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Identification of Mouse Langerin/CD207 in Langerhans Cells and Some Dendritic Cells of Lymphoid Tissues

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Human (h)Langerin/CD207 is a C-type lectin of Langerhans cells (LC) that induces the formation of Birbeck granules (BG). In this study, we have cloned a cDNA-encoding mouse (m)Langerin. The predicted protein is 66% homologous to hLangerin with conservation of its particular features. The organization of human and mouse Langerin genes are similar, consisting of six exons, three of which encode the carbohydrate recognition domain. The mLangerin gene maps to chromosome 6D, syntenic to the human gene on chromosome 2p13. mLangerin protein, detected by a mAb as a 48-kDa species, is abundant in epidermal LC in situ and is down-regulated upon culture. A subset of cells also expresses mLangerin in bone marrow cultures supplemented with TGF-β. Notably, dendritic cells in thymic medulla are mLangerin-positive. By contrast, only scattered cells express mLangerin in lymph nodes and spleen. mLangerin mRNA is also detected in some nonlymphoid tissues (e.g., lung, liver, and heart). Similarly to hLangerin, a network of BG form upon transfection of mLangerin cDNA into fibroblasts. Interestingly, substitution of a conserved residue (Phe²⁴⁴ to Leu) within the carbohydrate recognition domain transforms the BG in transfected cells into structures resembling cored tubules, previously described in mouse LC. Our findings should facilitate further characterization of mouse LC, and provide insight into a plasticity of dendritic cell organelles which may have important functional consequences. The Journal of Immunology, 2002, 168: 782–792.

Dendritic cells (DC) are APCs with a key function in the immune system. These cells are uniquely responsible for the stimulation of naive T lymphocytes. DC have the ability to capture and process foreign and self Ag, and to migrate and present processed Ag to T lymphocytes. DC have the ability to capture and process foreign and self Ag, and to migrate and present processed Ag to T lymphocytes (1). Langerhans cells (LC), located in epidermis and stratified mucosal epithelia, constitute a distinct population of immature DC of bone marrow derivation that play a sentinel role in the capture of Ags from the external environment (2). Following Ag capture, LC differentiate into mature DC as they migrate via lymphatics to the T cell areas of draining lymph nodes (3, 4). LC are characterized by Birbeck granules (BG), which are organelles consisting of superimposed pentalamellar and zippered membranes (5, 6). We previously identified human (h)Langerin/CD207, a transmembrane C-type lectin of LC (7). Langerin is a potent inducer of BG formation and is a nonconventional endocytic receptor routing into these organelles in LC, with potentially important consequences for Ag processing and presentation (7).

The DC system is continuously being defined both in human and mouse, and numerous subsets of DC with distinct phenotype and function have been described. The finding that murine thymic DC expressing CD8α derive from a CD4⁺CD8⁻ lymphoid progenitor has led to definition of the lymphoid DC lineage (8). It has recently been proposed that mouse LC originate from a lymphoid-committed progenitor on the basis of CD8α expression (9, 10). However, to date, CD8α has not been observed on human DC. Furthermore, CD1a, which represents a marker of human LC, is absent in the mouse. These examples illustrate the difficulties of translating functional findings between human and mouse DC subsets, which is key to optimizing preclinical models. In this work, we describe the identification of mouse (m)Langerin/CD207. The molecule displays functional features similar to the human counterpart and should be highly valuable for studying mouse LC. In addition, whereas wild-type mLangerin induces BG, a single amino acid substitution in the extracellular carbohydrate recognition domain (CRD) results in different cytoplasmic structures as observed by electron microscopy. This finding suggests that different organelles may form in LC depending on Langerin conformation and ligand interaction with the sugar-binding region.

Materials and Methods

Mice, media, and reagents

Mice of inbred strains C57BL/6 and BALB/c were purchased from Charles River Breeding Laboratories, Iffa-Credo (L’Arbresle, France), and used at 8–12 wk of age. All experiments involving mice were conducted according...
to institutional guidelines and all cell types were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% (v/v) heat-inactivated FBS (Flow Laboratories, Irvine, U.K.), 2 mM L-glutamine, 5 x 10^-5 M 2-mercaptoethanol (Sigma, St. Louis, MO), and gentamicin (Sigma-Aldrich, St. Louis, MO). M1 cells were purchased from Strathmann Biotech (Hamburg, Germany). Transfections were performed with the murine fibroblastic COP5 cell line (11) as described (7).

Identification of mLangerin cDNA and cloning

Bioinformatic searches were performed in GenBank EST databases. Two expressed sequence tags (ESTs) were used for PCR amplification of cDNA from a mouse lung cDNA library (Clontech Laboratories, Palo Alto, CA) using the RACE marathon kit (Clontech Laboratories). Primer sequences were for the 5' RACE (GSP1, TTTGAGCTCTACGGAGACGATC; and NSP1, GTTGTTCCTCGTGTGGTGTTTGTGC), and for the 3' RACE (GSP2, GGAGAATGTGCAAACTCGTCA; and NSP2, AAGCTCTACCTGACCTCAGTGG). PCR products were cloned in the PCRII TOPO plasmid (Invitrogen, San Diego, CA) and double-stranded plasmid DNA was sequenced on an ABI 373A sequencer (Applied Biosystems, Foster City, CA) using dye terminator technology. Sequence analysis was performed with the Gene Editor in vitro Site-Directed Mutagenesis kit (Promega, Madison, WI), according to instruction guidelines and using wild-type Langerin cDNAs as templates and the mutated oligonucleotides 5'-ATCTCTCCCTCAGCCACGGCTCC (hLangerin WPREF to WIREP) and 5'-GAACAAAGTTACCTCACAAG (mLangerin QKL5 to QKL5Y).

Northern and Southern analysis

Northern analysis was performed with Multiple Tissue Northern blots prepared from high-quality poly(A)+ RNA normalized for a β-actin hybridization signal (Clontech Laboratories, catalog no. 7761-2 and 7763-1), as described (12). For Southern analysis, DNA (5 μg) from primary amplified cDNA libraries constructed in our laboratory was digested with NotI and SalI (Boehringer Mannheim, Indianapolis, IN) to release the inserts, run on a 1% agarose gel, and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH). Hybridization was conducted using a PCR-generated probe labeled with 32P-dCTP using the High Prime kit (Boehringer Mannheim, Meylan, France). Unincorporated nucleotides were removed by spin column chromatography (Chromaspin-100; Clontech Laboratories). Hybridization and washes were performed in stringent conditions (0.1 x SSC/0.1% SDS at 65°C). Membranes were exposed for 21 days using Biomax MR film (Kodak, Rochester, NY). Characteristics (number of independent clones/percentage of clones with inserts) of the cDNA libraries used were as follows: wild-type thymus, 6.6 x 10^4/53%; rag-1/♂ thymus, 3.9 x 10^4/85%; pancreas, 2.2 x 10^4/97%; skin, 1.4 x 10^4/90%; spinal cord, 4.4 x 10^4/95%; spleen, 1.1 x 10^4/72%; lung, 3.7 x 10^4/85%; stomach, 4.0 x 10^4/73%; Peyer's patch, 8 x 10^4/72%; mesenteric lymph node, 5.8 x 10^4/83%; and colon, 5.4 x 10^4/90%.

Genomic organization and chromosomal localization of Langerin genes

A 900-bp EcoRI fragment of mLangerin cDNA was used as a probe to hybridize a 129/Ola mouse spleen genomic cosmid 121 library (Resource Center/Primary Database, Max Planck Institute for Molecular Genetics, Heidelberg, Germany). Hybridization was performed as described (11), and positive clones were selected and sequenced. Seven overlapping cosmid clones were found to be positive for the Langerin gene, as follows: MPMCg121B04216Q2, MPMCg121K01389Q2, MPMCg121K01387Q2, MPMCg121K01385Q2, MPMCg121D19640Q2, MPMCg121K20694Q2, and MPMCg121H03, 719Q2. One cosmid was used for fluorescence in situ hybridization (FISH) mapping of the mouse gene as described (14). The organization of the hLangerin gene was obtained by BLAST nucleotide using the GenBank high throughput genomic database. Chromosomal localization of the Langerin gene was performed with the Stanford RH medium resolution panel (Research Genetics, Huntsville, AL), using PCR with oligonucleotides that amplify an 850-bp fragment specific for the hLangerin gene (forward, 5'-ACGCCATTTCCAACAGGCTC; reverse, 5'-CAGCCCAAGACAGCAGGACTC). The result was scored manually and analysis was performed with the RHmapper program (http://shgc-www.stanford.edu).

Generation of Abs against mLangerin

mAbs were produced by immunizing BALB/c mice with three i.p. injections of lysate of COP5 cells transfected with mLangerin cDNA. Splenums were removed 3 days after a final i.v. injection and splenocytes were fused with SP2/0 cells using polyethylene glycol-1000 and cultured in 96-well plates using standard procedures. Hybridoma supernatants were screened for reactivity against mLangerin transfected vs nontransfected cells. After cloning of selected hybridomas, mAbs were produced as ascites fluids and purified. Data presented were obtained with mAb HD24 (306.G9) of IgG1 isotype. Polyclonal Ab was obtained by immunizing rabbits with mLangerin peptides followed by purification from antisera using affinity chromatography (Covalab, Oullins, France).

Biochemistry

COP5 fibroblastic cells transfected with mLangerin cDNA were washed three times with ice-cold PBS, pH 8 and then treated in a lysis buffer containing 50 mM Tris-HCl, pH 8, 1% Nonidet P-40, 150 mM NaCl, and protease inhibitors (Boehringer Mannheim). Lysates were incubated at 4°C for 20 min, and insoluble material was pelleted by centrifugation at 12,000 x g for 10 min at 4°C. Soluble extracts were preclarified three times with control mAb and protein G-agarose (Boehringer Mannheim). The extract was then incubated with mAb HD24 for 1 h before adding protein G-agarose for 3 h. Beads were washed three times in lysis buffer, resuspended in SDS-PAGE sample buffer with or without 5% 2-ME, boiled for 3 min, and centrifuged. Immunoprecipitates were separated by SDS-PAGE using 12% polyacrylamide gel and then transferred to a polycrylamide dinitrofluorobenzene membrane (Immobilon P; Millipore, Bedford, MA). Blots were blocked with 1% BSA and 0.1% Tween 20 in PBS, then incubated with HRP-conjugated mAb HD24 for 30 min. Proteins were detected by ECL (Boehringer Mannheim).

Epidermal sheets, epidermal cell suspensions, and bone marrow-derived dendritic cells

Epidermis from ear skin was separated from dermis by means of ammonium thiocyanate (15). Resulting sheets were fixed in acetone for 20 min at room temperature and rinsed in several changes of PBS and PBS containing 1% BSA. They were then subjected to an immunolabeling procedure as described (16). Bone marrow-derived DC were generated essentially as described by Inaba et al. (17), in the presence of GM-CSF supplemented or not with TGF-β. Cultures were analyzed on acetoxy-fixed cytopsins on days 6, 8, 10, 13, and 19 of culture.

Immunohistochemistry and cytofluorometry

For immunohistochemistry of sheets and cytopsins, the following mAbs were used: mouse IgG1 anti-mLangerin, clone HD24 (used at final concentrations of 1–2 μg/ml), mouse anti-I-A<sub>ab</sub> (clone 14-4-4S/HB32, mouse IgG2a, American Type Culture Collection, Manassas, VA), anti-I-A<sub>ab</sub>-conjugated goat anti-mouse IgG2a (Jackson Immunoresearch Laboratories, West Grove, PA). Enzyme activity was developed using 3-amin0-9 ethylcarbazol high sensitivity (DAKO) and slides were counterstained with Harris hematoxylin (Sigma-Aldrich).

The Journal of Immunology

783

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Flow cytometric analysis on COP5 fibroblasts was performed on a FACSCalibur (BD Biosciences, Mountain View, CA), following staining with 10 μg/ml anti-mLangerin mAb HD24 or anti-hLangerin mAb (DCGM4) (18) revealed by PE- or FITC-conjugated F(ab')2 goat anti-mouse Ig (DAKO). Nonspecific staining was determined using isotype-matched control mAb and all procedures were performed in the presence of 0.5 mM EDTA to avoid cell aggregation. Intracytoplasmic staining was performed in saponin (0.1% v/v).

FIGURE 1. mLangerin cDNA sequence and alignments. A. Nucleotide and predicted amino-acid sequences of mLangerin cDNA (accession no. AJ302711). The boxed sequence (hydrophobic amino acids 47–67) forms a signal anchor expected to represent the transmembrane domain. The C-type lectin domain with two cysteines forming a disulfide bridge (rounded) is shown in single underline. Broken and double underlines (positions 26–31, 90–92, and 116–118) indicate the proline-rich domain and potential N-glycosylation sites, respectively. B. Alignments of mouse and human Langerin. Conserved amino acids are shaded and the boxed sequence forms a signal anchor expected to represent the transmembrane domain.
Electron microscopy

Staining was performed as described (18). Briefly, COP5 transfected with mLangerin cDNA were fixed for 18 h with 2% glutaraldehyde in cacodylate buffer, followed by washing for at least 24 h in cacodylate buffer with sucrose. Samples were postfixed for 1 h with 1% osmium in cacodylate buffer with sucrose, dehydrated, and embedded in epoxy resin. Ultrathin sections were poststained with uranyl acetate and lead citrate and examined on a JEOL 1200 EX electron microscope (JEOL, Tokyo, Japan) (7).

Results

Identification of mLangerin/CD207 cDNA

Public databases were screened using a translated BLAST nucleotide alignment with the amino acid sequence of hLangerin/CD207 as query. Two mouse EST (AA76540 and AA423304) were identified and this sequence used in a 3′ and 5′ RACE PCR on a mouse lung cDNA library. A 1468-bp cDNA sequence was isolated, containing a 981-bp open reading frame (ORF) but lacking a start methionine. Using this cDNA as a probe, two cosmids clones were isolated and sequenced. Upstream of the 5′ end of the cDNA sequence, a potential initiation site (YCANTYY) followed by two methionines was identified. Thus, we isolated a 1565-bp full-length cDNA sequence encoding mLangerin/CD207 (accession no. AJ302711). It contains a 58-bp 5′ untranslated region, a 993-bp ORF, a 3′ untranslated region of 514 nt, and an AATAAA polyadenylation signal at position 1511 bp. The ORF predicts a novel polypeptide of 331 aa (Fig. 1A), with a molecular mass of 37.5 kDa. As described for hLangerin, amino acid sequence comparison in the human and mouse proteome demonstrated that residues 47–67 indicate the presence of an intracytoplasmic domain, the second exon encodes the end of the intracytoplasmic domain, and the third exon encodes the transmembrane domain, the third exon encodes the long neck domain, and three exons encode the CRD. Notably, the proline-rich motif is encoded by two exons. The size of the five introns is highly variable, ranging from 92 to 1.4 kb (intron 4). Using FISH, the mLangerin gene was mapped to chromosome 6D (Fig. 3C).

Furthermore, we obtained the organization of the hLangerin gene. A BAC clone (NH0504001) containing the entire hLangerin gene was identified by using the ESTs of mLangerin cDNA as a probe. Positive clones were propagated, digested, and hybridized to identify cosmids containing the entire mLangerin gene. Two cosmids clones of ~40 kb were used for subcloning and direct sequencing. The mLangerin gene spans 6.7 kb and consists of six exons varying in length between 117 and 624 bp (Fig. 3, A and B) (accession no. AJ313164). The first exon encodes half of the intracytoplasmic domain, the second exon encodes the end of the intracytoplasmic domain and the transmembrane domain, the third exon encodes the long neck domain, and three exons encode the CRD. Notably, the proline-rich motif is encoded by two exons. The size of the five introns is highly variable, ranging from 92 to 1.4 kb (intron 4). Using FISH, the mLangerin gene was mapped to chromosome 6D (Fig. 3C).

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mRNA (Fig. 4). These results indicate that mLangerin mRNA is mostly expressed in skin and hematopoietic tissues but is also detectable in lung, liver, and heart.

Characterization of mLangerin/CD207 protein and mAbs

By immunization of BALB/c mice with a murine cell line (COP5) transfected with mLangerin cDNA, we obtained a panel of mAbs against mLangerin (scoring selectively positive on the mLangerin transfectant vs mock-transfected cells). One mAb, named HD24 (clone 306G9, IgG1), was chosen for further studies. Immunoprecipitation revealed that HD24 mAb selectively recognizes a glycoprotein of 48 kDa in mLangerin-transfected COP5 cells, in accordance with the molecular mass predicted from the amino-acid sequence (Fig. 5A). Flow cytometric analysis on transfectants obtained with different constructs of mouse and human Langerin demonstrated that mAb HD24 cross-reacts with hLangerin and is directed against an intracellular epitope containing the proline-rich motif as reactivity is abolished following a site-directed mutation of WPREPP to WIREPP (Fig. 5B).

Expression of mLangerin/CD207 protein in vivo

Because hLangerin is selectively expressed by LC, we first performed immunohistology on mouse skin sections. Expression of mLangerin was observed by cells with LC morphology in the epidermis but not by cells in the dermis (Fig. 6A). As shown in Fig. 6B, bright staining was obtained on ear epidermal sheets of BALB/c (n = 3), C57BL/6 (n = 2), and 129 (n = 1) mice. Staining was highly specific to the LC, and keratinocytes were completely
negative. Under the fluorescence microscope, Langerin expression was visible as granules scattered internally throughout the cell, and no staining was observed on the cell surface. The granules were found even in the most distant parts of the dendrites where MHC class II molecules were too sparse to be visualized (Fig. 6B). Finally, this double-labeling revealed that virtually all LC (as identified by their MHC class II expression) were also Langerin positive. Inversely, all Langerin-positive cells were MHC class II positive, emphasizing the specificity of the Ab. In corresponding dermal sheets we observed only very few Langerin-positive cells, presumably LC in transit (data not shown).

Because mLangerin mRNA was also found in other lymphoid tissues, we analyzed the protein expression in thymus, spleen, and lymph nodes. Of interest, many cells expressing Langerin were observed in the thymic medulla (Fig. 6, C and E), localized by a serial staining with DEC205 (Fig. 6D; DEC205low areas). The Langerin+ cells in the medulla had a characteristic dendritic morphology (Fig. 6E). Some Langerin+ cells, with characteristic dendritic morphology, were occasionally found in the cortex (Fig. 6F). In spleen (Fig. 6G) and lymph node (Fig. 6H), only few Langerin+ cells were observed in perifollicular areas, expected to be marginal DC or interdigitating cells, as some of them possess dendrites. We never observed Langerin+ cells in germinal centers. Taken together, mLangerin is expressed by LC and a subset of DC in lymphoid tissues including thymic medulla.

**FIGURE 4.** mLangerin mRNA expression in a panel of lymphoid and nonlymphoid tissues. A, Northern blot analysis. B, Southern blot analysis.

**mLangerin/CD207 is expressed in bone marrow cultures supplemented with TGF-β**

DC can be obtained from mouse bone marrow progenitor cells in culture with GM-CSF (17). By FACS analysis of such permeabilized bone marrow-derived DC, only very weak if any Langerin staining could be detected. Indeed, inspection of

**FIGURE 5.** Characterization of anti-mLangerin mAb and mLangerin protein. A, Immunoprecipitation with mAb HD24 on mLangerin-transfected fibroblasts. B, Staining on COP5 fibroblasts transfected with various forms of mouse and human Langerin cDNA. Cells were permeabilized with saponin, stained with DCGM4 or HD24, and analyzed by FACS. Filled histograms were obtained with control fibroblasts (COP mock). M, Molecular mass; COP mock, control fibroblasts; COP mLg, COP transfected with mLangerin cDNA; WT, wild type.
cytospins from standard cultures in the presence of GM-CSF revealed only a very small number (1% of all cells) of brightly Langerin-positive cells (data not shown). TGF-β is a cytokine known to promote the differentiation of LC (29). When TGF-β was added to the cultures (at both 0.5 and 5 ng/ml), the number of Langerin-positive cells increased up to nearly 10% (Fig. 7). Of note, Langerin-expressing cells occurred preferably in cell aggregates. No obvious differences in the percentages of Langerin-positive cells were noted between various time points (day 6, 8, 10, 13, and 19).

LC from epidermal bulk cultures mature into potent immuno-stimulatory DC in 3 days and typically lose their BG, as demonstrated by electron microscopy (30). While fresh mouse LC expressed Langerin, 3 day-cultured epidermal cells expressed markedly reduced levels, and many cells became Langerin negative (data not shown), further confirming that Langerin is restricted to immature DC. Taken together, these results demonstrate that mLangerin is regulated in the same manner as its human counterpart, increased by TGF-β and decreased by the LC maturation process.

**Transfection of mLangerin/CD207 cDNA induces BG, whereas a Phe244 to Leu replacement induces structures resembling cored tubules**

Because transfection of hLangerin cDNA induced the formation of BG in fibroblasts (7), we analyzed by electron microscopy COP5 mouse fibroblasts transfected with mLangerin cDNA. In a manner reminiscent of hLangerin, COP5 cells transfected with the murine cDNA massively accumulated pentalamellar membranes with a central zippering typical of BG (Fig. 8, Langerin wild-type). Furthermore, a truncated hLangerin cDNA construct entirely devoid of the C-type lectin domain (CRD) did not induce BG or any

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**FIGURE 6. Expression of mLangerin protein in vivo.** mLangerin expression by LC on frozen skin sections with anti-mLangerin polyclonal Ab (A), or double staining with anti-mLangerin mAb HD24 (red) and FITC-anti-MHC class II mAb 2G9 (green) on epidermal sheet (B). In thymus, polyclonal anti-mLangerin (red) reveals mLangerin⁺ DC in the medulla (C and E) and some in the cortex as localized by a DEC205 serial staining (D) revealing cortical epithelial cells and indigitating cells. At higher magnification, the mLangerin⁺ DC show dendritic morphology (F). In spleen, some mLangerin⁺ cells (G) are observed in perifollicular areas, as in lymph node (H). No staining was detected with control Abs (not shown). Magnification: A, ×200; B, ×1000; C and D, ×40; E, ×200; F, ×400; G, ×100; and H, ×400.
detectable organelles in COP5 fibroblasts (data not shown). On this basis, we explored whether modifications within the CRD would have an impact on BG formation. We isolated a cDNA clone of mLangerin that displays a leucine at position 244 instead of the phenylalanine that is conserved in the CRD of type-II lectins (e.g., KCR, ASGPR) (F at position 48 in the CRDs depicted in Fig. 2). Strikingly, transfection of the mutated mLangerin cDNA resulted in accumulation of superimposed and thickened membranes clearly different from typical BG (Fig. 8, Langerin Leu 244). Reminiscent structures, referred to as cored tubules, have been described in mouse LC and related cells (31). No cytoplasmic structures were observed in mock-transfected COP5 cells (Fig. 8, control). Our findings demonstrate that mLangerin/CD207 induces BG formation, and that its lectin domain is highly plastic relative to the pattern of membrane superimposed structures that can be created.

Discussion

We previously described hLangerin/CD207, a LC-restricted C-type lectin that functions as an endocytic receptor inducing BG formation (7). In this work, we have identified mLangerin/CD207 through the use of ESTs in public databases. The predicted mLangerin protein displays an overall 66% sequence identity with the human counterpart, a figure well in agreement with the degree of conservation between other human and rodent C-type lectins.

The closest related lectin to mLangerin/CD207 is the murine KCR, expressed in the liver and also functioning as an endocytic receptor, although no BG-like structures have been demonstrated in Kupffer cells (32, 33). The phylogenetic tree determined from amino-acid sequences indicates that Langerin and the KCR belong to a subgroup of type-II proteins within the C-type lectin superfamily. This particular relationship is further emphasized by the fact that, as described for the rat KCR (28), the neck domain preceding the CRD of Langerin is encoded by a single exon. However, the KCR possesses an extra exon between the transmembrane and neck domains that can be explained by exon shuffling known to occur in other C-type lectins (34). It remains to be established whether Langerin and the KCR arose from duplication of an ancestral gene, because neither the human KCR nor the chromosomal localization of the mouse KCR is known.

In addition to the mouse and human Langerin and the KCR genes, the structure of several other genes that encode type-II C-type lectins has been determined. These include the hepatic H1 and H2 ASGPR (35), the low-affinity IgE receptor CD23 (36), and the HIV-binding lectins dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) and DC-SIGNR (37). Similarly to Langerin, the CRD of the above genes are encoded by three exons whose exon-intron boundaries are highly conserved. In addition, the insertion of a neck domain sequence between the transmembrane and CRD regions is the hallmark of a subfamily of the C-type lectins.
lectins (38). The neck domain of Langerin features a characteristic heptad repeat producing an α-helical coiled-coil stalk (38). Heptad repeats are important for dimerization/oligomerization, as illustrated for CD23 (39, 40) and the H1 and H2 subunits of the hepatic ASGPR (41). This raises the possibility that Langerin occurs as a dimer/oligomer, although we have no experimental evidence supporting this hypothesis.

The gene encoding mLangerin maps to chromosome 6D, in a region syntenic to the hLangerin gene on chromosome 2p13. The Langerin genes do not belong to any of the known C-type lectin gene clusters, such as the human DC-SIGN and CD23 genes (chromosome 19p13) (37), the human ASGPR (chromosome 17p11-13) (42), and the human KNR complex (chromosome 12p13-p12) (43). However, due to their particularly close relationship the KCR genes may turn out to cluster with Langerin.

One of the characteristic features of DC is the expression of many C-type lectins. DC express endocytic receptors for capturing exogenous carbohydrate-bearing material for pathogen recognition and Ag routing. Endocytic receptors are exemplified by the type-I lectin DEC205 (44, 45) and the type-II human lectins Langerin (18) and DC-SIGN, which seques-ter HIV through binding to gp120 (46). However, a given lectin may have more than one function, as demonstrated for DC-SIGN, which binds ICAM-3 and ICAM-2 and thus has an impact on DC/T cell interactions and DC migration (47, 48). In addition, type-II lectins such as DCIR (49) and dectin-1 (50) contain intracellular immunoreceptor sequences predictive of a role in inhibition or activation of DC function. Consequently, it is an important issue to determine whether multiple function is a general feature of DC lectins and whether lectins on a given DC subset have overlapping functions.

Langerin is abundantly expressed by mouse epidermal LC, both at mRNA and protein levels. Using mAb HD24, Langerin could not be detected in other epidermal cells, and could only be detected in scattered DC in the dermis. Langerin should thus be a highly useful molecular marker for identification and functional studies of mouse LC. This has been particularly hampered by paucity of reagents, because the mouse lacks CD1a, a useful marker of human LC. Mouse LC do express DEC205, but this molecule is also widely expressed by mature DC in lymphoid tissue (45). The co-expression of DEC205 and Langerin on mouse LC raises the possibility of some shared function on this DC subset. Langerin is down-regulated upon culture of mouse epidermal LC (data not shown), in line with the notion that it represents a feature of immature cells. Decreased expression of Langerin agrees with earlier observations describing the maturation of mouse LC in culture resulting in disappearance of BG (30). Scattered Langerin+ cells are observed in lymph nodes and spleen. Langerin+ CD11c+ cells expressing high levels of DEC205 are particularly conspicuous in skin-draining lymph nodes, where they represent a mature form of LC immigrated from the epidermis (51). Langerin+ cells in spleen have recently been shown to represent CD11c+CD8α+ DC (K. Inaba, personal communication). Langerin is also present on DC within the mouse thymic medulla. This finding warrants several comments and questions. First, the possibility that Langerin’s thymic DC and LC have a common origin. Mouse thymic DC, which include a major CD8α− subset (52), are essentially of lymphoid origin (8). Recently, lymphoid-committed CD4+ CD44high precursors were shown to generate LC upon adoptive transfer (9). Although CD8α is not a marker of lymphoid origin (53), it is striking that mouse LC can be induced to express this marker (9, 10). Second, the expression of Langerin by thymic DC is consistent with the description of BG in these cells, in both mouse (54) and human (55). Third, the presence of Langerin on thymic DC opens the interesting perspective that Langerin may play a role in T cell selection, possibly by endocytosis of self-derived mannosylated Ags. The fact that DEC205 is also expressed by thymic DC (52) further points to overlapping function between these two lectins. In addition to skin, mLangerin mRNA is detected in some nonlym-phoid organs. In particular, lung tissue was positive, similar to the expression of hLangerin in epithelium lining the human airways (18). The significance of mLangerin mRNA in liver and heart remains to be explored.

A subset (<10%) of Langerin+ cells was generated in cultures of mouse bone marrow in the presence of GM-CSF and TGF-β1. The role of TGF-β in expanding the Langerin+ subset is in line with the effects of this cytokine in up-regulation of hLangerin in human LC cultures (18) and its critical function in mouse LC development (29, 56). Langerin should be a highly useful marker to optimize conditions for in vitro generation of mouse LC that is currently a limitation to functional studies on this cell type.

Mouse and human Langerin share the capacity to induce the formation of pentalamellar membranes typical of BG. This property is conserved through evolution and emphasizes the potential importance of the molecule in LC function. Although introduction of Langerin cDNA does not create “tennis racket” structures sometimes observed in LC, it should be noted that these particular structures likely represent only a minority of the BG, probably resulting from fusion with endocytic vesicles that is not likely to occur in the transfected fibroblasts because most of the Langerin molecules will superimpose before reaching the cytomembrane. The prediction of our previous (7) and present findings is that Langerin plays a particular role in endocytosis of foreign Ags (e.g., mannosylated microorganisms) in the periphery, and of self Ags in the thymus. Routing of Ag into BG could represent the feature of a distinctive DC sublineage found in epidermis, stratified epithelia, and thymus. It would be of interest to examine the BG-inducing potential of other lectins (e.g., CD23, DEC205) expressed by the same cells. Deletion of the entire lectin domain of Langerin totally abolished the membrane superimposition effect in transfectants, emphasizing the role of carbohydrate ligand binding in BG formation. However, the nature of the physiological ligand(s) of Langerin remains to be determined. Strikingly, replacement of the highly conserved F244 by L in the α2 helix of the mLangerin CRD abrogated formation of the rod-shaped zipper membrane superimpositions typical of BG. The L244 form of mLangerin gave rise to structures reminiscent of cored tubules described in mouse LC-type cells (57, 58). As viewed by electron microscopy, cored tubules do have an inner central line, but their structure is different from the disc-shaped BG. Cored tubules have been observed in mouse LC and related cells (31), although their relationship with BG is unknown. We propose that the relationship between these different organelles is determined by the conformation of Langerin. Replacement of F244 by L, both hydrophobic residues, is not expected to disrupt the α2 helix of the mLangerin CRD (59). Rather, a three-dimensional model using the recently determined crystal structure of the H1 subunit of the ASGPR (60) indicates that absence of the aromatic ring of F244 is likely to abolish its interaction with the parallel and tightly juxtaposed aromatic ring of F205. The substitution of F244 with L may thus open the hydrophobic pocket in this region of the CRD, thus contributing to a change in the domain associated with receptor multimerization or with ligand binding. It has been suggested that CRD sequences flanking the coiled-coil stalk may also contribute to C-type lectin subunit assembly (41). The transition from BG to cored tubules could thus be a consequence of altered conformation of the
receptor-ligand complex. However, it remains to be determined whether the F244 to L substitution mimics a conformation change that occurs naturally in Langerin as a result of interaction with particular types of ligands.

In summary, our present findings identify mouse Langerin/CD207, which should be a useful molecule both as a marker and for functional studies of mouse LC and related cells. In addition, we have demonstrated a plasticity of the types of organelles that can form in DC, and that may have important consequences for the function of these cells.

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