

DNA Aptamers Block L-Selectin Function In Vivo

Inhibition of Human Lymphocyte Trafficking in SCID Mice

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Abstract

Selectins participate in the initial events leading to leukocyte extravasation from the blood into tissues. Thus the selectins have generated much interest as targets for antiinflammatory agents. Therapeutic molecules based on the monomeric carbohydrate ligand sialyl Lewis X (SLe^x) have low affinities and are not specific for a given selectin. Using SELEX (Systematic Evolution of Ligands by EXponential Enrichment) technology, we have generated aptamers specific for L-selectin that require divalent cations for binding and have low nanomolar affinity. In vitro, the deoxyoligonucleotides inhibit L-selectin binding to immobilized SLe^x in static assays and inhibit L-selectin-mediated rolling of human lymphocytes and neutrophils on cytokine-activated endothelial cells in flow-based assays. These aptamers also block L-selectin-dependent lymphocyte trafficking in vivo, indicating their potential utility as therapeutics. (*J. Clin. Invest.* 1996. 98:2688–2692.) Key words: oligonucleotide • inflammation • cell adhesion • therapeutics • SELEX

Introduction

The selectins, L-, E-, and P-selectin, are a family of calcium-dependent cell surface lectins that mediate cell adhesion by recognition of cell-specific carbohydrate ligands. In both normal and pathological conditions, selectins participate in leukocyte extravasation from the vasculature into tissues (1–7). L-selectin is constitutively expressed on most leukocytes (8). In contrast, E- and P-selectin are expressed inducibly on endothelial cells and/or platelets (1–7). All three selectins share similarity of structure, with an amino-terminal calcium-dependent lectin domain, an epidermal growth factor-like domain, and complement binding-like domains (1–7). Although all three selectins can recognize simple carbohydrate structures such as sialyl

Lewis X (SLe^x)¹ and sialyl Lewis A (9–12), most of the identified physiological ligands are mucin-like glycoproteins that present carbohydrate to the amino-terminal C-type lectin domain (3, 5, 12–16). Given the evidence that selectins play an important role in inflammation and in reperfusion injury, several groups have explored the therapeutic potential of small molecule derivatives of SLe^x (17–19). However, as monomers these antagonists have low affinity, require relatively large doses, and display little selectivity for a particular selectin (9, 10). Therefore, we used SELEX (Systematic Evolution of Ligands by EXponential Enrichment) technology (20–22) to isolate high affinity DNA aptamers that are specific for L-selectin. Here we report the selection of aptamers that bind with nanomolar affinity to L-selectin's lectin domain, prevent L-selectin from binding to SLe^x, and function in vivo to prevent the homing of human lymphocytes to lymph nodes in severe combined immunodeficiency (SCID) mice.

Methods

SELEX. An L-selectin-IgG fusion protein (LS-Rg) was produced and SELEX was performed as described (23 and references therein), with the following modifications. All selections were performed at 22°C except round 1 (4°C) and rounds 8, 13, 16, and 17 (37°C). Selected single-stranded DNAs (ssDNAs) were precipitated, PCR-amplified using a biotinylated 3' PCR primer, and the strands were separated using denaturing polyacrylamide gel electrophoresis. The starting ssDNA pool contained a 40-nucleotide randomized region flanked by fixed sequences for PCR primer annealing: 5'-ctacctacgatctgactagc-N₄₀-gcttactctcatgtatgtcc-3'. Individual ligands were cloned and sequenced by standard procedures.

DNA-protein equilibrium dissociation constants. *K_d*s were measured by nitrocellulose filter partitioning (20) in binding buffer (20 mM Hepes, pH 7.5, 125 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM KCl) plus 0.01% (wt/vol) human serum albumin (Sigma Chemical Co., St. Louis, MO) and were calculated by least square fits using Kaleidagraph (Synergy Software, Reading, PA).

Oligonucleotide synthesis and modification. DNAs were synthesized by Operon Technologies, Inc. (Alameda, CA) or at NeXstar Pharmaceuticals using standard procedures. Fluorescein labeling was accomplished by incorporation of an FITC phosphoramidite (Glen

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1. **Abbreviations used in this paper:** HUVEC, human umbilical vein endothelial cells; LS-Rg, L-selectin-Rg; MLN, mesenteric lymph nodes; PEG, polyethylene glycol; PLN, peripheral lymph nodes; SCID, severe combined immunodeficiency; SELEX, Systematic Evolution of Ligands by EXponential Enrichment; SLe^x, sialyl Lewis X; ssDNA, single-stranded DNA.

Research Corp., Sterling, VA) at the 5' or 3' end. Synthesis of modified oligonucleotides, at NeXstar, was initiated by coupling to a dT-5'-CE-polystyrene support (Glen Research Corp.), resulting in a 3'-3' terminal phosphodiester linkage, and ending with an Amino Modifier C6 dT (Glen Research Corp.) at the 5' end. For polyethylene glycol (PEG)-ylation, a 20,000 mol wt PEG2-*N*-hydroxysuccinimide ester (Shearwater Polymers, Huntsville, AL) was coupled to the oligonucleotide through the 5' amine moiety. Truncated forms of the three aptamers were synthesized: LD201t1: TAGCCAAGGTAACCAGTACAAGGTGCTAAACGTAATGGCTTCGGCTTAC; LD196t1: AGCTGGCGGTACGGGCCGTGCACCCACTTACCTGGGAA-GTGAGCTTA; LD174t1: TAGCCATTCACCATGGCCCCCTTCTACGTATGTTCTGCGGGTGGCTTA. A scrambled sequence control DNA was generated by randomizing the sequence of LD201t1 with an additional A: GATGTAGGGACAGTCAAATGGAGTGTTCAAACCGCCCATCTTCAACAAT.

SLe^x binding assay. SLe^x-BSA (Oxford GlycoSystems, Oxford, United Kingdom) in 1× PBS, without CaCl₂ and MgCl₂ (GIBCO-BRL, Gaithersburg, MD), was immobilized at 100 ng/well onto a microtiter plate by overnight incubation at 22°C. The wells were blocked for 1 h with the assay buffer consisting of 20 mM Hepes, 111 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 5 mM KCl, 8.9 mM NaOH, final pH 8, and 1% globulin-free BSA (Sigma Chemical Co.). The reaction mixtures, incubated for 90 min with orbital shaking, contained 5 nM LS-Rg, a 1:100 dilution of anti-human IgG peroxidase conjugate (Sigma Chemical Co.), and 0–50 nM of competitor in assay buffer. After incubation, the plate was washed with BSA-free assay buffer to remove unbound chimera-antibody complex and incubated for 25 min with *O*-phenylenediamine dihydrochloride peroxidase substrate (Sigma Chemical Co.) by shaking in the dark at 22°C. Absorbance was read at 450 nm on a Bio-Kinetics reader (model EL312e; Bio-Tek Instruments, Laguna Hills, CA).

Flow cytometry. 25-μl aliquots of heparinized whole blood were stained for 30 min at 22°C with 2 μg cyanine5-phycoerythrin-labeled anti-CD45 (generous gift of Ken Davis, Becton-Dickinson, San Jose, CA) and 0.15 μM FITC-LD201t1. The final concentration of whole blood was at least 70% (vol/vol). Stained, concentrated whole blood was diluted 1:15 in 140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 20 mM Hepes, pH 7.4, 0.1% BSA, and 0.01% NaN₃ immediately before flow cytometry on a Becton-Dickinson FACS[®] calibur.

Lymphocyte rolling. Human PBMC were isolated and human umbilical vein endothelial cells (HUVEC) were prepared in capillary tubes as described (24). HUVEC were then treated with human recombinant IL-1β for 1 h, washed, and kept at 37°C for 3 h before use. Modified aptamers (PEGylated and 3' capped) at 3 μM, or mAb DREG-56 at 60 nM, were preincubated with PBMC for 15 min at 37°C before infusion into the shear system loop. Flow rates simulated in vivo shear conditions at 2 dyn/cm² (24). PBMC were infused at 2 × 10⁶ cell/ml in Hepes-HBSS, containing MgSO₄ and CaCl₂ (GIBCO-BRL) plus 1% human serum. Rolling PBMC were monitored for the experiment's duration while being videotaped for off-line analysis using NIH IMAGE software within 350 μm by 250 μm video-microscopic fields.

Lymphocyte trafficking. Human PBMC were purified from heparinized blood by a Ficoll-Hypaque gradient, washed twice with HBSS (calcium/magnesium free) and labeled with ⁵¹Cr (Amersham, Arlington Heights, IL). Cells were then washed twice with HBSS (containing calcium and magnesium) and 1% BSA (Sigma Chemical Co.). Labeled cells (2 × 10⁶) were either untreated or mixed with either 15 pmol of antibody (DREG-56 or MEL-14) or 4, 1, or 0.4 nmol of PEGylated and 3' capped aptamer, before intravenous injection into female SCID mice (6–12 wk of age). Alternatively, the mice were injected with either 15 pmol DREG-56 or 4 nmol modified oligonucleotide 1–5 min before injecting the labeled cells. 1 h later the animals were anesthetized, a blood sample was taken, and the mice were killed. Peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN), Peyer's patches, spleen, liver, lungs, thymus, kidneys, and bone marrow were removed and the counts incorporated into the organs determined by a gamma counter (Packard Instruments, Meriden, CT).

Results and Discussion

Selection of aptamers from a combinatorial DNA library. The binding specificity of selectins for their ligands appears to be mediated predominantly by the amino-terminal calcium-dependent lectin domain (25–27). This calcium dependence provides a method for preferentially isolating inhibitory aptamers, since oligonucleotides bound at or near the carbohydrate binding site can be selectively eluted with low concentrations of EDTA (23). The 2' amino-pyrimidine oligonucleotides previously isolated by this procedure have high affinity at 4 and 22°C and much lower affinity at 37°C (23) and are hence unsuitable for in vivo testing. To isolate antagonists with improved thermal stability, we performed a SELEX experiment using deoxyoligonucleotides and higher selection temperatures, either 22 or 37°C. A starting pool of 10¹⁵ random sequence ssDNAs was incubated with human LS-Rg immobilized on protein A-Sepharose beads. After extensive washing, bound oligonucleotides were eluted with 5 mM EDTA and PCR-amplified. DNA strands were then separated and the cycle was repeated. After 15 iterations, the DNA pool bound to LS-Rg with a *K_d* of 0.9 ± 0.1 nM, versus > 5 μM for the starting random pool (data not shown).

Sequence, affinity, and specificity of aptamers. Cloning and sequencing of aptamers from the 15th and 17th rounds revealed three distinct sequence families (Hicke, B., and D. Parma, manuscript in preparation). Individual ligands had high affinity and specificity for LS-Rg; binding was divalent cation-dependent (data not shown). A representative aptamer from each sequence family is shown in Table I. Ligand LD201 bound to LS-Rg with a *K_d* of 1.8 nM at 37°C. The affinities of

Table I. High Affinity, Specific Binding of Deoxyoligonucleotide Ligands of L-Selectin: Sequences, Affinity, and Specificity of Three DNAs

Ligand	Sequence	<i>K_d</i> L (nM)	<i>K_d</i> E/ <i>K_d</i> L	<i>K_d</i> P/ <i>K_d</i> L
LD201	5'-CAAGGTAACCAGTACAAGGTGCTAAACGTAATGGCTTCG-3'	1.8 ± 0.2	300	9000
LD174	5'-CATTACCATGGCCCCCTTCTACGTATGTTCTGCGGGTG-3'	5.5 ± 5.1	600	8000
LD196	5'-TGCGGTACGGGCCGTGCACCCACTTACCTGGGAAGTGA-3'	3.1 ± 0.4	200	15000

Sequences, representative of the three sequence families, do not include the 5' and 3' fixed sequences (see Methods). Specificity is the ratio of *K_d*s for E-selectin-Rg (*E*) to L-selectin-Rg (*L*) and for P-selectin-Rg (*P*) to L, as measured by a nitrocellulose filter binding assay. Values shown represent the mean ± SE from ≥ 2 experiments.

LD174 and LD196 were comparable. No binding was detected to 700 nM concentrations of either wheatgerm agglutinin, an *N*-acetyl glucosamine/sialic acid binding plant lectin, or CD22-IgG chimera, a mammalian sialic acid binding lectin (28). These ligands bound to LS-Rg 200–600-fold more tightly than to human E-selectin–Rg and 8,000–15,000-fold more tightly than to human P-selectin–Rg (Table I). In contrast to SLe^x, the aptamers described here are specific for L-selectin and bind with low nanomolar affinity.

Inhibition of SLe^x binding and competition with a blocking mAb. We next determined that the DNAs inhibited L-selectin

binding to SLe^x. Shortened forms of LD201, LD174, and LD196 were prepared (see Methods for sequences). These truncated forms, LD201t1, LD174t1, and LD196t1 (data not shown) inhibited LS-Rg binding to immobilized SLe^x with IC₅₀s of ~ 3 nM (Fig. 1 A). This is a 10⁵–10⁶-fold improvement over the published IC₅₀ values for SLe^x in similar plate-binding assays (9–11, 23). A scrambled sequence based on LD201t1 showed no activity in this assay. Binding of all three DNA ligands to LS-Rg was blocked by DREG-56, an L-selectin blocking monoclonal antibody (29), but not by an isotype-matched control (Fig. 1 B). In competition experiments, LD201t1, LD174t1, or LD196t1 prevented radiolabeled LD201t1 from binding to LS-Rg, consistent with the premise that the ligands bind the same or overlapping sites (data not shown). The blocking and competition experiments, taken together with divalent cation dependence of binding, suggest that all three aptamers bind to the lectin domain. This conclusion has been verified for LD201 by cross-linking experiments (Hicke, B., and D. Parma, manuscript in preparation).

Aptamer binding to cell surface L-selectin. FITC-conjugated LD201t1 specifically bound human lymphocytes and neutro-

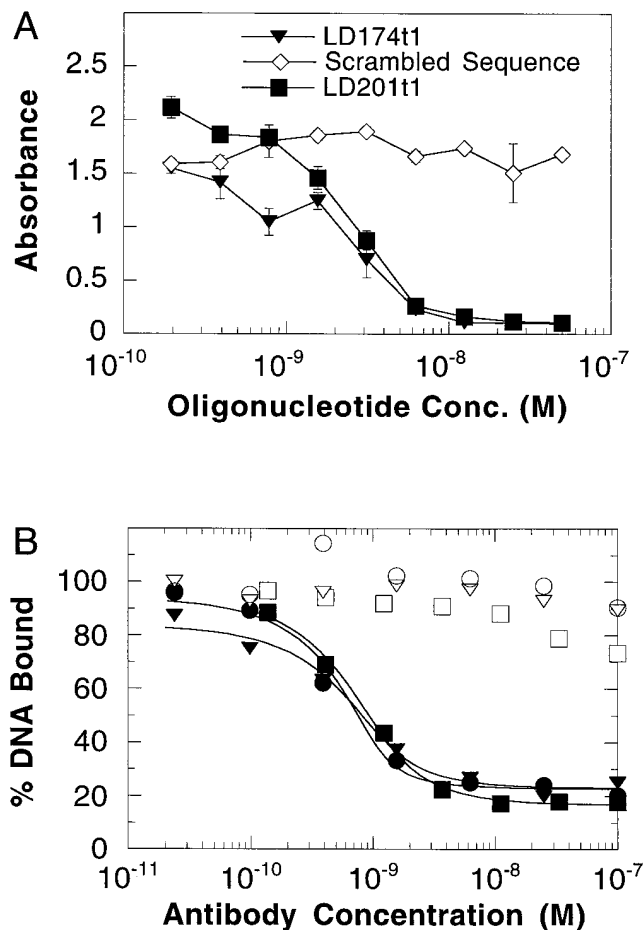


Figure 1. The oligonucleotides block L-selectin's SLe^x binding site. (A) Inhibition of LS-Rg binding to SLe^x. Immobilized SLe^x was incubated with LS-Rg and increasing concentrations of DNAs, along with a peroxidase-conjugated anti-human IgG. After washing, bound LS-Rg was indirectly quantified by addition of a peroxidase substrate and detection at 450 nM. Values shown represent the mean ± SE from duplicate, or triplicate, samples from one representative experiment. (B) Inhibition of oligonucleotide binding to LS-Rg by the adhesion blocking mAb DREG-56. 5'-³²P-labeled oligonucleotides (5 nM) were incubated with 1 nM LS-Rg and increasing concentrations of DREG-56 or an isotype-matched control. Reaction mixtures were incubated at 37°C for 15 min, partitioned by nitrocellulose filtration, and bound oligonucleotide was quantified. *Open squares*, LD201t1 plus control antibody; *filled squares*, LD201t1 plus DREG-56; *open triangles*, LD174t1 plus control antibody; *closed triangles*, LD174t1 plus DREG-56; *open circles*, LD196t1 plus control; *filled circles*, LD196t1 plus DREG-56. The data shown are representative of two experiments.

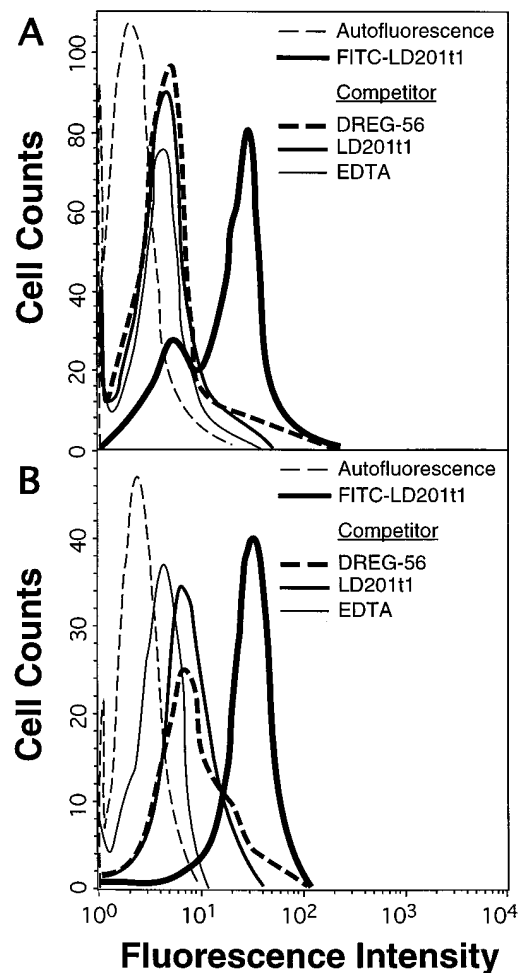


Figure 2. LD201t1 binds specifically to L-selectin on human lymphocytes (A) and granulocytes (B) in whole blood. Cells were stained with FITC-LD201t1 alone and in the presence of 0.3 μM DREG-56, 7 μM unlabeled LD201t1, or cells were reassayed after addition of 4 mM EDTA. Cells were gated using side scatter and CD45-Cy5PE staining.

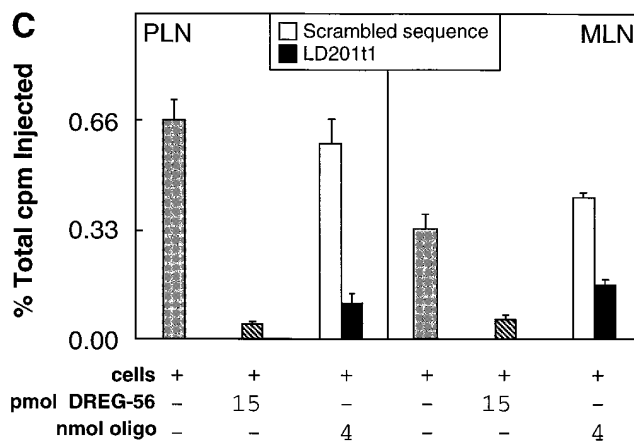
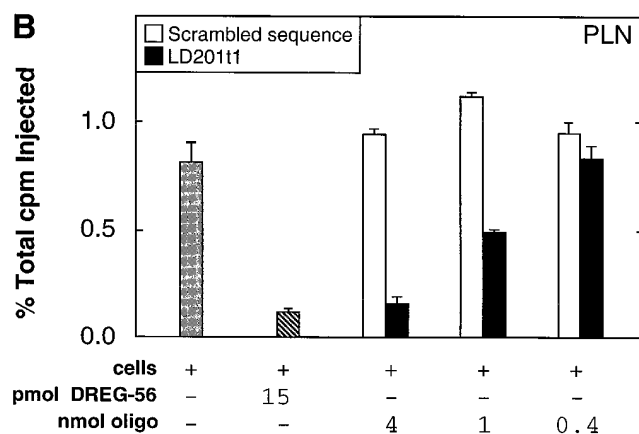
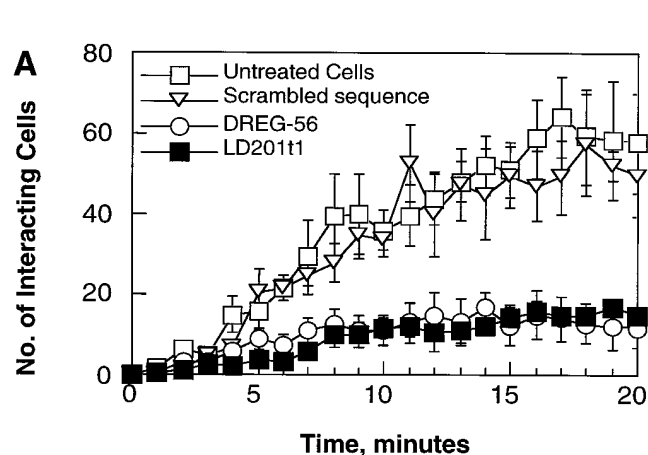


Figure 3. Modified oligonucleotides block L-selectin-mediated adhesion in shear dependent assays in vitro and in vivo. (A) In vitro, LD201t1 significantly reduces rolling of human PBMC on activated HUVEC. HUVEC were cultured in capillary tubes and activated with IL-1 β . Isolated PBMC were infused into a loop in which physiological shear forces were maintained, and rolling cells were monitored by video microscopy. (B) Ex vivo pretreatment of human PBMC with LD201t1 inhibits lymphocyte trafficking to SCID mouse PLN. ^{51}Cr -labeled human PBMC were incubated with modified (PEGylated and 3' capped) aptamer or antibody and then injected intravenously into SCID mice. After 1 h, mice were anesthetized, killed, and counts incorporated into organs were determined by a gamma counter. Values shown represent the mean \pm SE of triplicate samples, and are representative of three experiments. (C) Preinjection of LD201t1 inhibits human lymphocyte trafficking to SCID mouse PLN and MLN. 1–5 min before intravenous injection of ^{51}Cr -labeled human PBMC, modified aptamer or antibody was injected intravenously. Incorporated counts were determined as in B. Values shown represent the mean \pm SE of triplicate samples and are representative of two experiments.

phils in whole blood (Fig. 2); binding was inhibited by competition with DREG-56, unlabeled LD201, and by the addition of 4 mM EDTA (Fig. 2). In addition, human PBMC bound radiolabeled LD201. The binding was saturable, divalent cation-dependent, and competed by DREG-56 but not by an isotype-matched control antibody (data not shown). These cell binding studies demonstrated that the aptamers bound saturably and specifically to human L-selectin in the context of lymphocyte and neutrophil cell surfaces.

Inhibition of lymphocyte rolling on activated endothelial cells. To effectively block L-selectin-mediated adhesion in vivo, an antagonist must function in systems that are subject to hydrodynamic shear (5, 24). Accordingly, LD201t1 and the scrambled sequence, modified by addition of a 3'-capping group and a 5' 20,000 mol wt PEG (neither modification significantly alters aptamer affinity; data not shown), were studied in a flow system in vitro. In this system human PBMC "roll" on activated endothelial cells (HUVEC; activated with IL-1 β) and rolling is dependent on both L-selectin and E-selectin (24). LD201t1 and DREG-56 blocked rolling to a similar extent, 70% for PBMC (Fig. 3 A) and \sim 50% for neutrophils (data not shown). The scrambled sequence had no activity in this assay (Fig. 3 A).

Aptamer activity in vivo: lymphocyte trafficking in SCID mice. As the aptamers bound to human but not rodent L-selectin (data not shown), a xenogeneic lymphocyte trafficking sys-

tem was established to evaluate in vivo efficacy. Human PBMC, labeled with ^{51}Cr , were injected intravenously into SCID mice. In this system, human cells traffic to PLN and MLN. Lymphocyte accumulation in MLN and PLN is inhibited by DREG-56 (Fig. 3 B) but not by MEL-14 (data not shown), a monoclonal antibody that blocks murine L-selectin-dependent trafficking (30). Cell trafficking was determined 1 h after injection. For trafficking experiments, 3'-capped and 5'-PEGylated ssDNA aptamers were used because pharmacokinetic studies in rats indicate that their half-life in plasma is \sim 18 min, significantly longer than that of unmodified ssDNA aptamers (Gill, S.C., personal communication).

In initial trafficking experiments, cells were incubated with either DREG-56 or 3'-capped and 5'-PEGylated oligonucleotide before injection. LD201t1 inhibited trafficking of cells to PLN (Fig. 3 B) and MLN (data not shown) in a dose-dependent fashion but had no effect on the accumulation of cells in other organs (data not shown). At the highest dose tested (4 nmol), inhibition by the oligonucleotide was comparable with the effect of DREG-56 (15 pmol) in this system. LD174t1 had similar activity (data not shown), while the scrambled sequence had no significant effect (Fig. 3 B). We next assayed the effect of modified oligonucleotide when it was not preincubated with cells. DREG-56 (15 pmol/mouse) or the modified oligonucleotide (4 nmol/mouse) was injected intravenously into animals and 1–5 min later the radiolabeled human cells

were given intravenously. Again, both LD201t1 and DREG-56 inhibited trafficking to PLN and MLN while the scrambled sequence had no effect (Fig. 3 C). Therefore, the modified oligonucleotide did not require preincubation with the cells to effectively block trafficking. To our knowledge, these experiments are the first demonstration of *in vivo* efficacy of an aptamer directed against a cell surface receptor.

In summary, we have generated oligonucleotide antagonists that bind with high affinity and specificity, in a divalent cation-dependent fashion, to human L-selectin. *In vitro*, the aptamers block binding of soluble L-selectin to SLe^x, bind specifically to L-selectin on human leukocytes, and have 10⁵–10⁶-fold better affinity for L-selectin than does SLe^x. *In vivo*, the aptamers block the trafficking of human lymphocytes to murine peripheral lymphoid tissues, making them superior to the previously described aptamer antagonists of L-selectin, the temperature sensitivity of which rendered them unsuitable for use *in vivo* (23). We expect to improve aptamer performance *in vivo* by enhancing affinity for L-selectin (Ringquist, S., manuscript in preparation) or by increasing resistance to nuclease degradation, thereby providing high affinity, specific reagents that may block the interaction of L-selectin with its ligands in pathological states.

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References

1. Bevilacqua, M.P., and R.M. Nelson. 1993. Selectins. *J. Clin. Invest.* 91: 379–387.
2. Rosen, S.D., and C.R. Bertozzi. 1994. The selectins and their ligands. *Curr. Opin. Cell. Biol.* 6:663–673.
3. McEver, R.P., K.L. Moore, and R.D. Cummings. 1995. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J. Biol. Chem.* 270: 11025–11028.
4. Picker, L.J., and E.C. Butcher. 1992. Physiological and molecular mechanisms of lymphocyte homing. *Ann. Rev. Immunol.* 10:561–591.
5. Springer, T.A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 76:301–314.
6. Lasky, L.A. 1995. Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Ann. Rev. Biochem.* 64:113–139.
7. Carlos, T.M., and J.M. Harlan. 1994. Leukocyte-endothelial adhesion molecules. *Blood.* 84:2068–2101.
8. Bradley, L.M., G.G. Atkins, and S.L. Swain. 1992. Long-term CD4+ memory T cells from the spleen lack MEL-14, the lymph node homing receptor. *J. Immunol.* 148:324–331.
9. Berg, E.L., M.K. Robinson, O. Mansson, E.C. Butcher, and J.L. Magnani. 1991. A carbohydrate domain common to both sialyl Le(a) and sialyl Le(X) is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J. Biol. Chem.* 266:14869–14872.
10. Foxall, C., S.R. Watson, D. Dowbenko, C. Fennie, L.A. Lasky, M. Kiso, A. Hasegawa, D. Asa, and B.K. Brandley. 1992. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. *J. Cell Biol.* 117:895–902.
11. Nelson, R.M., S. Dolich, A. Aruffo, O. Cecconi, and M.P. Bevilacqua. 1993. Higher-affinity oligosaccharide ligands for E-selectin. *J. Clin. Invest.* 91: 1157–1166.
12. Varki, A. 1994. Selectin ligands. *Proc. Natl. Acad. Sci. USA.* 91:7390–7397.
13. Lasky, L.A., M.S. Singer, D. Dowbenko, Y. Imai, W.J. Henzel, C. Grimley, C. Fennie, N. Gillett, S.R. Watson, and S.D. Rosen. 1992. An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell.* 69:927–938.
14. Baumhueter, S., M.S. Singer, W. Henzel, S. Hemmerich, M. Renz, S.D. Rosen, and L.A. Lasky. 1993. Binding of L-selectin to the vascular sialomucin CD34. *Science (Wash. DC).* 262:436–438.
15. Sako, D., X.-J. Chang, K.M. Barone, G. Vachino, H.M. White, G. Shaw, G.M. Veldman, K.M. Bean, T.J. Ahern, B. Furie, et al. 1993. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell.* 75:1179–1180.
16. Norgard, K.E., K.L. Moore, S. Diaz, N.L. Stults, S. Ushiyama, R.P. McEver, R.D. Cummings, and A. Varki. 1993. Characterization of a specific ligand for P-selectin on myeloid cells. A minor glycoprotein with sialylated O-linked oligosaccharides. *J. Biol. Chem.* 268:12764–12774.
17. Albelda, S.M., C.W. Smith, and P.A. Ward. 1994. Adhesion molecules and inflammatory injury. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 8:504–512.
18. Bevilacqua, M.P., R.M. Nelson, G. Mannori, and O. Cecconi. 1994. Endothelial-leukocyte adhesion molecules in human disease. *Ann. Rev. Med.* 45: 361–378.
19. Lefer, A.M., A.S. Weyrich, and M. Buerke. 1994. The role of selectins, a new family of adhesion molecules, in ischemia-reperfusion injury. *Cardiovasc. Res.* 28:289–294.
20. Tuerk, C., and L. Gold. 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science (Wash. DC).* 249:505–510.
21. Gold, L. 1995. Oligonucleotides as research, diagnostic, and therapeutic agents. *J. Biol. Chem.* 270:13581–13584.
22. Gold, L., B. Polisky, O. Uhlenbeck, and M. Yarus. 1995. Diversity of oligonucleotide functions. *Ann. Rev. Biochem.* 64:763–797.
23. O'Connell, D., A. Koenig, S. Jennings, B. Hicke, H.-L. Han, T. Fitzwater, Y.-F. Chang, N. Varki, D. Parma, and A. Varki. 1996. Calcium-dependent oligonucleotide antagonists specific for L-selectin. *Proc. Natl. Acad. Sci. USA.* 93:5883–5887.
24. Bargatze, R.F., S. Kurk, G. Watts, T.K. Kishimoto, C.A. Speer, and M.A. Jutila. 1994. *In vivo* and *in vitro* functional examination of a conserved epitope of L- and E-selectin crucial for leukocyte-endothelial cell interactions. *J. Immunol.* 152:5814–5825.
25. Geng, J.-G., K.L. Moore, A.E. Johnson, and R.P. McEver. 1991. Neutrophil recognition requires a Ca(2+)-induced conformational change in the lectin domain of GMP-140. *J. Biol. Chem.* 266:22313–22318.
26. Erbe, D.V., B.A. Wolitzky, L.G. Presta, C.R. Norton, R.J. Ramos, D.K. Burns, J.M. Rumberger, B.N. Rao, C. Foxall, B.K. Brandley, and L.A. Lasky. 1992. Identification of an E-selectin region critical for carbohydrate recognition and cell adhesion. *J. Cell Biol.* 119:215–227.
27. Erbe, D.V., S.R. Watson, L.G. Presta, B.A. Wolitzky, C. Foxall, B.K. Brandley, and L.A. Lasky. 1993. P- and E-selectin use common sites for carbohydrate ligand recognition and cell adhesion. *J. Cell Biol.* 120:1227–1235.
28. Sgroi, D., A. Varki, S. Braesch-Andersen, and I. Stamenkovic. 1993. CD22, a B cell-specific immunoglobulin superfamily member, is a sialic acid-binding lectin. *J. Biol. Chem.* 268:7011–7018.
29. Kishimoto, T.K., M.A. Jutila, and E.C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 87:2244–2248.
30. Gallatin, W.M., I.L. Weissman, and E.C. Butcher. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.)* 304:30–34.