Internalizing Antibodies to the C-Type Lectins, L-SIGN and DC-SIGN, Inhibit Viral Glycoprotein Binding and Deliver Antigen to Human Dendritic Cells for the Induction of T Cell Responses

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References

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Internalizing Antibodies to the C-Type Lectins, L-SIGN and DC-SIGN, Inhibit Viral Glycoprotein Binding and Deliver Antigen to Human Dendritic Cells for the Induction of T Cell Responses

Naveen Dakappagari,* Toshiaki Maruyama,* Mark Renshaw,* Paul Tacken,† Carl Figdor,‡ Ruurd Torensma,§ Martha A. Wild,* Dayang Wu,‡ Katherine Bowdish,* and Anke Kretz-Rommel¹

The C-type lectin L-SIGN is expressed on liver and lymph node endothelial cells, where it serves as a receptor for a variety of carbohydrate ligands, including ICAM-3, Ebola, and HIV. To consider targeting liver/lymph node-specific ICAM-3-grabbing nonintegrin (L-SIGN) for therapeutic purposes in autoimmunity and infectious disease, we isolated and characterized Fabs that bind strongly to L-SIGN, but to a lesser degree or not at all to dendritic cell-specific ICAM-grabbing nonintegrin (DC-SIGN). Six Fabs with distinct relative affinities and epitope specificities were characterized. The Fabs and those selected for conversion to IgG were tested for their ability to block ligand (HIV gp120, Ebola gp, and ICAM-3) binding. Receptor internalization upon Fab binding was evaluated on human liver sinusoidal endothelial cells by flow cytometry and confirmed by confocal microscopy. Although all six Fabs internalized, three Fabs that showed the most complete blocking of HIVgp120 and ICAM-3 binding to L-SIGN also internalized most efficiently. Differences among the Fab panel in the ability to efficiently block Ebola gp compared with HIVgp120 suggested distinct binding sites. As a first step to consider the potential of these Abs for Ab-mediated Ag delivery, we evaluated specific peptide delivery to human dendritic cells. A durable human T cell response was induced when a tetanus toxide epitope embedded into a L-SIGN/DC-SIGN-cross-reactive Ab was targeted to dendritic cells. We believe that the isolated Abs may be useful for selective delivery of Ags to DC-SIGN- or L-SIGN-bearing APCs for the modulation of immune responses and for blocking viral infections. The Journal of Immunology, 2006, 176: 426–440.

Dendritic cell (DC)²-specific ICAM-grabbing nonintegrin (DC-SIGN) (CD209) and liver/lymph node-specific ICAM-3-grabbing nonintegrin (L-SIGN) (CD299/DCSIGNR) are closely related genes that map to chromosome 19p13.3. Both genes encode a member of the C-type lectin family of type II transmembrane proteins. The two receptors are 77% identical at the amino acid level, have similar three-dimensional structures, and share similar ligands (1). Despite their close evolutionary similarities at the gene and protein level, they are expressed in different tissues. Although DC-SIGN is expressed on DCs and macrophages (2, 3), L-SIGN is found in the endothelial cells of liver, lymph nodes, and placenta, and is absent on DCs and macrophages (4, 5). These receptors are composed of a C-terminal carbohydrate recognition domain (CRD) that is supported by a neck region made up of multiple highly conserved 23-aa repeats and a short cytoplasmic tail at the N terminus (1, 6). Both receptors have been shown to interact with ICAM-3 (2, 4), a molecule expressed on T cells. This interaction is thought to play a role in stabilizing the DC-T cell contact zone. Although L-SIGN and DC-SIGN interact with a number of similar ligands, the outcome of this interaction could potentially be different based on the unique tissue distribution of each receptor.

A number of viruses have been shown to bind to the CRDs of DC-SIGN and L-SIGN, but in a manner that is distinct from ICAM-3 (7–9). L-SIGN is able to bind and permit entry into the cell of HIV (4, 5), hepatitis C virus (HCV) (10), Ebola virus (11), severe acute respiratory syndrome (SARS) virus (12), CMV (13), and Sindbis virus (14). Current data suggest the idea that the interaction between HIV and HCV may be transmitted in trans to other cells, e.g., T cells and hepatocytes (4, 5, 15), whereas it is thought to result in direct infection by other viruses, e.g., Ebola and SARS (12, 16). Recently, L-SIGN has also been shown to serve as a receptor for bacterial pathogens, e.g., Mycobacterium tuberculosis (17) and parasites such as Schistosoma mansoni (18). Because liver sinusoidal endothelial cells (LSECs) are involved in tolerance induction, the use of L-SIGN receptor as a point of entry may explain the difficulty that the immune system has in eradicating these pathogens. Abs to L-SIGN that selectively inhibit pathogen attachment may be prophylactically or therapeutically useful in infectious disease by preventing pathogen entry or adhesion.

The liver is thought to play a central role in immunological tolerance. LSECs, which express L-SIGN and a number of immune cell recognition molecules, have been reported to actively...
capture potentially harmful antigenic proteins from the circulation and present the processed peptides efficiently to the trafficking leukocytes (19). The presentation of Ags by LSECs to both CD4+ and CD8+ T lymphocytes was found to result in immunological tolerance even in the presence of inflammatory mediators, e.g., IL-12 and IFN-γ (20, 21). Specific targeting of Ag to tolerance-inducing cells by linking an autoantigen to an L-SIGN Ab might result in the induction of T cell tolerance to the presented Ag. In contrast, specific targeting of Ag to DC-SIGN on DCs is expected to raise a stimulatory immune response.

Using phage display and screening technologies, we have successfully isolated a panel of L-SIGN-reactive Fabs with distinct relative affinities and epitope specificities. The ability of Fabs to block HIV binding correlated with their ability to block ICAM-3 binding, while blocking of Ebola binding required distinct Ab properties. We also demonstrated varying degrees of L-SIGN Ab internalization upon receptor binding. The ability of L-SIGN Abs that were cross-reactive with DC-SIGN and internalized by APCs was exploited further to specifically deliver Ag. We demonstrate induction of a robust human T cell response by targeted delivery of an Ag to autologous DCs using a L-SIGN/DC-SIGN-cross-reactive Ab embedded with a tetanus toxoid (TT) epitope.

**Materials and Methods**

**Cell lines and reagents**

Stable K562 cell lines expressing DC-SIGN (8) and L-SIGN (18) have been described previously. Fresh human liver nonparenchymal cells were purchased from CellzDirect. These cells are supplied after removal of hepatocytes from total liver cells. Before their use in assays, RBC were lysed and any remaining dead cells were further depleted using a dead cell removal kit (Miltenyi Biotec; catalog 130-090-101), per the manufacturer’s instructions. mAbs mAb162 (reactive only with L-SIGN), mAb1621 and mAb16211 (cross-reactive with DC-SIGN and L-SIGN) were purchased from R&D Systems. An allophycocyanin conjugate of mAb, mAb162 was prepared using a Mouse on mouse IgG2b labeling kit (Molecular Probes; catalog Z25525), according to the manufacturer’s instructions. All other Abs used in flow cytometry were purchased from eBioscience. Cytokines, IL-4, and GM-CSF were purchased from StemCell Technologies. Whole DC protein was purchased from Calbiochem. The universal HLA-DR-binding TT epitope, 632DR (aa 632–651, D1K6SD6V1TYPYG1ALN), was chemically synthesized by SynPep. The peptide was purified by reverse-phase chromatography to a single peak, and its identity was confirmed by mass spectrometry.

**Construction of human L-SIGN-Fc expression plasmid**

Human L-SIGN-Fc was generated by overlap PCR, fusing two PCR fragments derived from cDNA coding for human L-SIGN and the Fc portion of human Ig G1 (22). Flanking primers P1 EcoRV 5’-CAG ATG TGA TAT CTG GTA CGG CCC CAG CTC CCT AAG-3’ and P2 XhoI S’-TGG GCT CGA GGT GCT TGA AGC ACG CGG CG-3’ were used to amplify the extracellular domain of human L-SIGN from a human spleen cDNA library. The EcoRV site in the P1 primer allows fusion with the leader sequence. The XhoI site in the P2 primer was used to fuse the fragment with the hG2G4 Fc region. The primers P3 (forward), XhoI S’-AGA CGA ACT CGG CCA ATG TTG TGT GCA GT-3’ and P4 (reverse), stop codon NmgIV S’-TGG CCG CCC TGC TCA TTT ACC CAG AAG CAG GGA GGT GCT-3’ were used to amplify the hinge domain of Glu99 of the hinge domain to the C terminus by using a plasmid containing the hG2G4 C region. The PCR-amplified human L-SIGN and human hG2G4 Fc region fragments were cloned into vector pCR2.1. The resulting plasmid pcR2.1hL-SIGN was digested with EcoRV/XhoI, and the plasmid pcR2.1 hG2G4 was digested with XhoI and NmgIV. The resulting L-SIGN and hG2G4 Fc fragments were ligated into a modified Apex3P plasmid vector (23). The vector encodes a promoter with Kozak sequence and ATG codon for the initiating methionine.

**Expression and purification of recombinant L-SIGN-Fc and DC-SIGN-Fc fusion proteins**

The 293 EBNA human embryonic kidney cells were transfected with Apex3P-hL-SIGNhG2G4-FC expressing Effectene (Qiagen) and were grown in DMEM (Cellgro 10-013-CV) with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin, 250 µg/ml G418 sulfate, and 1 µg/ml puromycin at 37°C and 5% CO2. T-175 flasks at 90–95% confluence were washed with HBSS or Dulbecco’s PBS (DPBS) to remove serum proteins, and 30 ml of IS Pro serum-free medium (Irvine Scientific; catalog 91103) supplemented with l-glutamine and penicillin/streptomycin was added to each flask. The supernatant was concentrated and purified by protein A column chromatography. Cloning and expression of DC-SIGN-Fc fusion protein were described previously (8). Briefly, DC-SIGN-Fc consists of the extracellular portion of DC-SIGN (amino acids residues 64–404) fused at the C terminus to a human IgG1-Fc fragment and expressed in the Sig-pIgG1-Fc vector. DC-SIGN-Fc was produced in Chinese hamster ovary K1 cells by cotransfection of DC-SIGN-Sig-pIgG1 Fc (20 µg) and pEEl4 vector (5 µg).

**Immunization with human L-SIGN**

Four BALB/c mice were immunized twice at a 3-wk interval by i.p. administration of 10 µg of recombinant L-SIGN-Fc protein and 100 µl of the adjuvant ImmunEasys (Qiagen) in a total volume of 200 µl. After 3 wk, two of the mice received a third immunization similar to the first two rounds, and two were boosted with 5 × 106 K562/L-SIGN cells. Sera of all mice tested positive when analyzed for binding to K562/L-SIGN cells by flow cytometry. Mice were sacrificed and spleens were frozen immediately in liquid nitrogen.

**Phage display library construction**

Total RNA was isolated from L-SIGN-immunized mouse spleen samples using TRI Reagent (Molecular Research Center), according to the manufacturer’s protocol. mRNA was purified using Oligotex (Qiagen), digested with appropriate restriction endonucleases, and cloned into an IgG1 Fab expression vector. Library size was 1.5 × 109.

**Phage display library panning**

Two microtiter plates were coated with 100 µl of anti-human IgG Fc-specific Ab (Pierce) at 20 µg/ml in PBS at 4°C overnight. The first plate was washed five times with PBS and blocked with 1% BSA/PBS at 37°C for 1 h. The plates were washed with PBS and incubated with 100 µl of recombinant L-SIGN-Fc (10 µg/ml in PBS) at 37°C for 1 h. The second plate was washed with PBS and blocked with 1% BSA/PBS at 37°C for 1 h. The wells were washed five times with PBS and incubated with 100 µl of recombinant L-SIGN-Fc (10 µg/ml in PBS) at 37°C for 1 h. The plate was washed and the wells were incubated with 100 µl of library phage for 1 h at 37°C. The second plate was washed and library phage were transferred from the first plate to the second plate and incubated at 37°C for 1.5 h. The wells were washed with PBS with increasing stringency for each round of panning (3, 5, 10, and 10 times), each with a 5-min incubation and vigorous pipetting. The remaining phage were eluted and titrated on LB plates containing carbenicillin and glucose. Eluted phage were propagated in ER2738 cells overnight in the presence of antibiotics, 1 mM isopropyl β-D-thiogalactoside, and helper phage for the next round of panning.

**Phage ELISA**

Ninety-five single colonies from titration plates from panning rounds 2, 3, and 4 were grown in 1 ml of super broth medium with carbenicillin. Fab phage production was induced with 1 mM β-thiogalactoside and helper phage overnight at 30°C. The culture was spun down, and supernatants containing Fab phage were screened by ELISA. Microtiter plates were coated and incubated at 4°C overnight with either anti-human IgG Fc (Pierce) at 8 µg/ml in PBS to determine Ag binding or anti-mouse IgG F(ab)2 (Pierce) at 4 µg/ml in PBS to monitor Fab expression. The plates were washed three times with PBS and blocked with 100 µl of 1% BSA/PBS at 37°C for 1 h. The plates were washed three times with PBS and incubated with 50 µl of L-SIGN-Fc or DC-SIGN-Fc (5 µg/ml in PBS) at 37°C for 2 h before the next step. The plates were washed three times with PBS and incubated with the culture supernatant containing Fab phage at 37°C for 2 h. The plates were washed three times with PBS, and the bound Fab phage were detected with alkaline phosphatase-conjugated anti-mouse IgG Fab'1, Ab (Pierce) (1:500 in 1% BSA/PBS at 37°C for 1 h. The plates were washed three times with PBS, and the wells were developed with alkaline phosphatase substrate, p-nitrophenyl phosphate (Sigma-Aldrich).
**Screening of Fab phage on cells**

Ninety-five single colonies from titration plates (panning rounds 2, 3, and 4) were grown in 1 ml of super broth medium induced for Fab phage production, as described earlier. The cultures were spun down, and 50 μl of supernatant containing Fab phage was incubated with 0.5 × 10⁶ cells (K562 or K562/L-SIGN) in FACS buffer (DPBS with 1% BSA, 0.1% azide) for 1 h at 4°C, washed with FACS buffer, incubated with PE-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) at a 1/50 dilution in FACS buffer at 4°C for 30 min, washed and resuspended in 1% formaldehyde, and analyzed on a BD FACSCalibur (BD Biosciences).

**DNA sequence analysis**

All Fabs showing specific binding to L-SIGN were sent for DNA sequence analysis at Retrogen. The amino acid sequences were deduced and aligned by DNAsis software.

**Western blotting**

One million K562/L-SIGN cells were lysed in 50 μl of lysis buffer (150 mM NaCl, 25 mM Tris-HCl (pH 7.4), 2 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 1% Triton X-100, 0.5 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). Lysis was achieved by gentle rotation at 4°C for 20 min. Cell lysates were centrifuged (14,000 × g, 10 min) to remove cell debris and boiled for 5 min in SDS sample buffer containing 1 mM DTT. Protein lysates were resolved on 4 –15% SDS-PAGE gradient gels (Bio-Rad 116-1158), transferred to nitrocellulose membranes, and probed individually with L-SIGN-specific Fabs (1 μg/ml). Protein transfer was monitored with prestained molecular mass standards (Bio-Rad 161-0324). Immunoreactive bands were detected using HRP-conjugated goat anti-mouse IgG (Bio-Rad 170-6516) and ECL (Supersignal West Pico kit; Pierce).

**Competition ELISA**

Microtiter plates were coated with anti-human IgG Fc (Pierce) at 8 μg/ml in PBS and incubated at 4°C overnight. The plates were washed three times with PBS and blocked with 100 μl of 1% BSA/PBS at 37°C for 1 h. After three washes with PBS, plates were incubated with 50 μl of L-SIGN-Fc (5 μg/ml in PBS) at 37°C for 2 h. The plates were washed three times with PBS and incubated with a constant amount (25 nM) of mAb, mAb162, and 2-fold dilutions of L-SIGN Fabs at 37°C for 2 h. The plates were washed three times with PBS, and mAb162 binding was detected using alkaline phosphate-conjugated anti-mouse Fcy-specific secondary Ab (Jackson ImmunoResearch Laboratories), followed by alkaline phosphatase substrate, p-nitrophenyl phosphate.

**Ab internalization**

The assay was done, as described previously (25). Briefly, 0.5 × 10⁶ fresh human liver nonparenchymal cells or K562/L-SIGN cells were incubated with L-SIGN Fabs at 20 μg/ml for 30 min at 4°C in DPBS/1% BSA in duplicate. The unbound Fab was washed off; one sample was incubated at 37°C for an additional 2 h to enable internalization and the second sample was kept at 4°C for 2 h in DPBS/1% BSA/0.1% sodium azide as a non-internalizing control. At the end of the incubation period, cells were washed and incubated with PE-conjugated anti-mouse IgG for 30 min at 4°C in DPBS/1% BSA/0.1% sodium azide, washed, fixed in 1% paraformaldehyde, and analyzed on a BD FACSCalibur.

**Confocal microscopy**

A total of 10⁵ K562/L-SIGN and K562/DC-SIGN cells was incubated with 10 μg/ml various Fabs for 90 min at 37°C in RPMI 1640 supplemented with 10% FCS. Cells were then washed with PBS, fixed in PBS/4% paraformaldehyde, washed again, and adhered to poly(L-lysine)-coated coverslips (20 min at room temperature). Cells were incubated with blocking buffer (PBS/3% BSA/10 mM glucose/0.1% saponin) for 1 h at room temperature. Subsequently, cells were washed with blocking buffer and incubated with 10 μg/ml goat anti-mouse IgG Alexa 647 (Molecular Probes) in blocking buffer for 1 h at room temperature. Cells were then washed with blocking buffer, PBS, and finally with 50 mM Tris-HCl. Coverslips were mounted onto glass slides with Mowiol (Calbiochem, Omnilabo International). Fixed slides were imaged with a Bio-Rad MRC 1024 confocal system operating on a Nikon Optiphot microscope and a Nikon 60X Plan-Apochromat 1.4 oil immersion lens. Pictures were analyzed with Bio-Rad Lasersharp 2000 and Adobe Photoshop 7.0 software.

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**FIGURE 1.** Screening of phage-displayed Fabs for L-SIGN reactivity. A. Ninety-six Fab phage clones selected from three rounds of panning on recombinant DC-SIGN (negative selection) and L-SIGN (positive selection) proteins were screened for binding to K562 (negative control) and K562/L-SIGN-transfected cells by flow cytometry using goat anti-mouse IgG PE conjugate for detection. Only clones showing at least a 5-fold higher binding to K562/L-SIGN cells are shown. B. To select Fab phage clones uniquely reactive with L-SIGN, but not DC-SIGN, K562/L-SIGN cell-reactive clones were screened for reactivity with DC-SIGN-Fc and L-SIGN-Fc fusion proteins in a capture ELISA. mAb162 (L-SIGN specific), mAb1621, and mAb16211 (DC-SIGN-L-SIGN cross-reactive) were used as positive controls. Data are representative of two independent experiments.
Fluorescent bead adhesion assay for ligand blocking

Preparation of carboxylate-modified TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes) coated with ICAM-3 Fc (R&D Systems), HIVgp120 (strain JRCSF), or Ebola gp (strain Zaire Mayinga) (viral proteins were kindly provided by D. Burton, The Scripps Research Institute, La Jolla, CA) was previously described (26, 27). For adhesion to ligand-coated fluorescent beads, K562/L-SIGN and K562/DC-SIGN cells (5 × 10^5/ml) were resuspended in Tris-sodium-BSA buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM CaCl_2, 2 mM MgCl_2, and 0.5% BSA). Fifty thousand cells were preincubated with or without L-SIGN Fabs (20 μg/ml) for 10 min at room temperature in a 96-well V-bottom plate. The ligand-coated fluorescent beads (20 beads/cell) were added, and the suspension was incubated for 30 min at 37°C. After washing, the cells were resuspended in Tris-sodium-BSA buffer. The percentage of cells bound to ligand-coated beads was measured by FACSCalibur in FL-3.

Conversion of mouse Fabs to chimeric IgG

Overlap PCR was used to fuse the mouse κ V regions of the E10 and G10 Fabs with a human κ CH1 region. XbaI and NotI restriction sites at either end of the resulting fragments were used to clone these chimeric L chains into a Lonza vector mammalian expression system (28) adapted in-house for use with these sites. The Fab H chains were cloned by generating a PCR fragment containing the mouse V region with a short primer-derived segment containing human γ1 C_\text{H}1 sequence, including an existing NotI site upstream of the hinge region (DNA encoding aa 148–157 of the CH1 domain), in combination with the backward primer E10insertionRev: 5’-GGC GAT GTA GGG CAC GAT GGT GCT CAC GAT GCT TCT TCT CCC CAG GAG TGC TGG TGA GGA AGA-3’ that annealed to glycine 250 and downstream amino acids (250–258) and the reverse primer E10EcoRIRev 5’-GAT TAT GAT CAA TGA ATT CTG GCC GTC GCA CTC AT-3’ that annealed to a region spanning the stop codon and a unique EcoRI site within the vector at the end of the C_\text{H}3 region. For PCR, the expand high fidelity PCR system (Roche) was used. The two fragments were gel purified and combined for overlap extension PCR, and the PCR product was digested with AgeI and EcoRI and cloned into the similarly digested E10-IgG parental clone. The sequence of the final cloned product was confirmed by DNA sequencing.

For production of the Ab, plasmids were transiently transfected into 293 EBNA cells using Effectene (Qiagen), per manufacturer’s instructions. Briefly, 1.2 × 10^6 293 EBNA cells were seeded in 150 mM tissue culture dishes in DMEM/10% FBS. The following day, each dish was transfected with 1 μg of the IgG expression plasmid along with 4 μg of pAdVAntage (Promega) and 800 ng of pEGFP-1 (BD Clontech). The medium was changed to serum-free medium (IS PRO; Irvine Scientific) after 24 h. After

Cloning and expression of peptide epitope-embedded Abs

TT epitope 630DR was inserted by overlap PCR into the CH2 domain between glycines 249 and 250 (Kabat numbering; see Ref. 29) with two additional arginines upstream and three downstream of the epitope to give a final insertion of 25 total amino acids (RRIDKISDVSTIPYIG PALNIRRRA). The 5’ fragment was amplified using the forward primer E10Age5For: 5’-TTC CCC GAA CCG GTG ACG GTG TCG T-3’ that annealed to a region spanning the unique AgeI site upstream of the hinge region (DNA encoding aa 148–157 of the C_\text{H}1 domain), in combination with the backward primer E10insertionRev: 5’-GGC GAT GTA GGG CAC GAT GGT GCT CAC GAT GCT TCT TCT CCC CAG GAG TGC TGG TGA GGA AGA-3’ that annealed to 9 bases of the intron and the DNA encoding aa 244–249 of the C_\text{H}2 domain. The E10insertionRev primer contained a tail that encoded part of the insertion. The 3’ fragment was generated using the forward primer E10EcoRIFor: 5’-GTC AGC AAC ATC ATC CCT CCC AAC ATC CCT CCC GGC CTG AAC ATC AGA AGA GGA CCC TCA GTC TCC TCT CCC CCA-3’ that annealed to glycine 250 and downstream amino acids (250–258) and the reverse primer E10EcoRIRev 5’-GAT TAT GAT CAA TGA ATT CTG GCC GTC GCA CTC AT-3’ that annealed to a region spanning the stop codon and a unique EcoRI site within the vector at the end of the C_\text{H}3 region. For PCR, the expand high fidelity PCR system (Roche) was used. The two fragments were gel purified and combined for overlap extension PCR, and the PCR product was digested with AgeI and EcoRI and cloned into the similarly digested E10-IgG parental clone. The sequence of the final cloned product was confirmed by DNA sequencing.

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### Table I. Deduced amino acid sequences of loops comprising CDRs of L-SIGN Fabs

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*Sequences positions of CDR1, 2 and 3 as described in (Ref. 29).

Conditionally represents the same residue as in clone D12.

**Figure 2.** Receptor specificity analysis of soluble L-SIGN Fabs. A, 5 × 10^5 K562, K562/DC-SIGN, or K562/L-SIGN cells were incubated with purified soluble Fabs (20 μg/ml) representing six unique clones for 1 h, and the extent of their binding was assessed by flow cytometry using goat anti-mouse IgG PE conjugate. Values represent mean (bars, SD) of two independent experiments. B, Histograms showing similar expression of SIGN molecules on K562 cells after staining with SIGN-cross-reactive mAb16211 (20 μg/ml) and detecting with goat anti-mouse IgG PE conjugate.
an additional 24 h, 2.5 ml of 0.5 M HEPES/20% glucose was added. Cells were incubated 4 days, and the Ab in the medium supernatant was purified by protein A chromatography.

Human subjects and vaccination

Four normal, healthy volunteers were selected who had recently received the standard TT vaccine at their primary care physician’s office. Peripheral blood was drawn from these vaccinated donors after informed consent. The peripheral blood was used as a source to obtain both T cells and monocyte-derived DCs. The study protocol was approved by the institutional review board at Alexion Pharmaceuticals.

Monocyte-derived DCs and PBLs

Peripheral blood drawn from healthy TT-vaccinated individuals was separated on Ficoll density gradients to obtain mononuclear cells (PBMCs). PBMCs were allowed to adhere to flasks for 1 h at 37°C. Nonadherent cells (PBLs) were gently removed and washed, and CD3⁺ T cells (>95% pure) were magnetically isolated by negative selection (Pan T Cell Isolation Kit.
The adherent monocytes from the same donor were cultured in the presence of IL-4 (500 U/ml) and GM-CSF (800 U/ml) for 6–8 days to obtain immature DCs (iDCs) (>95% cells positive for CD11c, DC-SIGN, and HLA-DR, and negative for CD83 expression). X-VIVO 15 medium (Cambrex) supplemented with 2% human serum was used for all DC differentiation and T cell proliferation studies.

**Ab-targeted delivery of peptide epitopes**

iDCs were incubated with Abs E10 and E10-632DR for 1 h at 37°C, washed, and cocultured with purified CD3+ T cells (10,000 iDCs:100,000 T cells) at 37°C in a 96-well plate. After 4 days of coculture, tritiated thymidine (1 μCi/well; Amersham) was added to the cell cultures and...
thyidine incorporation was measured after 16–18 h on a microplate scintillation counter (PerkinElmer). Proliferation index was determined as (cpm of Ab treatment)/(cpm of medium treatment). For studies testing presentation of TT epitopes over time, similar procedures were performed, except that purified CD3+ T cells were added to the Ab-treated iDCs either immediately or 2 or 4 days later.

Statistical analysis

Differences between groups were analyzed by two-tailed unpaired Student’s t test. Significance was accepted when p < 0.05.

Results

Isolation and binding analysis of a panel of phage-derived Fabs recognizing C-type lectins, L-SIGN or DC-SIGN

An IgG1k phage-displayed Fab library derived from H and L chain coding sequences of mice immunized with human L-SIGN was first negatively selected in one round of panning on human DC-SIGN-Fc protein to remove Fabs with preferential binding to DC-SIGN or the Fc portion. The unbound phage were then used for selecting clones reactive with L-SIGN-Fc fusion protein in three rounds of positive selection. Of 95 clones selected from each of the three rounds of panning, 21 clones expressing phage-displayed Fabs showed a 5- to 100-fold higher binding to K562/L-SIGN compared with K562 cells (Fig. 1). As illustrated in Fig. 1, Fabs showed a 5- to 100-fold higher binding to K562/L-SIGN compared with K562 cells (Fig. 1A). As illustrated in Fig. 1B, 17 of the 21 Fabs showed specific binding in ELISA to recombinantly produced L-SIGN (O.D >1.0), demonstrating the success of the selection procedure, with two Fabs (D10 and E10) showing some level of cross-reactivity to recombinantly produced DC-SIGN. The relative magnitude of Fab binding to L-SIGN-transfected cells compared with Fab reactivity to recombinant protein can differ. For example, clone E10 showed a fairly robust signal to recombinant protein, but only a modest signal by FACS on cells. Recombinant proteins frequently have slightly different conformation and/or glycosylation patterns compared with the native form on the cell surface. Because the clones are derived from mice immunized with recombinant protein and the library was panned on recombinant protein, identification of a number of clones preferentially recognizing the recombinant protein might be expected.

All 21 clones reactive as phage-displayed Fabs with L-SIGN had unique DNA sequences (data not shown). Based on similarities among the H and L chain CDR3 sequences, a representative panel of six clones (C7, D12, E4, E10, G3, G10) was chosen for further characterization (Table I). Following subcloning to remove the phage gene III coat protein, the purified soluble Fabs of all six clones exhibited a 40- to 100-fold higher binding to K562/L-SIGN compared with K562 cells (Fig. 2A). Three purified Fabs, D12, G3, and E10, also reacted with K562/DC-SIGN cells, but at a lower level (Fig. 2A). This may represent some expected differences (due to expression or other differences that affect efficiency of display) in the analysis of Fabs when evaluated in a phage-displayed format compared with soluble Fabs. The expression of both DC-SIGN and L-SIGN receptors was found to be similar on K562 cells when assessed by SIGN-cross-reactive Ab mAb16211 (Fig. 2B).

Relative affinities and epitope specificities of L-SIGN Fabs

The six candidate Fabs were characterized further in terms of their relative affinities and epitope specificities. Although all Fabs displayed high affinity for L-SIGN as demonstrated by their reactivity in ELISA (>0.5 OD) at a concentration of 1 nM, Fab E10 exhibited strong binding even at picomolar concentrations (Fig. 3A). To determine epitope specificities, the ability of each of the six Fabs to inhibit the binding of the lone commercially available L-SIGN-specific Ab, mAb162, to L-SIGN-Fc fusion protein was assayed in a competition ELISA. As shown in Fig. 3B, four Fabs (C7, D12, G10, and E4) inhibited the binding of mAb162 in a concentration-dependent manner, while Fabs G3 and E10 did not compete, similar to the negative control Fab. These similarities and differences in epitope binding are also reflected by their sequences: clones D12, C7, E4, and G10 have closely related CDR, while those of clones E10 and G3 are unrelated to the set of four and to each other (see Table I). To further characterize and differentiate the epitopes recognized by the six Fabs, whole cell lysates of K562/L-SIGN cells prepared under denaturing and reducing conditions were separated by SDS-PAGE, and the membranes were probed with individual L-SIGN Fabs. As depicted in Fig. 3C, two Fabs (clone D12 and E10) recognized a protein band of ~42 kDa with good correlation to monomeric L-SIGN (5), while Fab G3 recognized a higher molecular mass protein band that may correspond to an oligomeric form of L-SIGN, possibly resulting from only partial denaturation of the sample. These data imply that the epitopes recognized by Fabs D12 and E10 are likely to be linear, and further, that although they recognize their epitope on L-SIGN-expressing cells, their epitope is inaccessible in the partially denatured oligomer. The epitopes recognized by Fabs C7, E4, and G10 are probably conformational because these epitopes are destroyed after partial or complete denaturation with concomitant loss of reactivity to either band. Because G3 only recognizes the presumed oligomeric form and not a monomeric form, it probably recognizes a conformational epitope formed on oligomerization. Our data suggest that under the denaturing conditions used (95°C), the oligomeric structure does not fall apart; such observations have been made with other proteins, which oligomerize via coiled-coil domains and require a high concentration of guanidinium chloride for separation (30).

<table>
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<tr>
<th>Clone</th>
<th>Receptor Specificity</th>
<th>Internalization (%)&lt;sup&gt;a&lt;/sup&gt; by K562/L-SIGN</th>
<th>Blocking (%)&lt;sup&gt;b&lt;/sup&gt; of ICAM-3</th>
<th>Blocking (%)&lt;sup&gt;b&lt;/sup&gt; of HIVgp120</th>
<th>Blocking (%)&lt;sup&gt;b&lt;/sup&gt; of Ebola gp</th>
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<tr>
<td>D12</td>
<td>L-SIGN/DC-SIGN</td>
<td>25</td>
<td>26</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
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<td>45</td>
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<td>L-SIGN</td>
<td>27</td>
<td>35</td>
<td>54</td>
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<td>L-SIGN</td>
<td>32</td>
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<td>L-SIGN</td>
<td>27</td>
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<td>50</td>
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<tr>
<td>G3</td>
<td>L-SIGN/DC-SIGN</td>
<td>46</td>
<td>45</td>
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<sup>a</sup> Percentage of internalization was determined as (mean fluorescence intensity at 4°C – mean fluorescence intensity at 37°C)/(mean fluorescence intensity at 4°C) × 100.

<sup>b</sup> Percentage of blocking was determined as (number of cells bound to ligand with Fab – number of cells bound to ligand without Fab)/(number of cells bound to ligand without Fab) × 100.
L-SIGN Fabs block ligand binding to the receptor

Several viruses, e.g., Ebola, SARS, HIV, and HCV, have been shown to use DC-SIGN and L-SIGN receptors for gaining entry into cells. Both ICAM-3 on T cells and envelope glycoproteins on viruses were found to interact with the CRD of the SIGN receptors in a manner unique to each ligand (7, 9). To determine whether we isolated CRD-reactive Fabs capable of blocking ligand binding, a ligand-coated fluorescent bead-blocking assay was performed. Ligand-coated fluorescent beads not only mimic multimeric binding of the ligand to the cell surface receptor, but also allow quantitation of ligand binding by flow cytometry. First, adhesion of fluorescent beads coated with envelope glycoproteins of Ebola and HIV to K562/DC-SIGN and K562/L-SIGN was assessed in the absence of Abs. As illustrated in Fig. 4A, while Ebola envelope glycoprotein bound equally well to both DC-SIGN- and L-SIGN-expressing cells, HIV envelope glycoprotein bound more strongly to DC-SIGN-expressing cells than to L-SIGN-expressing cells. These differences in viral protein binding to the SIGN molecules correlated with the ability of Fabs to block adhesion of the viral proteins. Although all six Fabs could block to some extent HIVgp120 binding to L-SIGN (39–62%), only two Fabs, C7 and E10, showed significant ($p < 0.001$) blocking of Ebola gp binding to L-SIGN (70 and 71%, respectively; Fig. 4B), despite recognizing different epitopes. Of the three DC-SIGN-cross-reactive Fabs, D12, E10, and G3, only E10 effectively blocked binding of both
viral proteins to DC-SIGN \((p < 0.0001; \text{see Fig. 4C})\). As expected, the other three Fabs uniquely reactive with L-SIGN had no blocking effect on ligand binding to DC-SIGN (Fig. 4C). In addition, three Fabs, C7, E10, and G10, which were most efficient at blocking the HIV gp120 viral protein binding to L-SIGN, also prevented the binding of ICAM-3 to K562/L-SIGN cells most efficiently (see Table II).

**Conversion of Fab to IgG enhances receptor binding and blocking of viral protein adhesion**

Based on the receptor-binding and ligand-blocking results, Fab clones E10 and G10 were converted into chimeric IgGs to increase avidity for the receptor and thereby enhance blocking of virus binding. As shown in Fig. 5A, Fab to IgG conversion greatly improved receptor binding of both clones E10 and G10, but also resulted in some cross-reactivity of G10 with DC-SIGN. This is not a general result of Fab conversion to IgG, because the IgG version of C7 was still uniquely reactive with L-SIGN (data not presented). As depicted in Fig. 5, B and C, full IgGs demonstrated enhanced blocking of viral protein binding compared with their Fab counterparts, for example, while full IgG forms of clones E10 and G10 produced >90% blocking of Ebola gp to L-SIGN, their Fab counterparts showed 71 and 22% blocking, respectively (see Fig. 5C).

**L-SIGN Fabs undergo internalization upon binding to the receptor on human liver sinusoidal endothelial cells and L-SIGN-transfected K562 cells**

Ab internalization is a prerequisite for delivering autoimmune Ags as Ab-linked cargo into L-SIGN-expressing liver sinusoidal endothelial cells (LSECs) (4, 21). The internalizing potential of the

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**FIGURE 7.** Confirmation of Ab internalization on transfected cells by confocal microscopy. K562/L-SIGN cells were incubated for 90 min with L-SIGN Fabs and imaged with a confocal system using goat anti-mouse IgG Alexa 647 as detection reagent. AZND2 is a SIGN-cross-reactive Ab used as a positive control. As additional specificity controls, two Fab clones, E4 (L-SIGN specific) and E10 (L-SIGN and DC-SIGN cross-reactive), were also incubated with K562 cells expressing DC-SIGN (see lowermost panels on the right). Data are representative of two independent experiments.
Fabs was assessed on freshly isolated human liver nonparenchymal cells containing ~55% cells that expressed L-SIGN (see Fig. 6A). L-SIGN-positive cells were further characterized using a panel of Abs against endothelial cell markers (CD31, CD54, CD106), immune cell recognition molecules (CD40, CD80, CD86, MHC class I and II), and myeloid cell markers (CD4, CD11c) (19, 31). The observed expression profile of these receptors correlated well with previously described studies on LSECs (19), which were characterized by strong expression levels of CD31, CD54, CD206, and MHC class I and moderate to weak expression of the other markers on LSECs (data not presented).

Internalization of Fabs after L-SIGN binding was determined by incubating freshly isolated human liver nonparenchymal cells with the six L-SIGN Fabs at 4°C and 37°C, respectively, and the level of Ab remaining on the cell surface after 2 h was determined by flow cytometry. As shown in Fig. 6B, >40% loss of signal was observed for three Fabs (C7, E10, and G10) and the L-SIGN-specific mAb162 at 37°C compared with 4°C. Three other Fabs (clone D12, E4, and G3) showed a slightly more modest (30–38%) loss of signal. Similar studies comparing the signal remaining on K562/L-SIGN cells further confirmed the observations made on LSECs (see Table II). In addition, confocal microscopy was used as an alternate method to further confirm Ab internalization. As illustrated in Fig. 7, all six L-SIGN Fabs were found inside K562/L-SIGN cells following 90-min incubation at 37°C. Furthermore, while the L-SIGN/DC-SIGN-cross-reactive Ab E10 was internalized...
by both DC-SIGN and L-SIGN transfectants, the L-SIGN-specific clone E4 was internalized only by L-SIGN transfectants (see bottom-most panels designated E4/DC-SIGN and E10/DC-SIGN). The microscopy images are illustrative of a specific internalization process as highlighted by the clustered vesicular staining under the cytoplasmic membrane. Interestingly, these studies show that while all of the L-SIGN Fabs are able to internalize to some extent, the degree of internalization correlated with the capacity of the Abs to block ICAM-3 and HIV binding, but not with their relative affinities.

Ab-targeted delivery of a peptide Ag induces a sustained human T cell response

To consider the use of these Abs in Ab-mediated Ag delivery, we embedded a universal Th epitope, 632DR (32, 33), from TT Ag within a peptide-embedded Ab, E10 (Fig. 8B), and was affinity purified to remove the partially formed Ab seen in crude preparations (Fig. 8, compare C with B). The relative affinity of the peptide-embedded Ab to the DC-SIGN and L-SIGN receptor was found to be similar to the native Ab (see Fig. 8, D and E).

As a first step to evaluate targeted delivery, processing, and presentation of the inserted epitope, we took advantage of the cross-reactivity of E10 to DC-SIGN as well as the relative ease of obtaining DCs and autologous T cells from human peripheral blood. iDCs were treated with the native and peptide-embedded Abs for 1 h, washed, and cocultured with autologous T cells from TT-vaccinated donors who had been prescreened for a proliferative T cell response to the whole TT protein (data not presented). As shown in Fig. 9, targeting with E10-632DR elicited a significant (p < 0.005 vs native Ab, E10) T cell proliferative response in donor 13 similar to the free 632DR peptide. However, no proliferative responses were induced in the other three donors by targeting with E10-632DR commensurate with lack of responses to the free peptide in these donors. Furthermore, when donor 13 iDCs were treated with different doses of the Ab, T cell responses directly correlated with the Ab dose, and a significant level (p < 0.00005 vs native Ab) of T cell proliferation was observed even at picomolar concentrations of the targeting Ab (see Fig. 10A). In addition, the proliferative responses induced by E10-632DR could be blocked with excess native Ab E10 (p = 0.00008 vs E10-632DR), but not a control Ab ALXN4100 (see Fig. 10A). Moreover, both the native Ab and peptide-embedded Ab showed equivalent binding to donor 13 iDCs used in the T cell activation assays (see Fig. 10B).

Delivery of Ags linked to Abs is expected to require internalization and proteasomal cleavage of the Ab to release the linked Ag for presentation to T cells. This type of processing could potentially prolong presentation of Ag to T cells, resulting in a long-lasting immune response. To test this notion, iDCs from donor 13 were treated with E10-632DR or free 632DR peptide for 1 h, washed, and cocultured with autologous T cells either immediately or 2 and 4 days after Ag pulsing. As shown in Fig. 11, only peptide-embedded Ab produced a significant T cell response even 4 days after Ag pulsing, demonstrating that delivering Ag via Abs can result in a sustained immune response, which was not achievable with free peptide. Based on this result, it is conceivable that in vivo targeting with the peptide-embedded Ab would produce a more heightened immune response compared with a free peptide vaccine.

Discussion

L-SIGN is a recently discovered C-type lectin expressed in lymph node and liver sinusoidal endothelial cells and placenta (4, 6). Although the expression of L-SIGN on cells implicated in tolerance induction vs the expression of DC-SIGN on cells potentially capable of raising a stimulatory immune response is very striking, neither functional consequences of the interaction of ICAM-3 on T cells with L-SIGN nor functional consequences of pathogen binding to the receptor are well defined (4, 5), with limited knowledge of the correlation between the two known roles (9). Identification of a panel of Abs that bind to different epitopes on the receptor may assist in elucidation of domains of the receptor involved in the various biologic processes. Abs that specifically recognize the ligand binding domain of L-SIGN may be useful for exploring the biological consequences of receptor activation. Furthermore, it may be feasible to use these same Abs therapeutically for the modulation of immune responses. Abs that block pathogen binding may find immediate therapeutic use in preventing transmission of disease in the host, or in the development of novel therapeutics based on epitope specificity.

Isolating Abs that react selectively with L-SIGN, but not DC-SIGN, is challenging due to the similarities between the two proteins. L-SIGN bears an overall amino acid sequence identity of 77% to DC-SIGN, with even greater identity (88%) in the extracellular domain, the target for Ab binding for therapeutic purposes. Furthermore, the 40 unique amino acids in the 330 aa extracellular domain of L-SIGN are not clustered in one region of the protein (1, 4). The ability to sequentially pan phage-displayed Fab libraries on DC-SIGN (negative selection) and L-SIGN (positive selection) provided us with a powerful tool to successfully identify a panel of high affinity Fab clones that are either uniquely reactive or preferentially reactive with the L-SIGN receptor. Competition ELISAs and Western blot studies revealed a number of interesting features in these Fabs. Fabs with at least three different specificities were identified in competition experiments. Although four of the Fabs competed with binding of L-SIGN-specific mAb162, they did so with differing kinetics, implying that either epitopes bound by these four Fabs are overlapping, but not identical, or their relative

![Graph](https://www.jimmunol.org)
affinities are different, or that competition was the result of steric hindrance. Further characterization of the panel demonstrated a variety of distinctions among this subset of Fabs. Western blot studies revealed at least three classes of epitopes recognized by the Fab panel. Although Fabs C7, E4, and G10 recognize conformational epitopes absent in denatured Western blots, Fabs D12 and E10 bound a linear epitope accessible in the monomeric receptor. In contrast, Fab G3 bound L-SIGN-transfected cells as well as or better than the other Fabs in the panel, and appeared to bind an epitope present only in the presumed oligomeric form of the receptor. This is an interesting finding, as crystal structure studies of the extracellular domain demonstrated that receptor oligomerization is required for the recognition of complex carbohydrate ligands by the SIGN molecules (36, 37). As a result of the differences in the epitopes recognized by the Fabs, the biological activities induced or prevented by them could potentially be diverse.

To be able to attribute specific biological functions to different domains of the receptor, we chose to explore two different properties: blocking of ligand binding and Ab internalization. Binding of the Abs to the CRD of the receptor is a prerequisite for their use in modulating the immune response and preventing viral transmission. Interestingly, while Fabs C7 and E10 consistently and effectively blocked the binding of ICAM-3 and both of the viral proteins, Fab G10 was able to block binding of ICAM-3 and HIVgp120, but not the binding of Ebola gp (summarized in Table

FIGURE 10. Dose-dependent responses to the targeting Ab and blocking of T cell activation by competition with native Ab. A, Ten thousand iDCs from donor 13 were incubated with Abs and free peptide for 1 h at 37°C, washed, and cocultured with 100,000 autologous T cells for 5 days. Proliferation was assessed by [3H]thymidine incorporation. **, Highly significant values; *p < 0.00005 when compared with medium or native Ab, E10. Values represent mean (SD) of eight well replicates. This experiment was repeated three times with similar outcomes. B, Dose-dependent binding of peptide-embedded and native Abs to donor 13 iDCs used in the T cell proliferation assays.
endosomal pH, while DC-SIGN does, Guo et al. (39) concluded that L-SIGN is not capable of mediating direct internalization. In contrast, studies using either pseudotype viruses of Ebola (16) or infectious strains of SARS (12) found that virus capture and internalization do occur by L-SIGN-positive cells in cis; this finding was demonstrated by the measurement of viral RNAs isolated from receptor-positive cells. More recently, Ludwig et al. (40) provided further support for L-SIGN-mediated internalization of HCV by monitoring its intracellular localization by confocal microscopy. Our studies demonstrating internalization of L-SIGN Abs on freshly isolated human LSECs by FACs analysis and on L-SIGN-transfected cells by confocal microscopy strongly support the latter virus internalization studies. The conservation of the di-leucine motif and the triadric cluster in the cytoplasmic tail of both DC-SIGN and L-SIGN, which are known to be required for receptor internalization, lends additional support for the internalization potential of L-SIGN (1, 4, 41). As LSECs are well designed to take up a wide variety of harmful Ags from circulation by receptor-mediated endocytosis (19), it is likely that L-SIGN may assist in the process of Ag internalization by LSECs.

Ab internalization is a prerequisite for the use of L-SIGN Ab to specifically deliver Ag to LSECs. LSECs appear to be directly involved in tolerance induction through active uptake of many Ags from blood circulation, followed by processing and presentation of the antigenic peptides efficiently to trafficking T cells (19, 42). CD8⁺ T cells, when exposed to an Ag presented by LSECs, stop producing IL-2 and IFN-γ to become tolerogenic. Furthermore, addition of inflammatory stimuli, e.g., TNF-α, IFN-γ, or IL-12, does not rescue the tolerogenic phenotype induced by LSECs (21). Likewise, CD4⁺ T cells stimulated by Ag-presenting LSECs show a regulatory phenotype characterized by the expression of IL-10 and IL-4 (20). If autoimmune Ags (e.g., proinsulin or myelin basic protein) could be delivered selectively to LSECs via L-SIGN-specific Abs, it might be possible to induce tolerance and thereby block the destructive functions of autoreactive T cells and might be of value in the treatment of autoimmune diseases such as type I diabetes or multiple sclerosis. Alternatively, specific targeting of Ag to DC-SIGN, expected to raise a stimulatory response, could be exploited therapeutically for the treatment of cancer. The ease of obtaining DCs and autologous T cells from human peripheral blood allowed us to first explore the feasibility of delivering Ag using L-SIGN/DC-SIGN Abs to DCs for the induction of Ag-specific T cell responses.

A significant obstacle in testing the targeted delivery approach is the production of Ag-linked Abs. Conventionally, this is achieved by chemical conjugation of Ag to the Ab; however, this method suffers from many problems, particularly in the context of large-scale therapeutic application in humans. It is difficult to control the number and the point of Ag attachment to the Ab and to ensure batch-to-batch production consistency. Ag has also been fused genetically to the C terminus of Fabs and IgGs (43, 44); however, such constructs resulted in significant to complete loss of expression of our peptide-fused Abs. Therefore, we developed a novel approach to embed peptide Ags into the C domain of L-SIGN Abs, expected to raise a stimulatory response, could be exploited therapeutically for the treatment of cancer. The use of Ags and Ag-specific T cells from human peripheral blood allowed us to first explore the feasibility of delivering Ag using L-SIGN/DC-SIGN Abs to DCs for the induction of Ag-specific T cell responses.
taken up specifically by human DCs and the embedded peptide epitope was subsequently processed and presented to autologous T cells for their robust activation. Importantly, the presentation of the embedded peptide was sufficient to activate significant T cell responses observed for up to 4 days after treatment with the targeting Ab. Tacken et al. (47) very recently validated DC-SIGN as a suitable candidate for targeted delivery of Ag to DCs by chemically conjugating keyhole limpet hemocyanin to a humanized anti-DC-SIGN Ab, resulting in a proliferative response of cells from a keyhole limpet hemocyanin-vaccinated donor. Our approach further expands on these studies, allowing for a more refined delivery of specific peptides and precise cleavage for presentation to the T cells, and is to our knowledge the first report of an Ab-targeted single epitope delivery from an infectious agent to human DCs that resulted in a productive T cell response. In further studies, we will address whether targeted delivery of Ag to L-SIGN will induce T cell tolerance to the presented Ags.

In conclusion, we report in this study the identification of human L-SIGN-specific Abs that appear to mediate therapeutically relevant functions, and represent a first step in evaluation of L-SIGN as a clinically important target. These Abs may find utility in exploring the biological function of the receptor, delivering Ags to target organs, modulating immune responses, and preventing the transmission of infectious agents.

Acknowledgments

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