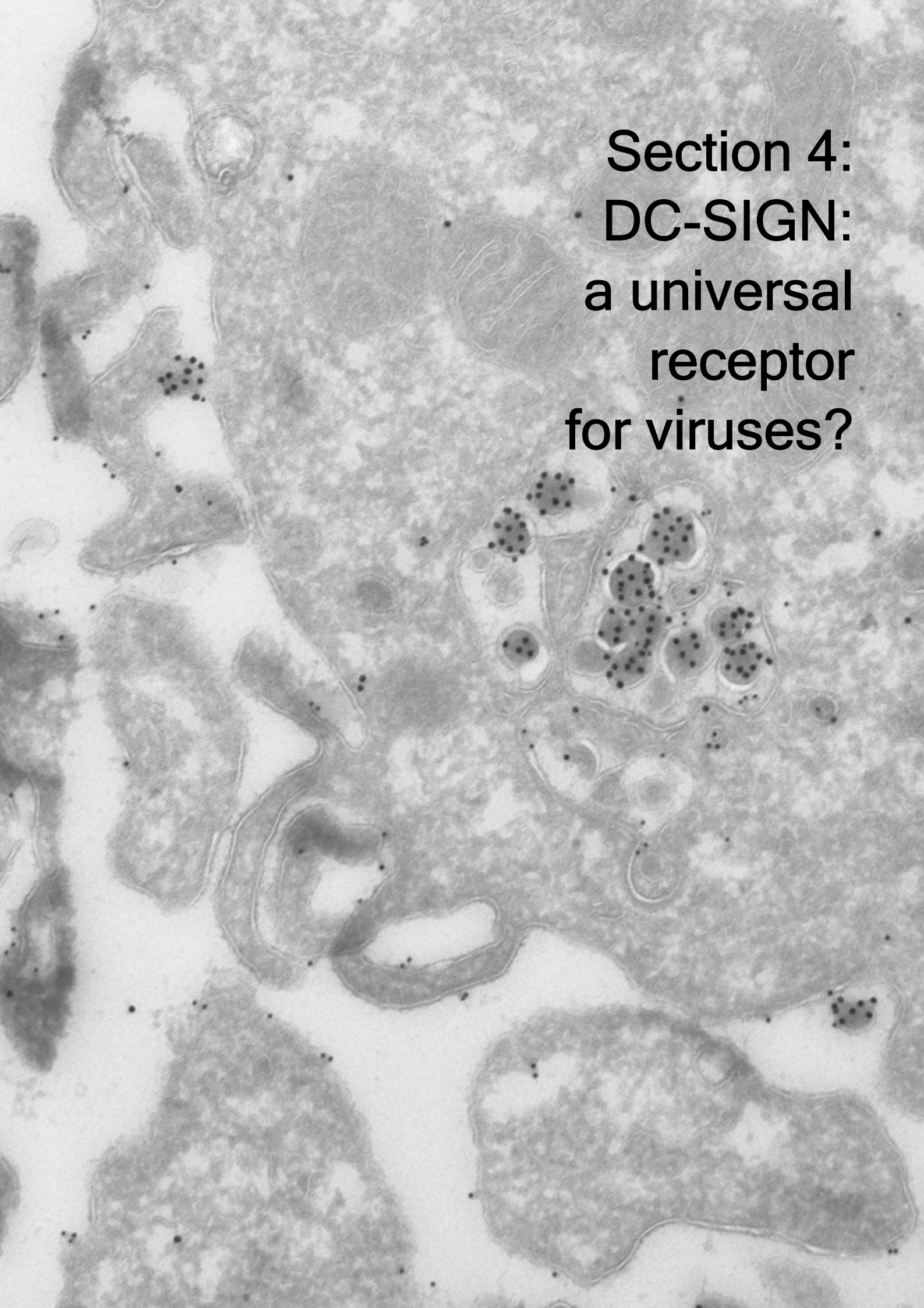

Section 4: DC-SIGN: a universal receptor for viruses?

Chapter 4.1

DC-SIGN specificity and viral glycosylation

Bridge

In **Section 2 & 3** we have demonstrated that DC-SIGN is a receptor for HIV-1 and MV. DC-SIGN mediates infection by these viruses of DCs *in cis* and of other target cell-types *in trans*. Furthermore, DC-SIGN contributes to antigen presentation in the context of MHC class molecules. In **Section 4** we have further investigated the specificity of DC-SIGN for viral ligands.

After the identification of DC-SIGN as the previously described HIV-1 receptor by Curtis *et al.*, it was thought that specific viruses had evolved a mechanism to gain entry into the host via DC-SIGN. However, the list of viruses that bind to DC-SIGN is still growing, whereas the list of viruses that do not interact with DC-SIGN is minimal. This requires a thorough re-evaluation of the role of DC-SIGN during viral infections. In this section we have investigated the binding of DC-SIGN to two additional viruses (herpes simplex virus and human papilloma virus L1 virus like particles). We will discuss the viral specificity of DC-SIGN and the role of this C-type lectin during viral infections.

DC-SIGN specificity

DC-SIGN (for more information, text box 4.1) has a single carboxyl terminal carbohydrate recognition domain (CRD)[10] that recognizes both internal branched mannose residues as well as terminal di-mannoses[5,16]. Moreover, DC-SIGN has also specificity for certain fucosylated glycan structures, such as Lewis antigens[1,28]. Due to this specificity DC-SIGN recognizes pathogens[8,9], self glycoproteins, such as ICAM-2, ICAM-3 and MAC-1[7,27], as well as tumour antigens[26].

Glycosylation of viral glycoproteins

Glycosylation of viral proteins depends on the machinery of the host cell[30]. Viral glycoproteins contain both *O*-linked glycosylation and *N*-linked glycosylation sites, which determine the folding and conformation of the protein[30]. In general, viral glycoproteins are more glycosylated than host glycoproteins, and often decorated with high mannose structures, but also fucose, GalNAc, GlcNAc and sialic acid-containing glycans are found[30]. Furthermore, viral *N*-linked glycans are relatively unprocessed, which might be due to the high production of viral glycoproteins in an infected cell and/or the high level of glycosylation on the proteins, which overload the function of the glycosyltransferases[22]. Furthermore, recent data revealed that viruses, such as cytomegalovirus and varicella-zoster virus influence the expression of glycosyltransferases[20].

Due to this glycan composition of viruses and the specificity of DC-SIGN for high mannose- and fucose-containing carbohydrates numerous viruses bind to DC-SIGN. During viral evolution, glycosylation sites are often added and deleted, thereby influencing viral infectivity, viral escape or recognition by the immune system, which might in certain cases involve altered binding of the virus to DC-SIGN.

Text box 4.1: Characteristics DC-SIGN

DC-SIGN:

- dendritic cell-specific intracellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin or CD209
- type-II transmembrane protein, belongs to the C-type lectin family.
- consists of an N-terminal intracellular tail, an extracellular stalk and a C-terminal carbohydrate recognition domain[10]
- binds carbohydrates in a calcium dependent manner
- specificity for high mannose and unsialylated Lewis-type antigens[1,28]
- functions as pattern recognition receptor, mediates antigen presentation on both MHC class-II molecules and cross-presentation on MHC class-I molecules[3,18,19,23]
- binds viruses and other pathogens, including bacteria, mycobacteria and helminths[2,8,9,29]
- is expressed on DCs in the peripheral tissues and DCs and specialized macrophage subsets in the lymphoid tissues and placenta[4,10,11]
- mediates binding of DCs to neutrophils[27] and to endothelial cells[7]
- modulates TLR signaling via Raf-1 kinase[12]

Glycosylation of HIV-1 gp120

HIV-1 gp120 has an average of 25 glycosylation sites and is thereby one of the most glycosylated natural proteins known[30]. HIV-1 glycans are composed of high mannose-type structures and complex-type structures[33]. Glycosylation sites are highly conserved components of gp120, suggesting an important role for glycan structures in the viral life cycle. These structures influence the interaction of HIV-1 with CD4[6,17] and C-type lectins, and might thereby affect infection or the induction of adaptive immune responses.

Heavy glycosylation of HIV-1 gp120 is thought to provide the virus with a 'glycan shield', which prevents an efficient production of antibodies against the envelope proteins of HIV-1. Fifty percent of the protein core of gp120 is invisible to the immune system by coverage with carbohydrates and is therefore referred to as the silent face[21,32]. Repositioning of glycans in HIV-1 gp120 enables HIV-1 to escape antibody responses directly, but also indirectly by affecting protein folding[31].

DC-SIGN interacts with viral glycoproteins and mediates antigen presentation

HIV-1 gp120 was the first viral ligand to be described for DC-SIGN[9]. However, since its first characterisation DC-SIGN has been shown not to be an exclusive HIV-1 receptor but a general pathogen receptor with a broad recognition pattern of viruses, including hepatitis C, dengue, measles and Ebola virus. This indicates that DC-SIGN has a universal function in binding and internalizing pathogenic glycoproteins, which in turn might lead to (viral) antigen presentation by DCs. Indeed, DC-SIGN captures viruses, resulting in internalisation of the virus for degradation and antigen presentation[3,19]. Moreover, DC-SIGN is involved in MHC class-I presentation by enhancing infection *in cis* or by cross-presentation of capture viruses[13,18].

DC-SIGN mediates infection in cis and in trans.

Although most of the internalized HIV-1 is degraded in DCs, a small amount of the virus escapes degradation and infects DCs *in cis* or other host cell types cells *in trans*[18,25], as discussed in **Section 2** and **3**. Enhancement of infection *in cis* by DC-SIGN is strong when the entry receptor:virus binding is weak or the expression of the entry receptors low. Blocking DC-SIGN in these cases abrogates viral infection of DCs. Therefore early reports concluded that DC-SIGN is an entry receptor for dengue virus[24] and SARS-coV[15]. It is now believed that an additional fusion receptor is needed to mediate entry of these viruses.

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