Altered IFNγ Signaling and Preserved Susceptibility to Activated Natural Killer Cell–Mediated Lysis of BCR/ABL Targets

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Abstract

Previous studies have shown that BCR/ABL oncogene, the molecular counterpart of the Ph1 chromosome, could represent a privileged target to natural killer (NK) cells. In the present study, we showed that activated peripheral NK cells killed high-level BCR/ABL transfectant UT-7/9 derived from the pluripotent hematopoietic cell line UT-7 with a high efficiency. To further define the mechanisms controlling BCR/ABL target susceptibility to NK-mediated lysis, we studied the effect of IFNγ, a key cytokine secreted by activated NK cells, on the lysis of these targets. Treatment of UT-7, UT-7/neo, and low BCR/ABL transfectant UT-7/E8 cells with IFNγ resulted in a dramatic induction of human leukocyte antigen class I (HLA-I) molecules and subsequently in their reduced susceptibility to NK-mediated cytolysis likely as a consequence of inhibitory NK receptors engagement. In contrast, such treatment neither affected HLA-I expression on transfectants expressing high level of BCR/ABL (UT-7/9) nor modulated their lysis by NK cells. Our data further show that the high-level BCR/ABL in UT-7/9 cells display an altered IFNγ signaling, as evidenced by a decrease in IFN regulatory factor-1 (IRF-1) and signal transducers and activators of transcription (STAT) 1 induction and activation in response to IFNγ, whereas this pathway is normal in UT-7 and UT-7/E8 cells. A decreased HLA-I induction and nuclear phospho-STAT1 nuclear translocation were also observed in blasts from most chronic myelogenous leukemia patients in response to IFNγ. These results outline the crucial role of IFNγ in the control of target cell susceptibility to lysis by activated NK cells and indicate that the altered response to IFNγ in BCR/ABL targets may preserve these cells from the cytokine-induced negative regulatory effect on their susceptibility to NK-mediated lysis. (Cancer Res 2005; 65(7): 2914-20)

Introduction

Chronic myeloid leukemia (CML) is characterized by the presence, in all leukemic stem cells and progenitors, of the chimeric BCR/ABL oncogene, which is thought to be responsible of the initiation of the leukemia (1). The development of a specific abl kinase inhibitor imatinib mesylate (Gleevec, STI571) has revolutionized the treatment of patients with chronic CML. Imatinib mesylate, by targeting BCR/ABL, induces hematologic and cytogenetic remissions in the large majority of patients in chronic phase (2, 3); it also displays major activity in accelerated phase and blast crisis (4, 5). Despite the major and unprecedented responses obtained in CML patients with imatinib as the first-line treatment, long-term outcome with the extended use of imatinib mesylate remains unknown and currently the only curative treatment especially in patients developing resistance to imatinib mesylate remains the allogeneic stem cell transplantation. CML is the most sensitive malignant disease to allogeneic adoptive immunotherapy as evidenced by both the high molecular remission rate after allogeneic bone marrow transplantation (6) and the efficacy of donor lymphocyte infusions in inducing remission in post-allogeneic bone marrow transplantation relapse (7). Despite recent reports outlining a role of allogeneic natural killer (NK) cells as potent antileukemic cytotoxic effectors of the graft-versus-leukemia effect (8, 9), the mechanisms controlling the leukemic target recognition and killing by NK cells are not yet well defined.

Besides the receptors involved in target–NK cell interaction and regulation, the production of cytokines is an important component of the activated NK cell response. In that context, IFNγ represent a key cytokine within the immune response. Through its role on MHC-I molecule up-regulation, it interferes with the recognition and killing of target by specific and NK cells. In that context, signaling pathway of IFNγ in transformed cells is an important feature to consider in the NK/target cell cross-talk.

Evidence has been provided that BCR/ABL dysregulates multiple signaling pathways that interfere with cytokine signaling, such as IFN. Thus, the transfection of the BCR/ABL gene in the primitive cell line UT-7 allowed the generation of several stably transfected sublines expressing low levels (UT-7/E8) or high levels (UT-7/9) of BCR/ABL protein (10). The high level of the BCR/ABL protein expression resulted in an acquired resistance to different apoptotic stimuli, their growth factor independence, and in their increased susceptibility to NK-mediated cytolysis (11).

In the present study, we investigated the role of IFNγ on the susceptibility to NK-mediated lysis of leukemic targets expressing low or high level of BCR/ABL. We show that UT-7/9 transfectants expressing high level of BCR/ABL exhibited an altered IFNγ signaling resulting in an impaired effect of this cytokine with respect to their NK-mediated lysis. An altered human leukocyte antigen class I (HLA-I) molecule induction as well as a decreased phospho–signal transducers and activators of transcription 1 (pSTAT1) nuclear translocation in primary CML cells from patients support the data obtained using the BCR/ABL transfectant model. Altogether, these results indicate that the altered...
IFN\(\gamma\) signaling in BCR/ABL targets unveils an increased immunogenicty of these targets to NK cells and emphasize the possible use of donor-derived activated NK cells or clones in adoptive cellular therapy after stem cells transplantation.

**Materials and Methods**

**Cell lines and reagents.** BCR/ABL clones of the pluripotent human cell line UT-7 that express high-level BCR/ABL (UT-7/J9) and low-level BCR/ABL (UT-7/E8) were obtained by the use of retroviral vectors as previously described (10). The UT-7 cell line and its transfectants expressing a low level of the BCR/ABL protein (UT-7/E8) as well as control UT-7/neo cell line (transfected with MSCV-Neo vector without BCR/ABL insert) propagated in RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 10% FCS and 10 ng/mL granulocyte macrophage colony-stimulating factor (GM-CSF, Amgen, Thousand Oaks, CA). The BCR/ABL transfectants (UT-7/J9) was expanded in medium without GM-CSF as previously reported (10). IFN\(\gamma\) was from Aventis Pharma (Romainville, France) and imatinib mesylate (previously named STI571) was from Novartis (Basel, Switzerland).

**Separation of natural killer cells and in vitro activation.** NK cells were sorted from the peripheral blood of healthy donors using RosetteSep System (StemCell Technologies, Meylan, France). Briefly, whole blood was diluted in an equal volume of normal saline buffer and incubated for 20 minutes with 50 \(\mu\)g/mL enrichment antibody cocktail at room temperature followed by centrifugation on a Ficoll gradient that eliminated rosette-forming cells. Resulting NK cells were activated by culture in human serum-supplemented RPMI 1640 with 10 ng/mL of interleukin (IL)-15 (R&D Systems, Minneapolis, MN) and 200 units/mL IL-2 for 4 to 18 days.

**Immunophenotypic study.** For the surface expression study of the NK cells and targets, FITC or phycoerythrin monoclonal antibodies (mAbs) specific for the following markers were used: CD56PE, CD16FITC, CD3FITC, CD8PE, CD94/NKG2A, and CD2FITC. Expression of natural cytotoxicity receptors (NCR) was analyzed using unconjugated antibodies against NKp46 (BAB281, IgG1), NKp44 (Z231, IgG1), NKp30 (Z25, IgG1), purchased from Immunotech (Marseilles, France) and NKG2D (IgG1, R&D Systems). Flow cytometry analyses were done on a FACSort flow cytometer (Becton Dickinson, Pont de Claix, France) using the CellQuest software (Becton Dickinson). For HLA-I molecule induction on UT-7 cells and its transfectant targets, the cells were incubated with medium supplemented or not with IFN\(\gamma\) (1,000 units/mL) before staining with anti-HLA-I mAb (W6/32, IgG1) and analyzed by flow cytometry.

**Cell proliferation.** Cell proliferation was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded 96-well plate (10,000 cells/well) in medium with or without increasing concentrations of IFNa, IFN\(\gamma\), or imatinib mesylate and incubated for 48 hours at 37°C. At the end of this period, 50 \(\mu\)L MTT solution was added to each well and the plate was incubated for an additional 4 hours at 37°C. The reaction was terminated by the addition of 50 \(\mu\)L MTT lysis buffer. Absorbance \((A)\), which was proportional to cell viability, was measured at 550 nm. Cell viability was evaluated using the following calculation: percent of viability = 100 \(\times\) \(A_1\) / \(A_2\), where \(A_1\) and \(A_2\) were the absorbances obtained after 48 hours culture with or without treatment.

**Cytotoxicity assays.** UT-7 cells and the transfectants UT-7/neo, UT-7/E8, and UT-7/J9 were used as targets of 4-hour \(^{51}\text{Cr}\) release assays. Effectors were resting or IL-2-activated NK cells immunoselected from peripheral blood. Phenotype and lytic potential of activated bulk NK cells was stable from 2 to 15 days of culture. In HLA-I blocking experiments, after chromium-51 labeling (C200), cell suspensions were incubated with anti-HLA-I mAb (A6-132, IgG3, provided by A. Moretta, Genova, Italy) at 1 to 5 \(\mu\)g/mL at room temperature for 30 minutes, and incubated with the appropriate number of effectors for the standard 4-hour interval. In some experiments, target cells were treated with 1,000 units/mL IFN\(\gamma\) for 48 hours before the assay. Triggering receptor–mediated activated NK cell lysis was assessed by redirected lysis assays using mouse mastocytoma P815 cells. Briefly, \(^{51}\text{Cr}\)-labeled P815 cells (3 \(\times\) 10\(^5\)) coated with anti-CD16, anti-NCR, or anti-NKG2D mAbs (10 ng/mL) were incubated with NK cells at 5:1 ratio. Data were expressed as the percentages of specific lysis at the indicated effector- to-target ratio. The percent-specific \(^{51}\text{Cr}\) release was calculated as (experimental release – spontaneous release) / (total release – spontaneous release) \(\times\) 100.

**Western blotting analysis.** For cytokine signaling studies, cells were treated or not by 1,000 IU/mL IFN\(\gamma\), washed thrice with PBS, then lysed for 20 minutes at 4°C in the following buffer: 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 5 mmol/L EDTA, and 1% (v/v) Triton X-100 containing a protease inhibitor cocktail (Roche Diagnostics, GmbH, Mannheim, Germany). After centrifugation (5,000 rpm, 15 minutes, 4°C), cytosolic fractions were carefully removed and total protein concentrations were determined by using the BCA Protein Assay kit (Pierce, Rockford, IL). Equal amounts of protein lysates were run on 10% SDS-PAGE then blotted onto nitrocellulose. Membranes were blocked for 1 hour at room temperature by using 5% w/v nonfat dry milk in TBS containing 0.1% (v/v) Tween 20 (Sigma-Aldrich, Saint-Quentin Fallavier, France), then probed with c-Abl (AB3, 1/1,000, Calbiochem, La Jolla, CA), IFN regulatory factor-1 (JF-1, 1/1,000, Santa Cruz Biotechnology, Santa Cruz, CA), STAT1, or pSTAT1 (1/2,000, Cell Signaling Technology, Beverly, MA) antibodies overnight at 4°C in TBS 0.1% Tween 20 containing 5% (w/v) bovine serum albumin (Sigma). Membranes were washed for 1 hour by TBS 0.1% Tween 20 then probed with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1/2,000, Rockland, Gilbertsville, PA) in TBS 0.1% Tween 20 containing 5% (v/v) nonfat dry milk. After extensive washings, blots were rinsed twice with distilled water. Immunoblotting were revealed by using the ECL system (Amersham Pharmacia, Little Chalfont, England). Quantification was done by using the Bio1D software (Vilber-Lourmat, Marne-la Vallée, France). For kinetics studies of IFN\(\gamma\) signaling, cytokine treatment was monitored in serum-free medium for 5 to 60 minutes at 37°C. Reaction was stopped on ice by adding an equal volume of cold PBS and cells were handled as described above.

**IFN\(\gamma\) response on primary chronic myeloid leukemia cells from patients.** Blood samples from CML patients were centrifuged on Ficoll Hypaque to separate mononuclear cells. These cells were treated with IFN\(\gamma\) (1,000 units/mL) for 16 to 20 hours and analyzed for HLA-I induction by flow cytometry following staining with W6/32 mAb. Alternatively, blasts were treated with IFN\(\gamma\) (1,000 units/mL) for 1 hour, stained for pSTAT1, and analyzed by confocal microscopy. Detection of pSTAT1 was done using a rabbit antiserum (Ozyme, Paris, France) followed by incubation with secondary antibody Alexa Fluor 488 GAR (Molecular Probes, Eugene, OR). Nuclei were stained using propidium iodide (red staining). Stained cells were washed with PBS, centrifuged in a cryoprotin 3 (Shandon, Pittsburg, CA), and analyzed on a Zeiss LSM-510 microscope (Carl Zeiss GmbH, Jena, Germany). HeLa cells were used as positive control.

**Results**

**Cytotoxic potential of peripheral activated natural killer cells toward UT-7 cell and its BCR/ABL transfectants.** Activated donor-derived NK cells represent powerful cytotoxic effectors that may participate to the antileukemic efficacy of donor lymphocyte-infusions post-allogeneic stem cell transplantation. We have first analyzed the expression of NCR on immunoselected NK cells (>90% of CD56\(^\text{dim}\)/CD3\(^\text{-}\) cells, mainly CD56\(^\text{dim}\)/CD16\(^\text{bright}\)) from peripheral blood of donors. All three NCR—NKp46, NKp44, and NKp30—expression was rapidly up-regulated on NK cells activated by IL-2 compared with resting NK cells. The activating receptor NKG2D was expressed on the surface of activated peripheral blood NK cells. The functional relevance of these activating receptors (NCR and NKG2D) was confirmed in redirected lysis experiments against murine Fe\(\text{R}\)^\(\gamma\) P815 cells (Fig. 1A). Although the respective involvement of the triggering receptors varies among donors \((n = 5)\), NKp46 was always the major triggering receptors of activated NK cells. IL-2-activated peripheral NK cells were used as cytotoxic effectors against...
the primitive hematopoietic UT-7 cell line and its BCR/ABL-expressing counterparts. Data shown in Fig. 1B indicate that activated peripheral NK cells killed the UT-7/9 clone, expressing high level of BCR/ABL protein with a high efficiency. Using activated peripheral NK cells derived from six different donors, there was no strict correlation between the BCR/ABL expression level of the transfectants (Fig. 1C) and their susceptibility to NK-mediated lysis (Fig. 1B). One representative experiment out of 10 is shown (Student’s t test with \( P = 0.201 \)). The level of cell survival in response to imatinib mesylate treatment discriminated low and high BCR/ABL transfectants, whereas the parental cell line and the UT-7.neo transfectant were resistant to high imatinib mesylate concentrations (Fig. 1D).

High-level BCR/ABL transfectants displayed an absence of human leukocyte antigen class I and a sustained natural killer cell–mediated lysis in response to IFNγ. It is well established that activated NK cells produce IFNγ and interactions between NK cells and leukemic targets occur in an environment with elevated concentrations of this cytokine. Therefore, the effect of IFNγ on the susceptibility of UT-7, UT-7.neo, UT-7/E8, and UT-7/9 cells to the NK cell–mediated lysis was evaluated by incubating the target cells with IFNγ (1,000 units/mL, 48 hours) before the cytotoxic assay. Results depicted in Fig. 2A show that treatment with IFNγ increased HLA-I molecule membrane expression of UT-7, UT-7.neo, and UT-7/E8 (mean fluorescent intensity \( \times 4-10 \) but not UT-7/9 cells (\( n = 6, P < 0.02; \) Fig. 2A). Moreover, treatment of UT-7, UT-7.neo, and UT-7/E8 cells with IFNγ decreased their sensitivity to the NK cell lysis, whereas the same treatment had no effect on UT-7/9 cell NK-mediated lysis (Fig. 2B). Similar results were obtained from activated NK cells derived from 10 different donors with a 35% to 60% lysis inhibition of UT-7, UT-7.neo, and UT-7/E8, whereas the effect on UT-7/9 was <7% (Student’s \( t \) test, \( P < 0.002 \)). Furthermore, whereas addition of blocking anti-HLA-I mAb (A6-136, IgM) had no effect on the lysis of nontreated cells, the lysis of IFNγ-treated UT-7, UT-7.neo, and UT-7/E8 targets was clearly restored by addition of anti-HLA-I mAb in a dose-dependent manner (Fig. 2C), whereas cIgM had no effect (<5%, data not shown). The lysis of UT-7/9 cells treated or not by IFNγ was not affected (modulation <5%) by anti-HLA-I mAb (Fig. 2C). The modulating effect of anti-HLA-I mAbs toward IFNγ-treated cells (UT-7 versus UT-7/9) was obtained with NK cell cultures derived from six donors and is statistically significant (Student’s \( t \) test, \( P < 0.002 \)). Thus, it is likely that the constitutive HLA-I expression on the different UT-7 cell lines is not sufficient to trigger inhibitory NK receptors and that their up-regulation by IFNγ is required to engage inhibitory NK receptors and counterbalance the positive signal. Altogether, these data indicate that absence of engagement of inhibitory NK receptors was likely responsible for the sustained susceptibility of IFNγ-treated UT-7/9 cells to NK cell lysis. It should be noted that IFNγ did not interfere with the survival of UT-7 and its transfectants as evaluated by an MTT assay (data not shown), excluding a direct cytostatic effect of IFNγ on target cells.

Alteration of IFNγ signaling in BCR/ABL transfectant UT-7/9 cells. To further dissect the altered IFNγ response in UT-7/9 cells that is likely to participate to their susceptibility to activated NK-mediated lysis, IFNγ signaling with respect to STAT1 and IRF-1 induction and activation was analyzed in UT-7 cells and its BCR/ABL transfectants. STAT1 basal expression was low in the parental UT-7 cells and its transfectants and clearly up-regulated in response to IFNγ. However, whereas the up-regulation of STAT1 was high in UT-7, UT-7.neo, and in UT-7/E8 (12-15), it was faint in UT-7/9 cells (<3) as shown in Fig. 3A. Furthermore, STAT1 activation in response to IFNγ (5-60 minutes, 100 units/mL) was analyzed by immunoblotting with anti-pSTAT1 antibody. Blots were stripped and reprobed with anti-pSTAT1 as control. As depicted in Fig. 3B, pSTAT1/STAT1 ratios were dramatically reduced in UT-7/9 compared with UT-7/E8 and parental cells. In high-level BCR/ABL transfectant UT-7/9 cells, STAT1 phosphorylation was weak and transient and rapidly decreased to its basal levels leading to the alteration of IFNγ signaling and the subsequent response in UT-7/9 cells.
unresponsiveness of the cells to this cytokine. In addition, IRF-1 basal expression was low in UT-7, UT-7/neo, and UT-7/E8, whereas it was hardly detectable in UT-7/9 cells. In response to IFNγ, there was a clear induction of IRF-1 in UT-7 and UT-7/E8 cells, whereas UT-7/9 did not respond (Fig. 3A). Altogether, these results indicate that the preserved susceptibility of UT-7/9 cells to activated NK-mediated lysis after IFNγ treatment is likely the result of their altered cytokine signaling associated with an up-regulation of HLA-I molecules in response to IFNγ secreted by activated NK cells.

Treatment with IFNs or imatinib did not restore the cellular response to IFNγ in UT-7/9 cells. In the light of our findings outlining the crucial role of IFNγ signaling in BCR/ABL target for the control of their susceptibility to NK-mediated lysis, we investigated

Figure 2. A, expression of HLA-I molecules on UT-7, UT-7/neo, UT-7/E8, and UT-7/9 cells treated (bold line) or not (thin line) with IFNγ (1,000 units/mL) for 48 hours. B, treatment by IFNγ decreases the susceptibility of UT-7 and its low-level BCR/ABL transfectants to NK-mediated lysis. Targets were treated with IFNγ for 48 hours (white symbols) before the cytolysis assay. C, modulation of lysis in percentage of IFNγ-treated UT-7/neo, UT-7/E8, and UT-7 cells by increasing doses of blocking anti–HLA-I mAbs. Cytotoxic assays were done in the presence of increasing doses (0.5, 1, and 2 μg/mL) of anti-HLA-I mAbs (A3.136, IgM) at an effector-to-target ratio of 5:1. MFI, mean fluorescent intensity.

Figure 3. Alteration of IFNγ signaling in BCR/ABL transfectant UT-7/9. A, basal expression of STAT1 and IRF-1 in UT-7, UT-7/neo, UT-7/E8, and UT-7/9 cells and induction in response to IFNγ (1,000 units/mL, 48 hours). B, activation of STAT1 measured by Western blotting of pSTAT1 in response to IFNγ (1,000 units/mL, 0-30 minutes) in UT-7, UT-7/E8, and UT-7/9 cells.
the possible interference of imatinib mesylate and IFNα with the cellular response to IFNγ in UT-7/9 cells. As depicted in Fig. 4, neither IFNα nor imatinib mesylate restored the IFNγ-induced cellular response in UT-7/9 transfectants. Imatinib mesylate had no effect on HLA-I molecule expression after IFNγ treatment on the high-BCR/ABL-level transfectants. Whereas imatinib mesylate slightly decreased the UT-7/9 susceptibility to NK-mediated lysis, the addition of IFNγ during the incubation with imatinib mesylate did not modify their sensitivity to NK lysis, in agreement with the absence of HLA-I up-regulation (Fig. 4A, left). Furthermore, IFNγ treatment did not restore the IFNγ-induced HLA-I up-regulation and did not modulate NK-mediated lysis of UT-7/9 cells (Fig. 4).

Altered human leukocyte antigen class I molecules induction and pSTAT1 nuclear translocation in IFNγ-treated primary chronic myeloid leukemia cells. To validate the hypothesis of an alteration of IFNγ signaling in BCR/ABL targets, we studied the cellular response of primary CML cells isolated from leukemic patients to this cytokine. From nine CML patients in advanced phase displaying hyperleucocytosis, mononuclear cells from peripheral blood were isolated. In these CML cells, IFNγ signaling was studied by examining HLA-I molecule induction and pSTAT1 nuclear translocation in response to this cytokine. Following treatment with 1,000 units/mL IFNγ for 16 to 20 hours, an absence of induction of HLA-I molecule was observed in six of nine blast cultures, whereas an IFNγ-mediated HLA-I molecule up-regulation was observed in three of nine patients (Fig. 5A; Table 1). In addition, activation of pSTAT1 was assessed by nuclear translocation in blast cultures from five patients. HeLa cells treated by IFNγ were used as control. In agreement with the results of HLA-I induction, there was a decreased nuclear translocation in four of five blast cultures (Fig. 5B). Absence of nuclear translocation in four patients not responding to IFNγ, whereas nuclear pSTAT1 was detected in IFNγ-treated cells of patient 1 further indicate the altered IFNγ signaling of this cytokine in most CML blasts (Table 1). These results further support the presence of a decreased IFNγ signaling and an altered cellular response to this cytokine in most CML patients.

Discussion

Activated NK cells play a prominent role in the antiviral and antitumor immune response by attacking infected or transformed cells that lack or have down-regulated some or all their HLA-I antigens (12, 13). These cells sense the presence of self-molecules using a panel of inhibitory receptors including killer immunoglobulin-type receptors (KIR) and the C-lectin–type receptors (e.g., CD94/NKG2A) that bind, respectively, to the HLA-I molecules and nonclassic HLA-E molecules on the surface of target cells (14, 15). When expression of HLA-I molecules on target cells is low or absent, the inhibitory receptors are not engaged and activating signals mediated by the NCR (16–19) and NKG2D (20, 21) initiate the lytic process. The sum of the interactions of all activating and inhibitory receptors with their respective ligands finally determines the outcome of the NK cell/target cell contact.

CML is the most susceptible disease to adoptive cellular therapy and there is growing evidence that donor-derived NK cells participate to the antileukemic effect of donor lymphocyte infusions following stem cell transplantation. Using stable BCR/ABL transfectants of the primitive hematopoietic cell line UT-7, we investigated the mechanisms regulating the increased susceptibility to NK-mediated lysis of high level BCR/ABL targets. We show that activated peripheral NK cells efficiently lyse the UT-7 cell line and its BCR/ABL transfectants. However, the data obtained from a large series of donors do not reveal a clear correlation between BCR/ABL level and NK cell–mediated lysis. Because our previous data showing that UT-7/9 cells displayed an increased susceptibility to in vitro CD34-differentiated NK cell–mediated lysis (11), it is tempting to speculate that activated peripheral NK cells and CD34-differentiated NK cells display distinct functional properties as reported (22). This also suggest that these two types of NK effectors may display different lytic potential and engage distinct cross-talk with BCR/ABL transfectants.

The present data provide further evidence that IFNγ produced by peripheral activated NK cells is able to modulate the recognition and killing of BCR/ABL cells and may, therefore, have implications for immunotherapy. Thus, in low-level BCR/ABL...
transfectants UT-7/E8, control UT-7/neo transfectants and parental UT-7 cells, IFNγ increased HLA-I expression and decreased susceptibility to NK cells as a consequence of inhibitory NK receptor engagement. Our results outline the requirement of high HLA-I molecule expression to trigger the inhibitory receptors as the basal expression of HLA-I was not sufficient to trigger NKR. It is likely that IFNγ present in the microenvironment may activate this inhibitory pathway, shifting the balance of triggering and inhibitory signals in activated NK cells. Such a paradoxical effect of IFNγ was recently described on short-term ovarian tumor cells, showing that resistance of IFNγ-treated tumor cells to CD8+ cytotoxic T lymphocyte was dependent on an enhanced expression of HLA-E mRNA and HLA-G protein by the tumor cells (23).

The altered cellular response to IFNγ in high-level BCR/ABL transfectants, UT-7/9, and UT-7/11 (data not shown) was likely associated with an altered IFNγ signaling in these cells and contributed to their high susceptibility to activated NK cells. Such alterations of the IFN signaling in advanced CML were reported and mainly involved Janus-activated kinase 1/STAT1 pathways (24, 25). The role of IRF-1 in the pathogenesis of CML was also outlined from IRF-invalidated mouse models reported to develop a blastic myeloid proliferation close to CML (26). In addition, reduced expression of some IRF gene family members, such as interferon consensus sequence-binding protein, was detected in CML patients (27–29). Interestingly, we showed that CML cells isolated from leukemic patients do not respond to IFNγ in terms of HLA-I induction and pSTAT1 nuclear translocation, further confirming our observation using the BCR/ABL transfectant, UT-7/9.

As our results outlined the role of IFNγ signaling in the susceptibility of high-level BCR/ABL transfectant to NK cell lysis,

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Abbreviations: R, responder; NR, nonresponder; MFI, mean fluorescent intensity.

Figure 5. Cellular response of blast cultures from CML responder (Pt1) and nonresponder (Pt2) patients to IFNγ. Circulating blasts from CML patients were incubated for 16 to 20 hours in medium supplemented or not with IFNγ (1,000 units/mL) before staining by anti–HLA-I mAb. For flow cytometry analysis, 10,000 events were evaluated and blasts cells were gated on FCS parameters (A). Alternatively, blasts were treated for 1 hour with IFNγ and stained with pSTAT1 antiserum and analyzed by confocal microscopy. Responder and nonresponder CML patients are depicted in A and B, respectively. HeLa cells were used as control for pSTAT1 nuclear translocation (C). MFI, mean fluorescent intensity.
we studied whether two cytostatic agents, IFNα and imatinib mesylate, efficient on BCR/ABL targets, modulate the IFNγ cellular response in UT-7/9 cells. Although the mechanism of IFNα on BCR/ABL targets is not clearly understood, it is known that IFNα exerts synergistic effect on IFNγ signaling through STAT1 activation (30). Our results showed that neither imatinib mesylate nor IFNα treatment affected the cellular response of UT-7/9 cells to IFNγ, including HLA-I expression and cytolysis. This suggests that such agents will not induce a negative signaling on NK cell cytolysis through engagement of inhibitory NK receptors by HLA-I molecules.

These results suggest that the unresponsiveness of high BCR/ABL transfectant to IFNγ has an important consequence for preserving their high susceptibility to activated NK cells. The crucial role of IFNs in the modulation of an ongoing immune response in CML patients was recently reported. It has been shown that only IFNα-sensitive CML patients have high avidity circulating cytotoxic T lymphocyte, suggesting that efficient IFN signaling allows CML-specific cytotoxic T-lymphocyte activation (31). Furthermore, IFNα but not imatinib mesylate has been shown to restore myeloblastin expression in CML, whereas remissions under imatinib mesylate are rarely associated with the emergence of myeloblastin-specific T cells (32). Our data provide additional proofs that CML, through overexpression of BCR/ABL, may shape the host immune system affecting the innate immune response by interfering with IFNγ signaling. Our data also suggest that altered IFNγ signaling may be a crucial determinant in the control of the BCR/ABL target susceptibility to NK-mediated lysis.

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References

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