

Human Langerhans cells capture measles virus through Langerin and present viral antigens to CD4⁺ T cells but are incapable of cross-presentation

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Langerhans cells (LCs) are a subset of DCs that reside in the upper respiratory tract and are ideally suited to sense respiratory virus infections. Measles virus (MV) is a highly infectious lymphotropic and myelotropic virus that enters the host via the respiratory tract. Here, we show that human primary LCs are capable of capturing MV through the C-type lectin Langerin. Both immature and mature LCs presented MV-derived antigens in the context of HLA class II to MV-specific CD4⁺ T cells. Immature LCs were not susceptible to productive infection by MV and did not present endogenous viral antigens in the context of HLA class I. In contrast, mature LCs could be infected by MV and presented de novo synthesized viral antigens to MV-specific CD8⁺ T cells. Notably, neither immature nor mature LCs were able to cross-present exogenous UV-inactivated MV or MV-infected apoptotic cells. The lack of direct infection of immature LCs, and the inability of both immature and mature LCs to cross-present MV antigens, suggest that human LCs may not be directly involved in priming MV-specific CD8⁺ T cells. Immune activation of LCs seems a prerequisite for MV infection of LCs and subsequent CD8⁺ T-cell priming via the endogenous antigen presentation pathway.

Key words: Cross-presentation/priming · DCs · Langerhans cells · T cells · Virology



See accompanying Commentary by Romani



Supporting Information available online

Introduction

DCs induce adaptive immunity by presenting antigens in the context of HLA class I (HLA-I) and II (HLA-II) to CD8⁺ and CD4⁺

T cells, respectively [1]. Virus-specific CD8⁺ cytotoxic T cells (CTLs) are crucial to clear viral infections and are classically induced by virus-infected APCs that present endogenous viral antigens in the context of HLA-I. However, DCs can also capture exogenous antigens and present these in the context of HLA-I

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molecules, a process referred to as cross-presentation which is thought to be important in both viral and tumor immunity [2, 3].

It is becoming evident that different DC subsets have specialized functions in anti-viral immunity, e.g. by inducing preferentially CD4⁺ or CD8⁺ T-cell responses or by inducing innate immunity against pathogens [4, 5]. Langerhans cells (LCs) are a subset of DCs that are, in humans, characterized by expression of CD1a, the C-type lectin Langerin and the presence of Birbeck granules [6]. LCs reside in the epidermis and stratified epithelial tissues [7–9]. Recently, we have shown that LCs form a barrier against HIV-1; LCs capture HIV-1 through Langerin, resulting in internalization of HIV-1 into Birbeck granules and preventing subsequent HIV-1 transmission [10]. However, little is known about the role of human LCs and Langerin in antigen presentation.

Capture of exogenous antigens by LCs leads to induction of CD4⁺ T-cell responses [11–13]. However, the ability of LCs to cross-present exogenous antigens to CD8⁺ T cells has been debated [14–17]. Klechevsky et al. [13] have shown that in vitro-generated human LCs from either CD34⁺ cells or monocytes are capable of cross-presenting influenza-virus proteins to CD8⁺ T cells. However, these in vitro-generated LCs might be different from primary immature LCs.

Here, we have investigated the antigen capture and presentation capacity of human primary LCs during measles virus (MV) infection. MV is the causative agent of measles, which remains an important cause of morbidity and mortality in developing countries. MV is a lymphotropic and myelotropic virus and DCs have been implicated in its transmission [18, 19]. MV is one of the most infectious viruses that infect humans and is transmitted via the respiratory route. CD150 is the primary receptor for wild-type MV [20], whereas the laboratory-adapted MV strain Edmonston also uses CD46 as entry receptor [21]. Wild-type MV initially replicates in mononuclear cells in the alveolar lumen, the bronchus-associated lymphoid tissue and the regional lymph nodes of the respiratory tract, followed by viremia and systemic disease [19, 22]. Although measles is associated with immune suppression, MV paradoxically induces a strong cellular and humoral immune response that efficiently clears the virus and results in lifelong immunity [23]. Human DC-SIGN⁺ DCs transmit MV to lymphocytes [18, 24] and DCs have been shown to become infected by MV in vivo in macaques [19]. Also, LCs have been shown to be susceptible to MV infection, albeit after exposure to mechanical stress [25, 26]. These data strongly suggest that different DC subsets are involved in MV dissemination, but little is known about the role of human LCs as professional APCs during measles.

Here, we show that Langerin functions as an attachment receptor for MV that is involved in virus capture but not infection of LCs. MV binding to both immature and mature LCs led to processing and presentation of viral antigens to MV-specific CD4⁺ T cells. Mature LCs, in contrast to immature LCs, were infected by MV and presented antigens in the context of HLA-I molecules to MV-specific CD8⁺ T cells. Notably, neither immature nor mature LCs were able to cross-present viral antigens derived from UV-inactivated MV or MV-infected apoptotic cells to MV-specific CD8⁺ T cells. This study shows that Langerin on LCs is involved in MV capture. Notably, we

show that primary human LCs are incapable of cross-presenting antigen from exogenous MV particles or MV-infected apoptotic cells. These results suggest that without prior activation, immature LCs do not induce CD8⁺ T-cell responses to MV.

Results

Langerin is a receptor for MV but does not mediate viral entry

Langerin interacted with MV in a dose-dependent manner as measured by a soluble Langerin-binding ELISA (Fig. 1A). This interaction is specific for the carbohydrate recognition domain of Langerin since binding was inhibited by mannan, which blocks Langerin (Fig. 1A). Next, Langerin-transduced THP-1 cells (Supporting Information Fig. 1) were incubated with FITC-labeled wild-type MV strain IC323 and laboratory-adapted MV strain Edmonston, and binding was measured by flow cytometry. Binding of both MV strains to Langerin-transduced THP-1 cells was higher compared to mock-transduced cells and could be blocked by mannan (Fig. 1B and C). Thus, Langerin is a receptor for both wild-type and laboratory-adapted MV strains.

Next, we investigated whether Langerin is involved in the interaction of human LCs with MV. Primary human mature LCs efficiently captured both MV strains, which was blocked by anti-Langerin and anti-CD150 to a similar extent (Fig. 1D). Moreover, mannan reduced binding to a similar extent as anti-Langerin. Thus, primary LCs capture MV through both Langerin and CD150.

To investigate whether Langerin mediates fusion and viral entry, Chinese hamster ovary (CHO) cells were transduced with Langerin (Supporting Information Fig. 1). CHO, CHO-Langerin and CHO-CD150 cells were incubated with wild-type MV (WTF) or recombinant MV strain IC323-eGFP [27]. Both viruses infected CHO-CD150 cells, but were unable to infect Langerin- or mock-transfected CHO cells (Fig. 1E, Supporting Information Fig. 2), demonstrating that Langerin is not an entry receptor for MV.

Next, we investigated infection of immature and mature LCs with MV strains Edmonston and IC323. Mature LCs, in contrast to immature LCs, were infected by both strains (Fig. 1F–I). Infection of mature LCs with the laboratory-adapted MV strain Edmonston was inhibited by anti-CD150 and anti-CD46, whereas the infection with the wild-type MV strain IC323 was inhibited by anti-CD150 (Fig. 1H and I). Anti-Langerin did not affect infection of LCs with either MV strain (Fig. 1H and I). Thus, Langerin is a binding receptor for MV that is involved in MV capture but does not mediate viral entry.

LCs are abundantly present in epithelium of the upper respiratory tract

LCs have been extensively characterized in mucosal tissues involved in HIV-1 infection [8, 28], but little is known about

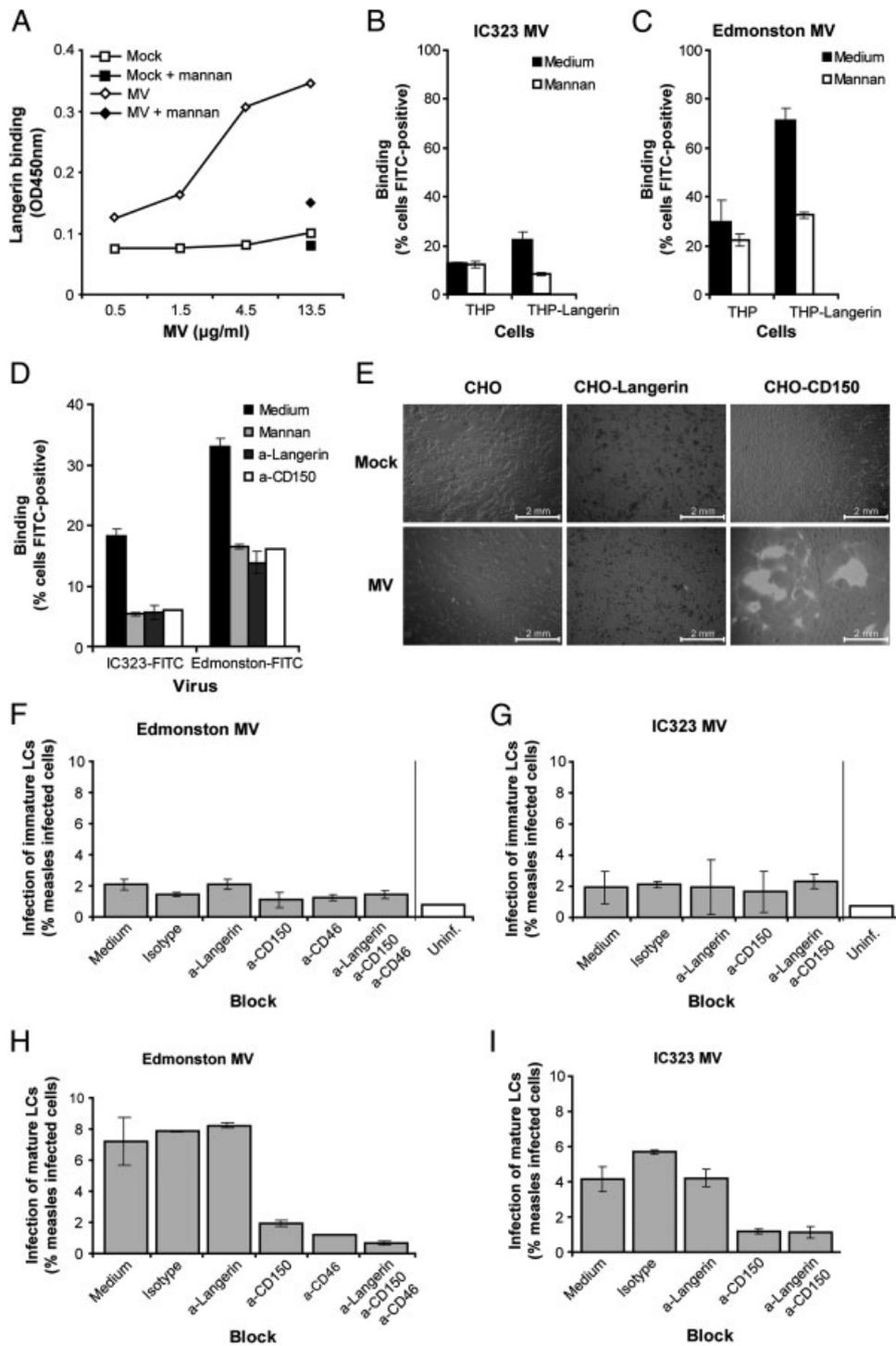


Figure 1. Langerin is a receptor for measles virus. (A) Different concentrations of wild type MV or a mock control were coated and Langerin binding was detected by anti-Langerin. Mannan was used to determine specificity of the interaction between Langerin and MV. Data are representative of two independent experiments. (B–D) Langerin-transduced THP-1 cells, mock-transduced THP-1 cells (B, C) and primary human mature LCs (D) were incubated with FITC-labeled IC323 (B, D) or Edmonston (C, D) and binding was determined by flow cytometry. Mannan (B–D), and anti-Langerin or anti-CD150 (D) were used to determine the specificity. Data show average \pm SD and are representative of two independent experiments. (E) CHO, CHO-Langerin and CHO-CD150 cells were infected with IC323-eGFP. Infection was assessed by syncytium formation and eGFP expression. Original magnification 10x. (F–I) Primary immature (F, G) or mature (H, I) LCs were infected with IC323-eGFP (G, I) or Edmonston-GFP (F, H). The percentages of CD1a⁺ GFP⁺ cells are depicted. Data show average \pm SD and are representative of two independent experiments.

the distribution of LCs in the respiratory tract where MV enters the host. We have analyzed the density and phenotype of LCs in the respiratory tract by staining different epithelial tissues for CD1a, Langerin and CD150. LCs were observed in tongue, buccal cavity, tonsil, pharynx and bronchi (Fig. 2A, data not shown). LCs were mainly located in the epidermis and epithelia. Furthermore, the density of LCs was high in the upper respiratory tract and decreased while descending toward the lungs, where LCs were only scarcely detected (data not shown).

Langerin was expressed on more than 95% of LCs in the skin and upper respiratory tract (Fig. 2A; data not shown). In lungs a donor variation was observed, since LCs from one donor expressed Langerin on the cell membrane and/or intracellular, whereas in two other donors, 80 and 20% of the CD1a⁺ cells expressed Langerin (data not shown). Only a small population of LCs (<10%; data not shown) in both upper and lower respiratory tracts was positive for CD150 (Supporting Information Fig. 3, arrows), indicating that the majority of LCs in respiratory tract are CD150-negative and that immature LCs are among the first cells to encounter MV in the upper respiratory tract.

Isolation of human immature LCs from the respiratory tract is not possible on a scale that allows functional experiments. Therefore, we have used epidermal LCs from skin because these cells are immature upon isolation and express Langerin and CD1a

but no CD150, similar to LCs observed in respiratory tract (Fig. 2). The isolation procedure did not activate the cells since expression of co-stimulatory molecules CD80, CD83 and CD86 remained very low or negative, whereas expression of HLA-I and HLA-II was intermediate (Fig. 2B). To obtain LCs with a more mature phenotype, epidermal sheets were cultured for two days and the emigrated LCs were isolated. Emigrated LCs were partially mature since these cells expressed CD150 and high levels co-stimulatory molecules as well as HLA-I and II molecules (Fig. 2B). Furthermore, Langerin expression was lower on emigrant LCs which further highlights the more mature phenotype compared to isolated immature LCs. Throughout this paper we will refer to emigrant LCs as mature LCs, and to isolated LCs as immature LCs.

Antibodies to Langerin are internalized and presented in the context of HLA-II

We next investigated the internalization pathway of human Langerin by using an antibody against Langerin to exclude the involvement of other receptors. Human Epstein–Barr virus-transformed B-lymphoblastic cell line (BLCL)-OUW expressing Langerin (Supporting Information Fig. 1) or mature primary

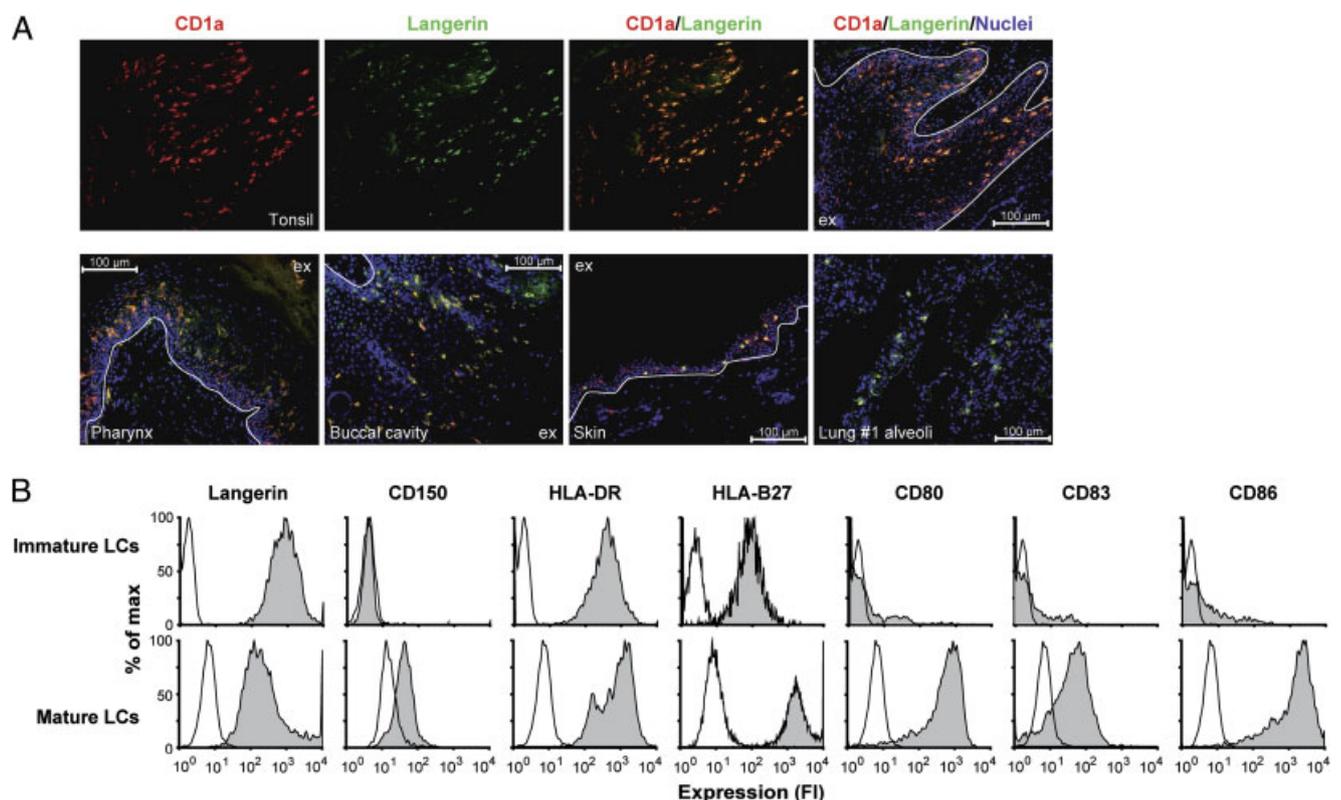


Figure 2. Human LCs are present in the upper respiratory tract and express Langerin. (A) Cryosections of human tissues were stained for CD1a (red), Langerin (green) and Hoechst (blue) and analyzed by fluorescence microscopy. The border between the epithelium/epidermis and subepithelium/dermis (white line), and the lumen or external environment (ex) are depicted. (B) Immature and mature LCs were analyzed by flow cytometry. Open histograms: isotype control; filled histograms: specific antibody. The data are representative for three donors.

human LCs were pulsed with a murine IgG1 monoclonal antibody specific for Langerin for 1 h at 37°C. The antibody against Langerin was internalized and co-localized with the lysosomal marker Lamp-1 (Fig. 3A). Immuno-electron-microscopic analysis showed that the anti-Langerin antibody was internalized into Birbeck granules (Fig. 3B) and the internalized antibody co-localized with a polyclonal antibody used to detect Langerin, suggesting that the internalized antibody was still attached to Langerin (Fig. 3B). A part of the internalized anti-Langerin antibody was observed in vesicles that were positive for Lamp-1 and/or HLA-DR (Fig. 3C and D). Moreover, the internalized antibody was also detected in multi-vesicular bodies (Fig. 3C and D, arrows) and incidentally in multi-lamellar bodies (Fig. 3D, complete left), which co-localized with HLA-DR (Fig. 3D). These data suggest that Langerin-captured antigens are internalized into Birbeck granules and are at least partly targeted to the lysosomes, where antigen processing can occur.

We investigated whether the antibodies against human Langerin are processed and presented in the context of HLA-II molecules to CD4⁺ T cells. We used the CD4⁺ T-cell clone Hd7 that recognizes a peptide derived from murine IgG1 presented in a HLA-DR1-restricted manner [29]. Both HLA-DR1-matched primary mature LCs and the Langerin-expressing B-cell line were pulsed with an anti-Langerin antibody or an isotype control antibody. Next, the cells were co-cultured with the CD4⁺ T-cell clone and IFN- γ production was measured to determine T-cell activation [29]. BLCL-OUW cells expressing Langerin incubated with the anti-Langerin antibody-induced T-cell proliferation that was neither observed with mock-transduced cells nor with the isotype control antibody (Fig. 3E). Primary LCs targeted with the anti-Langerin antibody-induced T-cell proliferation in a dose-dependent manner, which resulted from Langerin-mediated trafficking, since incubation with the isotype control antibody did not result in T-cell clone activation (Fig. 3E). Thus, antigens targeted to Langerin expressed by primary human LCs are processed and presented in the context of HLA-II to CD4⁺ T cells.

MV captured by LCs results in HLA-II-restricted antigen presentation

We investigated whether binding of MV by LCs results in antigen presentation to MV-specific CD4⁺ T cells using the CD4⁺ T-cell clone GRIM-F99 that recognizes a peptide derived from the MV envelope glycoprotein F in the context of HLA-DQw1 [30, 31]. Activation of the T-cell clone GRIM-F99 does not require full co-stimulation. Human LCs expressing HLA-DQw1 were pulsed with UV-inactivated Edmonston MV (MV-UV) and co-cultured with the MV-specific CD4⁺ T cells. Immature LCs directly isolated from skin were compared with mature LCs that had migrated out of the skin (Fig. 2B). Both immature and mature LCs stimulated the MV-F-specific CD4⁺ T-cell clone GRIM-F99 in a dose-dependent manner at comparable levels as monocyte-derived DCs (moDCs, Fig. 4A and B). The CD4⁺ T-cell clone alone did not produce IFN- γ . Activation of the T-cell clone

was comparable for both immature and mature LCs, which might be because activation of the T-cell clone is less dependent on co-stimulation than naïve T cells. We observed for different donors that mannan partially inhibited antigen presentation of MV-derived peptides to the CD4⁺ T-cell clone (Fig. 4C). Antibodies against Langerin inhibited antigen presentation to a similar extent as mannan, whereas the combination of antibodies against Langerin and mannan did not result in more inhibition than the inhibitors alone (Fig. 4D). These data confirm that Langerin is the only C-type lectin involved in MV capture on LCs. CD150 inhibition did not affect antigen presentation of MV, which is supported by the lack of CD150 expression on immature LCs (Fig. 2B). Other receptors or uptake mechanisms might also be involved since the inhibition with Langerin inhibitors was partial. These data demonstrate that human LCs capture MV resulting in efficient antigen processing and presentation in the context of HLA-II molecules, which is partially dependent on Langerin.

LCs do not cross-present MV antigens

Immature LCs, in contrast to mature LCs, were not efficiently infected by MV (Fig. 1F–I). We have used the MV-specific CD8⁺ T-cell clone WH-F40 that recognizes a peptide derived from the MV envelope glycoprotein F in the context of HLA-B27, to investigate the potential of LCs for HLA-I-restricted antigen presentation. HLA-B27⁺-typed immature LCs, mature LCs and moDCs were incubated with infectious MV or with the minimal 9-amino acid MV peptide. Next, the MV-F-specific CD8⁺ T-cell clone was added and T-cell activation was measured [32]. All peptide-loaded DCs and LCs were able to induce IFN- γ production by the MV-specific CD8⁺ T-cell clone (Fig. 5A and B), demonstrating that the expression of HLA-B27 and/or co-stimulatory molecules was sufficient in all three DC subsets. However, immature LCs did not activate the CD8⁺ T-cell clone upon incubation with infectious MV, whereas moDCs and mature LCs both activated the CD8⁺ T-cell clone (Fig. 5A and B). These data suggest that immature LCs do not present MV antigens to CD8⁺ T cells because these cells are not productively infected by MV.

To address the potential of LCs for cross-presentation, we tested all the three DC subsets for activation of the MV-specific CD8⁺ human T-cell clone after incubation with UV-inactivated MV. Inactivation was complete since the UV-inactivated viruses were not able to infect susceptible cells (data not shown). Under these conditions, moDCs reproducibly showed a dose-dependent potential for cross-presentation, which was enhanced when moDCs were matured by a maturation cytokine cocktail (Fig. 5C). In contrast to moDCs, neither immature nor mature LCs were capable of activating MV-specific CD8⁺ T cells after incubation with different concentrations of UV-inactivated MV. Repeated antigen pulse over several days or activation with a maturation cocktail also failed to induce cross-presentation by LCs (Fig. 5C; data not shown). These results demonstrate that antigen cross-presentation of cell-free MV by human primary LCs is inefficient and independent of activation state.

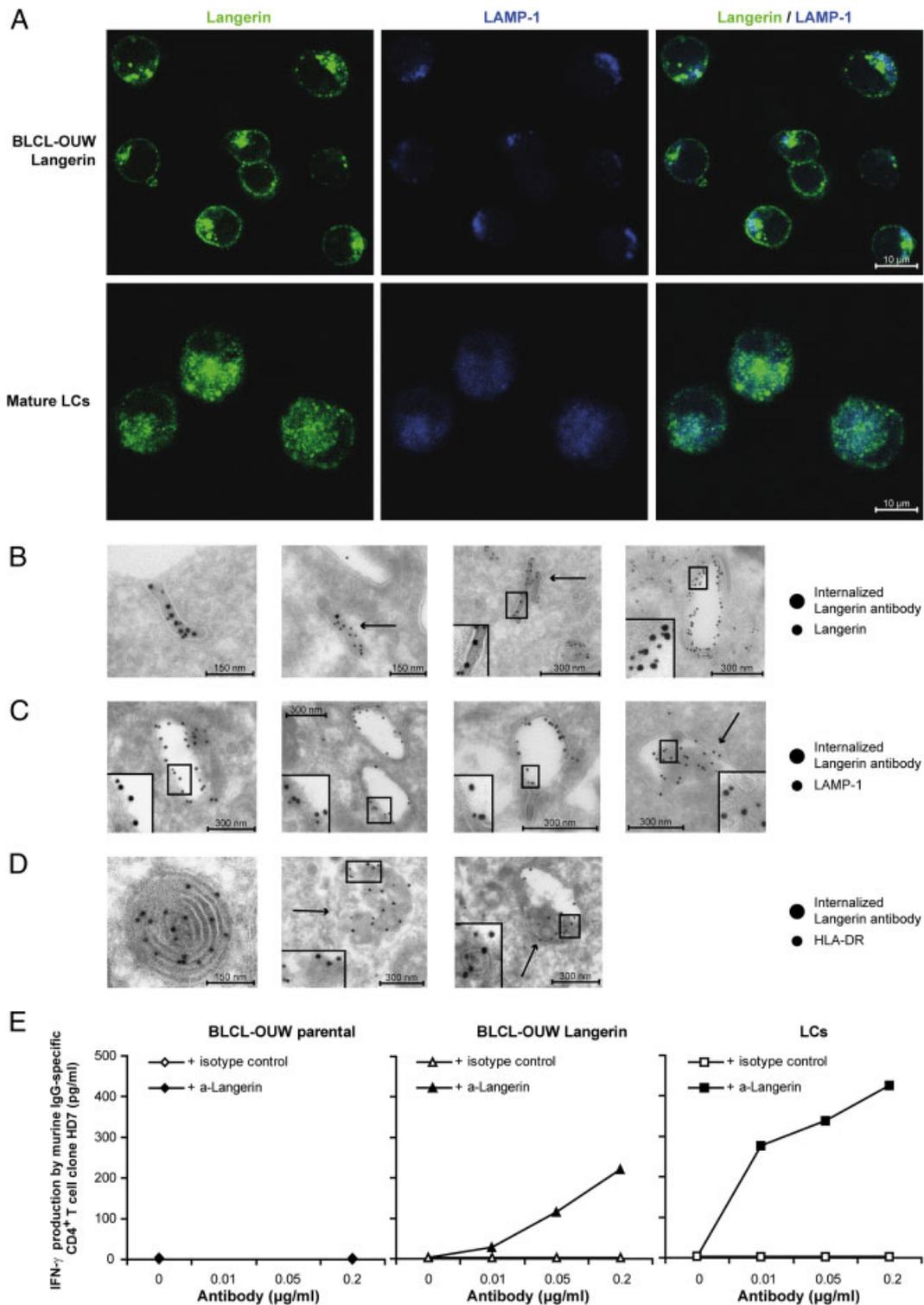


Figure 3. Langerin antibodies are internalized into Birbeck granules, Lamp-1⁺ and HLA-DR⁺ vesicles, and are presented in the context of HLA-II. (A) BLCL-OUW-Langerin cells and mature LCs were stained with anti-Langerin (green) and anti-Lamp-1 (blue) for confocal microscopy. Representative for two independent experiments, original magnification 63 \times . (B–D) Mature LCs were incubated with DCGM4. The internalized antibodies were detected using immuno-gold-labeling antibody (15 nm). Cells were stained with goat-anti-Langerin (B), Lamp-1 (C) or HLA-DR (D) and detected using immuno-gold-labeling (10 nm) for electron microscopy. Inlays: co-localization of 10 and 15 nm gold-labeling. Arrows: Birbeck granules (B), multi-vesicular bodies (C, D). (E) BLCL-OUW, BLCL-OUW-Langerin or mature LCs were incubated with DCGM4 or an isotype control and IFN- γ production of the co-cultured CD4⁺ HD7 T cells was measured. Data are representative of three independent experiments.

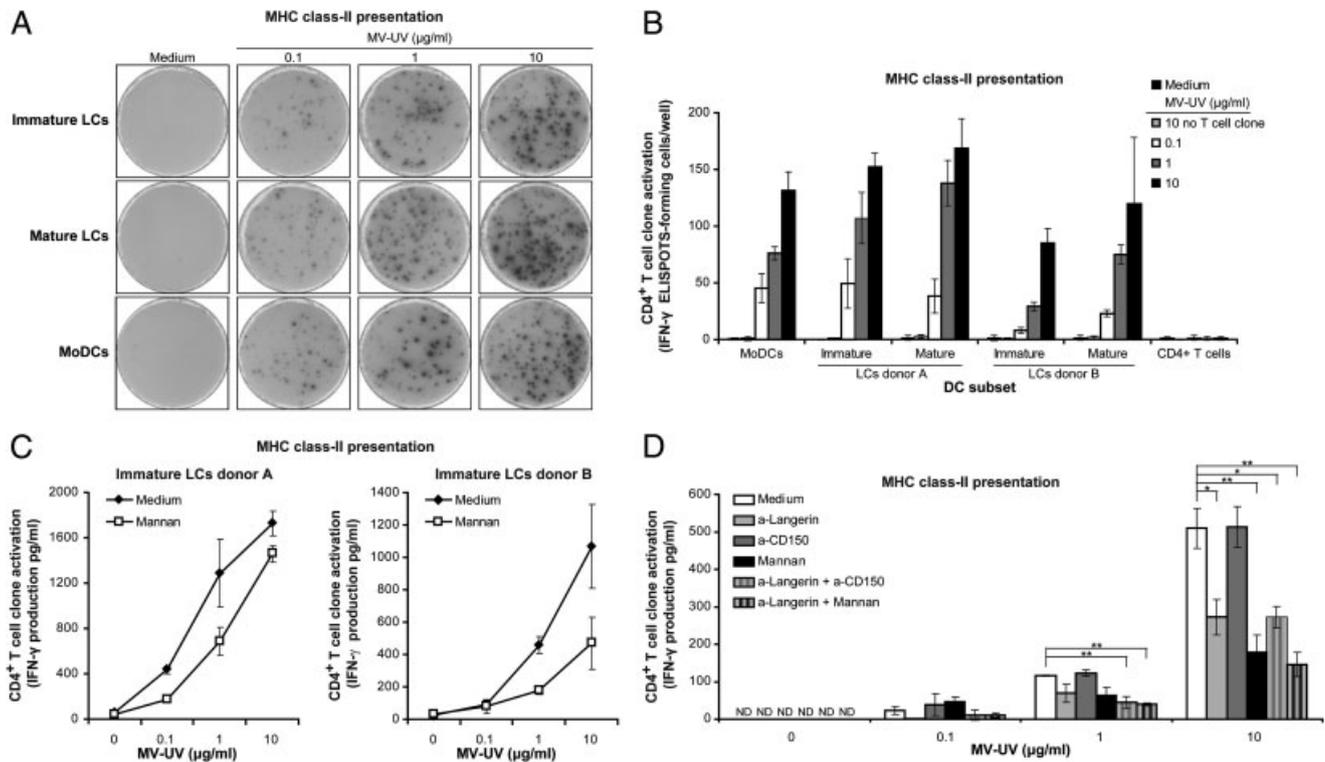


Figure 4. Human LCs process MV for antigen presentation to an HLA-II-restricted MV-specific CD4⁺ T-cell clone. Immature LCs, mature LCs or DCs were incubated with MV-UV. (A, B) Activation of the co-cultured CD4⁺ T-cell clone GRIM-F99 was measured by ELISPOT. (A) Representative for three experiments. (B) Data represent average \pm SD of duplicates. (C, D) Immature LCs were pre-incubated with (C) medium (●) or mannan (□), or (D) with anti-CD150, anti-Langerin, or mannan. Production of IFN- γ was determined by ELISA. Data represent average \pm SD of duplicates. * p <0.05, ** p <0.01, tested by one-way ANOVA. ND: not detectable.

Apoptotic virus-infected cells might be an important source for MV antigens in vivo. Therefore, we investigated cross-presentation of MV antigens derived from MV-infected cells by incubating the different DC subsets with UV-inactivated MV-infected BLCL-JP. The HLA-I type of the BLCL-JP was mismatched with the MV-specific T-cell clone to exclude antigen presentation by this MV-infected BLCL. Similar to that observed with cell-free virus particles, neither immature nor mature LCs were able to cross-present MV-derived peptides to the CD8⁺ T cells when incubated with MV-infected apoptotic cells (Fig. 5D). In contrast, moDCs efficiently cross-presented MV antigens derived from infected apoptotic cells to the specific CD8⁺ T-cell clone in a dose-dependent manner (Fig. 5D). Further activation of LCs by maturation stimuli did not induce cross-presentation. In summary, both immature and mature human primary LCs were incapable of cross-presentation of exogenous MV particles or MV-infected apoptotic cells to specific CD8⁺ T cells.

Discussion

Different DC subsets can have distinct functions and therefore might differ in their antigen presentation capacity. Here, we have investigated the function of human LCs in MV infection and their

ability to present MV-derived antigens to CD4⁺ and CD8⁺ T cells. We have identified Langerin as a binding receptor for MV on LCs that is involved in antigen presentation of MV-derived antigens to CD4⁺ T cells. Immature LCs were not infected by MV and did not present viral antigens in the context of HLA-I. In contrast, mature LCs were productively infected, resulting in activation of MV-specific CD8⁺ T cells by presentation of peptides derived from de novo synthesized MV proteins via the classical class I antigen presentation route. However, neither immature nor mature LCs were able to cross-present exogenous UV-inactivated MV particles or apoptotic MV-infected cells to CD8⁺ T cells. These data suggest that primary human LCs are not efficient in activating CD8⁺ T cells required for anti-viral immunity. Induction of naïve HLA-I-restricted T-cell responses is therefore more likely to be mediated by other DC subsets.

MV is transmitted via the respiratory route, and thus the initial targets must be present in or on respiratory mucosal surfaces. LCs that express the C-type lectin Langerin are abundantly present in the epithelium of the upper respiratory tract. Our data show that Langerin is an attachment receptor for MV on LCs. Viruses captured by Langerin, but not CD150, on immature LCs are processed and presented to CD4⁺ T cells. This is not specific to MV, because antibodies targeted to Langerin are also presented to CD4⁺ T cells. Since Langerin is also an attachment receptor for HIV-1 [10], Herpes Simplex Virus-2 [33] and fungi

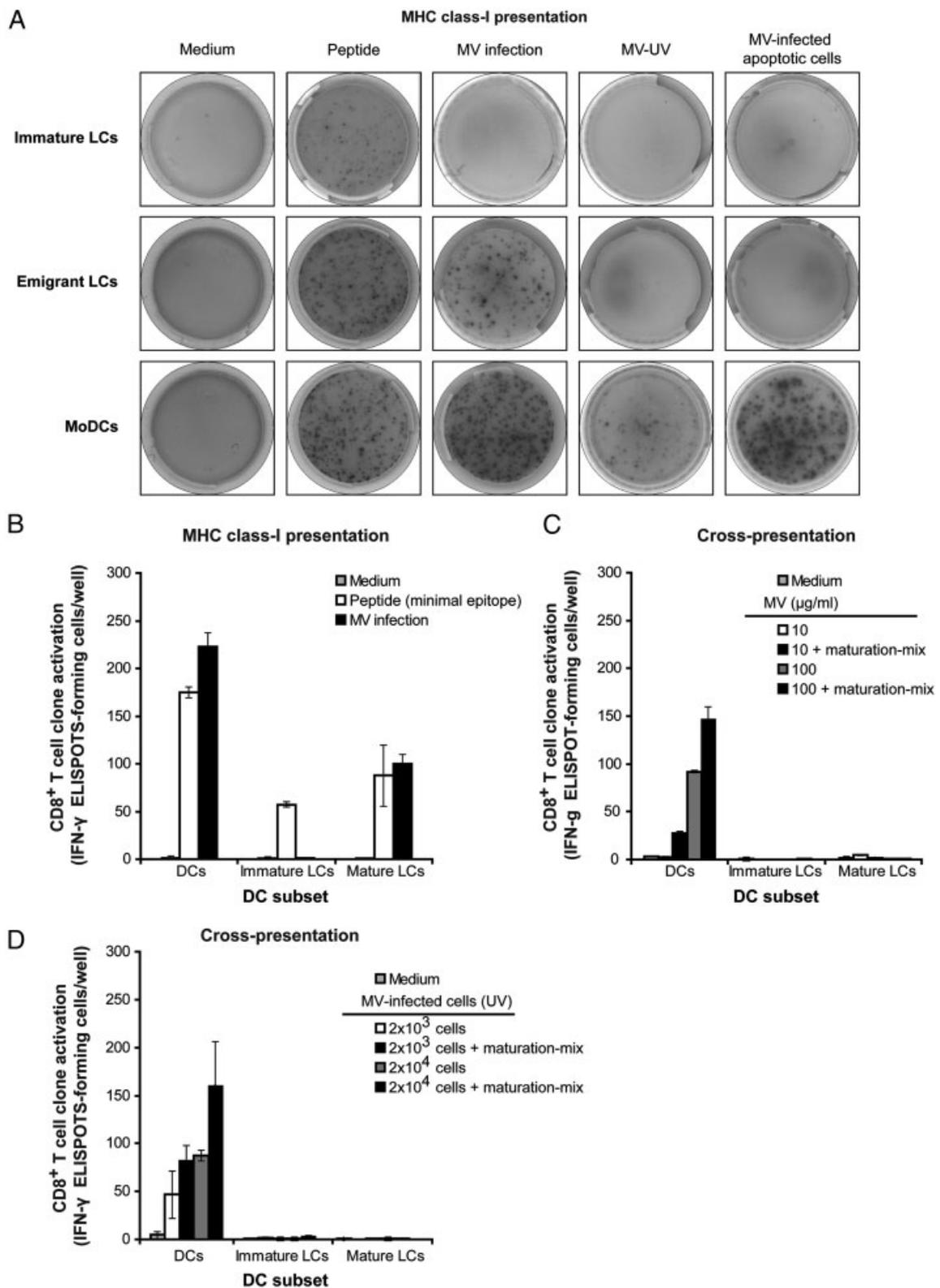


Figure 5. Human LCs are incapable of cross-presenting MV antigens to an HLA-I-restricted MV-specific CD8⁺ T-cell clone. (A–D) Immature LCs, mature LCs or DCs were incubated with MV Edmonston or a minimal-peptide (B), MV-UV (C) or MV-infected HLA-mismatched BLCL-JP (D) and activation of the co-cultured CD8⁺ T-cell clone WH-F40 was measured by ELISPOT. In (C, D) APCs were incubated with maturation stimuli (TNF- α , poly I:C and LPS) together with addition of the antigen. (A) Representative for two independent experiments. (B–D) Data are representative for two independent experiments and depict average \pm SD of duplicates.

[34], our data support a role for Langerin as a pathogen receptor. The MV envelope glycoproteins F and H contain high mannose structures [35] that are likely recognized by Langerin, similarly as shown for C-type lectin DC-SIGN [24]. This is further supported by the high similarity between the folding of the carbohydrate-binding domain of Langerin and DC-SIGN [36].

The fate of pathogens interacting with Langerin on LCs is not clear. Langerin recycles between the plasma membrane and early endosomes and is associated with Birbeck granules [6]. Langerin internalizes HIV-1 [10] and Langerin-antibodies into Birbeck granules [9]. In contrast to previous observations [9], we observed that internalized antibodies are routed into Lamp-1⁺ and HLA-II⁺ vesicles. The importance of Langerin in routing antigens to the HLA-II presentation pathway is supported by our data showing that both anti-Langerin and MV captured by Langerin are processed and presented to CD4⁺ T cells. Anti-Langerin as well as mannan partly inhibited presentation of MV antigens by HLA-II, suggesting that other receptors or other uptake mechanisms also contribute to antigen uptake. Our data suggest that pathogens captured by Langerin are processed for priming of CD4⁺ T cells.

Classically, exogenous antigens are presented in the context of HLA-II, whereas endogenous-derived peptides are presented in the context of HLA-I molecules. However, several DC subsets have the capacity to cross-present exogenous antigens in the context of HLA-I molecules [2], a process that is relevant in infection by viruses that circumvent the classical HLA-I processing pathways. Murine myeloid DCs cross-present viral antigens and induce anti-viral CD8⁺ T cells [37]. Murine LCs were reported to cross-present antigens [14] although a role for Langerin⁺ dermal DCs was not excluded. More recently, *in vivo* studies indicate that murine Langerin⁺ dermal DCs, but not LCs, are responsible for the induction of CD8⁺ T-cell responses [38, 39]. Our data show that human primary LCs cannot cross-present UV-inactivated MV and MV-infected apoptotic cells *in vitro*. Immature moDCs efficiently cross-presented UV-inactivated MV and infected apoptotic cells. In contrast, immature LCs were not able to cross-present exogenous-derived antigens to MV-specific CD8⁺ T cells. The inability to cross-present MV antigens was independent of activation state of LCs, since neither immature nor mature LCs were able to cross-present MV antigens despite stimulation with a maturation cocktail. In a previous study, *in vitro*-generated human LCs were shown to cross-present an influenza-virus matrix protein to CD8⁺ T cells [13]. The difference between both studies might be due to the origin of LCs. We have isolated primary LCs from epidermal sheets whereas Klechevsky et al. used *in vitro*-generated monocyte- or CD34⁺-derived LCs. It cannot be excluded that these *in vitro*-generated LCs differ from primary LCs in their antigen presentation pathways. Furthermore, cross-presentation by LCs might also depend on the uptake route: here, we used complete viruses and virus-infected cells that were internalized through receptors such as Langerin and CD150, whereas soluble proteins might be taken up via other routes. Further studies are required to investigate whether the inability of primary LCs to cross-present MV is a general phenotype, or specific for MV.

Our data show that immature LCs were not susceptible to MV infection whereas mature LCs were. Previously, immature LCs were found susceptible for MV [26], but they might have been more activated as others showed that mechanical stress facilitates LC activation and MV infection [25]. Therefore, differences in activation status of LCs determine susceptibility to MV.

MV infection has been shown to affect DC function; MV-infected DCs are unable to stimulate a mixed lymphocyte reaction and can induce lymphocyte unresponsiveness through expression of MV glycoproteins [40, 41]. Therefore, it is possible that MV interferes with the cross-presentation antigen-processing pathway of primary LCs. However, no MV replication could occur because the virus was UV-inactivated. Moreover, moDCs were able to cross-present to CD8⁺ T cells after uptake of both MV and MV-infected apoptotic cells. Furthermore, MV-infected mature LCs are capable of presenting endogenous MV antigens in the context of HLA-I molecules. Taken together these results strongly suggest that capture of MV by primary LCs results in presentation of antigens in the context of HLA-II molecules, but does not lead to cross-presentation of MV to CD8⁺ T cells.

Different studies have suggested a balance between efficient targeting of antigens for presentation in HLA-I or HLA-II [4, 15, 42]. Langerin internalizes antigens into Birbeck granules that might prevent antigens from entering the cross-presentation route or prevent triggering of pattern recognition receptors that could be required for cross-presentation [43]. However, we did not observe cross-presentation after activating LCs using different TLR ligands or other maturation stimuli.

In conclusion, our data suggest that LCs have a specialized function in MV infection; LCs are refractory to MV infection and do not induce MV-specific CD8⁺ T cells. These data suggest that other DC subsets are responsible for inducing CD8⁺ T-cell responses during MV infection. The finding that LCs are incapable of cross-presentation of MV *in vitro* might have implications for vaccine development where LCs are targeted to induce efficient CD8⁺ T-cell responses.

Materials and methods

Antibodies

The following antibodies were used: anti-CD150 (A12, AbD Serotec; 5C6 [44]), anti-Langerin (10E2, IgG1 [10]; DCGM4, Beckman Coulter; polyclonal goat, R&D Systems), anti-MV-F (A5047 [45]), anti HLA-DR (Q5/13) [46], anti HLA-DR1 (MEM-267, Genetex), rabbit anti-LAMP-1 polyclonal, anti-HLA-B27 FITC (HLA-ABC-m3, Abcam), anti-HLA-DQw1 (Genox 3.53, Novus Biologicals), rabbit anti-HLA-DR polyclonal (Santa Cruz biotechnology Inc), anti-CD1a (NA1/34, Dako Cytomation Denmark); anti-CD1a FITC or APC (HI149), anti-HLA-DR (Immu357), CD80 (B7-1) and CD86 (HA5.2B7) conjugated with PE (Pharmingen), anti-CD83 PE (HB15a, Immunotech), goat anti-mouse IgG PO, goat anti-mouse IgG Fcγ fragment (Jackson

Immunoresearch), goat anti-mouse IgG conjugated with FITC (Zymed Laboratories), Alexa488-, Alexa546-, Alexa594- and Alexa633-labeled isotype-specific anti-mouse antibodies (Molecular probes).

Cells

All research involving human tissues were approved by the VU University Medical Center Medical and Academic Medical Center review board. CHO-CD150 [24], Vero-CD150 cells [47], THP-1 and the Epstein–Barr virus-transformed B-lymphoblastic cell lines (BLCL-OUW, BLCL-GR, BLCL-WH, BLCL-JP) [30, 48] were cultured as described [10, 18, 49].

The CD4⁺ T-cell clone Hd7 [29], CD4⁺ T-cell clone GRIM-F99 [30] and the CD8⁺ T-cell clone WH-F40 [31] were cultured as described before.

Immature moDCs were cultured as previously described [50]. Primary LCs were isolated from normal healthy skin obtained from plastic surgery as described before [10].

Viruses

The wild-type MV strain WTF [51], the laboratory-adapted MV strain Edmonston B, the recombinant pathogenic MV strain IC323-eGFP [52] and the recombinant attenuated strain Edmonston-eGFP [53] were propagated on Vero-CD150 cells and purified by discontinuous sucrose gradient ultracentrifugation. Purified MV strains were UV-inactivated (30 min, 15 W, 312 nm, 1.5 J/cm²).

Immunofluorescence microscopy

Cryosections (7 μm) were stained with primary antibody against CD1a (10 μg/mL) in combination with either CD150 (10 μg/mL), Langerin (10E2, 10 μg/mL) or a buffer control for 18 h at 4°C as described before [18].

Langerin-binding assay

Different concentrations UV–MV WTF virions were coated onto Maxisorp plates (NUNC, Denmark), blocked with 5% BSA in 20 mM Tris; 150 mM NaCl; 1 mM CaCl₂; MgCl₂; pH 8 (TSA) and incubated with Jurkat-Langerin lysate (1 × 10⁸ cells/mL). Langerin binding was detected with DCGM4 (10 μg/mL) followed by goat anti-mouse-PO. Specificity was determined by pre-incubation with a saturating concentration of mannan (1 mg/mL).

FITC-labeled MV binding assay

Purified MV IC323 and Edmonston were dialyzed against 0.1% formalin and labeled with FITC (0.1 mg/mL in 0.5 mol/L

bicarbonate buffer, pH 9.5). Unbound FITC was removed by dialyzation against PBS. 2 × 10⁴ THP-1 or THP-Langerin were pre-incubated with medium, mannan (1 mg/mL) or blocking antibodies 10E2 or 5C6, before incubation with MV-FITC.

MV infection

CHO, CHO-DC-SIGN and CHO-CD150 cells (1 × 10⁵ cells) were infected with IC323-eGFP or WTF (multiplicity of infection (MOI): 1). Syncytia and eGFP expression were analyzed 2d post-infection using a DMIL microscope with a DFC320 camera (Leica Microsystems). For flowcytometry, the fusion inhibitory peptide Z-D-Phe-l-Phe-l-Gly (FIP [54]) (0.2 mM) (Bachem) was added. After 2d, cells were stained with anti-MV-F. Cells were fixed in 2% paraformaldehyde (PFA) in PBS. Immature LC suspensions (10–20% CD1a⁺) and mature LCs were infected with IC323-eGFP or Edmonston-GFP (MOI: 1) for 3d. LCs were stained for CD1a.

Electron microscopy

Mature LCs were incubated with DCGM4 (10 μg/mL) and fixed as described before [10]. Cryosections were immuno-labeled with anti-mouse antibodies (15 nm protein A gold label) to detect bound and internalized DCGM4 and either one of the following antibodies: goat anti-Langerin, anti-Lamp-1, rabbit anti-HLA-DR (10 nm protein A gold label) and analyzed as described before [10].

Internalization assay

Mature LCs or BLCL-OUW transfectants (2 × 10⁵) were incubated with DCGM4 (25 μg/mL) for internalization, washed and fixed with PFA (3% in PBS). Cells were permeabilized using saponine (0.1%), stained with anti-Lamp-1 (0.5 μg/mL) and analyzed by confocal microscopy (Leica AOBSP2 CSLM, DMIRE2 microscope, PL APO 63x/NA1.30). Images were acquired using Leica confocal software (version 2.61).

Antigen presentation assays

Primary LCs were screened to match HLA-profiles of the T-cell clones. Monocytes were isolated from an HLA-DQw1/HLA-B27-matched donor. APCs (1 × 10⁴ cells/well) were pre-incubated with serial dilutions of DCGM4 or AZN-D1 (isotype), washed and co-cultured with 2 × 10⁴ Hd7 T cells. After 24 h, IFN-γ production was measured by ELISA (Cytoset, Biosource). HLA-matched LCs or moDCs (5 × 10³ cells) were used as APCs in an IFN-γ ELISPOT as previously described [55]. Where indicated, the cells were incubated with a mix of TNF-α (100 ng/mL, Strathmann Biotec), poly I:C (10 μg/mL) and LPS (*Salmonella typhosa*, 100 ng/mL, Sigma).

In parallel, IFN- γ production in supernatant was measured by ELISA (Biosource International). To determine the contribution of Langerin, the APCs were pre-incubated with mannan (0.25 mg/mL), anti-Langerin (20 μ g/mL), anti-CD150 (20 μ g/mL) or an isotype control (20 μ g/mL).

Flow cytometry

Flow cytometry was performed on a FACScan or FACSCalibur (BD Biosciences). Gating strategies were as follows: live gated on forward and sideward scatter. Where indicated in the text, live cells were further gated on CD1a. Finally, infection and binding are depicted as percentage positive cells in the current gate.

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Abbreviations: BLCL: B-lymphoblastic cell line · DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin · LC: Langerhans cell · moDC: monocyte-derived dendritic cell · MV: measles virus · WTF: wild-type MV

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