The Binding Specificity and Selective Antagonism of Vedolizumab, an Anti-\(\alpha_4\beta_7\) Integrin Therapeutic Antibody in Development for Inflammatory Bowel Diseases

Dulce Soler, Tobias Chapman, Li-Li Yang, Tim Wyant, Robert Egan, and Eric R. Fedyk

Millennium Pharmaceuticals Inc., a Takeda Pharmaceuticals Company, Cambridge, Massachusetts

Received March 26, 2009; accepted June 8, 2009

ABSTRACT

Vedolizumab is a humanized monoclonal antibody that targets the \(\alpha_4\beta_7\) integrin exclusively, and modulates inflammation in the gastrointestinal tract without inducing the systemic immunosuppression that characterizes anti-\(\alpha_4\) chain monoclonal antibodies, such as natalizumab. This unique pharmacologic profile is largely attributable to four determinants. The first determinant is the restriction of the expression of the \(\alpha_4\beta_7\) integrin to subsets of leukocytes. Vedolizumab does not bind to the majority of memory CD4\(^+\) T lymphocytes (60%), neutrophils, and most monocytes. The highest level of vedolizumab binding is to a subset (~25%) of human peripheral blood memory CD4\(^+\) T lymphocytes that include gut-homing interleukin 17 T-helper lymphocytes. Vedolizumab also binds to eosinophils at high levels, and to naive T-helper lymphocytes, naive and memory cytotoxic T lymphocytes, B lymphocytes, natural killer cells, and basophils at lower levels; vedolizumab binds to memory CD4\(^+\) T and B lymphocytes with subnanomolar potency (\(EC_{50} = 0.3–0.4\) nM). The second determinant is binding specificity; vedolizumab binds exclusively to the \(\alpha_4\beta_7\) integrin, and not to the \(\alpha_4\beta_1\) and \(\alpha_4\beta_7\) integrins. The third determinant is selective antagonism; vedolizumab selectively inhibits adhesion of \(\alpha_4\beta_7\)-expressing cells to mucosal addressin cell adhesion molecule 1 (median inhibition concentration [IC\(_{50}\)] = 0.02–0.06 \(\mu\)g/ml) and fibronectin (IC\(_{50}\) = 0.02 \(\mu\)g/ml), but not vascular cell adhesion molecule 1. The fourth determinant is the gastrointestinal-specific tropism of the \(\alpha_4\beta_7\) integrin function. These pharmacologic properties of vedolizumab, in conjunction with the gastrointestinal tropism of \(\alpha_4\beta_7\) integrin function, may ultimately confer an improved risk-to-benefit profile for patients with inflammatory bowel diseases.

The inflammatory bowel diseases (IBDs), ulcerative colitis (UC) and Crohn’s disease (CD), are chronic diseases of the gastrointestinal (GI) tract characterized by an exacerbated inflammatory cell infiltrate in the gut mucosal tissue (Xavier and Podolsky, 2007). Multiple inflammatory cell types, including neutrophils, macrophages, dendritic cells, and lymphocytes, participate in the pathogenesis of IBD, with lymphocytes having a central role in the induction and maintenance of the chronic inflammatory process in the lamina propria (Xavier and Podolsky, 2007). Infiltration of the GI tract by T lymphocytes is a well documented pathogenic mechanism of IBD, and the molecular mechanisms by which these lymphocytes enter the gut are distinct from those in other peripheral tissues, such as the skin and central nervous system (Butcher and Picker, 1996; Engelhardt et al., 1998; Engelhardt and Briskin, 2005; Salmi and Jalkanen, 2005; Agace, 2006). The complex infiltration process in the GI tract requires the coordinated interaction of several adhesion and signaling molecules on the surface of T lymphocytes (selectins, integrins, chemokine receptors) with their corresponding ligands on the endothelium. The \(\alpha_4\beta_7\) integrin mediates the infiltration of the GI tract by memory T lymphocytes, binding to mucosal addressin cell adhesion molecule 1 (MAdCAM-1) on endothelial cells, and blockade of this interaction provides efficacy in animal models of IBD (Hesterberg et al., 1996; Picarella et al., 1997), and in patients with UC (Feagan et al., 2005) and CD (Feagan et al., 2008).

The \(\alpha_4\beta_7\) integrin is consequently an ideal therapeutic target for IBD and is currently being targeted through three different strategies. Two of these strategies target either the \(\alpha_4\) chain or the \(\beta_7\) chain. They are not specific for the \(\alpha_4\beta_7\)
integrin and bind to other integrins containing these chains, specifically the $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. The $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins mediate effects within and outside the GI tract. The $\alpha_4\beta_1$ integrin is postulated to locate and retain T lymphocytes within the epithelium of numerous tissues by binding E-cadherin on the basal surface of epithelial cells (Kilshaw, 1999). The $\alpha_5\beta_1$ integrin mediates extravasation of lymphocytes, monocytes, and eosinophils into numerous types of tissues by binding to vascular cell adhesion molecule 1 (VCAM-1) expressed on the luminal surface of endothelium, and to fibronectin within extracellular matrix (González-Amaro et al., 2005). Antagonizing the $\alpha_4\beta_1$ or $\alpha_5\beta_1$ integrin thus has a systemic effect.

The humanized anti-$\alpha_4$ antibody, natalizumab, elicits effects in numerous tissues, including leukocytosis (Ghosh et al., 2003; Sandborn et al., 2005; Targan et al., 2007), mobilization of hematopoietic stem cells (Bonig et al., 2008; Zohren et al., 2008), and inhibition of leukocyte trafficking into the central nervous system (del Pilar Martin et al., 2008). Indeed, antagonizing both the $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins may explain efficacy in the central nervous system (Miller et al., 2003) and the GI tract, respectively (Ghosh et al., 2003; Sandborn et al., 2005; Targan et al., 2007). However, administration of natalizumab is associated with systemic immunosuppression; for example, increased incidence of the fatal infectious disease progressive multifocal leukoencephalopathy (PML) (Berger and Koralnik, 2005; Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005; Ransohoff, 2005; Van Assche et al., 2005). It is postulated that the anti-inflammatory mechanism driving the efficacy of natalizumab in multiple sclerosis may also predispose patients to progressive multifocal leukoencephalopathy by decreasing immunosurveillance in the central nervous system (Berger, 2006; Berger and Houff, 2006; Koralnik, 2006; Niiro et al., 2006; Stève et al., 2006a,b; del Pilar Martin et al., 2008).

A third strategy is exclusive targeting of the $\alpha_4\beta_1$ integrin, which is used by vedolizumab (former versions known as MLN0002, MLN02, and LDP-02). Vedolizumab is a humanized version of Act-1, a mouse antibody (Lazarovits et al., 1984) that binds to a conformational epitope that is unique to the heterodimerization of the human $\alpha_4$ chain with the $\beta_7$ chain (Schweighoffer et al., 1993; Tidswell et al., 1997). Act-1 therefore binds specifically to the $\alpha_4\beta_7$ integrin, and administration to colitic cotton-top tamarins leads to the resolution of disease (Hesterberg et al., 1996). Vedolizumab binds to the $\alpha_4\beta_7$ integrin on peripheral blood lymphocytes and inhibits adhesion of the lymphocyte to MadCAM-1. Humanized Act-1 has demonstrated statistically significant efficacy in placebo-controlled phase 2 clinical trials of patients with moderately active UC (Feagan et al., 2005) and in patients with moderately active CD (Feagan et al., 2008). The enhanced specificity of vedolizumab may ultimately confer an improved risk-benefit ratio for patients with IBD. The data reported herein characterize the binding specificity and potency of vedolizumab and the associated selective antagonism of adhesion.

**Materials and Methods**

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Cell Lines and Culture Media.** The human B-cell lymphoma cell line, RPMI8866 (stably expressing $\alpha_4\beta_1$), was a kind gift from Dr. David Erle (University of California, San Francisco). The human B-cell lymphoma cell line, RAMOS (stably expressing $\alpha_4\beta_1$, originally sourced from the American Type Culture Collection, Manassas, VA), and $\alpha_4\beta_2$, L1.2 cell transfectants were generated in-house by use of cDNA provided by Christina Parker and Michael Brenner (Brigham and Women’s Hospital, Boston, MA). Culture medium for RPMI8866 and RAMOS cell lines consisted of RPMI 1640 medium supplemented with 1% penicillin/streptomycin and 1% l-glutamine (Invitrogen, Carlsbad, CA) and 10% US-defined fetal bovine serum (HyClone Laboratories, Logun, UT). Culture medium for $\alpha_4\beta_2$, transfectants consisted of RPMI 1640 medium supplemented with 1% penicillin/streptomycin, 1% sodium pyruvate, 1% l-glutamine, and 2 $\mu$g/ml puromycin (Invitrogen), 0.1% $\beta$-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), and 10% US-defined fetal bovine serum (HyClone Laboratories).

**Immunohistochemistry of Normal Human Tissues.** The binding specificity of vedolizumab in 38 different types of normal human tissues (three independent donors per tissue type) was investigated by immunohistochemistry. The quality of these tissues was verified by robust staining with a positive-control antibody against $\beta_2$-microglobulin (Dako North America, Inc.). Sections (5 $\mu$m) were cut from fresh-frozen tissue samples embedded in OCT Compound (Sakura Finetek USA, Inc., Torrance, CA) and fixed in acetone for 10 min at room temperature. Just before staining, slides were fixed for 10 s in 10% neutral buffered formalin. Acetone/formaldehyde-fixed cryosections were rinsed twice in phosphate-buffered saline (PBS) and incubated for 20 min with a protein block (PBS; 0.5% casein; 5% human gamma globulins; 0.02% goat IgG; 1 mg/ml heat-aggregated human IgG) designed to reduce nonspecific binding. Unconjugated vedolizumab or a negative control human IgG1 (Millipore Bioscience Research Reagents, Temecula, CA)

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-

---

**Binding and Inhibitory Properties of Vedolizumab**

---

**Proteins and Antibodies.** Ninety-six-well plates coated with recombinant human VCAM-1/fragment crystallizable (Fc) chimera protein, an alternatively spliced form of human fibronectin contain-
tion + Kit. All slides were rinsed with tap water, counterstained with hematoxylin, washed, “blued” in saturated lithium carbonate, washed, dehydrated through alcohols, cleared in xylene, and coverslipped. Staining intensity was graded semiquantitatively by a board-certified anatomic pathologist.

**Staining of Cell Lines and Whole Blood.** For the staining of cell lines, cells were resuspended at 2 × 10^6/ml in FACS buffer (5% fetal bovine serum and 0.05% sodium azide in D-PBS (Dulbecco’s phosphate buffers saline without calcium and magnesium) (VWR, West Chester, PA) and 200-μl samples were stained with the appropriate monoclonal antibodies (see “Proteins and Antibodies”) at 4°C for 30 min. Samples were washed with FACS buffer and analyzed by flow cytometry (FACSCalibur, BD). For the staining of human whole blood, 200-μl samples from healthy human volunteers were stained with the appropriate monoclonal antibody at 4°C for 30 min. Red blood cells were lysed with BD FACS lysis solution, and samples then washed with FACS buffer and analyzed by flow cytometry. In all cases, antibodies were used at saturating concentrations and, in many cases, up to four antibodies were used per sample. Appropriate single- or two-color control stains were also performed.

**Saturation and Competitive Binding Analyses.** The potency of vedolizumab binding to human leukocytes was examined through the generation of 1) antibody saturation binding curves with labeled vedolizumab or Act-1, and 2) antibody binding competition curves for the competing binding of labeled antibody with unlabeled antibodies (vedolizumab, Act-1, or isotype control IgG). Experiments were performed in 96-well v-bottom plates (Corning Inc., Corning, NY). For saturation binding experiments and determination of EC_{50} values, 100 μl of human peripheral blood from healthy human volunteers was incubated with the labeled antibody at the indicated range of concentrations in a final volume of 200 μl. In these saturation experiments, at all concentrations tested, specific binding was demonstrated by competition with 20-fold molar excess of unlabeled antibody. For binding competition experiments, 100 μl of human peripheral blood was incubated with the labeled antibody at its EC_{50} and the unlabeled antibody at the indicated range of concentrations in a final volume of 200 μl. Plates were incubated at 4°C for 30 min and cells were then washed. Red blood cells were lysed, washed, and stained with antibodies specific for memory CD4+ T lymphocytes (CD4, CD45RO) and B lymphocytes (CD19). Binding to either memory CD4+ T or B lymphocytes was examined by flow cytometry with use of a FACSCalibur flow cytometer and CellQuest Pro software. The geometric mean fluorescence intensity (GMFI) values of the positive memory CD4+ T-lymphocyte population or the entire B-lymphocyte population were plotted against antibody concentration.

In binding competition experiments, GMFI data were plotted as percentage inhibition versus antibody concentration. EC_{50} (for saturation binding curves) or median inhibition concentration (IC_{50}; for inhibition of binding curves) values were determined from these graphs by use of GraphPad Prism Version 4 regression curve fits.

**Inhibition of MAdCAM-1 Binding to CD4+ Memory T Lymphocytes.** Vedolizumab inhibition of high-affinity binding of MAdCAM-1 to human peripheral blood memory CD4+ T lymphocytes was tested. Peripheral blood (90 μl) was incubated with a saturating concentration (3 μg/ml) of MAdCAM-1-murine-Fc fusion protein and 4 mM MnCl_{2} in a final volume of 100 μl for 1 h at room temperature, in the presence or absence of vedolizumab. This saturating concentration was determined from previous MAdCAM-1-Fc protein binding saturation curves with three independent donors. After washing with assay buffer (25 mM Tris, 4 mM MnCl_{2}, 2.7 mM KCl, 150 mM NaCl, 0.5% BSA, pH 7.2), the cells were stained with fluorescent-labeled anti-mouse IgG for 15 min at room temperature. After washing again, cells were incubated with mouse serum for 10 min at room temperature, followed by staining with anti-CD4 and anti-CD45RO antibodies for 15 min at room temperature. After washing, red blood cells were lysed with BD FACS lysis solution and analyzed by flow cytometry in a FACSCalibur with CellQuest Pro software. Antibody dose-dependent inhibition curves were obtained by plotting the percentage of memory CD4+ T lymphocytes that bound MAdCAM-1 versus antibody concentration with use of GraphPad Prism Version 4. IC_{50} values were determined from these graphs with use of GraphPad Prism Version 4 nonlinear regression curve fits.

**Results**

**Binding of Vedolizumab to Human Tissue ex Vivo.** The binding specificity of vedolizumab was investigated in 38 different types of normal human tissue by immunohisto-
chemistry. Binding of vedolizumab was restricted to the cell membrane of mononuclear cells in lymphoid tissues, mononuclear infiltrates in tissues of the GI tract and bladder, and mononuclear cells in the lumens of blood vessels (Table 1). In GI tract tissues, vedolizumab bound mononuclear cells organized in submucosal lymphoid nodules and/or scattered in lamina propria. The large and small intestine contained the highest frequency of mononuclear cells that were bound by vedolizumab (data not shown).

**Binding Specificity of Vedolizumab to Human Leukocytes ex Vivo.** Flow cytometry experiments were performed on human peripheral blood stained with vedolizumab or Act-1, anti-α4- and anti-β7 monoclonal antibodies (mAbs), and markers of leukocyte subsets (including memory and naive CD4 and CD8 T lymphocytes, B lymphocytes, natural killer cells, monocytes, basophils, eosinophils, and neutrophils). Experiments were designed so that coexpression of the α4β7 and α4β1 integrins and binding of vedolizumab to subsets expressing the α4β7 integrin could also be examined. Vedolizumab bound to the majority of B lymphocytes, naive CD4 and CD8 T lymphocytes, natural killer cells, and basophils at low to intermediate levels (Fig. 1, A and B). Vedolizumab bound to B lymphocytes at intermediate levels uniformly, to natural killer cells and basophils at low levels uniformly, and to naive CD4 and CD8 T lymphocytes from low to intermediate levels. Vedolizumab bound to approxi-

### Table 1

<table>
<thead>
<tr>
<th>Tissue Region</th>
<th>Stromal elements</th>
<th>Mononuclear infiltrate</th>
<th>Platelets</th>
<th>Polymorphonuclear cells</th>
<th>Blood vessel endothelium</th>
<th>Bone marrow</th>
<th>Brain cerebrum</th>
<th>Brain cerebellum</th>
<th>Breast</th>
<th>Colon</th>
<th>Mononuclear infiltrate</th>
<th>Blood cells</th>
<th>Mononuclear cells</th>
</tr>
</thead>
</table>

*Neg.*, negative.
mately 25% of the memory (CD45RO⁺) CD4⁺ T lymphocytes at high levels (α4β7hi) and to 5 to 10% at intermediate levels, and not to the rest of the population (Figs. 1, A and B, and 2A). Vedolizumab bound to the majority of the naive (CD45RO⁻) CD4⁺ and CD8⁺ T lymphocytes, and to approximately 50% of the memory (CD45RO⁺) CD8⁺ T lymphocytes, at low to intermediate levels (Fig. 1, A and B, and 2A). Vedolizumab bound to all eosinophils at intermediate to high levels, and to approximately 15% of monocytes at low levels, but did not bind to neutrophils (Fig. 1, A and B). The highest level of binding by vedolizumab was observed on the α4β7hi population of memory CD45RO⁺ CD4⁺ T lymphocytes (Fig. 1B). The specificity of binding to the α4β7 integrin by vedolizumab was verified by competition with Act-1; unlabeled Act-1 completely inhibited binding of vedolizumab on all leukocyte subsets examined (data not shown). The same antigen specificity between these two antibodies was also demonstrated by the complete inhibition of labeled Act-1 binding to all leukocytes exposed to unlabeled vedolizumab.

The binding profile of vedolizumab contrasts the expression profile of the α4β7 integrin. Expression of the α4β7 integrin, in general, was more widespread than binding by vedolizumab. Vedolizumab bound naive CD4⁺ T lymphocytes at varying levels (Fig. 2A), whereas these cells expressed the α4β7 integrin uniformly (Fig. 2B). Vedolizumab bound to a specific subset of the memory CD4⁺ T-lymphocyte population; vedolizumab bound approximately 25% of memory CD4⁺ T lymphocytes at high levels (Fig. 2A), whereas most of these cells expressed relatively high levels of the α4 and/or β7 chains (Fig. 2B). Three major subsets of memory CD4⁺ T lymphocytes can be defined by α4β7 integrin expression: α4⁺, α4hiβ7hi, and α4loβ7hi. Vedolizumab binds to most (~80%) of the
\(\alpha_4^{hi}\beta_1^{lo}\) T-helper lymphocytes at high levels (Fig. 2C); this is the subset that contains the majority of the \(\alpha_4\beta_1^{lo}\) subset. In contrast, the majority (~90%) of \(\alpha_4^{hi}\beta_1^{hi}\) and all \(\alpha_4^{lo}\beta_1^{hi}\) T-helper lymphocytes are not bound by vedolizumab (Fig. 2C). Likewise, a majority (~85%) of monocytes are not bound by vedolizumab (Fig. 2D and E), but express relatively high levels of the \(\alpha_4\beta_1\) integrin (Fig. 2F).

Vedolizumab Specificity for the \(\alpha_4\beta_1\) Integriin Versus the \(\alpha_4\beta_1\) and \(\alpha_4\beta_7\) Integrins. The specificity of vedolizumab binding for the integrin \(\alpha_4\beta_1\) versus \(\alpha_4\beta_1\), and \(\alpha_4\beta_7\) antibodies. Vedolizumab binding was examined in the three \(\alpha_4\beta_1\) CD4+ memory T-cell populations of the total CD4 T-cell plot of B: \(\alpha_4\beta_1^{hi}\) (I), \(\alpha_4\beta_1^{lo}\) (II), and \(\alpha_4\beta_1^{lo}\) (III). D, blood was stained with anti-CD14, and anti-CD4, anti-CD45RO, and \(\alpha_4\beta_1\) antibodies. Vedolizumab binding was examined in the three \(\alpha_4\beta_1\) CD4+ memory T-cell populations of the total CD4 T-cell plot of B: \(\alpha_4\beta_1^{hi}\) (I), \(\alpha_4\beta_1^{lo}\) (II), and \(\alpha_4\beta_1^{lo}\) (III). E, specificity of binding was demonstrated by competition with Act-1. F, blood was stained with anti-CD14, anti-CD4, and \(\alpha_4\beta_1\) antibodies. E, specificity of binding was demonstrated by competition with Act-1. F, blood was stained with anti-CD14, anti-CD4, and \(\alpha_4\beta_1\) antibodies. This plot illustrates coexpression of \(\alpha_4\beta_1\) and \(\beta_1\) by monocytes. All data are representative of at least five unrelated, healthy donors.
Vedolizumab did not bind the vast majority of cells in either α4β7 populations (Fig. 2C, plot II, and D, respectively). The lack of binding by vedolizumab was maintained up to a concentration of 400 μg/ml (data not shown). The analysis of the specificity of vedolizumab for the α4β7 integrin versus αEβ7 in whole blood by flow cytometry was not possible because all the αEβ7-expressing cells are contained within the α4β7 hi subset. The specificity of binding to integrins containing the α4 and/or α7 chains by vedolizumab was investigated further with cell lines expressing the α4β1, α4β7, or αEβ7 integrins exclusively. Vedolizumab bound to RPMI8866 cells (Fig. 3A) that expressed the α4 and β7 chains (Fig. 3B) but not the β1 or αE chains (not shown). Incubation with Act-1 competed with the binding of vedolizumab (Fig. 3A) to RPMI8866 cells, confirming the specificity of binding to the α4β7 integrin (data not shown). Vedolizumab did not bind to RAMOS cells (Fig. 3C) that expressed the α4 and β1 chains (Fig. 3D) but not the β7 chain (data not shown), nor to mouse L12.2 cell transfectants (Fig. 3E) that expressed the human αE and β7 chains (Fig. 3E, F), but not the human α4 chain (data not shown).

Characterization of the Memory CD4 T Lymphocyte Population for Expression of the α4β1 and α4β7 Integrins and IL-17. A subset (~20%) of the Th17 cell population in peripheral blood was bound by vedolizumab (Fig. 4), and similar data were obtained for expression of the β7 chain (data not shown). In contrast, the majority (80%) of the memory Th17 cell population expressed the α4 chain (α4β1, α4β7; Fig. 4). The Th17 cell population therefore consists of three major subsets: α4β1 (20%), α4β7 (60%), and αEβ7 (20%).

Potency of Binding of Vedolizumab to B and Memory CD4+ T Lymphocytes. The potency of vedolizumab for binding to human peripheral blood B and memory CD4+ lymphocytes was estimated from saturation binding curves in experiments with labeled vedolizumab (Fig. 5A). The EC50 values were 0.067 μg/ml (0.4 nM) and 0.042 μg/ml (0.3 nM) for B and memory CD4+ lymphocytes, respectively. The potency of vedolizumab for binding to human peripheral blood B and memory CD4+ lymphocytes was also estimated from competitive binding experiments in which binding of labeled vedolizumab at its EC50 was competed off by unlabeled vedolizumab (Fig. 5B). The mean IC50 values were 0.045 μg/ml (0.3 nM) and 0.044 μg/ml (0.3 nM) for B and memory CD4+ lymphocytes, respectively. Similar results were obtained with Act-1; the IC50 values for Act-1 binding were 0.062 μg/ml (0.010 nM) and 0.059 μg/ml (0.008 nM) for B and memory CD4+ lymphocytes, respectively (data not shown).

Vedolizumab Inhibits Adhesion of α4β7 Cells to MadCAM-1 and Fibronectin but Not to VCAM-1. The neutralizing potency and specificity of vedolizumab was determined in cell adhesion assays with α4β7-expressing RPMI8866 cells and the cell adhesion proteins, MadCAM-1, VCAM-1, and the alternatively spliced form of fibronectin.
Fig. 4. Vedolizumab binds to a subset of Th17 lymphocytes. Flow cytometric analysis illustrates the percentage of human peripheral blood Th17 lymphocytes that bind vedolizumab and anti-α4 antibody. Data are the means of at least four unrelated, healthy donors with standard deviations indicated by the error bars. Values for percentage of total Th17 population for the positive (+) population were compared with the negative (−) population by use of a two-tailed, homoscedastic Student’s t test. ∗, p < 0.05; ∗∗, p < 0.01.

Fig. 5. Binding of vedolizumab to human peripheral blood B and memory CD4+ T lymphocytes. A, saturation binding curve of vedolizumab-alexa-647 binding to peripheral blood B lymphocytes (●) and memory CD4+ T lymphocytes (○). B, dose-dependent inhibition curve of vedolizumab-alexa-647 binding to B lymphocytes (●) and memory CD4+ T lymphocytes (○) by unlabeled vedolizumab. Control antibody for B lymphocytes (■) and memory CD4+ T lymphocytes (□). The data represent multiple donors.

containing the CS-1 peptide. Vedolizumab inhibited high-affinity adhesion of α4β7-expressing RPMI8866 cells (Mn2+-activated) to MAdCAM-1 (Fig. 6A) with a mean IC50 value of 0.058 ± 0.024 μg/ml (0.39 ± 0.16 nM; n = 6). Vedolizumab inhibited low-affinity adhesion of α4β7-expressing RPMI8866 cells (no Mn2+ activation) to MAdCAM-1 with similar potency (0.023 ± 0.012 μg/ml; 0.15 ± 0.08 nM; n = 7; Fig. 6B). Vedolizumab inhibited adhesion of α4β7-expressing RPMI8866 cells to fibronectin (Mn2+-activated, as required for fibronectin binding; Fig. 6F) with a mean IC50 value of 0.02 ± 0.012 μg/ml (0.14 ± 0.08 nM; n = 4). In contrast, vedolizumab did not inhibit adhesion of α4β7-expressing RPMI8866 cells to VCAM-1 under high- (Fig. 6, C, n = 4, and E) or low-affinity (Fig. 6, D, n = 3, and E) states, even at 400 μg/ml, the highest concentration tested (Fig. 6E, n = 3). The anti-α4-mAb, in contrast, inhibited all adhesion of α4β7-expressing RPMI8866 cells with MAdCAM-1, VCAM-1, and fibronectin with subnanomolar potency (Fig. 6, A–F). The humanized mAb negative control (Fig. 6, A, B, and F) and the murine isotype-matched negative control (Fig. 6, A–F) had no effect on adhesion. The effect of vedolizumab on the binding of MAdCAM-1 to T lymphocytes in human whole blood was also evaluated by flow cytometry. Vedolizumab inhibited the binding of soluble human MAdCAM-1-Fc fusion protein to the α4β7 CD4 memory cell population with similar potency (IC50 = 0.034 μg/ml, 0.225 nM mean of three donors) (Fig. 7).

Vedolizumab Has No Effect on Adhesion of the α4β7 Integrin to VCAM-1 or Fibronectin. The specificity of vedolizumab was further characterized in adhesion assays with the α4β7-expressing RAMOS cells. Vedolizumab did not inhibit high- (Mn2+-activated) or low-affinity (no Mn2+) adhesion of α4β7-expressing RAMOS cells to VCAM-1 (Fig. 8, A and C, respectively), even at the highest concentrations assayed, 400 μg/ml (Fig. 8, B and D, respectively). Conversely, anti-α4-mAb, anti-β7-mAb, and a combination thereof, inhibited all adhesion between α4β7-expressing RAMOS cells and VCAM-1 (Fig. 8, A–D). Vedolizumab did not inhibit adhesion of α4β7-expressing RAMOS cells to fibronectin at 400 μg/ml, the highest concentration assayed, whereas a combination of anti-α4- and anti-β7-mAbs inhibited adhesion (Fig. 8E). RAMOS cells did not bind to MAdCAM-1, even after integrin activation with Mn2+ (data not shown).

Discussion

Infiltration of the GI tract by T lymphocytes is a pathogenic mechanism of UC and CD (Xavier and Podolsky, 2007). Migration into the GI tract is a complex, multistep process requiring the coordinated interaction of several adhesion and signaling molecules (selectins, integrins, chemokine receptors) on the surface of T lymphocytes, with their corresponding ligands on the endothelium (Salmi and Jalkanen, 2005). The α4β7 integrin is a pivotal mediator of infiltration of GI tract by T lymphocytes and antagonizing its adhesion to MAdCAM-1 provides efficacy in animal models of IBD (Hesterberg et al., 1996; Picarella et al., 1997) and in patients with UC (Feagan et al., 2005, 2008) and CD (Feagan et al., 2005, 2008). These investigations also demonstrated that antagonizing the α4β7 integrin is well tolerated. The absence of overt deleterious effects is partially attributable to vedolizumab binding exclusively to leukocytes. This binding profile is consistent with the expression profile of the human α4β7 integrin (Schweighoffer et al., 1993; Erle et al., 1994; Farstad et al., 1997; Rott et al., 2000).
This desirable profile encouraged targeting the \( \alpha_4\beta_7 \) integrin by three different therapeutic strategies. One of these strategies targets the \( \beta_7 \) chain of the integrin and, consequently, inhibits both the \( \alpha_4\beta_7 \) and \( \alpha_4\beta_1 \) adhesion pathways thereby inhibiting the localization and retention of T lymphocytes within epithelial layers in numerous tissues (Kilshaw, 1999). This could potentially elicit systemic effects, perturbing immunosurveillance of normal epithelial tissue and reducing inflammation in diseased tissue. The second strategy targets the \( \alpha_4 \) chain and inhibits both the \( \alpha_4\beta_7 \) and \( \alpha_4\beta_1 \) integrins. The \( \alpha_4\beta_1 \) integrin is expressed by all leukocytes except neutrophils (González-Amaro et al., 2005), and antagonism by natalizumab inhibits adhesion of the \( \alpha_4\beta_1 \) integrin to VCAM-1, fibronectin, and osteopontin, and the adhesion of the \( \alpha_4\beta_7 \) integrin to MAdCAM-1, VCAM-1, and fibronectin (Biogen Idec, 2006). The \( \alpha_4\beta_1 \) integrin mediates adhesion in many different types of tissue, and inhibition by natalizumab therefore induces diverse systemic effects, such as mobilization of hematopoietic progenitor cells from the bone marrow (Bonig et al., 2008; Zohren et al., 2008), leukocytosis in the vasculature (Ghosh et al., 2003; Sandborn et al., 2005; Targan et al., 2007), and reduction in the number of leukocytes in cerebral spinal fluid (Stuve et al., 2006a,b) and cerebral tissue (del Pilar Martin et al., 2008). This diverse profile of physiologic effects may confer efficacy in pathologically distinct diseases, such as multiple sclerosis (Miller et al., 2003) and CD (Ghosh et al., 2003; Sandborn et al., 2005; Targan et al., 2007). Repeated administration of natalizumab to patients with multiple sclerosis and CD, however, is also associated with systemic immunosuppression, including an increased incidence of the fatal infectious disease, PML (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005; Van Asche et al., 2005). It is postulated that the anti-inflammatory mechanism that mediates efficacy of natalizumab in multiple sclerosis and CD may also predispose patients to PML by decreasing immunosurveillance in the
central nervous system (Berger and Houff, 2006; Stüve et al., 2006a,b; del Pilar Martin et al., 2008). Natalizumab may prevent the entry of memory T lymphocytes into the brain and perhaps into sites of viral infection, thereby precluding T lymphocytes from clearing or suppressing the viral infection (Berger, 2006; Stüve et al., 2006b; del Pilar Martin et al., 2008).

Vedolizumab uses a third strategy: exclusive targeting of the α4β7 integrin. Vedolizumab binds to the α4β7, but not to the α4β1 or α5β1 integrins (Figs. 2 and 3). This specificity was confirmed in functional assays demonstrating that vedolizumab inhibits adhesion of α4β7-expressing cells exclusively (Figs. 6 and 7), but not of α4β1-expressing cells exclusively (Fig. 8). These data agree with immunoprecipitation experiments that utilized Act-1 (Schweighoffer et al., 1993). Specifically targeting the α4β7 integrin enables vedolizumab to immunomodulate the GI tract without systemic effects, due to the GI-specific role of the α4β7 integrin in mediating infiltration by leukocytes (Butcher et al., 1999; Engelhardt and Briskin, 2005; Agace, 2006). This strategy does not elicit leukocytosis in UC and CD patients, and has demonstrated an excellent clinical safety profile to date (Feagan et al., 2005, 2008).

Another component of the unique pharmacologic activity of vedolizumab could be selective inhibition of cellular activity. Vedolizumab selectively inhibits the adhesion of cells expressing the α4β7 integrin to MadCAM-1 and fibronectin, but not to VCAM-1 (Figs. 6 and 7). These data are consistent with qualitative reports for the effect of Act-1 on adhesion of B and T lymphocytes to these ligands (Postigo et al., 1993; Schweighoffer et al., 1993; Erle et al., 1994; Walsh et al., 1996).

Differences in expression of integrin chains between memory CD4+ T lymphocytes also contribute to the unique clinical profile of vedolizumab. Circulating effector memory CD4+ T lymphocytes can be divided into three subpopulations according to expression of the α4 and β7 chains: 1) 20% that do not express the α4 chain (Fig. 2B); 2) 50% that express high levels of α4 and β7 chains (Fig. 2B) that would be bound by an anti-α4-therapeutic and not by vedolizumab (Fig. 2C), and 3) 30% that express high levels of the α4β7 integrin and low levels of the β7 chain (Fig. 2C) that would be bound by both an anti-α4-therapeutic and vedolizumab. The CD4+ memory α4β7hi population is postulated to be pathogenic in IBD (Butcher et al., 1999; Salmi and Jalkanen, 2005), and the clinical efficacy of natalizumab in CD and vedolizumab in CD and UC buttress this paradigm (Ghosh et al., 2003; Feagan et al., 2005, 2008; Sandborn et al., 2005). Vedolizumab specifically targets the CD4+ memory subpopulation that is pathogenic in IBD (α4β7hi), while sparing other CD4+ memory subpopulations (α4β7lo) and monocytes (Fig. 2) that are integral for immunosurveillance and host defense.

We discovered that vedolizumab binds to a subset (~20%) of Th17 cells that express the gut-tropic α4β7hi phenotype (Fig. 4). This proinflammatory subset is found in numerous types of inflamed tissue and is postulated to mediate many different autoimmune diseases, such as psoriasis, psoriatic arthritis, rheumatoid arthritis, type 1 diabetes, transplant rejection, and tumor immunotherapy (Iwakura and Ishigame, 2006). IL-23 plays an important role in the maintenance and function of the Th17 subset of CD4 memory T lymphocytes. Single-nucleotide polymorphisms of the human IL-23 receptor gene are associated with increased risk and protec-
tion from CD (Duerr et al., 2006). Ustekinumab is a therapeutic antibody targeting the human p40 subunit of the IL-12 and IL-23 cytokines, and is efficacious in CD (Sandborn et al., 2008) and psoriasis (Leonardi et al., 2008; Papp et al., 2008). It seems that vedolizumab targets only a subset (20%) of the Th17 cells, namely the GI-tropic, α5β7 subset and thus could provide efficacy in IBD more specifically than ustekinumab, conferring an improved clinical risk-to-benefit profile in patients with IBD.

Targeting systemic mediators of inflammation, such as α4β7 T cells and Th17 cells, inhibits systemic immunosurveillance, which may predispose patients to infection and/or neoplasia (Engelhardt and Briskin, 2005; Ransohoff, 2005; Iwakura and Ishigame, 2006; Wi et al., 2006; Mottet and Golshayan, 2007). The role of the αβ7 integrin in immunosurveillance, in contrast, is restricted to the GI tract (Butcher et al., 1999; Salmi and Jalkanen, 2005; Agace, 2006). Gut-specific immunomodulation by vedolizumab is hence likely less likely to predispose patients to infection and/or neoplasia outside of the GI tract than anti-α4, anti-β7, and anti-p40 (IL-12/IL-23) subunit therapeutics. The specificity of vedolizumab for GI-tropic T lymphocytes offers clinical efficacy in IBD (Feagan et al., 2005, 2008) without some of the undesirable systemic effects characteristic of natalizumab and ustekinumab. The ability to modulate inflammation specifically within the GI tract, without systemic immunosuppression, is likely to confer efficacy in IBD with an improved safety profile and risk-to-benefit ratio.

Acknowledgments

We thank Drs. Irving Fox, Asit Parikh, and Carl Alden, Veit Schmelmer and Dr. Jennifer Elliot, Karen Repetny, Brad Nohe, Dr. Charles Baum, Gretchen Bodun, Betsy Palmer, Yasushi Shimizu, Teruhisa Tanaka, Kenichiro Nogami, Ohta Sunao, Shaila Basappa, and Yasuo Tokifuji for insightful discussions and review of the manuscript.

References


Address correspondence to: Dr. Eric R. Fedyk, Millennium Pharmaceuticals Inc., 40 Landsdowne Street, Cambridge, MA 02139. E-mail: fedyk@mpi.com.