Polymorphisms in Human Langerin Affect Stability and Sugar Binding Activity*

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Langerhans cells are specialized skin dendritic cells that take up and degrade antigens for presentation to the immune system. Langerin, a cell surface C-type lectin of Langerhans cells, can be internalized and accumulates in Birbeck granules, subdomains of the endosomal recycling compartment that are specific to Langerhans cells. Langerin binds and mediates uptake and degradation of glycoconjugates containing mannose and related sugars. Analysis of the human genome has identified three single nucleotide polymorphisms that result in amino acid changes in the carbohydrate-recognition domain of langerin. The effects of the amino acid changes on the activity of langerin were examined by expressing each of the polymorphic forms. Expression of full-length versions of the four common langerin haplotypes in fibroblasts revealed that all of these forms can mediate endocytosis of neoglycoprotein ligands. However, sugar binding assays and differential scanning calorimetry performed on fragments from the extracellular domain showed that two of the amino acid changes reduce the affinity of the carbohydrate-recognition domain for mannose and decrease the stability of the extracellular domain. In addition, analysis of sugar binding by langerin containing the rare W264R mutation, previously identified in an individual lacking Birbeck granules, shows that this mutation abolishes sugar binding activity. These findings suggest that certain langerin haplotypes may differ in their binding to pathogens and thus might be associated with susceptibility to infection.

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3 The abbreviations used are: CRD, carbohydrate recognition domain; SNP, single nucleotide polymorphism; Man-BSA, mannose-BSA bovine serum albumin; MBP-A, serum mannose-binding protein; Mes, 4-morpholineethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and other DNA-modifying enzymes were obtained from New England Biolabs. Monosaccharides and Sepharose 4B were purchased from Sigma-Aldrich. Oligonucleotides were obtained from Invitrogen. Na 125I and isopropyl-β-D-thiogalactoside were from Amersham Biosciences. Mannose-BSA bovine serum albumin (Man-BSA) was purchased from E. Y. Laboratories (San Mateo, CA) and iodinated by the chloramine-T method (10). Human genomic DNA samples were obtained from the European Cell Culture Collection (Porton Down, UK). Immulon 4 96-well microtiter plates were from Dynex Technologies (Worthing, UK). Mannose-Sepharose and galactose-Sepharose were prepared by the divinyl sulfone method (11).

Expression and Purification of Polymorphic Forms of Langerin—Amino acid changes corresponding to the polymorphisms in the CRD of human langerin were introduced into the human langerin cDNA using synthetic oligonucleotides. The changes were verified by DNA sequencing. Expression and purification of polymorphic forms of the langerin CRD and of the whole extracellular domain using bacterial expression systems were performed as described previously (6).

Expression of Full-length Langerin in Fibroblasts—cDNAs coding for the polymorphic forms of full-length langerin were inserted into the retroviral expression vector pVc0s (12) at the EcoRI site. The neomycin resistance gene, under the control of the herpes virus thymidine kinase
promoter, was inserted into the resulting vector at the unique Clal site. The final plasmids were transfected into 9Cre packaging cells (13) to produce pseudo-virus that was used to infect Rat-6 fibroblasts as described previously (6). Cell lines stably expressing langerin were selected with G418. Langerin expression was detected by Western blotting of cell lysates. Cells were harvested using non-enzymatic cell dissociation solution (Sigma), and lysates were prepared by sonication in 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% Triton X-100. Following SDS-polyacrylamide gel electrophoresis and transfer to nitrocellulose, langerin was detected using a rabbit polyclonal antibody raised against bacterially expressed langerin CRD, and 125I-protein A. Radioactivity was detected using a PhosphorImager (Amersham Biosciences). Relative amounts of langerin expressed were quantified from Western blots using the ImageQuant software. Analysis of uptake and degradation of 125I-Man-BSA by fibroblasts expressing langerin was performed as described previously for cells expressing the chicken hepatic lectin (14).

Analysis of Sugar Binding by Polymorphic Forms of Langerin CRD—Samples (1 ml) of langerin CRD produced in bacteria, in loading buffer (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 20 mM CaCl2), were loaded on to 2-ml columns of mannos- or galactose-Sepharose equilibrated in loading buffer. After washing with eight 1-ml aliquots of loading buffer, the columns were eluted with six 1-ml aliquots of 20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% Triton X-100. Following SDS-polyacrylamide gel electrophoresis on 17.5% polyacrylamide gels followed by staining with Coomassie Blue.

Direct binding of langerin CRD to Man-BSA was determined using a solid phase binding assay described previously for the CRD of rat mannos-binding protein (15). Plastic microtiter plates with removable wells were coated with langerin CRD (50 μl/well of 100 μg/ml solutions in loading buffer). After incubation overnight at 4 °C, the wells were washed three times with cold loading buffer, filled with 5% (w/v) BSA in loading buffer, and incubated for 2 h at 4 °C. 125I-Man-BSA was diluted with unlabeled Man-BSA, and serial 2-fold dilutions were prepared in loading buffer containing 5% BSA and added to the wells in duplicate. Following incubation at 4 °C for 2 h, the wells were washed three times with cold loading buffer and counted on a gamma counter. Data were fitted to an equation for saturable binding superimposed on a linearly increasing background of nonspecific binding as shown in Equation 1.

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\text{Man-BSA bound} = \text{Bkg} + \text{Max} \cdot \frac{[\text{Man-BSA}]}{K_D} + \frac{[\text{Man-BSA}]}{\text{slope}} \cdot [\text{Man-BSA}] \quad (\text{Eq. 1})
\]

where Bkg is the background binding, Max is the saturation level for specific binding, slope is the linear increase in nonspecific binding, and \(K_D\) is the concentration of ligand at which half-maximal specific saturable binding is achieved (15, 16). Values for \(K_D\) are mean ± S.D. for two independent assays.

Differential Scanning Calorimetry—Samples of the extracellular domain of langerin were dialyzed extensively against 25 mM Na-HEPES, pH 7.8, 0.15 M NaCl, 1 mM CaCl2. Aliquots of sample (1 ml) and buffer were degassed for 15 min before loading the sample and reference cells of a Calorimetry Sciences Nano III calorimeter. All scans were preceded by 10 min of equilibration at the starting temperature. Initial scans from 2 to 35 °C were repeated until a stable baseline was obtained. Denaturation experiments were run from 20 to 90 °C. Base lines were calculated using a fourth order polynomial equation to fit the data between 25 and 90 °C using SigmaPlot. Protein concentrations were determined using the alkaline ninhydrin assay (17).

\(\text{pH Dependence Assays}\)—pH dependence of 125I-Man-BSA binding to langerin extracellular domain was determined using a solid phase binding assay described previously (6). Buffers used were 25 mM sodium acetate for pH 4.0–4.5 and 25 mM MES/MOPS for pH 5.0–8.5. Buffers contained either 1 or 20 mM CaCl2. Data were fitted, using SigmaPlot, to an equation for pH dependence (18).

Analysis of Sugar Binding by Langerin CRD with the W264R Mutation—The Arg264 form of the CRD of langerin was expressed in bacteria as described previously for the reference form (6). Periplasmic proteins, including the expressed CRD, were extracted from the bacteria in 20 ml of loading buffer and passed over 10-ml columns of mannos- or galactose-Sepharose. Columns were washed with ten 2-ml aliquots of loading buffer and eluted with eight 2-ml aliquots of elution buffer. All fractions were analyzed by SDS-polyacrylamide gel electrophoresis on 17.5% polyacrylamide gels followed by staining with Coomassie Blue or Western blotting and staining with an anti-langerin antibody.

RESULTS

Location of Polymorphic Residues in the CRD of Langerin—Examination of the SNP data base shows that three single nucleotide polymorphisms in the human langerin gene result in non-synonymous changes in amino acid residues in the CRD (www.ncbi.nlm.nih.gov/SNP). The changes are A278V (SNP data base cluster identification rs741326), N288D (SNP data base cluster identification rs810409), and A300P (SNP data base cluster identification rs2080391). For the A278V polymorphism the average allele frequencies have been determined as Ala 0.504 and Val 0.496, with an average estimated heterozygosity value of 0.500. Thus, at this position in langerin, Ala and Val appear to be equally represented in the populations studied. At position 288, Asp is less common than Asn. The average allele frequencies are Asn 0.886 and Asp 0.114 with an average estimated heterozygosity value of 0.202. No heterozygosity data are available for the A300P polymorphism, although this SNP has been identified multiple times. In our analysis of 136 genomic DNA samples from a variety of different populations (38 black African, 17 Japanese, 17 French, 15 Italian, 12 Thai, 12 Oriental, 10 Aboriginal, 9 South American Indian, 6 Ashkenazi Jewish) the Pro allele was not found (data not shown), suggesting that it is uncommon.

In the absence of a crystal structure of langerin, information about the likely positions of the polymorphic residues in the tertiary structure of the CRD can be obtained from analysis of the crystal structure of the homologous CRD of rat serum mannos-binding protein (MBP-A). Alignment of the sequences of the two CRDs and mapping of the three polymorphic residues in langerin onto the crystal structure of MBP-A are shown in Fig. 1. Position 288 of langerin, where the Asn/Asp change is found, corresponds to one of the residues of MBP-A, Asp188, which is a ligand for the auxiliary Ca2+ (Ca2+ 1). This Ca2+ 1 is important for stabilizing the loops at the top of the CRD that interact with the principal Ca2+ (Ca2+ 2), which in turn ligates directly to sugar (Fig. 1B).

Only two of the four residues that ligate Ca2+ 1 in MBP-A are conserved in langerin, so it is not yet clear whether langerin binds Ca2+ 2 at this auxiliary site. Some other C-type CRDs, for example those of the selectins, bind Ca2+ only at the principal site (21). However, regardless of whether residue 288 is involved in Ca2+ binding to langerin, it is positioned close to the conserved principal Ca2+ binding site where the sugar is predicted to bind, suggesting that the presence of aspartate rather than asparagine at this position might affect the ability of the CRD to bind sugar.

Position 278 of langerin corresponds to a residue located in the hydrophobic core of MBP. The presence of valine rather than alanine at this position would not be expected to have a direct effect on sugar binding by the CRD but might change the overall stability of the domain and thus have an indirect effect on the activity of langerin. Position 300
of langerin maps to a region of the CRD that forms an extended secondary sugar binding site in some C-type lectins, including the selectins and DC-SIGN (22). Binding studies with a Man9 oligosaccharide suggest that the langerin CRD might contain an extended binding site (6), in which case the Pro/Ala change at position 300 might affect sugar binding.

To test the effects of the polymorphic residues on the activity of langerin, mutations were introduced into the cDNA to produce amino acid changes at each of the three polymorphic positions in the CRD. Four forms of langerin were thus produced, one corresponding to the reference sequence Ala278, Asn288, Ala300 and three corresponding to versions each with an amino acid change at one of the polymorphic positions: Val278, Asn288, Ala300; Ala278, Asp288, Ala300; and Ala278, Asn288, Pro300. Full-length, membrane-bound versions of the four forms of langerin were expressed in fibroblasts. In addition, soluble fragments consisting of just the CRD and of the whole extracellular domain of each of the four forms of langerin were expressed in bacteria.

**Endocytosis by Variant Forms of Langerin**—The reference form of langerin, when stably expressed as the full-length molecule in fibroblasts, mediates uptake and degradation of 125I-Man-BSA (6). Stably transfected fibroblast cell lines expressing the other three forms of langerin were created so that the effects of the amino acid changes on endocytic activity could be determined. Western blotting of membrane extracts from the cell lines with an antibody specific for langerin showed a single band of the expected molecular weight for each form (Fig. 2A).

Although there was some variation in expression levels on multiple cell lines isolated for each form of langerin, there was no consistent correlation between amino acid sequence and the amount of protein expressed (data not shown). These findings indicate that the amino acid changes probably do not substantially affect the rates of langerin biosynthesis. The relative amounts of langerin expressed in the four cell lines used for endocytic assays are given in the legend to Fig. 2A.

Comparison of the abilities of cell lines expressing each form of langerin to endocytose 125I-Man-BSA showed that each of the four forms of langerin is able to mediate uptake and degradation of this neoglycoprotein ligand (Fig. 2B). In each case, ligand becomes associated with the cells and after a short lag period degradation products are released into the medium. Although each of the forms of langerin is functional in the endocytosis assay, there may be differences in the rate of endocytosis for the different forms. Taking into account the slightly different levels of langerin expressed in the four cell lines, the two cell lines expressing the Asp288 and Pro300 forms of langerin appear to process less 125I-Man-BSA over the course of the assay than does the cell line expressing the reference form of langerin (Table 1). Variability inherent in the assays makes it difficult to quantify small differences in endocytic rate, but it is clear that none of the amino acid changes abolishes the ability of langerin to mediate endocytosis.
Effect of Polymorphisms on Sugar Binding by the CRD of Langerin—
The effects of the polymorphic residues on sugar binding were tested by assessing the ability of CRDs of the four forms of langerin to bind to columns of mannosose-Sepharose, as well as in the fractions eluted with EDTA (Fig. 3), confirming that mannose binding activity is not abolished completely.

Stability of Variant Forms of Langerin—Differential scanning calorimetry was used to assess the stability of the different forms of langerin. Initial comparison of the thermal stabilities of the CRD alone with the whole extracellular domain (neck + CRD) of the reference form of langerin shows that each of these fragments of the protein denatures at a temperature of about 58°C (Fig. 5). The main denaturation peak for the extracellular domain corresponds to the major peak seen for the CRD (Fig. 5). The small shoulder seen at higher temperature in each case is due to precipitation of the denatured protein, as is often seen in these types of experiments. These results indicate that the protein is very stable, and that the CRD and the neck region may be closely packed. In this respect, langerin resembles serum mannose-binding protein, which differs from the tetrameric C-type lectins DC-SIGN and DC-SIGNR where the unfolding of the extracellular domain is accompanied by a linear increase in background binding activity.

The findings from the semiquantitative column binding assays were confirmed using a quantitative binding assay in which the affinity of the CRD for Man-BSA (Fig. 4 and Table 1), with very similar values being obtained for the Val278 form and the reference form. In contrast, the Pro300 forms of the CRD are still retarded on the mannosose-Sepharose column compared with the galactose-Sepharose column (Fig. 3), confirming that mannose binding activity is not abolished completely.

The affinity of the CRD for Man-BSA must be at least an order of magnitude lower than that of the reference form.

**TABLE 1**
Summary of the effects of amino acid changes due to single nucleotide polymorphisms on the function of langerin

| Langerin variant | Relative endocytic activity | Binding of CRD to Man-BSA | Melting temperature | pH₅₀ at 1 mM Ca²⁺ | pH₅₀ at 20 mM Ca²⁺ |
|------------------|----------------------------|--------------------------|--------------------|-----------------|-----------------|
| Ala²⁷⁸, Asn²⁸⁸, Ala³⁰⁰ | 1.4 | $K_D = 10.6 \pm 2.8$ | 57.4 | 5.0 ± 0.1 | 4.4 ± 0.1 |
| Val²⁷⁸, Asn²⁸⁸, Ala³⁰⁰ | 1.1 | $K_D = 10.6 \pm 4.4$ | 55.2 | 5.5 ± 0.4 | 4.5 ± 0.1 |
| Ala²⁷⁸, Asp²⁸⁸, Ala³⁰⁰ | 0.7 | $K_D > 100$ | 54.4 | 5.9 ± 0.4 | 4.7 ± 0.1 |
| Ala²⁷⁸, Asn²⁸⁸, Pro³⁰⁰ | 0.7 | $K_D > 100$ | 48.1 | 6.3 ± 0.2 | 4.7 ± 0.2 |

*Endocytic activities adjusted for the amounts of langerin expressed were calculated from the values for ligand molecules processed and released into the medium/cell at 4 h (values from Fig. 2B) divided by the relative amounts of langerin/cell detected on Western blots (values given in Fig. 2A).
Pro causes a large decrease in thermal stability (Fig. 6). The Pro300 form of langerin denatures at a temperature ~10 °C lower than the reference form. In contrast, replacement of Ala278 with Val or Asn288 with Asp results in decreases in denaturation temperature of ~2–3 °C, indicating that these amino acid changes have only a small effect on the stability of langerin. Thus, for the Pro300 form of langerin, the large decrease in stability correlates with the decrease in sugar binding activity by the CRD, but changing Asn288 to Asp has less effect on stability than on sugar binding. The correlation between effects on stability and sugar binding activity indicates that reduction of sugar binding activity caused by the presence of proline rather than alanine at position 300 is due to disruption of the overall structure of the CRD. In contrast, the reduction in sugar binding activity caused by the N288D change appears to be due to a more local change in structure that affects the sugar binding site without disrupting the rest of the structure.

**pH Dependence of Ligand Binding**—Determination of the pH dependence of ligand binding for the variant forms of langerin gives another means of assessing the effects of the amino acid changes. As is the case for other endocytic C-type lectins, sugar binding to langerin is pH dependent, with ligand release occurring at endosomal pH. pH-dependent release of sugar ligand is associated with a decrease in affinity for Ca2+ (6). Assays performed with the extracellular domains of each of the four forms of langerin showed that all of them exhibit pH dependence of ligand binding at physiological Ca2+ concentration (1 mM) (Fig. 7A). However, the Pro300 form is significantly more sensitive to pH change than the reference form. Half maximal binding for Pro300 langerin occurs at pH 6.3 ± 0.2 compared with pH 5.0 ± 0.1 for the reference form (Table 1). Replacement of Asn288 with Asp results in a smaller increase in sensitivity to pH, with half maximal binding occurring at pH 5.9 ± 0.4 for Asp288 langerin. Consistent with the lack of effect on sugar binding and stability, replacement of Ala278 with Val does not significantly alter pH sensitivity.

At 20 mM Ca2+, all four forms of langerin are much more resistant to change in pH and there is little difference in the behavior of the four forms (Fig. 7B and Table 1). As noted previously, it is likely that loss of sugar binding with decreased pH is due to decrease in affinity for Ca2+ at lower pH, so that at high Ca2+ the effect is abrogated (6). High Ca2+ abrogates pH sensitivity even for the two forms of langerin that show increased sensitivity to pH at physiological Ca2+. Thus, the increased sensitivity to pH of both the Pro300 and the Asp288 forms of langerin is likely to be related to effects on Ca2+ affinity. Compared with the other forms of langerin, affinity of these forms for Ca2+ must fall off more rapidly with decreasing pH, although this effect can still be overcome at high Ca2+.

**A Rare Mutation in the CRD of Langerin Abolishes Sugar Binding Activity**—In addition to the polymorphisms identified in the langerin CRD, a mutation found in the langerin gene of an individual lacking Birbeck granules in their Langerhans cells results in replacement of tryptophan at position 264 by arginine (9). Trp264 is predicted to be located within the hydrophobic core of the CRD, and an aromatic residue is conserved at this position in almost all C-type lectin-like domains (Fig. 1.). Changing this aromatic residue to a positively charged arginine residue would disrupt the structure of the domain and thus would be likely to affect sugar binding activity. The W264R form of langerin fused to green fluorescent protein and expressed in human fibroblasts fails to react with a langerin-specific monoclonal antibody, suggesting that the mutation causes a conformational change (9). Birbeck granules were not formed in the fibroblasts expressing W264R langerin, confirming that this mutation in langerin is responsible for the lack of these structures (9).
Large changes in the structure of the CRD of the W264R form of langerin would be expected to alter its sugar binding activity. To examine the effects of the W264R mutation on sugar binding by langerin, this form of the CRD was expressed in bacteria. Unlike the four forms of the langerin CRD described above, langerin CRD with arginine at position 264 could not be purified from the bacterial extracts by affinity chromatography on mannose-Sepharose. On a Coomassie Blue-stained gel of the wash and elution fractions from a 10-ml column of mannose-Sepharose, no Arg264 CRD was seen (not shown). However, Western blotting with a polyclonal anti-langerin antibody showed that the CRD is present in the first few fractions along with all the bacterial proteins that flow straight through the column (Fig. 8, upper panel). Whereas the CRD of the reference form of langerin, which contains tryptophan at position 264, is selectively retarded on mannose-Sepharose (Fig. 8, lower panel), the elution profile of the Arg264 CRD from the mannose-Sepharose column is identical to that seen with galactose-Sepharose (Fig. 8, middle panel), indicating that the W264R mutation abolishes mannose binding activity.

DISCUSSION

Both the Pro280 and the Asp288 forms of langerin would be expected to have decreased ability to interact with glycoconjugates on the surface of microorganisms, although the mechanism for the decrease in sugar binding activity is different for the two amino acid changes. In the Pro280 form of langerin, sugar binding activity is decreased as an indirect result of decreased stability of the CRD due to disruption of the core of the domain, whereas the presence of aspartate at position 288 at the surface of the domain appears to have a more local affect on the structure around the sugar binding site. Although neither of these amino acid changes abolishes the ability of langerin to endocytose a neoglycoprotein ligand, it seems likely that the interactions of langerin with physiological ligands, such as pathogens, would be impaired by these changes.

In contrast, the rare tryptophan to arginine mutation completely abolishes sugar binding activity, and this form of langerin would not be expected to bind to microorganisms. The presence of valine rather than alanine at position 278 has little, if any, effect on sugar binding activity or stability of langerin, which is consistent with the fact that the two alleles encoding these forms of langerin are approximately equally frequent in the populations analyzed.

Polymorphisms associated with susceptibility to infection have been well characterized in another human C-type lectin, mannose-binding protein. Three variant alleles of mannose-binding protein are associated with susceptibility to severe, recurrent infections, particularly in early childhood (24). However, in the case of mannose-binding protein, the single nucleotide polymorphisms result in amino acid changes in the collagenous region of the protein, not in the CRD, and the changes do not affect either the stability or the sugar binding activity of the CRD. Thus, the amino acid changes do not reduce the ability of serum mannose-binding protein to bind to microorganisms, but they affect the resulting downstream events that lead to killing of the microorganism. The amino acid changes destabilize the collagen-like domain of the variant forms of mannose-binding protein, resulting in decreased ability to interact with the mannose-binding protein-associated serine proteases that initiate the complement cascade (25). This situation is thus different from langerin because single nucleotide polymorphisms in langerin result in amino acid changes in the CRD that reduce sugar binding activity.

The finding that polymorphic forms of human langerin differ in stability and sugar binding activity might have implications for the susceptibility to infections of individuals with certain langerin haplotypes. The expressed forms of langerin examined in these studies mimic the homozygous case for each polymorphic residue, where every molecule of langerin has the same amino acid sequence. Because of the low frequency of the variant alleles, homozygotes for Asp288 or Pro280 are likely to be very rare. However, because langerin exists as a stable trimer held together by the neck region, even in heterozygotes, most langerin trimers would contain at least one polypeptide with aspartate rather than asparagine at position 288 or proline rather than alanine at position 300. If the two types of polypeptide in the heterozygotes are synthesized at the same rate and can associate freely, then only one in eight molecules of trimeric langerin will contain three polypeptides with the reference sequence. Because most langerin molecules in the heterozygotes would contain CRDs with the amino acid residues that affect sugar binding and stability, the physiological functions of langerin might be impaired even in heterozygotes. The individual with the W264R mutation is heterozygous for this change (9). The fact that this individual lacks Birbeck granules suggests that either the small fraction of langerin molecules without the mutation is not sufficient to allow the formation of Birbeck
granules or that the mutated molecules somehow affect the function of molecules without the mutation. Because this mutation completely abolishes sugar binding by langerin, it is possible that binding of langerin to a carbohydrate ligand is required for Birbeck granule formation. However, it is also possible that disruption in the structure of langerin due to the mutation prevents other interactions of langerin needed for Birbeck granule formation.

Although it is clear that some amino acid changes caused by single nucleotide polymorphisms in langerin affect langerin function, assessing the significance of the findings is hampered by a lack of evidence about the physiological roles of langerin. There is evidence consistent with langerin playing a role in host defense against infectious disease, but so far there is no direct evidence for such a role. Like other mannose binding C-type lectins found on immune cells, langerin is predicted to bind to microorganisms with mannose-containing surface glycoconjugates, including mycobacteria and yeast, although so far only binding to Candida albicans has been demonstrated (26). Knock-out mice for the langerin gene are healthy but lack Birbeck granules, like the individual with the W264R mutation (4). When challenged with C. albicans, Mycobacterium tuberculosis, Leishmania major, or Klebsiella pneumoniae, the knock-out mice are no more susceptible to infection with these microorganisms than are wild-type mice (4). The knock-out mice experiments suggest that langerin does not have an essential role in defense against these microorganisms (4). However, there are significant differences between the immune responses of mice and humans. Experiments with human Langerhans cells suggest that langerin presents non-peptide antigens from Mycobacterium leprae to CD1a-restricted T cells (8). Mice do not express the same CD1 molecules as humans, and there is no equivalent of CD1a in mice, so it is not possible to examine the importance of langerin in antigen presentation to CD1 molecules in the langerin knock-out mice. The fact that the individual with the W264R mutation is healthy does not rule out the possibility that langerin is important for defense against pathogens such as M. leprae, but it suggests that langerin is not essential for immunity against commonly encountered microorganisms. Thus, consequences of impaired langerin function due to polymorphisms may only become apparent if individuals encounter specific, less common pathogens.

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