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 NGK2D activating receptors and is blocked by imatinib mesylate. Indeed, BCR/ABL translocation enhances the expression levels of the NGK2D 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 CD56bright 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 NGK2D 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...
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 previously 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), anti-CD14, anti-CD1a, anti-CD83, HLA-DR and CD80, CD86, CD40 monoclonal antibodies (mAb; PharMingen, San Diego, CA) and human NKG2DFc (R&D Systems) 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).

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 cytochrome-conjugated anti-I-A<sup>β</sup> (AF6-120.1) and phycoerythrin-conjugated anti-CD11c (HL-3), phycoerythrin-conjugated anti-CD80 (16-10A1), CD86 (GL1), CD40 (5/23), 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).

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

<table>
<thead>
<tr>
<th>Patients</th>
<th>Treatment</th>
<th>Disease phase</th>
<th>CD83&lt;sup&gt;a&lt;/sup&gt; (Mixed lymphocyte reactions&lt;sup&gt;b&lt;/sup&gt; (CML dendritic cells + normal dendritic cells)</th>
<th>Lysis of K562&lt;sup&gt;c&lt;/sup&gt; (CML dendritic cells + NK)/ (normal dendritic cells + NK)</th>
<th>Lysis of DAUDI&lt;sup&gt;c&lt;/sup&gt; (CML dendritic cells + NK)/ (normal dendritic cells + NK)</th>
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Abbreviation: ND, not determined.
<sup>a</sup>CD83 expression was determined on CML dendritic cells in flow cytometry.
<sup>b</sup>Lysis of K562 or DAUDI was determined by a 20:1 effector/target ratio. 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).
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 CaPO₄ precipitation techniques. Transient retroviral supernatants were collected at days 2, 3, and 4 after the transfection, filtered and frozen or directly used to transduce 5-fluorouracil (5-FU) bone marrow. The titers obtained using this protocol varied between 5 × 10⁵ and 1 × 10⁶ CFU/ml of supernatant.

BCR/ABL Gene Transfer in Murine Bone Marrow
C57Bl/6 mice were injected with 150 μg/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), Flk3 ligand (100 ng/mL), WEHI-conditioned medium (10%), recombinant murine IL-6 (II-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 infections of 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 FACSscan using anti-CD3-FITC, NK1.1-phycocerythrin, or DX5-phycocerythrin before coculture with bone marrow–derived dendritic cells or spleen-derived dendritic cells. Up to 40% of such splenocytes were CD56+/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-NK22D 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 NK22D (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 cell cocultures were counted and used as effector cells. Cytotoxicity of NK cells was measured in a standard 4-hour ⁵¹Cr-release assay using Na₂¹⁶CrO₄-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 alloimmune 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 [³H]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 (×10⁶) from CML specimen versus 6.4 ± 3.4 (×10⁶) 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. 1A) 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. 1A), 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. 1B). 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 1C 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. 1C) 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. 1D) and up-regulation of the activation marker CD69 on CD3+/CD56− NK cells (Fig. 1E) 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. 1D 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.
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-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. 3A). 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. 3B). Importantly, not only bone marrow–derived BCR/ABL dendritic cells but also
spleen-derived BCR/ABL dendritic cells could boost NK cell lytic activity against YAC-1 cells in vitro (Fig. 3C), whereas the controls could not. In addition, autologous BCR/ABL+ NK cells which did not respond to short-term IL-2 stimulation for IFN production could be induced by autologous bone marrow–derived BCR/ABL+ dendritic cells (Fig. 3D).

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. 4A).

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 4B 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 gene (data not shown). As shown in Fig. 4C, 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. 4D).

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. 5A), 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](https://www.aacrjournals.org/CancerRes/article-pdf/65/14/6413/6116858/A001407.pdf)
NK cell activation (Fig. 5B). 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. 5C). 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. 5C).

Interestingly, incubation with STI571 reduced Rae-1 expression on bone marrow–derived BCR/ABL dendritic cells (Fig. 5C).

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. 5D). Moreover, the killing activity of NK cells promoted by CML dendritic cells was partially inhibited by STI571 (Fig. 5E) or by anti-NKG2D mAb but not by irrelevant isotype control antibody (Fig. 5F).

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 macrophagocytosis or via mannose receptors when compared with healthy individuals (30). Chemokine-induced migration of CML dendritic
cells in vitro was significantly reduced. Such defects may be related to underlying cytoskeletal changes induced by the p210BCR/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 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. 5A). 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. 5C and D). In accordance with this finding, STI571 inhibited Bae-1 expression on BCR/ABL dendritic cells in vitro (Fig. 5C).

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](https://example.com/figure4.jpg)

**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. 3A, 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 with similar results. B, similar experimental settings as in Fig. 3A, 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).
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

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9 A. Caignard, unpublished observation.
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 Ruggieri 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 CBR/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.

Acknowledgments

References

41. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
42. We remain indebted to the Institut Gustave Roussy animal facility staff for advice and support and to the Institut Gustave Roussy animal facility staff for advice and support. We remain indebted to the Institut Gustave Roussy animal facility staff for advice and support.
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