

Flow Cytometry Analysis of CXCR4 Expression on Circulating CD34+ Cells in Patients with Primary Myelofibrosis

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Abstract

Background: Primary myelofibrosis (PMF) is a distinct clonal myeloproliferative neoplasm characterized by marrow fibrosis and the accumulation of CD34 positive hematopoietic progenitor cells (HPC) in peripheral blood and other sites. CXCR4 chemokine receptor 4 (CXCR4), and its ligand CXCL12 (stromal derived factor-1, SDF-1) play key roles in HPC mobilization. To investigate if the HPC increase in PMF may be related to CXCR4/CXCL12 interactions, CXCR4 expression was evaluated in 14 well characterized patients as well as in 8 normal controls. **Methods:** CXCR4 expression on HPC was measured by five-color flow cytometry, using monoclonal antibodies against CD34, CD133, CD45, CXCR4 and CD38. Positivity was determined by comparison to isotype control antibody staining. The effects of sample storage and red cell lysis procedures on CXCR4 staining were also evaluated. CXCR4 expression was also correlated with morphologic, molecular, and cytogenetic data.

Results: As expected, the percentages of peripheral-blood HPC staining positively for CD34 were significantly higher in the PMF patients (mean 1.4%, range 0.065-7.15) compared to the controls (mean 0.05%, range 0.02-0.089). The percentages of HPC staining positively for CXCR4 from PMF patients were similar to those found in HPC from the normal bone marrow specimens, but were significantly higher than on HPC in normal peripheral blood specimens. However, CXCR4 expression on HPC in PMF patients showed an inverse correlation with the percentages of circulating HPC. A comparison of CXCR4 expression on HPC in samples stored for 2 and 24 hours after blood draw also did not indicate any significant differences.

Conclusions: Expression of CXCR4 appears to be increased on peripheral blood CD34+ HPC in PMF patients relative to normal controls, but is similar to normal marrow based HPC. This suggests that the increase release of HPC in PMF patients may be affected by factors besides CXCR4/CXCL12 interactions. However, the inverse correlation of CXCR4 levels with HPC numbers in the blood of PMF patients also suggests that CXCR4/CXCL12 may still be playing a role in HPC release from the marrow.

Materials and Methods

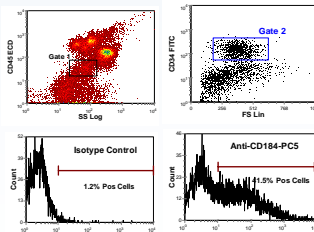
Case Selection: Peripheral blood samples from fourteen well characterized patients with PMF and 8 healthy controls, as well as 9 bone marrow samples without evident phenotyping abnormalities were included in the study.

Staining Procedure: White blood cells staining was performed in whole blood samples with sequential lysis with FACSllyse solution (Becton Dickinson, San Jose, CA), or after isolation with ammonium chloride lysing procedure, as previously described (Preobrazhensky, Bahler, Clinical Cytometry, 74B: 116, 2008) using anti-CD34-FITC (Becton Dickinson, San Jose, CA), anti-CD45-EC-D (Beckman Coulter, Immunotech, Marseille, France) and anti-CD184-PE-Cy5 or corresponding isotype antibody (BD Pharmingen) for 15 minutes at room temperature.

Flow Cytometry and Data Analysis: After washing with PBS and fixation with 0.5% formaldehyde solution stained samples were analyzed using FC 500 flow cytometer (Beckman Coulter, Miami, FL). Created listmode files were analyzed using CXP (Beckman Coulter, Miami, FL) and VenturiOne (Sheffield, UK) software. Populations of CD34-positive cells were identified with sequential gating using SS/CD45-EC-D and FSC/CD34-FITC histograms, as shown in Figure 1. Percentage of CD184 (CXCR4) positive cells was determined using negative threshold cutoff set up with isotype antibodies. Statistical data analysis was performed using Prism 4 GraphPad software (San Diego, CA).

Results

Figure 1. Sequential gating for detection of CXCR4 (CD184) positive, CD34 positive cells. Peripheral blood sample from PMF patient.



Introduction

Interaction of CXCR4 chemokine receptor 4 (CXCR4) with its ligand CXCL12 (stromal derived factor -1, SDF-1) retains CD34 positive hematopoietic progenitor cells (HPC) within marrow environment preventing their mobilization into peripheral blood. Since accumulation of HPC in peripheral blood is a characteristic symptom of primary myelofibrosis, a distinct clonal myeloproliferative neoplasm characterized by marrow fibrosis, we have investigated if accumulation of HPC in peripheral blood of PMF patients may be related to abnormal expression of CXCR4 receptor. Also, in previous studies (Shalekoff, Tiemessen; Clinical and Diagnostic Laboratory Immunology, 8, 432, 2001) it was shown that detection of CXCR4 receptor may be affected by duration of blood sample storage.

Results (Continued)

Figure 2. CD34 positive cell percentages are significantly increased in the peripheral blood of patients with primary myelofibrosis (P<0.0001)

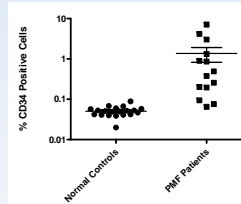


Figure 3. 24 hour storage of blood samples does not effect detection of CXCR4+ CD34+ cells

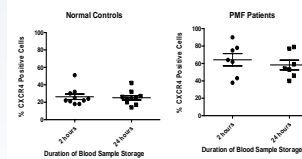


Figure 4. Red blood cell lysing procedure does not effect detection of CXCR4 positive, CD34 positive cells

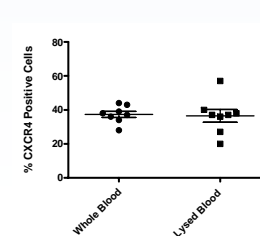


Figure 5. Percentages of CXCR4+/CD34+ cells in the peripheral blood of PMF patients are significantly (P<0.0001) higher compared to normal blood samples but are similar to the percentages in normal control bone marrows.

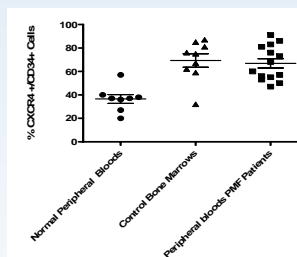
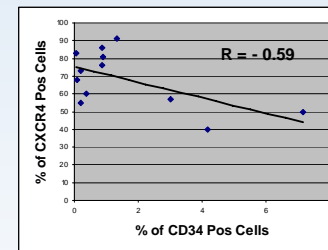


Figure 6. Negative correlation between percentages of CD34 positive cells and expression of CXCR4 in peripheral blood of PMF patients



Conclusions

- Blood sample storage for 24 hours and red blood cell lysis procedure do not effect measuring of CXCR4 expression on CD34 positive cells in peripheral blood.
- Percentage of CXCR4/CD34 positive cells in peripheral blood of patients with primary myelofibrosis is significantly (P< 0.0001) higher comparing to normal donors, and is similar to normal bone marrow.
- Level of CXCR4 expression in peripheral blood of patients with primary myelofibrosis inversely correlates with the percentage of circulating CD34 positive cells