Defective homing and impaired induction of cytotoxic T cells by BCR/ABL-expressing dendritic cells

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Chronic myelogenous leukemia (CML) is a malignant myeloproliferative disease arising from a hematopoietic stem cell expressing the BCR/ABL fusion protein. Leukemic and dendritic cells (DCs) develop from the same transformed hematopoietic progenitors. How BCR/ABL interferes with the immunoregulatory function of DCs in vivo is unknown. We analyzed the function of BCR/ABL-expressing DCs in a retroviral-induced murine CML model using the glycoprotein of lymphoctic choriomeningitis virus as a model leukemia antigen. BCR/ABL-expressing DCs were found in bone marrow, thymus, spleen, lymph nodes, and blood of CML mice. They were characterized by a low maturation status and induced only limited expansion of naive and memory cytotoxic T lymphocytes (CTLs). In addition, immunization with in vitro–generated BCR/ABL-expressing DCs induced lower frequencies of specific CTLs than immunization with control DCs. BCR/ABL-expressing DCs preferentially homed to the thymus, whereas only few BCR/ABL-expressing DCs reached the spleen. Our results indicate that BCR/ABL-expressing DCs do not efficiently induce CML-specific T-cell responses resulting from low DC maturation and impaired homing to secondary lymphoid organs. In addition, BCR/ABL-expressing DCs in the thymus may contribute to CML-specific tolerance induction of specific CTLs. (Blood. 2009;113:4681-4689)

Introduction

Chronic myelogenous leukemia (CML) is a malignant clonal myeloproliferative disease. The BCR/ABL fusion protein results from the reciprocal chromosomal translocation t(9;22) forming the Philadelphia chromosome (Ph). BCR/ABL is responsible for the malignant phenotype of leukemia cells in CML and increases cell proliferation, inhibits apoptotic processes, and alters cellular adhesion of myeloid cells.¹,² CML is characterized by an initial chronic phase with a massive expansion of all stages of the granulocyte cell lineage. Eventually, hematopoietic differentiation becomes arrested and CML progresses to blast crisis with immature blast cells accumulating in the periphery.¹

Several studies have shown that cytotoxic T lymphocytes (CTLs) are involved in the immunosurveillance of CML. BCR/ABL is a leukemia-specific antigen, and CTLs specific for peptides derived from its sequence could recognize CML cells in vitro and in vivo.³,⁴ This suggests that there is efficient intracellular processing and presentation of BCR/ABL-derived peptides by CML cells. In addition, overexpressed self-proteins, such as proteinase-3, Wilms tumor 1 protein, and minor histocompatibility antigens, can act as leukemia-specific antigens for T cells.⁵

Dendritic cells (DCs) are professional antigen-presenting cells and are key mediators for the initiation and regulation of both innate and adaptive immune responses.⁶ DCs are a heterogeneous population that can be divided into myeloid and plasmacytoid DCs based on their origin, expression of surface markers, and function.⁷ As CML mainly affects cells of the myeloid lineage, it is probable that myeloid BCR/ABL-expressing DCs are circulating in CML patients. Indeed, BCR/ABL-expressing DCs could be detected in the peripheral blood of CML patients.⁸,⁹ However, CML patients in chronic phase had reduced numbers of circulating myeloid and plasmacytoid DCs compared with healthy persons.¹⁰ Contradictory data regarding the maturation status and function of BCR/ABL-expressing DCs have been published. BCR/ABL-expressing DCs had either a normal maturation status or lower expression of the costimulatory molecules CD80/CD83/CD40 compared with control DCs.⁷,⁸,¹¹,¹² In acute myeloid leukemia, the plasmacytoid DCs were immature and could not elicit the proliferation of naïve CD4⁺ T cells.¹³ Moreover, in vitro–generated BCR/ABL-expressing DCs have been reported to be defective in antigen processing.⁷,¹¹ In contrast, other studies suggested that BCR/ABL-expressing DCs are able to effectively stimulate the proliferation of allogeneic and autologous T cells.⁸,¹⁴ Similarly, vaccination with autologous, nonirradiated leukemic DCs induced antileukemic T-cell responses in some CML patients.¹⁵

The function of BCR/ABL-expressing DCs in vivo is unknown. Therefore, we analyzed the function of BCR/ABL-expressing DCs in a murine retroviral-induced bone marrow transplant model.¹⁶ To study antigen-specific immune responses, we used H8 transgenic mice, ubiquitously expressing the glycoprotein gp33 of the lymphocytic choriomeningitis virus (LCMV), as bone marrow donor mice. In CML mice, a large fraction of DCs expressed BCR/ABL. BCR/ABL-expressing DCs displayed a low maturation status and were functionally impaired. Immunization with bone marrow–derived BCR/ABL-expressing DCs induced only limited CTL expansion. Interestingly, BCR/ABL-expressing DCs did not home to the spleen. They preferentially migrated to the thymus where they induced a deletion of antigen-specific CD8⁺ T cells. Therefore, the low maturation status and impaired homing of BCR/ABL-expressing DCs to the spleen result in defective CTL induction. Moreover, the preferential homing to the thymus may induce central tolerance to some leukemia
antigens. In summary, functional deficiencies of BCR/ABL-expressing DCs contribute to escape of CML from immunosurveillance.

Methods

Mice

C57BL/6 mice were purchased from Harlan (Horst, The Netherlands). H8 transgenic mice, with ubiquitously expressing amino acids 1 to 60 of the LCMV glycoprotein, p14 T-cell receptor transgenic mice specific for the LCMV-gp33 (line 318, ~60% specific CD8+ T cells; line 327, ~90% specific CD8+ T cells), and p14 (line 327) × RAG1−/− × CD45.1+ were obtained from the Institute for Laboratory Animals (Zurich, Switzerland). CD45.1+ mice were obtained from C. Mueller (Berne, Switzerland). Animal experiments were performed with sex- and age-matched mice. They were approved by the Experimental Animal Committee of the canton of Berne and were performed according to Swiss laws of animal protection.

Virus, virus detection, peptides, and retroviral vectors

LCMV, strain WE, was provided by R.M. Zinkernagel (Zurich, Switzerland) and propagated on L929 fibroblasts. The LCMV glycoprotein, amino acids 33 to 41 (gp33; KAVYNFATM) and LCMV nucleoprotein, amino acids 396 to 404 (np396; FQPQNGQFI), were purchased from NeoMPS (Strasbourg, France). The retroviral vectors pMSCV-p121 BCR/ABL-IRES-GFP (MSCV, mouse stem cell virus; IRES, internal ribosomal entry site; GFP, green fluorescent protein) and pMSCV-IRES-GFP (empty vector, vector containing GFP) were a gift from J. Schwaller (Basel, Switzerland). Antibodies and flow cytometry

Cells and retroviral particle production

Retroviral particles were generated by transient cotransfection of 293 T cells with the respective MSCV vector and the packaging vector pK6, using Superfect transfection reagent (Qiagen, Basel, Switzerland) according to the manufacturer’s protocol. Forty-eight hours after transfection, virus-containing supernatant was harvested, filter-sterilized, and stored at −80°C. For determination of retroviral titers,莴/FACS analysis. CML model

H8 bone marrow donor mice were pretreated with 150 mg/kg 5-fluorouracil intraperitoneally (Sigma-Aldrich) dissolved in phosphate-buffered saline (PBS; Biochron AG, Berne, Switzerland) 6 days before bone marrow harvest. Two days before bone marrow transplantation, femurs and tibias were collected from 5-fluorouracil–treated mice and flushed with RPMI 10% fetal calf serum (FCS). Erythrocytes were removed by Puregen red blood cell lysis solution (Bioconcept, Basel, Switzerland), and the bone marrow cells were incubated in transplantation media (RPMI 10% FCS with 6 ng/mL recombinant murine interleukin-3 [IL-3], BD Biosciences, San Jose, CA; 10 ng/mL recombinant murine stem cell factor, Biocoba, Reinach, Switzerland; and 10 ng/mL recombinant human IL-6, BD Biosciences) in bacteriologic Petri dishes for 24 hours at 37°C in an atmosphere with 5% CO2. A total of 4 × 10^6 bone marrow cells were injected intravenously into previously irradiated (4.5 Gv) syngeneic recipient mice.

Blood smears and cytopsins

For cytopsins, the erythrocytes were removed with red blood cell lysis solution from 100 μL blood of CML mice or C57BL/6 control mice. Then, the cells were resuspended in 50 μL PBS/0.5% human serum albumin (Stem Cell Laboratory, Inselspital, Berne, Switzerland), loaded on a funnel, and spun down to a microscope slide (20g for 15 minutes). Air-dried and unfixed peripheral blood smears and cytopsins were stained with a Pappenheim staining (Merck Grogg Chemie, Berne, Switzerland) according to the manufacturer’s protocol. The images were captured by a Nikon Eclipse 800 (Tokyo, Japan).

DC generation

H8 bone marrow cells were spin infected as described in “CML model” with retroviral particles containing BCR/ABL-GFP or empty GFP vector as a control. A total of 2 × 10^6 transduced bone marrow cells were cultivated in Petri dishes for 10 days in DC medium (RPMI 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM t-glutamine, 50 μM b-mercaptoethanol; Sigma-Aldrich) and 10% supernatant of a granulocyte-macrophage colony-stimulating factor (GM-CSF) hybridoma, provided by T. Rolink, (University of Basel, Basel, Switzerland). DCs were matured in tissue culture dishes for 1 or 2 days in the presence of lipopolysaccharide (LPS, 10 μg/mL; Sigma-Aldrich) or 10% supernatant of an αCD40 hybridoma (provided by T. Rolink) in DC medium containing 5% GM-CSF supernatant. DCs were collected from the supernatant and sorted by flow cytometry (FACS Vantage, BD Biosciences) for living cells and stained for CD11c and α-B7. To generate nontransduced DCs from C57BL/6 mice, bone marrow was harvested, cultivated for 12 days in DC medium, and matured for 1 day with LPS. For immunization, nontransduced DCs were pulsed with LCMV-gp33 (10^-7 M) and -np396 (10^-6 M) peptides at 37°C for 90 minutes and washed 3 times with Hank balanced salt solution (Sigma-Aldrich). A total of 2 × 10^5 pulsed DCs were injected intravenously into recipient mice.

Antibodies and flow cytometry

αCD8-phycoerythrin (PE), αCD4-biotin, αB220-biotin, αA*-major histocompatibility complex (MHC) class II-biotin and phycoerythrin-cyanin5 (PE-Cy5), αCD11c-biotin, -PE and PE-Cy7, αCD86-PE, αCD80-PE, αCD70-PE, αCD83-PE, αCXC4-PE, αCD44-biotin, α-interferon (IFN-γ)-fluorescein isothiocyanate, streptavidin-PE and allophycocyanin (APC), αCD45.1-APC and PE-Cy7, αCD45.2-PE, αCCR7-Cy7, αCD19-biotin, αCD3-biotin, αNK1.1-biotin, rat IgG2b isotype-PE, rat IgG2b isotype-PE-Cy5, Golden Syrian hamster IgG isotype-PE, rat IgG2a isotype-APC, and αVLA-4-PE were purchased from eBioscience (San Diego, CA), αGR-1-PE, αCD8-PerCP-Cy5.5, and αCD162-PE were purchased from BD Biosciences PharMingen (San Diego, CA). MHC class I (H-2D^d)-tetramer-PE complexed with gp33 was purchased from Beckman Coulter (Fullerton, CA) and used according to the manufacturer’s protocol.

For intracellular staining, spleenocytes were restimulated with LCMV-gp33 peptide (10^-6 M per well) or np396 (10^-6 M per well) in the presence of 25 μM L rabinomat synthase 2 and 5 μg/mL brefeldin A (Sigma-Aldrich) for 5 hours. Cells were stained for CD11c with fixed cells and fixed with 4% paraformaldehyde (Sigma-Aldrich) in PBS. Then, cell membranes were permeabilized with Perm-buffer (PBS, 2% FCS, 5 mM ethylene-diaminetetraacetic acid, 0.1% saponin, 0.1% NaN3; Sigma-Aldrich) and stained with anti-IFNγ-fluorescein isothiocyanate. Relative fluorescence intensities were measured on a BD LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR).

Proliferation assays

Memory p14 T cells were generated as described previously. Naive and memory p14 spleenocytes were purified for CD8+ T cells by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). A total of 2 × 10^5 purified CD8+ T cells were restimulated for 4 days with 2 × 10^5, 1 × 10^5, 0.4 × 10^5, or 0.2 × 10^5 in vitro–generated, LPS-matured, and
FACS-sorted irradiated GFP+ BCR/ABL-expressing or control DCs (empty GFP vector–transduced). Alternatively, CD11c+ DCs from the spleen of CML mice and naive H8 mice were isolated with MACS. DCs from CML mice were sorted by flow cytometry for living GFP-transduced cells. Eighteen hours later, thymus and spleen were collected and the number of CD11c+CD45.1+GFP+ or CD11c+empty GFP vector–transduced DCs was determined by flow cytometry.

Statistical analysis
Statistical significance was determined using the unpaired Student t test (2-tailed). P values less than .05 were considered significant.

Results
BCR/ABL-expressing DCs in CML
Bone marrow cells of H8 transgenic donor mice were transduced with retroviral particles expressing BCR/ABL-GFP and injected into sublethally irradiated (4.5 Gy) C57BL/6 recipient mice. In previous studies, recipient mice were lethally irradiated.16,24 This eliminates most lymphocytes, destroys the architecture of secondary lymphoid organs, and prevents the induction of a leukemia-specific immune response by the host. In contrast, sublethally irradiated mice (4.5 Gy) have a largely intact immune system from the recipient mouse with leukemia cells originated from donor bone marrow (S.M., unpublished results, 2008). CML developed in approximately 2 weeks, and animals died within 40 days (Figure 1A). Microscopy of blood smears and cytospin preparations showed an increased number of segmented granulocytes in the circulation (Figure 1B), and CML mice developed splenomegaly (Figure 1C), pulmonary hemorrhage, and granulocyte infiltrations in different organs. Control C57BL/6 mice receiving donor bone marrow transduced with an empty GFP vector did not develop CML. The frequency of GFP-transduced cells in these mice in the blood was low with 6.6% plus or minus 3.5% 19 days after transplantation and further decreased to 2.6% plus or minus 2.4% 34 days after transplantation.

Bone marrow, thymus, spleen, inguinal and mesenterial lymph nodes, and blood of CML mice were analyzed for the presence of...
BCR/ABL-GFP-expressing cells. BCR/ABL-expressing GR-1+ granulocytes were found in all organs analyzed, confirming the development of CML (Figure 1D). A preferential accumulation of high numbers of granulocytes was found in spleen and blood. Interestingly, BCR/ABL-expressing DCs were also present in all organs (Figure 1E). BCR/ABL expression was detected in approximately 50% of DCs in the thymus, in 6% of DCs in spleen and inguinal lymph nodes, and in 12% to 20% of DCs in bone marrow, blood, and mesenterial lymph nodes. In addition, BCR/ABL expression was also found in few CD8+ and CD4+ T cells, B cells, and NK cells (data not shown). These data suggest that CML in mice as well as CML in human patients developed from a pluripotent stem cell with the ability to differentiate to various cell lineages. However, the preferential differentiation to the myeloid lineage resulted in large granulocyte counts and a high percentage of BCR/ABL-expressing DCs.

BCR/ABL-expressing DCs in CML display a low maturation status

DCs have several functions in innate and adaptive immunity, such as activating NK cells, priming T-cell responses, or polarizing immune responses to either T helper 1- or 2-type reactions. To fulfill all these functions, DCs must undergo terminal differentiation and maturation. To determine the maturation status of BCR/ABL-expressing DCs in CML mice, we analyzed the expression of MHC class II molecules and the costimulatory molecule CD86 and CD80 on DCs in spleen, thymus, blood, inguinal and mesenterial lymph nodes, and bone marrow (Figure 2; and data not shown). We compared the fluorescence shifts of MHC class II molecules, CD86 and CD80 staining vs isotype staining. The x-fold increase in mean fluorescence intensity was calculated (Figure 2D). The expression of MHC class II molecules and CD86, but not CD80, on BCR/ABL-expressing DCs in the spleen and thymus was significantly reduced compared with DCs isolated from naive C57BL/6 mice (Figure 2D). Therefore, BCR/ABL-expressing DCs display a low maturation status in vivo. The expression level of MHC class II molecules and CD86 on DCs isolated from blood of naive C57BL/6 mice and from CML mice was very low, indicating that most DCs circulating in blood are phenotypically immature DCs independent of the expression of BCR/ABL.

BCR/ABL-expressing DCs in CML are functionally impaired

To determine the capacity of BCR/ABL-expressing DCs of CML mice to induce proliferation of antigen-specific naïve and memory CD8+ T cells, BCR/ABL-expressing DCs were isolated from CML mice. 3H-Thymidine incorporation of naive and memory p14 CD8+ T cells was measured after restimulation with titrated numbers of isolated BCR/ABL-expressing DCs and DCs from naive H8 transgenic mice. The expansion of naive and memory p14 CD8+ T cells after stimulation with BCR/ABL-expressing DCs was significantly lower compared with DCs isolated from H8
transgenic mice (Figure 3). Therefore, DCs isolated from CML mice are functionally impaired.

In vitro–generated BCR/ABL-expressing DCs display low maturation and are functionally impaired

To analyze the effect of BCR/ABL on the maturation of DCs and the functional consequences thereof, we generated BCR/ABL-expressing and control DCs from H8 bone marrow cells in vitro. Control DCs were transduced with an empty GFP vector. Immature DCs transduced with BCR/ABL-GFP or control DCs were phenotypically similar when analyzed after 10 days of in vitro culture in DC medium (Figure 4A). In contrast, similar to BCR/ABL-expressing DCs in vivo, expression of MHC class II molecules and CD86 was reduced on in vitro–generated and matured BCR/ABL-expressing DCs. This was independent of the maturation protocol used. After maturation with LPS, BCR/ABL-expressing DCs expressed significantly lower levels of MHC class II molecules and the costimulatory molecules CD86, but similar levels of CD80 and CD70 compared with control DCs (Figure 4B,D). BCR/ABL-expressing DCs matured with αCD40 antibody expressed lower levels of MHC class II molecules and CD86 compared with control DCs (Figure 4C). In contrast, expression of CD83 on BCR/ABL-expressing DCs was higher than in control DCs independently of the maturation status (Figure 4B,D).

We next tested the ability of in vitro–generated BCR/ABL-expressing DCs to induce proliferation of antigen-specific naive CD8+ T cells and to reexpand antigen-specific memory CTLs in vivo. These from H8 bone marrow cells in vitro–generated DCs expressed LCMV-gp33 on MHC class I molecules, which served as model leukemia antigen to stimulate transgenic gp33-specific p14 CD8 T cells. GFP+ DCs were sorted by FACS from in vitro–generated and LPS-matured BCR/ABL-expressing and control DCs (empty GFP vector–transduced). Stimulation with titrated numbers of BCR/ABL-expressing DCs induced only a very limited expansion of naive or memory p14 CD8+ T cells (Figure 5A). In contrast, control DCs efficiently stimulated proliferation of naive and memory p14 CD8+ T cells. Therefore, DCs isolated from CML mice and in vitro–generated BCR/ABL-expressing bone marrow derived DCs are phenotypically and functionally comparable.

To determine whether the reduced maturation of BCR/ABL-expressing DCs affects the induction of antigen-specific primary
the frequency of np396-specific IFN-γ-secreting CD8+ T cells was comparable in all groups (Figure 5C).

In the same experimental setting, CTLs were isolated 10 days after the challenge immunization to analyze functional differences in vitro. CTLs from mice pre-immunized with LPS-matured BCR/ABL-expressing DCs did not lyse peptide-pulsed target cells (Figure 5D). Thus, immunization with BCR/ABL-expressing DCs induces only a reduced frequency of specific CTLs in vivo compared with empty vector-transduced control DCs. A challenge immunization with nontransduced DCs did not improve the gp33-specific CTL frequency but induced a normal CTL response against a nonrelated control peptide.

Preferential homing of BCR/ABL-expressing DCs to the thymus

After capturing antigens in the periphery, DCs mature and migrate to secondary lymphoid organs.25 In addition, it has been shown that circulating DCs can be recruited to the thymus.26 We therefore analyzed the homing of BCR/ABL-expressing DCs to the spleen and thymus. First, the expression of the thymus homing markers VLA-4 (CD49d) and P-selectin ligand (PSGL-1, CD162)26 as well as the spleen homing marker CCR7 was determined on in vitro–generated and LPS-matured BCR/ABL-expressing DCs, control DCs (empty GFP vector–transduced), and nontransduced DCs. In addition, expression of homing markers that are of relevance in the homing of BCR/ABL+ myeloid progenitor cells was analyzed (CD44 and CXCR4, receptor for SDF-1). The expression of VLA-4, PSGL-1, and CD44 was increased on BCR/ABL-expressing DCs compared with control DCs (empty GFP vector–transduced); P, mice receiving only challenge immunization; LCMV-immune mice; and naive C57BL/6 mice. Symbols represent 51Cr release of gp33-pulsed target cells. Specific 51Cr release of unpulsed target cells was less than 10%. CTL activity is given as mean plus or minus SEM of 4 mice per group, except for LCMV-immune and naive C57BL/6 mice; ns indicates not significant.

Figure 5. Primary and secondary immune responses are reduced after immunization with BCR/ABL-expressing DCs. (A) Naive and memory p14 CD8+ T cells were restimulated in vitro for 4 days with titrated numbers of LPS-matured BCR/ABL-expressing (●) or control DCs (▲), empty GFP vector–transduced) generated from H8 bone marrow cells. 3H-Thymidine incorporation was measured during the last 14 hours of culture. Results are mean plus or minus SEM of 3 to 4 samples per group. Pooled data from 2 independent experiments are shown. (B) Naive C57BL/6 mice were immunized intravenously with 2 × 106 LPS-matured BCR/ABL-expressing (●) or control DCs (▲), empty GFP vector–transduced). Ten days later, the frequency and absolute number of gp33-specific CD8+ T cells in the spleen were determined by tetramer staining and by intracellular IFN-γ staining after in vitro restimulation with gp33. Results are mean plus or minus SEM of 4 mice per group. One representative experiment of 3 is shown. (C) C57BL/6 mice previously immunized with 2 × 106 LPS-matured BCR/ABL-expressing (●) or control DCs (▲), empty GFP vector–transduced) and naive C57BL/6 mice (square dotted) were immunized 14 days later with gp33- and np396-pulsed DCs generated from C57BL/6 mice. Ten days later, the frequency of gp33- and np396-specific CD8+ T cells was determined by intracellular IFN-γ staining after in vitro restimulation with gp33 and np396. (D) Splenocytes were isolated 10 days after challenge immunization and analyzed in a standard 51Cr-release assay. ● represents mice primarily immunized with BCR/ABL-expressing DCs; ▲, mice with control DCs (empty GFP vector–transduced); P, mice receiving only challenge immunization; ¶, LCMV-immune mice; and △, naive C57BL/6 mice. Symbols represent 51Cr release of gp33-pulsed target cells. Specific 51Cr release of unpulsed target cells was less than 10%. CTL activity is given as mean plus or minus SEM of 4 mice per group, except for LCMV-immune and naive C57BL/6 mice; ns indicates not significant.
control the progression of the disease. Various mechanisms may explain the escape of CML from the immunosurveillance, including the expression of FasL during blast crisis,27 the deletion of high-avidity CTLs specific for a leukemia-associated self-antigen,28 as well as the development of functional blocks in the caspase activation pathway in acute myeloid leukemia cells.29

We now analyzed the role of BCR/ABL-expressing DCs in the induction of specific CTLs in a murine CML model. The murine CML model recapitulates cardinal features of human CML, such as markedly elevated leukocyte counts with granulocyte predominance, splenomegaly, multiple organ involvement, and the expression of BCR/ABL in affected tissues.10,24,30

So far, the role of BCR/ABL-expressing DCs in vivo is poorly defined. In our CML model, 6% to 50% of all DCs expressed BCR/ABL. We found that in vitro–generated BCR/ABL-expressing DCs as well as BCR/ABL-expressing DCs in CML mice had a lower maturation status compared with control DCs. In addition, the immunization with BCR/ABL-expressing DCs resulted in impaired CTL responses. Importantly, CTL priming was only defective for antigens that are selectively expressed on BCR/ABL-expressing DCs, including leukemia-specific antigens. The presence of a large proportion of normal non–BCR/ABL-expressing DCs explains why CML patients and mice with CML-like disease develop normal CTL responses to infectious pathogens.

Myeloid DCs isolated directly from CML patients expressed lower levels of CD83, CD80, and CD40 compared with DCs from healthy donors.8 This is comparable with our results in the murine CML model. Although the isolated human CML DCs efficiently stimulated alloreactive T cells in vitro and induced cytokine production, they failed to induce proliferation of autologous T cells.8 In contrast, DCs generated from human peripheral blood mononuclear cells (PBMCs)7 or bone marrow12 of CML patients can be matured in vitro to express costimulatory molecules comparable with those DCs generated from healthy donors. Similarly, in vitro–generated and matured CML DCs from human PBMCs were able to induce CML-specific CTL responses in vitro and in vivo.15,31 The differences in maturation may be explained by different maturation protocols. Human CML DCs generated and matured with GM-CSF, IL-4, and GM-CSF, IL-4, and LPS led to a reduced maturation status of CML DCs.11 Together, these results indicate that the maturation of DCs in vivo in human CML patients and in CML mice is reduced. However, depending on the maturation protocol used, mature human DCs can be generated in vitro and probably used for immunotherapy.7,12

BCR/ABL can interfere with the maturation of DCs via different mechanisms. First, cytokines produced by BCR/ABL-expressing cells may inhibit DC maturation. The maturation of DCs can be inhibited efficiently by transforming growth factor-β (TGF-β) or IL-10 through blockade of the nuclear factor-κB pathway.32 It has been demonstrated that BCR/ABL expression dramatically up-regulates TGF-β signaling in transduced cells in vitro.33 Genes of the TGF-β signaling pathway are significantly up-regulated in CD34+ bone marrow cells of untreated CML patients.34 In addition, human CML cells express IL-10 mRNA35 and produce high levels of IL-10 in vitro.36 Therefore, the secretion of TGF-β and/or IL-10 by leukemic cells may contribute to a reduced maturation status of BCR/ABL-expressing DCs. Second, BCR/ABL activates the signal transducer and activator of transcription (STAT) pathway.34,37 BCR/ABL constitutively activates STAT3 through the Janus kinase (JAK) and mitogen-activated protein-kinase

Discussion

Although CTLs specific for BCR/ABL and other leukemia antigens can be detected in CML patients, eventually these CTLs fail to
DC differentiation, constitutive activation of STAT3 inhibits DC maturation. Interestingly, IL-10 signals also through STAT3.

Primary antigen-specific immune responses are induced exclusively in secondary lymphoid organs. During maturation, DCs acquire the capacity to migrate to secondary lymphoid organs and to prime naive T cells. Recently, it has been demonstrated that immature peptide-presenting DCs preferentially migrate to the thymus and induce clonal deletion of antigen-specific CD4+ T cells. The circulating DCs were recruited to the thymus through a 3-step adhesion cascade involving P-selectin, the integrin VLA-4, and chemoattractant signaling by pertussis toxin. We now found a 3-step adhesion cascade involving P-selectin, the integrin VLA-4, and various cytoskeletal elements important for normal thymus-homing molecules VLA-4 and PSGL-1 were higher whereas the expression of the spleen-homing molecule CCR7 was lower compared with control DCs. This resulted in an impaired homing of BCR/ABL-expressing DCs to the spleen. Instead, BCR/ABL-expressing DCs preferentially migrated to the thymus. Differences in the migration of DCs from CML patients vs DCs from healthy donors have been documented before in vitro. DCs generated from CD34+ PBMCs of CML patients had altered actin organization and reduced migratory ability to a chemokine gradient of MIP-1α. We documented differences in cell surface expression of PSGL-1, VLA-4 (β1-integrin), CD44, CXCR4, and CCR7. It has been shown that CD44 surface expression is regulated by BCR/ABL and is required for the homing of BCR/ABL-expressing leukemic cells. In contrast, other studies documented functional defects in β1 integrins. The BCR/ABL oncogene product p210 interacts with a variety of cytoskeletal elements important for normal integrin signaling. Moreover, BCR/ABL alters the chemotactic response of myeloid cells to SDF-1, not by regulating the expression of its receptor CXCR-4 but probably by inhibition of receptor function. The observed change of expression of β1-integrins and P-selectin ligands in the present study may be a consequence of impaired DC maturation because this phenotype was also found in immature non–BCR/ABL-expressing DCs. In addition, BCR/ABL may contribute to an impaired homing by interfering with integrin signaling. In summary, besides the reduced expression of costimulatory molecules on BCR/ABL-expressing DCs, the impaired migration to the thymus explains the inefficient CTL induction in vivo.

Central tolerance is maintained through the clonal deletion of high affinity T cells specific for antigens presented in the thymus. Central tolerance was originally thought to be restricted to proteins that are expressed by thymic medullary epithelial cells, or thymic DCs, or to proteins that enter the thymus through blood circulation.


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