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Disruption of the langerin/CD207 Gene Abolishes Birbeck Granules without a Marked Loss of Langerhans Cell Function

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Langerin is a C-type lectin expressed by a subset of dendritic leukocytes, the Langerhans cells (LC). Langerin is a cell surface receptor that induces the formation of an LC-specific organelle, the Birbeck granule (BG). We generated a langerin−/− mouse on a C57BL/6 background which did not display any macroscopic aberrant development. In the absence of langerin, LC were detected in normal numbers in the epidermis but the cells lacked BG. LC of langerin−/− mice did not present other phenotypic alterations compared to wild-type littermates. Functionally, the langerin−/− LC were able to capture antigen, to migrate towards skin draining lymph nodes, and to undergo phenotypic maturation. In addition, langerin−/− mice were not impaired in their capacity to process native OVA protein for I-A restricted presentation to CD4+ T lymphocytes or for H-2K restricted cross-presentation to CD8+ T lymphocytes. langerin−/− mice inoculated with mannosylated or skin-tropic microorganisms did not display an altered pathogen susceptibility. Finally, chemical mutagenesis resulted in a similar rate of skin tumor development in langerin−/− and wild-type mice. Overall, our data indicate that langerin and BG are dispensable for a number of LC functions. The langerin−/− C57BL/6 mouse should be a valuable model for further functional exploration of langerin and the role of BG.

Dendritic cells (DC) are the most potent leukocytes to mediate the rapid initiation of a primary immune response (7). DC are bone marrow-derived leukocytes, localized in most tissues including primary and secondary lymphoid organs. In the periphery, most DC are in an immature state and are able to capture antigenic molecules via unique endocytic receptors or by fluid-phase macropinocytosis. This process generally leads to a first step of DC maturation, concomitant with their migration to secondary lymphoid organs. DC can subsequently activate naive CD4+ T or CD8+ T lymphocytes if peptides processed from native antigenic molecules are displayed on cell surface major histocompatibility complex (MHC) class II or I molecules in conjunction with costimulatory molecules (40).

DC heterogeneity is a common feature of mice and humans. Precursor cell populations, anatomical localization, morphology, phenotype, and functions determine the type of DC. However, the origin of different DC subsets is still controversial (4). A particular subset of DC is represented by Langerhans cells (LC), which are immature DC present in the epidermis and mucosal epithelium (53, 71). LC can be generated either from myeloid precursors (77, 78) or from CD4low lymphoid precursors (2). LC express a number of cell surface receptors including CD205/DEC205, Fcε and Fcε receptors, and langerin/CD207 (70, 72).

Langerin is a C-type lectin oriented in a type II configuration and featuring a single carbohydrate recognition domain in its extracellular region (72). Langerin molecules oligomerize as trimers at the cell surface and display Ca2+-dependent binding specificity for mannose, N-acetyl-glucosamine, and fucose (61, 72). Langerin is a potent inducer of Birbeck granules (BG), the hallmark organelles of LC, which consist of pentalaminar and zippered membranes at the electron microscopic level (12, 75). In addition to inducing BG formation, langerin is an endocytic receptor involved in the trafficking of exogenous mannosylated ligands from the cell surface into intracellular BG compartments (71).

To further explore the role of langerin and BG, we generated C57BL/6 mice with a targeted disruption of the langerin gene. Although MHC class II-positive LC were detected in normal numbers in the epidermis of these mice, they did not have detectable cytoplasmic BG. Yet LC functions, such as migration, endocytosis (capture of macromolecules), antigen processing, and presentation to MHC class I- and class II-restricted T lymphocytes, were not altered. Finally, langerin−/− and C57BL/6 wild-type control mice responded similarly to infections by microorganisms such as Mycobacterium tubercu-
loss and Leishmania major or to chemically induced skin carcinogenesis. Together, our data demonstrate that langerin and BG are dispensable for a number of LC functions.

MATERIALS AND METHODS

Mice. C57BL/6J mice were purchased from Charles River Breeding Laboratories (Saint Germain sur l’Arbresle, France) and were used as wild-type controls. OT-I mice express a transgenic Vα2Vβ5 T-cell receptor (TCR) specific for the OVA257-264 epitope in the context of H-2K^b (29). OT-II mice express a transgenic Vα2Vβ5 TCR specific for the OVA233-249 epitope in the context of I-A^b (6) (both were kind gifts from Francis R. Carbone, University of Melbourne, Melbourne, Australia), and langerin^{−/−} mice were bred and housed in the Charles River facility under specific-pathogen-free conditions. All experiments involving mice were conducted in accordance with protocols approved by French laws and European or Australian directives. Mice were used at 8 to 12 weeks of age.

langerin-null targeting vector construction. The langerin targeting vector was constructed from a cosmided genomic clone isolated from a 129/Oia cosmid library as previously described (70). Briefly, a 2.9-kb KpnI-XhoI fragment (5′ homology arm, 1.5 kb from sequence and 1.4 kb from sequence) and a 2.2-kb BglII fragment (3′ homology arm containing 128 bp of exons 4 and 5 and 155 bp of exon 6) were simultaneously cloned into KpnI and BamHI sites of pBluescript II KS+ (Stratagene, La Jolla, Calif.). To assure complete elimination of langerin expression, the unique XhoI site was blunted by using a Klenow with exonuclease activity, and a pgk-neomycin cassette flanked by loxP sites was inserted blunt into the targeting vector. Sequence analysis of the neomycin insertion site revealed complete deletion of exon 1 (70 bp upstream of the ATG start codon) and partial deletion of exon 4. Finally, the targeting vector was abutted to a thymidine kinase expression cassette and linearized by a unique NotI site.

ES cell electroporation and generation of langerin^{−/−} mice. 129/Sv CK35 embryonic stem (ES) cells (35) were electroporated with 30 μg of linearized targeting vector at 250 V and 500 μF of capacitance. After selection in G418 and ganciclovir, colonies were screened for homologous recombination by Southern analysis by an external probe. The 5′ single-copy probe corresponded to a 0.38-kb EcoRV-KpnI fragment isolated from a langerin genomic subclone. When tested on EcoRV-digested DNA, it hybridized either to a 7.0-kb wild-type fragment or to a 4.8-kb recombinant fragment. Positive recombinant ES clones were further screened by Southern analysis using an internal probe and with a neo external probe to ensure that adventitious nonhomologous recombination events had not occurred in the selected clones.

Mutant ES clones were injected into BALB/c blastocysts. Male chimeras exhibiting over 60% of chimerism, as judged by coat color, were crossed with deleter transgenic mice (backcrossed on C57BL/6) to eliminate the neo^+ flanked Neo^+ cassette (58). The resulting F1 progeny was tested for both germ line deleter transgenic mice (backcrossed on C57BL/6) to eliminate the expressing over 60% of chimerism, as judged by coat color, were crossed with secondary backcrosses. All experiments involving mice were conducted in accordance with protocols approved by French laws and European or Australian directives. Mice were used at 8 to 12 weeks of age.

F1 progeny was homogenized by a unique NotI site.

Expression of OVA257-264 on new isotype.

The langerin targeting vector was recombined to a 4.8-kb recombinant fragment. Positive recombinant ES clones were further screened by Southern analysis using an internal probe and with a neo external probe to ensure that adventitious nonhomologous recombination events had not occurred in the selected clones.

Mutant ES clones were injected into BALB/c blastocysts. Male chimeras exhibiting over 60% of chimerism, as judged by coat color, were crossed with deleter transgenic mice (backcrossed on C57BL/6) to eliminate the neo^+ flanked Neo^+ cassette (58). The resulting F1 progeny was tested for both germ line deleter transgenic mice (backcrossed on C57BL/6) to eliminate the expressing over 60% of chimerism, as judged by coat color, were crossed with secondary backcrosses. All experiments involving mice were conducted in accordance with protocols approved by French laws and European or Australian directives. Mice were used at 8 to 12 weeks of age.

F1 progeny was homogenized by a unique NotI site.
purified naïve LN CD4+ T lymphocytes from OT-II mice, in medium containing IL-2 to improve the sensitivity and ensure that the limiting factor is antigen presentation, not differences in the ability to induce IL-2 production. After 3 days, the cultures were pulsed for 8 h with [3H]thymidine and DNA radioactivity was estimated by scintillation counting.

**Immunolabeling protocol for flow cytometry.** Cell surface staining was performed in PBS buffer with 1% BSA and 0.02% sodium azide. For surface and intracellular staining, the Fix&Perm kit (BD PharMingen) was used according to the manufacturer’s recommendations. Antibodies were incubated for 30 min, followed by three washes. When required, second steps were done similarly. Flow cytometric analysis was performed on a FACSCalibur (BD Biosciences, Mountain View, Calif.) with CellQuest software.

**Immunohistochemistry of epidermal sheets.** Epidermis from ear skin was separated from the dermis by means of ammonium thioxytanate (31). Resulting sheets were fixed in acetone for 20 min at room temperature and washed in several changes of PBS and PBS containing 1% BSA and 0.02% sodium azide. They were then subjected to an immunolabeling procedure as described previously (74). Sheets were placed on a slide and mounted under coverslips with one drop of Fluoromount G (Electron Microscopy Sciences, Fort Washington, Pa.). Specimens were viewed on a Zeiss Axiovert epifluorescence microscope. Pictures were taken with an Optronics MagnaFire digital camera (Optronics, Goleta, Calif.).

**Electron microscopy.** Ear skin of langerin−/− mice and of their age-matched C57BL/6 littermates was fixed with Karnovsky's half-strength formaldehyde-glutaraldehyde fixative. Finely minced pieces of tissue were postfixed in 3% aqueous osmium tetroxide, followed by en bloc staining in Veronal-buffered 1% uranyl acetate. After dehydration in a graded series of ethanol, specimens were infiltrated and embedded in Epon 812 resin. Ultrathin sections were inspected with a Philips EM400 (Fei Company, Eindhoven, The Netherlands) at an operating voltage of 80 kV.

**In vivo delivery of microorganisms.** Klebsiella pneumoniae ATCC strain 43816, serotype 2 (American Type Culture Collection, Rockville, Md.) was grown in 100 ml of tryptic soy broth (BioMérieux, Marcy-l’Étoile, France) for 2 h, washed twice in PBS at 5,000 rpm for 15 min, and resuspended in PBS to calculate the concentration by measuring the absorbance at 600 nm. Bacteria were then diluted in PBS at the desired concentration for inoculation. The inoculation dose was verified retrospectively by plating serial dilutions on tryptic soy agar plates and enumerating colonies. Mice were anesthetized with ketamine (Imalgene 500; Merial, Lyon, France). The tra chea was exposed, and 50 µl of inoculum or PBS was administered. Mice were checked on a daily basis to monitor death rate and survey physical condition.

An M. tuberculosis H37Rv isolate was grown to mid-log phase in Middlebrook 7H9 broth (Difco) supplemented with 10% ADC enrichment (5% BSA fraction V, 2% dextrose, 0.003% beef catalase, 0.85% NaCl) and 0.05% Tween 80. Aliquots were then frozen at −80°C in the presence of 15% glycerol. Before infection, one aliquot was thawed, washed, and resuspended in PBS (pH 7.4) with 0.1% Tween 80 at 3 × 104 CFU ml−1. Eight-week-old female langerin−/− mice and C57BL/6 control mice were infected by the aerosol route with M. tuberculosis H37Rv. Mice were exposed to aerosols for 15 min, which delivered approximately 200 CFU per lung. On days 1, 8, 21, and 98 postinfection, the lungs and spleen were removed aseptically and homogenized in a Potter apparatus. Serial dilutions were performed to Sauton medium and plated on solid agar Middlebrook 7H11 medium (Difco) supplemented with OADC (0.05% oleic acid, 5% BSA fraction V, 2% dextrose, 0.004% beef catalase, 0.85% NaCl). CFU were counted 14 to 21 days later. Five langerin−/− and five C57BL/6 control mice were used for each time point.

**RESULTS**

**Targeted disruption of the langerin gene.** To characterize the role of langerin in LC, we used targeted homologous recombination to generate langerin−/− mice. To completely eliminate the expression of langerin, a targeting vector was constructed which would create a deletion in the langerin gene locus, spanning from 70 bp upstream of exon 1 to most of exon 4 (Fig. 1a). After electroporation and selection of CK35 ES cells, 105 colonies were screened by genomic Southern analysis with a 5′ external probe (Fig. 1b). Seven recombinant clones were found to have been correctly targeted, and after further verification with a 3′ external and neomycin probe (data not shown), two clones were injected into mouse blastocysts. One ES clone was found capable of germ line transmission as determined by PCR (Fig. 1c). Heterozygotes were crossed among themselves to establish a langerin−/− mouse line which demonstrated stable, Mendelian transmission of the null mutation. Three cycles of accelerated backcrosses by microsatellite-based selection were performed to generate langerin−/− mice on a C57BL/6 genetic background.

The langerin−/− mice backcrossed on the C57BL/6 background bred and developed normally. In addition, the mice did
not present any morphological abnormalities at the macroscopic level. As expected from the targeted deletion in the langerin gene locus, the epidermis of the null mice completely lacked reactivity with anti-langerin antibodies (Fig. 2).

We analyzed the phenotype and distribution of leukocyte populations in the spleen, thymus, and peripheral and mesenteric LN of langerin\(^{-/-}\) mice. The qualitative and quantitative composition of DC subsets based on the expression of CD11c, CD11b, CD8, and DEC205 was not modified compared to wild-type control mice (data not shown). No quantitative or qualitative differences were observed with respect to other cell types in lymphoid organs, i.e., B lymphocytes (CD19), T lymphocytes (CD3, CD4, CD8, TCR-\(\alpha\beta\), and TCR-\(\gamma\delta\)), NK cells (NK1.1 and pan-NK MAb), and other non-T, non-B, non-NK leukocytes (data not shown). DC expressing langerin are notably present in the thymus, and it has been speculated that langerin could play a role in thymic T-cell selection (70). We found that the different intrathymic T-cell populations, i.e., double negative, double positive, or single positive, were present in similar percentages and absolute numbers in langerin\(^{-/-}\) mice compared to C57BL/6 controls (data not shown).

Furthermore, the langerin\(^{-/-}\) mouse strain was crossed to both the P14 and SMARTA transgenic TCR mouse strains to assess positive selection of an MHC class I- and II-restricted TCR, respectively (36, 48). No difference was found in TCR selection in the langerin\(^{-/-}\) mouse compared to wild-type littermates (data not shown).

Therefore, mice deficient in langerin and backcrossed on a C57BL/6 background were devoid of any remarkable alterations in their development, morphology, and leukocyte distribution in the thymus as well as secondary lymphoid organs.

**MHC class II-positive DC are present in the epidermis of langerin\(^{-/-}\) mice.** Epidermal LC express high levels of langerin (70). Thus, we addressed whether this DC subset is present or altered in the epidermis of the langerin\(^{-/-}\) mouse. Epidermal sheets from langerin\(^{-/-}\) mice stained with an anti-MHC class II MAb showed a network of DC similar to the one observed in C57BL/6 control mice (Fig. 2A, panels c and f). Even though these cells did not express langerin, as shown by epidermal sheet staining (Fig. 2A, panel e) or immunostaining of trypsin-isolated LC (Fig. 2B), they displayed a normal dendritic morphology with normal expression of MHC class II molecules (Fig. 2A, panel f, and B). Dendritic epidermal \(\gamma\delta\)-T cells (DETC), as detected by CD3 expression, were also present in normal numbers in the langerin\(^{-/-}\) mice (Fig. 2A, panel f).

Thus, LC are present in normal numbers in the epidermis of langerin\(^{-/-}\) mice and display a morphology and expression of MHC class II molecules similar to that in C57BL/6 control mice.

Epidermal Langerhans cells of langerin\(^{-/-}\) mice lack BG but otherwise do not display an altered phenotype. The cytoplasm of LC contains a unique organelle not found in other cell lineages, the BG (12). Langerin has been shown to be part of Cy5), I-A\(^\alpha\) (PE), and cell surface (MAb 205C1 FITC) or intracellular (MAb 929F3 FITC) langerin and analyzed by flow cytometry. Cells were gated on CD11b (gate R2 in left panels) and then analyzed for I-A\(^\alpha\) and the presence of langerin, analyzed either at the cell surface (middle panels) or intracellularly (right panels).
these organelles and to induce their formation (72). Inspection of ultrathin sections of the epidermis from normal mice readily revealed the presence of unequivocal BG in virtually every LC at first glance (Fig. 3B and E). The BG occurred sometimes in the shape of tennis rackets but more often as rods. In the skin of langerin−/− mice, long stretches of epidermis were examined. In 22 unequivocally identified LC (some of which were examined two or three times on serial sections) and on numerous small profiles of LC dendrites, we were unable to detect any BG at all (Fig. 3A, C, and D). DETC could readily be distinguished from LC by their characteristic electron-dense round granules (54).

LC from ear epidermis of C57BL/6 and langerin−/− mice were isolated after trypsin treatment. The phenotype of these freshly isolated LC was identical for both mouse lines with high expression of MHC class II, CD11c, CD11b (Fig. 2B), DEC-205, and E-cadherin (data not shown) but no detectable CD8α, CD40, or CD86 (data not shown). When cultured for a few days in the presence of granulocyte-macrophage colony-stimulating factor, LC from both langerin−/− and wild-type mice matured and became activated, expressing CD40, CD80, and CD86 (data not shown).

In summary, our results show that langerin is essential for BG formation, i.e., that no other gene can substitute for this function, but that LC from langerin−/− mice otherwise display an identical phenotype to that of wild-type control littermates.

Antigen capture and migration of epidermal Langerhans cells is not altered in langerin−/− mice. Due to their physical location, LC are sentinels for contact with microbial or environmental antigens. They capture and process exogenous material and subsequently migrate into skin draining LN to present antigen to specific T lymphocytes. We thus studied whether these functions would be affected by the absence of langerin.

In vitro, freshly isolated LC from langerin−/− epidermis were able to phagocytose zymosan particles as efficiently as LC from wild-type mice (data not shown). Furthermore, LC were able to crawl out from dispase-treated epidermis of langerin−/−
Processing and presentation of exogenous protein antigen is not impaired in *langerin*−/− mice. Since lack of langerin hampered neither antigen capture nor the migratory capability of LC, we investigated next whether langerin plays a role in the processing of a model antigenic protein (OVA) and the subsequent presentation of derived peptides to specific T lymphocytes in the context of MHC class I and II molecules.

LC that had crawled out of the epidermis were harvested, mixed with purified OVA-specific naïve T lymphocytes from OT-I or OT-II mice, and cultured in the presence of different concentrations of OVA protein. Results depicted in Fig. 5A indicate that *langerin*−/− LC were able to process OVA protein and to present peptides on MHC class I and II molecules. Moreover, no difference was observed when compared with LC derived from C57BL/6 control mice (Fig. 5A).

To test the capacity of the DC to capture, process, and present OVA in vivo, we adoptively transferred OT-I or OT-II spleen cells previously labeled with CFSE to C57BL/6 and *langerin*−/− mice. Forty-eight hours later, we injected soluble OVA protein into the footpads of the reconstituted mice. Four days later, the draining LN were harvested and CFSE fluorescence was analyzed as a maker of OVA-reactive T-lymphocyte divisions. As shown in Fig. 5B, both CD8+ (OT-I) and CD4+ (OT-II) OVA-specific T lymphocytes had divided in vivo, and no difference in T-lymphocyte proliferation was detected between C57BL/6 and *langerin*−/− mice.

Langerin has been previously described in spleen CD8α+ DC (39). Furthermore, it is known that the CD8α+ DC subset in the spleen is responsible for cross-presentation of exogenous OVA to MHC class I-restricted T lymphocytes (20, 49). To investigate whether lack of langerin affects cross-presentation by CD8α+ DC, we injected soluble OVA protein intravenously and collected spleens 18 h later. Spleen DC were then enriched and sorted into CD8α+ and CD4+ subpopulations. Each of these populations was cultured for 3 days with OVA-specific naive T lymphocytes from either OT-1 or OT-II mice in the presence of exogenous IL-2. The response of OT-II T lymphocytes assesses the immunostimulating features of DC displaying OVA peptides bound to MHC class I I-A^d^ while the response of OT-I T lymphocytes assesses the immunostimulating features of DC displaying OVA peptides bound to MHC class I H-2K^d^ as a result of cross-presentation. As depicted in Fig. 5C, there was no difference in cross-presentation between the *langerin*−/− mice and the controls. Thus, the CD8α+ DC of the *langerin*−/− mice do retain their cross-presentation capacity to OT-I cells. Furthermore, the CD4+ spleen DC is the major population subset presenting OVA to the OT-II T lymphocytes in both mouse lines. A series of culture experiments with soluble and cell-bound OVA confirmed this result (data not shown).

Our data thus indicate that lack of langerin does not influence protein antigen processing and presentation, as evaluated either in vitro or in vivo.

*langerin*−/− mice do not display altered susceptibility to pathogenic microorganisms. Langerin is an endocytic receptor of the C-type lectin family, with specificity for mannos, GlcNac, and fucose residues (61, 72). Langerin-expressing cells are found in the epidermis and in epithelia of pluristratified mucosa, such as in the respiratory tract. Previous observations (70; unpublished data) have validated the presence of langerin in airway epithelium. Based on these considerations, we inco-
FIG. 5. Protein antigen presentation and cross-presentation is not altered in langerin−/− mice. (A) In vitro OVA presentation. Langerhans cells were allowed to crawl out from the epidermis of C57BL/6 control and langerin−/− mice. After 72 h, cells were counted and placed in 96-well plates with different concentrations of OVA protein and OVA-specific CD8+ T lymphocytes from OT-I (MHC class I restricted) or CD4+ T lymphocytes from OT-II (MHC class II restricted) mice for 48 h. Triplicate supernatants were collected and added to the IL-2-dependent CTLL2 cell line, and proliferation was assessed with [3H]thymidine. (B) In vivo OVA presentation. C57BL/6 control and langerin−/− mice were reconstituted with CFSE-labeled spleen cells from OT-I or OT-II mice. After 48 h, the recipient mice were injected with OVA protein or PBS in the footpads. Four days later, draining LN cells were harvested and analyzed for CFSE staining by flow cytometry to assess the specific proliferation of the adoptively transferred T lymphocytes. (C) OVA cross-presentation. C57BL/6 and langerin−/− mice were injected intravenously with OVA protein and sacrificed 18 h later for spleen recovery. DC were enriched from spleens, and CD8+ or CD4+ subpopulations were sorted. Graded numbers of each DC subpopulation were then cultured in duplicate for 3 days with 10,000 OVA-specific T lymphocytes from OT-I or OT-II mice. Each well was pulsed with [3H]thymidine for 8 h, and the incorporated radioactivity was counted.
lated langerin \(^{-/-}\) and C57BL/6 mice with different pathogenic microorganisms to assess differences in susceptibility.

Klebsiellae are enterobacteria that normally produce prominent polysaccharide capsules with mannose residues (5). Cell wall-associated mannoproteins have been described for Candida albicans and are implicated in immunogenicity (15, 32). We inoculated langerin \(^{-/-}\) and wild-type control mice with K. pneumoniae (10\(^4\) CFU) and C. albicans (10\(^6\) CFU) by intratracheal delivery. Mice were monitored every day for survival, and our results indicate that both mouse lines displayed a similar death rate in response to either K. pneumoniae (Fig. 6A) or C. albicans (data not shown).

Given the diversity of the polymannosylated constituents of the envelope of mycobacteria (22) and the description that DC play a role in mycobacterial infection (19), we analyzed whether a lack of langerin influences susceptibility to M. tuberculosis. Thus, 200 CFU of M. tuberculosis H37Rv was delivered to mice by aerosol. The M. tuberculosis load in the spleen and lungs was then monitored at different time points. As shown in Fig. 6B, M. tuberculosis developed within the tissues of mice of both lines with similar kinetics and multiplication rates. As expected from the natural route of delivery, live bacilli were recovered from lungs at all time points and were detected in the spleen 21 days after aerosol delivery, which corresponds to a hematogenous spread of M. tuberculosis. Thus, langerin does not play a dominant role in M. tuberculosis uptake in the airway tract or in dissemination of the bacillus in distant mouse tissues.

Leishmania are protozoan parasites, transmitted to mammalian hosts by small blood-feeding female insects known as sand flies, such as Phlebotomus papatasi for L. major. Due to their physical location, LC may play a particular role in the early processes following dermal delivery of metacyclic promastigotes, which represent the infective developmental stage of the parasite (6). We delivered 1,000 L. major metacyclic promastigotes intradermally in the right ears (ventral side) of langerin \(^{-/-}\) and wild-type control mice. Every week, the thickness of both ears was measured, with the left ears being the controls. As shown in Fig. 6C, we observed ear swelling in both mouse lines, with similar kinetics and size. As expected from previous reports, the ear thickness of C57BL/6 mice went back to a normal shape and size, and the same result was observed for langerin \(^{-/-}\) mice. When 10\(^3\) L. major promastigotes were delivered intradermally to the cured mice in the opposite ears, no swelling was observed in either of the two mouse lines (data not shown).

Taken together, we did not observe any differences between langerin \(^{-/-}\) and C57BL/6 mice in response to the microorganisms we studied. langerin \(^{-/-}\) and control mice died at a similar rate when infected with K. pneumoniae and C. albicans. In addition, M. tuberculosis proliferated to the same extent in the organs of both mouse lines, and intradermal inoculation of a low dose of L. major metacyclic promastigotes led to an identical phenotype, namely the development of a transient cutaneous lesion that heals, as well as the absence of a lesion at a second site of delivery, i.e., the development of so-called resistance to a second inoculum.

Chemically induced skin carcinogenesis is not affected in langerin \(^{-/-}\) mice. To test whether langerin is involved in the development of an experimental skin tumor, C57BL/6 and langerin \(^{-/-}\) mice were exposed to a two-stage chemical carcinogenesis protocol. First, mice received a single skin application of the carcinogen DMBA, and then biweekly, TPA was applied as a tumor promoter. Mice were visually examined twice a week, and the number of mice exhibiting tumors was counted. This carcinogenesis protocol leads directly to the development of predominantly squamous cell carcinomas. As shown in Fig. 7, the kinetics of tumor development in langerin \(^{-/-}\) mice was similar to that of C57BL/6 control mice. Moreover, the number of tumors per mouse was not different between the two mouse lines (2 to 3 per mouse) (data not shown). It is known that the protocol used in our study entails tumor initiation in epidermal keratinocytes with the oncogenic activation of the c-Ha-ras gene (50). Taken together, these results indicate that langerin-dependent functions of LC do not affect ras-mediated keratinocyte oncogenic processes in vivo.

DISCUSSION

Langerin is a transmembrane type II Ca\(^{2+}\)-dependent lectin with a single carbohydrate recognition domain displaying specificity for mannose, N-acetyl-glucosamine, and fucose (61, 72). Langerin is an endocytic receptor that translocates ligands from the cell surface into BG. To get further insight into the role of langerin in LC functions, we performed targeted disruption of the langerin gene. The resulting langerin \(^{-/-}\) mouse was backcrossed on a C57BL/6 background. These mice developed and bred normally. An epidermal MHC class II\(^{\text{high}}\) dendritic network was observed in langerin \(^{-/-}\) mice, indistinguishable from that of C57BL/6 control mice. Our results indicate that langerin is not necessary for the establishment of the LC system in the epidermis and support a recent description that, even though some MHC class II-positive cells are present in the epidermis of newborn mice, langerin expression is detectable only 2 to 3 days after birth (67). The phenotype of freshly isolated epidermal LC from langerin \(^{-/-}\) mice was further identical to that of C57BL/6 controls, including expression of the type I lectin DEC205/CD205 and E-cadherin. Other leukocyte cell populations such as DETC or dermal DC were also normally detected in langerin \(^{-/-}\) skin. Thus, absence of langerin does not perturb the development of leukocytes known to display immune functions in the skin. In this context, the development of LC is abolished in tgfβ1\(^{-/-}\) mice (14) or in id2\(^{-/-}\) mice (26), with Id2 being a transcription factor induced by transforming growth factor β1. Notably, even though the skin of these mice is totally devoid of LC, no defect has yet been reported in immune responses against pathogenic microorganisms.

A major feature of the langerin \(^{-/-}\) mouse was the absence of BG in the LC. Transfection of fibroblasts with langerin cDNA is known to induce the formation of BG (72). Our present data demonstrate that langerin is essential for this function, i.e., that no molecular redundancy exists for inducing the development of BG. BG have been described as a component of the endocytic pathway (28, 66) and implicated in the processing of antigenic molecules (63). Langerin is internalized into BG
when cross-linked by an antibody (72). It is likely that BG represent membrane domains of tubular recycling endosomes where langerin accumulates (38). Langerin belongs to a family of lectin receptors that capture carbohydrate-bearing molecules (23). Interestingly, in human LC, the subcellular routing of langerin largely overlaps with that of CD1a (27, 57), a molecule related to MHC class I products and implicated in the presentation of antigenic lipids and glycolipids (45). A recent study has shown that human langerin and CD1a interact for the uptake and presentation of glycolipids derived from Mycobacterium leprae, resulting in the induction of a cellular immune response (30, 41). However, a parallel between human and mouse LC is not obvious at this level. Thus, group I CD1 molecules (CD1a, CD1b, and CD1c) are notoriously absent in mice (18), which only express the group II CD1 molecule CD1d, with an intracellular routing quite different from that of human CD1a. Indeed, mouse CD1d traffics deeper into late lysosomes in a pattern that resembles human CD1b (18, 64), thus not suggestive of a routing into BG.

Despite the lack of BG, we were not able to show any significant functional differences between langerin−/− mice and their wild-type littermates. The migration of LC to skin draining LN was not impaired in langerin−/− mice, implying that the absence of BG formation does not perturb this function. Furthermore, OVA protein was processed and the derived peptides presented to CD4-positive and CD8-positive T lymphocytes with normal efficiency in langerin−/− mice. Exogenous antigens are normally processed and presented by MHC class

FIG. 6. langerin−/− and C57BL/6 mice are equally susceptible to pathogenic microorganisms. (A) K. pneumoniae. K. pneumoniae (10⁹ CFU) or PBS was delivered directly into the tracheas of anesthetized C57BL/6 control and langerin−/− mice. The survival curves are presented. (B) M. tuberculosis. C57BL/6 control and langerin−/− mice were exposed to M. tuberculosis-loaded aerosols. Five mice per group were sacrificed on days 1, 8, 21, and 98. Spleens and lungs were homogenized, and M. tuberculosis CFU were counted after culture on solid medium. Mean CFU ± standard deviations are represented. (C) L. major. L. major metacyclic promastigotes (10⁷) were delivered intradermally into the right ear of C57BL/6 control and langerin−/− mice (ventral side). The thickness of both ears was recorded weekly and plotted ± standard deviation. Pictures show that epidermally isolated LC from both langerin−/− and C57BL/6 control mice were able to capture parasites. Cells (2 × 10⁶) were cultured at 34°C in 5% CO₂ overnight with 2 × 10⁷ L. major promastigotes. After adhesion to poly-L-lysine-coated coverslips and fixation, cells were stained with an anti-I-Aβ biotinylated MAb and L. major was stained with a hamster polyclonal immune serum. Primary antibodies were detected with streptavidin-Alexa Fluor 488 (green) and anti-hamster antibody–Texas Red (red). Original magnification, ×1,000.
II molecules to CD4+ T lymphocytes, whereas antigens presented to MHC class I molecules are generally of endogenous origin. However, exogenous antigens can enter the MHC class I presentation pathway in professional antigen-presenting cells and, thereby, prime CD8+ T lymphocytes, a process termed cross-presentation. This has been originally described for cell-associated antigens (11, 16) as well as for soluble antigens (46). It has been shown that splenic CD8α+ DC are responsible for the cross-presentation of exogenous antigens to CD8+ T lymphocytes (49). Interestingly, although langerin is expressed exclusively by the CD8α+ DC subset in the mouse spleen (39), our results show that cross-presentation of soluble OVA by splenic CD8α+ DC was not affected in langerin−/− mice.

Immature DC, including LC, are leukocytes that recognize surface molecules displayed by microorganisms or the soluble components they release. They capture and internalize bound microorganisms or microbial molecules through several mechanisms, including endocytosis following lectin-mediated recognition of sugar patterns displayed by the microorganisms (23). In this context, human langerin has been shown to bind mannosylated gp120 from human immunodeficiency virus (68) and was recently reported to capture glycolipids derived from M. leprae (30). We thus tested the susceptibility of langerin−/− mice to the live mannose-targeted pathogenic microorganisms K. pneumoniae, C. albicans, and M. tuberculosis, administered to reach the airway epithelium expressing langerin (70; unpublished data). K. pneumoniae is the most common gram-negative pathogen causing community-acquired bacterial pneumonia (17), and is involved in the outbreak of nosocomial infections, mainly in immunocompromised individuals (56). C. albicans is a dimorphic, opportunistic fungal pathogen that is responsible for the majority of fungal infections in immunocompromised hosts. DC are able to interact with C. albicans and to discriminate between virulent and nonvirulent forms of the fungus (21). Although DC are not the primary targets for infection by mycobacteria, they play a role in modulating the cellular immune response induced by the bacilli (47). Thus, the control of M. tuberculosis infection requires a coordinated interaction of macrophages, DC, and T lymphocytes acting as essential effectors (24). The lectin DC-SIGN is a receptor for M. tuberculosis (25, 65). It has been suggested that after binding of mannose-containing components of the M. tuberculosis envelope, DC-SIGN+ DC from the respiratory tract may transport bacilli to the draining LN and thus contribute to chronic infection (65). Since langerin and DC-SIGN share similar carbohydrate specificity, we reasoned that pulmonary LC may also play an important role in early steps of infection in mice by means of M. tuberculosis capture by langerin. However, langerin−/− mice reacted similarly to C57BL/6 controls to each of the pathogenic microorganisms we tested. These findings could suggest that other LC molecules may compensate for lack of langerin. Alternatively, DC in the lamina propria of the airway epithelium may play a dominant role in shaping the response to these pathogens.

Mice were also inoculated intradermally with L. major, a parasite displaying skin tropism. In their natural habitat, the parasites are inoculated by a sand fly during blood feeding in the ears of rodents. Even though Leishmania have been essentially described to be routed into phagolysosomes of macrophages, it is clear from recent studies that DC also play an important role in shaping the immune response (6, 51, 52, 59, 69). LC in the epidermis are potential candidates for parasite phagocytosis, and we speculated that langerin could be involved in this process and/or in the subsequent development of the parasitic processes, whether those are clinically silent or assessed by transient cutaneous lesions. Indeed, it has been described that Leishmania can be taken up by LC in vitro and in vivo (13) and that they play a role in parasite transport and initiation of the immune response against L. major (42, 43), at least after subcutaneous delivery of very high numbers of stationary-phase promastigotes. We observed that both the langerin−/− and C57BL/6 mouse lines developed reversible cutaneous lesions, indicating that langerin does not play a dominant role in this process. We also observed that LC from both mouse lines were able to phagocytose L. major in vitro. It should again be noted that LC express numerous cell surface receptors, and lack of langerin could be overcome by alternative receptors. In addition, recent contradictory reports have been published regarding the cells that shuttle the parasite into draining LN after skin infection (6, 52). Finally, it is well known that C57BL/6 mice are resistant to L. major (55). In this context, our results do not exclude that infection of langerin−/− mice on a susceptible background (e.g., BALB/c) may reveal a role for langerin not seen on the present C57BL/6 background.

Overall, our data show that the absence of mouse langerin does not lead to any phenotypical, morphological, or functional perturbations in vivo and in the ex vivo systems studied here. These observations are interestingly corroborated by a study reported by Mommaas and colleagues who described a human case of epidermal LC lacking BG (44). The BG-negative individual did not show any signs of pathology. Furthermore, LC were present in normal numbers and morphology and displayed a normal antigen-presenting capacity. Thus, the absence of langerin, and consequently, that of BG, does not yet reveal the role of this molecule and the resulting organelles in the shaping of the immune response by LC. In this context, a study with herpes simplex virus has recently reconsidered the potential role of LC in in vivo T-cell priming (1), suggesting that LC may play a carrier or shuttle function, but are not the key antigen-presenting cells for induction of an immune response. However, other reports have here described a role for LC, such as in infection with Dengue virus (76) or encephalitis virus (37). Even though it is known which carbohydrates bind to langerin (61), the range of its biological ligand(s) is still not known. Other lectins are expressed by LC, including DEC205, and redundancy for the capture of pathogens bearing carbohydrate residues cannot be excluded. Further studies involving double-deficient mice, such as langerin-DEC205 knockouts, could help to unravel unique functions of LC. The langerin−/− mouse line should represent a valuable tool for further investigation of the function of langerin and BG and, by extension, the role of LC in particular models that have not been addressed in the present study.

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