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Antisense oligonucleotide blockade of alpha 4 integrin prevents and reverses clinical symptoms in murine experimental autoimmune encephalomyelitis

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Abstract

We investigated the use of an antisense oligonucleotide (ASO) specific for mRNA of the alpha chain (CD49d) of mouse VLA-4 to down-regulate VLA-4 expression and alter central nervous system (CNS) inflammation. ISIS 17044 potently and specifically reduced CD49d mRNA and protein in cell lines and in ex-vivo-treated primary mouse T cells. When administered prophylactically or therapeutically, ISIS 17044 reduced the incidence and severity of paralytic symptoms in a model of experimental autoimmune encephalomyelitis (EAE). This was accompanied by a significant decrease in the number of VLA-4+ cells, $CD4^+$ T cells, and macrophages present in spinal cord white matter of EAE mice. ISIS 17044 was found to accumulate in lymphoid tissue of mice, and oligonucleotide was also detected in endothelial cells and macrophage-like cells in the CNS, apparently due to disruption of the blood–brain barrier during EAE. These results demonstrate the potential utility of systemically administered antisense oligonucleotides for the treatment of central nervous system inflammation.

Keywords: EAE/MS; Adhesion molecules; Cell trafficking; Inflammation; Neuroimmunology

1. Introduction

Integrins are heterodimeric adhesion molecules that play key roles in leukocyte activation, trafficking, and signaling (Hynes, 1992). The VLA-4 integrin consists of an α4 chain noncovalently linked to the β1 molecule. It is expressed on most leukocytes, whether they occur in peripheral blood, lymphoid tissue, or at sites of inflammation in various organs (Hemler et al., 1990). The α4β7 integrin is another leukocyte integrin predominantly known for its expression on a set of gut-homing $CD4^+$ memory T cells (Schweighoffer et al., 1993). Both α4β1 and α4β7 bind to VCAM-1 on activated endothelium and to the CS1 segment of fibronectin found in extracellular matrix (Carlos and Harlan, 1994). α4β7 additionally binds to MadCAM in gut mucosal tissue (Berlin et al., 1993). These interactions are critical for leukocyte migration across endothelium and into inflamed tissues.

Ligand binding by α4 integrins has diverse biological consequences. α4 ligation can deliver costimulatory signals to T cells (Burkly et al., 1991), alter apoptotic pathways (Meerschaert et al., 1999), and modulate cellular secretion of cytokines, proteinases, and other inflammatory mediators (Romanic and Madri, 1994; Yurochko et al., 1992). Perhaps the best-known role for α4, however, is its function as an adhesion molecule guiding leukocytes across vascular endothelium and into sites of inflammation. Leukocytes are recruited from the blood and into tissues by a multistep

Abbreviations: ASO, antisense obligonucleotide; MIF, mean intensity of fluorescence; MOE, methoxyethyl; P=S ODN, phosphorothioate oligodeoxynucleotide.

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process that involves an initial transient rolling of cells along the vascular endothelium followed by firm adhesion and subsequent transendothelial migration (Butcher, 1991). The α4 integrin is unique among adhesion molecules in that it can support both the rolling and firm adhesion steps (Berlin et al., 1995).

α4 integrin-dependent adhesion pathways represent critical intervention points in a wide variety of inflammatory conditions. Studies using several different monoclonal antibodies specific for α4 integrin have revealed beneficial effects of α4 blockade in at least seven different species, with demonstrated inhibition of leukocyte trafficking into a wide variety of organ systems, including arthritic joints, skin, lung, gut, kidney, and pancreas (reviewed in (Lobb and Hemler, 1994)). α4-specific monoclonal antibodies have been effective in modulating organ-specific autoimmune disease, with efficacy seen in three classical models of T-cell-dependent autoimmunity, including skin contact hypersensitivity (Chisholm et al., 1993; Ferguson and Kupper, 1993), type I diabetes, (Baron et al., 1994), and experimental autoimmune encephalomyelitis (EAE) (Baron et al., 1993; Yednock et al., 1992).

The latter model, EAE, is the most commonly used animal model for multiple sclerosis. Studies in this model suggest that α4 blockade could benefit MS patients since monoclonal antibodies targeting VLA-4 can moderate or reverse signs of paralysis in rat, mouse, and guinea pig models of EAE (Yednock et al., 1992; Baron et al., 1993; Kent et al., 1995).

We have adopted a different approach to VLA-4 inhibition using an antisense oligonucleotide (ASO) specific for the α4 chain to reduce α4 mRNA and protein expression. We examined the effects of this oligonucleotide in a direct immunization model of EAE, where it proved effective at inhibiting paralysis when administered either before or after clinical symptoms were apparent.

2. Materials and methods

2.1. Antisense oligonucleotide design and synthesis

20-mer phosphorothioate oligonucleotides containing 2′-methoxyethyl (2′-MOE) modifications (Baker et al., 1997) were used in all experiments. ISIS 17044 (base sequence CCGCAGCCATGCGCTCTTGG) is an antisense oligonucleotide hybridizing to a sequence just 3′ of the translation initiation codon of murine α4 integrin mRNA. ISIS 17614 (base sequence GCCGACACCCGTTCGTTCGG) and ISIS 17048 (base sequence GCCGACACCCGTTCGTTCGG) are scrambled versions of ISIS 17044. 2′-MOE modifications occur at the underlined positions; cytosines within the 2′-MOE "wings" of all oligonucleotides are methylated to reduce potential immunostimulatory properties (Krieg et al., 1995). ISIS 29848 is a universal control for 20-mer phosphorothioate 2′-MOE-modified oligonucleotides. It consists of a library of 20-mer ASOs containing a random mixture of A, T, C, and G residues at each nucleotide position, with MOE modifications at nucleotide positions 1–5 and 16–20. All oligonucleotides were synthesized using an Applied Biosystems 380B automated DNA synthesizer and purified as previously described (Baker et al., 1997).

2.2. Mice

EAE-susceptible female CSJLF1 mice [(Balb/c×SJL)F1] were purchased from Jackson Laboratories (Bar Harbor, ME) and used at approximately 7–10 weeks. Mice were provided easier access to food and water during periods of paralysis.

2.3. RT–PCR and flow cytometry analysis of α4 integrin expression

2.3.1. bEND cells

Activity of the α4 integrin ASO was initially characterized in the bEND.3 cell line provided by Dr. Werner Risau, Max Planck Institute, Planegg-Martinsreid, Germany. Cells were plated at 5000 cells/cm^2 in DMEM high-glucose media containing 10% FBS the day before ASO treatment. Cells were washed extensively in Opti-MEM (Gibco, Grand Island, NY) just prior to antisense treatment. Oligonucleotides (ISIS 17044 or ISIS 17614) were mixed with 10 μg/ml Lipofectin (Gibco) at indicated concentrations and incubated with the cells 4 h at 37 °C. Following antisense treatment, the Lipofectin/ASO/Opti-MEM solution was replaced with complete growth media.

α4 integrin mRNA levels were measured 24 h after antisense treatment by a quantitative RT–PCR assay using the Applied Biosystems PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). All primers and probes were synthesized by Operon Technologies (Alameda, CA) except for the G3PDH-probe made by Applied Biosystems. The α4 integrin probe was multiplexed with the G3PDH probe to measure relative mRNA levels. One hundred nanograms total RNA was purified from cells using the Qiagen RNeasy kit (Qiagen, Valencia, CA). Primer and probe sequences are as follows: α4 integrin forward primer 5′-CGTCGCATCCTGTGCAACT-3′; α4 integrin reverse primer 5′-CCTGGTCTGCACCTCCGTT-3′ ; α4 integrin probe 5′-TGGCCGTTTTGTGTTGAATGTTCTCCAC-3′; G3PDH forward primer 5′-GGCAAATTCAACGGCACAGT-3′; G3PDH reverse primer 5′-GGGTCTCGCTCCTGGAAGAT-3′; and G3PDH probe 5′-AAGGCCGAGAATGGGAAGCTTGTCATC-3′.

Cell membrane expression of α4 integrin was determined by flow cytometry in trypsinized washed bEND.3 cells 48 h after antisense treatment, using an FITC-conjugated anti-α4 integrin mAb (clone 9C10) or a rat IgG2a isotype control antibody, both from Pharmingen (San Diego, CA). Stained cells were fixed in 0.5% paraformaldehyde and analyzed on a FACScan (Becton-Dickinson,