Natalizumab and Impedance of the Homing of CD34⁺ Hematopoietic Progenitors

Christian Saure, MD; Clemens Warnke, MD; Fabian Zohren, MD; Thomas Schroeder, MD; Ingmar Bruns, MD; Ron P. Cadeddu; Christian Weigelt, MD; Ute Fischer, MD; Guido Kobbe, MD; Hans-Peter Hartung, MD; Ortwin Adams, MD; Bernd C. Kieseier, MD; Rainer Haas, MD

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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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 [n=17], 49.6% [7.4%]; G-CSF, 99.4% [2.2%]; 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%]), coexpression of CD133 was reduced in the NAT group (NAT, 37.5% [1.7%]; G-CSF, 54.3% [1.9%]; 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%–

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Figure 1. Expression of CD49d, CD184, and CD133 on CD34+ cells. A, Representative fluorescence-activated cell-sorting dot plots showing the expression of CD49d on CD34+ cells from the peripheral blood (PB) and bone marrow (BM) of patients with multiple sclerosis treated with natalizumab (NAT). For comparison, dot plots of granulocyte colony-stimulating factor (G-CSF)-mobilized CD34+ cells and BM-derived CD34+ cells of healthy control (HC) patients are shown. B, Percentage of CD34+ cells expressing CD49d, CD184, and CD133 as measured by fluorescence-activated cell sorting. Gray and black bars on the left side represent G-CSF–mobilized CD34+ cells from 5 HCs and PB-derived CD34+ cells from 17 patients with multiple sclerosis receiving NAT therapy. Gray and black bars on the right side represent BM-derived CD34+ cells from 7 HCs and from 9 patients with multiple sclerosis treated with NAT. Error bars indicate mean (SEM). FITC indicates fluorescein isothiocyanate; PE, phycoerythrin.

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RESULTS

Of CD34+ separates were performed as previously published.3 Flow cytometric analysis of the cell cycle and cellular DNA content as well as of the phosphatidylserine expression were performed as described previously16,17 to detect apoptotic CD34+ cells. Statistical analysis was performed using SPSS statistical software, version 18 (IBM, Armonk, New York).
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% [5.11%]; 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, 98.3% [0.8%], mean fluorescent intensity, 66.1% [8.5%]; HC, 99.7% [0.2%], mean fluorescent intensity, 97.4% [34.3%]; P = .03) (Figure 1). There was no significant difference in the median (SEM) BM cellularity between 9 MS patients and 6 HCs (NAT, 16,000 [3000] mononuclear cells/µL; HC, 9000 [2100] mononuclear cells/µL; P = .15). The median (SEM) proportion of CD34+ cells (2.03% [0.42%]) as measured by fluorescence-activated cell sorting analysis and of blasts (1% [0.2%]) as obtained by cytomorphologic analysis were within the normal range (Figure 3).

The adhesive abilities of BM CD34+ cells of NAT-treated patients remain unaffected, whereas the migratory properties are impaired. We observed no significant difference in the median (SEM) adhesive abilities of CD34+ cells obtained from BM between the 2 groups (NAT [n=9], 28.2% [0.7%]; HC [n=6], 23.3% [1.5%]), whereas the median migratory capacity was significantly reduced in the NAT group (NAT [n=9], 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. 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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Correspondence: Christian Saure, MD, Department of Hematology, Oncology, and Clinical Immunology, University of Dusseldorf, Moorensstr. 5, 40225 Dusseldorf, Germany (Christian.Saure@med.uni-duesseldorf.de).

Author Contributions: Study concept and design: Saure, Zohren, Schroeder, Kobbe, and Haas. Acquisition of data: Saure, Zohren, Schroeder, Bruns, Cadeddu, Weigelt, Fischer, Hartung, and Adams. Analysis and interpretation of data: Saure, Warnke, Zohren, Schroeder, Cadeddu, Fischer, Kobbe, Adams, Kieseier, and Haas. Drafting of the manuscript: Saure, Warnke, Schroeder, and Fischer. Critical revision of the manuscript for important intellectual content: Warnke, Zohren, Schroeder, Bruns, Cadeddu, Weigelt, Kobbe, Hartung, Adams, Kieseier, and Haas. Statistical analysis: Zohren. Obtained funding: Saure and Schroeder. Administrative, technical, and material support: Saure, Bruns, Cadeddu, Weigelt, Fischer, Kobbe, and Kieseier. Study supervision: Zohren, Weigelt, Kobbe, Hartung, and Haas.

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