GLAXOSMITHKLINE/MRS PAPER

DC-SIGN (dendritic cell-specific ICAM-grabbing non-integrin) and DC-SIGN-related (DC-SIGNR): friend or foe?*

Elizabeth J. SOILLEUX
Medical Research Council Cancer Cell Unit, Hutchison/M.R.C. Research Centre, Hills Road, Cambridge CB2 2XY, U.K.

ABSTRACT

C-type lectins are calcium-dependent carbohydrate-binding proteins with a wide range of biological functions, many of which are related to immunity. DC-SIGN (dendritic cell-specific ICAM-grabbing non-integrin, where ICAM is intercellular adhesion molecule) is a recently described mannose-specific C-type lectin expressed by dendritic cells. Dendritic cells are potent antigen-presenting cells capable of activating T-lymphocytes. DC-SIGN, which is expressed by dendritic cells, binds to ICAM-3 on T-lymphocytes, therefore playing an important role in the activation of T-lymphocytes. DC-SIGN can also bind HIV, and the virus may remain bound to DC-SIGN for protracted periods. DC-SIGN may deliver bound HIV to permissive cell types, mediating infection with high efficiency. A closely related C-type lectin, DC-SIGN-related molecule (DC-SIGNR) has also been described. DC-SIGNR is expressed by restricted subsets of endothelial cells, but has similar ICAM-3 and HIV-binding properties to DC-SIGN. This review describes the mapping of DC-SIGN and DC-SIGNR to chromosome 19p13.3 adjacent to the previously described C-type lectin, CD23 [the low-affinity receptor for immunoglobulin E (FcERII)]. The similar genomic organization of these three genes is discussed and consideration is given to the evolutionary duplications that may underlie this arrangement. Both DC-SIGN and DC-SIGNR possess a neck region, made up of multiple repeats, which supports the ligand-binding domain. Consideration is given to the biological reasons underlying the considerable polymorphism in the numbers of repeats in DC-SIGNR, but not DC-SIGN. The expression patterns of both DC-SIGN and DC-SIGNR are discussed in detail, with particular attention to the expression of both molecules in the placenta, which may have implications for the vertical transmission of HIV. Since dendritic cells may be important in determining the phenotype of many immune responses, via effects on T-lymphocytes, the differential expression of DC-SIGN by particular dendritic cell subsets may have important implications for the immunobiological functions of DC-SIGN. Similarly, the expression of DC-SIGNR by very restricted subsets of endothelial cells may give clues to the function of DC-SIGNR. Finally, the role of DC-SIGN in pathology, particularly in infective and neoplastic processes, is discussed, followed by speculation about likely future developments in this field.

* This paper was presented at the GlaxoSmithKline/MRS Young Investigator session at the MRS meeting, Royal College of Physicians, London, on 4 February 2002.

Key words: dendritic cell, dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN), DC-SIGN-related (DC-SIGNR), endothelial cell, HIV, intercellular adhesion molecule (ICAM)-3, lectin, placenta, vertical transmission.

Abbreviations: CCR5, chemokine receptor 5; CRD, carbohydrate-recognition domain; DC, dendritic cell; DC-SIGN, DC-specific ICAM-grabbing non-integrin; DC-SIGNR, DC-specific ICAM-grabbing non-integrin related; ICAM, intercellular adhesion molecule; IL, interleukin; Th1 and Th2, T-helper 1 and 2.

Correspondence: Dr Elizabeth Soilleux (e-mail ejs17@cam.ac.uk).

C-TYPE LECTINS IN IMMUNITY

Lectins are unique among proteins in that they bind to carbohydrates with considerable specificity [1]. Several subtypes of animal lectin have been defined, including one large group of calcium-dependent carbohydrate-binding molecules, known as the C-type lectins [1]. This group includes conglutinin, the surfactant proteins, mannose-binding lectin, CD23, the natural killer cell receptors, the selectins and an emerging group of dendritic cell (DC) lectins [2–4].

C-type lectins possess one or more carbohydrate-recognition domains (CRDs). CRDs of C-type lectins frequently have a number of conserved amino acid residues and a conserved overall secondary structure, the C-type lectin fold [4]. The crystallographic structures of an increasing number of lectins have been determined [4].

Certain motifs within the CRDs of C-type lectins allow the prediction of carbohydrate specificity, such as mannose/*N*-acetylglucosamine versus glucose/galactose [4]. A number of type II integral membrane proteins with C-type lectin domains have regions that are predicted to form coiled-coil neck structures [5]. In many cases, the potential for oligomerization through the neck regions remains to be investigated. One report suggests that the human asialoglycoprotein receptors 1 and 2 are able to form a hetero-oligomer via their neck regions. Similarly CD23 and mannose-binding proteins can form homotrimers [5,6].

Within an oligomer, these coiled-coil region neck structures may form a relatively rigid neck support for the CRD, thus altering the angles and distances between the carbohydrate-binding sites within the each of the constituent monomers [5]. This may have a profound impact upon the ligand specificity of the lectin [5].

This review outlines the recent discovery of two mannose-binding C-type lectins, DC-SIGN (DC-specific ICAM-grabbing non-integrin, where ICAM is intercellular adhesion molecule) and DC-SIGNR (DC-SIGN-related molecule). Both are able to bind HIV and faciliate HIV infection of various human cells. The cloning and genomic, expression, structural and functional analysis of DC-SIGN and DC-SIGNR will be discussed.

THE DISCOVERY OF DC-SIGN

DCs are highly specialized antigen-presenting cells, capable of activating naïve and memory T-lymphocytes. A number of adhesive interactions between the DC and T-lymphocyte are important during T-lymphocyte activation [7]. The DC-expressed β-integrin, leucocyte function associated molecule-1 ('LFA-1'), was originally described as the major ligand for ICAM-3 on T-lymphocytes. However, antibodies against leucocyte function

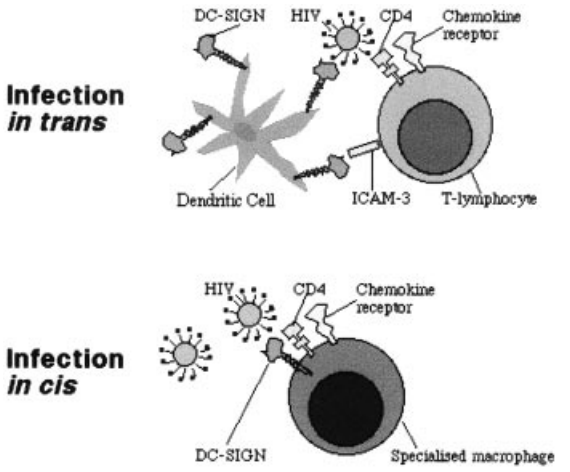

Figure 1 DC-SIGN-mediated enhancement of HIV infection *in trans* and *in cis*

Upper panel: DCs can adsorb HIV and, despite very thorough washing, can mediate infection of T-lymphocytes *in trans* during subsequent co-culture [11]. HIV entry requires the expression of CD4 and a chemokine receptor, such as CCR5 [11]. DC-SIGN also interacts with ICAM-3 on T-lymphocytes, thus being important for the activation of naïve T-lymphocytes [8]. Lower panel: whereas infection *in trans* occurs when DC-SIGN is expressed on a separate cell from that which becomes infected, infection *in cis* may occur when DC-SIGN is co-expressed with CD4 and chemokine receptor (e.g. CCR5) on a permissive cell type, such as a macrophage [13]. Reprinted from Int. J. Biochem. Cell Biol., vol. 35, E. J. Soilleux and N. Coleman, "Transplacental transmission of HIV: a potential role for HIV binding", pp. 283–287, © (2003), with permission from Elsevier Science.

associated molecule-1 failed to abrogate T-cell–DC clustering, whereas anti-(ICAM-3) antibodies completely abrogated this clustering. A search began for the DC-expressed molecule responsible for this ICAM-3-binding activity. Anti-DC hybridoma supernatants were screened for the ability to inhibit ICAM-3 binding to DCs and a C-type lectin was eventually cloned and designated DC-SIGN [8]. This review describes the genomic organization, expression pattern, postulated functions and role in pathology of DC-SIGN and a closely related molecule designated DC-SIGNR {also called liver/lymph node ('L')-SIGN and DC-SIGN2 by other groups [9,10]}.

The sequence designated DC-SIGN was identical to that of a C-type lectin cloned due to its ability to bind the HIV envelope protein gp120 in 1992 [11,12]. Subsequent work by the group which described DC-SIGN demonstrated that it could bind HIV gp120 with high affinity and for up to several days. DC-SIGN-bound virus could infect other cells with appropriate HIV receptors and co-receptors, and this process was described as HIV infection *in trans* (Figure 1) [11]. At this time, DC-SIGN expression was reported to be restricted to a subset of DC with an immature phenotype. Analogous to ligand binding by other C-type lectins, DC-SIGN binding to

both ICAM-3 and HIV can be abrogated by mannose and calcium-chelating agents [8,11]. Our more recent work has demonstrated the ability of DC-SIGN to mediate HIV infection of permissive cell types *in cis* (Figure 1) [13].

GENOMIC CLONING OF DC-SIGN AND A NOVEL TRANSCRIPT, DC-SIGNR

DC-SIGN and a closely related (73% identical) transcript, DC-SIGNR, were cloned from placental cDNA [8,9,14,15]. The genes encode 77% identical type II integral membrane proteins, which have tracts of repeats of 23 amino acids, predicted to form a coiled-coil neck region (Figure 2) [14]. This supports a C-type lectin domain, which possess motifs known to bind mannose in a calcium-dependent fashion [14], consistent with the observed calcium-dependent binding of DC-SIGN to HIV gp120 and ICAM-3 [8,11], both of which possess high-mannose structures [16–18]. DC-SIGN and DC-SIGNR have since been crystallized in the presence of high-mannose oligosaccharides [19]. The predicted α-helical structure of the neck regions and the high level of identity between repeats, both within each molecule and between DC-SIGN and DC-SIGNR, suggested that both homo- and hetero-oligomerization might occur via this region [14]. Subsequent structural analysis has demonstrated that the extracellular domains of both DC-SIGN and DC-SIGNR form tetramers [20]. The neck region of DC-SIGN and DC-SIGNR may also be important in determining the orientation of the carbohydrate-binding C-type lectin domain and may therefore have an impact on ligand specificity [20].

Polymorphism analysis of the numbers of repeats present in this neck region was undertaken (Table 1) [9]. Alleles of *DC-SIGNR* have between 3–9 repeats in the neck region at the genomic level [9]. This variation may have important consequences for both the function and the expression levels of the DC-SIGNR protein, particularly in individuals with two non-identical alleles of *DC-SIGNR*. It is possible that the higher level of polymorphism seen in DC-SIGNR reflects a function that is less critical for survival or reproduction.

Table 1 Results of DC-SIGNR polymorphism analysis from Bashirova et al. [9]

This group did not find any significant polymorphism in the numbers of neck repeats in DC-SIGN [9]. Reproduced from The Journal of Experimental Medicine, 2001, vol. 193, pp. 671–678, by copyright permission of The Rockefeller University Press.

Number of repeats	Allele frequency	Percentage frequency
3	1	0.3
4	25	3.6
5	202	28.9
6	86	12.2
7	377	53.9
8	2	0.3
9	7	1.0
Total	700	100

DC-SIGN AND *DC-SIGNR* FORM A TIGHT CLUSTER WITH CD23 ON chromosome 19p13.3

Radiation hybrid panel mapping [21] of *DC-SIGN* and *DC-SIGNR* gave a localization on chromosome 19p13.3 [14]. Subsequent PAC library [22] screening by hybridization and PCR showed *DC-SIGN* and *DC-SIGNR* to be tightly linked to *CD23* [14], a C-type lectin known to map to 19p13 [23]. These results concur with preliminary high-throughput genomic sequence data from previous studies [9,15]. All three C-type lectin genes have analogous genomic organization (Figure 3)

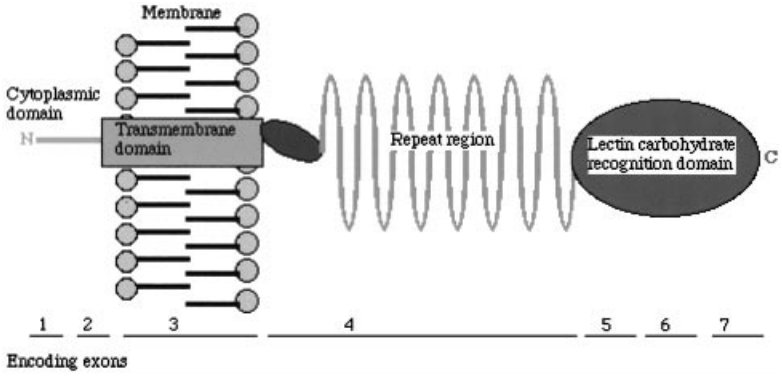

Figure 2 Predicted protein domains in DC-SIGN and DC-SIGNR

The exons encoding these are shown below their respective domains. The repeat region forms a putative neck region, supporting the CRD above the membrane [14].

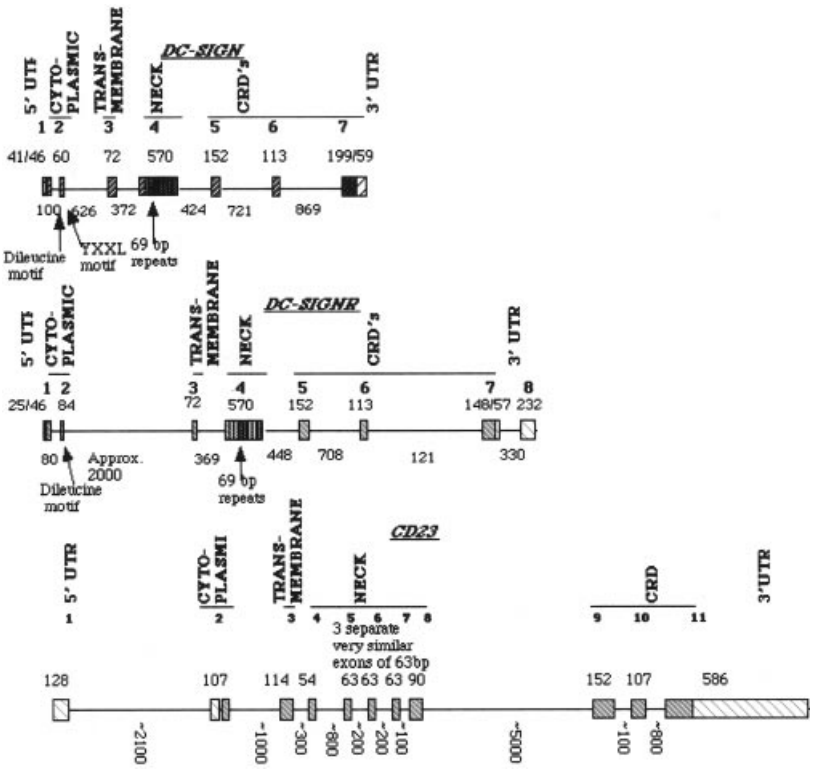

Figure 3 ***DC-SIGN*** **and** ***DC-SIGNR*** **gene structures assembled by comparison of the full genomic sequence of** ***DC-SIGN*** **and partial sequence of** ***DC-SIGNR***

The gene structure of closely linked *CD23* is included for comparison [24]. *DC-SIGN* and *DC-SIGNR* have very similar gene structures, except for the presence of an additional 3′ untranslated exon in *DC-SIGNR*. Exon sizes are shown in nucleotides above the figure and intron sizes are below [14]. In *CD23*, the three repeats of the neck region are encoded by three separate 63 bp exons [24]. The seven 69 bp repeats of both *DC-SIGN* and *DC-SIGNR* are each encoded by a single 570 bp exon [14]. UTR, untranslated region.

[24], suggesting that they were duplicated from a single ancestral gene. Indeed, recent murine data [25] suggest that duplication may have occurred in an ancestor of modern day mammals, since mice have five *DC-SIGN*/*DC-SIGNR* genes of which four may represent *DC-SIGNR* homologues and one a *DC-SIGN* homologue. The murine homologues also show close genetic linkage to *CD23* [25]. Further evidence for gene duplication includes: (i) the ability of mannose to provide incomplete inhibition of the binding of CD23 to certain of its ligands [26,27], analogous to the mannose-mediated inhibition of ligand binding by DC-SIGN and DC-SIGNR [8,9,11,28], and (ii) recent data demonstrating that DC-SIGN expression, similar to that of CD23, is critically regulated by interleukin (IL)-4 [29].

DC-SIGN AND PARALOGOUS REGIONS: THE CHICKEN STORY

In addition to the proposed duplication of an ancestral lectin gene on chromosome 19p13.3 to give *DC-SIGN*, *DC-SIGNR* and *CD23*, a larger scale and more ancient duplication may have resulted in these lectins being on chromosome 19p13.3. Kasahara and co-workers [30–32] proposed that duplications of the entire genome took place in an ancestor of jawless fish, underpinning the observed paralogy between human chromosome 19p13 and parts of human chromosomes 6p21 (the region encoding the MHC) and parts of chromosome 12p (the site of the natural killer cell lectin locus) [30–32]. The chicken MHC (the B locus), which is encoded by a region syntenic to human 6p21, contains two lectin genes, whereas no lectins are encoded in the human MHC region [33]. The predicted proteins from the chicken B locus lectins show between 27 and 31% identity at the amino acid level across the CRD with DC-SIGN and DC-SIGNR. It may be that homologues of the chicken B locus lectins have been lost from the human MHC (6p21), while remaining in a paralogous region on human chromosome 19p13. The lectins in the human natural killer cell lectin locus on chromosome 12p show lower identity to DC-SIGN and DC-SIGNR, but retain a similar gene structure. Therefore the chicken B locus lectins, the natural killer cell lectins and the DC-SIGN gene family may have arisen from a single ancestral gene,

which has undergone multiple duplications during evolution.

DC-SIGN EXPRESSION ON RESTRICTED ANTIGEN-PRESENTING CELLS: EVIDENCE FOR A ROLE IN IMMUNOMODULATION?

DCs are a very heterogeneous group of antigen-presenting cells. Different subpopulations differ in the efficacy with which they activate naïve T-lymphocytes, besides inducing the development of phenotypically different T-lymphocyte responses [34]. Defining the exact DC subpopulations that express DC-SIGN might give clues to the biological functions of DC-SIGN. DCs express DC-SIGN at all mucosal surfaces and ubiquitously within fibrous connective tissue, whereas epidermal Langerhans cells are consistently DC-SIGN-negative [8,11,35,36]. However, a closely related mannose-binding lectin, Langerin, may complement DC-SIGN's role in Langerhans cells [35].

DC-SIGN$^+$ DCs are present in all lymphoid organs [8,11,36]. In lymph nodes, the majority of DC-SIGN$^+$ DCs are located near the entry points for lymph in the cortical sinuses, whereas most DCs in the paracortex fail to express DC-SIGN. Concomitantly, DCs expressing appreciable levels of activation/maturation markers, such as CD83, CD86 and cmrf-44 (located almost exclusively in lymph node paracortex), are negative for DC-SIGN. In addition to their pronounced dendritic morphology, DC-SIGN$^+$ cells are CD3$^-$ CD79a$^-$ CD56$^-$ CD68$^+$ CD14^{weak} HLA-DR^{weak} S100$^{+/-}$, consistent with an immature DC phenotype (Figure 4). Foetal tissues show a very similar distribution of DC-SIGN expression to that in the adult, suggesting that at the majority of sites antigenic stimulation is unlikely to be critical for DC-SIGN expression [36]. However, two sites deserve special consideration.

The adult lung contains a population of DCs located within the connective tissue between alveoli, in addition to the alveolar macrophage population within alveolar airspaces. These populations are normally distinguished by their anatomical localization, rather than by the expression of specific markers. Both the lung DCs and more surprisingly the alveolar macrophages are DC-SIGN$^+$ [36]. Alveolar macrophages also express appreciable levels of the HIV-entry receptors CD4 and chemokine receptor 5 (CCR5), suggesting that DC-SIGN could mediate HIV infection of alveolar macrophages *in cis* (Figure 1) [13]. This may explain why alveolar macrophages provide a viral reservoir during chronic HIV infection [37]. In the neonate, smaller numbers of alveolar macrophages are present, but these are universally DC-SIGN$^-$. Therefore, although no antigenic stimulation appears to be required to induce expression of DC-SIGN on DCs, macrophages may require environmental instruction to express DC-SIGN [36]. In support of this

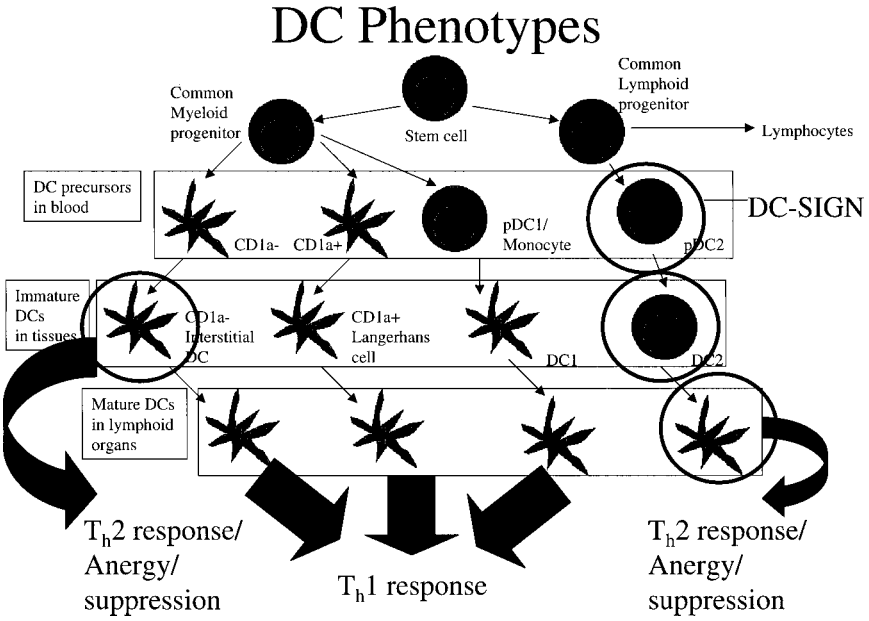

Figure 4 Summary of DC phenotypes expressing DC-SIGN

A number of phenotypically and functionally different DC lineages have been defined and are summarized here. DC precursors are generated in bone marrow and then enter the blood. They may then leave the blood and enter tissues, where they continually take up both self and foreign antigens. Certain stimuli, such as tissue injury, can induce migration to local lymph nodes where the DCs interact with and activate T-lymphocytes. The most likely T-cell phenotypes generated by these interactions are included [34]. DC phenotypes expressing DC-SIGN are encircled [13,35,36].

hypothesis, IL-13 treatment of macrophages in culture can induce DC-SIGN RNA production [36].

DC-SIGN is expressed by DCs in the thymus, located predominantly in the cortex. The thymus is the site of maturation of all T-lymphocytes. Therefore, thymic DCs could bind HIV particles for protracted periods via DC-SIGN and could mediate HIV infection of thymocytes *in trans*. The expression of CD4 by all thymocytes at the double positive ($CD4^+$ $CD8^+$) stage, in addition to a number of chemokine receptors permissive for HIV infection, could substantially increase the thymocyte death toll. DC-SIGN expression in the thymus may therefore significantly diminish the potential for T-lymphocyte repopulation despite maximal highly active anti-retroviral therapy ('HAART') and minimal viral load [36].

A number of DC lineages have been defined (Figure 4) [34]. Besides DC-SIGN expression on immature/unactivated DCs, one further DC subset appears to express DC-SIGN under certain circumstances. Plasmacytoid or DC2 DCs induce T-lymphocytes to become T-helper 2 (Th2; anti-parasite and pro-allergic) cells under many circumstances [34]. Use of a combination of the DC2 markers blood DC antigen-2 ('BDCA-2') and CD123 demonstrated DC2 expression of DC-SIGN protein in allergic nasal polyps and some lymph nodes [36]. Furthermore, thymic cortical DCs, which we have shown to express DC-SIGN [36], have previously been reported to have a DC2 phenotype [38]. The IL-4-dependent expression of DC-SIGN [29], the ability of the Th2 cytokine IL-13 to induce DC-SIGN RNA in macrophages [36] and the close genetic linkage between DC-SIGN CD23 (an integral part of the Th2 axis of immunity) suggest that DC-SIGN too may play a critical role in the Th2 axis of immunity.

As discussed above, in addition to DCs, macrophages can express DC-SIGN, and IL-13 treatment of macrophages *in vitro* can induce DC-SIGN RNA production [13,36]. DC-SIGN expression appears to be restricted to alveolar and decidual macrophages (see below) [13,36]. IL-13 may be abundant at both these sites and may therefore be important in the induction of DC-SIGN expression by macrophages [36]. Finally, up to 20 % of lymph node sinus endothelial cells are $DC\text{-}SIGN^+$. Concomitant DC-SIGN and DC-SIGNR expression occurs at this site. Heterotetramerization of DC-SIGN and DC-SIGNR may allow modulation of the binding and/or trafficking properties of the two molecules [39].

DC-SIGNR EXPRESSION ON RESTRICTED ENDOTHELIA

DC-SIGNR was shown to bind HIV and mediate HIV infection *in trans* in a very similar manner to DC-SIGN (Figure 1) [28]. DC-SIGNR binding to ICAM-3 has also been demonstrated, although the functional outcome of this interaction remains unclear [9]. Therefore, detailed expression analysis of DC-SIGNR was paramount to give clues to its function *in vivo*.

DC-SIGNR is expressed by lymph node sinus endothelium and hepatic sinusoidal endothelium, in addition to placental capillaries [9,28]. DC-SIGNR is therefore unlikely to be important in the early stages of HIV infection, but may potentiate the infection of T-lymphocytes *in trans* in the blood and lymph nodes once HIV infection is established. Additionally, the role of DC-SIGNR in hepatotropic and other infections remains to be determined. In primates, DC-SIGNR expression is similar to that in man, although DC-SIGNR has also been detected on a proportion of capillaries in the distal ileum [40].

Factors determining the expression pattern of DC-SIGNR are difficult to elucidate, although preliminary work has yielded some clues. Whether the endothelial cells line blood vessels or lymphatics appears to be unimportant. In addition, endothelial activation is not required. The specialization of the endothelium varies between $DC\text{-}SIGNR^+$ sites [39]. However, the endothelial microenvironment appears to be important. Hyperplasia of $DC\text{-}SIGNR^+$ endothelium, both in lymph node sinus hyperplasia and cat scratch disease, allows the maintenance of intensely positive DC-SIGNR immunostaining. However, neoplasms of DC-SIGNR-positive endothelial cell origin (hepatic angiomas and angiosarcomas), which induce their own stroma and thus microenvironment, are $DC\text{-}SIGNR^-$ [39].

DC-SIGN AND DC-SIGNR MAY FACILITATE THE INTRAUTERINE VERTICAL TRANSMISSION OF HIV

Around 1.5–2 % of cases of vertical transmission of HIV appear to occur across the placenta, and no current obstetric or pharmacological measures have been able to reduce this [41]. The mechanisms underlying this presumed transplacental transmission of HIV are poorly understood. Phenotypic characterization of the exact cell type(s) responsible for DC-SIGN RNA expression in the placenta was undertaken using immunohistochemistry. DC-SIGN was expressed by $CD68^+$ $HLA\text{-}II^+$ $CD14^{low}$ $S100^{+/-}$ $CD83^-$ $CD86^-$ $cmrf\text{-}44^-$ cells within chorionic villi, consistent with Hofbauer cells (specialized foetal macrophages), and also by $CD68^+$ $HLA\text{-}II^+$ $CD14^{high}$ $S100^-$ $CD83^-$ $CD86^-$ $cmrf\text{-}44^-$ decidual macrophages (Figure 5) [42]. Two other studies have also demonstrated DC-SIGN expression on Hofbauer cells [15,43]. The $DC\text{-}SIGN^+$ Hofbauer cells co-expressed CD4 and the chemokine receptors CCR5 and chemokine receptor of the CXC family ('CXCR4'), observations that may

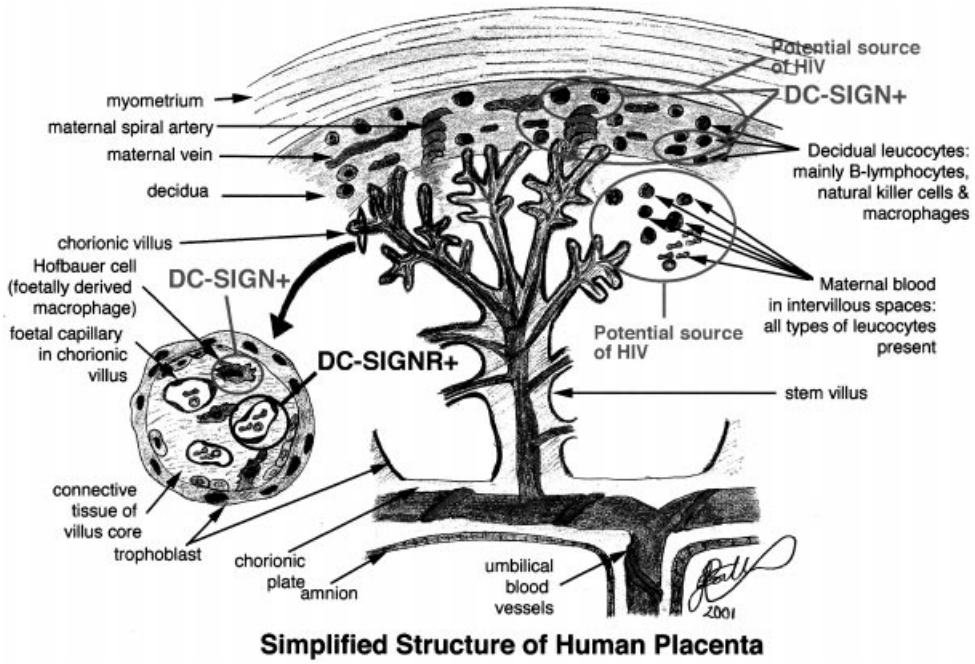

Figure 5 Summary of the placenta and salient features that may be involved in transplacental transmission of HIV

The intimate relationship between foetal and maternal tissues can be appreciated. Resident leucocyte populations on both the foetal and maternal sides are indicated [57,58]. DC-SIGN is expressed both on foetally derived Hofbauer cells found within chorionic villi and on maternally derived decidual macrophages. These cell populations are in very close proximity. Closely related DC-SIGNR is expressed on placental capillary endothelium. Reprinted from Int. J. Biochem. Cell Biol., vol. 35, E. J. Soilleux and N. Coleman, "Transplacental transmission of HIV: a potential role for HIV binding", pp. 283–287, © (2003), with permission from Elsevier Science.

account for the ability of these cells to become infected with HIV. DC-SIGN could therefore potentiate HIV infection of Hofbauer cells *in cis* [13,42]. Foetal DC-SIGN$^+$ Hofbauer cells are separated by only a layer of trophoblast from both DC-SIGN$^+$ maternal cells and maternal blood, potential sources of HIV in infected mothers (Figure 5) [42]. Previous studies have suggested that this trophoblast layer is frequently breached during pregnancy [44]. DC-SIGN$^+$ cells were also detectable in umbilical cord blood, suggesting that they may traffic from the placenta into the foetus (E. J. Soilleux, unpublished work). In addition, DC-SIGNR is expressed at high levels on the majority of placental blood vessels (Figure 5). DC-SIGN and DC-SIGNR may facilitate the transplacental transmission of HIV by one or more of the following mechanisms. (1) Hofbauer cells either infected with HIV or with virions adsorbed to their surface may enter the umbilical vein and migrate into the foetus. (2) Hofbauer cells, similarly either infected with HIV or with virions adsorbed to their surface, may remain *in situ* within chorionic villi, presenting antigen to and infecting T-lymphocytes. The observation that T-lymphocytes are inconspicuous within chorionic villi suggests that this mechanism is less likely. (3) Hofbauer cells may become infected with HIV [45–50] and may release infectious viral particles, which may become adsorbed to DC-SIGNR on immediately adjacent placental capillary endothelium. The endothelium may, in turn, mediate infection of HIV receptor-positive T-lymphocytes circulating in the blood. Infected T-lymphocytes or Hofbauer cells either productively infected with HIV or simply with the virus adsorbed to their surface may then travel between the placenta and the foetus in umbilical cord blood.

WHAT'S IT ALL FOR? BIOLOGICAL ROLES OF DC-SIGN AND DC-SIGNR

Both DC-SIGN and DC-SIGNR can bind ICAM-3 and are thus likely to be important in the early phase of T-lymphocyte activation. The outcome of these interactions still requires further investigation. However, several common themes emerge. The Th2 cytokines IL-4 and IL-13 are important in inducing DC-SIGN expression under specific conditions, and DC-SIGN is expressed on DC2 DCs, as well as on immature DCs [29,36]. DC-SIGN is closely linked to CD23, a molecule of great important in the Th2 axis of immunity [14]. Neither immature (DC-SIGN$^+$) DCs nor (DC-SIGN$^+$) DC2s are likely to induce strong Th1 responses in T-lymphocytes, erring more towards Th2 or tolerogenic responses [34]. DC-SIGNR is expressed at sites known to present antigen to T-lymphocytes, but likely to induce tolerance to these

antigens [28]. Therefore, this novel lectin family may be important in the induction of Th2 and/or immunomodulatory responses.

PATHOBIOLOGY OF DC-SIGN

As discussed above, both DC-SIGN and DC-SIGNR can mediate efficient HIV infection *in trans* of cells expressing appropriate entry receptors (Figure 1) [11]. Additionally, DC-SIGN can mediate a similar process *in cis* (Figure 1) [13]. The presence of DC-SIGN at mucosal surfaces suggests that it may be important early in the chronology of HIV infection, perhaps potentiating viral entry to deeper tissues [11]. The expression of DC-SIGNR in liver and lymph nodes may potentiate the infection of other cell types once HIV infection is more established [28]. Both DC-SIGN and DC-SIGNR may be important in facilitating intrauterine vertical transmission of HIV. HIV is not the only infection in which these molecules may play a role. Recent data demonstrate the capacity of both DC-SIGN and DC-SIGNR to mediate cellular entry of Ebola virus both *in cis* and *in trans* [51]. Additionally, *Leishmania* amastigotes may use DC-SIGN as an entry receptor [52].

DC-SIGN may also have an important immunomodulatory role in pathobiology. Our recent work has demonstrated large numbers of DC-SIGN$^+$ DCs in soft tissue tumours [53]. These DC-SIGN$^+$ cells may be involved in the induction of tolerance to neo-antigens. However, the very recent demonstration that DC-SIGN may traffic from the DC surface to endosomal and lysosomal compartments raises the possibility that DC-SIGN may act as an endocytic receptor in a manner analogous to many other C-type lectins [54,55]. The crystallographic structure of DC-SIGN suggests that it may bind more avidly to highly mannosylated self proteins than to microbial antigens [19]. Therefore, DC-SIGN could also be important for the endocytosis of neoplasm-derived neo-antigens, facilitating their presentation to T-lymphocytes.

DC-SIGN, DC-SIGNR AND THE FUTURE

Much remains to be clarified about the physiological and pathophysiological roles of DC-SIGN and DC-SIGNR. However, a number of potential therapeutic strategies based on DC-SIGN can be envisaged. One very pertinent question is how HIV remains DC-SIGN bound and viable for such long periods. The ability of DC-SIGN to enter endosomal and lysosomal compartments suggests that the virus particles may risk degradation. However, DCs possess a number of rapid recycling pathways within the superficial part of their cytoplasm [56]. Furthermore, recent work has demonstrated that DC-SIGN-bound HIV is rapidly internalized into a low pH non-lysosomal compartment and that such internalization is necessary for the virus to retain competence to infect target cells [55]. One therapeutic strategy to prevent the early stages of HIV infection via mucosal surfaces would be to interrupt this pathway. Similar mechanisms may help prevent the transplacental transmission of HIV. However, any disruption of the physiological functions of DC-SIGN may have deleterious consequences. In particular, decidual macrophages, which are also DC-SIGN$^+$, may play a critical immunomodulatory role in preventing maternal immune responses directed against the placenta, and disruption of this function may lead to abortion.

Phenotypes of DC-SIGN$^+$ DCs are becoming more clearly defined. Where antigen presentation by DC-SIGN$^+$ DCs may have beneficial immuological consequences, antigen delivery could be targeted via specific anti-(DC-SIGN) antibodies. Additionally, where DC-SIGN$^+$ DCs are deemed phenotypically inappropriate, molecules, such as cytokines or even cytotoxic agents, might be targeted to DC-SIGN$^+$ cells to modulate the phenotype of the immune response. The potential for future use of DC-SIGN as a molecular target is the result of its relative specificity for DCs. There is, however, one simpler and more immediate application, in both research and histopathological diagnosis. This is the use of anti-(DC-SIGN) antibodies for the identification of DCs.

ACKNOWLEDGMENTS

E.J.S. was supported by a Medical Research Council Clinical Training Fellowship and by the Sackler Foundation.

REFERENCES

1 Vasta, G. R., Ahmed, H., Fink, N. E. et al. (1994) Animal lectins as self/non-self recognition molecules. Biochemical and genetic approaches to understanding their biological roles and evolution. Ann. N.Y. Acad. Sci. **712**, 55–57

2 Bezouska, K., Crichlow, G. V., Rose, J. M., Taylor, M. E. and Drickamer, K. (1991) Evolutionary conservation of intron position in a subfamily of genes encoding carbohydrate-recognition domains. J. Biol. Chem. **266**, 11604–11609

3 Hartgers, F. C., Figdor, C. G. and Adema, G. J. (2000) Towards a molecular understanding of dendritic cell immunobiology. Immunol. Today **21**, 542–545

4 Weis, W. I., Taylor, M. E. and Drickamer, K. (1998) The C-type lectin superfamily in the immune system. Immunol. Rev. **163**, 19–34
5 Drickamer, K. (1999) C-type lectin-like domains. Curr. Opin. Struct. Biol. **9**, 585–590
6 Beavil, R. L., Graber, P., Aubonney, N., Bonnefoy, J. Y. and Gould, H. J. (1995) CD23/Fc epsilon RII and its soluble fragments can form oligomers on the cell surface and in solution. Immunology **84**, 202–206
7 Hart, D. N. (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. Blood **90**, 3245–3287
8 Geijtenbeek, T. B., Torensma, R., van Vliet, S. J. et al. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell (Cambridge, Mass.) **100**, 575–585
9 Bashirova, A. A., Geijtenbeek, T. B., van Duijnhoven, G. C. et al. (2001) A dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J. Exp. Med. **193**, 671–678
10 Mummidi, S., Catano, G., Lam, L. et al. (2001) Extensive repertoire of membrane-bound and soluble DC-SIGN1 and DC-SIGN2 isoforms: Inter-individual variation in expression of DC-SIGN transcripts. J. Biol. Chem. **276**, 33196–33212
11 Geijtenbeek, T. B., Kwon, D. S., Torensma, R. et al. (2000) DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. Cell (Cambridge, Mass.) **100**, 587–597
12 Curtis, B. M., Scharnowske, S. and Watson, A. J. (1992) Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. Proc. Natl. Acad. Sci. U.S.A. **89**, 8356–8360
13 Lee, B., Leslie, G., Soilleux, E. et al. (2001) *cis* Expression of DC-SIGN allows for more efficient entry of human and simian immunodeficiency viruses via CD4 and a co-receptor. J. Virol. **75**, 12028–12038
14 Soilleux, E. J., Barten, R. and Trowsdale, J. (2000) Cutting edge: DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J. Immunol. **165**, 2937–2942
15 Mummidi, S., Catano, G., Lam, L. et al. (2001) Extensive repertoire of membrane-bound and soluble dendritic cell-specific ICAM-3-grabbing nonintegrin 1 (DC-SIGN1) and DC-SIGN2 isoforms. Inter-individual variation in expression of DC-SIGN transcripts. J. Biol. Chem. **276**, 33196–33212
16 Shenoy, S. R., O'Keefe, B. R., Bolmstedt, A. J., Cartner, L. K. and Boyd, M. R. (2001) Selective interactions of the human immunodeficiency virus-inactivating protein cyanovirin-N with high-mannose oligosaccharides on gp120 and other glycoproteins. J. Pharmacol. Exp. Ther. **297**, 704–710
17 Sanders, R. W., Venturi, M., Schiffner, L. et al. (2002) The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. J. Virol. **76**, 7293–7305
18 Funatsu, O., Sato, T., Kotovuori, P., Gahmberg, C. G., Ikekita, M. and Furukawa, K. (2001) Structural study of N-linked oligosaccharides of human intercellular adhesion molecule-3 (CD50). Eur. J. Biochem. **268**, 1020–1029
19 Feinberg, H., Mitchell, D. A., Drickamer, K. and Weis, W. I. (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science (Washington, D.C.) **294**, 2163–2166
20 Mitchell, D. A., Fadden, A. J. and Drickamer, K. (2001) A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR. Subunit organization and binding to multivalent ligands. J. Biol. Chem. **276**, 28939–28945
21 Gyapay, G., Schmitt, K., Fizames, C. et al. (1996) A radiation hybrid map of the human genome. Hum. Mol. Genet. **5**, 339–346
22 Ioannou, P. A. and De Jong, P. J. (1996) Construction of a bacterial artificial chromosome library using the modified P1 (PAC) system. In Current Protocols in Human Genetics (Dracopoli, N. C., Haines, J. L., Korf, B. R., Moir, D. T., Morton, C. C., Seidman, C. E., Seidman, J. G. and Smith, D. R., eds), pp. 5.15.1–5.15.24, John Wiley and Sons, New York
23 Wendel-Hansen, V., Riviere, M., Uno, M. et al. (1990) The gene encoding CD23 leukocyte antigen (FCE2) is located on human chromosome 19. Somat. Cell. Mol. Genet. **16**, 283–286
24 Suter, U., Bastos, R. and Hofstetter, H. (1987) Molecular structure of the gene and the 5′-flanking region of the human lymphocyte immunoglobulin E receptor. Nucleic Acids Res. **15**, 7295–7308
25 Park, C. G., Takahara, K., Umemoto, E. et al. (2001) Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN. Int. Immunol. **13**, 1283–1290
26 Bajorath, J. and Aruffo, A. (1996) Structure-based modeling of the ligand binding domain of the human cell surface receptor CD23 and comparison of two independently derived molecular models. Protein Sci. **5**, 240–247
27 Richardson, D. R., Cameron, K., Robinson, B. and Turner, K. J. (1993) The mechanisms of IgE uptake by human alveolar macrophages and a human B-lymphoblastoid cell line (Wil-2wt). Immunology **79**, 305–311
28 Pohlmann, S., Soilleux, E. J., Baribaud, F. et al. (2001) DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection *in trans*. Proc. Natl. Acad. Sci. U.S.A. **98**, 2670–2675
29 Relloso, M., Puig-Kroger, A., Pello, O. M. et al. (2002) DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-β, and anti-inflammatory agents. J. Immunol. **168**, 2634–2643
30 Kasahara, M. (1997) New insights into the genomic organization and origin of the major histocompatibility complex: role of chromosomal (genome) duplication in the emergence of the adaptive immune system. Hereditas **127**, 59–65
31 Kasahara, M. (1999) The chromosomal duplication model of the major histocompatibility complex. Immunol. Rev. **167**, 17–32
32 Kasahara, M., Nakaya, J., Satta, Y. and Takahata, N. (1997) Chromosomal duplication and the emergence of the adaptive immune system. Trends Genet. **13**, 90–92
33 Kaufman, J., Milne, S., Gobel, T. W. et al. (1999) The chicken B locus is a minimal essential major histocompatibility complex. Nature (London) **401**, 923–925
34 Liu, Y. J. (2001) Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell (Cambridge, Mass.) **106**, 259–262
35 Soilleux, E. J. and Coleman, N. (2001) Langerhans cells and the cells of Langerhans cell histiocytosis do not express DC-SIGN. Blood **98**, 1987–1988
36 Soilleux, E. J., Morris, L. S., Leslie, G. et al. (2002) Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations *in situ* and *in vitro*. J. Leukocyte Biol. **71**, 445–457
37 Sierra-Madero, J. G., Toossi, Z., Hom, D. L., Finegan, C. K., Hoenig, E. and Rich, E. A. (1994) Relationship between load of virus in alveolar macrophages from human immunodeficiency virus type 1-infected persons, production of cytokines, and clinical status. J. Infect. Dis. **169**, 18–27
38 Vandenabeele, S., Hochrein, H., Mavaddat, N., Winkel, K. and Shortman, K. (2001) Human thymus contains 2 distinct dendritic cell populations. Blood **97**, 1733–1741
39 Soilleux, E. J., Morris, L. S., Rushbrook, S., Lee, B. and Coleman, N. (2002) Expression of human immunodeficiency virus (HIV)-binding lectin DC-SIGNR: consequences for HIV infection and immunity. Hum. Pathol. **33**, 652–659

40 Jameson, B., Baribaud, F., Pohlmann, S. et al. (2002) Expression of DC-SIGN by dendritic cells of intestinal and genital mucosae in humans and rhesus macaques. J. Virol. **76**, 1866–1875
41 Dorenbaum, A. (2001) Report of results of PACTG 316: An international phase III trial of standard anti-retroviral (ARV) prophylaxis plus nevirapine (NVP) for prevention of perinatal HIV transmission. Abstract LB7, 8th Conference on Retroviruses and Opportunistic Infections, Chicago, IL, U.S.A.
42 Soilleux, E. J., Morris, L. S., Lee, B. et al. (2001) Placental expression of DC-SIGN may mediate intrauterine vertical transmission of HIV. J. Pathol. **195**, 586–592
43 Geijtenbeek, T. B., van Vliet, S. J., van Duijnhoven, G. C., Figdor, C. G. and van Kooyk, Y. (2001) DC-SIGN, a dentritic cell-specific HIV-1 receptor present in placenta that infects T cells *in trans* – a review. Placenta **22**, S19–S23
44 Newell, M. L. (1998) Mechanisms and timing of mother-to-child transmission of HIV-1. AIDS **12**, 831–837
45 Backe, E., Jimenez, E., Unger, M., Schafer, A., Jauniaux, E. and Vogel, M. (1992) Demonstration of HIV-1 infected cells in human placenta by *in situ* hybridisation and immunostaining. J. Clin. Pathol. **45**, 871–874
46 Jimenez, E., Unger, M., Blass, G. and Schafer, A. (1989) Immunohistologic study of the significance of the Hofbauer cell for the transplacental HIV infection chain. Arch. Gynecol. Obstet. **245**, 171–172
47 Kesson, A. M., Fear, W. R., Kazazi, F. et al. (1993) Human immunodeficiency virus type 1 infection of human placental macrophages in vitro. J. Infect. Dis. **168**, 571–579
48 Martin, A. W., Brady, K., Smith, S. I. et al. (1992) Immunohistochemical localization of human immunodeficiency virus p24 antigen in placental tissue. Hum. Pathol. **23**, 411–414
49 Lewis, S. H., Reynolds-Kohler, C., Fox, H. E. and Nelson, J. A. (1990) HIV-1 in trophoblastic and villous Hofbauer cells, and haematological precursors in eight-week fetuses. Lancet **335**, 565–568
50 Sheikh, A. U., Polliotti, B. M. and Miller, R. K. (2000) Human immunodeficiency virus infection: *in situ* polymerase chain reaction localization in human placentas after in utero and in vitro infection. Am. J. Obstet. Gynecol. **182**, 207–213
51 Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L. and Delgado, R. (2002) C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus *in cis* and *in trans*. J. Virol. **76**, 6841–6844
52 Colmenares, M., Puig-Kroger, A., Muniz Pello, O., Corbi, A. L. and Rivas, L. (2002) Dendritic-cell specific ICAM-3 grabbing nonintegrin (DC-SIGN, CD209), a C-type surface lectin in human dendritic cells, is a receptor for *Leishmania amastigotes*. J. Biol. Chem. **277**, 36766–36769
53 Soilleux, E. J., Rous, B., Love, K. et al. (2003) Myxofibrosarcomas contain large numbers of infiltrating immature dendritic cells. Am. J. Clin. Pathol., in the press
54 Engering, A., Geijtenbeek, T. B., van Vliet, S. J. et al. (2002) The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. J. Immunol. **168**, 2118–2126
55 Kwon, D. S., Gregorio, G., Bitton, N., Hendrickson, W. A. and Littman, D. R. (2002) DC-SIGN-mediated internalization of HIV is required for *trans*-enhancement of T cell infection. Immunity **16**, 135–144
56 Thery, C. and Amigorena, S. (2001) The cell biology of antigen presentation in dendritic cells. Curr. Opin. Immunol. **13**, 45–51
57 Sadler, T. W. (1990) Langman's Medical Embryology. Williams and Wilkins, Baltimore
58 Vince, G. S. and Johnson, P. M. (2000) Leucocyte populations and cytokine regulation in human uteroplacental tissues. Biochem. Soc. Trans. **28**, 191–195