Natalizumab and Impedance of the Homing of CD34+ Hematopoietic Progenitors

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Background: Treatment with natalizumab, an antibody blocking the α4-integrin, is associated with increased numbers of circulating CD34+ cells in the peripheral blood of patients with multiple sclerosis.

Objective: To determine whether natalizumab mobilizes CD34+ cells from or inhibits homing to the bone marrow (BM).

Design: Fifty-two patients with relapsing-remitting multiple sclerosis treated with natalizumab were included. Flow cytometric analyses; polymerase chain reaction assays for JC (John Cunningham) virus DNA detection; and adhesion, migration, and apoptosis assays of immunomagnetically enriched peripheral blood and BM CD34+ cells were conducted. A comparison was made with CD34+ cells from granulocyte colony-stimulating factor–mobilized peripheral blood or steady-state BM of age- and sex-matched healthy donors.

Results: We found adhesion and migration of peripheral blood–derived CD34+ cells to be reduced. In BM aspirates from natalizumab-treated patients, the cellularity, the proportion, and the adhesive capacity of CD34+ cells were normal. The JC virus was undetectable.

Conclusions: Natalizumab mediates an increase in circulating CD34+ cells by interfering with homing to the BM. Thus, CD34+ cells appear unlikely to represent a source mobilizing JC virus out of the BM in patients treated with natalizumab.

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Natalizumab (NAT), an approved treatment for relapsing-remitting multiple sclerosis (MS) (co-marketed by Biogen Idec and Elan as Tysabri), is a recombinant humanized monoclonal IgG4 antibody directed against the α4-subunit of VLA-4. We and others1-3 have shown that management of patients with relapsing-remitting MS treated with NAT leads to a rapid and sustained increase in circulating CD34+ cells. A recent study4 in a small cohort of 8 MS patients was suggestive of mobilization of CD34+ cells out of the bone marrow (BM).

However, increased numbers of CD34+ cells in the peripheral blood (PB) could alternatively be the result of impaired homing of CD34+ cells to, rather than true mobilization of, the BM in patients treated with NAT. We addressed this by assessing the adhesive and migratory capacity of CD34+ cells in PB relevant for transendothelial egress in a cohort of MS patients treated with NAT. In addition, we studied the effect of NAT on coexpression of CD49d (α4-subunit of VLA-4) on and adhesive properties of BM CD34+ cells, the BM cellularity, the proportion of BM CD34+ cells, and JC (John Cunningham) virus DNA expression in BM aspirates of 9 MS patients and 8 healthy age- and sex-matched control patients. From our results, we conclude that CD34+ cells exposed to NAT in the PB are functionally impaired, whereas the CD34+ cells in the BM appear to be only marginally affected by the antibody. This argues against the use of NAT as an agent to mobilize hematopoietic progenitor cells. Furthermore, patients with relapsing-remitting MS appear not to be at risk of exhaustion of the CD34+ hematopoietic progenitor pool as a potential adverse effect of long-term treatment. In addition, the present findings add to recent data arguing against a BM release hypothesis in the pathogenesis of progressive multifocal leukoencephalopathy observed in MS patients treated with NAT.3,5-7

Methods

Fifty-two patients with relapsing-remitting MS, receiving NAT once every month (median...
[range] number of infusions, 7 [2-33]), were included. Informed consent was obtained following the guidelines of the local ethics committee of Heinrich-Heine-University (Düsseldorf, Germany) in accordance with the Declaration of Helsinki. Before NAT infusion, 50 mL of EDTA-anticoagulated PB was collected.

Peripheral blood mononuclear cells were obtained by density centrifugation using density gradient media (lymphoprep; Technoclone GmbH, Vienna, Austria) as previously described. The CD34+ cells were separated by midiMACS magnetic separation system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) as previously published. The CD34+ cells purified from leukapheresis products of 24 healthy donors, who had received a 4- to 5-day course of granulocyte colony-stimulating factor (G-CSF)–mobilized CD34+ cells from healthy control (HC) patients are shown. Flow cytometric analysis of the cell cycle and cellular DNA content as well as of the phosphatidylserine expression were performed as described previously to detect apoptotic CD34+ cells. Statistical analysis was performed using SPSS statistical software, version 18 (IBM, Armonk, New York).

Circulating CD34+ cells of patients treated with NAT were more mature, showed impaired adhesive and migratory properties, and showed no increase in the rate of apoptosis. Circulating CD34+ cells of MS patients treated with NAT (NAT group) were compared with those of HCs mobilized by G-CSF (G-CSF group). The phenotype of circulating CD34+ cells was assessed by multicolor immunofluorescence analysis. As expected, the median (SEM) coexpression rate of CD49d was reduced in the NAT group (NAT, 45.2% [5.4%] vs G-CSF, 40.0% [13.3%]; P=.007) (Figure 1). Whereas median (SEM) CD184 (CXCR4) expression was similar between groups (NAT, 45.2% [5.4%] vs G-CSF, 40.0% [13.3%]), expression of CD133 was reduced in the NAT group (NAT, 45.2% [5.4%] vs G-CSF, 40.0% [13.3%]; P<.001), suggestive of a more mature subpopulation (Figure 1). In line with this finding, the ability of NAT-exposed circulating CD34+ cells to initiate long-term cultures was poor (data not shown). The median (range) proportion of adhering CD34+ cells (NAT [n=8], 15.6% [12.9%-

![Figure 1](https://example.com/figure1.png)
21.6%; G-CSF [n=9], 25.5% [16.4%-35.3%]; P = .003) and the migratory capacity of circulating CD34+ cells (NAT, 1% [0.7%]; G-CSF, 32.3% [5.1%]; P = .003) (Figure 2) were reduced in the NAT group compared with those of HCs. The labeling of apoptotic cells showed no increase in the proportion of apoptotic CD34+ cells in the NAT group (NAT [n=5], 4.5% [1.7%]; G-CSF [n=4], 8.1% [1.4%]), confirmed by a small proportion (1.5% [1.7%]) of apoptotic cells in DNA content analysis of CD34+ cells of 5 patients treated with NAT.

The BM cellularity and the proportion of BM CD34+ cells are not affected by treatment with NAT. The CD34+ cells derived from the BM of 9 MS patients treated with NAT were compared with those of 7 HCs. The median (SEM) CXCR4-expression rate (NAT, 62.1% [5.1%]; HC, 54.8% [5.2%]) and CD133-expression rate (NAT, 73.3% [5.3%]; HC, 67.8% [3.8%]) were similar between the 2 groups. Median coexpression of CD49d was lower in the NAT group (NAT [n=8], 0.4% [0.1%]; HC [n=7], 1.7% [0.6%]; P = .02) (Figure 2).

The JC virus DNA was not detectable in BM CD34+ cells of 9 MS patients treated with NAT. This was demonstrated by polymerase chain reaction assay.

**COMMENT**

Treatment of MS patients with NAT leads to a rapid and sustained increase in circulating CD34+ cells in the PB.1-3 Here, we present data showing that this increase is the result of impaired homing of CD34+ cells to, rather than...
true mobilization of, the BM. Therefore, we believe that the increased concentration of CD34+ cells in the PB is the result of a gradual accumulation of cells unable to return to homing sites, such as the BM. The following data support this view: (1) circulating CD34+ cells of MS patients treated with NAT revealed reduced adhesive and migratory properties; thus, transendothelial egress relevant for homing is most likely to be impaired; (2) adhesive properties of BM CD34+ cells are not impaired, whereas migratory capacity is reduced; thus, BM CD34+ cells are less likely to get mobilized out of the BM; (3) the BM cellularity and the proportion of BM CD34+ cells are not affected by treatment with NAT; thus, NAT treatment appears to have only a marginal influence on BM in our cohort.

These findings have implications for the clinical use of NAT in the treatment of MS, as well as for NAT as a potential agent to mobilize hematopoietic progenitor cells in hematology. First, coexpression analysis demonstrates that circulating CD34+ cells of MS patients treated with NAT are more mature and therefore have a poor ability to initiate long-term cultures. Thus, NAT is not a promising candidate to mobilize hematopoietic progenitors in hematology. Second, our findings argue against an antibody-induced exhaustion of the CD34+ progenitor pool as a potential adverse effect of long-term application of NAT in MS patients. Third, the BM has been hypothesized to be a relevant reservoir for the JC virus and the cases of progressive multifocal leukoencephalopathy observed in MS patients treated with NAT.3,6,7 We were unable to detect the JC virus in BM CD34+ cells within this study. We cannot exclude a sampling error in only 9 MS patients. However, the fact that NAT treatment impairs homing strongly argues against a hypothesis claiming NAT induced mobilized JC virus–infected BM cells to be involved in progressive multifocal leukoencephalopathy pathogenesis. This finding is in line with negative JC virus DNA findings in circulating CD34+ cells in a recently published study.3 Thus, other mechanisms of JC virus reactivation and central nervous system infection should be addressed to understand NAT-associated cases of progressive multifocal leukoencephalopathy.

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