Characterization of a Human Homologue of the Murine Peripheral Lymph Node Homing Receptor

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Abstract. Lymphocyte trafficking is a fundamental aspect of the immune system that allows B and T lymphocytes with diverse antigen recognition specificities to be exposed to various antigenic stimuli in spatially distinct regions of an organism. A lymphocyte adhesion molecule that is involved with this trafficking phenomenon has been termed the homing receptor. Previous work (Lasky, L., T. Yednock, M. Singer, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel, and S. Rosen. 1989. *Cell.* 56:1045–1055) has characterized a cDNA clone encoding a murine homing receptor that is involved in trafficking of lymphocytes to peripheral lymph nodes. This molecule was found to contain a number of protein motifs, the most intriguing of which was a carbohydrate binding domain, or lectin, that is apparently involved in the adhesive interaction between murine lymphocytes and peripheral lymph node endothelium. In this study, we have used the murine cDNA clone to isolate a human homologue of this peripheral lymph node–specific adhesion molecule. The human receptor was found to be highly homologous to the murine receptor in overall sequence, but showed no sequence similarity to another surface protein that may be involved with human lymphocyte homing, the Hermes glycoprotein. The extracellular region of the human receptor contained an NH_2 terminally located carbohydrate binding domain followed by an EGF-like domain and a domain containing two repeats of a complement binding motif. Transient cell transfection assays using the human receptor cDNA showed that it encoded a surface glycoprotein that cross reacted with a polyclonal antibody directed against the murine peripheral lymph node homing receptor. Interestingly, the human receptor showed a high degree of sequence homology to another human cell adhesion glycoprotein, the endothelial cell adhesion molecule ELAM.

THE ability of lymphocytes to be exposed to antigenic stimuli located in lymphoid tissues in distant regions of an organism is, in part, due to an adhesive interaction between a receptor on the lymphocyte surface and a ligand on the surface of the specialized endothelial venules that are found within the lymph nodes (4, 9, 12, 31). The glycoprotein receptor on the surface of the lymphocyte has been termed the homing receptor, since it allows these cells to home to specific regions of the lymphoid system. A number of lines of evidence suggest that this adhesive interaction can be subdivided into at least three specificities that include peripheral lymph nodes (pln), gut-associated lymphoid tissue (galt), and synovial tissue (6, 7, 8, 18). The nature of this tissue specificity is currently not understood, although it has been hypothesized that a combination of recognition events between diverse lymphocyte homing receptors and endothelial cell ligands may allow for a tissue-specific lymphocyte distribution (5, 17, 19).

Molecules that may mediate lymphocyte homing have been identified in several rodent species (8, 11, 22, 24). Much has recently been learned regarding the molecular nature of the murine lymphocyte homing receptor that is required for pln trafficking. A monoclonal antibody specific for this receptor, termed Mel 14, was initially shown to block the adhesive interaction between lymphocytes and the high endothelium of pln but not galt or synovium (11). This antibody recognized a surface glycoprotein of ∼90,000 D that was both developmentally regulated and specifically localized on immune cells (12). It was subsequently shown that the glycoprotein recognized by this monoclonal antibody also bound to various sugar compounds and that the interaction between lymphocytes and pln endothelium could be blocked by these compounds, suggesting that the murine pln homing receptor was a lectin and that the adhesive interaction was due to this lectin-like activity (28, 29, 33, 34). In agreement with this hypothesis, a cDNA clone that encoded the antigen recognized by Mel 14 was found to contain a domain that was homologous to a number of carbohydrate binding proteins or lectins (23a, 27). These results strongly suggested that the pln specificity of lymphocytes expressing the

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1. *Abbreviations used in this paper:* galt, gut-associated lymphoid tissue; pln, peripheral lymph nodes.

glycoprotein recognized by Mel 14 was imparted by the lectin domain contained within this protein and a carbohydrate found specifically on the endothelial cell surface of pln.

In contrast to the murine case, the nature of the human pln-specific homing receptor is less clear. Stoolman et al. (30) have clearly shown that the interaction between human lymphocytes and pln endothelium appears to be mediated by a lectin that has identical carbohydrate specificity to that found in the murine case. The character of a surface glycoprotein that may be involved in human pln homing was investigated with a series of monoclonal and polyclonal antibodies generically termed Hermes. While the initial monoclonal antibody, Hermes 1, was unable to block the lymphocyte–pln interaction, a polyclonal antibody directed against the Hermes 1 antigen was able to block both pln and galt binding while a monoclonal antibody, Hermes 3, was able to block only galt binding, although at relatively high antibody levels (15, 16, 20). These results suggested that the Hermes antigen(s) was involved in the adhesive interaction between lymphocytes and lymphoid endothelium in humans. The Hermes antibodies recognized a ∼90,000-D surface glycoprotein that was found on a large number of both immune and nonimmune cell types and that, by antibody preclearing experiments, appeared to be related to the Mel 14 antigen, suggesting that the Hermes antigen was the human equivalent of the murine pln-specific homing receptor.

We have used a murine pln-specific homing receptor cDNA clone to isolate a human homologue of this glycoprotein from a peripheral blood lymphocyte cDNA library. The sequence of the human homologue revealed a high degree of homology with the murine receptor, especially within the carbohydrate binding and EGF-like domains. No homology was found with the Hermes protein sequence, but a significant degree of homology was found with another adhesion molecule found on human endothelial cells, the endothelial leukocyte adhesion molecule, ELAM. These results suggest that the human pln homing receptor is a lectin and that the mechanism of pln recognition by lymphocytes is the same in both the human and murine immune systems.

Materials and Methods

cDNA Isolation and Sequencing

The 2.2-kb Eco RI insert of the murine Mel 14 antigen cDNA clone (23a) was isolated, labeled to high specific activity by randomly primed DNA polymerase synthesis with ^{32}P triphosphates, and used to screen 600,000 clones from an oligo dT primed lambda gt10 cDNA library derived from human peripheral blood lymphocyte mRNA (the kind gift of K. Fisher and R. Lawn). The filters were hybridized overnight at 42°C in 40% formamide, 5× SSC (1× SSC is 30 mM NaCl, 3 mM trisodium citrate), 50 mM sodium phosphate (pH 6.8), 10% dextran sulfate, 5× Denhardt's solution, and 20 micrograms/ml sheared, boiled salmon sperm DNA. They were washed twice for 40 min in 0.2× SSC, 0.1% SDS at 55°C. 12 clones (approximately one positive per plate of 50,000 phage) were picked, and the largest Eco RI insert (∼2.2 kb) was isolated and the DNA sequence was determined by didoxynucleotide sequencing in the bacteriophage M13 using sequence-specific primers. Sequence homologies were analyzed using the hom.global and align programs.

Transient Expression of the Human pln-specific Homing Receptor

The Eco RI insert encoding the full-length human sequence was isolated and ligated to an expression vector that contains a cytomegalovirus early promoter (pRK5) (10). This vector has previously been shown to direct the efficient synthesis of inserted genes when transfected onto a transformed human embryonic kidney line (293 cells) (23). A plasmid in the correct orientation relative to the promoter was isolated and transfected into 293 cells using the calcium phosphate transfection procedure. After 2 d, cells were stained using either a rabbit polyclonal antibody directed against a purified preparation of the murine pln homing receptor (the gift of S. Rosen, Department of Anatomy, University of California, San Francisco) and a phycoerythrene-labeled goat anti-rabbit antibody or the Mel 14 monoclonal antibody and phycoerythrene-labeled goat anti-rat antiserum for FACS (fluorescence activated cell sorter) analysis.

Results

Experiments by Stoolman, Rosen, and colleagues (28, 29, 33, 34) initially suggested that human lymphocytes bound to pln endothelium by using a lectin-like activity that was found on the cell surface. In addition, recent work by Butcher and colleagues (32) clearly demonstrated that the binding activity of lymphocytes to pln endothelium was conserved between humans and rodents in that both species bound specifically to each other's lymphoid endothelium. We, therefore, screened a human peripheral blood lymphocyte cDNA library with a cDNA encoding the murine pln-specific homing receptor at mildly reduced hybridization stringency (see Materials and Methods). The largest of 12 positive cDNA clones was isolated, and its nucleotide sequence is shown in Fig. 1. This ∼2.2-kb clone encoded an open reading frame of 372 amino acids with a molecular mass of ∼42,200 D that began with a methionine which was preceded by a eukaryotic translational start homology (21). The encoded protein contained 26 cysteine residues and 8 potential N-linked glycosylation sites (14). A highly hydrophobic region at the NH_2 terminus of the protein (residues 20–32) was a potential signal sequence, while another highly hydrophobic COOH-terminally located region of 22 amino acids in length (residues 333–355) was a potential stop transfer or membrane anchoring domain (3). This COOH-terminal hydrophobic region was followed by a charged, presumably cytoplasmic, region.

Comparison of the nucleotide sequence of this human clone with that previously found for the murine pln homing receptor (23a, 27) showed a high degree of DNA sequence identity (∼71%) overall. As can be seen in Fig. 2, the amino acid sequences of the human homologue of the murine pln-specific homing receptor is also highly homologous. Previous work had shown that the murine receptor contained three protein motifs: an NH_2-terminal carbohydrate binding domain or lectin, an EGF-like domain, and two repeats of a complement binding domain, followed by a transmembrane anchor sequence (23a, 27). The human receptor shows an identical overall structure. The degrees of identity between the domains found in the human versus the mouse receptor, however, are variable. For example, the degree of sequence conservation between the murine and human receptors in both the lectin and EGF domains is 83%, while the degree of conservation in the first complement binding repeat is 79% and is 63% in the second repeat. Comparison of the homologies of the individual complement binding repeats with each other showed that the two repeats are identical to each other in the murine receptor while those in the human receptor show differences between themselves as well as with the murine repeats (Fig. 3). Interestingly, the degree of conservation between the two receptors in the transmembrane sequence and surrounding regions is virtually identical, with

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GAATTCCAGTGTGCTGGCTTCCTCACCTGCAGCACAGCACACTCCCTTTGGCAAGGACCTGAGACCCTTGTGCTAAGTC

                                                    1
                                         met ile phe pro trp lys cys
AAGAGGCTCAATGGGCTGCAGAAGAACTAGAGAAGGACCAAGCAAAGCC  ATG ATA TTT CCA TGG AAA TGT

   10                                        20      Signal Sequence
gln ser thr gln arg asp leu trp asn ile phe lys | leu trp gly trp thr met leu cys
CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC TTC AAG   TTG TGG GGG TGG ACA ATG CTC TGT

   30                              Probable N-Terminus
cys asp phe leu ala | his his gly thr tyr cys trp thr tyr his tyr ser glu lys pro
TGT GAT TTC CTG GCA  CAT CAT GGA ACC TAC TGC TGG ACT TAC CAT TAT TCT GAA AAA CCC

   50                            60
met asn trp gln arg ala arg arg phe cys arg asp asn tyr thr asp leu val ala ile
ATG AAC TGG CAA AGG GCT AGA AGA TTC TGC CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA

   70                            80
gln asn lys ala glu ile glu tyr leu glu lys thr leu pro phe ser arg ser tyr tyr
CAA AAC AAG GCG GAA ATT GAG TAT CTG GAG AAG ACT CTG CCC TTC AGT CGT TCT TAC TAC

   90                            100
trp ile gly ile arg lys ile gly gly ile trp thr trp val gly thr asn lys ser leu
TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG GTG GGA ACC AAC AAA TCT CTC

   110                           120
thr glu glu ala glu asn trp gly asp gly glu pro asn asn lys lys asn lys glu asp
ACT GAA GAA GCA GAG AAC TGG GGA GAT GGT GAG CCC AAC AAC AAG AAG AAC AAG GAG GAC

   130                           140
cys val glu ile tyr ile lys arg asn lys asp ala gly lys trp asn asp asp ala cys
TGC GTG GAG ATC TAT ATC AAG AGA AAC AAA GAT GCA GGC AAA TGG AAC GAT GAC GCC TGC

   150                           160
his lys leu lys ala ala leu cys tyr thr ala ser cys gln pro trp ser cys ser gly
CAC AAA CTA AAG GCA GCC CTC TGT TAC ACA GCT TCT TGC CAG CCC TGG TCA TGC AGT GGC

   170                           180
his gly glu cys val glu ile ile asn asn his thr cys asn cys asp val gly tyr tyr
CAT GGA GAA TGT GTA GAA ATC ATC AAT AAT CAC ACC TGC AAC TGT GAT GTG GGG TAC TAT

   190                           200
gly pro gln cys gln leu val ile gln cys glu pro leu glu ala pro glu leu gly thr
GGG CCC CAG TGT CAG CTT GTG ATT CAG TGT GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC

   210                           220
met asp cys thr his pro phe gly asn phe ser phe ser ser gln cys ala phe ser cys
ATG GAC TGT ACT CAC CCC TTT GGA AAC TTC AGC TTC AGC TCA CAG TGT GCC TTC AGC TGC

   230                           240
ser glu gly thr asn leu thr gly ile glu glu thr thr cys gly pro phe gly asn trp
TCT GAA GGA ACA AAC TTA ACT GGG ATT GAA GAA ACC ACC TGT GGA CCA TTT GGA AAC TGG

   250                           260
ser ser pro glu pro thr cys gln val ile gln cys glu pro leu ser ala pro asp leu
TCA TCT CCA GAA CCA ACC TGT CAA GTG ATT CAG TGT GAG CCT CTA TCA GCA CCA GAT TTG

   270                           280
gly ile met asn cys ser his pro leu ala ser phe ser phe thr ser ala cys thr phe
GGG ATC ATG AAC TGT AGC CAT CCC CTG GCC AGC TTC AGC TTT ACC TCT GCA TGT ACC TTC

   290                           300
ile cys ser glu gly thr glu leu ile gly lys lys thr ile cys glu ser ser gly
ATC TGC TCA GAA GGA ACT GAG TTA ATT GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA

   310                           320
ile trp ser asn pro ser pro ile cys gln lys leu asp lys ser phe ser met ile lys
ATC TGG TCA AAT CCT AGT CCA ATA TGT CAA AAA TTG GAC AAA AGT TTC TCA ATG ATT AAG

   330                           Stop Transfer Sequence
glu gly asp tyr asn pro leu phe ile pro val ala val met val thr ala phe ser gly
GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA GTG GCA GTC ATG GTT ACT GCA TTC TCT GGG

   350                           360
leu ala phe ile ile trp leu ala arg arg leu lys lys gly lys lys ser lys arg ser
TTG GCA TTT ATC ATT TGG CTG GCA AGG AGA TTA AAA AAA GGC AAG AAA TCC AAG AGA AGT

   370    372
met asn asp pro tyr OC
ATG AAT GAC CCA TAT TAA ATCGCCCTTGGTGAAAGAAAATTCTTGGAATACTAAAAATCATGAGATCCTTTA

AATCCTTCCATGAAACGTTTTGTGTGGTGGCACCTCCTACGTCAAACATGAAGTGTGTTCCTTCAGTGCATCTGGGAA

GATTTCTACCCGACCAACAGTTCCTTCAGCTTCCATTTCGCCCCTCATTTATCCCTCAACCCCCAGCCCACAGGTGTT

TATACAGCTCAGCTTTTTGTCTTTTCTGAGGAGAAACAAATAAGACCATAAGGGAAAGGATTCATGTGGAATATAAAG

ATGGCTGACTTTGCTCTTTCTTGACTCTTGTTTTCAGTTTCAATTCAGTGCTGTACTTGATGACAGACACTTCTAAAT

GAAGTGCAAATTTGATACATATGTGAATATGGACTCAGTTTTCTTGCAGATCAAATTTCACGTCGTCTTCTGTATACT

GTGGAGGTACACTCTTATAGAAAGTTCAAAAAGTCTACGCTCTCCTTTCTTTCTAACTCCAGTGAAGTAATGGGGTCC

TGCTCAAGTTGAAAGAGTCCTATTTGCACTGTAGCCTCGCCGTCTGTGAATTGGACCATCCTATTTAACTGGCTTCAG

GCCTCCCCACCTTCTTCAGCCCACCTCTCTTTTTCAGTTGGCTGACTTCCACACCTAGCATCTCATGAGTGCCAAGCAA

AAGGAGAGAAGAGAGAAATAGCCTGCGCGGTTTTTTAGTTTGGGGGTTTTGCTGTTTCCTTTTATGAGACCCATTCCT

ATTTCTTATAGTCAATGTTTCTTTTATCACGATATTATTAGTAAGAAAACATCACTGAAATGCTAGCTGCAAGTGACA

TCTCTTTGATGTCATATGGAAGAGTTAAAACAGGTGGAGAAATTCCTTGATTCACAATGAAATGCTCTCCTTTCCCCT

GCCCCCAGAACTTTTATCCACTTACCTAGATTCTACATATTCTTTAAATTTCATCTCAGGCCTCCCTCAACCCCACGG

GGCCGCCAGCACACTGGAATTC
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Figure 1. DNA and protein sequence of a human homologue of the murine pln homing receptor. The figure shows the DNA and translated protein sequence of a cDNA clone isolated from a human peripheral blood lymphocyte library by virtue of its homology to the murine homing receptor. The human protein sequence starts with a methionine encoded by nucleotides 131–133 that is preceded by a eukaryotic translational initiation site homology (5'AAAGCC3') (21). A 372–amino acid open reading frame comes after this initiation codon. An NH_2-terminally located hydrophobic core region is shown (amino acid residues 20–32) that could function as a signal sequence (3). The probable NH_2 terminus (trp residue number 39) is suggested by analogy to that determined by amino acid sequencing of the isolated murine pln homing receptor (23a, 27). The protein contains 26 cysteines (shaded), four of which are found in the putative signal sequence and eight potential N-linked glycosylation sites (boxed) (asn X ser/thr) (14). A COOH-terminally located hydrophobic domain (amino acid residues 333–355) is shown underlined with dots and contains a highly hydrophobic region that appears to encode a transmembrane anchor domain (3).
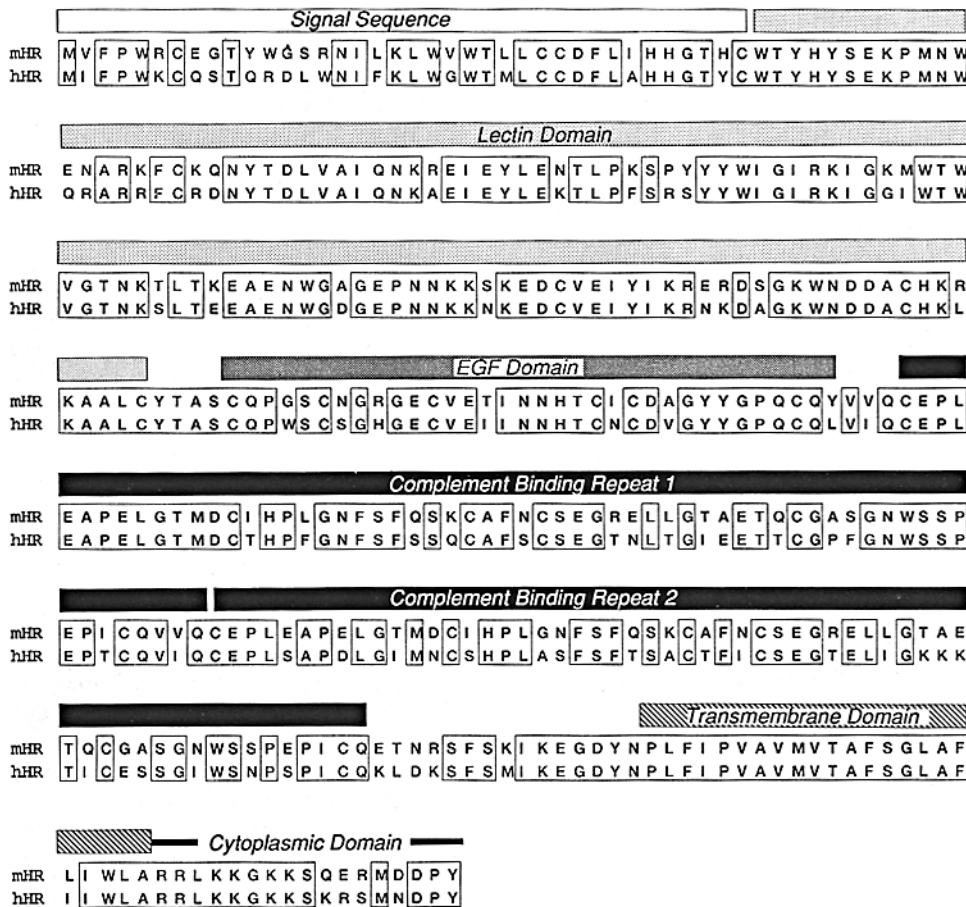
Signal Sequence

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mHR  M V F P W R C E G T Y W G S R N I L K L W V W T L L C C D F L I H H G T H C W T Y H Y S E K P M N W
hHR  M I F P W K C Q S T Q R D L W N I F K L W G W T M L C C D F L A H H G T Y C W T Y H Y S E K P M N W
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Lectin Domain

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mHR  E N A R K F C K Q N Y T D L V A I Q N K R E I E Y L E N T L P K S P Y Y Y W I G I R K I G K M W T W
hHR  Q R A R R F C R D N Y T D L V A I Q N K A E I E Y L E K T L P F S R S Y Y W I G I R K I G G I W T W
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mHR  V G T N K T L T K E A E N W G A G E P N N K K S K E D C V E I Y I K R E R D S G K W N D D A C H K R
hHR  V G T N K S L T E E A E N W G D G E P N N K K N K E D C V E I Y I K R N K D A G K W N D D A C H K L
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EGF Domain

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mHR  K A A L C Y T A S C Q P G S C N G R G E C V E T I N N H T C I C D A G Y Y G P Q C Q Y V V Q C E P L
hHR  K A A L C Y T A S C Q P W S C S G H G E C V E I I N N H T C N C D V G Y Y G P Q C Q L V I Q C E P L
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Complement Binding Repeat 1

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mHR  E A P E L G T M D C I H P L G N F S F Q S K C A F N C S E G R E L L G T A E T Q C G A S G N W S S P
hHR  E A P E L G T M D C T H P F G N F S F S S Q C A F S C S E G T N L T G I E E T T C G P F G N W S S P
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Complement Binding Repeat 2

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mHR  E P I C Q V V Q C E P L E A P E L G T M D C I H P L G N F S F Q S K C A F N C S E G R E L L G T A E
hHR  E P T C Q V I Q C E P L S A P D L G I M N C S H P L A S F S F T S A C T F I C S E G T E L I G K K K
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Transmembrane Domain

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mHR  T Q C G A S G N W S S P E P I C Q E T N R S F S K I K E G D Y N P L F I P V A V M V T A F S G L A F
hHR  T I C E S S G I W S N P S P I C Q K L D K S F S M I K E G D Y N P L F I P V A V M V T A F S G L A F
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Cytoplasmic Domain

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mHR  L I W L A R R L K K G K K S Q E R M D D P Y
hHR  I I W L A R R L K K G K K S K R S M N D P Y
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Figure 2. Comparison of the murine and human pln homing receptor sequences. The human and murine pln homing receptor sequences were analyzed by the hom.global and align programs. The figure illustrates the previously identified protein motifs found in the murine pln homing receptor (23a). The relative conservation of amino acid residues between the murine and human homologues in each of these domains is: carbohydrate binding domain, 83%; EGF-like domain, 82%; complement binding repeat 1, 79%; complement binding repeat 2, 63%; transmembrane domain, 96%.

only one conservative hydrophobic substitution, probably within the transmembrane anchor region. Finally, comparison of the amino acid sequence found for the human pln homing receptor with that recently found for the human Hermes/CD44 antigen (13, 27a) showed no significant homology between these proteins (data not shown). These results suggested that human lymphocytes encoded a glycoprotein that is highly homologous to the previously well-characterized murine pln homing receptor (the Mel 14 antigen) but that is not homologous to the human Hermes glycoprotein. This high degree of sequence homology suggests that the human clone isolated here encodes the human peripheral lymph node homing receptor.

Transient transfection assays were performed with the hu-

man cDNA clone to examine the in vivo expression of the encoded sequence. As can be seen from the FACS analysis shown in Fig. 4 A, cells transfected with an expression vector containing the human sequence expressed a surface form of the glycoprotein that was recognized by a polyclonal antiserum directed against the murine receptor, but not by a monoclonal antibody (Mel 14) directed against the murine pln homing receptor homologue (11). These results suggest that the human cDNA clone encodes a surface glycoprotein with epitopes that were cross-reactive with the murine homing receptor. The lack of recognition by the Mel 14 monoclonal antibody suggests that the epitope recognized by this antibody is not conserved between the human and murine receptors.

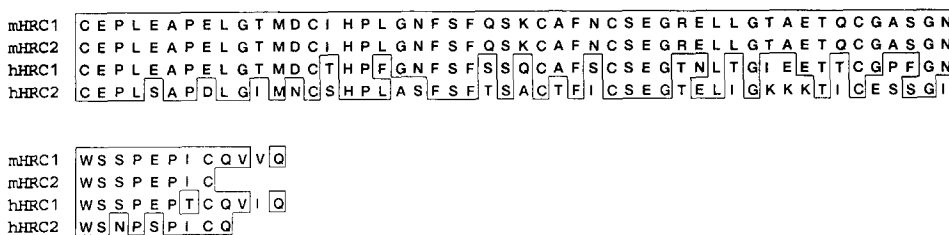Recently, the sequence for human ELAM, a neutrophil


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mHRC1  C E P L E A P E L G T M D C I H P L G N F S F Q S K C A F N C S E G R E L L G T A E T Q C G A S G N
mHRC2  C E P L E A P E L G T M D C I H P L G N F S F Q S K C A F N C S E G R E L L G T A E T Q C G A S G N
hHRC1  C E P L E A P E L G T M D C T H P F G N F S F S S Q C A F S C S E G T N L T G I E E T T C G P F G N
hHRC2  C E P L S A P D L G I M N C S H P L A S F S F T S A C T F I C S E G T E L I G K K K T I C E S S G I
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mHRC1  W S S P E P I C Q V V Q
mHRC2  W S S P E P I C
hHRC1  W S S P E P T C Q V I Q
hHRC2  W S N P S P I C Q
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Figure 3. Homologies within the repeated complement binding motifs of the murine and human pln homing receptors. The two complement binding motifs from each receptor were compared with one another using the hom.global and align programs. *mHRC1*, murine complement binding motif one (amino acids 197–258);

mHRC2, murine complement binding motif two (amino acids 259–316); *hHRC1*, human complement binding motif one (amino acids 197–258); *hHRC2*, human complement binding motif two (amino acids 259–316).
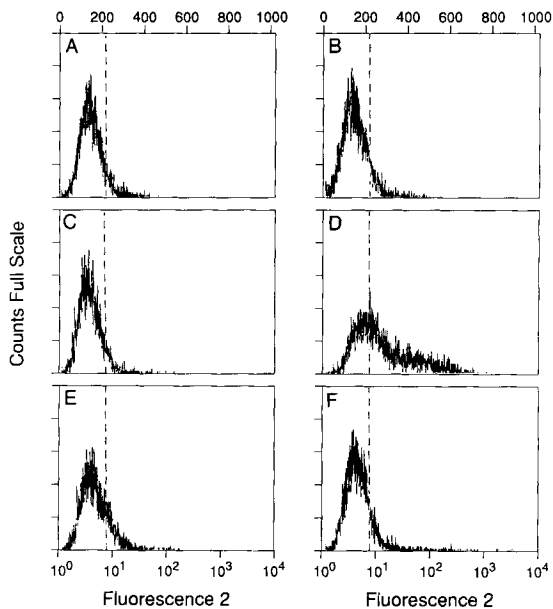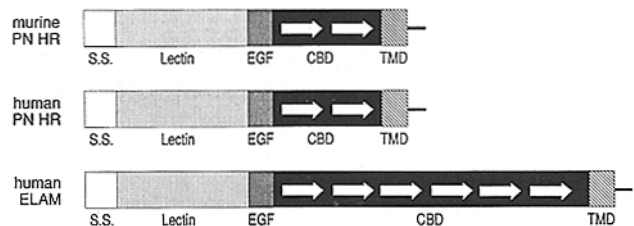
Figure 5. Comparison of the domain structures of the lectin-containing cell adhesion molecules. The figure shows the domain structures of the murine pln homing receptor (23a), the human pln homing receptor, and the human endothelial cell adhesion molecule, ELAM (1, 2). All three molecules begin with an NH₂-terminal signal sequence (*S.S.*) followed by a carbohydrate binding domain, an EGF-like domain, a domain containing either two (the murine or human pln homing receptors) or six (the human ELAM receptor) repeats of a complement binding motif (*CBD*) followed by a transmembrane anchor domain (*TMD*), and a charged cytoplasmic domain.

Figure 4. FACS (fluorescence activated cell sorter) analysis of transient expression of the human pln homing receptor homologue. The Eco RI insert encoding the sequence shown in Fig. 1 was ligated into an expression plasmid containing the cytomegalovirus early promoter (resulting in the plasmid pRK5-hHR) and transfected onto transformed human embryonic kidney cells (293 cells) (10, 23). (*A*) The cells were analyzed by FACS analysis using various sera and phycoerythrene-labeled goat anti–rabbit antiserum (*A–D*) or phycoerythrene-labeled goat anti–rat antiserum (*E* and *F*). (*A*) Mock-transfected 293 cells, preimmune rabbit serum. (*B*) pRK5-hHR-transfected 293 cells, preimmune serum. (*C*) Mock-transfected cells, rabbit anti–murine pln homing receptor serum. (*D*) pRK5-hHR-transfected 293 cells, rabbit anti–murine pln homing receptor. (*E*) Mock-transfected 293 cells, murine pln homing receptor monoclonal antibody (Mel 14). (*F*) pRK-hHR-transfected 293 cells, murine pln homing receptor monoclonal antibody (Mel 14) (11).

binding adhesion molecule that is found on cytokine-activated endothelial cells (1), became available (2). This glycoprotein shows a striking overall structural similiarity to that found for both the human and the murine pln-specific homing receptors. As can be seen in Fig. 5, all three receptors contain a lectin domain followed by an EGF-like domain and, in the case of ELAM, a domain that contains six repeats of the complement binding motif, as opposed to two repeats

in the human and murine homing receptors. Perhaps most interesting is the fact that both the lectin and EGF domains of the human pln homing receptor and ELAM show a high degree of overall conservation. As can be seen in Fig. 6, both the EGF and lectin domains of these two adhesion molecules show a ~62% overall sequence homology, as compared to a much lower degree of homology when these motifs are compared with lectin or EGF domains found in other EGF- or lectin-containing proteins (~25–30%) (23a). Interestingly, the homology at the NH₂ terminus of the lectin regions in these two adhesion molecules is far lower than in the rest of this domain, suggesting that these lectin domains may not recognize identical carbohydrates (see Discussion). Finally, the homologies of the complement binding repeats are also much lower, with an average of only ~42% between those found in the pln receptor and ELAM (data not shown).

Discussion

The work reported in this article describes a human homologue of the murine pln-specific homing receptor. The human receptor shows an identical overall domain organization to that found in the mouse, with an NH₂-terminal lectin or carbohydrate binding protein motif followed by an EGF-like domain, two complement binding repeats, and a transmem-
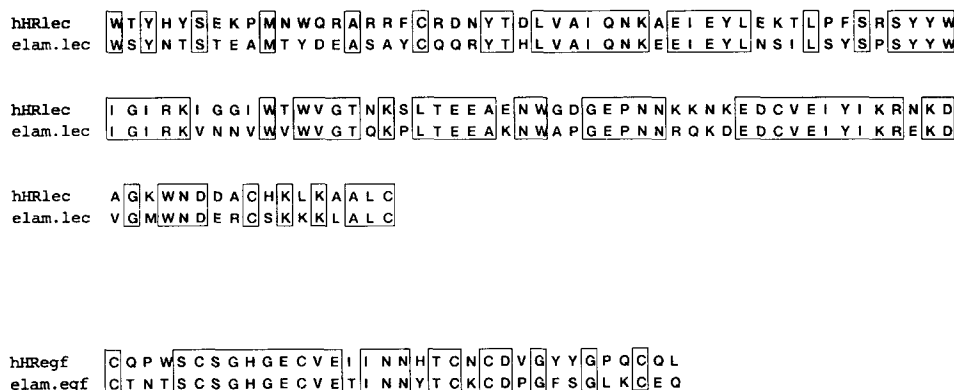

Figure 6. Sequence homologies between the lectin and EGF-like domains of the human pln homing receptor and the human endothelial cell adhesion molecule, ELAM. The figure shows the sequence conservation in the lectin domains (*hHRlec* and *elam.lec*) and EGF-like domains (*hHRegf* and *elam.egf*) for the pln homing receptor (*hHR*) and ELAM. The sequences were analyzed by the hom.global and align programs.

brane or membrane anchor domain (Fig. 5). The striking similarity in sequences within the carbohydrate binding domains of these two receptors correlates well with previous findings which showed that both murine and human lymphocyte-high endothelial cells interactions could be blocked with identical sugar-containing compounds as well as the demonstration of cross-recognition between the lymphocytes and high endothelial cells of both human and rodent species (28, 29, 32, 33, 34). However, the fact that the overall homology between these two domains is not complete (~83%), with a number of nonconservative substitutions, may suggest that this region can withstand a certain degree of sequence divergence, yet can still endow the lymphocyte with the ability to recognize the appropriate carbohydrate residue(s). Alternatively, since we have yet to determine if the human receptor described here recognizes pln-high endothelial cells, it is also possible that the human cDNA that we have isolated encodes a lectin that has yet another carbohydrate specificity, with this different recognition due to the amino acid changes between the human and mouse species (6, 7, 8, 14, 18). We are currently testing these possibilities by in vivo and in vitro homing studies with cell lines produced by transfection of these lymphocyte receptors.

The actual sequence requirements within the lectin domain for appropriate carbohydrate recognition can now be analyzed with the isolation and sequence comparison of the human and murine lymphocyte homing receptors. For example, previous work has shown that treatment of high endothelial venules with neuraminadase, which removes the sialic acid groups from carbohydrates, abolishes the ability of the treated endothelium to bind to lymphocytes, suggesting the prospect that positively charged residues on the homing receptor may be involved in endothelial adhesion (25). The conservation of 12 lysine residues in the lectin region of the human receptor, in comparison to 16 in the same region of the murine receptor, implies the possibility of this positively charged amino acid's participation in the recognition of the appropriate carbohydrate on the high endothelial cell. Moreover, analysis of other members of the type C lectin family, a family of animal lectins that requires Ca^{+2} for binding activity (23a, 27), shows that these proteins have, on average, much lower lysine contents (about five per lectin domain) than either the human or murine homing receptors, further strengthening the possibility that these basic residues are involved in the lectin interaction, perhaps with negatively charged sugars on the high endothelial cell surface. Analysis of the effects of various in vitro mutants on the ability of these receptors to recognize the carbohydrates presented by the high endothelial venules should allow the determination of the relative importance of various residues within the lectin domain for appropriate lymph node localization in vivo.

Other homologies between the human and murine forms of the pln receptor suggest further interesting functional possibilities. The high degree of protein conservation between the EGF-like domains of these molecules (82%) is much greater than the average homology between the murine or human homing receptor EGF domains and the EGF domains in other proteins (23a). By contrast to the much lower conservation found in the complement binding repeats, this suggests a potential structural role for this domain that is specific to the workings of the homing receptor. Such roles include the use of this domain as a structure-inducing link between the carbohydrate binding motif and the rest of the molecule as well as the possibility that this region may bind to an EGF-like receptor on the endothelial cell surface, thus strengthening the adhesive interaction. The conservation of the two complement-binding motifs in both the human and murine receptors suggests that these domains also have an important functional role. The fact that the murine repeats are identical at both the nucleotide and protein levels contrasts with the nonidentical conservation found in the complement-binding motifs of the human receptor. This result suggests that these repeats need not be identical, but that their overall length, gross structure, and repeat number must be conserved for appropriate homing receptor function. Finally, the almost identical amino acid sequences found in the transmembrane anchor and surrounding regions suggests that the primary sequence of this region, in addition to its hydrophobic, alpha helical character involved in membrane interaction, is required for appropriate receptor function. One interesting functional possibility for this region is to allow for homo- or heteropolymeric complex formation involving the homing receptor.

The results of this work are difficult to reconcile with previous studies that showed a degree of immunological cross-reactivity between the murine Mel 14 antigen and the human Hermes antigen (15, 16, 20). Comparison of the Hermes sequence (13, 27a) with the human pln receptor shown here revealed no significant sequence homology. It thus appears, by analogy with the murine system, that the cDNA clone described here may be the human form of the pln homing receptor, although definitive proof for this contention will require that this glycoprotein endow transfected cells with the ability to bind to human pln high endothelial cells. This possibility, then, calls into question the role(s) of the Hermes antigen in pln recognition by lymphocytes. Studies with both monoclonal and polyclonal anti–Hermes antibodies have clearly shown that these antibodies block the interaction between lymphocytes and high endothelial cells of both peripheral as well as Peyer's patch lymphoid tissue, suggesting that Hermes may, indeed, play a role in this adhesion event. Thus, it is possible that the lectin-containing glycoprotein described here, in addition to the Hermes antigen, are both involved in lymphocyte-high endothelial cell adhesion. This latter possibility might argue for a close physical (although noncovalent) association between the pln homing receptor described here and the Hermes antigen in a manner analogous to that found for the integrin adhesion family (26), a hypothesis that also might help to explain the previous results obtained in antibody preclearing experiments. Further experiments investigating the ability of these various adhesion receptors, either singly or together, to endow high endothelial cell binding characteristics on transfected cells will help to resolve these questions.

The finding of a high degree of protein sequence homology in both the lectin and EGF-like domains of the human homing receptor described here and the endothelial cell adhesion molecule ELAM, together with other similiarities in their overall domain structures, suggests that these two glycoproteins are members of a family of carbohydrate binding adhesion proteins. The fact that one of these molecules, the pln homing receptor, is on circulating immune cells (11, 12) while the other, ELAM, is induced on the endothelium during inflammation (1, 2) suggests that this type of adhesive

configuration may be a general one in the immune system. While experiments regarding the carbohydrate specificity of ELAM binding have not yet been reported, the lack of conservation between this carbohydrate-binding domain and that found in the pln homing receptor at their respective NH_2 termini suggests that they may, in fact, recognize different types of carbohydrates. If this is the case, it suggests that this general type of carbohydrate-binding motif may be used for specific recognition of structurally different carbohydrates, perhaps in specific tissue locations. In addition, as is the case for the human and murine pln receptors, the high degree of conservation found in the EGF-like domain strongly suggests that this region performs a function that is specific for this family of carbohydrate binding adhesion proteins.

In summary, the work reported here demonstrates that human lymphocytes express a carbohydrate binding adhesion molecule that is highly homologous to the murine pln homing receptor and that, from a number of previous studies, may function in an analogous manner. The relative contributions of the various protein motifs to the role of the homing receptor in pln localization can now be investigated by in vitro mutagenesis and in vivo analysis of the homing patterns of such mutants. In addition, investigations into whether the pln homing receptor and ELAM are the exclusive representatives of this type of adhesion molecule, or are only two members of a larger family of such proteins, will shed further light on the role of lectin-carbohydrate adhesive interactions in the immune system.

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