Imatinib and plasmacytoid dendritic cell function in chronic myeloid leukemia patients*

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Abstract

Plasmacytoid dendritic cells (PDCs) are crucial effectors in innate immunity. In this study, we show that imatinib, a potent inhibitor of BCR/ABL tyrosine kinase activity, in the presence of Flt3-Ligand, could induce CD34+ progenitors from chronic myeloid leukemia (CML) to give rise in vitro to typical BDCA-2+ type I interferon-producing PDCs. The impact of imatinib on PDC generation was related to upregulation of Flt3 on leukemic CD34+ progenitors. Moreover, CML patients who were in complete cytogenetic or molecular response after imatinib treatment restored their blood PDCs both quantitatively and functionally comparable to healthy donors, in contrast to patients not responding to imatinib, further confirming that disease response to imatinib is accompanied by restoration of PDC function in vivo. These findings provide evidence that response to imatinib is capable to restore some DC-related immune functions in CML that might be beneficial for long term disease control.
Introduction

Chronic myelogenous leukemia (CML) is a malignant clonal disorder of primitive hematopoietic progenitors characterized by a balanced translocation between chromosomes 9 and 22, leading to the fusion of a portion of the ABL gene with the BCR gene. The resulting BCR/ABL gene has been shown to play a critical role in the pathogenesis of CML. The BCR/ABL gene product demonstrates constitutive activation of tyrosine kinase activity that appears critical for BCR/ABL-induced transformation. Imatinib mesylate (Gleevec, STI571) has been demonstrated to be a potent inhibitor of ABL tyrosine kinases. Several studies demonstrated high levels of imatinib activity against BCR/ABL-containing cells with remarkable results in the treatment of CML. Despite the exciting success of this targeted cancer therapy, it is still needed to determine whether imatinib can also influence the immune functions in CML patients, allowing for immune-mediated durable responses as it has been already shown after allogeneic stem cell transplantation. Dendritic cells (DCs) are responsible for the initiation and regulation of immune responses. The plasmacytoid DC (PDC) subset is a crucial effector in innate immunity. We and others have reported previously multiple abnormalities in blood DC subsets from leukemic patients, especially PDCs in CML. Although the exact role of PDCs in antitumor immunity has yet to be established, these results suggest that restoration of PDC function and other immune effectors function may be a goal for CML immunotherapy. Here, we investigated whether imatinib can impact PDC differentiation from CML patients in vitro, and whether response to imatinib in vivo is accompanied by restoration of quantitative and functional properties of PDCs from CML patients.
Materials and methods

 Patients and controls

CML cells samples were obtained after informed consent from 6 patients in chronic phase at diagnosis, and from 9 patients in chronic phase after treatment with imatinib. CML peripheral blood mononuclear cells (PBMC) analyzed in this study were derived from patients treated at the Institut Paoli-Calmettes (Marseille) or at the CHU de Nimes (Nimes). PBMC from patients and healthy controls (Etablissement Français du Sang, Marseille, France) were isolated by standard density gradient centrifugation with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) prior to cryopreservation.

Blood DC detection and flow cytometry

Blood DCs were identified by three-color staining performed on PBMC as previously described. The following monoclonal antibodies were also used for standard flow cytometry: CD3, CD4, CD11c, CD14, CD16, CD19, CD34, ILT3, Flt3 (Beckman-Coulter, Marseille, France), CD56, CD123 (PharMingen, San Diego, CA) and BDCA-2 (Miltenyi Biotec, Bergisch-Gladbach, Germany). Stained cells were analyzed on a FACSCalibur cytometer using CellQuest software (Becton-Dickinson, San Jose, CA).

Generation of PDCs from leukemic CD34+ circulating progenitors

Circulating CD34+ progenitors from CML patients were immunomagnetically purified with CD34 mAb-conjugated microbeads (Miltenyi Biotec). Cultures were established in 24-well culture plates in RPMI 1640 medium containing 10% fetal calf serum (FCS, BioWhittaker, Verviers, Belgium), 0.55 mM L-arginine, 0.24 mM L-asparagine, 2 mM L-glutamine (Life Technologies, Paisley, Scotland), in the presence of 100 ng/ml Flt3-ligand
all purchased from R&D Systems, with or without 1 µM imatinib added once at the beginning of culture (kind gift of Dr. P. Dubreuil, INSERM U119, Marseille, France). Except for imatinib, the medium was replenished with cytokines every 5 days. For the IFN-α secretion assay, cells were plated in triplicates in 96-well round-bottomed plates either with medium alone, or with herpes simplex virus 1 (HSV, kind gift of Dr C. Zandotti, Hôpital La Timone, Marseille, France) as described previously. Supernatants were collected after 48 hours and tested for their IFN-α content by ELISA (ELISA kits, Beckman-Coulter).

**Statistical analysis**

Statistical comparisons were performed as previously described.⁹
Results and discussion

Several reports in humans and mice demonstrated that Flt3L can induce the generation or mobilize IFN-α producing PDCs from CD34+ hematopoietic progenitors both in vitro and in vivo. Since elevated numbers of CD34+ progenitors have been previously described in the blood of CML patients, we isolated circulating CD34+ from 6 CML patients in chronic phase at diagnosis, and cultured them with SCF, TPO and Flt3L, a condition that is known capable to give rise to functional PDCs from CD34+ hematopoietic progenitors. After 17 to 21 days of culture, CML-derived CD34+ stem cells could not give rise to PDCs as shown by phenotypic analysis (Figure 1A), in line with previous data showing altered cell differentiation from CML-derived CD34+ progenitors. In contrast, the addition of imatinib at the beginning of culture allowed to generate significant numbers PDCs as ascertained by the simultaneous expression of CD4, CD123, and the typical PDC marker BDCA-2 (Figure 1B). These cells could secrete significant amounts of IFN-α following exposure to HSV (Figure 1C), further confirming that CD34+ leukemic progenitors can be induced to differentiate in vitro towards genuine type I IFN-producing PDCs in the presence of imatinib. In order to elucidate the mechanisms underlying the impact of imatinib on PDC generation, we could show that a 24h exposure of CML cells to imatinib upregulated the expression of Flt3 on CML CD34+ progenitors (Figure 1D), in accordance with recent data suggesting that Flt3 expression and signaling might represent a prerequisite for DC development.

The impact of imatinib on PDC development in vitro prompted us to investigate whether response to imatinib in vivo could restore PDC numbers and function in CML patients. Analysis of 7 patients who were in complete cytogenetic or molecular response under imatinib treatment demonstrated that these patients restored normal PDC numbers in
comparison to healthy donors \( (P=\text{NS}; \text{Figure } 2A) \). In contrast, results obtained from 2 patients who could not reach cytogenetic responses under imatinib treatment, showed that these 2 patients still had a severe depletion and altered function of their circulating blood PDC compartment (Figure 2A and 2B). In addition, we found that PDCs from patients responding to imatinib could secrete significant amounts of IFN-\( \alpha \), comparable to healthy donors \( (P=\text{NS}; \text{Figure } 2B) \), further confirming that response to imatinib is accompanied by restoration of the major PDC function in vivo.

Potential consequences pertaining to both PDC physiology and CML therapy can arise from the above observations. “Dendritopoiesis” in CML is likely to be affected by the leukemic process\(^9,10,19\) that induces a marked expansion of granulopoietic progenitors to the detriment of other populations, especially DCs. Flt3L was shown to drive PDC development both in vitro and in vivo.\(^11,20\) The impact of imatinib on Flt3 expression and PDC development correlates with data showing that Flt3+ hematopoietic progenitors have DC developmental potential, whereas Flt3- fractions do not.\(^21,22\) However, it remains uncertain whether Flt3 expression and signaling is mandatory for all DC subsets development, since Flt3- monocytes for example generate DCs in vitro and likely in vivo.\(^23\) Thus, the PDC/CML/imatinib model might represent a valuable tool to test whether overexpression or stimulation of Flt3 or other cytokine receptors can “rescue” progenitors to the DC lineage that normally have lost DC developmental potential. On the therapeutic level, in addition to inhibition of BCR-ABL activity, our findings provide some evidence that response to imatinib is accompanied by restoration of important DC-related immune functions in CML. It is yet still unclear whether imatinib can completely eliminate malignant CML primitive progenitors abrogating the risk of disease relapse.\(^24\) However, in addition to its beneficial action on the attachment of CML cells to bone marrow
stroma, and although the exact role of PDCs in antitumor immunity has yet to be established, reconstitution of the DC system after response to imatinib might be beneficial for long term disease control.
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References

Figure Legends

Figure 1. Generation of functional PDCs from CML CD34+ progenitors in the presence of imatinib. CD34+ hematopoietic progenitors from CML patients in chronic phase at diagnosis were cultured in the presence of Flt3L as described in materials and methods, (A) in the absence or (B) in the presence of imatinib. CD4, CD123 and BDCA-2 expression was analyzed by flow cytometry. Empty histograms show the background staining with isotype control monoclonal antibodies, and solid histograms represent specific staining of the indicated cell-surface markers. Representative of 4 independent experiments. (C) IFN-α secretion by PDCs generated from CD34+ CML progenitors in the presence of imatinib. 10⁶ cells from cultures described in A and B were stimulated with HSV without prior PDC sorting or any additional cytokine. Supernatants were harvested after 48 hours of stimulation with HSV. IFN-α secretion was analyzed by ELISA. Results are represented as the mean and SEM of IFN-α concentration obtained from 3 independent experiments. (D) Expression of Flt3 by flow cytometry is shown on CD34+ CML progenitors before (left panel), and after 24h of incubation of PBMCs from CML with imatinib. Empty histogram show the background staining with isotype control monoclonal antibody, and solid histogram represent specific staining of Flt3. Representative of 6 different CML patients in chronic phase at diagnosis.

Figure 2. Imatinib restores PDC function in vivo. (A) PBMCs isolated from healthy volunteers or CML patients treated with imatinib were analyzed by flow cytometry after three-color staining with a combination of FITC-labeled monoclonal antibodies against lineage markers (CD3, CD14, CD16, CD19 and CD56), PE-labeled anti-CD11c and PC5-labeled anti-ILT3. Two distinct populations of lin-/ILT3+ cells were observed with respect
to the expression of CD11c with the phenotypes of lin-/CD11c+/ILT3+ (myeloid DCs) and lin-/CD11c-/ILT3+ (PDCs). Examples of results obtained from >15 healthy volunteers (left panel), 7 CML patients in complete cytogenetic or molecular remission after imatinib treatment (middle panel), and 2 CML patients not reaching cytogenetic or molecular remission after imatinib treatment (right panel). $P$ = not significant for comparison of PDC numbers between healthy donors (left panel) and CML patients in complete cytogenetic or molecular remission after imatinib treatment (middle panel). (B) IFN-$\alpha$ secretion by PDCs from healthy donors and CML patients receiving imatinib treatment following stimulation with HSV. Cells were stimulated with HSV without any additional cytokine. Supernatants were harvested after 48 hours of stimulation. IFN-$\alpha$ secretion was analyzed by ELISA. Concentrations obtained from $10^4$ PDCs are represented as the mean and SEM of IFN-$\alpha$ secretion obtained from 5 healthy donors, 5 CML patients in complete cytogenetic or molecular remission after imatinib treatment, and 2 CML patients not reaching cytogenetic or molecular remission after imatinib treatment. $P$ = not significant for comparison of IFN-$\alpha$ secretion between healthy donors and CML patients in complete cytogenetic or molecular remission after imatinib treatment. Control conditions that were not stimulated with HSV did not contain detectable or significant amounts of IFN-$\alpha$. 

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Figure 1

A

CD123

CD123

B

CD4

BDCA-2

C

CD4

BDCA-2

IFN-alpha (UI/ml)

0

25

50

75

100

125

STI571

HSV

+ + - -

D

Flt3
Figure 2

A

<table>
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<th>CML not in complete remission</th>
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B

CD11c

IFN-alpha (UI/ml)

Healthy donons
CML patients in complete remission
CML patients not in complete remission

HSV

+ + + - -
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