Summary

The C-type lectin receptors (CLRs) DC-SIGN and L-SIGN are both expressed on antigen presenting cells; dendritic cells (DCs) and liver sinusoidal endothelial cells (LSECs) respectively. These cells capture and process ligands for antigen presentation to T cells.
DC-SIGN and L-SIGN recognize pathogens and self-ligands through recognition of specific carbohydrate moieties on glycoconjugates. Due to a high degree of homology between the carbohydrate recognition domains of DC-SIGN and L-SIGN, both CLRs have affinity for high mannose structures and the fucose containing Lewis^{a} (Le^{a}), Le^{b}, and Le^{y} antigens, with the exception of Le^{x}, which is only bound by DC-SIGN.
In **chapter 2** a novel L-SIGN specific carbohydrate ligand is described, which is not recognized by DC-SIGN. This ligand is neuraminidase sensitive, suggesting that L-SIGN recognizes a sialicacid containing glycan structure that is not recognized by DC-SIGN. This makes L-SIGN comparable to its murine homologue mSIGNR1 that similar to L-SIGN is expressed on LSECs in the liver and also is able to bind sialylated carbohydrates.

Cellular ligands for DC-SIGN and L-SIGN
DC-SIGN recognizes several cellular ligands on different cells, for example ICAM-2 on endothelial cells and ICAM-3 on T cells. Furthermore, DC-SIGN interacts with neutrophils via Mac-1 binding. Early during infection neutrophils are involved in killing of pathogenic bacteria and regulation of innate immune responses at the site of infection. It has become clear that neutrophils also modulate adaptive immunity through interactions with DCs. Upon activation, neutrophils release TNF-α and combined with Mac1-DC-SIGN interactions, induce maturation of DCs that enables these APCs to stimulate T cell proliferation and to induce T helper 1 polarization. In **chapter 7**, we demonstrate that also CEACAM1 is an important ligand for DC-SIGN on neutrophils. Binding of DC-SIGN to both CEACAM1 and Mac-1 is required to establish cellular interactions with neutrophils. L-SIGN on the other hand, only recognizes Mac-1 on neutrophils. Neutrophils are able to adhere to and roll over L-SIGN positive cells, indicating a role for L-SIGN in neutrophil homing to the liver sinusoid.
Besides the interaction with neutrophils, L-SIGN also interact with a yet unknown ligand on monocytes (**chapter 8**). These cells are amongst others precursors of resident tissue macrophages like the Kupffer cells in the liver. Using an *in vivo* model we demonstrate that the murine L-SIGN homologue mSIGNR1 expressed on LSECs influences Kupffer cell (KC) numbers in the liver, because mSIGNR1 deficient mice have reduced numbers of KCs. The effect of mSIGNR1 on KC recruitment appears after the initial phase of KC repopulation, indicating that L-SIGN could be involved in the recruitment of KC precursors in the human liver.
We furthermore demonstrate in **chapter 2** that L-SIGN recognizes a carbohydrate ligand on hepatoma cells. Hepatocytes express high amounts of sialylated glycans, which are abrogated in metastasizing hepatocellular carcinoma cells. The interaction between LSEC and hepatocytes, which are not connected with extracellular matrix, could contribute to maintenance of hepatic sinus architecture. The loss of sialylated ligands would loosen the L-SIGN-hepatocellular carcinoma cell interaction and the hepatocellular carcinoma cells would

more easily transmigrate into the bloodstream and form metastases at distant sites. Indeed this loss of sialic acid expression is indicative for a poor patient survival.
DC-SIGN mediates interactions with colon carcinoma cells, which express high levels of Le^y- and Le^x-decorated CEA and CEACAM1. In **chapter 2** we show that, similar to DC-SIGN, L-SIGN recognizes tumour derived CEA, probably because of the presence of Le^y on CEA, and therefore could participate in colon cancer liver metastasis. Furthermore, in contrast to hepatocellular carcinoma cells, colon cells display an increased or *de novo* expression of sialylated carbohydrates upon malignant transformation. The binding of these tumour-associated glycoconjugates to L-SIGN could, in addition to getting constricted by the narrow vasculature of the sinus, explain the frequent metastasis formation of colon carcinomas in the liver.

DC-SIGN/L-SIGN-pathogen interactions
Besides self ligands, DC-SIGN and L-SIGN also recognize a range of pathogens. DC-SIGN was originally identified as a human immunodeficiency virus 1 (HIV-1) gp120 binding receptor. DC-SIGN plays a key-role in the dissemination of HIV-1 by DCs through HIV-1 gp120 binding. Upon binding the virus is internalized and with the DC transported to the lymph node where it infects T cells efficiently.
Since the capture of HIV-1 by DC-SIGN enhances T cell infection, the interaction of DC-SIGN with gp120 provides an attractive target for intervention of HIV-1 transmission. In **chapter 5** and **6** we describe two ways to inhibit the DC-SIGN-gp120 interactions. In **chapter 6** we describe that human milk contains a component that inhibits the DC-SIGN-mediated transfer of HIV-1 to T cells. This Le^x containing inhibitory factor directly interacted with DC-SIGN, and prevented the HIV-1 gp120 envelope protein from binding to DC-SIGN thereby prevent the capture and subsequent transfer of HIV-1 to T cells. The presence of such a DC-SIGN-binding molecule in human milk may both influence antigenic presentation and interfere with pathogen transfer in breastfed infants. Interactions of L-SIGN with HIV-1 gp120 are not affected by this milk component since L-SIGN can not bind Le^x carbohydrates.
In **chapter 5**, we have investigated the potency of gp120 antibodies to inhibit the DC-SIGN-gp120 interaction. We demonstrate that antibodies against the V3 loop partially inhibit DC-SIGN binding, suggesting that these antibodies sterically hinder DC-SIGN binding to gp120. Polyclonal antibodies raised against non-glycosylated gp120 inhibited DC-SIGN-gp120 interactions in contrast to polyclonal antibodies raised against glycosylated gp120. Thus, glycans present on gp120 may prevent the generation of antibodies that block the DC-SIGN-gp120 interactions. Moreover, the polyclonal antibodies against gp120 efficiently inhibited HIV-1 capture by both DC-SIGN transfectants and immature dendritic cells. Therefore, non-glycosylated gp120 may be an attractive immunogen to elicit gp120 antibodies that block the binding to DC-SIGN. Furthermore, we demonstrate that DC-SIGN binding to gp120 enhanced CD4 binding, suggesting that DC-SIGN induces conformational changes in gp120, which may provide new targets for neutralizing antibodies.

In **chapter 3** and **4** the interaction of DC-SIGN and L-SIGN with the hepatitis C virus (HCV) glycoproteins E1 and E2 is described. HCV targets hepatocytes in the liver. However, the mechanism of hepatocyte infection is largely unknown. DC-SIGN and L-SIGN are important receptors for HCV E1 and E2. DC-SIGN is the main HCV receptor on DCs and L-SIGN is the main HCV receptor in liver since interaction can be inhibited by specific anti-DC-SIGN and L-

SIGN antibodies. Mutagenesis analyses demonstrates that both HCV E1 and E2 bind the same binding site on DC-SIGN as the pathogens HIV-1 and mycobacteria, which is distinct from the cellular ligand ICAM-3.
HCV virus-like particles (VLPs) are efficiently captured and internalized by DCs through binding of DC-SIGN. Interestingly, internalized HCV-VLPs are partially targeted to non-lysosomal compartments within DCs, where they are protected from degradation similarly as was demonstrated for HIV-1. This suggests that HCV may target DC-SIGN to 'hide' within DCs and to facilitate viral dissemination. L-SIGN expressed by APC cell-lines internalizes HCV particles into similar non-lysosomal compartments, suggesting that L-SIGN on LSECs may capture HCV from blood and transmit it to hepatocytes, the primary target for HCV. However, in **chapter 4** we describe that HCV-VLPs are also susceptible for degradation by DCs, and degradation is more efficient in the presence of HCV-specific antibodies. We demonstrate that degradation of the HCV-antibody complexes leads to MHC class II presentation by DCs. Moreover, induction of DC maturation by HCV-VLPs on an aluminium carrier strongly enhances this antigen presentation by DCs. Thus, antibody targeting and maturation-induction directs DC-SIGN-captured ligands into lysosomal compartments and efficient antigen presentation. Moreover, we demonstrate that L-SIGN captures HCV-antibody complexes leading to antigen presentation to $CD4^+$ T cells, demonstrating that L-SIGN is an antigen receptor on LSECs. Human LSECs express both L-SIGN and MHC class II molecules and our data suggest that LSECs are important as antigen presentation cells of viral antigens in the liver. However this presentation may lead to erroneous T cell activation since LSEC are known to induce T cell tolerance.

Final conclusion

DC-SIGN mediates several interactions with self-ligands, tumours and pathogens. The function of DC-SIGN on primary cells has been extensively studied and it has become clear that there is a delicate balance in the outcome of the DC-SIGN-mediated interactions. Therefore one should be careful with interfering in DC-SIGN-pathogen interactions at the side of DC-SIGN.
We have described here that L-SIGN also mediates important interactions with cells, such as neutrophiles, monocytes and tumour cells, and with pathogens. However research into the function of L-SIGN on primary cells has been hampered by the difficulty to isolate sufficient amounts of L-SIGN-positive LSECs from human liver. Therefore, most studies have used cell lines expressing L-SIGN as a model. Here we demonstrate that L-SIGN function *in vivo* can be investigated using a mouse model. mSIGNR1 is expressed on homologues cells in the murine liver and has a comparable carbohydrate recognition profile. Therefore, the mSIGNR1 deficient mice will be important in further exploring the function of L-SIGN.