incubated with a phycoerythrin (PE)-conjugated anti-CXCR4 monoclonal antibody (mAb) (12G5 clone, Pharmingen, San Diego, CA) and were costained with fluorescein isothiocyanate (FITC)-conjugated mAb directed against CD34, CD33, CD61, CD105, and glycophorin-A.8

CD34+ cells mobilized by chemotherapy and G-CSF homogeneously expressed CD33, CD13, and HLA-DR antigens, thus confirming previous observations (Figure, A).9 CXCR4 was expressed on the majority of circulating G-CSF–mobilized CD34+ cells (90%, range 86 to 93). CD34+ cells were next fractionated into a CD105+ and a CD105− population, as previously described; surprisingly, no differences in CXCR4 expression levels were detected when comparing the 2 cell subsets (> 90% CXCR4+; Figure, B). Coreceptor expression was also investigated during unilineage E, GM, and Mk differentiation pathways. CXCR4 expression was rapidly down-regulated during erythroid differentiation, and a negligible percentage of glycophorin-A+ cells were found to coexpress CXCR4 after 7 days of culture (< 2%). CXCR4 levels moderately decreased along the GM pathway; conversely, high levels of coreceptor were found in Mk cultures, and increasing CD61 staining intensities were associated with increasing CXCR4 levels (Figure, C).

Chemokine receptors belong to a family of 7 membrane-spanning molecules coupled to G-proteins, and their activation leads to calcium flux and intracellular and extracellular signaling; the surface expression of different chemokine receptors on leukocytes and the biological functions of their cognate ligands have been thoroughly investigated.9 CXCR4 is abundantly expressed on peripheral blood lymphocytes, monocytes, thrombocytes, pre-B cells, and dendritic cells; stromal cell-derived factor 1 (SDF-1), the natural ligand for CXCR4, initially characterized as a pre-B-cell growth-stimulating factor receptor, CXC chemokine receptor 4, on CD34+ human bone marrow cells is a phenotypic alteration for committed lymphoid progenitors.10

Circulating CD34+ cells contain a progenitor pool supporting prompt and durable trilineage hematopoietic reconstitution after myeloablative chemotherapy.8 G-CSF has been recently shown to upregulate CXCR4 expression on peripheral blood CD34+CD38− cells from normal donors10; interestingly, CXCR4 expression is crucial for the engraftment of human stem cells and for the population of NOD/SCID mice.11 The present investigation suggests that G-CSF might up-regulate CXCR4 expression on peripheral blood CD34+ cells, which differ profoundly from their bone marrow counterpart, where CXCR4 is specifically associated with lymphoid commitment; in addition, the bone marrow microenvironment might easily accomodate immigrating progenitor cells that express high levels of CXCR4 following G-CSF mobilization or stress conditions. The different functional significance of CXCR4 on G-CSF–mobilized cells compared with bone marrow CD34+ cells is further suggested by the finding of similar CXCR4 levels on CD34+CD105+ and CD34+CD105− cell subsets, which represent functionally distinct subpopulations of circulating progenitors.8,12 Following the observation that CXCR4 might be required for the migration of CD34+ progenitor cells from the fetal liver to the bone marrow microenvironment during fetal development,2 the dynamic alterations of CXCR4 expression on human CD34+ hematopoietic progenitors during growth factor-induced mobilization compared with steady-state hematopoiesis could confer an enhanced homing capacity and deserve further investigation.

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References


To the editor:

High incidence of symptomatic cytomegalovirus infection in multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation

Holmberg et al1 report on 268 cases of malignant diseases treated with autologous peripheral blood stem cell transplantation (APBSCT); an increased incidence of cytomegalovirus (CMV) disease was observed in patients who had autologous CD34+ selected cell infusion: 7 out of 31 cases (22.6%) developed CMV disease and 4 of them died. In univariate and multivariate analysis, only CD34 selection was significant for the development of CMV disease. Holmberg et al1 hypothesize that the delayed immune reconstitution observed after the infusion of CD34-selected cells increases susceptibility to CMV infection and disease.
In patients receiving allogeneic bone marrow transplantation, the incidence of CMV infection, defined as either evidence of any level of quantitative PP65 antigenemia or a positive blood or mouth culture, ranges from 42% to 69%. The incidence of CMV disease was up to 23% in patients with concomitant GvHD; posttransplant immunosuppression, concomitant GvHD, and immunosuppressive therapy to treat such complications may be partially responsible. Wingard et al reported a 45% incidence of CMV infection in a cohort of 143 autologous BMTs, which was similar to the infection rate observed in patients undergoing autologous transplant. Nevertheless, the incidence of CMV disease in autologous transplantation was only 2%. In a retrospective study of the EBMT group, the incidence of CMV pneumonia in autologous transplant was 0.8%.

The high incidence of CMV disease noticed by Holmberg et al had never been observed in previous series of patients undergoing autologous transplant; Holmberg et al indicate the CD34 cell selection as the only predictive factor of CMV infection and disease; nevertheless, the incidence of CMV disease in multiple myeloma (MM) patients was 5 of 32 (15%); 4 of 5 had received selected cell transplant.

At our institution, 106 CMV seropositive patients affected with hematological malignancies received APBSCT after massive chemotherapy. They were affected with MM (33 cases), non-Hodgkin lymphomas (NHL) (42 cases), Hodgkin disease (HD) (18 cases), acute nonlymphoid leukemia (ANLL) (8 cases), and chronic granulocytic leukemia (CGL) (5 cases). The median age was 42 years (range, 18 to 61). Sixty patients were males, and 46, females. All patients received unselected peripheral blood stem cells mobilised with G-CSF and cyclophosphamide (CY) given at the dose of 4000 mg/m². Conditioning regimen was as follows: patients with MM received melphalan and thiotepa (14 cases) or a modified BEM regimen including carbustine 600 mg/m², etoposide 30 mg/kg, and melphalan 200 mg/m² (19 cases); patients with HD or NHL had carbustine, etoposide, and CY in association (BCV). Patients with ANLL or CGL had busulphan (BU) and CY at standard dose. The weekly screening of PP65 antigenemia was not routinely performed because the low incidence of CMV disease usual in patients undergoing autologous bone marrow transplantation. CMV antigenemia was effected only in patients with fever unresponsive to a wide spectrum of antibiotic therapy, joint pain, weakness or diarrhea, cough, or unexplained dyspnea or leucopenia. Patients with positive CMV antigenemia received prompt treatment with ganciclovir at the dose of 5 mg/kg every 12 hours over 3 weeks; in these selected patients, the weekly screening of antigenemia was thereafter performed.

One episode of CMV symptomatic infection was noticed in 8 patients (7.5%). The median time to CMV reactivation ranged from day +15 to day +374. The main characteristics of patients who developed CMV infection are reported in the Table. Six out of 8 patients (75%) were affected with MM; the crude incidence of CMV infection in MM patients was 18%, and only 2 of 73 patients (2.7%) affected with NHL, HD, or ANLL had CMV complication (P = .011). In our experience, the diagnosis of MM seems to be predictive of CMV infection; we can hypothesize that the immunosuppressive status related to the underlying disease may have a role.

In previous studies, the incidence of CMV infection was never related to the diagnosis of MM probably because the low number of autologous bone marrow transplants performed before the use of peripheral blood stem cells become more general. Although many patients affected with MM receive APBSCT, there are not prospective studies exploring the incidence of CMV infection and disease in this subset of patients. We believe that, given the increased incidence of symptomatic CMV infection observed in our study, close monitoring and anti-CMV therapy are needed in patients with MM receiving autologous peripheral blood progenitor cell transplantation and particularly in those receiving CD34 selected cell transplant.

<table>
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<tr>
<th>UPN</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Day CMV infection diagnosed</th>
<th>Symptoms</th>
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<td>125</td>
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<td>+16</td>
<td>Fever, nausea, and vomiting</td>
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</tbody>
</table>

UPN, unique patient number; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; ANLL, acute nonlymphoid leukemia; PMN, number of positive cells on 200 000 leucocytes.

References