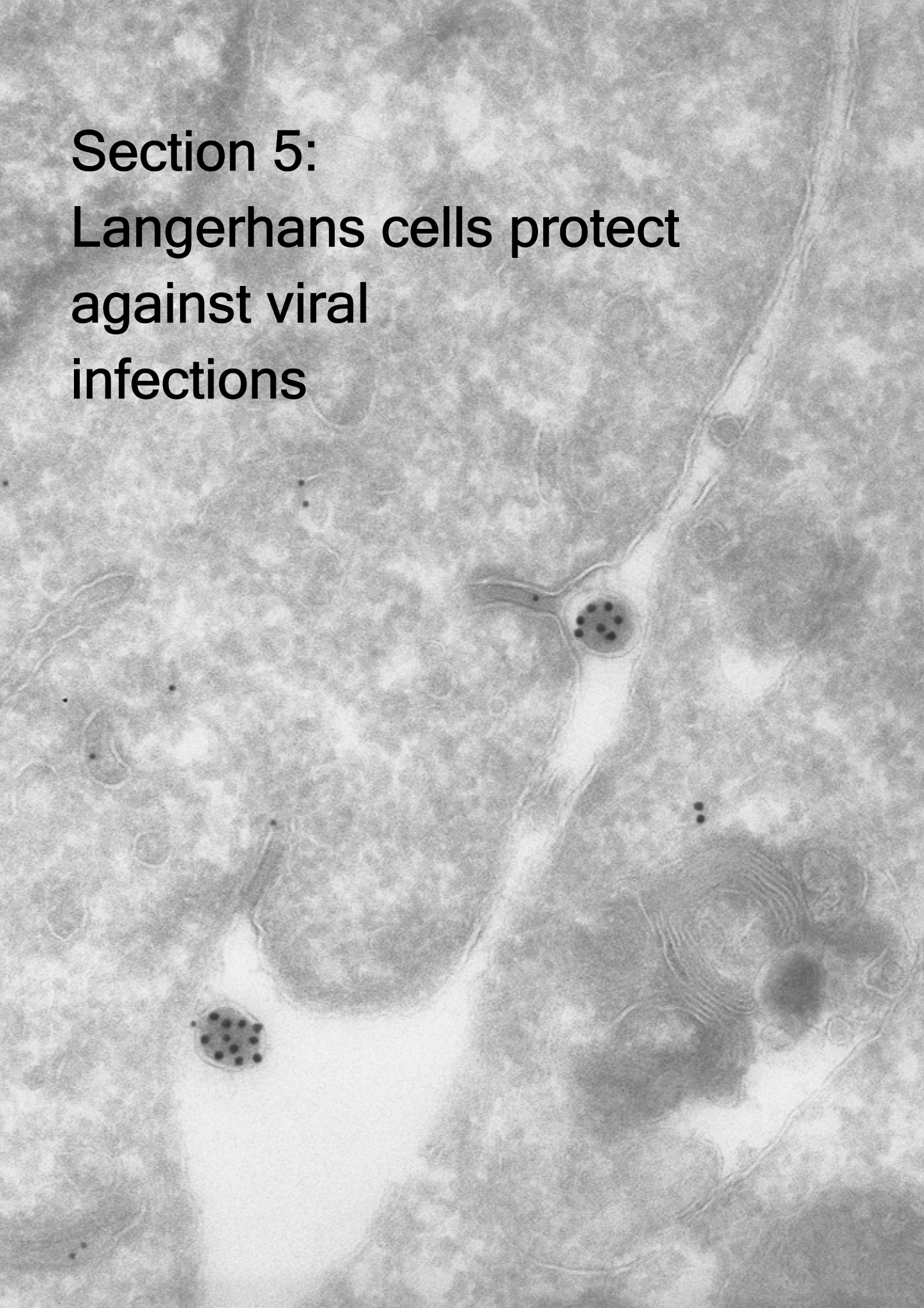
Section 5: Langerhans cells protect against viral infections



Chapter 5.1

Langerhans cells, Birbeck granules and Langerin

Bridge

In **Section 2, 3 and 4** we have demonstrated that a subset of DCs, the DC-SIGN$^+$ DCs, mediates viral transmission of both MV and HIV-1. The C-type lectin DC-SIGN plays a crucial role in this process and is a general receptor for viruses. However, in skin and various stratified epithelial tissues DC-SIGN$^+$ DCs are present in the subepithelial tissue/dermis, whereas another subset of DCs, the LCs, is present in the epithelial tissues and the epidermis. In these tissues, LCs are the first cells to encounter invading viruses, when the epithelium is not breached. This section describes the role of LCs during viral transmission of lymphotropic viruses, such as HIV-1 and MV, and the potency of LCs to present viral antigens to T cells.

Langerhans cell discovery

In 1868, Paul Langerhans identified a cell type in the epidermis of human skin, which he claimed to be nerve cells, due to their dendritic morphology[33]. More than one hundred years later, it slowly became comprehensible that Langerhans had misunderstood the neurological function of the cells that were named after him. LCs were demonstrated to express Fc- and complement receptors, MHC class-II and ATPase and to have antigen presenting capacities [6]. In 1985, Schuler and Steinman *et al*, provided their niche in the DC family of the immune system, which they still have: dendritic cells of the epidermis and epithelia [49].

Langerhans cell ontogeny

LCs are derived from a haematopoietic precursor and murine studies indicated that they have a life cycle distinct from other DC subtypes[42,50,54]. LCs have a long life-span compared to other subsets of DCs and constitutively migrate from the skin to the draining lymph nodes[21,38,48,50]. A self-renewing population of LCs has been described in the epidermis[38], which is thought to supply the tissues with new LCs during steady-state conditions. However, during inflammation, monocytes migrate towards the skin, where they actively proliferate and differentiate into epidermal LCs[17].

Langerhans cell localization and characterisation

Langerhans cells are localized in the epidermis and epithelial tissues. At some places epithelia lack LCs, such as the columnar epithelium of the rectum, endocervix, bronchi and gut[24,25]. With their dendrites, epidermal and epithelial LCs generate a fine web-like cellular network, throughout the body surfaces. LCs express specific markers that distinguish them from other dendritic cell subsets, such as CD1a and the C-type lectin Langerin[59]. Moreover, LCs are characterized by specific organelles, the Birbeck granules. Classical descriptions refer to LCs as the antigen presenting cells of the epidermis[46]. In this thesis we have included the $CD1a^+$ cells from the mucosal epithelia to the group of LCs[25]. In Section 6 we will demonstrate that both mucosal and skin LCs express Langerin, HLA-DR and contain Birbeck granules. However, experiments comparing isolated oral and skin LCs suggested that these different LC subtypes might have different T cell stimulating capacities[1,19,20].

Langerin structure and cellular localization

Langerin is a 40 kDa transmembrane protein that belongs to the family of type II Ca_2^+-dependent (C-type) lectins and consists of an intracellular region, a short transmembrane segment and an extracellular moiety[58,59]. The cytoplasmic tail of Langerin contains an interesting proline-rich motif (WPREPPP), which might be involved in signalling and Birbeck granule formation[44,59,60]. The extracellular domain of Langerin consists of a single C-terminal carbohydrate recognition domain (CRD) and a neck-region that is involved trimerization of the protein[51] (Figure 4.1.1). We and others [36,59] have observed that Langerin is present at the cell surface, in racket shaped organelles, as well as in vesicles and tubular structures just below the cell membrane, structures that might all be part of the Birbeck granule network.

Langerin specificity

Langerin has specificity for mannose, fucose and *N*-acetyl-glucosamine monosaccharides[51] (www.functionalglycomics.org), and requires multivalency to mediate high-affinity binding to oligosaccharides or glycoproteins[68]. Recent crystallography of Langerin demonstrated large similarities in the folding of the CRD between Langerin and DC-SIGN. Both lectins display a Ca_2^+-dependent sugar binding site, which is conserved in the C-type lectin family. Strikingly, Langerin contains an additional

calcium-independent carbohydrate binding site[9]. In contrast to the CRD of *DC-SIGN* that is highly conserved, polymorphisms in the CRD of *Langerin* have been described[66]. These polymorphisms are situated in proximity of the carbohydrate binding sites[9] and affect carbohydrate binding, and in a specific polymorphisms abolishes birbeck granule formation[65,66]. Our recent analysis revealed additional polymorphisms in the *Langerin* gene (M. van der Vlist, personal communications).

Langerin versus DC-SIGN

Langerin and DC-SIGN are both type II C-type lectins. However, the receptors are expressed by specific subtypes of DCs. The similarities and differences are summarized in text box 5.1.

Box 5.1. DC-SIGN versus Langerin, the similarities and the differences

Similarities

- **Family:** DC-SIGN and Langerin belong to the family of C-type lectins: receptors that recognize carbohydrates in a calcium dependent manner[10,16,59].
- **Structure:** DC-SIGN and Langerin are both type II transmembrane proteins with an extracellular region, consisting of a neck involved in multimerization and a C-terminal C-type carbohydrate-recognition domain[15,59].
- **Specificity for high mannose:** Both DC-SIGN and Langerin recognize high mannose-type carbohydrate structures, and therefore both bind HIV-1 gp120[15,55].

Differences

- **Expression:** Langerin has a very unique expression pattern, since it is only expressed by LCs, which reside abundantly in epidermis and mucosal stratified epithelia. DC-SIGN is expressed by DCs in dermis, submucosa, rectal epithelia, but also on DCs in other tissues, such as the lymphoid tissues. Moreover, DC-SIGN is expressed by a population of macrophages during inflammation[15,18,59].
- **Multimerization:** Langerin forms a trimer, whereas DC-SIGN molecules are clustered in tetramers[12,51].
- **Polymorphisms:** The sequence coding for the carbohydrate recognition domain of DC-SIGN is highly conserved, whereas *Langerin* polymorphisms are present that result in different carbohydrate binding capacities. Future research needs to further elucidate whether *Langerin* polymorphisms result in different HIV-1 transmission rates[34,35,66].
- **Murine homologues:** In contrast to DC-SIGN, the murine homologue of Langerin shows large similarities to human Langerin, including the formation of Birbeck granules and expression on LCs. This allows future research on Langerin function in small animal models [4,31,32,40,53,57].
- **Carbohydrate recognition:** The carbohydrate recognition spectrum of both C-type lectins partly overlaps (both recognize high mannose and fucose structures), but also differs (www.glycomics.com)[3,51,63].
- **Intracellular domain:** The intracellular domain of DC-SIGN and Langerin is very distinct and might reflect differences in function. Langerin induces the formation of the Birbeck granules in Langerin$^+$ cells. Future research will demonstrate whether the extracellular domains of Langerin and DC-SIGN, which are similar in the respect binding mannose and fucose containing structures, might also contribute to the differences in function[15,59].

Langerin induce Birbeck granule formation

In 1961, Michael S.C. Birbeck described the presence of rod- or tennis racket-shaped organels within LCs in the skin[8]. These granules that were named after Birbeck, are exclusively found in LCs and are composed of two superimposed membranes that are characterized by zipper like striations[69]. 3D reconstruction after electron tomography analysis of LCs, demonstrated that the Birbeck granules are composed of disc-like structures with a spherical vesicle-like formation at one of its ends. Moreover, Birbeck granules seem to interconnect with each other via tubular structures (M. Mommaas, personal communications).

Transfection of Langerin is sufficient to induce the formation of Birbeck granules in fibroblastic cells[59], suggesting that Langerin is equally involved in Birbeck granule formation in human LCs. Moreover, a cytomembrane sandwiching structure, the initiation of a Birbeck granule, appears to form where Langerin accumulates on the LC membrane[4,5,59] (Figure 4.1.1). However, the formation of these structures is still unclear. They could either form by cross-linking of Langerin:Langerin, Langerin and another cell-surface protein, or Langerin:Langerin via a soluble ligand (Figure 4.1.1). We observed that ligands such as HIV-1 induce the formation of birbeck granules and cytomembrane sandwiching structures (data not shown), supporting the latter hypothesis.

Function of Birbeck granules

The role or Birbeck granules, their biogenesis and the intracellular trafficking and function of Langerin are not fully elucidated. Due to the involvement of the pattern-recognition receptor Langerin, that mediates uptake and degradation of antigen[56,59], they have been implicated in antigen processing. Moreover, Langerin has been shown to be involved in the presentation of microbial lipid antigens to CD1a-restricted T cells[23].

Birbeck granules display a remarkable association with Rab11, which is a member of the Rab GTPase family which are known membrane traffic regulators[71]. Therefore, McDermott *et al.*[36,37] concluded that Birbeck granules are involved in the endosomal recycling pathway. Langerin is internalized into $CD1a^{+}/Rab11^{+}$ early/sorting recycling compartment and not in late-endosomal compartments. Inhibitors of endocytosis increase Langerin expression and the presence of pulsed-antibody at the cell surface within one hour, suggesting recycling of this receptor to the cell membrane[36]. Knock-down of Rab11 results in the disappearance of Birbeck granules and decreased steady-state levels of Langerin[56]. These results indicate that Rab11 is involved in the biogenesis of Birbeck granules and the intracellular trafficking of Langerin. In the absence of Rab 11A, Langerin is targeted to the lysosomes for degradation.

Langerin, Birbeck granules and disease

Although several attempts have been made to understand the immunological role of Langerin, its precise physiological function remains obscure. A person who lacks Birbeck granules, due to a mutation in *Langerin* was discovered coincidentally, and showed no signs of pathology. Murine Langerin is 66% homologous to the human variant, and induces the formation of Birbeck granules [57]. *Langerin* knock-out mice lack Birbeck granules, but apart from that do not have an apparent phenotype and respond similar as wild-type mice to tumours, (myco) bacteria, yeast and parasites [31]. Thus, Langerin might not have a crucial role in the immune system or the function of Langerin might not have been challenged in the Langerin-deficient person and the knock-out mice. Interestingly, the response of *Langerin* knock-out mice to viral infection has yet not been reported. Thus, question remains whether Langerin contributes to disease by either protecting against incoming pathogens, mediating an adaptive immune response, or by being subverted by pathogens, similar to DC-SIGN [62].

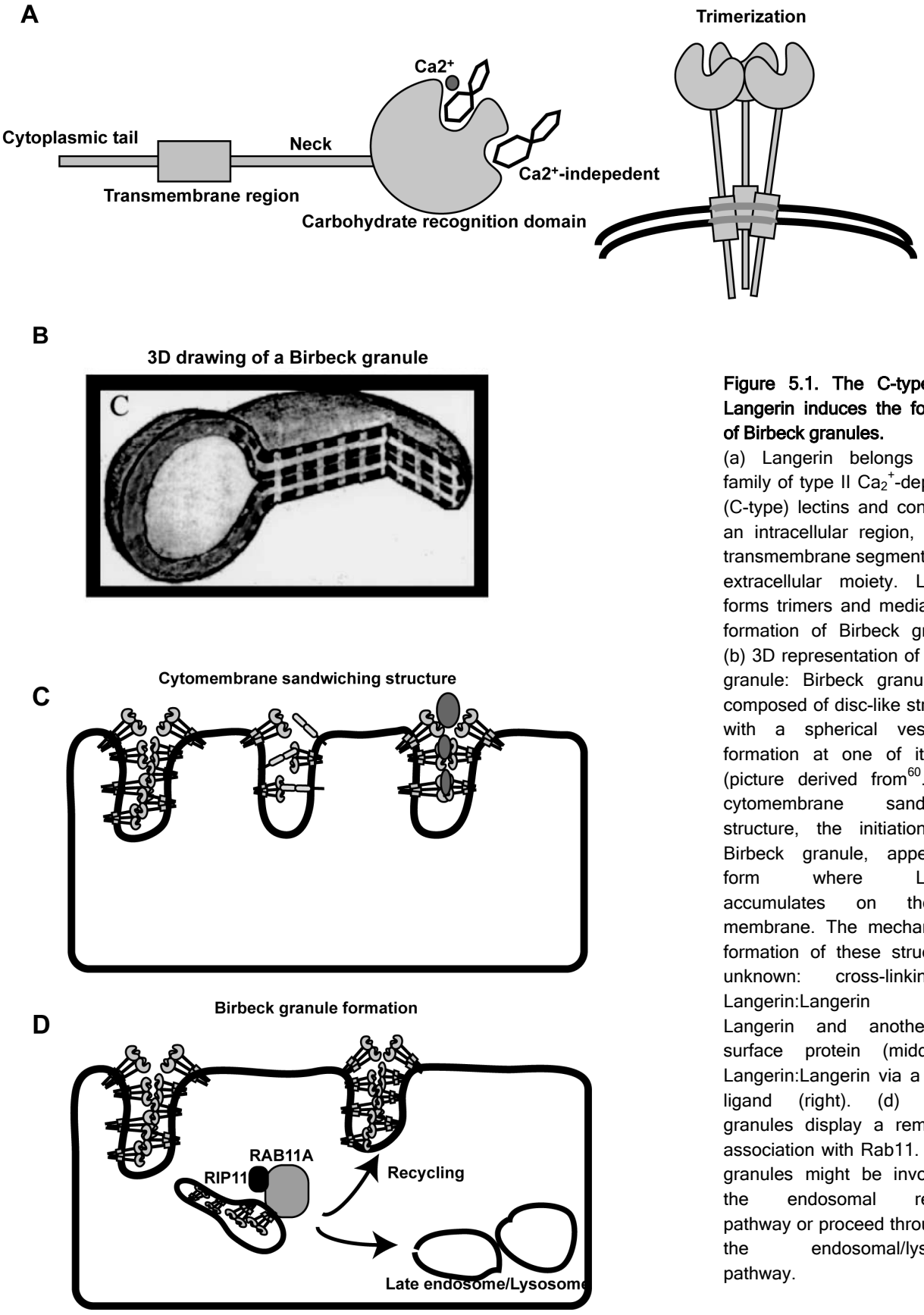


Figure 5.1. The C-type lectin Langerin induces the formation of Birbeck granules.
(a) Langerin belongs to the family of type II Ca_2^+-dependent (C-type) lectins and consists of an intracellular region, a short transmembrane segment and an extracellular moiety. Langerin forms trimers and mediates the formation of Birbeck granules. (b) 3D representation of Birbeck granule: Birbeck granules are composed of disc-like structures with a spherical vesicle-like formation at one of its ends (picture derived from[60]. (c) A cytomembrane sandwiching structure, the initiation of a Birbeck granule, appears to form where Langerin accumulates on the LC membrane. The mechanism of formation of these structure is unknown: cross-linking of Langerin:Langerin (left), Langerin and another cell-surface protein (middle) or Langerin:Langerin via a soluble ligand (right). (d) Birbeck granules display a remarkable association with Rab11. Birbeck granules might be involved in the endosomal recycling pathway or proceed through into the endosomal/lysosomal pathway.

The role of Langerhans cells in the immune system

LCs were always assumed to be classical antigen presenting cells inducing adaptive immune responses to pathogens invading the skin or epithelia. This is supported by data, demonstrating that LCs express pathogen recognition receptors (C-type lectins and TLRs), antigen presenting receptors (MHC class-I/II, CD1a), mediate MHC class-II presentation, migrate towards the lymph node upon activation and are important for antigen presentation of cutaneous antigens[7,13,30,47,59,61].

However, recent reports questioned the role of LCs in the generation of adaptive immune responses[2,26,30,45,72]. This together with the unique localisation of LCs in the body, and their subset-specific characteristics, suggests that LCs fulfil a particular, but yet unknown, function or subspecialisation in the DC family. Due to their localisation at the border of the body surfaces, LCs might play a role in the induction of tolerance against commensal bacteria, fungi and yeast[11] or might direct would healing. Furthermore, LCs express a unique TLR-profile (TLR 1-3, 6, 10, and maybe 7/8) with a specificity for viruses[13,61] and respond to viruses but not bacteria, which might point to the direction of a specific role in viral infections.

Langerhans cells and HIV-1

In intact vagina, ectocervix and penis tissues, epithelial LCs are the first cells encountered by HIV-1 that express its entry receptors CD4 and CCR5. This suggests that HIV-1 target LCs in these tissues for transmission. Several *ex vivo* data support a role for LC infection in HIV-1 transmission[29]. LCs are infected by HIV-1 within epidermal sheets and after the cells migrate out of the tissues, they mediate HIV-1 transmission to T cells[28,43]. In contrast to dermal DC-SIGN$^+$-DCs, LCs are primarily infected R5-viruses and not with X4-viruses[27,43,70], and infectivity is associated with CCR5 genotype[28]. These studies support a role for selective R5-transmission at the level of the LCs[64,73]. Moreover, LCs are infected within human cervical, vaginal and foreskin tissue[22,41,41]. Vaginal simian immunodeficiency virus (SIV) infection of rhesus macaques resulted in infected LCs beneath the vaginal epithelium within the first day of infection[39]. These reports definitely demonstrate that LCs can be infected *ex vivo*, however the circumstances are not entirely clear, and might not reflect the *in vivo* situation, since high virus concentrations and spinoculation was used. Moreover, detection of HIV-1 infected LCs in the genital mucosal tissues *in situ* and *in vivo* is complicated, since infection is a rare event and the existence of HIV-1 specific proteins cannot distinguish between viral uptake and virus replication in LCs. Finally, the fact that HIV-1 infectivity per sexual act is low and the chance an LC will encounter HIV-1 in the genital tissues is high, suggests that more is going on.

Langerhans cells and measles virus

Reports on the interaction of MV with LCs are limited. LCs isolated from skin can be infected by both vaccine and wildtype MV, and infection results in suppression of T cell activation in a mixed leukocyte reaction[52]. Moreover, CD14$^+$-derived LC-like cells, sharing characteristics with primary LCs, are infected with MV after mechanical stress, which might coincide with the upregulation of the entry receptor CD150[67].

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