BCR-ABL Activity Is Critical for the Immunogenicity of Chronic Myelogenous Leukemia Cells

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Abstract

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder caused by excessive granulopoiesis due to the formation of the constitutively active tyrosine kinase BCR-ABL. An effective drug against CML is imatinib mesylate, a tyrosine kinase inhibitor acting on Abl kinases, c-KIT, and platelet-derived growth factor receptor. Recently, a study revealed that patients treated with imatinib showed impaired CTL responses compared with patients treated with IFN-α, which might be due to a treatment-induced reduction in immunogenicity of CML cells or immunosuppressive effects. In our study, we found that inhibition of BCR-ABL leads to a down-regulation of immunogenic antigens on the CML cells in response to imatinib treatment, which results in the inhibition of CML-directed immune responses. By treating CML cells with imatinib, we could show that the resulting inhibition of BCR-ABL leads to a decreased expression of tumor antigens, including survivin, adipophilin, hTERT, WT-1, Bcl-x1, and Bcl-2 in correlation to a decreased development of CML-specific CTLs. In contrast, this reduction in immunogenicity was not observed when a CML cell line resistant to the inhibitory effects of imatinib was tested, but could be confirmed by transfection with specific small interfering RNA against BCR-ABL or imatinib treatment of primary CML cells. [Cancer Res 2007;67(11):5489–97]

Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disease characterized by excessive proliferation and differentiation of the myeloid cells in the bone marrow. The characteristic cytogenetic hallmark in ~90% of the patients is a shortened chromosome 22, the so-called Philadelphia chromosome. It results from a reciprocal translocation of the long arms of chromosomes 9 and 22 [t(9;22) (q34;q11)]. This translocation results in the chimeric BCR-ABL oncogene, which is translated into a cytoplasmic protein with a strong constitutive tyrosine kinase activity (1).

The mechanisms involved in malignant transformation are not yet fully understood, but the BCR-ABL translocation is believed to be the etiologic event in the development of CML (2). Recently, the introduction of imatinib mesylate (Gleevec/Grivec, Novartis), an inhibitor of the BCR-ABL tyrosine kinase activity, has strongly improved the therapy of CML, leading to a significant prolongation of hematologic and cytogenetic remissions and the mean survival time without inducing major adverse effects (1, 3–5).

Nevertheless, resistance to imatinib treatment is a problem. In most cases, point mutations or an amplification of the BCR-ABL gene are responsible for the loss of efficacy, but also BCR-ABL– independent mechanisms like a differential gene expression in resistant cells have been described (6–9).

Alternative treatment options to achieve remissions are therefore still needed to combat CML, especially in patients resistant to the standard forms of therapy, including IFN-α or imatinib. For this purpose, immunotherapy trials using mostly dendritic cell–based vaccination strategies have been conducted (10, 11). In IFN-α–treated patients, antigen-specific CTLs recognizing a peptide derived from the overexpressed antigen proteinase 3 (PR3) could be detected. As anticipated, these cells were able to contribute to the elimination of malignant cells (12, 13).

Today, the first class therapy of CML relies on imatinib rather than on IFN-α. Consequently, the compatibility of this kind of treatment with immune therapeutic strategies has to be examined. In the present study, we therefore investigated the influence of imatinib treatment on the inducibility of CTL responses to CML-associated antigens using dendritic cells electroporated with RNA from K-562 CML cells for CTL induction.

In Western blot and real-time PCR experiments we also analyzed the expression of several antigens shown to be expressed in CML and to potentially be associated with the immunogenicity of CML cells (14–18) and tested the specificity of the CTLs in Elispot assays. In our experiments, we were able to show that the down-regulation of the expression or inhibition of the activity of BCR-ABL in CML cells strongly impedes the generation of CTLs recognizing antigens from the K-562 cell line as well as primary CML cells and is associated with a down-regulation of several tumor antigens. These results indicate that BCR-ABL is critical for the immunogenicity of CML cells, probably due to up-regulation of immunogenic antigens by its uncontrolled tyrosine kinase activity. Imatinib treatment is therefore likely to interfere with immunotherapeutic approaches due to an inhibition of the priming of CTL responses in patients.

Materials and Methods

Tumor cell lines. The tumor cell line K-562 (CML in blast crisis; American Type Culture Collection) was grown in RP10 medium (RPMI 1640 with glutamax I, supplemented with 10% heat-inactivated FCS, 100 units/mL penicillin, and 100 µg/mL streptomycin, all purchased from Gibco). K-562R (K-562 cell line resistant to low doses of imatinib, kindly provided by the group of J. Griffin, Dana-Farber Cancer Institute, Boston, MA) was grown in RP10 medium supplemented with 0.5 µmol/L imatinib (19).

Flow cytometric measurement of apoptosis. DNA fragmentation in apoptotic nuclei was measured by the method of Nicoletti et al. (20). Briefly, cells were lysed in hypotonic buffer (1% sodium citrate, 0.1% Triton X-100, 50 µg of propidium iodide per milliliter) to release apoptotic nuclei and subsequently analyzed by flow cytometry on a FACSCalibur flow cytometer (Becton Dickinson) using CellQuest analysis software. Nuclei
healthy volunteers were obtained from the blood bank of the University of Technology), and mTOR (Cell Signaling Technology). Membranes were reprobed with antibody to human actin (Santa Cruz Biotechnology), Bcl-xL (BD Biosciences), x-linked inhibitor of apoptosis protein (xIAP; BD Biosciences), PTEN (BD Biosciences), phosphorylated mammalian target of rapamycin (p-mTOR; Cell Signaling Technology), and mTOR (Cell Signaling Technology). Membranes were reprobed with antibody to human actin (Santa Cruz Biotechnology) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; HyTest Ltd.) to confirm that equal amounts of protein were present in every lane.

Dendritic cell generation from adherent peripheral mononuclear cells. Dendritic cells were generated from adherent peripheral blood monocytes as described before (21–25). Buffco preparations from healthy volunteers were obtained from the blood bank of the University of Tübingen (Tübingen, Germany). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll/Paque (Biochrom) density gradient centrifugation. Cells were resuspended in serum-free X-VIVO 20 medium (BioWhittaker, Walkersville, MD) for 30 min and then labeled with a 10% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (Schleicher & Schuell). The blot was probed with antibodies to human survivin (R&D Systems), adipophilin (Progen Biotechnik), Bcl-2 (Santa Cruz Biotechnology), Bcl-xL (BD Biosciences), x-linked inhibitor of apoptosis protein (xIAP; BD Biosciences), PTEN (BD Biosciences), phosphorylated mammalian target of rapamycin (p-mTOR; Cell Signaling Technology), and mTOR (Cell Signaling Technology). Membranes were reprobed with antibody to human actin (Santa Cruz Biotechnology) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; HyTest Ltd.) to confirm that equal amounts of protein were present in every lane.

Induction of specific CTLs using dendritic cells transfected with RNA. Dendritic cells were electroporated with RNAs of different sources and cultured for 24 h as described above. For induction of specific CTLs, 5 × 10^5 dendritic cells were cocultured with 2 × 10^5 autologous nonadherent cells in RP10 medium. After 7 days of culture, the CTLs were harvested and 5 × 10^5 CTLs were restimulated in coculture with 5 × 10^5 electroporated dendritic cells and 2 × 10^5 irradiated nonadherent cells (30 Gy, Gammacell 1000, Atomic Energy of Canada, Ltd.) per well. Human recombinant IL-2 (2 ng/mL; R&D Systems) was added on days 1, 3, and 5. Further restimulations were done on day 7 after the last restimulation as described above. The specificity of the CTLs was analyzed in standard 51Cr-release assays. Standard 51Cr-release assays (CTL assays). CTL assays were done as described previously. Briefly, target cells were prepared by transfection of dendritic cells with RNA 1 day before the assay. If needed, they were treated with monoclonal antibodies PA2.1 (10 μg/mL) directed against HLA class I molecules or Tu39 (10 μg/mL) directed against HLA class II molecules (kindly provided by S. Stevanovic, Department of Immunology, University of Tu 39. Tübingen, Germany)–coated plates. 51Cr–labeled target cells were seeded into each well. Varying numbers of CTLs were added to a final volume of 200 μL. Plates were incubated for 4 h at 37°C, 5% CO2 before the supernatants were harvested (50 μL/well), transferred to scintillator-coated plates (LumaPlate-96, Perkin-Elmer), and counted in a B-counter plate. The percentage of specific lysis was calculated as follows: 100 × (experimental release – spontaneous release) / (maximal release – spontaneous release). Spontaneous and maximal release were determined in the presence of either RP10 medium or 2% Triton X-100, respectively.

IFN-γ Ellispot assay. CTLs were harvested after the third restimulation and incubated in an anti–IFN-γ antibody (10 μg/mL, Mabtech AB)–coated 96-well plate (Multiscan-HA, Millipore Corporation) at a ratio of 4:1 and 1:1 with 5 × 10^5 autologous stimulators per well. Dendritic cells, which had been electroporated with different RNA species 24 h before the assay as described above, were used as stimulators. For the detection of spots, a biotin-labeled anti-human IFN-γ antibody (2 μg/mL, Mabtech AB) was used. Spots were counted using an automated Ellispot reader (Immunospot Analyzer, CTL Analyzers LLC).

Results

Imatinib impairs CTL induction to K-562 antigens. To analyze the influence of imatinib treatment on the immunogenicity of CML cells, we subjected K-562 and imatinib-resistant K-562R cells...
cells to either DMSO or imatinib treatment at 3 μmol/L for 48 h. The number of apoptotic nuclei as analyzed by Nicoletti staining was <35% in K-562 and <25% in K-562R cells (data not shown). Total RNA was extracted from the treated cells and used for electroporation of dendritic cells to generate CML-specific CTL induction. The cytolytic activity and specificity of the generated CTLs were determined in standard 51Cr-release assays done after two restimulations. As target cells, we used dendritic cells electroporated with RNA from DMSO- or imatinib-treated K-562 or K-562R cells. As negative control targets, dendritic cells transfected with EGFP-IVT were included as negative controls. K-562 cells were used as targets to exclude NK cell activity. HLA class I restriction was confirmed by inhibition of the cytolytic activity with an anti-HLA class I antibody. A, induction of CTLs with dendritic cells (DC) electroporated with RNA from DMSO-treated K-562 cells. E:T, effector-to-target ratio. B, treatment of K-562 cells with imatinib impairs the generation of specific CTLs. C, induction of CTLs with dendritic cells electroporated with RNA from DMSO-treated K-562R. D, induction of CTLs using dendritic cells electroporated with RNA from imatinib-treated K-562R cells.

Figure 1. CTL induction using dendritic cells electroporated with RNA from imatinib-treated K-562 cells. Representative results of three independent experiments. In the 51Cr-release assays, dendritic cells electroporated with RNA from DMSO- or imatinib-treated K-562 or K-562R cells were used as targets and dendritic cells transfected with EGFP-IVT were included as negative controls. K-562 cells were used as targets to exclude NK cell activity. HLA class I restriction was confirmed by inhibition of the cytolytic activity with an anti-HLA class I antibody. A, induction of CTLs with dendritic cells (DC) electroporated with RNA from DMSO-treated K-562 cells. E:T, effector-to-target ratio. B, treatment of K-562 cells with imatinib impairs the generation of specific CTLs. C, induction of CTLs with dendritic cells electroporated with RNA from DMSO-treated K-562R. D, induction of CTLs using dendritic cells electroporated with RNA from imatinib-treated K-562R cells.
K-562 cells with hydroxyurea at a concentration of 1 mmol/L, which corresponds to the plasma concentration reached in patients. After 48 h, this therapeutic agent only induced apoptosis in 20% of K-562 cells, but led to cell cycle arrest in the G1 phase as seen by a strong increase of the 2N peak and a corresponding decrease of the 4N peak in Nicoletti staining (data not shown). CTLs generated using RNA from hydroxyurea-treated cells specifically lysed targets with the antigenic profile of untreated K-562 cells, demonstrating that interfering with the cell cycle as well as inducing apoptosis does not influence the immunogenicity of CML cells (data not shown).

Specific siRNA directed against BCR-ABL impairs CTL induction to K-562 antigens. Imatinib is not only effective in inhibiting BCR-ABL but also affects c-ABL, platelet-derived growth factor (PDGF) receptor, and c-KIT kinase activity (27, 28). To further confirm that the impaired CTL response we observed is due to direct inhibition of BCR-ABL, we down-regulated BCR-ABL expression in K-562 and K-562R cells with specific siRNA directed against the fusion site b3a2 (sib3a2_1) using varying amounts of siRNA ranging from 120 to 320 pmol per 2 \times 10^6 cells for electroporation (Figs. 2A and 3A). To exclude secondary effects like an IFN response in the treated cells, we included a mismatch control siRNA (simis) at the same concentrations, as well as a sequence-unrelated siRNA (siGL2) and nontransfected cells as controls.

Total RNA extracted from siRNA-transfected K-562 and K-562R cells was used to electroporate dendritic cells that were then used as antigen-presenting cells (APC) for CTL induction. As mentioned above, standard \textsuperscript{51}Cr-release assays were done to analyze the cytolitic activity and specificity of the generated CTLs. As target cells, we used dendritic cells electroporated with RNA from siRNA-transfected K-562 cells. K-562 cells and dendritic cells electroporated with an EGFP-IVT were included as negative controls. As shown in Fig. 2B, CTLs generated using total RNA from mismatch control siRNA-transfected (simis-transfected) K-562 cells showed effective killing of dendritic cells electroporated with RNA from simis-transfected K-562 cells, whereas they were incapable of lysing targets presenting antigens from K-562 cells transfected with BCR-ABL–specific siRNA. HLA class I restriction was proven by inhibition of target cell lysis with an anti-HLA class I antibody. The same lysis pattern was observed using CTLs generated using RNA from simis-transfected K-562R cells, as shown in Fig. 3B.

Moreover, when RNA from K-562 cells, which were transfected with 0.8 \mu mol/L of the specific siRNA, was used to generate CTLs,
this resulted in a complete unresponsiveness of the CTLs (Fig. 2C). Treatment of the K-562R cells with 0.8 μmol/L sib3a2_1 only led to a reduction in the reactivity of induced CTLs compared with transfection of these cells with simis (Fig. 3C). When resistant cells were transfected with 1.6 μmol/L of the specific siRNA, which corresponds to a down-regulation of the expression of BCR-ABL to a BCR-ABL/c-ABL ratio achieved in the K-562 cells using 0.8 μmol/L siRNA, specific killing of the induced CTLs was completely abolished, as shown in Fig. 3D.

In line with the findings from the experiments using imatinib to inhibit BCR-ABL function, a down-regulation of the kinase expression by administration of specific siRNA had a similar effect on CTL induction. Inhibition of the function by down-regulation of BCR-ABL kinase expression led to a complete loss of immunogenicity in K-562 cells, whereas in K-562R cells kinase inhibition via imatinib did not have a measurable effect. Detectable reduction of protein levels in these cells by siRNA resulted in a diminished responsiveness of CTL and a reduction of the BCR-ABL/c-ABL ratio using higher concentrations of siRNA to the ratio seen in fully suppressed K-562 cells led to a complete loss of specific killing.

**Influence of imatinib on the expression of tumor antigens.**

From the previous experiments, it became evident that imatinib-treated K-562 cells carry an altered antigen profile compared with DMSO-treated cells. This brought up the question as to which antigens might be up-regulated by BCR-ABL and therefore be involved in CTL generation directed against CML cells. We examined the expression of several tumor antigens that have been shown to be expressed in CML cells and involved in the recognition by CTLs, including survivin (15, 29, 30), adipophilin (31), Bcl-2 (32, 33), Bcl-xL (14), hTERT (18), and WT-1 (16). In Western blot experiments, we were able to show that survivin, adipophilin, Bcl-2, and Bcl-xL are increasingly down-regulated in K-562 CML cells in response to treatment with increasing concentrations of imatinib (Fig. 4A). As we were not able to obtain antibodies suitable for detection of the antigens WT-1 and hTERT in Western blot, we did Real-time quantitative PCR experiments and were able to show that mRNA levels of WT-1 as well as hTERT are also strongly down-regulated in K-562 cells in response to treatment with increasing concentrations of imatinib (Fig. 4B). As we were not able to obtain antibodies suitable for detection of the antigens WT-1 and hTERT in Western blot, we did Real-time quantitative PCR experiments and were able to show that mRNA levels of WT-1 as well as hTERT are also strongly down-regulated in K-562 cells in response to treatment with increasing concentrations of imatinib (Fig. 4B). 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Imatinib. The expression of the xIAP and the phosphatase PTEN, which opposes phosphatidylinositol 3-kinase signaling and is therefore regulating one of the most important pathways activated by BCR-ABL (34), was not altered in response to imatinib treatment. It has been described that imatinib causes a reduced phosphorylation of mTOR, whereas compensatory activation of the phosphatidylinositol 3-kinase/mTOR pathway can be associated with resistance development in BCR-ABL–positive cells (34–36). Therefore, we investigated the expression of mTOR in response to imatinib treatment and found that the overall protein expression in contrast to phosphorylation levels remained unchanged (Fig. 4).

**Imatinib reduces the immunogenicity of primary CML cells.** In line with the results above, we were able to detect a reduction of hTERT in primary CML cells from patients in blast crisis (patients 1 and 2) or chronic phase (patient 3; Fig. 5A). We were able to obtain enough cells from patient 2 to additionally carry out Western blot analysis and found a down-regulation of survivin, whereas Bcl-2 and adipophilin were not detectable (Fig. 5B). These findings corresponded to a decreased lysis of dendritic cells electroporated with RNA from imatinib-treated cells from all three patients in standard 51Cr-release assays compared with the lysis of dendritic cells electroporated with RNA from DMSO-treated primary CML cells by CTLs generated using K-562-RNA (Fig. 5C).

**Imatinib impairs induction of CTLs specific for several tumor antigens.** To define the reactivity and specificity of the CTLs generated with K-562-RNA, the effector function of T cells was analyzed in IFN-γ Elispot assays. Autologous dendritic cells electroporated with IVT coding for several tumor antigens that have been described to be recognized by CTLs in myeloid leukemias, such as survivin, PRAME, PR-3, and WT-1, were used as stimulators. As shown in Fig. 6A, transfection of dendritic cells with RNA extracted from DMSO-treated K-562 cells resulted in the induction of T cells that specifically recognized autologous dendritic cells transfected with K-562-RNA, survivin-IVT, PRAME-IVT, PR-3-IVT, or WT-1-IVT as shown by their secretion of IFN-γ. When RNA extracted from imatinib-treated K-562 cells was used to transfect dendritic cells used for the generation of CTLs, the resulting CTLs did not show any reactivity against these antigens (Fig. 6B).

**Discussion**

In CML, the constitutive tyrosine kinase activity of the BCR-ABL fusion protein causes malignant transformation of the CML blasts through deregulation of proliferation, apoptosis, and adhesion control pathways. In addition, BCR-ABL represents an antigen uniquely expressed in these cells and offers the opportunity to be specifically targeted by drug-based or immunotherapeutic strategies. Imatinib mesylate, an inhibitor of the BCR-ABL kinase activity, has strongly improved the treatment and outcome of CML patients. In contrast to CML patients treated with IFN-α, in which the antileukemic effect in part relies on the induction of T-cell responses against leukemic cells, it has been recently found that patients treated with imatinib show impaired CTL responses (37). This might reflect a negative effect of the drug on the function of the immune system or be due to a reduced immunogenicity of the CML cells. Previously, imatinib has been described to influence the function and differentiation of APCs and inhibit the effector function of T lymphocytes and might therefore strongly interfere with immune therapeutic strategies based on dendritic cell vaccination and the induction of CTL responses in vivo (38–41).

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**Figure 4.** Expression of antigens in K-562 and K-562R cells in response to imatinib treatment. Representative results of three independent experiments. A, Western blot analysis showing down-regulation of the tumor antigens survivin and adipophilin as well as the antiapoptotic proteins Bcl-2 and Bcl-xL in K-562 cells after imatinib treatment. B, real-time PCR analysis showing down-regulation of the tumor antigen WT-1 in K-562 cells. Columns, mean; bars, SE. C, real-time PCR showing down-regulation of the tumor antigen hTERT in K-562 cells. Columns, mean; bars, SE.
Here, we show that BCR-ABL activity plays an important role in the immunogenicity of CML cells as its inhibition by imatinib impairs the generation of CTLs against CML-associated antigens as well as their detection by CML-specific CTLs.

Transfection of dendritic cells with RNA isolated from malignant cells or coding for tumor-associated antigens was recently shown to be a powerful tool to generate specific T cells with the ability of recognizing tumor cells (23, 24, 26, 42–44).

To analyze the effects of BCR-ABL inhibition on the expression of CML-associated antigens, we treated the CML cell line K-562 and an imatinib-resistant K-562 variant, K-562R, with imatinib or DMSO and generated CTLs using dendritic cells electroporated with RNA from these cells. CTLs elicited using RNA from DMSO-treated K-562 cells as well as DMSO- or imatinib-treated K-562R cells showed a robust cytolytic activity and were able to recognize several CML-associated antigens, like survivin, PRAME, WT-1, and PR3, in Elispot assays. CTLs induced using RNA from imatinib-treated K-562 cells were incapable of specific killing of targets with different CML-associated antigen profiles. To exclude that the inhibitory effects observed are due to apoptosis and therefore overall down-regulation of protein synthesis, we additionally used etoposide, rapamycin, and \( \gamma \)-irradiation to induce apoptosis rates of \(~40\%\), which was even higher than achieved with imatinib at \(3 \mu \text{mol/L}\). CTLs induced using RNA from these cells displayed a specific killing capacity comparable with CTLs induced using RNA from DMSO-treated cells. Similarly, the CML therapeutic hydroxyurea, which leads to a cell cycle arrest, does not impair the generation of specific CTLs.

Besides BCR-ABL, imatinib inhibits c-KIT and PDGF receptor kinases. To further prove that the observed effect is mediated by BCR-ABL inhibition, we used specific siRNA against the BCR-ABL fusion site b3a2 to down-regulate the protein expression and found essentially the same results.

Although K-562 cells are fully responsive to imatinib when it is administered in the range of the therapeutic concentration, the resistance of K-562R cells is at least in part caused by a 2- to 3-fold overexpression of BCR-ABL. This explains the refractiveness of these cells to the imatinib concentration used here (19). However, targeting the expression of the protein directly by specific siRNA shows that the extent of down-regulation achieved with specific siRNA correlates with the effect on CTL inducibility. When using \(0.8 \mu \text{mol/L}\), the less pronounced down-regulation compared with K-562 cells evokes an impairment of CTL induction, whereas a concentration leading to a similar BCR-ABL/c-ABL ratio as in K-562 cells also has a similar detrimental effect on the induction of specific CTLs.

Taken together, these results indicate that the expression and activity of BCR-ABL directly determine the immunogenicity of CML cells. This supports the assumption that BCR-ABL activity might mediate the up-regulation of immunogenic antigens and that its inhibition, in turn, leads to the corresponding reduction of their expression.

To confirm and expand these studies, we analyzed the expression of antigens connected to immune responses to CML in imatinib-treated cells using Western blotting and real-time PCR. We found that imatinib-mediated inhibition of BCR-ABL leads to a decreased expression of tumor antigens and cellular proteins, including survivin, adipophilin, hTERT, WT-1, Bcl-x\(_L\), and Bcl-2 in correlation to a decreased development of specific CTLs, whereas the expression levels of several other proteins remained unchanged. In line with the previous results, the down-regulation effects were not observed in K-562R cells. Similarly, in primary CML...
cells subjected to imatinib, a down-regulation of hTERT and survivin could be detected, which also corresponded to a decreased lysis of dendritic cells electroporated with RNA from these cells in standard 51Cr-release assays.

It has been shown before that survivin expression is regulated through BCR-ABL downstream cascades and that survivin is critical for the survival of BCR-ABL–positive cells (15). We were able to show that it is also involved in the recognition of CML cells by CTLs. The down-regulation in K-562 cells as well as in primary cells after imatinib treatment matched the missing recognition of targets carrying the antigen profiles of these cells by CTLs recognizing K-562 antigens in 51Cr-release as well as Elispot assays. However, other tumor-associated antigens that have not been investigated in this study might contribute to the immunogenicity of CML cells.

Our results point out that BCR-ABL is critical for the immunogenicity of CML cells as the inhibition of its activity by imatinib as well as a reduction of its expression by specific siRNA lead to a loss of inducibility of specific CTL responses. This might be due to down-regulation of immunogenic antigens that are up-regulated by the uncontrolled tyrosine kinase activity.

As the concentrations used here are in the range of plasma levels of treated patients, imatinib treatment could therefore interfere with immunotherapeutic approaches due to an inhibition of CTL responses as well as the lacking recognition of the CML cells by specific CTLs.

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Figure 6. Elispot assays showing the reactivity of CTLs to different antigens. The specificity of the CTLs generated as described above was determined after three weekly restimulations in an IFN-γ Elispot assay. A, induction of CTLs with dendritic cells electroporated with K-562-DMSO-RNA leads to a strong reactivity against survivin-, PRAME-, WT1-, and PR3-IVT. B, CTLs induced with dendritic cells electroporated with RNA from imatinib-treated K-562 show no increased reactivity compared with EGFP.
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