vivo*. Nat Med 1999;5:405–11.
12. Andrews DM, Scalzo AA, Yokoyama WM, Smyth MJ, Degli-Esposti MA. Functional interactions between dendritic cells and NK cells during viral infection. Nat Immunol 2003;4:175–81.
13. Valteau-Couanet D, Leboulaire C, Maincent K, et al. Dendritic cells for NK/LAK activation: rationale for multicellular immunotherapy in neuroblastoma patients. Blood 2002;100:2554–61.
14. Fernandez NC, Flament C, Crepineau F, Angevin E, Vivier E, Zitvogel L. Dendritic cells (DC) promote natural killer (NK) cell functions: dynamics of the human DC/NK cell cross talk. Eur Cytokine Netw 2002;13:17–27.
15. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. J Exp Med 2002;195:327–33.
16. Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. J Exp Med 2002;195:335–41.
17. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Munz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. J Exp Med 2002;195:343–51.
18. Zitvogel L. Dendritic and natural killer cells cooperate in the control/switch of innate immunity. J Exp Med 2002;195:F9–14.
19. Eibl B, Ebner S, Duba C, et al. Dendritic cells generated from blood precursors of chronic myelogenous leukemia patients carry the Philadelphia translocation and can induce a CML-specific primary cytotoxic T-cell response. Genes Chromosomes Cancer 1997;20:215–23.
20. Dietz AB, Bulur PA, Erickson MR, et al. Optimizing preparation of normal dendritic cells and bcr-abl+ mature dendritic cells derived from immunomagnetically purified CD14+ cells. J Hematother Stem Cell Res 2000;9:95–101.
21. Eisendle K, Lang A, Eibl B, et al. Phenotypic and functional deficiencies of leukaemic dendritic cells from patients with chronic myeloid leukaemia. Br J Haematol 2003;120:63–73.
22. Smit WM, Rijnbeek M, van Bergen CA, et al. Generation of dendritic cells expressing bcr-abl from CD34-positive chronic myeloid leukemia precursor cells. Hum Immunol 1997;53:216–23.
23. Heinzinger M, Waller CF, von den Berg A, Rosenstiel A, Lange W. Generation of dendritic cells from patients with chronic myelogenous leukemia. Ann Hematol 1999;78:181–6.
24. Westermann J, Kopp J, Korner I, et al. Bcr/abl+ autologous dendritic cells for vaccination in chronic myeloid leukemia. Bone Marrow Transplant 2000;25 Suppl 2:S46–9.
25. Choudhury A, Gajewski JL, Liang JC, et al. Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 1997;89:1133–42.
26. Mannering SI, McKenzie JL, Hart DN. Optimisation of the conditions for generating human DC initiated antigen specific T lymphocyte lines *in vitro*. J Immunol Methods 1998;219:69–83.
27. Nieda M, Nicol A, Kikuchi A, et al. Dendritic cells stimulate the expansion of bcr-abl specific CD8+ T cells with cytotoxic activity against leukemic cells from patients with chronic myeloid leukemia. Blood 1998;91:977–83.
28. Osman Y, Takahashi M, Zheng Z, et al. Generation of bcr-abl specific cytotoxic T-lymphocytes by using dendritic cells pulsed with bcr-abl (b3a2) peptide: its applicability for donor leukocyte transfusions in marrow grafted CML patients. Leukemia 1999;13:166–74.
29. Muller L, Provenzani C, Faul C, Pawelec G. Recognition of chronic myelogenous leukaemia cells by autologous T lymphocytes primed *in vitro* against the patient's dendritic cells. Br J Haematol 2001;112:740–8.
30. Dong R, Cwynarski K, Entwistle A, et al. Dendritic cells from CML patients have altered actin organization, reduced antigen processing, and impaired migration. Blood 2003;101:3560–7.
31. Wang C, Al-Omar HM, Radvanyi L, et al. Clonal heterogeneity of dendritic cells derived from patients with chronic myeloid leukemia and enhancement of their T-cells stimulatory activity by IFN-α. Exp Hematol 1999;27:1176–84.
32. Passegue E, Jochum W, Schorpp-Kistner M, Mohle-Steinlein U, Wagner EF. Chronic myeloid leukemia with increased granulocyte progenitors in mice lacking junB expression in the myeloid lineage. Cell 2001;104:21–32.
33. Holtschke T, Lohler J, Kanno Y, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell 1996;87:307–17.
34. Mayordomo JI, Loftus DJ, Sakamoto H, et al. Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. J Exp Med 1996;183:1357–65.
35. Berthier R, Martinon-Ego C, Laharie AM, Marche PN. A two-step culture method starting with early growth factors permits enhanced production of functional dendritic cells from murine splenocytes. J Immunol Methods 2000;239:95–107.
36. Ahmed M, Dusanter-Fourt I, Bernard M, et al. BCR-ABL and constitutively active erythropoietin receptor (cEpoR) activate distinct mechanisms for growth factor-independence and inhibition of apoptosis in Ba/F3 cell line. Oncogene 1998;16:489–96.
37. Pear WS, Miller JP, Xu L, et al. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood 1998;92:3780–92.
38. Elefanty AG, Hariharan IK, Cory S. bcr-abl, the hallmark of chronic myeloid leukaemia in man, induces multiple haemopoietic neoplasms in mice. EMBO J 1990;9:1069–78.
39. Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 2002;100:1935–47.
40. Salih HR, Antropius H, Gieseke F, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003;102:1389–96.
41. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature 2001;413:165–71.
42. Gabriele L, Borghi P, Rozera C, et al. IFN-α promotes the rapid differentiation of monocytes from patients with chronic myeloid leukemia into activated dendritic cells tuned to undergo full maturation after LPS treatment. Blood 2004;103:980–7.
43. Koka R, Burkett P, Chien M, Chai S, Boone DL, Ma A. Cutting edge: murine dendritic cells require IL-15Rα to prime NK cells. J Immunol 2004;173:3594–8.

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BCR/ABL Promotes Dendritic Cell–Mediated Natural Killer Cell Activation

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