The monoclonal anti-VLA4 Antibody Natalizumab mobilizes CD34+ Hematopoietic Progenitor Cells in Humans

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Short title: VLA-4 blockage mobilizes CD34+ cells
Abstract

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). Blood samples were taken four weeks following the last infusion. With a median proportion of 7.6 CD34+ cells/µl (range 2.2-30.4) these patients had a significantly higher (p=0.003) amount of circulating CD34+ cells compared to 5 healthy volunteers (median 1.4, range 0.6-2.4) and 5 untreated MS patients (median 1.0, range 0.5-1.7) (p=0.001). Serial measurements in four patients receiving their first Natalizumab infusion showed a maximal significant increase in circulating CD34+ cells from 3.3/µl (range 1.6-4.8) to 10.4/µl (range 7.5-12.04) 72 hours following Natalizumab infusion (p=0.0005), representing pluripotent progenitors in colony-forming assays. These mobilizing ability of Natalizumab might be useful for patients with poor response to G-CSF based protocols.

Introduction

Essential parts of the functional repertoire of hematopoietic stem cells (HSC) 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)\(^1\)\(^2\)\(^3\). Very late activation antigen 4 (VLA-4), the \(\alpha\)-4 submember of the \(\beta\)-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)\(^4\)\(^5\)\(^6\). Bone marrow derived CD34+ cells express VLA-4 at a higher level and different functional state compared to 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 \(\alpha\)-4 subunit of the \(\alpha\)4-\(\beta\)1 integrin and inhibits the \(\alpha\)-4 mediated adhesions of leukocytes to their counter receptors. It has been introduced for the treatment of autoimmune diseases such as MS and Crohn’s 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.

Patients and Methods

A total of 20 patients with relapsing-remitting MS (15 females/ 5 males; median age 32 years, range: 21-43) were included into the study after informed consent following the guidelines of our local ethical committee of Heinrich Heine-University. The patients were treated with Natalizumab on 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), 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) at 300mg i.v. monthly. Blood samples were taken before and 1h after Natalizumab infusion. In 4 patients who received their first natalizumab infusion a sequential measurement was performed before, 1h, 24h, 48h, 72h 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, 40ml of 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 colour FACS analysis on a Becton Dickinson flow cytometer (BD FACSCaliburTM system, BD Bioscience). Colony-forming units were determined by plating 4 x 10^5 MNC in 24-well plates as described before^{10} and granulocyte-marrow colony-forming units (CFU-GMs), granulocyte-erythroid-marrow-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’s t test using SPSS statistical software.

**Results and Discussion**

The major hematological finding relates to the number of circulating CD34+ cells. With a median proportion of 0.07% CD34+ cells (range: 0.03 - 0.3) and a corresponding median concentration of 7.6 CD34+ cells/µl blood (range: 2.2 - 32.4) the MS patients receiving Natalizumab showed significantly higher CD34+ cell numbers compared to 5 healthy volunteers (0.03% CD34+ cells [range: 0.01-0.03]; 1.4 CD34+ cells/µl blood [range: 0.6 - 2.5]; (p=0.003)) and 5 untreated MS patients (0.02% CD34+ cells [range: 0.01-0.02]; 1.06 CD34+ cells/µl blood [range: 0.57 - 1.68]; (p=0.001)) (Figure 1a). We also performed a second CD34+ cell measurement in twelve patients one 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]; 1h after Infusion: 5.6 CD34+ cells/µl [range: 1.76- 30.9]).

In order to get a better idea on the kinetics of the Natalizumab induced mobilization we measured the concentration of CD34+ cells on three consecutive days in the PB of four patients who received the first cycle of Natalizumab. A gradual increase of circulating CD34+ cells was noted with a maximal median concentration of 10.4 CD34+ cells/µl following 72 hours after cessation of the infusion. Significant increases in circulating CD34+ cells were observed 24h (p=0.016), 48h (p=0.0006), 72h (p=0.0005) and 1 month (p=0.0029) 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 24h, 48h and 72h 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\textsuperscript{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 colour 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 less 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-positive 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 mice\textsuperscript{13-15}. 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 HSC were capable of reconstituting haematopoiesis 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 to 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 hematological malignancies responding poorly to G-CSF based mobilization protocols.

**Authorship:**

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

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

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Reference List


Table 1: Peripheral blood myeloid progenitor cell mobilizing effects of Natalizumab 300 mg iv. in 4 MS patients. Colony-forming activity was determined after plating $4 \times 10^5$ MNC in 24-well plates before and 1, 24, 48 and 72 hours following the initial infusion of Natalizumab.

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* Indicates, that in these particular culture plates at least 100 single BFU-Es were discernible. In fact the plating efficacy was unexpectedly higher, so that some of the BFU-E growth was not clearly attributable to individual colonies.

# Statistical significance expressed as p-value compared with baseline (time = before) using the two-sided paired t-test.

Figure legends:

Figure 1a: CD34+ cells/µl in the peripheral blood of 20 MS patients receiving Natalizumab 300 mg i.v. monthly. Natalizumab patients were examined 28 days following the last infusion. For comparison 5 healthy volunteers and 5 untreated MS patients were analysed too. (Diamonds indicate the individual amount of CD34+ cells per patient; bars indicate the median number of CD34+ cells/µl in each group, brackets indicate statistical significance between groups using Mann Whitney test)

Figure 1b: Sequential analysis of Natalizumab induced mobilization of CD34+ cells into peripheral blood of 4 MS patients before and 1h, 24h, 48h, 72h and 1 month following infusion. MS patients received a single intravenous infusion of 300mg Natalizumab, peripheral venous blood was taken at time intervals after drug administration, FACS analysis were performed to determine the concentration of CD34+ cells. Brackets indicate statistical significance expressed as p-value compared with baseline (time = before) using two-sided paired t-test.
Figure 1a:

![Graph showing CD34+ cells/µl in peripheral blood for Natalizumab treated MS patients, healthy volunteers, and untreated MS patients. The graph includes statistical significance levels (p=0.003 and p=0.001) for comparisons between groups.]

Figure 1b:

![Graph showing CD34+ cells/µl in peripheral blood over time after Natalizumab infusion for four patients and the median. Statistical significance levels are indicated for each comparison point.]

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