Chapter 8.3

Cellular level: Do dendritic cells fool lymphotropic viruses?

DC-SIGN$^+$ DCs versus Langerhans cells

DC-SIGN$^+$ DCs and LCs are the major DC populations in the peripheral tissues. The distinct localisation within these tissues and the specific features of these two subsets suggest a differential function or sub-specialisation in the immune system. LCs are present at the frontline of the human tissues and will often encounter pathogens. These pathogens have not yet invaded and are often harmless. We hypothesize that LCs must be strictly controlled in their response, since overreaction could result in allergy and hypersensitivity. Indeed, different stimuli, including TLR ligands, induce strong maturation of DC-SIGN$^+$ DCs but not LCs (Marein de Jong, personal communications) and the production of pro-inflammatory cytokines by these cells is low[59]. Moreover, LCs may be actively involved in the induction of tolerance to commensal bacteria[77].

In contrast, pathogen encounter of DC-SIGN$^+$ DCs, in the dermis and subepithelium requires an adequate response, since encounter of these deeper located cells is a sign of infection. Indeed dermal DCs, but not LCs, are important for the induction an of immune response against HSV and *Leishmania*[4,89,116]. Here we have demonstrated an additional, innate sub-specialisation of LCs: clearance of virus particles, reminiscent of the function of macrophages.

Langerhans cells clear invading virus particles

In **Section 5** we have demonstrated that LCs capture HIV-1 and MV via Langerin, resulting in viral clearance and inhibition of LC infection by these viruses. These data indicate that body surfaces are equipped with a unique innate defence mechanism. LCs with their dendrites sense incoming viruses, and subsequently degrade these viruses via Langerin. Moreover, data in chapter 5.3 indicate that degradation via Langerin results in an antiviral adaptive immune response that defends the body during systemic infection.

It was previously demonstrated that epithelial DCs reach with their dendrites into the lumen of the gut and respiratory tract[88]. This suggests that epithelial LC-dendrites clear virus via Langerin that is shed into the genital and respiratory tract, which is transmitted to an uninfected person, and as such protect the population against infection.

The protective function of Langerin is not able to prevent new measles infections *in vivo*. In fact, this virus is one of the most contagious viruses that infects humans[36]. In **Section 3**, we have demonstrated

that the subepithelial DC-SIGN$^+$ DC subset, efficiently mediates MV transmission. Thus, these data suggest that MV invades the body at a site where the LC barrier is not “virus-proof” and with easy access to the DC-SIGN$^+$ DCs, such as in the bronchi. Other factors such as LC activation, inhibition of Langerin function, CD150 upregulation may be involved.
Although HIV-1 is less contagious than MV, Langerin function has not prevented the pandemic[113]. Possible explanations are discussed in chapter 8.4. To determine the role of Langerhans cells in protection against invading viruses the sensitivity of the MV IC323-EGFP macaque model might allow us to go back in time to just hours after entry of the virus to determine where MV invades the body. Entry in an LC-rich tissue would indicate that the efficiency of the LC barrier is not sufficient for invading MV, and in LC-poor tissues would suggest a role for LCs in protection against MV infection. Small animal models with conditional LCs knockout[9] could address LC function for viral transmission *in vivo*.

Is this innate function essential?
The functional niche of LCs within the DC family is still unclear. Here, we have demonstrated that LCs protect against viral infections *in vitro.* The Birbeck granule seems to be involved in this process. Birbeck granules are found in LCs in mammals, including mice and guinea pigs but also in chicken. Furthermore, Birbeck-like granules are detected in salmon[69] but not in frogs[19], indicating that these granules have evolved during the early vertebrate ancestors and have an important role in the immune system. This is supported by the fact that *Langerin* is highly polymorphic (M. Carrington, personal communications), which suggests a benefit of a broad pathogen specificity of Langerin variants within the population. However, the function might be subtle or only challenged during certain viral epidemics, since a person lacking Birbeck granules and the Langerin knock-out mice do not have specific phenotype[55,107]. Importantly, the Langerin knockout-mice were not evaluated for susceptibility to viral infections, which could answer the question for Langerin function during viral infections *in vivo*.

DC subsets and adaptive immune responses
DC-SIGN$^+$ DCs and Langerhans cells are both professional antigen presenting cells and as such crucial for the immune system. We have demonstrated in chapter 3.4 and 5.3 that DCs are indeed professional antigen presenting cells, since antigen presentation in the context of MHC-class-II is much more efficient than in B cells. These results together with their localization in the peripheral tissues, suggest that these subsets of DCs are involved in the initiation of CD4$^+$ T cells responses.
Until recently, it was thought that MHC class-I ligands are derived from endogenous cytosolic proteins, whereas MHC class-II ligands are derived from exogenous proteins that are captured by endocytosis. Although this paradigm is still correct, we now know that these pathways are not as restricted as was assumed[99]. We have demonstrated that infection of DC-SIGN$^+$ DCs and, although to a lesser extent LCs, leads to MHC I presentation to CD8$^+$ T cells. And although presentation of exogenous antigens in the context of MHC class-II was efficient, cross-presentation of inactivated virus particles was highly inefficient by both DC subsets. Furthermore, cross-presentation of virus-infected cells was efficient by DC-SIGN$^+$ DCs, but not by LCs. Thus internalisation of ligands via DC-SIGN allows escape of the antigen into the cytosol during the internalisation route. Our results indicate that the specific internalisation pathway of ligands via Birbeck granules reflect a pathway that does not allow escape for both transmission and cross-presentation, leading directly to degradation for MHC class-II presentation (Figure 8.3). These results need to be further explored, including the question whether cross-presentation of inactivated viruses is indeed inefficient and whether activation of these DC subsets confers the ability to mediate cross-presentation.

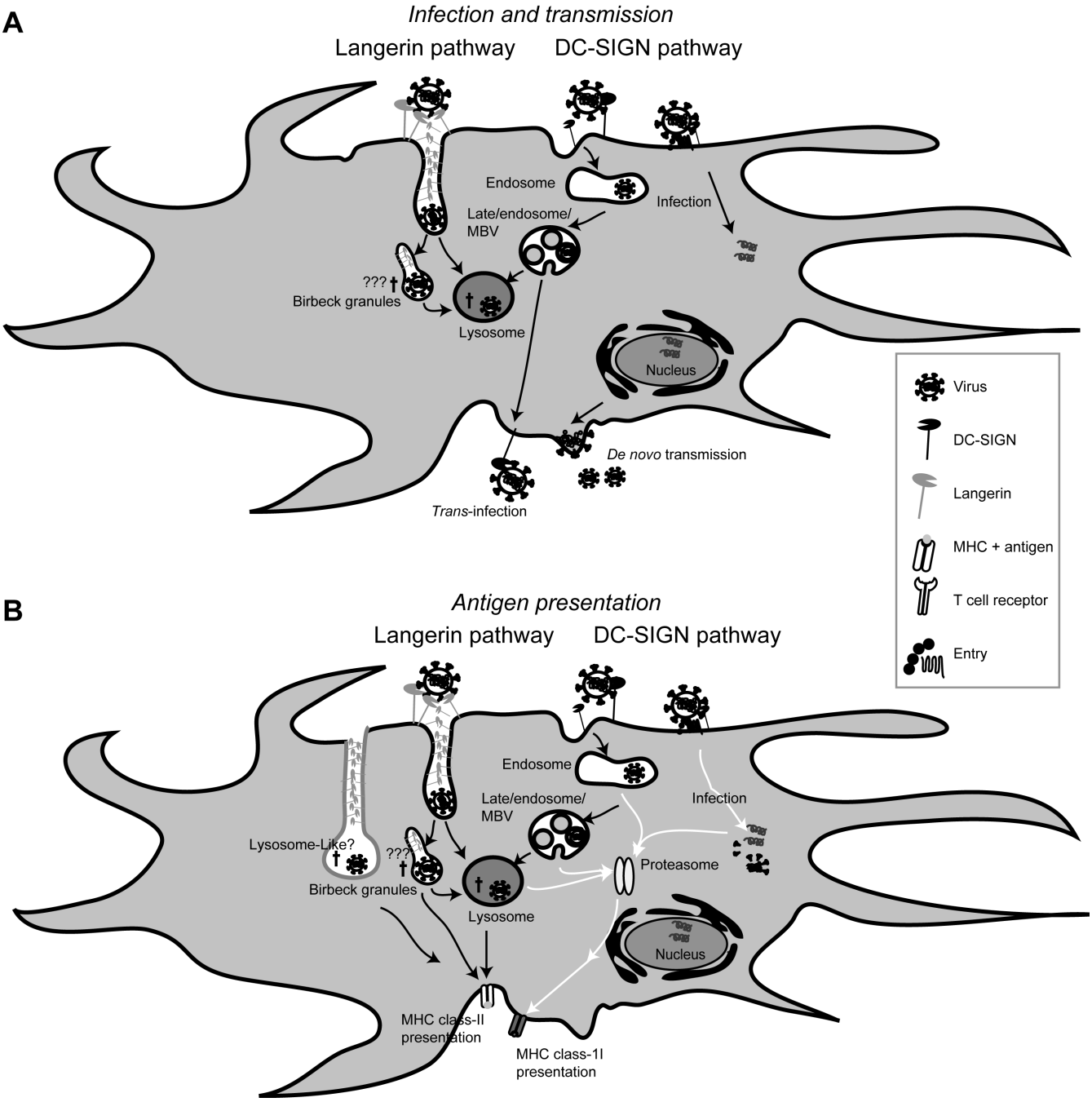

Figure 8.3 Interaction of DC-SIGN and Langerin results in different internalization pathways (adapted from[99]) **.** (a) Interaction of viruses with DC-SIGN results in infection in cis and subsequent de novo transmission (right) and internalization of the virus via endosomes into late endosomes or lysosomes. A part of the virus can escape degradation and is transmitted to target cells (middle). (b) Interaction of viruses with Langerin results in internalisation.of viruses into Birbeck granules The compartment where the virus is degraded is not elucidated yet. Possibly, the virus is degraded in the Birbeck granules, transported from the Birbeck granules into the lysosomes or directly ‘zippered’ into lysosome-like structures, which are normally observed as the vesicles at one site of the birbeck granule. (b) Interaction of viruses with DC-SIGN results in MHC class-II presentation via the endosomal/lysosomal pathway (black arrows) but might also result in presentation in the context of MHC-class I via either infection or cross-presentation (white arrows), where proteins escape from the endosomal-lysomal pathway into the cytosol. Subsequently, peptides will processed and loaded via the proteasome on MHC-class I. Internalisation via Langerin results in virus degradation and MHC-class II presentation (black arrows). However, this does pathway not result in cross-presentation, indicating that the virus can not escape from the endosomal/lysosomal pathway after interaction with Langerin, similar to DC-SIGN (left).

LCs as specific virus-fighters?

Three different observations suggest a sub-specialisation of LCs in the antiviral immune response: i) LCs clear virus particles via Langerin ii) LCs respond to viruses but not to bacterial ligands due to a specific expression profile of TLRs, suggesting that these cells have an important function in the adaptive antiviral immune system[30,103]. iii) LCs present only endogenous antigens in MHC class-I. Cross-presentation may be especially important for tumour antigens or microbes that do not infect DCs, such as yeast or extracellular bacteria[99]. Thus, LCs might have specificity for inducing innate and adaptive immune response against viruses, controlling their response against other microbes to avoid overreaction. In contrast with this hypothesis, murine studies indicated that LCs produce more Th-2 molecules[59], which is favourable for clearing extracellular infections. In conclusion, others and we have observed functional differences between the different DC subsets in the peripheral tissues, which indicates a further sub-specialisation of LCs and DC-SIGN$^+$ DCs within the DC family to maximize immune response and to prevent auto-immunity and allergy.