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Human homologue of mouse lymph node homing receptor: Evolutionary conservation at tandem cell interaction domains

(lymphocyte homing receptor/cell adhesion/lymphocyte recirculation/protein structure/DNA sequence)

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ABSTRACT **A cDNA clone homologous to the mouse lymph node homing receptor core protein ($mLHR_c$) was isolated from a cDNA library derived from stimulated human peripheral blood lymphocytes. Human RNA blot analysis shows a tissue and cell-line distribution of transcript expression generally parallel to that seen in the mouse, with expression confined to lymphoid tissues and some cell lines. Genomic DNA analysis suggests a low-copy gene under high-stringency conditions. The complete nucleotide sequence predicts a mature protein of 334 amino acids, identical in length to $mLHR_c$. The protein shows striking conservation globally between human and mouse sequences. In particular, all three genre of protein interaction domains identified in the mouse—an animal lectin domain, an epidermal growth factor (EGF)-like domain, and two homologous repeat units preserving the motif of complement regulatory proteins (CRP)—are present in the human protein ($hLHR_c$), and maintain the same tandem arrangement. The lectin and EGF-like regions are the most homologous, while the CRP domains are less conserved between species. The two CRP units in $hLHR_c$ are distinct from those in $mLHR_c$ in that they are homologous to one another rather than identical, suggesting strong pressure for maintenance of two repeats in this molecule. $hLHR_c$ is distinct from other kinds of lymphocyte adhesion molecules represented by VLA-4 (integrin) or CD44/$gp90^{Hermes}$ and, together with $mLHR_c$ and two other recently described molecules having a similar domain motif, defines a novel class of adhesion molecules exhibiting distinct evolutionary features. We propose that $hLHR_c$ likely represents the protein core of the human homologue of $mLHR_c$ functionally as well as structurally.**

An initial and fundamental event determining the distribution of lymphocytes within an organism occurs at the interface between blood vessel wall and surrounding tissue. The role of high endothelial venule (HEV)–lymphocyte interactions in the nonrandom migration of lymphocytes has been demonstrated (1–6). In the mouse, a monoclonal antibody, MEL-14 (7), defines the peripheral lymph node homing receptor (LHR) to be a 90- to 95-kDa glycoprotein, $gp90^{MEL-14}$. The working model of the receptor complex is that of a core protein that is highly glycosylated and apparently conjugated to ubiquitin in isopeptide linkage (8, 9). In contrast, a Peyer's patch-specific lymphocyte homing receptor, LPAM-1, has been shown to be an α/β heterodimer closely related to the VLA-4 member of the family of integrins (10, 11).

We have recently cloned the cDNA encoding the core polypeptide of the mouse LHR ($mLHR_c$), which reveals a transmembrane protein with an unusual protein mosaic architecture (12). It contains a tandem collection of four domains—an animal lectin domain, an epidermal growth factor (EGF)-like domain, and two precisely identical repeat units conforming to the homologous repeat structures of complement regulatory and other proteins. This structure has been confirmed (Δ3), and the primary structure of two additional molecules has recently been elucidated, both of which are associated with endothelial cells and conform to a quite similar protein mosaic makeup—endothelial adhesion molecule ELAM-1 (13) and GMP-140 (14).

In the human, a cell-surface molecule, $gp90^{Hermes}$, has also been identified that structurally and functionally has been thought to represent the human counterpart to $gp90^{MEL-14}$ (15) and to bear antigenic relationship to it (16). Molecular cloning in both the human and primate has recently shown this molecule to be equivalent to CD44, Pgp-1, and the ECMIII receptor (17–19). To determine whether a homologous protein to $mLHR_c$ is expressed in human lymphocytes and to address evolutionary issues posed by the unusual $mLHR_c$ composition, we sought to clone the human cDNA encoding homologues to $mLHR_c$ by cross-hybridization.

MATERIALS AND METHODS

A cDNA phage library (a gift of Lewis Lanier, Becton Dickinson Immunocytometry Systems) was constructed by using the phage λgt11 vector and cDNA inserts essentially as described (20). The source of oligo(dT)-selected RNA was human peripheral blood lymphocytes (PBL) stimulated with phytohemagglutinin/phorbol myristate acetate for 24 hr. The phage library was screened by plaque hybridization with hexamer-primed (21) full-length $mLHR_c$ cDNA. Recombinant phages were plaque-purified and grown, and DNA was extracted by the formamide extraction method (22). cDNA inserts were excised with *Eco*RI and subcloned into the phage vector M13mp19. Restriction endonucleases were obtained from New England Biolabs and used as directed. Horizontal gel electrophoreses of RNA and DNA, transfer to nylon membranes, and blot analysis were performed as described (23). Nucleotide sequence analysis on both strands was determined as described (24) by using restricted fragments and oligonucleotides complementary to various regions spanning the cDNA.

RESULTS AND DISCUSSION

Screening of 1.0×10^6 λgt11 recombinant phage plaques from a cDNA library prepared from stimulated normal human PBL resulted in the isolation of six independent strongly hybridizing phage clones. *Eco*RI digestion of these revealed a single insert in each phage, all hybridizing to the $mLHR_c$ clone. The size of four of the clones was 2.4–2.5 kilobases (kb) and of two was 1.5 kb. The length of the shorter clones was later determined to be a 3′ truncated form of the larger clone. The

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Abbreviations: LHR, lymph node homing receptor; $mLHR_c$, mouse lymph node homing receptor core protein; EGF, epidermal growth factor; CRP, complement regulatory protein(s); PBL, peripheral blood lymphocytes; HEV, high endothelial venule; ELAM, endothelial adhesion molecule.

2.5-kb insert was designated $hLHR_c$, a putative human LHR, and was utilized for subsequent studies.

Expression of $hLHR_c$ Is Confined to Lymphoid Cells. Since RNA blot analysis for $mLHR_c$ showed distribution of transcripts limited to lymphoid tissues and lymphoma cell lines with cell-surface expression of $gp90^{MEL-14}$ (12), an RNA blot was performed with RNA from a panel of normal human tissues and cell lines by using full-length $hLHR_c$ clone as probe (Fig. 1). All lanes in Fig. 1 showing a hybridizing band have a transcript of about 2.5 kb. This stands in contrast to the pattern in the mouse (12), in which two additional hybridizing species of 1.5 and 5.2 kb exist. Nonetheless, in precise analogy to the mouse, expression of transcripts was limited to blood and lymphoid tissues tested. PBL (lane A), tonsil (lane F), and thymus (lane G) were positive for transcripts, whereas detectable transcripts were absent from liver (lane B), brain (lane D), and placenta (lane E). Though a barely perceptible identically sized band was present in lung (lane C), this is likely attributable to the large amount of blood and passenger lymphocytes nonspecifically associated with this tissue. Alternatively, it is conceivable that lymphocytes exit the lung vasculature via specific adhesion mechanisms utilizing $hLHR_c$ or a homologous receptor. It is also of note that the thymus transcript was 200 to 300 base pairs (bp) larger than all other transcripts from tissues or cell lines. Since the MEL-14 epitope in the mouse is transiently expressed at high levels in the thymus during embryonic development at a stage when mature $T_{\gamma/\delta}$ cells are poised to emigrate to the periphery including the skin (25) and expression is restricted architecturally in the adult mouse thymus, altered transcript size could have functional significance.

Among cell lines, hybridization was not observed (Fig. 1) in nonlymphoid human cells, hepatocyte cell-line Hepa-2 (lane H), or RNA from cultured foreskin fibroblasts (lane L). MOLT-4, a T-cell lymphoma line described as negative for HEV binding (lane J), and CCRF-CEM (lane K), a lymphoma cell line characterized as binding barely detectably (<0.05 relative adherence ratio) to HEV (15), were both negative at the level of sensitivity of this analysis. However, PGF-2 (lane I), known to bind HEVs of both mucosal and peripheral lymph node sites, was positive. In addition, another lymphoblastoid cell line, IBW4, positive for HEV binding also showed the presence of the single 2.5-kb transcript (data not shown). Therefore, in all tissues and cell lines examined, the pattern of transcript expression paralleled that seen in the mouse, with restriction to lymphoid tissues and lymphoid cell lines that exhibit significant HEV binding capacity.

Genomic blotting of an acute lymphoblastic leukemia cell line, AE, was performed and probed with the 2.4-kb cDNA insert. All restriction enzymes used that did not cleave within the cDNA sequence itself showed two hybridizing bands (data not shown). The results are most consistent with the presence of no more than two gene copies under high-stringency hybridization.

$hLHR_c$ cDNA Encodes a Protein Highly Conserved with the Mouse $mLHR_c$ Counterpart. The complete nucleotide sequence of this 2354-bp-long cDNA was determined on both strands (Fig. 2).* The apparent initiator ATG, seen in the context of a consensus initiator sequence (26), is preceded by 115 bp of 5′ untranslated region and is followed by an uninterrupted reading frame of 1002 bp; 1123 bp of 3′ untranslated region succeed the TAA stop codon. A common polyadenylylation site, AATAAA, is present and ends 24 bp from the *Eco*RI site ending the 3′ extent of the clone. The length of the predicted protein including the signal sequence is identical to that of the mouse protein and is 374 amino acids long. The mature core protein is 334 amino acids with a predicted unmodified polypeptide size of 37,402 Da. Both species contain 22 completely conserved cysteine residues (6.6 mol %). Nineteen of these are concentrated in the CRP and EGF-like domains, constituting an extremely cysteine-rich membrane-proximal extracytoplasmic region. The overall identity of mouse and human sequences is 77% at the amino acid level and 79% at the nucleotide level. For the mature protein, there is 80% identity and 85% similarity when considering conservative substitutions (Fig. 2).

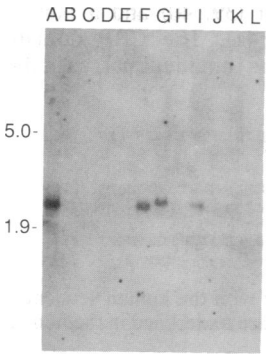

FIG. 1. RNA blot analysis of human tissue (lanes A–G) and cell line (lanes H–L) RNA using $hLHR_c$ cDNA. Lanes: A, PBL; B, liver; C, lung; D, brain; E, placenta; F, tonsil; G, thymus; H, Hepa-2; I, PGF-2; J, MOLT-4; K, CCRF-CEM; L, cultured human fibroblasts. Positions of 24S and 18S ribosomal RNA markers are indicated.

Potential N-linked glycosylation sites number 8 in the human, whereas 10 have been identified in the mouse (Fig. 3) (12). Five of these in the human sequence are identical in position to those in the mouse sequence; in particular, both sites in the lectin domain and the single site in the EGF domain align. As in the mouse sequence, no serine/threonine-rich regions are present to suggest the presence of O-linked glycosylation. The least conserved region with respect to potential glycosylation sites is localized to the carboxyl-terminal CRP homologous repeat.

The leader sequence is 38 amino acids long, identical in length to the mouse protein and, as we have noted (12), is unusually long for a eukaryotic secreted or cell-surface protein. Outside of a short stretch of 9 amino acids from −31 to −24, where the sequences diverge considerably, the leader sequences are quite conserved for this evolutionary distance. There is overall 63% identity and 82% similarity when conserved substitutions are included. Among the conserved residues are the three cysteines and two of the three histidines of the mouse. The peculiar features of this signal sequence do not appear to be requisite for this class of cell-surface adhesion molecule, since ELAM-1, an endothelial adhesion molecule, has a very similar structure (13), yet its leader sequence has no significant homology to those of $hLHR_c$ and $mLHR_c$. GMP-140 has a 41-amino acid leader sequence (14), but the sequence is otherwise quite dissimilar from those of the LHRs. The degree of conservation of length and sequence between $hLHR_c$ and $mLHR_c$ in this region suggests possible stringent structural constraints for the function of this leader, perhaps reflecting specialized pathways of intracellular traffic. The putative transmembrane region (positions 296–317) shows complete identity between the mouse and human forms with the exception of a single conservative isoleucine–leucine interchange at position 313. The 8 residues preceding and initial 9 intracytoplasmic residues (with positively charged residues consistent with a stop transfer sequence) following the hydrophobic transmembrane region are also completely conserved, resulting in a 39-amino acid stretch of virtual identity, the longest region of identity between the molecules. In both species the carboxyl terminus of the molecule ends with a tyrosine, an amino acid implicated in recognition events initiating internalization into clathrin-coated pits (27). This may have implications for some biosynthetic properties of $gp90^{MEL-14}$ (28).

Variety, Number, and Relationship of Extracytoplasmic Domains Are Preserved Between $hLHR_c$ and $mLHR_c$. The lectin

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M25280).

FIG. 2. DNA sequence and predicted amino acid sequence of $hLHR_c$. The hydrophobic transmembrane region is indicated by a double underline, and the polyadenylylation signal in the 3′ untranslated region is indicated by a single underline. Numbering to the right indicates the nucleotide and protein positions, respectively.

family of proteins has been distinguished by its carbohydrate receptor activity. Primary structural analysis of many of these has shown a common structural basis (29–32). The animal lectin domain identified structurally in $mLHR_c$ has been shown to have a functional correlate in that $gp90^{MEL-14}$ has been inferred to have lectin properties (33–35). $hLHR_c$ and $mLHR_c$ sequences show 86% identity over this entire region (Fig. 4*a*). Focally there are concentrated stretches of greater similarity—namely, residues 1–12 (100% identity), 22–43 (95%), 48–97 (94%), and 102–120 (95%), regions that show increased conservation with ELAM-1 and GMP-140 as well. The ELAM-1 sequence in the same region is 59% identical to $hLHR_c$ and 60% identical to $mLHR_c$. The GMP-140 sequence shows 65% identity to both $hLHR_c$ and $mLHR_c$ sequences in this domain. In addition, the ELAM-1 and GMP-140 sequences, unlike all the other animal lectin sequences compared, require no insertions or deletions for alignment with $hLHR_c$ or $mLHR_c$ sequences (Fig. 4*a*). Comparisons to the animal lectins most similar to $hLHR_c$ show the human IgE receptor sequence and the human hepatic lectin sequence to show 32% and 28% identity, respectively. We have suggested that a relative lysine-rich region in the LHR_c domain (the carboxyl-terminal 45 amino acids of this domain) may provide clues to possible sites of ubiquitination (12). All of the seven lysine residues in this region in $mLHR_c$ are precisely preserved in the human sequence. An additional eighth lysine is present in $hLHR_c$ at position 99. Remarkably, the ELAM-1 sequence is also relatively lysine-rich in this region with seven residues, including a cluster of three at 111–113. Four of these are shared with $hLHR_c$ but not other animal lectins. In this regard, it is of interest that 14 kDa of the mature ELAM-1 protein in biosynthetic studies is as yet unexplained by glycosylation differences or other posttranslational modifications (13), allowing the possibility of ubiquitination in this cell-surface adhesion molecule. We have shown a similar 8- to 10-kDa (a ubiquitin equivalent) discrepancy in biosynthetic and *in vitro* translation studies of $mLHR_c$ (28).

Another protein motif comprising residues 122–154 is related to proteins containing the EGF-like sequence (36) (Fig. 4*b*). $hLHR_c$ and $mLHR_c$ sequences share 82% identity in this region. In contrast to the lectin domain, comparison of the corresponding EGF-like region of ELAM-1 to that of $hLHR_c$ shows somewhat greater similarity to the human sequence than to the mouse sequence, with 73% and 60% identity, respectively. GMP-140 shows about 60% identity to $hLHR_c$ in this region. Unlike the lectin domain, although $hLHR_c$ and $mLHR_c$ are more related to each other overall, the human sequences ELAM-1 and $hLHR_c$ show more similarity in the EGF-like domain than ELAM-1 shows to $mLHR_c$. Within this domain, there is a 17-amino acid stretch of 100% identity (126–142) between $hLHR_c$ and ELAM-1, which is 82% conserved between $mLHR_c$ and both human proteins, perhaps suggesting possible common species-specific structural requirements for function in this domain. For example, it is thought that the EGF domain in the low density lipoprotein receptor is essential for recycling of the receptor back to the surface after endocytosis in clathrin-coated pits (37). Alternatively, the EGF domain in LHRs may subserve a separate protein–protein, cell–cell, or cell–matrix interactive role in the process of adhesion to HEVs or translocation through the vessel wall into lymph node parenchyma. It is striking that four neurogenic loci in *Drosophila melanogaster* provide some of the best alignments and similarities to the LHRs. These apparently mediate early cell–cell interaction events in embryogenesis controlling the developmental choice of ectodermal cells to either neural or epidermal pathways.

CRPs generally share the property of binding to C3b and C4b of the complement cascade (reviewed in ref. 38), thereby dampening the cascade at key amplification junctures, perhaps to protect host cells from complement lysis. Proteins apparently unrelated to this system also contain this motif, including the interleukin 2 receptor (39, 40) and a human serum protein β_2 glycoprotein I (41). The CRP domain (155–278) consists of two homologous [not identical, as in the

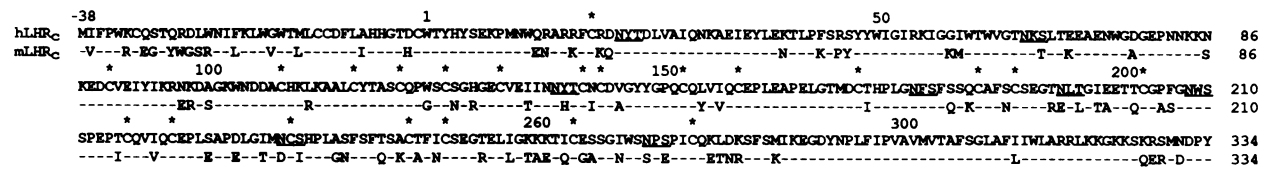

FIG. 3. Comparison of the predicted protein sequences of $hLHR_c$ and $mLHR_c$ (14). A dash indicates identity with the human sequence. Cysteines in the mature protein are marked with a star. Canonical asparagine-linked carbohydrate attachment sites are underlined in the human sequence.

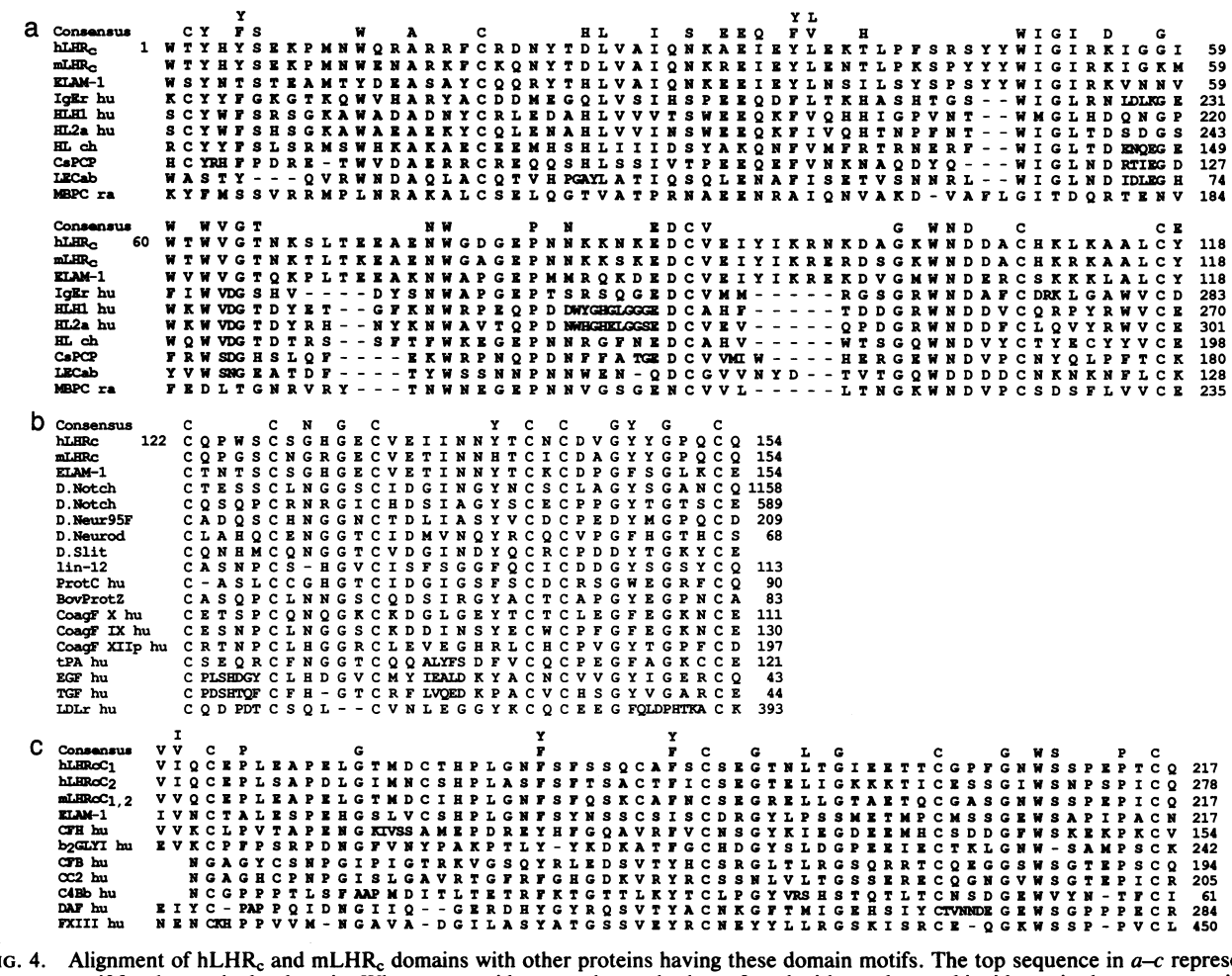

FIG. 4. Alignment of hLHR$_c$ and mLHR$_c$ domains with other proteins having these domain motifs. The top sequence in *a–c* represents a consensus motif for the particular domain. Where two residues are shown, both are found with nearly equal incidence in the representative set of proteins. (*a*) Lectin domain. hLHR$_c$ and mLHR$_c$ residues 1–118 are compared to other carbohydrate binding protein domains. (*b*) EGF-like domain. LHR residues 122–154 are compared to other EGF-like proteins. (*c*) CRP domains. The two domains (155–217 and 218–278) of hLHR$_c$ and mLHR$_c$ are compared to complement regulatory proteins and other proteins having this domain. hu, Human; mo, mouse; ra, rat; bo, bovine; ch, chicken; IgEr, immunoglobulin E receptor; HLH1 and HL2a, (human) hepatic lectins 1 and 2; HL, (chicken) hepatic lectin; CsPCP, cartilage-specific proteoglycan core protein; LECab, acorn barnacle lectin; MBPC, mannose binding protein c; CFH, complement factor H; b_2GLYI, β_2 glycoprotein I; CFB, complement factor B; CC2, complement component 2; C4Bb, C4B binding protein; DAF, decay-accelerating factor; FXIII, coagulation factor XIII; D. Notch, notch, *Drosophila*; D. neur95F, position 95F homologous to neurogenic locus, *Drosophila*; D. Neurod., neurogenic locus δ, *Drosophila*; D. Slit, slit, *Drosophila*; lin-12, lin-12, *Caenorhabditis elegans*; ProtC, protein C; BovProtZ, bovine protein Z; CoagF X, coagulation factor X; CoagF IX, coagulation factor IX; CoagF XIIp, coagulation factor XII precursor; TGF, transforming growth factor α; LDLr, low density lipoprotein receptor.

mouse (12)] repeat units of 63 amino acids each in hLHR$_c$ (Fig. 4*c*). The human CRP$_1$ and CRP$_2$ are quite divergent, sharing only 60% of positions. However, the hLHR$_c$ does retain the six-cysteine pattern in both repeats, in common with ELAM-1 and GMP-140, but distinct from other CRP subunits which contain four (Fig. 4*b*). Comparisons between hLHR$_c$ and mLHR$_c$ sequences show 74% conservation to CRP$_1$ and only 60% to CRP$_2$. If the more conserved amino-terminal 38 amino acids are considered alone, the mouse sequence shows 86% identity to CRP$_1$ and 67% to CRP$_2$. This suggests possible duplication of an ancestral segment related to the mouse CRP sequence, with relative preservation of this sequence in CRP$_1$ of the human, particularly amino-terminally, and relative drift of the CRP$_2$ subunit. An alternative explanation of the identical repeats in the mouse is that there was an early domain duplication, with a recent gene conversion event in the mouse resulting in the identical repeat. In any case, there appears to be a strict requirement for two CRP subunits in the LHRs. Alignment of the first CRP unit of ELAM-1 with CRP$_1$ and CRP$_2$ of hLHR$_c$ shows 41% and 40% identity, respectively. While less similar to the LHRs than in the lectin and EGF domains, the first ELAM-1 CRP sequence is nonetheless more related to the LHRs than the most related other proteins in this region, with human complement factor H showing 35% and β_2 glycoprotein I showing 27% identity. It is attractive to consider that the CRP segment in LHRs has a complement regulatory function, which might seem an appropriate role for structures on cells recruited to and at inflammatory sites—i.e., lymphocytes (LHRs) and activated endothelial cells (ELAM-1 and GMP-140). Indeed, the MEL-14 epitope has also been identified on mouse neutrophils and is implicated in participating in extravasation of these cells at inflammatory sites (42).

The characteristic protein mosaic architecture, consisting of an amino-terminal lectin domain followed by an EGF-like region and a variable number of CRP homology repeats, distinguishes a novel class of cell-surface molecules. Three members have recently been described: mLHR$_c$ on mouse lymphocytes, effecting adhesion of lymphocytes to HEV (12, 43); and two human endothelial markers, ELAM-1, which mediates adhesion of leukocytes (44), and GMP-140, a protein found in secretory granules in platelets and endothelial cells and on cell surfaces on induction (14). This establishes an apparently new family of adhesion molecule structurally

unrelated to the immunoglobulin (45–47) or integrin (48) gene superfamilies.

We have now isolated a fourth member of this family, a human cDNA encoding a predicted amino acid sequence highly conserved with that of $mLHR_c$. The high degree of structural conservation with a molecule known to confer specific lymphocyte homing activity in another species and the restricted lymphoid expression of transcript, in strict analogy to the mouse, strongly suggest a cell-surface molecule likely mediating similar function in the human, which we provisionally designate $hLHR_c$. An apparent tissue-specific lymphocyte–HEV adhesion system in the human has been described that also involves a cell-surface gp90 species, designated $gp90^{Hermes}$ (15). The molecule is sulfated and modified by O-linked as well as N-linked glycosylation (16) in contrast to $gp90^{MEL-14}$, which we have shown to contain glycosylation only in N-linked form (28). Expression of $gp90^{Hermes}$ is not restricted to hematolymphoid cell types (49). However, the $gp90^{Hermes}$ class apparently participates in specific binding to both mucosal and peripheral lymph node sites. In contrast, specific binding of human and mouse lymphocytes to mucosal site HEVs in the mouse utilizes an α/β integrin heterodimer related to VLA-4 (50); there is no current evidence that there is a requirement for or participation of any gp90 species in Peyer's patch binding in the mouse. Molecular cloning of $gp90^{Hermes}$ (17) and its counterpart in the primate $gp90^{Hutch}$ (19) reveal identity to previously described molecules with a wide distribution, CD44, Pgp-1, and the ECMIII receptor (51–53). The cloned molecule is related to regions of the cartilage proteoglycan core and link proteins (17–19). It contains no sequences related to any of the three major homologous domains of $hLHR_c$ and $mLHR_c$ and shows no significant sequence homology to $hLHR_c$. It has been shown that the monoclonal antibody MEL-14, used to define peripheral lymph node homing in the mouse (7), can block adhesion of human lymphocytes to HEV *in vitro* and that $gp90^{Hermes}$ apparently bears the MEL-14 epitope (16). Since the primary structure of $gp90^{Hermes}$ and $hLHR_c$ are so disparate, it is unclear whether this represents commonality of some posttranscriptional modification such as ubiquitination or fortuitous cross-reaction. It is unlikely that mouse and human utilize completely different systems governing lymphocyte–HEV adhesion, since the adhesive event is preserved across these species (54). If $hLHR_c$ has a function homologous to that of the murine protein, it is possible that both $gp90^{Hermes}$ and $hLHR_c$ act in conjunction as a receptor complex. However, this suggestion conflicts with our observations in the mouse, which has shown frequent and completely discordant expression of $gp90^{MEL-14}$ and Pgp-1 (28). Indeed, some MEL-14-positive peripheral lymph node HEV-binding cell lines are completely negative for Pgp-1 antigen, mitigating against a necessary role for this molecule in adhesion to HEVs in the mouse. Resolution of these issues awaits further characterization of the protein encoded by $hLHR_c$ and functional studies to address the role of the protein in lymphocyte homing and adhesion to endothelium.

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