Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder that accounts for approximately 15% of newly diagnosed cases of adult leukemia (1, 2). The causal molecule of this disease is BCR-ABL, a 210 kDa oncoprotein encoded by the BCR-ABL fusion gene, which is generated by a t(9;22) chromosomal translocation (3). BCR-ABL possesses deregulated ABL tyrosine kinase activity that stimulates many downstream pathways. These signals lead to stimulation of cell proliferation, inhibition of apoptosis, and alteration of adhesion to stroma cells and extracellular matrix. CML begins with a chronic phase in which clonal BCR-ABL+ hematopoietic stem cells give rise to increased numbers of their progenies, particularly myeloid precursors and mature cells (mainly neutrophils) in the bone marrow, blood, and extramedullary tissues. After 3 to 5 years, if not treated, the disease progresses into a fatal blast crisis, characterized by accumulation of immature leukemic stem cells, including dendritic cells. CD8+ dendritic cells, CD4+ dendritic cells, and pDC both in humans and mice (9). CD8α+ chronic phase in which clonal BCR-ABL+ hematopoietic stem cells give rise to increased numbers of their progenies, particularly myeloid precursors and mature cells (mainly neutrophils) in the bone marrow, blood, and extramedullary tissues. After 3 to 5 years, if not treated, the disease progresses into a fatal blast crisis, characterized by accumulation of immature leukemic stem cells, including dendritic cells.

The Transcription Factor IRF8 Counteracts BCR-ABL to Rescue Dendritic Cell Development in Chronic Myelogenous Leukemia

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Abstract

BCR-ABL tyrosine kinase inhibitors (TKI) have dramatically improved therapy for chronic myelogenous leukemia (CML). However, several problems leading to TKI resistance still impede a complete cure of this disease. IFN regulatory factor-8 (IRF8) is a transcription factor essential for the development and functions of immune cells, including dendritic cells. IRF8−/− mice develop a CML-like disease and IRF8 expression is downregulated in patients with CML, suggesting that IRF8 is involved in the pathogenesis of CML. In this study, by using a murine CML model, we show that BCR-ABL strongly inhibits a generation of dendritic cells from an early stage of their differentiation in vivo, concomitant with suppression of IRF8 expression. Forced expression of IRF8 overrode BCR-ABL (both wild-type and T315I-mutated) to rescue dendritic cell development in vitro, indicating that the suppression of IRF8 causes dendritic cell deficiency. Gene expression profiling revealed that IRF8 restored the expression of a significant portion of BCR-ABL–dysregulated genes and predicted that BCR-ABL has immunostimulatory potential. Indeed, IRF8–rescued BCR-ABL–expressing dendritic cells were capable of inducing CTLs more efficiently than control dendritic cells. Altogether, our findings suggest that IRF8 is an attractive target in next-generation therapies for CML. Cancer Res; 73(22); 6642–53. ©2013 AACR.
IRF8 Overrides BCR-ABL in Dendritic Cell Development

Dendritic cells (BDCA3+ in humans) are a unique subset capable of priming CTLs by cross-presentation of dead cell materials and are thus critical for tumor immunity. pDCs produce a large amount of type I IFN, that is, IFN-α/β, upon Toll-like receptor (TLR)-7/8/9 signaling. Type I IFN elicits antiviral and antitumor responses. Of note, IFN-α was once used as a major therapeutic agent for CML (10, 11).

CML has long been suggested to be highly sensitive to T cell-mediated tumor immunity (12). CML responds to immune-mediated therapies, such as IFN-α, allogeneic stem cell transplantation (alloSCT), and donor lymphocyte infusion (13). IFN-α induces a specific T-cell response in CML (10, 11). In alloSCT, T-cell depletion from donor bone marrow cells results in a significant increase in relapse, especially in patients with CML. Yet, the fact that the disease develops in patients with CML suggests that tumor immunity is insufficient in patients with CML. Because T cells per se in patients with CML rarely express BCR-ABL, it is reasonable to suspect dendritic cells as a culprit. Indeed, several studies have shown that patients with CML have reduced numbers of cDCs and pDCs in the chronic phase, as compared with healthy individuals (14, 15). Other studies have reported that dendritic cells generated in vitro from monocytes or CD34+ cells of patients with CML are functionally defective in multiple aspects, such as actin organization, antigen processing, migration, maturation, and cytokine production (16, 17). In a murine CML model, defective homing and impaired induction of CTLs by BCR-ABL–expressing dendritic cells have been reported (18). However, the mechanism of impaired dendritic cell development in CML remains unknown.

IFN regulatory factor-8 (IRF8) is a hematopoietic transcription factor that regulates the development of multiple immune cell types (19). IRF8 is required for differentiation of mouse cDCs (particularly CD8α+ dendritic cells: refs. 20, 21), pDCs (22), and monocytes (particularly Ly6C+ monocytes: ref. 23), while inhibiting myeloid cell proliferation and neutrophil differentiation (24). IRF8 is also indispensable for the differentiation of CTLs (25). Thus, IRf8−/− mice develop immunodeficiency and a CML-like syndrome (26). Importantly, mutations in the human IRF8 gene are associated with dendritic cell immunodeficiency (27). Furthermore, IRF8 expression is dramatically decreased in patients with CML (28, 29). IRF8 also overcomes the mitogenic activity of BCR-ABL in differentiating myeloid progenitors in vitro (30). Coexpression of IRF8 in bone marrow progenitors can ameliorate BCR-ABL–induced myeloproliferative disorder in mice (31). Coexpression of IRF8 in a BCR-ABL–transformed mouse pro-B cell line induces a chemokine-dependent antileukemia immunity in mice (31–33). These findings imply that there is an antagonistic relationship between IRF8 and CML pathogenesis. However, this idea has not been tested in terms of dendritic cell biology yet. In this study, we have investigated how BCR-ABL and IRF8 are involved in the development and function of dendritic cells in CML using a murine model.

Materials and Methods

**Mice**

C57BL/6-Ly5.1 or -Ly5.2 congenic mice and OT-I or OT-II T cell receptor transgenic mice (The Jackson Laboratory) were used at 8 to 12 weeks of age. All animal experiments were carried out in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan), and all protocols were approved by the Institutional Review Boards of Yokohama City University (Yokohama, Japan; Protocol #F11-85).

**Flow cytometry**

Flow cytometry was performed using FACSCanto II (BD Biosciences), and data were analyzed using the FlowJo software (TreeStar). For antibodies and their clone names, see Supplementary Materials and Methods.

**Separation of hematopoietic progenitors**

Murine lineage marker–negative (Lin−) cells were purified from bone marrow cells by the magnetic-activated cell sorting (MACS) system using the Lineage Cell Depletion Kit (Miltenyi Biotec) and anti-interleukin (IL)-7 receptor (IL-7R/CD127) antibody. For isolation of Lin−Sca-1−c-Kit+ (LSK) cells, MACS-purified Lin− cells were stained with antibodies against Sca-1 and c-Kit, and were then purified by the fluorescence-activated cell sorting (FACS) system using a FACSaria II (BD Biosciences).

**Retroviral transduction and CML model mice**

The following murine stem cell virus (MSCV) retroviral vectors were used: MIG [MSCV-internal ribosome entry site (ires)-GFP], MIG-p210BCR-ABL, MIG-p210BCR-ABL-IRES-GFP, MIGΔ8 [MSCV-IRES-human truncated CD8 (hCD8)], and MIGΔ8-IRF8 (MSCV-IRF8-IRES-hCD8). Lin− cells were precultured for 24 hours with stem cell factor (SCF) and thrombopoietin (TPO) for transplantation, or SCF, IL-6, and IL-3 for in vitro experiments, and were then transduced with MSCVs by spinoculation for 2 consecutive days. All cytokines were purchased from PeproTech. For generating CML model mice, C57BL/6-Ly5.1 LSK cells transduced with MIG or MIG-p210BCR-ABL were injected intravenously into lethally irradiated syngenic C57BL/6-Ly5.2 recipients.

**qRT-PCR, ELISA, and immunoblot analysis**

Quantitative PCR (qPCR) with quantitative reverse transcription PCR (qRT-PCR) was performed using RNAiso Plus (Takara Bio), DNase I (Invitrogen), PrimeScript (Takara Bio), Thunderbird SYBR qPCR Mix (Toyobo), and an ABI PRISM 7900 sequence detection system (Applied Biosystems) according to the manufacturers’ protocols. Primer sequences are described in Supplementary Materials and Methods. Data were analyzed using the ΔΔCt method and normalized against Gapdh levels. ELISA for IFN-α and IL-12p40 was performed using commercially available kits (PBL and BioLegend, respectively). For immunoblot analysis, anti-IRF8 (C-19; Santa Cruz Biotechnology), c-ABL (Cell Signaling Technology), phosphotyrosine (4G10; Millipore), and β-actin (AC-74; Sigma-Aldrich) antibodies were used.

**Dendritic cell culture**

Bone marrow Lin− cells transduced with MSCVs were washed 24 hours after the last spinoculation and cultured with human...
fms-like tyrosine kinase-3 (Flt3)-ligand (Flt3L) for 7 days to induce dendritic cell differentiation, TKIs and a STAT5 inhibitor [N-(4-oxo-4H-chromen-3-yl)methylene]nicotinohydrazide] were purchased from Santa Cruz Biotechnology. Trichostatin A (TSA) and 5-azacytidine (5-Aza) were purchased from Focus Biomolecules and Sigma-Aldrich, respectively.

Microarray
RNAs from two independent experiments were analyzed using a Whole Mouse Genome 8 × 60 K Microarray (Agilent) according to the manufacturer’s protocol. Microarray data are available at the GEO/NCBI database (GSE44920). For details, see Supplementary Materials and Methods.

T-cell response assays
Antigen presentation capability of dendritic cells via MHC class II (MHC-II) was evaluated using OT-II T cells essentially as previously described (21). In vitro CTL assays were performed using OT-I T cells essentially by the method previously established (34).

Results

BCR-ABL inhibits dendritic cell development and Irf8 expression
To investigate how dendritic cell development is affected in CML, we first used a murine CML model in which hematopoietic progenitors (LSK cells) were transduced with bicistronic MIG-p210BCR-ABL retrovirus (carrying cDNAs encoding BCR-ABL and GFP) in the presence of SCF and TPO, and then transplanted these cells into lethally irradiated mice. These mice (BCR-ABL mice), but not mice transplanted with empty MIG-transduced cells (MIG mice), exhibited splenomegaly and died by 4 to 8 weeks after the transplantation (Fig. 1A and Supplementary Fig. S1A). Wright–Giemsa staining revealed that while many of the MIG-transduced cells showed typical dendritic cell morphology, transduced cells showed a greater increase in cell number percentages and absolute cell counts, even though BCR-ABL expression was not significantly affected in BCR-ABL mice (data not shown), consistent with the fact that monocyte counts are not reduced in patients with CML. The developmental route of these BCR-ABL+ monocytes is unknown, but one possibility is that they are derived from upstream myeloid progenitors without transiting MDPs. Overall, these data suggest that BCR-ABL affects the early stage of dendritic cell differentiation, particularly the generation of MDPs.

We analyzed Irf8 expression in BCR-ABL and MIG mice. qRT-PCR analysis revealed that BCR-ABL suppressed Irf8 mRNA expression both in Lin− bone marrow progenitors and splenic cells (Fig. 1F). Immunostaining for Irf8 in splenic dendritic cells demonstrated that the remaining few BCR-ABL+ dendritic cells expressed lower levels of Irf8 than control dendritic cells (Fig. 1G and Supplementary Fig. S1F).

IRF8 overrides BCR-ABL to rescue dendritic cell differentiation
To further investigate the mechanisms involved in this process, we examined whether BCR-ABL inhibits dendritic cell differentiation in vitro. We transduced MIG-p210BCR-ABL or empty MIG retrovirus into bone marrow Lin− progenitors in the presence of SCF, IL-6, and IL-3, and the transduced cells were then cultured with Flt3L for 7 days to induce differentiation toward cDCs (CD11c+CD11b−MHC-II+) and pDCs (CD11c−CD123−CD8+CD4−; ref. 21). In MIG-transduced control cultures, cDCs and pDCs were efficiently generated (Fig. 2A). In BCR-ABL–transduced cultures, however, the development of both cDCs and pDCs was significantly inhibited in terms of percentages and absolute cell counts, even though BCR-ABL–transduced cells showed a greater increase in cell number during the Flt3L culture than MIG-transduced cells. Wright–Giemsa staining revealed that while many of the MIG-transduced cells showed typical dendritic cell morphology,
BCR-ABL–transduced cells exhibited neutrophil-like, macrophage-like, or immature morphologies (Fig. 2B). Furthermore, the induction of If8 mRNA expression during the Flt3L culture was almost completely abrogated by BCR-ABL (Fig. 2C). When a kinase-dead K1172R mutant of BCR-ABL was transduced into cells, neither dendritic cell development nor IRF8 expression was affected, suggesting that the kinase activity of BCR-ABL is required for the observed inhibitory effects (Fig. 2D). The expression levels of wild-type (WT) and K1172R BCR-ABL were comparable, and K1172R lacked autophosphorylation at tyrosine residues.

We next asked whether restoration of IRF8 expression could improve dendritic cell differentiation impaired by BCR-ABL. To this end, we cotransduced Lin− cells with MICD8-IRF8 (that expresses IRF8 and hCD8t) together with MIG-p210BCR-ABL, and treated them with Flt3L. hCD8t, used as a transduction marker, does not transmit any signals because it contains no cytoplasmic domain. Flow-cytometric analysis of doubly transduced (i.e., GFP+GFP+) cDCs and pDCs determined by qRT-PCR in triplicate. G, IRF8 protein expression levels in splenic GFP+ cells determined by flow cytometry. The mean fluorescent intensity (MFI) was calculated by subtracting the autofluorescence intensity. All values in bar graphs are the mean ± SD. *, P < 0.05 and **, P < 0.01 (Student t test).
IRF8-transduced BCR-ABL+ cells were comparable with those of endogenous IRF8 in control cells (Fig. 3B and C, compare the first and fourth lanes). Importantly, IRF8 also efficiently overrode the TKI-resistant T315I-mutant BCR-ABL to rescue dendritic cell differentiation (Fig. 3D). WT and T315I BCR-ABL were expressed at comparable levels (Fig. 3B and C). These results indicate that the suppression of IRF8 is a cause, rather than a result, of dendritic cell deficiency by BCR-ABL in CML.

**IRF8 normalizes the expression of a significant portion of BCR-ABL-dysregulated genes**

We next performed transcriptome analysis by microarray in bone marrow Lin− cells transduced with empty vectors, MIG-p210BCR-ABL+ or MIG-p210BCR-ABL+MIC8-IRF8 followed by 5-day Flt3L culture (see Supplementary Fig. S2A and S2B for quality control data). We first calculated the number of genes whose expression was significantly affected by BCR-ABL [fold change (FC) > 3; false discovery rate (FDR) < 0.05] and those normalized by IRF8 (Fig. 4A and Supplementary Tables S1 and S2). The criteria of normalization were as follows: IRF8 significantly reverses the expression in BCR-ABL+ cells (FC > 2; FDR < 0.05), and expression of the gene becomes comparable between control dendritic cells and IRF8-rescued BCR-ABL+ dendritic cells (i.e., FC is no longer > 3 and FDR < 0.05). BCR-ABL decreased the expression of 1,433 genes and increased the expression of 1,915 genes (FC > 3). Among these genes, despite the stringent criteria, IRF8 normalized the expression of 688 genes (48%) and 564 genes (29%), respectively. These results indicate that BCR-ABL impairs the expression of many genes via suppressing IRF8 expression.

Figure 2. Inhibition of dendritic cell development and IRF8 expression by BCR-ABL in vitro. Bone marrow Lin− cells were transduced with MIG or MIG-p210BCR-ABL and then cultured for 7 days in the presence of Flt3L to induce dendritic cell differentiation. A, flow-cytometric analysis of dendritic cells. Numbers in middle left indicate percentages of pDCs relative to GFP+CD11c+ cells (without parentheses) and those relative to total GFP+ cells (in parentheses). For evaluating cell numbers, FACs-purified GFP+transduced cells were seeded at 2×106 cells/mL at the beginning of Flt3L culture. B, Wright-Giemsa stains of FACs-sorted transduced GFP+ cells determined by qRT-PCR. D, flow cytometry, qRT-PCR, and immunoblot analysis in cells transduced with K1172R BCR-ABL. Tyr-P BCR-ABL indicates tyrosine-phosphorylated BCR-ABL. β-Actin expression is shown as a loading control. All values in bar graphs are the mean ± SD from three independent experiments. *P < 0.05 and **P < 0.01 (Student t test).
Gene set enrichment analysis (GSEA; ref. 36) showed that expression of dendritic cell signature genes (37) was indeed significantly suppressed by BCR-ABL, and was efficiently reinduced by coexpression of IRF8 (Fig. 4B). qRT-PCR analysis of several of these genes (Ciita, H2-Eb2, and H2-Au) in the same set of transduced cells as well as cells transduced with IRF8 alone on day 7 and in freshly isolated Lin− cells confirmed the findings (Fig. 4C, top). Expression of other genes critical for the function of CD8α+ dendritic cells (e.g., cross-presentation), such as Clec9a, Xcr1, and Tlr3, was also suppressed by BCR-ABL and restored by IRF8 (Fig. 4C, bottom). Conversely, GSEA showed that expression of granulocyte signature genes (see Supplementary Materials and Methods) was significantly induced by BCR-ABL, but suppressed by IRF8 (Supplementary Fig. S2C).

We also paid attention to the genes dysregulated by BCR-ABL but not normalized by IRF8. We used Ingenuity Pathway Analysis (IPA) software to predict transcription factors that may act upstream of these genes. The results showed that the genes induced by BCR-ABL, irrespective of IRF8 supplementation, were significantly enriched with those inducible by NF-kB, a transcription factor activated by BCR-ABL and involved in many activation signals in dendritic cells (Supplementary Fig. S2D). Furthermore, GSEA showed that
IRF8-rescued BCR-ABL⁺ dendritic cells expressed higher levels of dendritic cell maturation-related genes (38) than control dendritic cells (Fig. 4D, right). This was even more apparent in IRF8-untransduced BCR-ABL⁺ cells compared with control dendritic cells and IRF8-rescued BCR-ABL⁺ dendritic cells (Fig. 4D, left and middle), suggesting that dendritic cell maturation-related genes were upregulated by BCR-ABL rather than exogenous IRF8. These results lead us to speculate that IRF8-rescued, BCR-ABL–expressing dendritic cells may be in a "preactivated" state. Other predicted transcription factors include GATA1 and STAT5 (see Discussion). Indeed, the expression of Gata1 itself was 15-fold higher in BCR-ABL–transduced cells than in control dendritic cells, regardless of IRF8 supplementation.

Figure 4. Gene expression profiling of cells transduced with BCR-ABL and/or IRF8. Bone marrow Lin⁻ cells were cotransduced with empty MIG or MIG-p210BCR-ABL and empty MICD8 or MICD8-IRF8, and FACS-sorted GFP⁺CD8t⁺ cells were then cultured for 5 (for microarray) or 7 days (for qRT-PCR) in the presence of Flt3L. Microarray and qRT-PCR were performed in biologic duplicates. A, Venn diagrams for the numbers of genes that displayed more than a 3-fold change in expression by BCR-ABL and those normalized by IRF8. B, GSEA for dendritic cell (DC) signature genes. NES, normalized enrichment score. C, qRT-PCR. Values are from two independent experiments, in which each PCR was performed in triplicate. D, GSEA for dendritic cell maturation-related signature genes.
IRF8-rescued BCR-ABL⁺ dendritic cells have higher cytokine production and CTL induction capacities

We next evaluated the functionality of the rescued dendritic cells. To examine their ability to produce IFN-α and IL-12p40, transduced Lin⁻ cells followed by 7-day Flt3L culture were stimulated with TLR9 ligands CpG-A (for pDCs) or CpG-B (for cDCs), or a TLR4 ligand lipopolysaccharide (LPS; Fig. 5A). TLRs stimulated with TLR9 ligands CpG-A (for pDCs) or CpG-B (for cDCs), or a TLR4 ligand lipopolysaccharide (LPS; Fig. 5A). TLRs recognize not only the pathogen-associated molecular patterns (PAMP) but also damage-associated molecular patterns (DAMP) that may be involved in antitumor immunity (39). BCR-ABL almost completely abolished production of these cytokines, but their expression was restored by coexpression of BCR-ABL. Interestingly, IRF8-rescued BCR-ABL⁺ dendritic cells produced even higher levels of these cytokines than control dendritic cells, especially for IL-12p40, whose expression is dependent on NF-κB and IRF8 (19, 40).

We also examined the expression of MHC-related molecules by flow cytometry. BCR-ABL strongly inhibited MHC-II expression, but increased MHC-I expression 1.1-fold (Fig. 5B and Supplementary Fig. S3). When IRF8 expression was supplemented, MHC-II expression was recovered, and MHC-I expression remained high. We also found that expression of the costimulatory molecules CD80 and CD86 was significantly induced by BCR-ABL and remained high in IRF8-cotransduced cells. We noted that the mRNA levels of MHC-I, CD80, and CD86 did not correlate with protein expression levels on the cell surface, suggesting the occurrence of posttranscriptional control. The expression level of another costimulatory molecule CD40 was low and not affected by BCR-ABL. Thus, IRF8-rescued BCR-ABL⁺ dendritic cells expressed higher levels of MHC-I and some costimulatory molecules than regular dendritic cells.

CD8⁺ dendritic cells have been shown to be critical for eliminating tumor cells by priming CTLs via cross-presentation in mice (41). In addition, CD8⁺ dendritic cells require type I IFN to induce CTLs that reject tumors (42, 43). These previous findings, together with the above data on cytokine production and MHC-related molecules, prompted us to examine the ability to cross-present an antigen (OVA) to induce a CTL response (Fig. 5C). Transduced Lin⁻ cells followed by 7-day Flt3L culture were pulsed with OVA protein, and then mixed with T cells from OT-1 mice. The primed T cells were then mixed with target splenocytes loaded with OVA peptide, and OVA-specific CTL activity was calculated. Although control dendritic cells efficiently induced CTLs, BCR-ABL–transduced cells failed to do so. Strikingly, IRF8-rescued BCR-ABL⁺ dendritic cells regained the ability to induce CTLs, and the observed response was even stronger than that of control dendritic cells.

Transduction of IRF8 without BCR-ABL did not augment the above functional parameters. Thus, these results suggest that BCR-ABL harbors an immune-stimulatory potential that is...
manifested when dendritic cell differentiation is restored by supplementation with IRF8.

Imatinib partially restores Irf8 expression, but cancels the enhancement of functionality in IRF8-rescued BCR-ABL+ dendritic cells

To investigate whether clinically available TKIs can restore Irf8 expression as well as dendritic cell differentiation and function, imatinib, nilotinib, and dasatinib were added at concentrations two times higher than those required for 90% inhibition (IC50) of BCR-ABL kinase activity during the Flt3L treatment of transduced Lin- cells. These TKIs partially restored the expression of Irf8 in BCR-ABL-transduced cells (Fig. 6A, top). Efficient inhibition of BCR-ABL kinase activity by imatinib was confirmed by immunoblot analysis for autophosphorylation of BCR-ABL in BCR-ABL-rescued dendritic cells (Fig. 6A, bottom). Presumably, due to the modest but significant increase in Irf8 expression, imatinib restored cDC differentiation (Fig. 6B). However, the recovery of pDC differentiation and MHC-II expression was not complete (Fig. 6B and C). In addition, the recovery of cytokine production and CTL induction was very modest (Fig. 6D). Interestingly, imatinib canceled the enhancement of dendritic cell functionality by BCR-ABL in IRF8-rescued BCR-ABL+ dendritic cells: MHC-I, CD80, and CD86 expression, cytokine production, and CTL induction all returned to unenhanced levels. Thus, BCR-ABL augments dendritic cell functionality via its tyrosine kinase activity. We also noted that in control dendritic cells, TKIs modestly inhibited Irf8 expression, and that imatinib slightly suppressed the ability of control dendritic cells to induce CTLs (Fig. 6E). Overall, these data suggest that dendritic cells rescued by IRF8 are more functional than those rescued by TKIs, at least under the condition used in this study.

To elucidate the pathway through which BCR-ABL inhibits Irf8 expression, we tested whether a STAT5 inhibitor, a histone deacetylase inhibitor (TSA), or a DNA methyltransferase inhibitor (5-Aza) improved Irf8 expression and dendritic cell differentiation. TSA and 5-Aza were tested because we speculated that epigenetic mechanisms may be involved, given the discrepancy between the effects of the kinase-dead BCR-ABL–mutant and of imatinib treatment after BCR-ABL expression on Irf8 expression. However, none of these agents restored Irf8 expression or dendritic cell differentiation in BCR-ABL–transduced cells, and instead tended to suppress these events in control cells (Supplementary Fig. S4). Combinations of TSA or 5-Aza with imatinib also failed to rescue Irf8 expression and dendritic cell differentiation.

Discussion

In this study, the antagonistic relationship between BCR-ABL and IRF8 was investigated in the context of dendritic cell development in CML. Our results revealed that BCR-ABL abolishes dendritic cell development at the level of generating MDPs. This causes severe reductions in dendritic cell counts for all subsets in CML mice, consistent with previous findings in patients with CML (14, 15). BCR-ABL inhibits Irf8 expression, and supplementation with IRF8 restores the expression of a significant portion of BCR-ABL–dysregulated genes, leading to efficient recovery of dendritic cell differentiation. Thus, the suppression of Irf8 expression is the cause of impaired dendritic cell development by BCR-ABL, which we infer accounts, at least in part, for the inability of patients with CML to establish antileukemic immunity.

Gene expression profiling indicated that the expression of a significant numbers of NF-kB target genes was increased by BCR-ABL, and predicted that IRF8-rescued BCR-ABL+ dendritic cells may be in a preactivated state. In fact, we found that the rescued dendritic cells had higher cytokine production and CTL induction capacity than normal dendritic cells, and such enhancement was completely canceled by imatinib. These results indicate that BCR-ABL harbors an immune-stimulatory potential, which would not be elicited in the natural course of CML or during the therapy with TKIs. Thus, IRF8 seems to “convert” BCR-ABL from a dendritic cell suppressor to a dendritic cell activator.

We have previously shown that IRF8 overrides the mitogenic activity of BCR-ABL in differentiating myeloid progenitor cells (30). It has also been shown that IRF8 antagonizes the anti-apoptotic effect of BCR-ABL (44). Moreover, coexpression of IRF8 inhibits BCR-ABL–induced myeloid hyperplasia in vivo (31). Neutrophil differentiation is promoted by BCR-ABL (45), but potently inhibited by IRF8 (24). Therefore, we expect the restoration of IRF8 to have two distinct effects. One is to directly reduce tumor burden by inhibiting abnormally enhanced growth and survival of myeloid cells as well as disproportionate neutrophil differentiation. The other is to induce effective anti-CML immunity by restoring dendritic cell development and by cooperating with BCR-ABL to strengthen dendritic cell functionality. In this regard, IRF8 may be the key to establishing multidisciplinary therapy for CML.

We propose that the selective restoration of IRF8 will serve as a hallmark for new CML therapies that may overcome the problems with current TKI therapy. First, the effects of IRF8 supplementation are not affected by TKI-resistant mutations in BCR-ABL. Second, unlike TKIs, IRF8 therapy would generate BCR-ABL+ dendritic cells with enhanced functionality to induce anti-CML CTL responses. Finally, once anti-CML CTLs are induced, it would be possible to discontinue the IRF8 therapy. It should be noted, however, that the possibility of autoimmune reactions by these hyperfunctional dendritic cells will need to be evaluated.

To develop a method for restoration of IRF8 expression, it will be essential to understand the mechanism through which BCR-ABL inhibits Irf8 expression and dendritic cell development. Our data seem to provide some insights into these mechanisms. Unlike Irf8−/− mice, which show severe reduction in CDP counts but not in MDP counts (23, 46), CML model mice lack both MDPs and CDPs. Thus, the dendritic cell differentiation stage affected by BCR-ABL is upstream of that affected by the loss of IRF8. This suggests that BCR-ABL acts on machinery that is required for the expression of Irf8 (the mechanism of which has not been clarified yet) as well as the generation of MDPs.

One possibility raised from the above context is inhibition of the transcription factor PU.1 by GATA1. PU.1 is expressed in
Figure 6. The effects of TKIs on dendritic cell differentiation, function, and Irf8 expression. Lin⁻ cells were singly transduced as in Fig. 2 or doubly transduced as in Fig. 3 and cultured in the presence of Flt3L for 7 days. TKIs (imatinib at 2 μmol/L, nilotinib at 50 nmol/L, or dasatinib at 5 nmol/L) were added during the Flt3L culture. Their differentiation status and function were analyzed as in Figs. 2 and 5. In A, D, and E, transduced cells were FACS-sorted before the Flt3L culture. A, qRT-PCR (top) and immunoblot analysis (bottom). B, representative data of flow-cytometric analysis. C, expression levels of antigen presentation–related molecules. D, cytokine production. E, induction of CTLs. Imatinib was added to the Flt3L culture of dendritic cells but not to the coculture with T cells. All values in bar graphs are from three independent experiments. *, P < 0.05 and **, P < 0.01 (Student t test).
similar sets of hematopoietic cell lineages as IRF8 and is required for the generation of MDPs (35). PU.1 activity is inhibited by GATA1, which is essential for the generation of MEPs (47). Our data showed that BCR-ABL strongly induced Gata1 expression. Moreover, GATA1 targets were indeed significantly enriched in genes induced by BCR-ABL and that the percentage of MEPs was increased in BCR-ABL mice. Interestingly, GATA1 has been shown to inhibit IFβ expression, possibly by preventing PU.1 recruitment to the Irf8 promoter in dendritic cells (48). Moreover, GATA1 activity can be augmented via PI3K/AKT, a signaling pathway stimulated by BCR-ABL (49). Therefore, inhibition of PU.1 through induction and activation of GATA1 by BCR-ABL is an attractive possibility. Other possibilities included the involvement of STAT5. It has been reported that GM-CSF inhibits Irf8 expression via STAT5 (50). Thus, it was tempting to speculate that BCR-ABL–activated STAT5 suppresses IRF8 expression. However, our experiments using the STAT5 inhibitor did not support this. Finally, even though we observed that TSA or 5-Aza, as single agents or in combination with imatinib, failed to rescue even though we observed that TSA or 5-Aza, as single agents or in combination with imatinib, failed to rescue even though we observed that TSA or 5-Aza, as single agents or in combination with imatinib, failed to rescue

**References**

IRF8 Overrides BCR-ABL in Dendritic Cell Development


The Transcription Factor IRF8 Counteracts BCR-ABL to Rescue Dendritic Cell Development in Chronic Myelogenous Leukemia

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