

Expression of C-type lectin receptors by subsets of dendritic cells in human skin

Susanne Ebner, Zita Ehammer, Sandra Holzmann, Philipp Schwingshackl, Markus Forstner, Patrizia Stoitzner, Georg M. Huemer¹, Peter Fritsch and Nikolaus Romani

Departments of Dermatology and ¹Plastic and Reconstructive Surgery, Innsbruck Medical University, Innsbruck, Austria

Keywords: DC-SIGN, DEC-205, dermal dendritic cells, Langerhans cells, Langerin

Abstract

C-type lectins are cell surface receptors that recognize carbohydrate structures which are often part of microbial pathogens. Several of these molecules are expressed on dendritic cells and are involved in antigen uptake. Expression of C-type lectins on dendritic cells of the human skin, i.e. Langerhans cells of the epidermis and dermal dendritic cells, has been incompletely studied to date. We therefore investigated C-type lectins *in situ* and on dendritic cells obtained by migration from skin explants by immunofluorescence and flow cytometry. Emphasis was laid on expression patterns of DEC-205/CD205 and BDCA-2, a marker for plasmacytoid dendritic cells. Langerhans cells *in situ* expressed low levels of DEC-205. Expression was upregulated upon maturation in skin explant organ culture. Most dermal dendritic cells were found to be positive for DEC-205 and DC-SIGN/CD209. Few BDCA-2-expressing cells were found in most skin samples. They were located in small groups in the dermis close beneath the basement membrane. The vast majority of all types of dendritic cells in normal human skin was of immature phenotype, i.e. did not express DC-LAMP/CD208. It is concluded that normal appearing human skin harbors different subsets of dendritic cells including few scattered BDCA-2-expressing cells, presumably plasmacytoid dendritic cells, expressing variable sets of C-type lectin receptors. This may critically contribute to the capacity of the skin immune system to flexibly respond to the world of microbial pathogens.

Introduction

Dendritic cells are highly specialized antigen presenting cells. They have developed and optimized several functions that enable them to fulfill their prime task, i.e. to initiate primary immune responses (1–3) but also to maintain peripheral tolerance (4). This functional spectrum comprises antigen uptake and processing capacities, which are exerted mostly in peripheral organs and tissues, as well as T cell sensitizing skills, which are performed in the lymphoid organs.

C-type lectin-like receptors are a set of cell surface receptors that are involved in the uptake of microbes or microbial products (5). They bind to carbohydrate structures, often of the mannose type and are thus thought to facilitate the adsorptive endocytosis of pathogens into dendritic cells. As opposed to their similar, though not identical binding preferences, their intracellular pathways upon ligand binding appear diverse. For instance, the DEC-205/CD205 receptor is efficiently routed to the MHC II-rich compartments of dendritic cells (6). As a consequence, antigens that are

targetted to this receptor are very efficiently presented to T cells, both for the generation of immunity (6) and for the maintenance of peripheral tolerance (7,8). The Langerin/CD207 receptor, on the other hand, does not reach the MHC II-rich compartment upon internalization (9). Its functional implications are not yet clear. It is an important molecular constituent of Birbeck granules, the conspicuous organelles that characterize Langerhans cells (10). The DC-SIGN/CD209 receptor is critically involved in the uptake of HIV (11) and other viruses, bacteria and even fungi (12–14). The BDCA-2 receptor is exclusively expressed on plasmacytoid dendritic cells where it is involved in the down-regulation of virus-triggered interferon-alpha/beta production (15).

Whereas an antibody against mouse DEC-205 [NLDC-145 (16)] has long existed, antibody tools to study expression of DEC-205 and other C-type lectins on human cells have only recently become available. The skin harbors at least two populations of dendritic cells, Langerhans cells of the

epidermis (17) and dermal dendritic cells (18). Expression of C-type lectin-like receptors in healthy human skin has not been systematically studied yet (5). Therefore, we investigate here the expression of these receptors in human skin *in situ* and in dendritic cells isolated from human skin.

Methods

Human skin

Clinically normal appearing skin included excised skin from plastic surgery of the breast ($n = 5$), normal skin used for autologous skin transplantation ($n = 7$) and foreskins ($n = 2$). Furthermore, biopsies of lichen ruber planus ($n = 2$) were examined. This common inflammatory skin disorder is characterized by epidermal damage presumably caused by a dense lymphocytic infiltrate in the underlying dermis. The majority of lymphocytes are CD8⁺ memory (CD45RO⁺) T cells. The nature of the antigenic stimulus responsible for this condition is not known (19).

Immunoreagents

The following unconjugated primary mouse mAbs were used: anti-CD205/DEC-205 [clone MG38 (20), IgG2b, gift of Dr R.M. Steinman, The Rockefeller University, New York], anti-CD207/Langerin [clone DCGM4 (9), IgG1, Beckman-Coulter, Fullerton, CA], anti-CD208/DC-LAMP [clone 104.G4 (21), IgG1, Beckman-Coulter], anti-CD209/DC-SIGN [clones AZN-D1 (22), IgG1, R&D Systems, Minneapolis, MN and DCN46, IgG2b, BD Biosciences, San Diego, CA] and anti-BDCA-2 [clone AC144 (15), IgG1, Miltenyi Biotec, Bergisch-Gladbach, Germany]. In addition, FITC-conjugated anti-CD207 (clones DCGM4 and 8080E) and anti-CD208, clone 104.G4 were generous gifts of Dr S. Lebecque, Schering-Plough Corporation, Dardilly, France. FITC-conjugated anti-CD209, FITC-anti-HLA-DR, and FITC-anti-CD14 were purchased from BD Biosciences. FITC-BDCA-2 was obtained from Miltenyi.

Preparation of specimens for immunofluorescence

Skin and, for control purposes, tonsils were snap-frozen in liquid nitrogen and sectioned on a cryostat (Frigocut, Leica, Wetzlar, Germany). Sections (5 μ m) were mounted onto glass slides, fixed in acetone and immunostained. Alternatively, skin was floated dermal side down on 0.5 M ammoniumthiocyanate for 20–30 min at 37°C (23). The epidermis was peeled off the dermis, cut into 5×5 mm pieces and fixed in acetone for 20 min at ambient temperature. Sheets were immunostained as described (24).

Labeling protocol for immunofluorescence

Primary mouse monoclonal antibodies were visualized using species-specific biotinylated anti-mouse Ig (Amersham-Pharmacia, Amersham, UK) followed by Streptavidin-FITC (Amersham). For double-labeling, Streptavidin-Texas Red (Amersham) was used (instead of Streptavidin-FITC), followed in sequence by mouse Ig (100 μ g/ml; Jackson Immunoresearch, Avondale, PA), for blocking residual binding sites of preceding antibodies, and FITC-conjugated mouse monoclonal antibodies. Immunolabeled specimens were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and viewed on a conventional fluorescence microscope (Olympus).

Skin explant culture

This was based on established methods (18,25,26). Standardized pieces of normal skin were prepared by means of an 8 mm punch from split-thickness skin (0.3 mm). These explants were floated on 1.5 ml culture medium (RPMI-1640 supplemented with 10% FCS; no cytokines added) in 24-well plates (one explant per well) for 2–5 days at 37°C. For immunofluorescence, epidermal sheets were prepared from cultured skin as described above for fresh skin. Cells that had emigrated into the culture medium during this time were harvested, counted and further evaluated by flow cytometry without further enrichment. Dendritic cells in these populations are consistently enriched to a variable, often high degree (10–80%) (18). In some experiments epidermis and dermis were split before the onset of culture by the enzyme dispase (Dispase I, 30 min/37°C; Roche Applied Sciences, Basel Switzerland) as described (18), and Langerhans cells and dermal dendritic cells were obtained from the cultured epidermal and dermal sheets, respectively.

Epidermal cell suspensions

They were procured by trypsinization of split thickness skin as described in detail (26,27). Trypsin-EDTA (Cat. No. 25300; Gibco-Invitrogen, Paisley, UK) was used at a final concentration of 0.05% trypsin and 0.53 mM EDTA at 4°C for 16 h.

Labeling protocol for flow cytometry

Cell suspensions were incubated on ice for 45 min each with primary mouse monoclonal antibodies followed by phycoerythrin-conjugated rabbit F(ab)₂ fragments anti-mouse Ig (DAKO-Cytomation, Glostrup, Denmark). Subsequently, cells were permeabilized by Fix&Perm™ (BD) and the incubation sequence was extended by mouse Ig (100 μ g/ml; Jackson) for blocking residual binding sites of preceding antibodies, and diverse FITC-conjugated mouse monoclonal antibodies. Specificity of staining was assessed by the use of control immunoglobulins of the same isotype with irrelevant specificity (Dako-Cytomation). Samples were evaluated on a FACScalibur instrument using CellQuest™ software (BD Biosciences, San Diego, CA). In some experiments leukocytes were specifically labeled with anti-CD45-PerCP (BD).

Results

Expression of DEC-205, Langerin, DC-SIGN and BDCA-2 in normal healthy skin in situ

CD205/DEC-205 expression. Expression on epidermal Langerhans cells was weak to absent on cryostat sections (Fig. 1). On epidermal sheets, however, Langerhans cells, as identified by either MHC class II or Langerin expression were unequivocally, though weakly, reactive with the anti-DEC-205 mAb (Fig. 1). In the underlying dermis, many DEC-205⁺ cells were present in the cryosections. Most of them were located in the upper, papillary dermis. They were irregular and dendritic in shape, and they were MHC class II⁺ in double labeling experiments (Fig. 1). MHC II⁺ blood vessels did not express DEC-205. Double-labeling with anti-DC-SIGN, a marker for dermal dendritic cells showed that

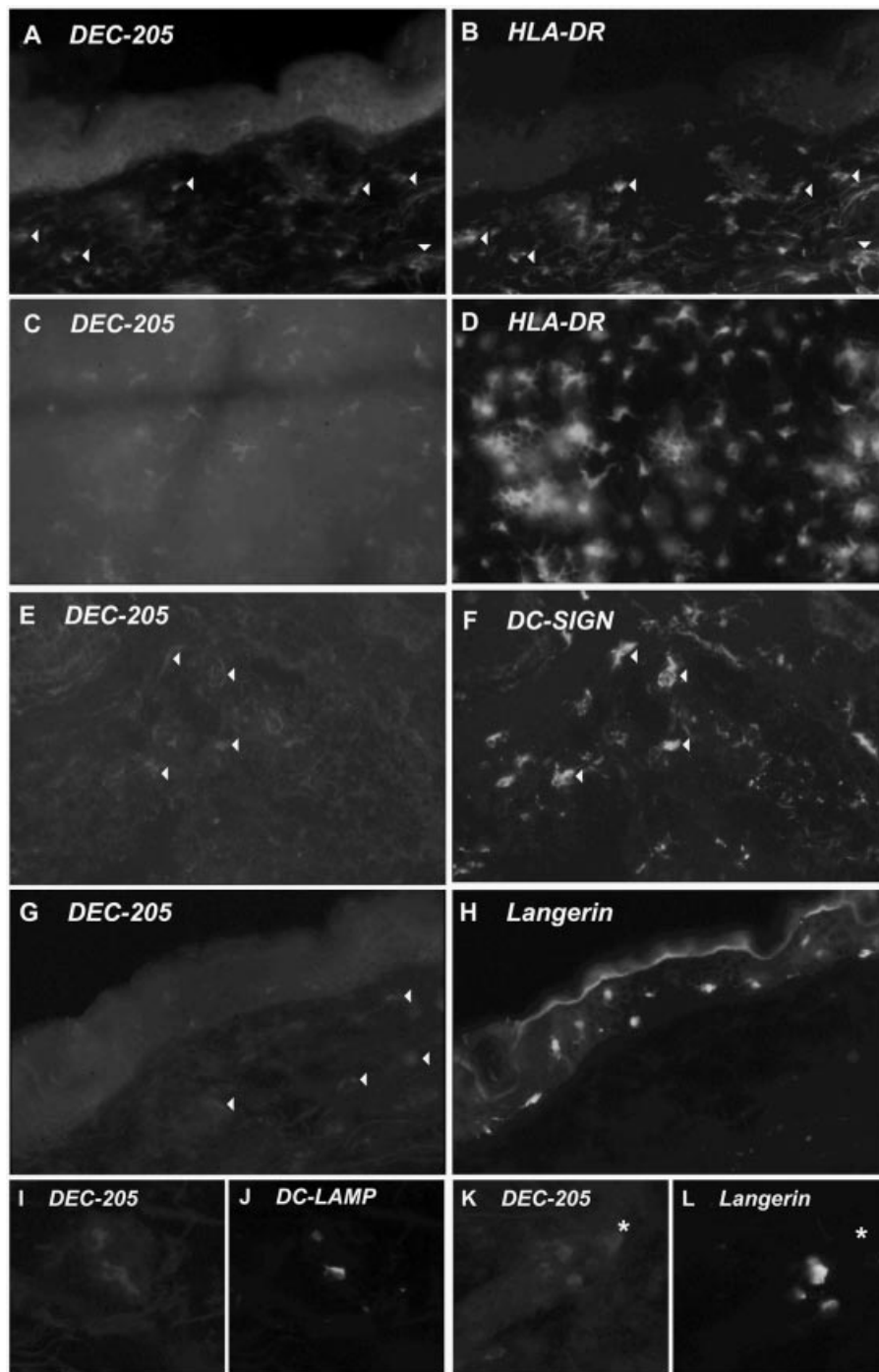


Fig. 1. DEC-205 is expressed on Langerhans cells and dermal dendritic cells. Cryostat sections (all panels but C and D) and epidermal sheets (C and D) of fresh human skin were double-labeled as indicated. Pairs of pictures in each row (A–B, C–D, E–F, G–H, I–J, K–L) represent double-labeling of the same area. Some dendritic cells are marked with arrowheads in the corresponding panels. HLA-DR⁺ Langerhans cells in the epidermis express DEC-205 weakly. DEC-205-expressing cells also co-express HLA-DR (A–D). To the most part they co-express DC-SIGN in the dermis (E and F). DEC-205-expressing cells are Langerin-positive in the epidermis but Langerin-negative in the dermis (G and H), with the exception of few cells that do co-express Langerin (K and L). Likewise, only a few scattered dermal DEC-205⁺ cells were DC-LAMP⁺ (I and J). Magnification, $\times 230$ (A–H), $\times 450$ (I–L).

most, though not all DEC-205⁺ cells co-expressed DC-SIGN (Fig. 1). Additional double-labeling experiments using FITC-conjugated anti-Langerin and anti-DC-LAMP mAbs showed that most DEC-205⁺ dermal cells were Langerin- and DC-

LAMP-negative, indicating that they were not Langerhans cells and they were not mature. Inversely, the very few Langerin⁺ and DC-LAMP⁺ dermal cells co-expressed DEC-205 (Fig. 1).

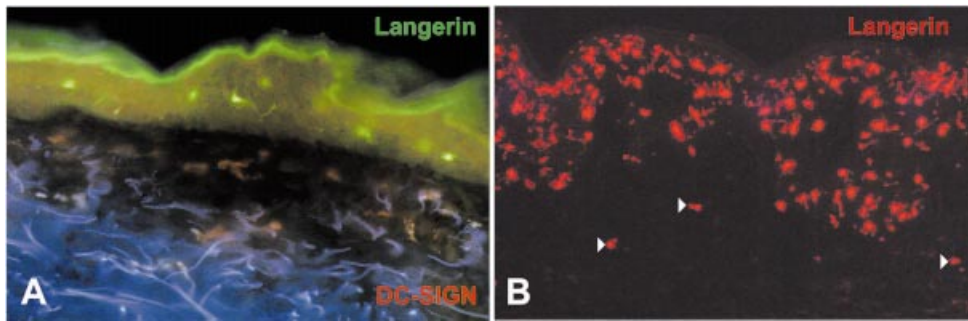


Fig. 2. Langerin and DC-SIGN are expressed in a mutually exclusive fashion. (A) Cryostat sections of normal human skin were immunolabeled for DC-SIGN (red fluorescence) and Langerin-FITC (green fluorescence). Langerhans cells in the epidermis express Langerin but not DC-SIGN. Inversely, dermal dendritic cells express DC-SIGN but not Langerin. Dermal collagen autofluoresces in blue. (B) Single immunofluorescence for Langerin. Most Langerhans cells are concentrated in the epidermis. Note the few strongly Langerin-expressing cells in the dermis (arrowheads), presumably Langerhans cells migrating in the steady state. Magnification, $\times 230$ in (A), $\times 70$ in (B).

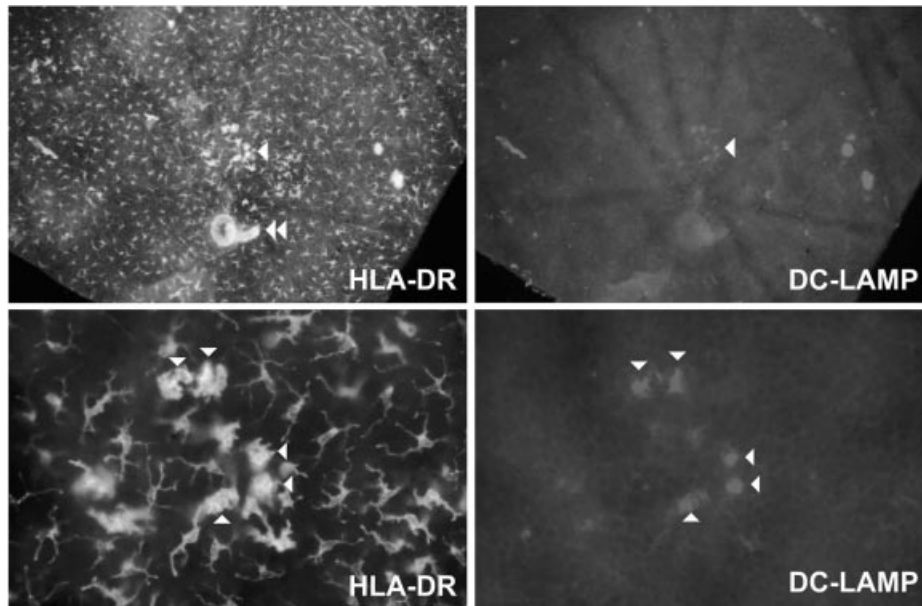


Fig. 3. Maturing Langerhans cells can be detected in normal human epidermis. Epidermal sheets from fresh skin were double-labeled for HLA-DR (left) and DC-LAMP (right). Singly scattered or grouped DC-LAMP⁺ cells (arrowheads) can be found both at low (upper row) and at high (lower row) magnifications. The double arrowhead indicates an HLA-DR⁺ duct of an eccrine sweat gland. Magnifications, $\times 30$ (upper row), $\times 125$ (lower row).

CD207/Langerin expression. Expression was brilliant in Langerhans cells of the epidermis as described originally (9). Occasional Langerin⁺ cells became apparent in the upper dermis (Figs 1 and 2B). In double-labeling experiments both epidermal and dermal Langerin-positive cells turned out to be uniformly MHC class II⁺, too. DC-LAMP was negative in the epidermis on sections. In epidermal sheets, however, few singly scattered or small groups (up to five cells) of Langerhans cells expressed DC-LAMP (Fig. 3).

CD209/DC-SIGN expression. Monoclonal antibodies against DC-SIGN yielded a reciprocal pattern to Langerin. Langerhans cells in the epidermis were negative but dermal dendritic cells could readily be visualized by this mAb

(Fig. 2A). They were found mostly in the upper dermis. They co-expressed MHC II but were negative for Langerin, as expected. Only few of them were mature as defined by their expression of DC-LAMP. As described above, many DC-SIGN⁺ cells co-expressed DEC-205.

BDCA-2 expression. Sections of clinically normal appearing skin from 14 different donors were examined. No unequivocal staining was found in the epidermis except occasional single cells. However, BDCA-2-expressing cells could consistently be found in the dermis of all samples. They were few in number and their staining intensity was lower than what was observed for the other C-type lectins. They were often aggregated in small groups of up to five cells and they were always located just beneath the

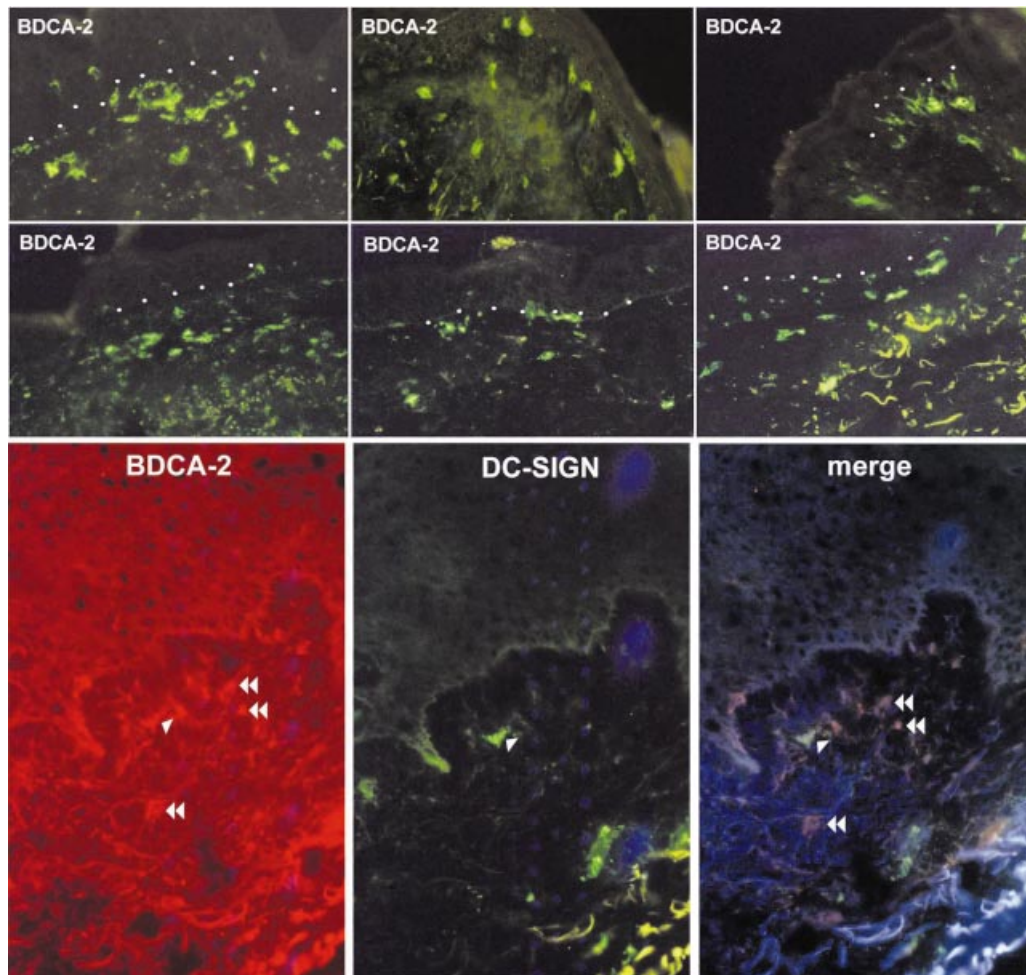


Fig. 4. Expression of BDCA-2 in normal human skin. Cryostat sections of normal human skin were immunolabeled for BDCA-2 using FITC-conjugated detection reagents. Examples from six different donors are depicted (six top panels). The dermo-epidermal junction is marked with dots in some panels. Yellow structures in the bottom right panel are autofluorescent connective tissue fibers. The three bottom panels show a nest of BDCA-2⁺ cells in the dermis subjacent to the basement membrane. One of the BDCA-2⁺ cells co-expresses DC-SIGN (arrowhead); three examples of BDCA⁺/DC-SIGN⁻ cells are marked with double arrowheads. Magnification $\times 150$ (top panels) and $\times 300$ (bottom panels).

basement membrane of the epidermis (Fig. 4). No correlations could be established between the source of the skin (e.g. slightly inflamed foreskin vs. not inflamed excess skin from transplantations) and the frequency of BDCA-2⁺ cells in the dermis. Double-labeling experiments showed that BDCA-2⁺ cells in the dermis did not express Langerin nor DC-LAMP. As described for dendritic cells in nasal polyps (28) there was some overlap between BDCA-2 and DC-SIGN (Fig. 4).

Expression of DEC-205, Langerin and DC-SIGN in inflamed skin in situ

DEC-205 expression in human dendritic cells is upregulated upon maturation [our unpublished observations and (20,29,30)]. Maturation of dendritic cells is typically brought upon by inflammatory stimuli (31). We therefore studied two types of inflamed skin; firstly an inflammatory skin disease proper (lichen ruber planus) (19), and secondly skin explant

cultures that may be regarded as a model for a strong cutaneous inflammation (32,33).

Staining of cryostat sections of lichen ruber revealed distinct MHC class II expression of keratinocytes overlying the dermal infiltrates (Fig. 5). This may be taken as a sign that inflammatory mediators are present and exert their effects even in the epidermis (34). In spite of this cytokine milieu Langerhans cells within the epidermis did not detectably upregulate DEC-205 expression, at least as judged by the immunofluorescence method applied. They were physically present, though, as revealed by a completely overlapping Langerin and MHC II labeling (Fig. 5). The inflammatory infiltrates contained conspicuous nests of DC-LAMP⁺ cells, i.e. phenotypically mature dendritic cells. DC-SIGN as well as Langerin staining identified markedly more positive cells in the infiltrated areas than in healthy normal skin.

Skin explants were cultured (i.e. floated) for 48–72 hours. At the end of the culture period epidermal sheets were prepared

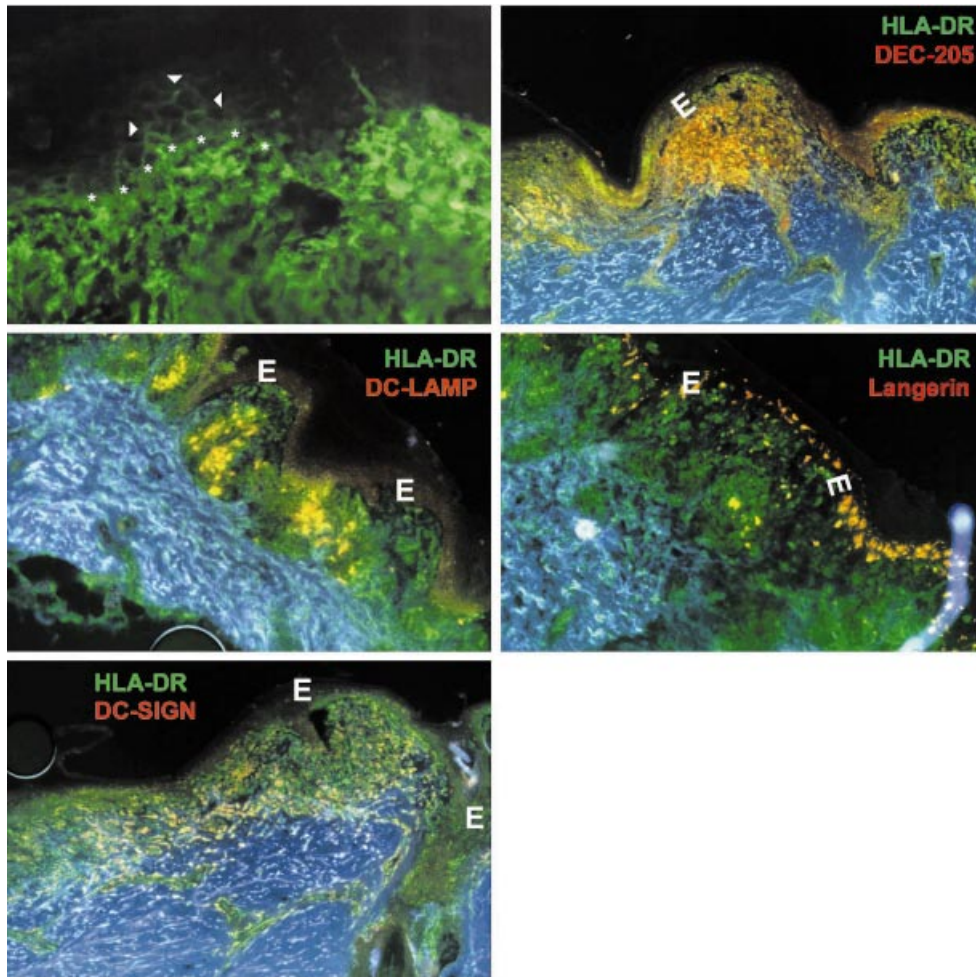


Fig. 5. Expression of DEC-205 in an inflammatory skin disease. Cryostat sections of lichen ruber planus were double-immunolabeled for MHC class II (HLA-DR) and DEC-205, DC-LAMP, Langerin and DC-SIGN as indicated. Upper left panel shows an HLA-DR single labeling. Note HLA-DR⁺ epidermal keratinocytes (arrowheads) and the dense HLA-DR-expressing dermal infiltrate. The basement membrane is marked with asterisks. Amidst the dermal infiltrate of HLA-DR⁺ leukocytes accumulations of DEC-205⁺ cells and DC-LAMP⁺ cells can be detected (orange color). Epidermal LC do not upregulate DEC-205 or DC-LAMP. HLA-DR staining of epidermal Langerhans cells is weak because a directly FITC-conjugated antibody had to be used for the double-labeling experiments. Dermal collagen fluoresces in blue. Magnification, $\times 230$ (upper left) and $\times 60$ (other panels); E, epidermis.

and analysed. Langerhans cells were identified by MHC class II and/or Langerin double-labeling. As described previously (32), the numbers of Langerhans cells in the epidermis decreased markedly during culture. The remaining intra-epidermal Langerhans cells were enlarged and had strongly up-regulated their MHC II expression. Like in the mouse (24) they retained their Langerin expression. DEC-205 expression was also stronger as compared to uncultured epidermis (Fig. 6). This was particularly true for those large Langerhans cells with long dendrites. Interestingly, a sizeable epidermal population of round to oval Langerin⁺ cells was DEC-205-negative. This population was not further analyzed. Since it also occurred in cultured epidermal explants these cells need not necessarily have come from dermal precursors. Rather, they may not have responded to the stimulus of being placed in culture.

Expression of DEC-205, Langerin and DC-SIGN in mature dendritic cells obtained by emigration from cultured skin explants

Cells that had migrated out of whole skin or epidermal explants over a culture period of 2–5 days were analyzed by flow cytometry. Dendritic cells, as defined by their strong MHC II expression were uniformly CD86⁺ and CD83⁺ (not shown) indicating that they were mature as described previously (18,25,35). This was confirmed here in that virtually all emigrant dendritic cells, be they Langerhans cells or dermal dendritic cells strongly expressed the maturation marker DC-LAMP (Fig. 7). Two-color analyses revealed that both Langerin⁺ emigrant cells (i.e. Langerhans cells) and Langerin⁻ emigrant cells (i.e. dermal dendritic cells) expressed cell surface DEC-205 at similar levels (Fig. 7). All DEC-205⁺ cells co-expressed DC-LAMP, indicating that they

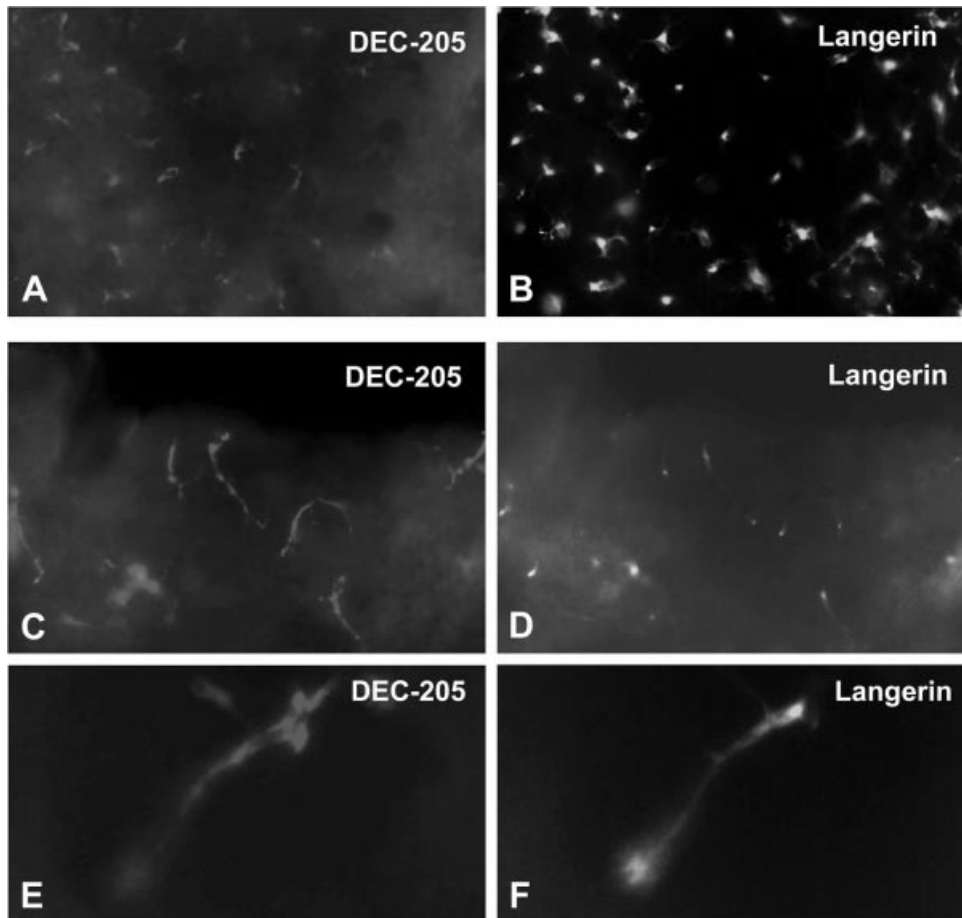


Fig. 6. DEC-205 is upregulated in Langerhans cells during skin explant culture. Epidermal sheets were prepared from fresh (A and B) and cultured (C–F) skin and were double-labeled for DEC-205 (left) and Langerin (right). Magnification, $\times 125$ (A–D); $\times 325$ (E and F).

were mature, and they did not express CD14. BDCA-2-expressing cells were not found. As described by Larregina *et al.* (35) we often, though not consistently detected a small population of CD14⁺/MHC II⁺ cells. These cells did not co-express Langerin (see Supplementary fig. 1, available at *International Immunology Online*) nor DEC-205 (Fig. 7).

Attempts to determine cell surface expression of DEC-205 on Langerhans cell in the steady state

Immunohistochemistry on cryosections and/or epidermal or dermal sheets does not allow to unequivocally determine whether the receptor molecules are expressed on the cell surface. This can only be done by flow cytometry. Classical trypsinization techniques (26,27) for the generation of fresh epidermal cell suspensions digest the murine DEC-205 molecule to a large extent (36). We therefore tested sensitivity of human DEC-205 surface expression to trypsin (0.05%, 30 min, 37°C) and to dispase (1.2 U/ml, 30 min, 37°C) with mature monocyte derived dendritic cells. Similar trypsin treatment as used for the procurement of epidermal cell suspensions (16 h at 4°C for skin trypsinization vs. 30 min at 37°C for trypsinization of monocyte-derived DC) only partially reduced DEC-205 surface expression. Interestingly, dispase was markedly more powerful in digesting away DEC-205

molecules (Supplementary fig. 2). Langerhans cells in freshly trypsinized epidermal cell suspensions did not express DEC-205 on their surfaces ($n = 2$; not shown).

Discussion

We describe here the expression of four C-type lectin receptors on dendritic cells of healthy and inflamed human skin (Table 1). The systematic analyses of DEC-205 and BDCA-2 expression are new to the best of our knowledge. Our observations with regard to Langerin and DC-SIGN confirm and extend previous analyses (9,28,29,37,38).

Epidermis

Langerhans cells express DEC-205 and Langerin. Low levels of DEC-205 mRNA have previously been reported for human Langerhans cells (39). Here we show that this mRNA indeed translates into the DEC-205 protein, albeit at low levels. It remains uncertain whether the low expression of DEC-205 in Langerhans cells *in situ* means true surface expression. The relative resistance of DEC-205 against trypsin suggests that our failure to detect surface DEC-205 on freshly isolated Langerhans cells would not be due to trypsin-induced loss of the molecule but rather reflect absence or very low levels of

surface DEC-205 on resident Langerhans cells. Clearly, however, surface expression of DEC-205 is upregulated upon maturation.

An additional C-type lectin receptor on human Langerhans cell is Dectin-1 (40). As shown here and as described previously they do not express DC-SIGN (29,38,41), BDCA-2

(42) and the mannose receptor/CD206 (43,44). Langerin, DEC-205, and Dectin-1 may enable Langerhans cells to bind mannose- or β -glucan-containing microbes or microbial components. Within the epidermis these receptors may mediate the uptake of microbes that have been found in Langerhans cells such as Dengue virus (45), *Leishmania* (46,47) or probably *Schistosoma* (48) that otherwise use DC-SIGN for entering or binding to dendritic cells, as described for Dengue virus (49), *Leishmania* (13,50), and *Schistosoma* (13). Whether this also applies for HIV (11) is not clear. It was shown recently that HIV can enter and infect (at least part of) epidermal Langerhans cells in a manner that could not be blocked by mannan, suggesting a Langerin-independent uptake (51). Binding of soluble HIV gp120, however, to Langerin-transfected cells was observed, indicating that the Langerin receptor may still be somehow involved in HIV uptake (38). No data are available with regard to DEC-205. Clearly, HIV appears to be flexible in its choice of co-receptors that it needs for binding to dendritic cells (38). However, given the paucity of available data on the ligands for Langerin, DEC-205 or Dectin-1 (as opposed to those for DC-SIGN) the above considerations are largely speculative.

DEC-205 and Langerin on Langerhans cells appear to be inversely regulated. Whereas upon maturation the expression of Langerin is moderately down-regulated, although not abrogated, expression levels of DEC-205 increase. This is similar as described for mouse Langerhans cells (24,36).

Dermis

Dermal dendritic cells are equipped with a different set of C-type lectin receptors as compared to Langerhans cells. We show here that dermal dendritic cells, as defined by their expression of DC-SIGN (22,28,29,38) are largely reactive with antibodies to DEC-205, both *in situ* and—even more so—in response to skin explant culture. This has also been described for dendritic cells in the human decidua (52) and for mature

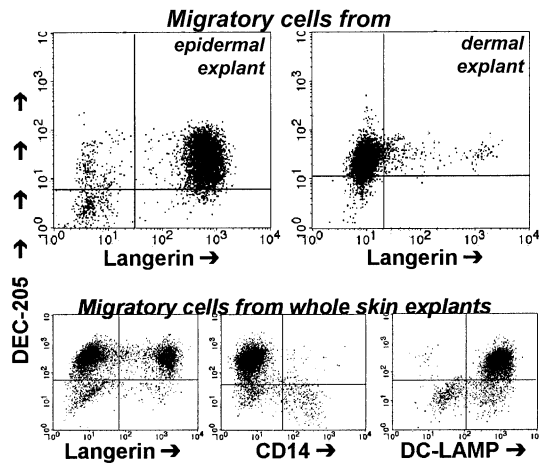


Fig. 7. Flow cytometry. Dendritic cells emigrated from explant cultures over a period of 3–4 days were analysed. Markers were set according to staining with isotype-matched control Ig. Top panels show emigrant populations obtained from epidermal explants where Langerhans cells are highly enriched (left panel) and from corresponding dermal explants where Langerhans cells constitute only a minor contaminant population and dermal dendritic cells are enriched (right panel). Bottom panels depict emigrants from whole skin explants that comprise both Langerhans cells (Langerin⁺) and dermal dendritic cells (Langerin⁻). Note that both Langerhans cells and dermal dendritic cells (but not the few CD14⁺ cells in whole skin emigrant populations) express DEC-205 and DC-LAMP. FITC-conjugated anti-Langerin and anti-DC-LAMP antibodies were a kind gift of Dr Serge Lebecque.

Table 1. Expression of C-type lectin receptors on Langerhans cells (LC) and dermal dendritic cells (DC)

| Langerhans cells | | | | | Dermal dendritic cells | | | | |
|-------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------|----------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|----|
| Immunofluorescence on sections and sheets | | | | Flow cytometry | Immunofluorescence on sections and sheets | | | Flow cytometry | |
| Immature LC <i>in situ</i> | Mature LC <i>in situ</i> in fresh epidermis ^a | Mature LC <i>in situ</i> in epidermis from cultured skin | LC <i>in situ</i> in inflammatory skin disease | Mature LC emigrated from explants ^b | Immature dermal DC <i>in situ</i> | Mature dermal DC <i>in situ</i> ^c | Dermal DC <i>in situ</i> in inflammatory skin disease | Mature dermal DC emigrated from explants ^b | |
| DEC-205 | + | n.t. | ++ | + | ++ | ++ | n.t. | + | ++ |
| Langerin | ++ | ++ | ++ | ++ | ++ | - | n.t. | - | - |
| DC-SIGN | - | - | n.t. | - | - | ++ | + | ++ | ++ |
| BDCA-2 ^d | - | - | n.t. | n.t. | - | - | n.t. | n.t. | - |

^aMaturity was defined by expression of DC-LAMP in epidermal sheets. There are only very few DC-LAMP-expressing cells in fresh epidermis.

^bMaturity was defined as described previously (18) and was confirmed by DC-LAMP expression. Langerhans cells were defined by their Langerin expression; dermal dendritic cells by their lack of Langerin expression. Explant cultures may be regarded as a model for inflammation of the skin.

^cMaturity was defined by expression of DC-LAMP in cryostat sections. These are only very few cells.

^dBDCA-2 was only investigated in uncultured healthy human skin and in cells obtained by migration from skin explants. Mature dendritic cells have been reported to lose BDCA-2 expression (15). n.t., not tested.

dermal dendritic cells in the mouse (18). With regard to mouse dermal dendritic cells *in situ* it is not entirely clear whether they express DEC-205 at low levels (53) or not at all. It would appear from other data that dermal dendritic cells also co-express the C-type lectins mannose receptor/CD206 (29), asialo-glycoprotein receptor (54), and DCIR/dendritic cell immunoreceptor (55). However, for the latter two receptors this has not been confirmed *in situ* yet.

Larregina *et al.* (35) have described CD14⁺/Langerin⁺ cells that migrated from human whole skin explants and could serve as precursors for Langerhans cells. We also found variable percentages of such migrated CD14⁺ cells. They expressed neither DEC-205 nor Langerin.

Plasmacytoid dendritic cells

The distribution of this cell type has not been extensively studied in normal human skin. Normal skin was used as a control in a recent report that showed the presence of plasmacytoid dendritic cells (pDCs) (i.e. BDCA-2-expressing cells) in epidermal cell suspensions from inflamed but not from normal skin (42). Furthermore, pDCs (i.e. CD123-expressing cells) were shown in melanoma but not in few samples of normal control skin (56). Similarly, in the first description of pDCs in lesions of cutaneous lupus erythematosus, normal skin was also found not to contain pDCs (i.e. CD123-expressing cells) (57). Finally, in a recent report suggesting an involvement of pDCs in allergic contact dermatitis, few CD123- and BDCA-2-expressing pDCs were also detected in the dermis of normal control skin samples (58). Thus, to the best of our knowledge, our findings represent the first systematic analysis of normal human skin using the pDC-specific marker BDCA-2. Clearly, normal skin does not harbor a large and regularly distributed population of pDCs. Rather, pDCs in the dermis are rare. They are close beneath the basement membrane and they are immature. From these observations one may conclude that pDCs are yet another constitutive dendritic cell population of the skin, in addition to Langerhans cells and dermal dendritic cells. Alternatively, and probably more likely, the observed few BDCA-2-expressing cells may have been recruited from the blood in response to a locally confined pathogenic stimulus.

In summary, our studies show that distinct carbohydrate recognition receptors are expressed on subsets of epidermal, dermal and plasmacytoid dendritic cells of intact skin, and that both Langerhans cells and dermal dendritic cells undergo maturation during skin inflammation and explant cultures.

Supplementary data

Supplementary data for this paper are available at *International Immunology* Online.

Acknowledgements

S.E., M.F. and N.R. are members of the KMT (Kompetenzzentrum Medizin Tirol, project no. 3b) that supported this work. We thank Dr G. Sprinzl from the ENT Department for providing us with tonsils, and Drs C. Rainer and M. Oehlbauer from the Department of Plastic Surgery for also supplying us with normal human skin.

Abbreviations

| | |
|-----|-----------------------------|
| DC | dendritic cell |
| LC | Langerhans cell |
| pDC | plasmacytoid dendritic cell |

References

- Banchereau, J. and Steinman, R. M. 1998. Dendritic cells and the control of immunity. *Nature* 392:245.
- Mellman, I. and Steinman, R. M. 2001. Dendritic cells: Specialized and regulated antigen processing machines. *Cell* 106:255.
- Guermonprez, P., Valladeau, J., Zitvogel, L., Théry, C. and Amigorena, S. 2002. Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol.* 20:621.
- Steinman, R. M., Hawiger, D. and Nussenzweig, M. C. 2003. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21:685.
- Figdor, C. G., Van Kooyk, Y. and Adema, G. J. 2002. C-type lectin receptors on dendritic cells and Langerhans cells. *Nature Rev. Immunol.* 2:77.
- Mahnke, K., Guo, M., Lee, S., Sepulveda, H., Swain, S. L., Nussenzweig, M. and Steinman, R. M. 2000. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. *J. Cell Biol.* 151:673.
- Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J. V., Steinman, R. M. and Nussenzweig, M. C. 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* 194:769.
- Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M. C. and Steinman, R. M. 2002. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8⁺ T cell tolerance. *J. Exp. Med.* 196:1627.
- Valladeau, J., Duvert-Frances, V., Pin, J. J., Dezutter-Dambuyant, C., Vincent, C., Massacrier, C., Vincent, J., Yoneda, K., Banchereau, J., Caux, C., Davoust, J. and Saeland, S. 1999. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells and is rapidly internalized from the cell surface. *Eur. J. Immunol.* 29:2695.
- Valladeau, J., Ravel, O., Dezutter-Dambuyant, C., Moore, K., Kleijmeer, M., Liu, Y., Duvert-Frances, V., Vincent, C., Schmitt, D., Davoust, J., Caux, C., Lebecque, S. and Saeland, S. 2000. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12:71.
- Geijtenbeek, T. B. H., Kwon, D. S., Torensma, R., Van Vliet, S. J., Van Duinhoven, G. C. F., Middel, J., Cornelissen, I. L. M. H., Nottet, H. S. L. M., KewalRamani, V. N., Littman, D. R., Figdor, C. G. and Van Kooyk, Y. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell* 100:587.
- Tailleux, L., Schwartz, O., Herrmann, J. L., Pivert, E., Jackson, M., Amara, A., Legres, L., Dreher, D., Nicod, L. P., Gluckman, J. C., Lagrange, P. H., Gicquel, B. and Neyrolles, O. 2003. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med.* 197:121.
- Appelmelk, B. J., Van Die, I., Van Vliet, S. J., Vandenbroucke-Grauls, C. M. J. E., Geijtenbeek, T. B. H. and Van Kooyk, Y. 2003. Cutting edge: Carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J. Immunol.* 170:1635.
- Cambi, A., Gijzen, K., De Vries, J. M., Torensma, R., Joosten, B., Adema, G. J., Netea, M. G., Kullberg, B. J., Romani, L. and Figdor, C. G. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol.* 33:532.
- Dzionek, A., Sohma, Y., Nagafune, J., Cella, M., Colonna, M., Facchetti, F., Günther, G., Johnston, I., Lanzavecchia, A., Nagasaka, T. *et al.* 2001. BDCA-2, a novel plasmacytoid dendritic cell-specific type IIC-type lectin, mediates antigen

- capture and is a potent inhibitor of interferon α/β induction. *J. Exp. Med.* 194:1823.
- 16 Kraal, G., Bree, M., Janse, M. and Bruin, G. 1986. Langerhans' cells, veiled cells and interdigitating cells in the mouse recognized by a monoclonal antibody. *J. Exp. Med.* 163:981.
 - 17 Romani, N., Holzmann, S., Tripp, C. H., Koch, F. and Stoitzner, P. 2003. Langerhans cells—dendritic cells of the epidermis. *APMIS* 111:725.
 - 18 Lenz, A., Heine, M., Schuler, G. and Romani, N. 1993. Human and murine dermis contain dendritic cells. Isolation by means of a novel method and phenotypical and functional characterization. *J. Clin. Invest.* 92:2587.
 - 19 Daoud, M. S. and Pittelkow, M. R. 2003. Lichen Planus. In Freedberg, I. M., Eisen, A. Z., Wolff, K., Austen, K. F., Goldsmith, L. A. and Katz, S. I. eds, *Fitzpatrick's Dermatology in General Medicine*, pp. 463–477. McGraw-Hill, New York.
 - 20 Guo, M., Gong, S. C., Maric, S., Misulovin, Z., Pack, M., Mahnke, K., Nussenzweig, M. C. and Steinman, R. M. 2000. A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Hum. Immunol.* 61:729.
 - 21 De Saint-Vis, B., Vincent, J., Vandenabeele, S., Vanbervliet, B., Pin, J. J., Ait-Yahia, S., Patel, S., Mattei, M. G., Banchereau, J., Zurawski, S., Davoust, J., Caux, C. and Lebecque, S. 1998. A novel lysosome-associated membrane glycoprotein, DC-LAMP, induced upon DC maturation, is transiently expressed in MHC class II compartment. *Immunity* 9:325.
 - 22 Geijtenbeek, T. B. H., Torensma, R., Van Vliet, S. J., Van Duijnhoven, G. C. F., Adema, G. J., Van Kooyk, Y. and Figdor, C. G. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100:575.
 - 23 Juhlin, L. and Shelley, W. B. 1977. New staining techniques for the Langerhans cell. *Acta Derm. Venereol. (Stockh.)* 57:289.
 - 24 Stoitzner, P., Holzmann, S., McLellan, A. D., Ivarsson, L., Stössel, H., Kapp, M., Kämmerer, U., Douillard, P., Kämpgen, E., Koch, F., Saeland, S. and Romani, N. 2003. Visualization and characterization of migratory Langerhans cells in murine skin and lymph nodes by antibodies against Langerin/CD207. *J. Invest. Dermatol.* 120:266.
 - 25 Pope, M., Betjes, M. G. H., Hirmand, H., Hoffman, L. and Steinman, R. M. 1995. Both dendritic cells and memory T lymphocytes emigrate from organ cultures of human skin and form distinctive dendritic-T-cell conjugates. *J. Invest. Dermatol.* 104:11.
 - 26 Romani, N., Bhardwaj, N., Pope, M., Koch, F., Swiggard, W. J., O'Doherty, U., Witmer-Pack, M. D., Hoffman, L., Schuler, G., Inaba, K. and Steinman, R. M. 1997. Dendritic Cells. In Herzenberg, L. A., Weir, D. M., Herzenberg, L. and Blackwell, C. eds, *Weir's Handbook of Experimental Immunology*, pp. 156.1–156.14. Blackwell Science, Oxford.
 - 27 Koch, F., Kämpgen, E., Schuler, G. and Romani, N. 2001. Isolation, enrichment and culture of murine epidermal Langerhans cells. In Stagg, A. J. and Robinson, S. eds, *Dendritic Cell Protocols*, pp. 43–62. Humana Press, Totowa, NJ.
 - 28 Soilleux, E. J., Morris, L. S., Leslie, G., Chehimi, J., Luo, Q., Levroney, E., Trowsdale, J., Montaner, L. J., Doms, R. W., Weissman, D., Coleman, N. and Lee, B. 2002. Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations *in situ* and *in vitro*. *J. Leukoc. Biol.* 71:445.
 - 29 Engering, A., Geijtenbeek, T. B., Van Vliet, S. J., Wijers, M., van Liempt, E., Demaurex, N., Lanzavecchia, A., Fransen, J., Figdor, C. G., Piguët, V. and Van Kooyk, Y. 2002. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J. Immunol.* 168:2118.
 - 30 Mehlhop, E., Villamide, L. A., Frank, I., Gettie, A., Santisteban, C., Messmer, D., Ignatius, R., Lifson, J. D. and Pope, M. 2002. Enhanced *in vitro* stimulation of rhesus macaque dendritic cells for activation of SIV-specific T cell responses. *J. Immunol. Methods* 260:219.
 - 31 Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. T., Pulendran, B. and Palucka, K. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18:767.
 - 32 Lukas, M., Stössel, H., Hefel, L., Imamura, S., Fritsch, P., Sepp, N. T., Schuler, G. and Romani, N. 1996. Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model. *J. Invest. Dermatol.* 106:1293.
 - 33 Romani, N., Ratzinger, G., Pfaller, K., Salvenmoser, W., Stössel, H., Koch, F. and Stoitzner, P. 2001. Migration of dendritic cells into lymphatics—The Langerhans cell example: Routes, regulation and relevance. *Int. Rev. Cytol.* 207:237.
 - 34 Auböck, J., Romani, N., Grubauer, G. and Fritsch, P. 1986. Expression of HLA-DR antigens on keratinocytes is a common feature of diseased skin. *Br. J. Dermatol.* 114:456.
 - 35 Larregina, A. T., Morelli, A. E., Spencer, L. A., Logar, A. J., Watkins, S. C., Thomson, A. W. and Falo, L. D. Jr. 2001. Dermal-resident CD14+ cells differentiate into Langerhans cells. *Nature Immunol.* 2:1151.
 - 36 Inaba, K., Swiggard, W. J., Inaba, M., Meltzer, J., Mirza, A., Sasagawa, T., Nussenzweig, M. C. and Steinman, R. M. 1995. Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse leukocytes. *Cell. Immunol.* 163:148.
 - 37 Engering, A., Geijtenbeek, T. B. H., Van Vliet, S. J., Wijers, M., van Liempt, E., Demaurex, N., Lanzavecchia, A., Fransen, J., Figdor, C. G., Piguët, V. and Van Kooyk, Y. 2002. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J. Immunol.* 168:2118.
 - 38 Turville, S. G., Cameron, P. U., Handley, A., Lin, G., Pohlmann, S., Doms, R. W. and Cunningham, A. L. 2002. Diversity of receptors binding HIV on dendritic cell subsets. *Nature Immunol.* 3:975.
 - 39 Kato, M., Neil, T. K., Fearnley, D. B., McLellan, A. D., Vuckovic, S. and Hart, D. N. J. 2000. Expression of multilectin receptors and comparative FITC-dextran uptake by human dendritic cells. *Int. Immunol.* 12:1511.
 - 40 Yokota, K., Takashima, A., Bergstresser, P. R. and Ariizumi, K. 2001. Identification of a human homologue of the dendritic cell-associated C-type lectin-1, dectin-1. *Gene* 272:51.
 - 41 Soilleux, E. J. and Coleman, N. 2001. Langerhans cells and the cells of Langerhans cell histiocytosis do not express DC-SIGN. *Blood* 98:1987.
 - 42 Wollenberg, A., Wagner, M., Günther, S., Towarowski, A., Tuma, E., Moderer, M., Rothenfusser, S., Wetzel, S., Endres, S. and Hartmann, G. 2002. Plasmacytoid dendritic cells: A new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J. Invest. Dermatol.* 119:1096.
 - 43 Wollenberg, A., Mommaas, M., Oppel, T., Schottdorf, E. M., Günther, S. and Moderer, M. 2002. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J. Invest. Dermatol.* 118:327.
 - 44 Mommaas, A. M., Mulder, A. A., Jordens, R., Out, C., Tan, M. C. A. A., Cresswell, P., Kluin, P. M. and Koning, F. 1999. Human epidermal Langerhans cells lack functional mannose receptors and a fully developed endosomal/lysosomal compartment for loading of HLA class II molecules. *Eur. J. Immunol.* 29:571.
 - 45 Wu, S. J. L., Grouard-Vogel, G., Sun, W., Masciola, J. R., Brachtel, E., Putvatana, R., Louder, M. K., Filgueira, L., Marovich, M. A., Wong, H. K. et al. 2000. Human skin Langerhans cells are targets of dengue virus infection. *Nat. Med.* 6:816.
 - 46 Elhassan, A. M., Gaafar, A. and Theander, T. G. 1995. Antigen-presenting cells in human cutaneous leishmaniasis due to *Leishmania major*. *Clin. Exp. Immunol.* 99:445.
 - 47 Blank, C., Fuchs, H., Rappersberger, K., Röllinghoff, M. and Moll, H. 1993. Parasitism of epidermal Langerhans cells in experimental cutaneous Leishmaniasis with *Leishmania major*. *J. Infect. Dis.* 167:418.
 - 48 Angeli, V., Faveeuw, C., Roye, O., Fontaine, J., Teissier, E., Capron, A., Wolowczuk, I., Capron, M. and Trottein, F. 2001. Role of the parasite-derived prostaglandin D₂ in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J. Exp. Med.* 193:1135.
 - 49 Tassaneitripath, B., Burgess, T. H., Granelli-Piperno, A., Trumpheller, C., Finke, J., Sun, W., Eller, M. A., Pattanapanyasat, K., Sarasombath, S., Bix, D. L., Steinman, R. M., Schlesinger, S. and Marovich, M. A. 2003. DC-SIGN (CD209)

- mediates dengue virus infection of human dendritic cells. *J. Exp. Med.* 197:823.
- 50 Colmenares, M., Puig-Kröger, A., Pello, O. M., Corbí, A. L. and Rivas, L. 2002. Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN, CD209), a C-type surface lectin in human DCs, is a receptor for *Leishmania* amastigotes. *J. Biol. Chem.* 277:36766.
- 51 Kawamura, T., Gulden, F. O., Sugaya, M., McNamara, D. T., Borris, D. L., Lederman, M. M., Orenstein, J. M., Zimmerman, P. A. and Blauvelt, A. 2003. R5 HIV productively infects Langerhans cells and infection levels are regulated by compound *CCR5* polymorphisms. *Proc. Natl Acad. Sci. USA* 100:8401.
- 52 Gardner, L. and Moffet, A. 2003. Dendritic cells in the human decidua. *Biol. Reprod.* 69:1438.
- 53 Duraiswamy, N., Tse, Y., Hammerberg, C., Kang, S. and Cooper, K. D. 1994. Distinction of class II MHC⁺ Langerhans cell-like interstitial dendritic antigen-presenting cells in murine dermis from dermal macrophages. *J. Invest. Dermatol.* 103:678.
- 54 Valladeau, J., Duvert-Frances, V., Pin, J. J., Kleijmeer, M. J., Ait-Yahia, S., Ravel, O., Vincent, C., Vega, F. Jr., Helms, A., Gorman, D. *et al.* 2001. Immature human dendritic cells express asialoglycoprotein receptor isoforms for efficient receptor-mediated endocytosis. *J. Immunol.* 167:5767.
- 55 Bates, E. E. M., Fournier, N., Garcia, E., Valladeau, J., Durand, I., Pin, J. J., Zurawski, S. M., Patel, S., Abrams, J. S., Lebecque, S., Garrone, P. and Saeland, S. 1999. APCs express DCIR, a novel C-type lectin surface receptor containing an immunoreceptor tyrosine-based inhibitory motif. *J. Immunol.* 163:1973.
- 56 Vermi, W., Bonecchi, R., Facchetti, F., Bianchi, D., Sozzani, S., Festa, S., Berenzi, A., Cella, M. and Colonna, M. 2003. Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J. Pathol.* 200:255.
- 57 Farkas, L., Beiske, K., Lund-Johansen, F., Brandtzaeg, P. and Jahnsen, F. L. 2001. Plasmacytoid dendritic cells (natural interferon- α/β -producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am. J. Pathol.* 159:237255.
- 58 Bangert, C., Friedl, J., Stary, G., Stingl, G. and Kopp, T. 2003. Immunopathologic features of allergic contact dermatitis in humans: Participation of plasmacytoid dendritic cells in the pathogenesis of the disease? *J. Invest. Dermatol.* 121:1409.