Natalizumab Effects on Immune Cell Responses in Multiple Sclerosis

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Objective: Our objective was to study in vivo biological effects of natalizumab on immune cell phenotype and function in multiple sclerosis (MS) patients.

Methods: Blood was obtained before and after serial monthly natalizumab infusions to track functional expression of VLA-4 and migratory capacity of immune cells. The impact of infusion on activation thresholds of immune cells was evaluated.

Results: Preinfusion VLA-4 expression differed across immune cell subsets. Natalizumab significantly, albeit partially, diminished VLA-4 expression on circulating immune cells. Cell subsets were differentially affected. Treatment significantly decreased migratory capacity of immune cells, correlating well with changes in VLA-4 expression. Effects of a single dose were not saturating and did not persist through the monthly dose interval. Infusion effect varied across patients but was remarkably stable in individual patients, over multiple infusions. Treatment significantly modulated proliferative responses of immune cells.

Interpretation: To our knowledge, we provide first proof of concept that natalizumab diminishes migratory capacity of immune cells. Our prospective study further shows that effects of therapy likely (1) differ for distinct immune cell subsets, (2) are not sustained over current dose interval, (3) have unique profiles in individual patients, and (4) include modulation of activation threshold of immune cells. Monitoring these parameters could be relevant to ongoing safety and efficacy considerations.

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Natalizumab (Tysabri, Biogen Idec Inc., Cambridge, MA and Elan Pharmaceuticals, Inc., San Diego, CA), a humanized monoclonal antibody directed against the α4-integrin of the adhesion molecule VLA-4, was approved by the Food and Drug Administration in November 2004 for treatment of relapsing-remitting MS based on promising phase II[1] and 1-year efficacy data from two phase III clinical trials.[2] The drug was voluntarily withdrawn and the trials stopped in February 2005 when two treated MS patients (and later a Crohn's disease patient) were found to develop progressive multifocal leukoencephalopathy (PML), a rare and potentially fatal central nervous system (CNS) reactivation of the JC virus.[3,4]

The strong hope in the MS community that natalizumab will be reintroduced is mitigated by the need to identify who may benefit most from therapy and who may be at particular risk for complications such as PML. These outcomes are likely to relate to the effects of the drug on particular immune cell subsets. Although inhibition of T-cell trafficking has been thought to underlie both drug efficacy and toxicity,[5,6] the impact on other cell subsets may be quite relevant.[7] Furthermore, animal model studies suggest that the effects of VLA-4 inhibition may extend beyond trafficking and also impact immune cell activation.[8,9] Nonetheless, surprisingly little is known about the in vivo biological effects of natalizumab on immune cell responses in treated patients.

Here, we prospectively studied the impact of serial natalizumab infusions on immune cell phenotype and functional responses in MS patients. We provide the first biological proof of concept that natalizumab diminishes functional α4-integrin expression and migratory capacity of immune cells and identify several unexpected response parameters that may prove to be relevant to both the efficacy and safety profile of natalizumab in patients.

Subjects and Methods

Patients

Patients with clinically definite[10] relapsing-remitting MS who were transitioning into the open-label phase of the na-

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talizumab clinical trial program, and normal volunteers were recruited after institutional review board approval at the Hopital Charles-LeMoyne and the Montreal Neurological Hospital, Quebec, Canada. All patients were studied while treated with the standard 300mg monthly intravenous regimen of natalizumab. For each patient, blood samples were collected just before and 1.5 hours after each treatment (pretreatment and postinfusion, respectively), based on published pharmacokinetic data.[11] A prospective patient cohort (n = 8) was studied up to five times over a 13-month period.

Cell Isolation and Migration Assays

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples using Ficoll-Paque (Amersham Biosciences, Uppsala Sweden). Migration assays were conducted blindly on preinfusion and postinfusion PBMCs in parallel, using Boyden chambers, as previously described.[12] In this system, VLA-4 on immune cells is known to contribute to migration by interacting with the CS-1 fragment of fibronectin, implicated as one of the functional binding partners of VLA-4 in migration across human brain endothelial cells.[7,13] In brief, 5μm chambers were precoated with 50μg/ml fibronectin (BD PharMingen, Bedford, MA) and inserted into one well of a 24-well plate. Ex vivo isolated PBMCs (1×10^6) were added to the top compartment in 500μl of RPMI-1640 supplemented with 2.5% fetal calf serum. After 1 hour, cells were collected from the bottom compartment and counted. For blocking experiments, monoclonal mouse anti–human VLA-4 or control mouse IgG1 were added (both at 15μg/ml; R&D Systems, Minneapolis, MN).

Flow Cytometry

Three-color staining was performed as previously described[7] by incubating whole blood with saturating amounts of directly conjugated monoclonal antibodies noted below (all from BD PharMingen) for 30 minutes at 4°C in the dark. Isotype-matched antibodies were used as negative controls (BD PharMingen). Red blood cells were lysed using FACS Lysing Solution (BD Biosciences, Mississauga, Canada) for 10 minutes at room temperature. Stained cells then were washed with phosphate-buffered saline and acquired with a BD FACScan (Becton Dickinson). Analysis of mean fluorescence intensity (MFI) of α_4-integrin (CD49d) on immune cell subsets was performed by a blinded operator with the FlowJo software (Treestar, Ashland, OR). Antibodies targeted CD49d; CD11a (LFA-1); CD3, CD4, and CD8 T cells; CD19 B cells; CD14 monocytes; and memory markers CD45RA/CD45 RO (T cells) and CD27 (B cells).

In Vitro Stimulation Assays

Total PBMCs were cultured at 37°C/5% CO_2 in 96-well plates at 1×10^5 viable cells/well in RPMI-1640 supplemented with 100U/ml penicillin/streptomycin and 2mM L-glutamine (Invitrogen, La Jolla, CA). Effects of in vitro anti–VLA-4 on PBMC proliferation were assessed by culturing PBMC in parallel either with a dose titration of anti-CD3 antibody alone (OKT3; ATCC, Rockville, MD) or with the OKT3 as well as dose titrations of anti–VLA-4 or mouse IgG1 isotype control. Each subcondition was done in triplicate. At the indicated times, proliferation was assessed as 18-hour tritiated thymidine uptake (1μCi; ICN Biochemicals, Mississauga, Ontario, Canada) using a beta scintillation counter. The effect of in vivo infusion of anti–VLA-4 on PBMC proliferation was evaluated using the above approach to compare in parallel responses of PBMC obtained from patients, before and after natalizumab infusion. In addition, the proliferative responses of normal PBMC were evaluated upon the addition, in parallel, of preinfusion or postinfusion serum from treated patients (dilutions of 1:15 or 1:5 in RPMI-1640).

Statistics

Data from migration and FACS assays comparing averages of samples obtained before and after natalizumab infusions were analyzed using the Student's paired *t* test. Analysis between cell phenotypes and MFI utilized analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). *p* values less than 0.05 were considered statistically significant.

Results

We first confirmed that in vivo infusion of the standard dose of natalizumab (300mg IV) significantly decreased the migratory capacity of peripheral blood mononuclear cells (PBMC) in patients with multiple sclerosis (Fig 1A; n = 24; $p = 0.0001$). The migration of postinfusion PBMC could be further inhibited by the addition of anti–VLA-4 antibody in vitro (see Fig 1; $p < 0.0001$), suggesting that the in vivo infusion of natalizumab did not fully block the functional expression of VLA-4 on the surface of circulating immune cells. We evaluated this using whole-blood flow cytometry to compare MFI of the α4-integrin (CD49d) of VLA-4 on the surface of immune cells in preinfusion and postinfusion samples. CD49d MFI of circulating immune cells was significantly, although only partially, reduced after natalizumab treatment (see Fig 1C; n = 30; $p < 0.0001$). This effect was specific, because the expression of CD11a (the α-integrin of LFA-1) on the same cells was not impacted by in vivo natalizumab. A strong correlation ($r = 0.6508$; $p = 0.0006$) was seen between the effects of natalizumab infusion on the CD49d α4-integrin surface expression and on the migratory capacity of the same immune cells (see Fig 1D), reinforcing the functional relevance of the flow cytometry measurement of α4-integrin expression.

Both the efficacy and safety profiles of natalizumab are likely to depend on which immune cell subsets are most impacted by anti–VLA-4 therapy. This may be influenced by the basal (preinfusion) expression of α4-integrin on distinct subsets, as well as by the relative decrease in surface expression after treatment. Examination of immune cell subsets in the circulation of patients preinfusion demonstrated that $CD19^+$ B cells and $CD14^+$ monocytes expressed significantly higher basal levels of α4-integrin compared with $CD3^+$ T

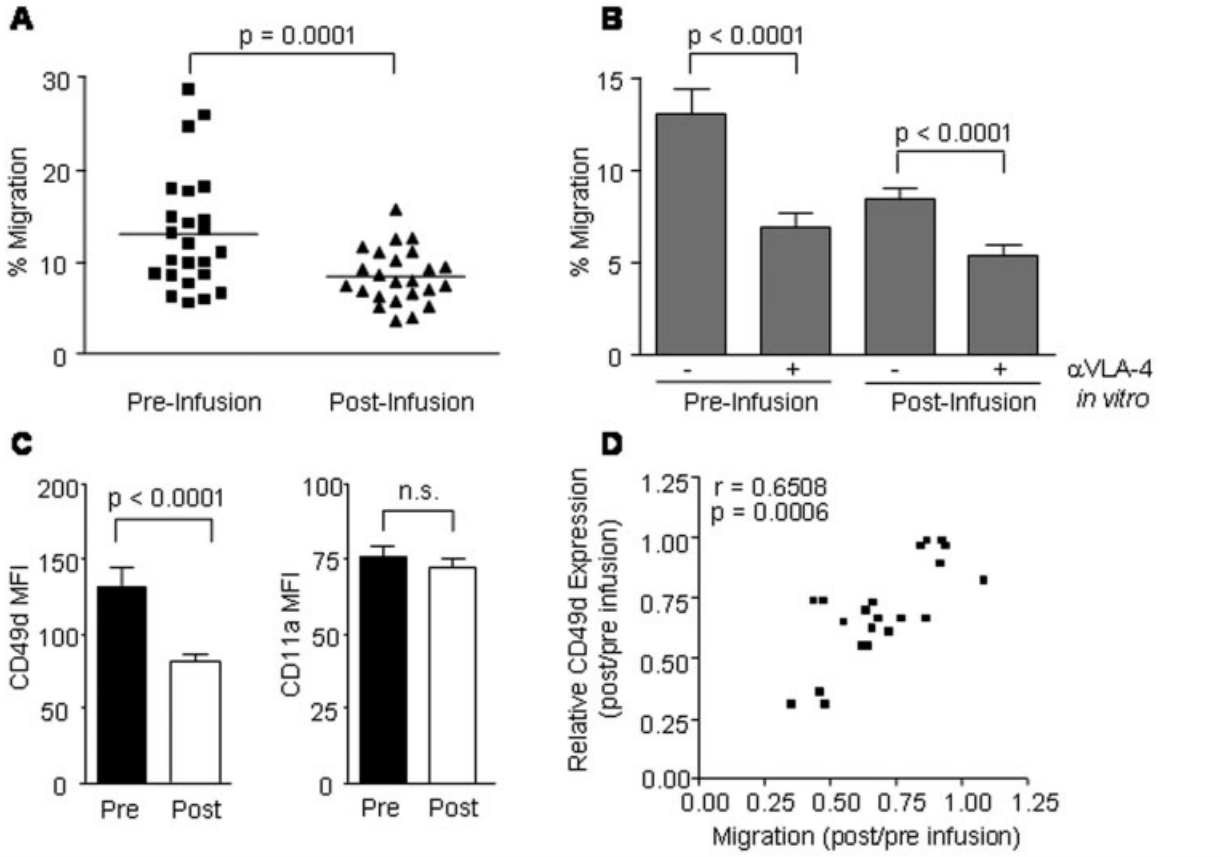

Fig 1. Effects of in vivo natalizumab infusion on migration and functional expression of the α4-integrin in patients with multiple sclerosis. (A) Significant reduction in percent migration of postinfusion peripheral blood mononuclear cells (PBMCs) compared with preinfusion PBMCs (n = 24 independent Boyden chamber experiments, performed in duplicate). (B) Migration of postinfusion PBMCs is further reduced upon in vitro addition of anti–VLA-4 (n = 24). (C) Natalizumab infusion significantly decreases available surface expression of the α4-integrin of VLA-4, but not of the α-integrin of LFA-1 (n = 30); MFI = mean fluorescence intensity by flow cytometry. (D) Strong correlation between the effects of in vivo natalizumab infusion on α4-integrin expression (MFI) and migratory capacity of corresponding PBMC.

cells (Fig 2A; n = 30; $p < 0.0001$ for both). Among T cells, $CD8^+$ cells expressed more than twice as much α4-integrin as did $CD4^+$ T cells (see Fig 2B; $p = 0.0002$). Consistent with a previous report,[14] memory ($CD45RO^+RA^-$) T cells expressed higher levels of the α4-integrin compared with naive ($CD45RO\text{-}RA^+$) T cells (see Fig 2B; $p = 0.0059$). Memory ($CD27^+$) B cells expressed significantly higher basal levels of α4-integrin than did naive ($CD27^-$) B cells ($p < 0.0001$). We next questioned the extent to which in vivo natalizumab treatment impacted the available α4-integrin on the different immune cell subsets. Although the absolute decrease in α4-integrin expression (absolute change in MFI) was smaller for T cells compared with B cells and monocytes (data not shown), the relative impact of infusion (percent decrease in MFI) was greatest for T cells (49%) compared with the B cells (29%; $p = 0.006$) or monocytes (approximately 25%; $p = 0.0034$). There were no differences in the relative impact of natalizumab infusion on α4-integrin expression comparing CD4 to CD8 T cells, memory to naive T cells or memory to naive B cells (data not shown).

We next asked whether the effect of a single standard natalizumab infusion (300mg IV) persisted until the next infusion. α4-Integrin surface expression on ex vivo PBMCs was prospectively monitored in preinfusion and postinfusion samples from MS patients undergoing serial monthly infusions of natalizumab over a 10-month period (see Fig 2C, example of an individual patient plotted over time, and summary histogram of n = 8 patients). We observed that the available surface expression of the α4-integrin was decreased by each infusion, although this effect did not persist over the 1-month dosing interval, because subsequent preinfusion levels tended to recover. The same transient effect was seen at the level of individual $CD3^+$, $CD19^+$, and $CD14^+$ cell subsets (data not shown), and also at the functional level when serially studying the migratory capacity of PBMC. Specifically, we found that the percentage of inhibition of migration

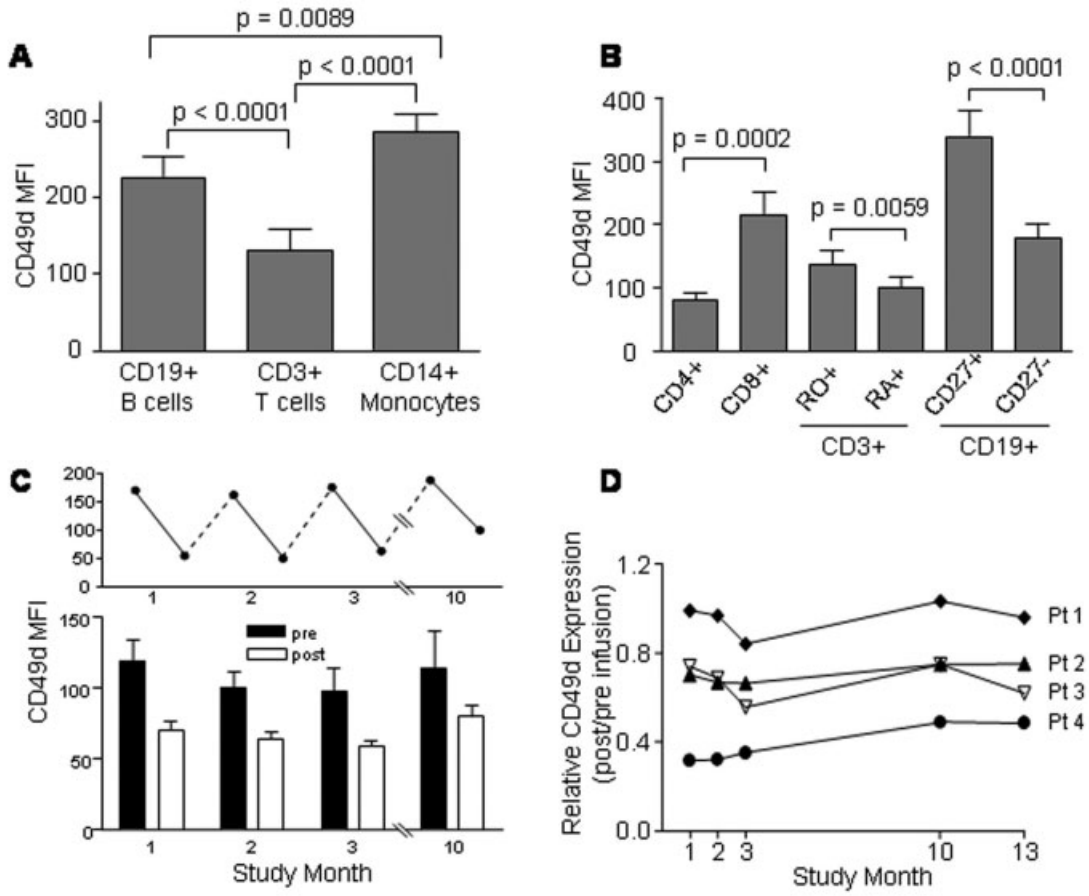

Fig 2. α4-Integrin expression on immune cell subsets at baseline and during prospective follow-up of serial infusions in patients with multiple sclerosis. (A) Significantly higher baseline (preinfusion) levels of α4-integrin on circulating B cells ($CD19^+$) and monocytes ($CD14^+$) compared with T cells ($CD3^+$). (B) Baseline α4-integrin expression on T-cell and B-cell subsets. (C) The decreased α4-integrin expression on peripheral blood mononuclear cells mediated by natalizumab infusion does not persist through the monthly dosing interval. Example of serial studies are shown for individual patient and summarized for n = 8. (D) Effect of natalizumab infusion on α4-integrin can be different across patients yet tends to be similar within individual patients over multiple infusions.

averaged across patients was 26%, 34%, and 29% around the first, second, and third study infusions, respectively. An interesting pattern was revealed when looking at individual patient profiles over time (see Fig 2D; data for four patients shown for ease of visualization). The magnitude of drug effect is depicted as the ratio of CD49d expression on PBMC postinfusion divided by the expression preinfusion, such that a value of 1.0 reflects no drug effect on this measure and lower fractions reflect greater drug effect. We found that the magnitude of the infusion effect on the α4-integrin expression could vary significantly across patients but tended to be quite similar within individual patients, over multiple infusions as confirmed through month 13 of treatment (see Fig 2D). This relatively consistent effect of infusions in individual patients was also true at the level of immune cells subsets (T cells, B cells, and monocytes; data not shown).

In addition to its role in trafficking, VLA-4 is also recognized as potentially involved in costimulation of T cells.[14,15] To study the potential effects of natalizumab on human T cells, we first confirmed that in vitro addition of anti–VLA-4 significantly modulated the activation of normal human PBMC. At a relatively low concentration (0.1μg/ml), anti–VLA-4 significantly enhanced PBMC proliferation to anti-CD3 (OKT3; Fig 3A). Increasing concentrations resulted in successively lower enhancement of proliferation (see Fig 3B). Similar to results from normal PBMC above, in vitro anti–VLA-4 enhanced proliferation of PBMC obtained from MS patients before natalizumab infusion, but this effect was abrogated in postinfusion PBMC of the same patients (see Fig 3C). We also found that, compared with preinfusion serum, serum obtained from patients after natalizumab infusion was able to significantly enhance the anti–CD3-mediated proliferation of normal PBMC (see Fig 3D). We confirmed a similar costimulatory effect of anti–VLA-4 on

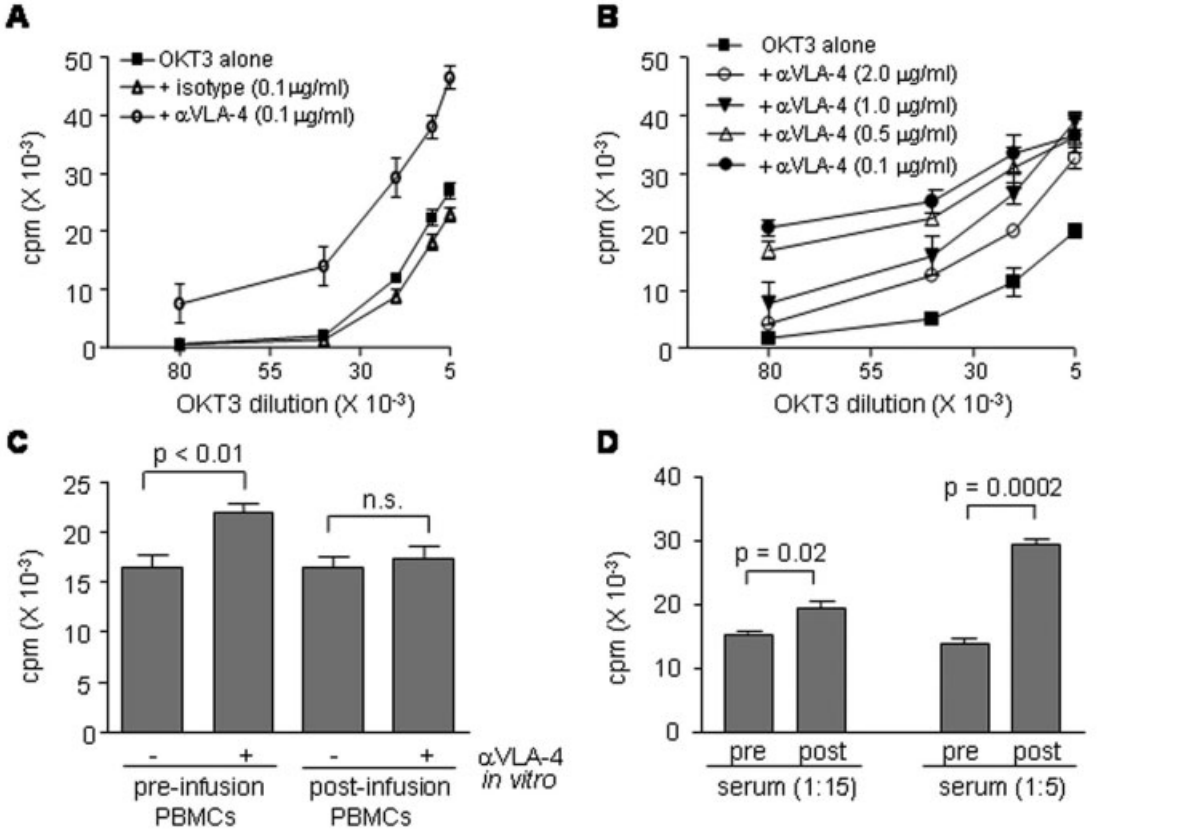

Fig 3. Natalizumab therapy modulates peripheral blood mononuclear cell (PBMC) activation. (A) Normal PBMC proliferation to OKT3 (anti-CD3) is significantly enhanced by in vitro addition of anti–VLA-4. (B) Dose–response of anti–VLA-4 effect on normal PBMC proliferation. (C) Postinfusion PBMC from natalizumab-treated patients no longer exhibit enhanced proliferative response to in vitro anti–VLA-4 compared with preinfusion PBMCs. (D) Serum obtained from patients postinfusion with natalizumab can significantly enhance proliferative responses of normal PBMCs compared with preinfusion serum.

the proliferative responses of PBMC to a dose titration of glatiramer acetate (GA), a random mix of four amino acids known to induce T-cell receptor–mediated and major histocompatibility complex class II–restricted responses across a broad range of T-cell specificities[16,17] (see supplementary online Figure A). PBMC exhibited enhanced proliferation to GA also when exposed to serum obtained from patients after infusion with natalizumab, compared with preinfusion serum from the same patients (data not shown). Finally, we found that anti–VLA-4 could induce proliferative responses to a myelin basic protein (MBP) peptide pool that were not otherwise detectable in a primary proliferation assay (see supplementary online Figure B).

Discussion

To our knowledge, we provide the first biological proof of concept of the expected effects of in vivo natalizumab on cell trafficking in MS patients, demonstrating that therapy diminishes the functional surface expression of the α4-integrin and the migratory capacity of circulating immune cells. Several of our findings, however, were less expected.

Although studies of anti–VLA-4 therapy have tended to focus on targeting T cells,[1,5,12,14] our preinfusion studies demonstrate that basal levels of VLA-4 are significantly higher on circulating B cells and monocytes compared with T cells, reinforcing reports implicating VLA-4 in trafficking of these cell types across the blood brain barrier endothelium.[18,19] These findings also point to potentially different effects of anti–VLA-4 on these cells and their subsets. It is not known whether such therapy will have a greater, or lesser, effect on the trafficking of a cell expressing lower levels of VLA-4. On one hand, cells expressing lower levels of VLA-4 may be more affected by a given concentration of anti–VLA-4. On the other hand, lower levels of VLA-4 expression may reflect a lesser reliance of particular cells for VLA-4 in trafficking. In the context of PML risk, it is of interest to speculate on the particularly high levels of VLA-4 expressed on B cell subsets, because B cells have been implicated as main carriers of JC virus in the circulation and may represent a mode of transport of

the virus into the CNS.[7,20,21] Similarly, the higher levels of VLA-4 on CD8 compared with CD4 T cells may translate into differential effects on trafficking of these subsets, and on their respective roles in CNS anti-viral immune surveillance.[22–24]

Also noteworthy is the observation that the standard (300mg) intravenous infusion of natalizumab did not fully block functional surface VLA-4, because adding more anti–VLA-4 in vitro to postinfusion PBMCs could further diminish migration. Although anti–VLA-4 has been reported to induce apoptosis in T cells,[25] we found that this occurred when cells were concurrently activated, but not in unstimulated cells such as those used in our migration assays (data not shown). Thus, the diminished migration we observed after both in vivo and ex vivo addition of anti–VLA-4 was unlikely to be confounded by apoptosis. We further observed that the effects of the standard dose on both α4-integrin expression and cell migration were not sustained over the monthly dose interval. These findings suggest that with the standard regimen, natalizumab effect on trafficking may be neither complete, nor persistent, features that actually may be desirable because they would allow for intermittent immune surveillance. Patients in whom functional VLA-4 expression may fail to recover between infusions could be at higher risk for complications.

A surprising finding that emerged from careful prospective monitoring of natalizumab-treated patients was that the magnitude of the infusion effect could differ considerably across individuals, although was remarkably stable for the same patient across multiple infusions. Although our cohort is too small to meaningfully correlate biological, clinical, and neuroimaging responses, these results suggest that patients may exhibit individualized response profiles to natalizumab. We hope that the relatively simple immune readouts described here will be applied to larger patient cohorts as part of biomarker development and validation initiatives.

Although discussion of the presumed mode of action of anti–VLA-4 therapy has tended to focus on the drug's impact on cell trafficking, VLA-4 is also recognized as playing a role in costimulation of T cells, and anti–VLA-4 therapy in the experimental autoimmune encephalomyelitis animal model of MS appears to impact T-cell activation.[8,9,14] Our results confirm reports that anti–VLA-4 and anti-CD3 signals can integrate to enhance human T cell activation.[15,26] We further demonstrate that in vivo natalizumab therapy of MS patients can modulate the properties of patients' serum as well as the activation threshold of their circulating immune cells, including CNS-autoreactive T cells.

The implication of such modulation remains to be elucidated yet underscores the multiple biological effects that need to be considered in the context of anti–VLA-4 therapy. The future of natalizumab will be predicated on our ability to eventually identify those patients who will benefit most from therapy, and those who may be at particular risk for severe complications such as PML. We describe several simple immune response parameters that may be relevant to both the efficacy and safety profiles of anti–VLA-4 therapy and suggest that immune monitoring during future exposure may be a valuable investment. Ultimately, validation and careful correlation of reliable biological measures, with rigorously obtained clinical and neuroimaging data, will be critical to elucidation of important responder/suboptimal responder issues, toxicity profiles, and future phramacogenomic considerations.

References

1. Miller DH, Khan OA, Sheremata WA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 2003;348:15–23.
2. Available at: U.S. Food and Drug Administration. FDA News. http://www.fda.gov/bbs/topics/news/2004/NEW01141.html. Accessed Nov 23, 2004.
3. Langer-Gould A, Atlas SW, Green AJ, et al. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. N Engl J Med 2005;353:375–381.
4. Kleinschmidt-Demasters BK, Tyler KL. Progressive multifocal lekoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. N Engl J Med 2005; 353:369–374.
5. Yednock TA, Cannon C, Fritz LC, et al. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 1992;356:63–66.
6. Cada DJ, Levien T, Baker DE. Natalizumab. Hospital pharmacy. 2005;40:336–346.
7. Alter A, Duddy M, Hebert S, et al. Determinants of human B cell migration across brain endothelial cells. J Immunol 2003; 170:4497–4505.
8. Theien BE, Vanderlugt CL, Eagar TN, et al. Discordant effects of anti-VLA-4 treatment before and after onset of relapsing experimental autoimmune encephalomyelitis. J Clin Invest 2001; 107:995–1006.
9. Theien BE, Vanderlugt CL, Nickerson-Nutter C, et al. Differential effects of treatment with small-molecule VLA-4 antagonist before and after onset of relapsing EAE. Blood 2003;102: 4464–4471.
10. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
11. Sheremata WA, Vollmer TL, Stone LA, et al. A safety and pharmacokinetic study of intravenous natalizumab in patients with MS. Neurology 1999;52:1072–1074.
12. Prat A, Biernacki K, Lavoie JF, et al. Migration of multiple sclerosis lymphocytes through brain endothelium. Arch Neurol 2002;59:391–397.
13. Wong D, Prameya R. Dorovini-Zis K. In vitro adhesion and migration of T lymphocytes across monolayers of human brain microvessel endothelial cells: regulation by ICAM-1, VCAM-1, E-selectin and PECAM-1. J Neuropathol Exp Neurol 1999;58: 138–152.

14. Sato T, Tachibana K, Nojima Y, et al. Role of the VLA-4 molecule in T cell costimulation. Identification of the tyrosine phosphorylation pattern induced by the ligation of VLA-4. J. Immunol 1995;155:2938–2947.
15. Ennis E, Isberg RR, Shimizu Y. Very late antigen 4-dependent adhesion and costimulation of resting human T cells by the bacterial beta 1 integrin ligand invasin. J Exp Med 1993;177: 207–212.
16. Fridkis-Hareli M, Neveu JM, Robinson RA, et al. Binding motifs of copolymer 1 to multiple sclerosis- and rheumatoid arthritis-associated HLA-DR molecules. J Immunol 1999;162: 4697–4704.
17. Duda PW, Krieger JI, Schmied MC, et al. Human and murine CD4 T cell reactivity to a complex antigen: recognition of the synthetic random polypeptide glatiramer acetate. J Immunol 2000;165:7300–7307.
18. Bar-Or A, Nuttall RK, Duddy M, et al. Analyses of all matrix metalloproteinase members in leuckocytes: monocytes as major inflammatory mediators in multiple sclerosis. Brain 2003;126: 2738–2749.
19. Seguin R, Biernacki K, Rotondo RL, et al. Regulation and functional effects of monocyte migration across human brain-derived endothelial cells. J Neuropathol Exp Neurol 2003;62: 412–419.
20. Tornatore C, Berger JR, Houff SA, et al. Detection of JC virus DNA in peripheral lymphocytes from patients with and without progressive multifocal leukoencephalopathy. Ann Neurol 1992;31:454–462.
21. Sabath BF, Major EO. Traffic of JC virus from sites of initial infection to the brain: the path to progressive multifocal leukoencephalopathy. J Infect Dis 2002;186(suppl 2):S180–S186.
22. Koralnik IJ, Du Pasquier RA, Kuroda MJ, et al. Association of prolonged survival in HLA-A2+ progressive multifocal leukoencephalopathy patients with a CTL response specific for a commonly recognized JC virus epitope. J Immunol 2002;168: 499–504.
23. Gasnault J, Kahraman M, de Goer de Herve MG, et al. Critical role of JC virus specific CD4 T cell responses in preventing progressive multifocal leukoencephalopathy. AIDS 2003;17: 1443–1449.
24. Du Pasquier RA, Kuroda MJ, Schmitz JE, et al. Low frequency of cytotoxic T lymphocytes against the novel HLA-A*0201-restricted JC virus epitope VP1(p36) in patients with proven or possible progressive multifocal leukoencephalopathy. J Virol 2003;77:11918–11926.
25. Leussink VI, Zettl UK, Jander S, et al. Blockade of signaling via the very late antigen (VLA-4) and its counter ligand vascular cell adhesion molecule-1 (VCAM-1) causes increased T cell apoptosis in experimental autoimmune neuritis. Acta Neuropathol 2002;103:131–136.
26. Maguire JE, Danahey KM, Burkly LC, van Seventer GA. T cell receptor- and beta 1 integrin-mediated signals synergize to induce tyrosine phosphorylation of focal adhesion kinase (pp125FAK) in human T cells. J Exp Med 1995;182:2079–2090.