We investigated the role of adhesion molecule VLA-4 in CD34+ blood stem-cell mobilization. Therefore, we examined 20 patients with multiple sclerosis (MS) who were treated with the anti–VLA-4 antibody natalizumab. Treated patients had received a median number of 4 natalizumab infusions (range: 2-9 infusions). Blood samples were taken 4 weeks following the last infusion. With a median proportion of 7.6 CD34+ cells/μL (range: 2.2-30.4 cells/μL), these patients had a significantly higher \((P = .003)\) amount of circulating CD34+ cells compared with 5 healthy volunteers (median: 1.4/μL; range: 0.6-2.4/μL) and 5 untreated MS patients (median: 1.0/μL; range: 0.5-1.7/μL) \((P = .001)\). Serial measurements in 4 patients receiving their first natalizumab infusion showed a maximal significant increase in circulating CD34+ cells from 3.3/μL (range: 1.6-4.8/μL) to 10.4/μL (range: 7.5-12.04/μL) 72 hours following natalizumab infusion \((P = .001)\), including pluripotent cells in colony-forming assays. This mobilizing ability of natalizumab might be useful for patients with poor response to granulocyte colony-stimulating factor (G-CSF)–based protocols. (Blood. 2008;111:3893-3895) © 2008 by The American Society of Hematology

## Introduction

Essential parts of the functional repertoire of hematopoietic stem cells (HSCs) such as migration, circulation, and proliferation depend on adhesive interactions between membrane-bound receptors and their respective ligands on the various components of the extracellular matrix (ECM).1,3 Very late activation antigen 4 (VLA-4), the α-4 submember of the β-1 integrin family expressed on all mononuclear hematopoietic cells, is an adhesion molecule involved in mobilization and migration of CD34+ cells. The cognate receptor for VLA-4 is the vascular cell adhesion molecule-1 (VCAM-1). In addition, VLA-4 binds to osteopontin and connecting segment 1 (CS-1).4-6 Bone marrow–derived CD34+ cells express VLA-4 at a higher level and different functional state compared with circulating CD34+ cells, suggesting that the ability to circulate is related to the expression level and avidity of VLA-4.7

Natalizumab is a recombinant humanized IgG4 monoclonal antibody that binds to the α-4 subunit of the α4-β1 integrin and inhibits the α4-β1–mediated adhesions of leukocytes to their counter-receptors. It has been introduced for the treatment of autoimmune diseases such as multiple sclerosis (MS) and Crohn disease. Having bound to the leukocytes, natalizumab prevents their transmigration through the blood-brain and the endothelial cell barrier leading to a diminished inflammatory activity in the target organ.8,9 Based on these observations, we examined the levels of CD34+ cells in the peripheral blood (PB) of patients with MS during natalizumab treatment.

## Methods

A total of 20 patients with relapsing-remitting MS (15 females/5 males; median age: 32 years; range: 21-43 years) were included into the study after informed consent following the guidelines of our local ethical committee of Heinrich Heine-University and in accordance with the Declaration of Helsinki. The patients were treated with natalizumab on an outpatient basis of monthly visits at the Department of Neurology, at Heinrich Heine University Düsseldorf.

The median time from first diagnosis was 36 months (range: 1-50 months); previous MS treatment was interferon beta-1a/b (n = 16), glatirameracetat (n = 5), and mitoxantrone (n = 5). In all patients, blood counts, liver and kidney function tests, as well as the findings on physical examination were normal. None of the patients took concomitant medication during natalizumab treatment. At the time of examination, the patients had received a median number of 4 natalizumab infusions (range: 2-9 infusions) at 300 mg intravenously each month.

Blood samples were taken before and 1 hour after natalizumab infusion. In 4 patients who received their first natalizumab infusion, a sequential measurement was performed before, 1 hour, 24 hours, 48 hours, 72 hours, and 1 month thereafter.

Peripheral blood samples from 5 healthy volunteers (4 females/1 male) and 5 untreated MS patients (4 females/1 male) served as controls.

In principal, 40 mL ethylenediaminetetraacetic acid–anticoagulated venous blood samples were obtained for blood cell counts, CD34+ cell count, and immunophenotype fluorescence-activated cell sorter (FACS) analysis.

CD34+ cells were counted according to a protocol of the International Society for Hematotherapy and Graft Engineering (ISHAGE) using a dual-color FACS analysis on a Becton Dickinson flow cytometer (BD FACSCalibur system; BD Bioscience, San Jose, CA). Colony-forming units were determined by plating \(4 \times 10^5\) mononuclear cells (MNCs) in 24-well plates as described before,10 and granulocyte-macrophage colony-forming units (CFU-GMs), granulocyte-erythroid-macrophage-megakaryocyte colony-forming units (CFU-GEMMs), and erythroid burst-forming units (BFU-Es) were counted using an inverted microscope.

Statistical analysis were performed by Mann-Whitney and paired Student t test using SPSS statistical software (Chicago, IL).
Results and discussion

The major hematologic finding relates to the number of circulating CD34+ cells. With a median proportion of 0.07% CD34+ cells (range: 0.03-0.3 cells) and a corresponding median concentration of 7.6 CD34+ cells/μL blood (range: 2.2-32.4/μL), the MS patients receiving natalizumab showed significantly higher CD34+ cell numbers compared with 5 healthy volunteers (0.03% CD34+ cells [range: 0.01-0.03 cells]; 1.4 CD34+ cells/μL blood [range: 0.6-2.5/μL]; P = .003) and 5 untreated MS patients (0.02% CD34+ cells [range: 0.01-0.02 cells]; 1.06 CD34+ cells/μL blood [range: 0.57-1.68/μL]; P = .001) (Figure 1A). We also performed a second CD34+ cell measurement in 12 patients 1 hour following the end of the natalizumab infusion without noting a significant change in the number of circulating CD34+ cells (before infusion: 5.6 CD34+ cell/μL [range: 2.08-11.76/μL]; 1 hour after infusion: 5.6 CD34+ cells/μL [range: 1.76-30.9/μL]).

To get a better idea on the kinetics of the natalizumab-induced mobilization, we measured the concentration of CD34+ cells on 3 consecutive days in the PB of 4 patients who received the first cycle of natalizumab. A gradual increase of circulating CD34+ cells was observed (Table 1).

Table 1. Peripheral blood myeloid progenitor cell–mobilizing effects of 300 mg intravenous natalizumab in 4 MS patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before infusion</th>
<th>1 h after infusion</th>
<th>24 h after infusion</th>
<th>48 h after infusion</th>
<th>72 h after infusion</th>
<th>Median BFU-E colonies, no.</th>
<th>Median CFU-G/M/GM colonies, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>100*</td>
<td>88</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>15.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4</td>
<td>34</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>44</td>
<td>0.009</td>
</tr>
<tr>
<td>Patient 3</td>
<td>10</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>15.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Patient 4</td>
<td>50</td>
<td>100</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>15.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Median</td>
<td>7</td>
<td>67</td>
<td>100*</td>
<td>100*</td>
<td>100*</td>
<td>15.5</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Colony-forming activity was determined after plating 4 × 10^5 PB mononuclear cells/24-well plate before and 1, 24, 48, and 72 hours following the initial infusion of natalizumab. Colony-forming activity was determined after plating 4 × 10^5 PB mononuclear cells/24-well plate before and 1, 24, 48, and 72 hours following the initial infusion of natalizumab. Colony-forming activity was determined after plating 4 × 10^5 PB mononuclear cells/24-well plate before and 1, 24, 48, and 72 hours following the initial infusion of natalizumab.
noted with a maximal median concentration of 10.4 CD34+ cells/µL 72 hours after cessation of the infusion. Significant increases in circulating CD34+ cells were observed 24 hours (P = .016), 48 hours (P = .001), 72 hours (P = .001) and 1 month (P = .003) following natalizumab (Figure 1B).

In vitro colony-forming unit assays demonstrated that a single dose of natalizumab increased the levels of circulating myeloid and erythroid progenitor cells (Table 1). Significant increase of colony-forming activity was observed at 24, 48, and 72 hours following natalizumab infusion. The greatest relative increase was observed in the number of circulating BFU-Es assayed at 24 and 48 hours after a single dose of natalizumab.

Therefore, blocking the VLA-4-mediated interactions of a CD34+ cell with their respective binding partners of the ECM and endothelial cells leads to a relatively rapid egress of CD34+ cells from the marrow cavity into the PB. But it is also conceivable that there is no true mobilizing effect, but an antibody-associated inhibition of homing once the CD34+ cells enter the peripheral blood. This view is in line with the current model proposed for lymphocyte trafficking.11,12 It was interesting to note that the concentration of CD34+ cells observed 4 weeks later on the occasion of the second natalizumab infusion did not differ significantly from the concentration observed 72 hours following the first infusion. Thus, despite the estimated half-life of natalizumab of 11 (± 4) days, the mobilizing stimulus of the antibody was still effective.

For CD34+ subset analysis, we performed dual color phenotyping. The majority (> 85%) of circulating CD34+ cells belonged to the subset of more committed progenitors coexpressing CD38.

In the entire group of 20 patients, the median proportion of circulating CD34+ cells coexpressing CD49d was 33% (range: 0%-100%). In 15 patients who had received more than 5 consecutive infusions of natalizumab, the proportion of CD34+CD49d+ cells was 11% (range: 0%-33%), whereas in 5 patients with fewer than 5 infusions, the median proportion was 67.5% (range: 0%-100%). This finding indicates an inverse relationship between the duration of treatment with natalizumab and the proportion of CD34+CD49+ cells and suggests that patients with greater amounts of double-positive cells might need a higher dose of antibody.

Monoclonal antibodies directed against VLA-4 were already used for HSC mobilization in primates and mice13-15. Our study demonstrates that the results obtained in these animal models are conferrable with the findings that we made in our patients. In that respect, it is worth noting that the anti–VLA-4–exposed HSCs were capable of reconstituting hematopoiesis in recipient mice following myeloablative conditioning. Furthermore, the combination of anti-α4 antibodies and G-CSF was found to exert an even greater mobilizing effect in comparison with anti-α4 or G-CSF treatment alone, which implies a synergy between cytokine-mediated effects on adhesion molecules and their direct blocking. This finding could be advantageous for patients with hematologic malignancies responding poorly to G-CSF–based mobilization protocols.

Authorship

Contribution: F.Z. and R.H. designed the study, analyzed data, and wrote the paper; D.T. collected patient samples and performed experiments; V.K. analyzed data and performed experiments; H.-P.H. and B.K. wrote the paper and analyzed data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

The monoclonal anti–VLA-4 antibody natalizumab mobilizes CD34+ hematopoietic progenitor cells in humans

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