Antagonizing the $\alpha_4\beta_1$ Integrin, but Not $\alpha_4\beta_7$, Inhibits Leukocytic Infiltration of the Central Nervous System in Rhesus Monkey Experimental Autoimmune Encephalomyelitis

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doi: 10.4049/jimmunol.1202490

http://www.jimmunol.org/content/190/5/1961
Antagonizing the $\alpha_4\beta_1$ Integrin, but Not $\alpha_4\beta_7$, Inhibits Leukocytic Infiltration of the Central Nervous System in Rhesus Monkey Experimental Autoimmune Encephalomyelitis

Krista G. Haanstra,* Sam O. Hofman,* Dave M. Lopes Estêvão,* Erwin L. A. Blezer,† Jan Bauer,‡ Li-Li Yang,§ Tim Wyant,§ Vilmos Csizmadia,§ Bert A. ‘t Hart,*§ and Eric R. Fedyk¶

The immune system is characterized by the preferential migration of lymphocytes through specific tissues (i.e., tissue tropism). Tissue tropism is mediated, in part, by the $\alpha_4$ integrins expressed by T lymphocytes. The $\alpha_4\beta_1$ integrin mediates migration of memory T lymphocytes into the CNS, whereas the $\alpha_4\beta_7$ integrin mediates migration preferentially into gastrointestinal tissue. This paradigm was established primarily from investigations in rodents; thus, the objective of this investigation was to determine if blocking the $\alpha_4\beta_1$ integrin exclusively would affect migration of T lymphocytes into the CNS of primates. The effects of the dual $\alpha_4\beta_1$ and $\alpha_4\beta_7$ antagonist natalizumab were compared with those of the $\alpha_4\beta_7$ antagonist vedolizumab on experimental autoimmune encephalomyelitis in the rhesus monkey. Animals received an initial i.v. bolus of placebo, natalizumab (30 mg/kg), or vedolizumab (30 mg/kg) before intracutaneous immunization with recombinant human myelin oligodendrocyte glycoprotein and then Ab once weekly thereafter. Natalizumab prevented CNS inflammation and demyelination significantly ($p < 0.05$), compared with time-matched placebo control animals, whereas vedolizumab did not inhibit these effects, despite saturating the $\alpha_4\beta_7$ integrin in each animal for the duration of the investigation. These results demonstrate that blocking $\alpha_4\beta_7$ exclusively does not inhibit immune surveillance of the CNS in primates. The Journal of Immunology, 2013, 190: 1961–1973.

The immune system is characterized by regionalization at multiple levels, and immunosurveillance in particular is characterized by the preferential migration of lymphocyte subsets through specific tissues. Naive lymphocytes primarily recirculate through secondary lymphoid organs, for example, whereas differentiated memory T cells migrate preferentially through tissues in which Ag was initially encountered, such as the skin, CNS, or gut. This adaptive response is postulated to enhance the efficiency with which the immune system responds to pathogens (1–3).

Tissue-tropic migration is mediated in part by vascular lymphocytes firmly adhering to the endothelial lumen and diapedesing into surrounding tissue. This process requires the formation of shear-resistant attachments, which are mediated by the binding of lymphocyte integrins to Ig superfamily members expressed on the endothelial lumen. Integrins are obligate heterodimers containing two distinct chains, called the $\alpha$ and $\beta$ subunits. In mammals, 18 $\alpha$ and 8 $\beta$ subunits have been characterized. These chains heterodimerize and create at least 24 unique integrins, many of which have distinct functions. The $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins in particular are expressed by discrete subsets of memory T lymphocytes (4, 5), and these subsets exhibit distinct patterns of migration in vivo (1–3). Mechanistic investigations have demonstrated that the $\alpha_4\beta_1$ integrin expressed on memory T lymphocytes mediates migration into the CNS, bone marrow, and skin, via firm adhesion to VCAM-1 (1, 2). In contrast, memory T lymphocytes expressing the $\alpha_4\beta_7$ integrin preferentially migrate into the gastrointestinal tract via firm adhesion to mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) (1–3, 6, 7). These tissue-tropic mechanisms were primarily elucidated in mice, and it is unknown to what extent $\alpha_4\beta_7$ may contribute to immune surveillance of the CNS in primates.

These mechanisms also mediate inflammation. Blocking the $\alpha_4$ integrins with Abs or small-molecule inhibitors reverses the clinical and pathological hallmarks of experimental autoimmune encephalomyelitis (EAE) in mice and guinea pigs (8–13). Natalizumab is an antagonist of the human $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins and delays the accumulation of physical disability, as well as reducing the frequency of clinical exacerbations in patients with relapsing forms of multiple sclerosis (14). Natalizumab specifically inhibits the firm adhesion of human T cells to the inflamed spinal cord microvasculature of mice with acute EAE (15). Natalizumab also alleviates gastrointestinal inflammation in Crohn’s disease (CD) (16) and thus is a pleiotropic anti-inflammatory agent. The clinical utility of natalizumab is limited, however, by progressive multifocal leu-
koencephalopathy (PML), a life-threatening brain infection characterized by progressive damage of brain white matter at multiple locations (17–19). It is caused by recrudescence of the JC virus in immunosuppressed patients, presumably due to impaired immune surveillance of the brain by memory T lymphocytes (17–19). This theory cannot be directly tested, however, because an appropriate model of PML does not exist (17–19).

Inflammatory bowel disease (IBD) comprises a group of inflammatory conditions of the gastrointestinal tract, of which CD and ulcerative colitis (UC) are the most common forms (20). An improved therapeutic strategy for IBD could be to antagonize the \( \alpha_4 \beta_7 \) integrin exclusively, based on the premise that this would provide anti-inflammatory activity in the gastrointestinal tract without compromising immune surveillance of the CNS. Blockade of the \( \alpha_4 \beta_7 \) integrin exclusively with the Act-1 mAb induced anti-inflammatory effects and remission of disease in spontaneously colitic cotton-top tamarins (21). Vedolizumab (former versions: MLN0002, MLN02, LDP-02) is also a highly selective mAb that binds exclusively to the gut-tropic \( \alpha_4 \beta_7 \) integrin; it does not bind to other \( \alpha_4 \) or \( \beta_7 \) integrins, such as the \( \alpha_4 \beta_1 \) integrin or the \( \alpha_6 \beta_4 \) integrin (22). It inhibits the functional activity of the \( \alpha_4 \beta_7 \) integrin by selectively antagonizing binding and adhesion to MadCAM-1 and to the extracellular matrix glycoprotein fibronectin, does not antagonize binding to VCAM-1 (22). It elicited anti-inflammatory effects selectively in the gastrointestinal tract of cynomolgus monkeys (23) and demonstrated statistically significant efficacy in placebo-controlled phase 2 clinical trials of patients with active UC (24) and CD (25). Therefore, vedolizumab was used in this investigation to specifically examine whether exclusive antagonism of the \( \alpha_4 \beta_7 \) integrin would compromise immune surveillance of the CNS in rhesus monkey EAE.

Materials and Methods

**Abs**

Natalizumab (Tysabri, Biogen Idec, Cambridge, MA) is a humanized IgG4 mAb that binds to the \( \alpha_4 \) chain of the human \( \alpha_4 \beta_1 \) and \( \alpha_4 \beta_7 \) integrins (14, 16). Vedolizumab is a humanized IgG1 version of the Act-1 mAb, which binds to the \( \beta_7 \) chain of the human \( \alpha_4 \beta_7 \) integrin, but not to the \( \alpha_4 \) or \( \alpha_6 \) integrins (22).

Natalizumab exhibited a mean concentration producing 50% maximal binding (EC50) of 18.6 ± 11.4 ng/ml and IC50 of 70.4 ± 37.2 ng/ml for binding to rhesus monkey memory helper T lymphocytes, which is consistent with a human EC50 of 11.4 ng/ml and IC50 of 37.2 ng/ml (Table I) and with a previous investigation (20). Vedolizumab exhibited a mean EC50 of 27.6 ± 21.3 ng/ml and IC50 of 12.2 ± 8.4 ng/ml for binding to rhesus monkey memory helper T lymphocytes, which is consistent with a human EC50 of 21.3 ng/ml and IC50 of 8.4 ng/ml (Table I). In contrast, natalizumab did not bind to marmoset, rabbit, rat, or mouse lymphocytes (data not shown), and vedolizumab did not bind to guinea pig, rat, or mouse lymphocytes (data not shown).

**Animals**

Naive, adult rhesus monkeys (Macaca mulatta) were randomly selected from the purpose-bred colony of the Biomedical Primate Research Centre and housed under conventional, non–specific pathogen–free conditions. Animals were included only after a complete physical, hematological, and biochemical checkup had been performed. During the study, monkeys were pair housed and remained under intensive veterinary care. The daily diet consisted of commercial food pellets for nonhuman primates (Sniff, Soest, Germany), supplemented with rice, raisins, peanuts, and fresh fruit. Drinking water was provided ad libitum. Ethically responsible use of nonhuman primates was further ensured by modeling the pharmacodynamic (PD) responses of cynomolgus monkeys to natalizumab and vedolizumab and then extrapolating the minimum number of rhesus monkeys required per treatment group to yield statistically significant differences in PD responses after exposure to natalizumab and vedolizumab. This modeling predicted that statistically significant PD results would require at least seven animals per treatment group. This experimental design, all study protocols, and experimental procedures were reviewed and approved by the Biomedical Primate Research Centre’s Ethics Committee, in accordance with Dutch law on animal experimentation.

**Experimental design**

The rhesus monkey recombinant human myelin oligodendrocyte glycoprotein (rhMoG) EAE model (27) was specifically chosen because T cells are required for induction of the disease (28), the expression profiles of rhesus monkey \( \alpha_4 \beta_7 \) and \( \alpha_4 \beta_1 \) integrins are similar to those of humans (29), it is the only EAE model in which both natalizumab and vedolizumab are pharmacologically active, and finally, all animals develop disease in this model (27).

Twenty-two animals were stratified over three groups, ensuring comparable (i.e., no significant differences in group means) age, weight, and sex distribution (Table II). Placebo animals (\( n = 8 \)) were administratively coupled in a 1:1 (doublet) or 1:2 (triplet) ratio with natalizumab (\( n = 7 \)) or vedolizumab-treated (\( n = 7 \)) animals before EAE induction (Table III). The experiment was executed in two phases. The objective of the first phase was to determine if antagonizing the \( \alpha_4 \) integrins would inhibit development of EAE. Seven natalizumab-treated animals were randomly coupled with four placebo-treated animals, yielding 4 groups (Table III). When one animal of a doublet or triplet was diagnosed with EAE score ≥ 2 (the indicator animal), the remaining coupled animals were euthanized either the same day or, at maximum, 48 h later to ensure time-matched comparators for subsequent postmortem analyses of samples. The objective of the second phase was to determine if specifically antagonizing the \( \alpha_4 \beta_7 \) integrin would (also) inhibit the development of EAE. This phase was conducted identical to the first phase, except that vedolizumab-treated animals (\( n = 7 \)) were coupled with four additional placebo-treated animals (Table III). Natalizumab and vedolizumab were dosed at 30 mg/kg on days 0, 7, 14, and 21, and placebo animals received an equivalent volume of saline.

**EAE induction and monitoring**

EAE was induced using rhMoG encompassing the extracellular domain, amino acids 1–125, which was produced in E. coli and purified as described previously (30). Animals were immunized on day 0 with 300 μg rhMoG dissolved in 500 μl PBS, and emulsified in an equal volume of CFA (Difco Laboratories, Detroit MI).

Clinical signs were scored daily by observers blinded to the treatment, using a previously described semiquantitative scale (27, 31, 32): 0 = no clinical signs; 0.5 = loss of appetite, vomiting; 1 = substantial reduction of general condition; 2 = ataxia, sensory loss, and/or visual problems; 2.5 = incomplete paralysis of one (hemiparesis) or both sides (paraparesis); 3 = complete paralysis of one (hemiplegia) or both sides (paraplegia); 4 = complete paralysis (quadriplegia); 5 = moribund. The rhMoG-induced EAE model in rhesus monkeys is characterized by acute onset and rapid disease progression, with the monkeys reaching a moribund state within 24 h. To avoid suffering, monkeys were euthanized at EAE score ≥ 2.5, or at score 2 when the animal was not expected to survive until the next day.

**PK monitoring**

The concentrations of natalizumab and vedolizumab in serum samples from rhesus monkeys were quantified using ELISA by a Good Laboratory Practices (GLP) methodology for vedolizumab (Quest Pharmaceutical Services, Wilmington, DE) and by a non-GLP methodology for natalizumab (Millennium Pharmaceuticals) per testing facility standard operating practice (SOP). Briefly, the assays used a goat anti-human IgG heavy and light chain, macaque-adsorbed, capture Ab (Bethyl Laboratories Montgomery, TX) and a mouse anti-human IgG4 monoclonal IgG4 Ab conjugated donkey anti-rabbit IgG (Alpha Diagnostics, Owings Mills, MD). After the addition of a chromogenic HRP substrate (3,3',5,5'-tetramethylbenzidine; Pierce Biotechnology Rockford, IL), color development was measured at 450 nm on a Wallac 1420 Victor 2 Microplate Reader (PerkinElmer, Cambridge, MA). Data were analyzed with SoftMax Pro software, version 4.8.4, from MindVision Software (Lincoln, NE), and all statistics were calculated using Microsoft Office Excel (version 11.0.0.0, Microsoft, Redmond, WA). The intensity of the color was proportional to the serum natalizumab concentration that was interpolated by four-parameter logistic regression from a standard curve ranging from 0.125 to 8.0 μg/ml. The lower limit of quantitation for natalizumab was determined to be 0.125 μg/ml, and the upper limit of quantitation was 8 μg/ml in the ELISA assay. The calculated concentration of natalizumab was within 100% ± 20% of the nominal value, and precision values of the assay parameters were ≤ 20%. An analogous quantitative ELISA assay was used for detection of vedolizumab in serum samples, via GLP methodology, per testing facility SOP (Quest Pharmaceutical Services).
**PD monitoring**

PD effects were monitored by flow cytometry. The PD assays were whole-blood competition binding assays between the therapeutic mAb administered in vivo (natalizumab or vedolizumab) and the corresponding directly labeled mAb (natalizumab–Alexa 647 or vedolizumab–Alexa 647) incubated in whole blood ex vivo. Venous blood samples were collected in K3-EDTA vacutainers [Becton Dickinson (BD), Mountain View, CA]. Whole-blood samples were washed to remove excess Ab. Samples were stained with anti-CD3, anti-CD4, CD8, CD45RA (clones SP34-2, L200, SK1, and SH9, respectively; all from BD), and CD14 (TUK4; Milteny) and with Alexa Fluor 647–labeled natalizumab or vedolizumab, followed by lysis of the RBCs. Samples were analyzed on an LSR-II (BD), using DIVA software (BD).

**Primate anti-human Ab monitoring**

A non-GLP evaluation of primate anti-human Ab (PAHA) to natalizumab was performed by semi-quantitative ELISA of serum samples (Millennium Pharmaceuticals). Briefly, natalizumab (Biogen Idec) was immobilized to the plate. Bound anti-natalizumab Abs were detected with HRP-conjugated anti-macaque IgG, IgA, and IgM Abs (Rockland Immunochemicals, Gilbertsville, PA) after the addition of a chromogenic HRP substrate (3,3′,5′,5′-tetramethylbenzidine, Pierce Biotechnology). Qualification assays were conducted, and the precision (percent coefficient of variation) of the assays was calculated to be <20% acceptance range for both intra- and interassay runs, indicating good assay reproducibility. The detection cutoff point was determined using a panel of negative control serum samples from 10 individual rhesus monkeys (LAMPIRE Biological Laboratories, Pipersville, PA). An interference test showed that the assay was specific for natalizumab. An analogous GLP evaluation of PAHA to vedolizumab was performed by semiquantitative ELISA of serum samples, per testing facility SOP (Quest Pharmaceutical Services).

**Cellular immune responses against rhMOG**

PBMCs were isolated before and once weekly after EAE induction and at the time of necropsy. At necropsy, mononuclear cells (MNCs) were isolated from the spleen. Isolated MNCs were dispensed in quadruplicate at 1 × 10^7 cells per well in 96-well round-bottom microtiter plates with 5 μg/ml rhMOG. Stimulation by Con A (5 μg/ml) was used as a positive control. MNC proliferation was assayed by the incorporation of [3H]-thymidine (0.5 μCi per well) during the final 18 h of a 5-d culture. Cells were harvested for β-scintillation counting (TopCount NXT; Packard, Ramsey, MN). The results obtained from the different culture conditions were expressed as 6 counts per minute (Δcpm): (cpm of MNC proliferation with peptide) – (cpm of MNC proliferation without peptide).

**Humoral immune responses against rhMOG**

Serum samples were collected prior to EAE induction, once weekly thereafter, and at necropsy. CSF samples obtained as described below and the sera were tested by ELISA in 96-well microtiter plates for the presence of Abs against rhMOG. Plates were coated with rhMOG (5 μg/ml) and incubated overnight at 4°C. After washing and blocking with PBS/1% BSA, the wells were incubated in duplicate with 1:100 or 1:1000 diluted sera or CSF. Bound rhMOG Abs were detected with alkaline phosphatase–labeled goat–anti-human IgG (1:2000; cat. no. AH11305; Invitrogen) or alkaline phosphatase–labeled goat–anti-human IgM (1:10,000; cat. no. A9794; Sigma-Aldrich, Zwijndrecht, The Netherlands). Conjugate binding was quantified with p-nitrophenyl phosphate (Sigma-Aldrich). OD values were converted to arbitrary units using the same positive control on all plates as reference.

**Postmortem magnetic resonance images**

Postmortem magnetic resonance images (MRI) of formalin-fixed hemispheres were recorded on a 9.4-T horizontal-bore nuclear magnetic resonance spectrometer (Varian, Palo Alto, CA), equipped with a quadrature coil (RAPID Biomedical, Rimpar, Germany). Formalin-fixed hemispheres were submerged in a nonmagnetic oil (Fomblin; perfluorinated polyether, Solvay Solexis, Weesp, The Netherlands) to prevent unwanted susceptibility artifacts. On a sagittal scout image, 111 contiguous coronal slices of 0.75 mm covering the complete hemisphere were defined, with the following characteristics: field of view = 55 × 55 mm; matrix = 256 × 256; voxel volume = 34.6 × 10^3 μm^3; two transitions. The following MRI datasets were collected to analyze the size (qualitative), spatial distribution, and characteristics of white matter lesions:

1. T2 relaxation images. These maps were calculated by a monoexponential fit of five spin echo images with increasing echo times (TE). Repetition time = 7550 ms; TE = 12.5+4 × 12.5 ms. The sequence image in this sequence, that is, TE = 25 ms, was selected as the T2-weighted image. T2-weighted and T2 relaxation time images are highly sensitive for changes in water distribution, such as the occurrence of vasogenic edema induced by inflammation.

2. Magnetization transfer ratio (MTR) images were calculated from two T1-weighted spin echo images with and without an MT-saturation pulse. Repetition time = 4250 ms; TE = 20 ms; MT-pulse: 8.19 ms gaussian-shaped pulse, nominal flip angle 1000°, offset –9.4 kHz. MTR values thus represent reduction of the MR signal as a result of the off resonance saturation pulse. Reduction of the MTR value of a tissue occurs when the density of tissue macro-molecules decreases, such as by demyelination or when the tissue water content increases (inflammation).

The presence of white matter lesions was semiquantitatively graded between 0 (no lesions in white matter structures) and 10 (total white matter is affected by the lesion). The rater (E.B.) was blinded for the treatment while scoring.

**Histological examination of formalin-fixed tissues**

Following MRI scanning, the formalin-fixed hemispheres were processed for histopathological examination. Three samples were excised from standardized regions of each brain and embedded in paraffin. For histo-chemical staining, 5- to 5-μm-thick paraffin sections were deparaffinized in xylene and transferred to 90% ethanol. H&E staining for inflammation and Klüver–Barrera staining for demyelination were performed. Immunohis-tochemical stainings for CD3, CD20, and MRP14 were performed as described previously (33).

**Quantification of demyelination and cells**

Klüver–Barrera–stained sections (three sections per animal) were scanned with an Agfa DuoScan Scanner at 1000 dpi resolution. Recorded images were then analyzed with Image J (version 1.44p, a public domain image processing and analysis program developed by Wayne Rasband at the National Institutes of Health, Bethesda, MD). For this procedure, white matter areas in the sections were selected with the selection tools and quantified. The same was done for demyelinated areas. Finally, demye-lination was given as a percentage of total white matter. Quantification of parenchymal CD3+ T cells, CD20+ B cells, and MRP14+ macrophages in lesions was performed in consecutive sections, using an ocular morpho-metric grid covering an area of 4 mm^2 at 100-fold magnification.

**CSF sampling and processing**

CSF samples were taken prior to EAE induction, on days 4 or 5 and days 11 or 12 post EAE induction and prior to euthanasia, from sedated monkeys with a 23-gauge needle via the cisterna magna. When this method proved unsuccessful, a sample was taken via lumbar puncture. Typically, 0.5 ml clear CSF was obtained from each monkey.

Manual WBC and RBC counting was performed. Samples with >0.01 × 10^3/RBC, indicative of blood contamination, were excluded from further analysis. The samples were centrifuged. Supernatants were stored for determination of anti-rhMOG Abs. Pelleted cells were analyzed by FACS for expression of CD3, CD4, CD8, CD16, and CD20. The mAb clones used are specified under Subset analysis.

**Subset analysis**

Venous blood samples, collected as explained above, were washed and stained for CD3, CD4, CD8, CD14, and CD45RA as described earlier for the PD monitoring. In addition, cells were stained with anti-CD16 and anti-CD20 clones (3G8 and L27, respectively; all from BD). Samples were analyzed on an LSR-II (BD), using DIVA software (BD).

**Statistical analysis**

Statistical analysis was performed using Prism 5 for Mac OS X (GraphPad, San Diego, CA). Survival curves were compared using the log-rank test (Mantel–Cox). Significance of differences between groups was calculated using the nonparametric one-way ANOVA (Kruskal–Wallis test).

**Results**

The pharmacokinetics, immunogenicity, and PD of natalizumab and vedolizumab in rhesus monkeys

Natalizumab and vedolizumab exhibited conventional pharmaco-kinetcis properties for humanized IgGs administered to monkeys.
Substantial exposure was achieved in each animal after administration of natalizumab or vedolizumab, compared with placebo controls. All seven animals exposed to natalizumab exhibited trough levels that exceeded the EC50 for α4 integrin saturation in vitro (Table I) between days 7 and 14, and four of six animals exhibited trough levels that exceeded this EC50 between days 14 and 21 (Fig. 1A). All seven animals exposed to vedolizumab exhibited trough levels that exceeded the EC50 for α4β7 saturation in vitro (Table I) between days 7 and 21 (Fig. 1B). Higher exposures were generally achieved with vedolizumab than with natalizumab throughout the investigations (Fig. 1A, 1B). The mean concentration of vedolizumab in animals at day 14 was 557.8 ± 63.8 μg/ml, for example, whereas for natalizumab it was 184.4 ± 187.3 μg/ml. PAHA responses reduced exposure to natalizumab in some animals. All seven monkeys dosed with natalizumab developed PAHA responses by day 14 (Fig. 1C), and corresponding decreases in exposure were observed in two animals (i.e., N6 and N7, see Table II for animal demographics) on days 21 and 26 (Fig. 1A). Six monkeys dosed with vedolizumab developed PAHA responses by day 14 (Fig. 1D); however, corresponding decreases in exposure were not observed (Fig. 1B). Neutralizing activity of PAHA was identified by monitoring saturation of the target or targets expressed by T lymphocytes and monocytes by the therapeutic mAbs. Prior to exposure to therapeutic Ab, natalizumab–Alexa 647 bound to 75–92% of the total population of memory helper T lymphocytes (CD3+CD4+CD45RA2) in peripheral blood of rhesus monkeys (Fig. 1E). On day 7 post dose, this binding was completely blocked in each animal because the α4 integrins were saturated by the dosed natalizumab (Fig. 1E). A partial restoration of natalizumab–Alexa 647 binding (14–36%) was observed in animals N6 and N7 on days 14–21 (Fig. 1E), illustrating that desaturation of α4 integrins

### Table I. Binding affinities of natalizumab and vedolizumab to rhesus and human memory helper T lymphocytes

<table>
<thead>
<tr>
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<th>Natalizumab</th>
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<th>Vedolizumab</th>
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<tr>
<td></td>
<td>EC₅₀ SD IC₅₀ SD</td>
<td></td>
<td>EC₅₀ SD IC₅₀ SD</td>
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<tr>
<td>Rhesus (ng/ml)</td>
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<td></td>
<td>27.6 13.3 12.2 4.0</td>
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<tr>
<td>Human (ng/ml)</td>
<td>11.4 ND 37.2 ND</td>
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<td>21.3 ND 8.4 ND</td>
<td></td>
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</table>

Competitive binding assay used the saturating concentrations of natalizumab-Alexa647 and vedolizumab-Alexa647 at 100 and 200 ng/ml, respectively.

ND, The SD could not be calculated for these donors because only two donors were analyzed. These data are nonetheless consistent with more thorough investigations of binding affinities (23, 27).
Immune responses to rhMOG

Immune responses to rhMOG were monitored to determine if antagonizing the α4β1 integrins affected the generation of pathogenic cells. Peripheral blood was collected before immunization with rhMOG, once weekly thereafter, and at the time of necropsy. The anti-rhMOG proliferative responses of PBMCs were monitored weekly and in splenocytes at necropsy. An anti-rhMOG proliferative response was observed in splenocytes (Fig. 3A) and PBMCs (data not shown) from each animal. The group means for the placebo control, natalizumab, and vedolizumab groups were comparable. EAE developed in the two animals exhibiting the weakest anti-rhMOG proliferative responses at necropsy (Fig. 3A), indicating that all animals generated sufficient numbers of pathogenic cells for inducing EAE.

An Ab response to rhMOG was also detected in the serum of each animal at necropsy (Fig. 3B). The anti-rhMOG IgG means of the placebo, natalizumab, and vedolizumab groups were also comparable (Fig. 3B), and both animals exhibiting the weakest anti-rhMOG IgG response also developed EAE (Fig. 3B), illustrating that each animal had an anti-MOG response capable of inducing EAE. These data indicate that the mechanism causing the inhibition of EAE by natalizumab was not attributable to inhibiting the induction of anti-rhMOG responses.

Effects on cerebral inflammation and demyelination

Clinical signs of EAE result from lesions within brain white matter. These pathological changes were quantified by postmortem MRI of formalin-fixed brain hemispheres. Conventional T2-weighted (Fig. 4A, 4D), quantitative relaxation time (Fig. 4B, 4E), and MTR (Fig. 4C, 4F) images were obtained for each animal and used by a blinded imaging specialist (E. B.) to quantify the magnitude of cerebral lesions. Compared with white matter, lesions showed increased T2 relaxation time values and decreased MTR values. The mean semiquantitative values for lesion loads in brain hemispheres from the natalizumab group trended lower than those from the placebo and vedolizumab groups were also comparable (Fig. 4F), and both animals exhibiting the weakest anti-rhMOG proliferative responses at necropsy (Fig. 3A), indicating that all animals generated sufficient numbers of pathogenic cells for inducing EAE.

In contrast, desaturation of the α4β7 integrin was not observed in animals dosed with vedolizumab. Vedolizumab–Alexa 647 bound to 25–42% of total memory helper T lymphocytes (CD3+CD45RA+), naive and memory cytotoxic T lymphocytes (CD3+CD8+CD45RA+), naive helper T lymphocytes (CD3+CD4+CD45RA+), and monocytes (CD14+) (data not shown) from each animal. The group means for the placebo control, natalizumab, and vedolizumab groups were comparable (Fig. 4F), and both animals exhibiting the weakest anti-rhMOG proliferative responses at necropsy (Fig. 3A), indicating that all animals generated sufficient numbers of pathogenic cells for inducing EAE.

Table II. Overview of animal demographics

<table>
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<tr>
<th>Group</th>
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occurred in these animals. In contrast, desaturation of the α4β7 integrin was not observed in animals dosed with vedolizumab. Vedolizumab–Alexa 647 bound to 25–42% of total memory helper T lymphocytes prior to dosing with vedolizumab, and this binding remained blocked post dose, on days 7, 14, and 21 (Fig. 1F). No restoration of binding was observed, indicating that the α4β7 integrin remained saturated by vedolizumab for the duration of the investigation in each animal. Comparable results were obtained for both Abs on naive helper T lymphocytes (CD3+CD4+CD45RA+), naive and memory cytotoxic T lymphocytes (CD3+CD8+CD45RA+), total T lymphocytes (CD3+), and monocytes (CD14+) (data not shown).

Effects on clinical signs of EAE

In this investigation, four of eight placebo-dosed animals developed clinical signs of EAE. This finding contrasts with the natalizumab group, in which one of seven animals developed clinical signs of EAE (Table III). The mean time of onset of clinical signs of EAE was significantly shorter (p = 0.0336) for the placebo control animals, compared with animals exposed to natalizumab (Fig. 2A). In contrast, four of seven animals in the vedolizumab group developed clinical signs of EAE (Table III), and the onset of clinical signs in these animals was not significantly delayed (p = 0.1350), compared with the placebo-treated animals (Fig. 2B). The time to onset of clinical signs of EAE observed in this study was also compared with control group data from a previous investigation (27) (Fig. 2C). Both the historical control group and the vedolizumab group have a median survival of 21 d to time of onset of EAE symptoms (p = 0.3100). The placebo group has a median survival of 25 d, which is also not statistically different from that of the historical control group (p = 0.4284).

Table III. Experiment phases, groups, and individual EAE scores

<table>
<thead>
<tr>
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<th>Day of Sacrifice</th>
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Following MRI, the formalin-fixed hemispheres were processed for histological examination. Three blocks of tissue were excised from the same regions of each hemisphere, representative sections were prepared, and cerebral demyelination (Fig. 5A–C) and inflammation (Fig. 5D–L) were quantified by an anatomic pathologist (J.B.). Four animals in the placebo control group, one animal in the natalizumab group, and three animals in the vedolizumab group exhibited demyelinating lesions, and each of these animals also exhibited clinical signs of EAE (Fig. 5M). Five animals in the placebo control group, one animal in the natalizumab group, and five animals in the vedolizumab group exhibited inflammation (Fig. 5N). The animals with the largest brain infiltrates exhibited demyelinating lesions and clinical signs of EAE, whereas two animals with mild infiltrates (P8 and V5) did not demonstrate demyelination and EAE. These data indicate that the animals P8 and V5 were also developing EAE; however, they may have been euthanized prior to demyelination and the development of clinical signs of EAE because they were time-matched comparators to an animal already exhibiting clinical signs of EAE. The composite group mean score for demyelination (Fig. 5M) and inflammation (Fig. 5N) was nonetheless significantly lower ($p = 0.0098$) in the natalizumab group than in the placebo group, whereas the vedolizumab composite group mean score was not significantly different from those for the placebo group. Collectively, these data indicate that natalizumab inhibited inflammation and the development of demyelinating lesions, whereas vedolizumab had no effect.

The cerebral lesions contained high numbers of neutrophilic polymorphonuclear granulocytes (PMN), some eosinophilic PMNs, and mononuclear leukocytes (Fig. 5D–F), which is characteristic of the rhesus monkey EAE model (27). The mononuclear leukocytes were qualitatively similar between groups and consisted of CD3+ T lymphocytes (Fig. 5D–F), a few CD20+ B lymphocytes (Figure 5G–I), and numerous MRP14++ macrophages (Fig. 5J–L). Quantification of immunohistochemical staining revealed that cerebral sections from animals exposed to natalizumab contained lower levels of CD3+ T lymphocytes, MRP14++ macrophages, and CD20+ B lymphocytes than did comparable sections from animals exposed to placebo or vedolizumab (Table IV). The infiltrates of the two animals that did not demonstrate demyelination and EAE (P8 and V5) contained proportionately more CD3+ T lymphocytes and fewer MRP14+ macrophages (Table IV), indicating that T cells may arrive at the site of a potential lesion prior to macrophages, PMNs, and demyelination. Taken together, these data demonstrate that natalizumab inhibited cerebral inflammation and demyelination, whereas vedolizumab did not; thus, it can be inferred that the $\alpha_4\beta_1$ integrin mediates the inflammation and formation of cerebral lesions in EAE.

Infiltration of the CSF by leukocytes

Leukocytes migrate into the CSF of monkeys developing EAE, and the level of various subsets was measured as an additional assessment of immune surveillance of the CNS. Serial CSF samples were collected before and after exposure to placebo control, natalizumab, or vedolizumab. A relative increase in CSF leuko-
cytes from baseline levels was observed in animals that developed clinical signs of EAE primarly at the time of necropsy. Elevations in CSF leukocytes were observed in eight of nine animals that developed EAE, and no elevations were observed in animals that did not develop EAE (Fig. 6A–C). Three placebo control animals (P1, P3, and P4), one natalizumab animal (N2), and four vedolizumab animals (V1, V2, V4, and V6) exhibited elevations in CSF leukocytes and EAE (Fig. 6A–C). Collectively, CSF samples taken from animals without overt neurological deficit (EAE score 0) contained a median of 0.00 (range 0.00–0.07) \times 10^9/l leukocytes (n = 68). Samples taken from animals with an EAE score ≥ 2 (n = 9) contained a significantly (p < 0.0001) higher number of leukocytes, with a median of 0.15 (0.10–0.44) \times 10^9/l. The absence of CSF infiltrates in animals exposed to natalizumab indicates that natalizumab blocks migration of leukocytes into the CSF. Conversely, the presence of infiltrates in animals exposed to vedolizumab demonstrates that this Ab does not block migration of leukocytes into the CSF.

To determine the phenotype of leukocytes infiltrating the CSF in diseased animals, the relative percentages of leukocyte subsets in the CSF of animals exhibiting signs of EAE were compared with relative percentages of subsets in peripheral blood of the same animal (Fig. 6D–H). The relative percentage of CD3+ T cells is higher in CSF than in peripheral blood (Fig. 6D), indicating that the CSF infiltrate contains disproportionately more T cells than other leukocyte subsets. The CD4/CD8 ratio is generally similar in peripheral blood and CSF (Fig. 6E), indicating that proportional amounts of CD4+ and CD8+ cells migrated into the CSF. The relative percentage of CD14+ monocytes is also generally similar in CSF compared with blood (Fig. 6F), suggesting this subset is also migrating into the CNS. In contrast, the relative percentages of CD3+CD16+ NK cells and CD20+ B lymphocytes are lower in the CSF than in peripheral blood (Fig. 6G, 6H), indicating that disproportionately fewer of these subtypes are migrating into the CSF. These collective data thus illustrate that helper (CD3+CD4+) and cytotoxic (CD3+CD8+) T cells and monocytes (CD14+) migrated preferentially into the CSF, whereas B lymphocytes (CD20+) and NK cells (CD3+CD16+) did not migrate as readily into the CSF in animals developing EAE.

Similar to infiltration of the CSF by leukocytes, anti-rhMOG Abs were found only in the CSF of animals with EAE, although not all animals with EAE had Abs in the CSF (data not shown). Moreover, anti-rhMOG Abs were not found in CSF samples taken at time points prior to necropsy (data not shown).

Elevation of leukocytes in the vasculature corresponds to inhibition of EAE

The MRI and histology analyses demonstrated that natalizumab blocked migration of leukocyte subsets into the CNS. A consequence of this mechanism of action, in conjunction with continued homeostatic production, would be the accumulation of these subsets within the vasculature of these animals. Exposure to natalizumab induced significant (p < 0.05) elevations in the level of mature WBCs in the vasculature of animals, within 5 d of initial exposure (the shortest duration examined), compared with pre-exposure baselines and with time-matched placebo controls (Fig. 7A). This leukocytosis occurred without significant changes in RBC indices (data not shown) or neutrophils (Fig. 7B). The natalizumab-induced leukocytosis consisted of significant (p < 0.05) elevations in monocytes (Fig. 7C), eosinophils (Fig. 7D), and lymphocytes (Fig. 7E), compared with pre-exposure baselines and with time-matched placebo controls. The lymphocytosis consisted of elevations in total T lymphocytes, total and memory helper T lymphocytes, total and memory cytotoxic T lymphocytes, and total B lymphocytes, but not NK cells (Fig. 7F). Each animal exposed to natalizumab exhibited elevations in these subsets, and the one animal that developed EAE (N2) and exhibited CNS infiltrates showed the smallest overall elevation in vascular lymphocytes (data not shown). These data indicate that exposure to natalizumab sequesters specific subsets of leukocytes in the vasculature and that this effect may explain the inhibition of EAE.

In contrast to natalizumab, vedolizumab did not affect levels of total leukocytes, monocytes, eosinophils, or lymphocytes, compared with pre-exposure baselines and with time-matched placebo controls, in any animal (Fig. 7A–E). Moreover, exposure to vedolizumab did not affect levels of lymphocyte subsets that were elevated by natalizumab, including memory helper T lymphocytes, memory cytotoxic T lymphocytes, and B lymphocytes (Fig. 7F). These data demonstrate that natalizumab elicited a broader PD profile than did vedolizumab and this difference is consistent with the distinct effects of these Abs on the development of EAE in these animals.
Discussion

The use of therapeutics can be limited by adverse events associated with modulating a pleiotropic target. The utility of natalizumab in multiple sclerosis and CD indications, for example, is limited by an association with PML, a severely debilitating, often fatal opportunistic infection of the brain caused by reactivation of latent JC virus (17). The anti-inflammatory activity of natalizumab in multiple sclerosis is attributed to blocking transmigration of leukocytes, including T lymphocytes, across the endothelium into inflamed parenchymal tissue of the brain (Tysabri, U.S. package insert, 2011). It has also been postulated that this mechanism of action predisposes patients to PML because it could also block immune surveillance for reactivated virus by protective memory T lymphocytes (17–19). This theory remains largely untested, however, because an appropriate model of PML does not exist (17).

EAE is an experimental model of immune surveillance of the CNS that resembles some aspects of multiple sclerosis and is often used to assess potential perturbations of immune surveillance resulting from pharmacological intervention. In one version of this model, effector memory T lymphocytes are initially generated in the lymph nodes draining the dermal sites of Ag exposure. These primed, anti-MOG effector T cells subsequently survey additional organs for the presence of Ag. Upon encountering endogenous MOG in the brain parenchyma, T lymphocytes initiate a pathological response that destroys oligodendrocytes, demyelinates axons, and causes EAE. Natalizumab is an $\alpha_4\beta_1$ and $\alpha_4\beta_7$ dual antagonist and inhibits the development of EAE in guinea pigs by reducing the migration of leukocytes into brain parenchyma and by reducing plaque formation (10, 13). Natalizumab also inhibits the firm adhesion of human T cells to the inflamed blood–brain barrier in mice with acute EAE, by blocking adhesion of the $\alpha_4\beta_7$ integrin to VCAM-1 (15). These data are corroborated by additional EAE investigations using other $\alpha_4\beta_1$ and $\alpha_4\beta_7$ antagonists (8–13) and, moreover, in mice lacking expression of the $\alpha_4$ or $\beta_7$ integrin chains (34). Collectively, these data conclusively demonstrate that the $\alpha_4\beta_1$ integrin mediates the migration of leukocytes into the CNS.

Studies specifically addressing the role of $\alpha_4\beta_7$ integrin in EAE pathogenesis have produced inconsistent results. A seminal investigation demonstrated that the $\alpha_4\beta_7$ integrin was not required for the development of EAE in a SJL/N mouse model (9). Blocking the $\alpha_4\beta_7$ integrin exclusively or in conjunction with the $\alpha_4\beta_1$ integrin did not affect the development of EAE. In contrast, blocking the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins or VCAM-1 inhibited clinical and histopathological signs of EAE (9). A different model (i.e., C57BL/6 mouse), however, indicated that $\beta_7$ integrins and MAdCAM-1 contributed to development of EAE. Blocking the $\alpha_4\beta_7$ and $\alpha_4\beta_7$ integrins partially impaired development of EAE, but less so than blocking the $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins (35). Blocking MAdCAM-1 in the same model also ameliorated EAE.
(36). Conversely, ectopic expression of MAdCAM-1, specifically on the endothelial lumen of the blood–brain barrier, did not enhance αβ7 integrin–mediated immune-cell trafficking into the CNS, nor aggravate EAE in the C57BL/6 mouse model (37). These observed differences have been attributed to the different mouse strains investigated and/or the different protocols for EAE induction used in these studies (2). They nonetheless illustrate that the αβ1 integrin mediates immune surveillance of the CNS in mice.

To date, comparable investigations have not been conducted in primates. Therefore, we assessed the effects of antagonizing the α4 integrins in the rhesus monkey EAE model because this is the only established EAE model (28) in which both vedolizumab and natalizumab are pharmacologically active (Table I). This specific rhMOG protocol was chosen because all animals develop EAE (27) and generally do so prior to the development of strong neutrophil infiltration of the brain (Table IV).

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The primary objective of the investigation was to determine if vedolizumab decreased infiltration of the brain by leukocytes, as assessed by histopathological examination, and achieving this required comparing time-matched control and therapeutic samples from euthanized animals. Animals were consequently grouped together and the entire group was euthanized, once the indicator animal had developed clinical signs of EAE. A limitation of this experimental design was that it confounded assessment of the onset of clinical signs for treatment groups because nonindicator animals were euthanized before they developed EAE. Animals P8 and V5 are specific examples, neither of which exhibited clinical signs of EAE or demyelination at necropsy, but did demonstrate T cell infiltration of the brain (Table IV).

In addition, a consequence of the experimental design is that a strong inhibitory effect in one phase, but not in the other, would cause the incidence of disease in the placebo animals in both phases to differ. This difference results from euthanizing all animals in a group once one exhibits clinical signs of EAE (Table III). For example, a strong inhibitory effect by a mAb would result in only placebos exhibiting EAE (i.e., phase 1). No effect would result in one to two placebos exhibiting EAE (e.g., phase 2), based on the unbalanced pairing of placebo to mAb-exposed animals (Table III). Conversely, a stimulatory effect would result in all mAb-exposed animals exhibiting EAE. Therefore, the difference in the incidence of EAE between placebos in each phase is primarily attributable to natalizumab having a strong inhibitory effect on the development of EAE, an effect not shared by vedolizumab. This conclusion is supported by the observation that the time of EAE onset in the placebo animals and historical controls described by Kerlero de Rosbo et al. (27) was not significantly different (Fig. 2C). Half of the animals exposed to placebo (four of eight) and vedolizumab (four of seven) developed clinical signs of EAE and, moreover, did so with similar kinetics (Fig. 2). The time to onset of EAE symptoms of the historical controls and the vedolizumab group were also not significantly different (Fig. 2C). These data contrast with those of the natalizumab group, in which 14% of animals (one of seven) developed clinical signs of EAE (Fig. 2A, 2C). It can thus be inferred that blocking the αβ1 integrin, but not the αβ2 integrin, delayed the onset of clinical signs of EAE.

The delay in onset of EAE by natalizumab was not attributable to inhibition of the generation of autoreactive memory T lymphocytes; the two animals exhibiting the weakest anti-MOG proliferative responses developed EAE (Fig. 3). The delay was attributable to blocking infiltration of the CNS by leukocytes.

The MRI revealed that the mean values for lesion loads in brain hemispheres from the natalizumab group was not significantly different from those of the natalizumab group, in which 14% of animals (one of seven) developed clinical signs of EAE (Fig. 2A, 2C). It can thus be inferred that blocking the αβ1 integrin, but not the of EAE by preventing inflammation and the formation of acute inflammatory cerebral lesions.
The clinical manifestations of EAE are associated with inflammation and demyelinating lesions in histological sections of brain white matter. Comparable levels of inflammation and demyelination were observed in the white matter of animals exposed to placebo control or vedolizumab, but significantly less (p < 0.01) was observed in animals exposed to natalizumab (Fig. 5). These data are consistent with similar reductions in brain inflammation in postmortem analyses of brain tissue from patients with multiple sclerosis and a patient with PML exposed to natalizumab (39).

Levels of leukocyte subsets in the CSF of rhesus monkeys were also measured as an independent assessment of immune surveillance of the CNS. An increase in the level of CSF leukocytes took place concurrently with the onset of clinical signs of EAE in this investigation, and animals in the placebo and vedolizumab groups exhibited higher levels than animals in the natalizumab group (Fig. 6). These data are consistent with clinical studies demonstrating that a single dose of vedolizumab does not alter CD4⁺ and CD8⁺ T lymphocyte levels or ratio in the CSF of healthy volunteers (40). These data contrast with those from multiple sclerosis patients exposed to natalizumab; they exhibit significantly lower levels of leukocytes and CD4⁺ and CD8⁺ T lymphocytes in CSF (41). These data collectively demonstrate that infiltration of the primate CNS by leukocytes in general is mediated by the α_4β_1 integrin and not the α_4β_7 integrin.

Immunohistochemical analysis of the lesions revealed that a prominent feature of the lesions of animals with EAE is acute inflammation and demyelination, with mostly granulocytes in the core. At the rim, T cells and macrophages are found, but no B cells (Fig. 5). No significant differences between the groups were found with regard to the type of infiltrating cells. It is noteworthy that the two animals with inflammation but without demyelination or EAE (P8 and V5) may represent earlier stages of lesion formation in the pathogenesis of EAE. Presumably, memory T lymphocytes are the initial type of cells arriving at a site of lesion formation. These
cells recognize endogenous MOG and initiate an autoimmune reaction, which triggers an inflammatory cascade that subsequently recruits neutrophils and monocytes, culminating in a necrotic lesion. It is important to note that neutrophils do not express $\alpha_\beta_2$ or $\alpha_\beta_7$; thus, natalizumab does not inhibit their recruitment directly. It is likely that neutrophil recruitment is induced indirectly by natalizumab, perhaps by preventing generation of chemotactic stimuli, which result from immune surveillance by memory T cells. Although the clinical features of EAE are also determined by lesions in the spinal cord, the clear dichotomy of demyelination in the brain of animals with EAE, and no demyelination in animals without EAE, suggests that this is a very acute process. This is unlike the marmoset EAE model, in which demyelination can be found in animals without clinical EAE (42, 43).

Dose-dependent increases in the level of mature lymphocytes in the vasculature upon exposure to natalizumab, without concomitant elevations of more immature forms, has been attributed to inhibiting extravasation of lymphocytes from the circulation into parenchymal tissues (26, 44). The blockade of CNS infiltration in monkeys exposed to natalizumab (Fig. 5) was accompanied by a significant ($p < 0.05$) increase in the absolute level of leukocyte subsets in peripheral blood of these animals (Fig. 7). Natalizumab did not significantly affect levels of neutrophils (Fig. 7B), which is consistent with the lack of expression of $\alpha_4$ integrins by these leukocytes (4, 5, 22, 29). Rather, the natalizumab-induced leukocytosis consisted of significant ($p < 0.05$) elevations in monocytes, eosinophils, and lymphocytes, including total T lymphocytes, total and memory helper T lymphocytes, total and memory cytotoxic T lymphocytes, and total B lymphocytes (Fig. 7). These effects are consistent with expression of $\alpha_4$ integrins by these subsets (4, 5, 22, 29). Of interest, exposure to natalizumab did not affect levels of NK cells (Fig. 7), despite expression of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ (4, 22, 29). Similar overall results have been observed in healthy cynomolgus and rhesus monkeys exposed to natalizumab (26). These data illustrate that the expression pattern of these integrins is not an accurate predictor of potential functional effects of corresponding antagonists (1–3). Finally, these effects in monkeys are consistent with the vascular leukocytosis, lymphocytosis, monocytosis, basophilia, and eosinophilia observed in multiple sclerosis and IBD patients exposed to natalizumab (45, 46), and further illustrate that elevation of vascular leukocyte levels is a useful biomarker of the scope of PD activity of integrin antagonists.

A fundamental difference in blocking both $\alpha_4$ integrins versus the $\alpha_4\beta_7$ integrin exclusively is the proportion of the total leukocyte population that is affected, given that the $\alpha_4\beta_1$ integrin is more widely expressed than the $\alpha_4\beta_7$ integrin (4, 5, 22). The population of leukocytes affected by natalizumab in this investigation was indeed larger and more diverse in composition than that affected by vedolizumab. Exposure to natalizumab elevated $\sim 40\%$ of the total leukocyte population in the vasculature, whereas vedolizumab did not affect these subsets of leukocytes (Fig. 7). Vedolizumab does elevate a gut-homing ($\alpha_4\beta_7$$^{\text{high}}$) subpopulation of memory (CD45RA$^-$$^-$) T lymphocytes in cynomolgus monkeys, which represents $\sim 1\%$ of vascular leukocytes (23). Similar data have emerged from clinical trials. Natalizumab induced significant leukocytosis, lymphocytosis, monocytosis, basophilia, and eosinophilia in CD patients (16, 47), whereas vedolizumab did not affect levels of total leukocytes, lymphocytes, monocytes, basophils, or eosinophils in peripheral blood of CD or UC patients (24, 25). The relatively broad target population and PD effects of natalizumab may thus explain the pleiotropic effects observed to date, including those in the bone marrow (48, 49), the central and peripheral nervous systems (50), the upper and lower respiratory system, the urinary system, the musculoskeletal system, and the skin (Tysabri, U.S. package insert, 2011).

Overall, these data illustrate that blocking the $\alpha_4\beta_1$ integrin sequesters a larger and more diverse population of leukocytes in the vasculature, and consequently impairs immunosurveillance more broadly, than blocking the $\alpha_4\beta_7$ integrin. This investigation in particular demonstrates that blocking the $\alpha_4\beta_7$ integrin exclusively does not impair immune surveillance of the CNS in rhesus EAE. This same therapeutic approach (i.e., blocking $\alpha_4\beta_7$) elicited anti-inflammatory activity in the gastrointestinal tract of colitic monkeys (21) and in UC (24) and CD (25) patients. Thus, targeting the $\alpha_4\beta_7$ integrin exclusively may provide efficacy in UC and CD patients without impairing immune surveillance of the CNS.
Acknowledgments We thank Carl Alden, Robert Egan, Vivek Kadambi, Cathy Milch, Asit Parikh, and Henk van Westbroek for technical assistance, formative discussions, preparing the figures, and/or review of the manuscript.

Disclosures L.-Y., T.W., V.C., and E.R.F. were employed by Millennium Pharmaceuticals during this investigation. The other authors have no financial conflicts of interest.


23. Hesterberg, P. E., D. Winsor-Hines, M. J. Briskin, D. Soler-Ferran, C. Merrill, and Henk van Westbroek for technical assistance, formative discussions, preparing the figures, and/or review of the manuscript.


