CD1d is Expressed on Dermal Dendritic Cells and Monocyte-Derived Dendritic Cells

Gianni Gerlini,*† Hans Peter Hefti,* Martin Kleinhans,* Brian J. Nickoloff,‡ Günter Burg,* and Frank O. Nestle*

*Department of Dermatology, University of Zürich Medical School, Zürich, Switzerland; †Department of Dermatological Sciences, University of Florence Medical School, Florence, Italy; ‡Department of Pathology and Skin Cancer Research Laboratories, Cardinal Bernardin Cancer Center, Loyola University of Chicago, Maywood, Illinois, U.S.A.

CD1 proteins are a family of cell surface molecules that present lipid antigens to T cells. We investigated skin dendritic cells and monocyte-derived dendritic cells for expression of CD1 molecules using a panel of 10 different monoclonal antibodies focusing on the recently described CD1d molecule. By immunohistochemical analysis, CD1d expression in normal human skin was restricted to dendritic appearing cells in the papillary dermis mainly located in a perivascular localization. Langerhans cells did not show detectable CD1d expression *in situ*. Epidermal/dermal cell suspensions analyzed by flow cytometry demonstrated distinct subpopulations of HLA-DR positive dermal dendritic cells expressing CD1a, CD1b, and CD1c. CD1d was expressed on HLA-DR^{bright} dermal antigen-presenting cells in dermal suspensions (16% ± 3.6%), as well as on highly enriched dermal dendritic cells migrating out of skin explants (60.5% ± 8.0%). Migrated mature dermal dendritic cells coexpressed CD83 and CD1d. Western blot analysis on microdissected skin sections revealed the presence of a 50–55 kDa CD1d molecule in dermis, suggesting that CD1d is highly glycosylated in skin. Both immature and mature monocyte-derived dendritic cells cultured in autologous plasma expressed CD1d molecules. In contrast, culture in fetal bovine serum downregulated CD1d expression. In conclusion, antigen-presenting cells in skin express different sets of CD1 molecules including CD1d and might play a role in lipid antigen presentation in various skin diseases. Differential expression of CD1 molecules depending on culture conditions might have an impact on clinical applications of dendritic cells for immunotherapy. *Key words: antigen-presenting cells/CD1molecules/glycolipids/Langerhans cells/lipids. J Invest Dermatol 117:576–582, 2001*

Dendritic cells are professional antigen-presenting cells (APC) specialized for initiation of primary immune responses. Dendritic APC capture, process, and finally present protein antigens in the form of peptide major histocompatibility complexes (MHC) to effector T cells. For this purpose dendritic cells are well equipped with MHC, adhesion, and costimulatory molecules (Cella *et al*, 1997; Banchereau and Steinman, 1998). Recently, several studies have identified a nonclassical antigen-presenting pathway involving molecules of the CD1 family of surface proteins (Balk *et al*, 1989; Blumberg *et al*, 1991; Porcelli *et al*, 1998; Porcelli and Modlin, 1999). In contrast to MHC molecules CD1 proteins are nonpolymorphic and are able to present lipids and glycolipids to T cells generating a specific cell mediated immune response (Grant *et al*, 1999; Rosat *et al*, 1999). CD1-restricted T cells play a crucial role in immune responses against mycobacteria (Sieling *et al*, 1999; Stenger and Modlin, 1999) and tumors (Cui *et al*, 1997; Kawano *et al*, 1998; 1999; Toura *et al*, 1999). The CD1 family is divided in two groups on the basis of biochemical and functional similarities. Group I includes CD1a, CD1b, and CD1c proteins, whereas group II includes CD1d (Balk *et al*, 1989; Yu and Milstein, 1989). It has been demonstrated that the CD1d molecule enables cells to interact with natural killer T cells, a subset of lymphocytes able to produce high levels of interleukin-4 (IL-4) and interferon-γ (IFN-γ) upon stimulation (Exley *et al*, 1997; Kawano *et al*, 1997; Brossay *et al*, 1998a, b; Exley *et al*, 1998). Although numerous studies have been carried out on the expression of group I CD1 molecules in skin (Furue *et al*, 1992; Elder *et al*, 1993; Meunier *et al*, 1993, 1996; Nestle *et al*, 1993; Fivenson and Nickoloff, 1995; Larregina *et al*, 1997) and monocyte-derived dendritic cells (mo-DC) (Kasinrerk *et al*, 1993), few data are available on the expression of CD1d by skin APC in humans (Bonish *et al*, 2000). The aim of this study was therefore to investigate the expression of CD1 molecules on skin APC, focusing in particular on CD1d. We extended our investigation also to mo-DC, as these cells are classical *in vitro* models for immature and mature dendritic cells. Furthermore, mo-DC are increasingly used for immunotherapy of cancer such as melanoma (Nestle *et al*, 1998).

Here we show that in normal human skin dermal dendritic cells (DDC) both *in vivo and in vitro* express all members of the CD1 family, including CD1d. CD1d expression on mo-DC was influenced by the presence of culture factors such as autologous plasma (AP) or fetal bovine serum (FBS). In the presence of AP immature and mature mo-DC expressed CD1d, whereas downregulation of this molecule was induced by FBS. Thus mo-DC and

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Reprint requests to: Dr. Frank O. Nestle, Department of Dermatology, University of Zurich Medical School, Gloriastrasse 31, CH-8091 Zurich, Switzerland. Email: nestle@derm.unizh.ch

Abbreviations: APC, antigen-presenting cells; AP, autologous plasma; DDC, dermal dendritic cells; mo-DC, monocyte-derived dendritic cells.

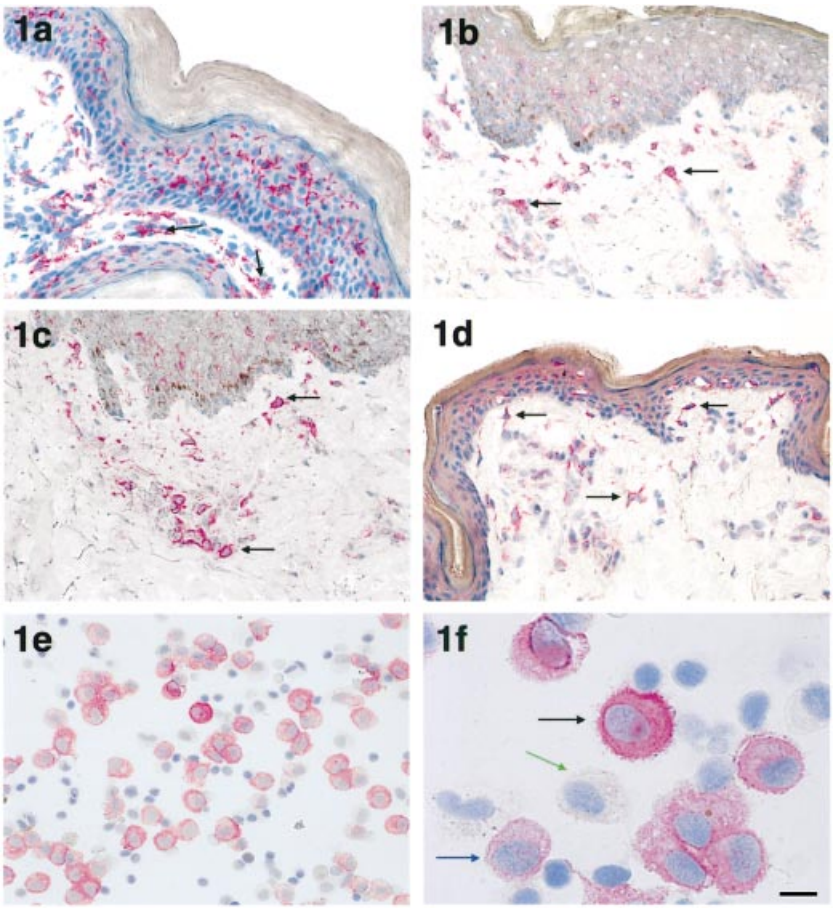

Figure 1. Expression of CD1 molecules, including CD1d, on skin dendritic APC. Representative immunohistochemical staining of normal skin. (*a*) Epidermal dendritic cells and DDC (*arrow*) stained with anti-CD1a (NA1/34). (*b*)–(*d*) Dendritic dermal APC express CD1b (*b*, BCD1b3.1.6), CD1c (*c*, L161), and CD1d (*d*, polyclonal rabbit antibody). (*e*)–(*f*) A subset of cultivated DDC express CD1d. Immunohistochemical staining of migrated DDC using anti-CD1d antibody (*e*, 51.1.3). There is strong (*f*, *black arrow*), intermediate (*blue arrow*), and negative (*green arrow*) expression of CD1d. Note the clustering of small lymphocytes with CD1d positive DDC (*black arrow*). *Scale bar*: (*a–e*) 50 μm; (*f*) 10 μm.

DDC express surface molecules that facilitate the presentation of lipids and glycolipids and potential cross-talk with natural killer T cells.

MATERIALS AND METHODS

Reagents and antibodies RPMI 1640 (Life Technologies, Eggenstain, Germany) and X VIVO 15 (BioWhittaker, Belgium) were supplemented with 1% penicillin/streptomycin (Gibco, Basel, Switzerland), 2% glutamin (Seromed, Berlin, Germany), and 10% heat-inactivated FBS. In some experiments FBS was replaced by 1% heat-inactivated (45 min at 56°C) AP. The following monoclonal (MoAb) or polyclonal antibodies were used: CD1a PE and CD1a (NA1/34, IgG2a) from Dako (Glostrup, Denmark); CD1b FITC (M-T101, IgG1) and CD86 FITC (2331 FUN-1, IgG1) from Pharmingen (San Diego, CA); CD1b (BCD1b3.1.6, IgG1) kindly provided by Dr. S.A. Porcelli; CD1c (L161, IgG1) and CD83 PE (HB15a, IgG2b) from Immunotech (Marseilles, France); CD1d (42.1, IgG1; D5, IgG2b; 51.1.3, IgG2b; 27.1, IgG1; rabbit anti-CD1d) kindly provided by Dr. S.P. Balk; CD1d (NOR3.2, IgG1) from Biosource International (Camarillo, CA); HLA-DR FITC, HLA-DR PE (L243, IgG2a), and CD80 PE (L307.4, IgG1) from Becton Dickinson (Mountain View, CA). Goat antimouse phycoerythrin (PE) and fluorescein isothiocyanate (FITC) from Dako were used as secondary antibodies for flow cytometry. Goat antimouse conjugated with Texas Red (Southern Biotechnology Associates, Birmingham, AL) was used for immunofluorescence staining.

Generation of mo-DC Peripheral blood mononuclear cells were obtained from buffy coats by Ficoll-Paque gradient (Amersham Pharmacia Biotech, Uppsala, Sweden) and frozen in 90% FBS and 10% dimethylsulfoxide until use. Dendritic cells were generated from peripheral blood mononuclear cells as previously described with minor modifications (Cella *et al*, 1997; Jonuleit *et al*, 1997; Nestle *et al*, 1998). Briefly, 50×10^6 peripheral blood mononuclear cells were thawed and cultured in 10 cm diameter Petri dishes (Falcon-Becton Dickinson, Franklin Lakes, NJ) in complete RPMI with either 1% AP or 10% FBS for 30–45 min. After removing the floating cells and washing with phosphate-buffered saline (PBS) the adherent cells were cultured overnight in complete RPMI. The following day RPMI was removed and replaced with X VIVO 15 supplemented with 800 U per ml granulocyte macrophage colony stimulating factor (GM-CSF; Leucomax, Sandoz-Wander Pharma, Bern) and 1000 U per ml IL-4 (R&D System, Europe). At days 3 and 5 fresh medium supplemented with the cytokines was added. At day 6 the cells were stimulated with a maturation cytokine cocktail including tumor necrosis factor α (TNF-α) (10 ng per ml), IL-6 (1000 U per ml), and IL-1β (10 ng per ml) (R&D System, Europe). Cells were analyzed at different maturation days by flow cytometry.

Isolation of skin dendritic cells Skin dendritic cells were obtained from normal human skin tissue belonging to healthy patients undergoing breast reduction. The skin was incubated in Dispase type 2 (Boehringer-Mannheim, Mannheim, Germany) and RPMI 1:1. After 1 h at 37°C epidermis was separated from dermis, washed three times with PBS, and cut in small pieces (1–10 mm). A portion of the epidermal and dermal sheets was cultured in RPMI supplemented with 10% FBS for 2 d in order to allow the cells to migrate out as described previously (Nestle *et al*, 1993). The remaining epidermal sheets were incubated with trypsin (Biochrom, Berlin, Germany), whereas the dermal sheets were incubated with an enzyme cocktail consisting of collagenase, hyaluronidase (Sigma-Chemie, Switzerland), and DNAse (Boehringer-Mannheim, Mannheim, Germany) for 1 h at 37°C on a shaker in order to obtain a cell suspension. Fresh epidermal and dermal suspensions were either immediately analyzed by fluorescence-activated cell sorter (FACS) analysis or cultured for 2 d in RPMI supplemented with 10% FBS, 1000 U per ml IL-4, and 800 U per ml GM-CSF. After 2 d both migrated cells and cells in suspension were collected and used for FACS analysis. In addition cytospins (Shandon, Pittsburgh, PA) were also prepared using the migrated cells.

Immunohistochemistry Biopsy samples of normal skin ($N = 10$) were chosen from the cryopreserved tissue archived in the Department of Dermatology of Zurich. Representative 5–7 μm serial cryostat

sections as well as migrated DDC spun down on cytospins were stained with the APAAP technique as described previously (Nestle *et al*, 1997). Unconjugated primary polyclonal antibodies or MoAb, used at 10 μg per ml (except MoAb 27.1 supernatant at 1:1, MoAb D5 ascitis at 1:250), are described in *Reagents and antibodies*.

Immunofluorescence Migrated DDC spun down on cytospins were fixed in acetone, incubated 15 min with normal rabbit serum (Dako Diagnostic, Switzerland), and stained with anti-CD1d (42.1, IgG1) followed by goat antimouse conjugated with Texas Red (Southern Biotechnology Associates) for 1 h. After three washing steps in PBS the anti-HLA-DR FITC-conjugated MoAb was added for an additional hour. Afterwards they were washed again, covered with a cover slip, and analyzed by microscopy (Leica Mikroscopie, Wetzlar, Germany) using the TCS4D confocal laser scanning system (Leica Lasertechnik, Heidelberg, Germany).

Flow cytometry analysis All MoAb dilutions and washing steps were done in PBS containing 2% FBS. 1×10^5 cells per reaction were stained with the conjugated or unconjugated MoAb described in *Reagents and antibodies* followed by a secondary goat antimouse PE or FITC MoAb at 10 μg per ml. In order to block the Fc mediated unspecific binding cells were incubated with FBS and PBS (1:1) for 30 min at 4°C.

To control for nonspecific staining the appropriate isotype controls were used. For single staining cells were labeled with anti-CD1 MoAbs. To identify dendritic cells cells were double stained with HLA-DR and CD1 MoAb. All the incubations were done at 4°C for 30 min. The specificity of the anti-CD1 MoAb was checked using C1R mock and C1R CD1d transfectant (kindly provided by Dr. S Balk, Boston) and C1R CD1a, CD1b, CD1c transfectants (kindly provided by Dr. S. Porcelli).

Western blot Dermis from microdissected skin sections was boiled in sodium dodecyl sulfate sample buffer. Equal amounts of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The Western blot was developed using D5 as anti-CD1d antibody (gift from Dr. S. Balk, Boston). C1R mock and C1R CD1d transfectant cell lines (kindly provided by Dr. S. Balk, Boston) were used as negative and positive controls, respectively.

Microdissection Cryostat sections were stained with hematoxylin–eosin and dermis microdissection was performed under light microscope visualization (magnification 200×) using a 30 gauge needle.

RESULTS

Expression of CD1d in normal human skin Immunohistochemistry was performed on human skin sections using a panel of MoAb directed toward different members of the CD1 family. CD1a expression was mostly confined to dendritic appearing cells in the epidermis corresponding to Langerhans cells (**Fig 1*a***). There were few cells expressing CD1a in dermis. CD1b and CD1c expression was demonstrated on dendritic cells in dermis, mostly in a perivascular localization (**Fig 1*b*, *c***). These cells correspond to DDC as published previously (Lenz *et al*, 1993; Meunier *et al*, 1993; Nestle *et al*, 1993). Investigation of CD1d expression was performed with a panel of six antibodies. Consistent and equivalent immunohistochemical staining was obtained with antibodies D5, 51.1.3, and polyclonal rabbit anti-CD1d, which revealed positive staining on large cells with dendritic processes in the dermis (**Fig 1*d***). Staining with the MoAb NOR 3.2 revealed strong staining of keratinocytes as well as a few dendritic cells in dermis as published previously (Bonish *et al*, 2000). Most of these cells were located in the upper dermis in the papillary area and in a perivascular localization. We did not observe any CD1d expression on epithelial Langerhans cells. Cytospin preparations from migrated DDC and Langerhans cells revealed expression of CD1d in various intensities on DDC (**Fig 1*e*, *f***) but not Langerhans cells (data not shown). There were DDC with strong expression (*black arrow*), intermediate expression (*blue arrow*), and no expression (*green arrow*) of CD1d.

DDC express CD1d To further investigate the expression of CD1d on epidermal cells and DDC, cell suspensions were prepared from normal skin using a cocktail of enzymes as described above and analyzed by flow cytometry. Double staining with HLA-DR was performed to identify HLA-DR positive APC. In epidermal cell suspensions no expression of CD1b or CD1d was observed on HLA-DR positive cells (data not shown). CD1a and CD1c expression on HLA-DR^{bright} cells was observed as previously described (Meunier *et al*, 1993; data not shown). In fresh dermal cell suspensions HLA-DR positive cells were found to express CD1a, CD1b, CD1c, and CD1d (**Fig 2*a***). These cells exhibited

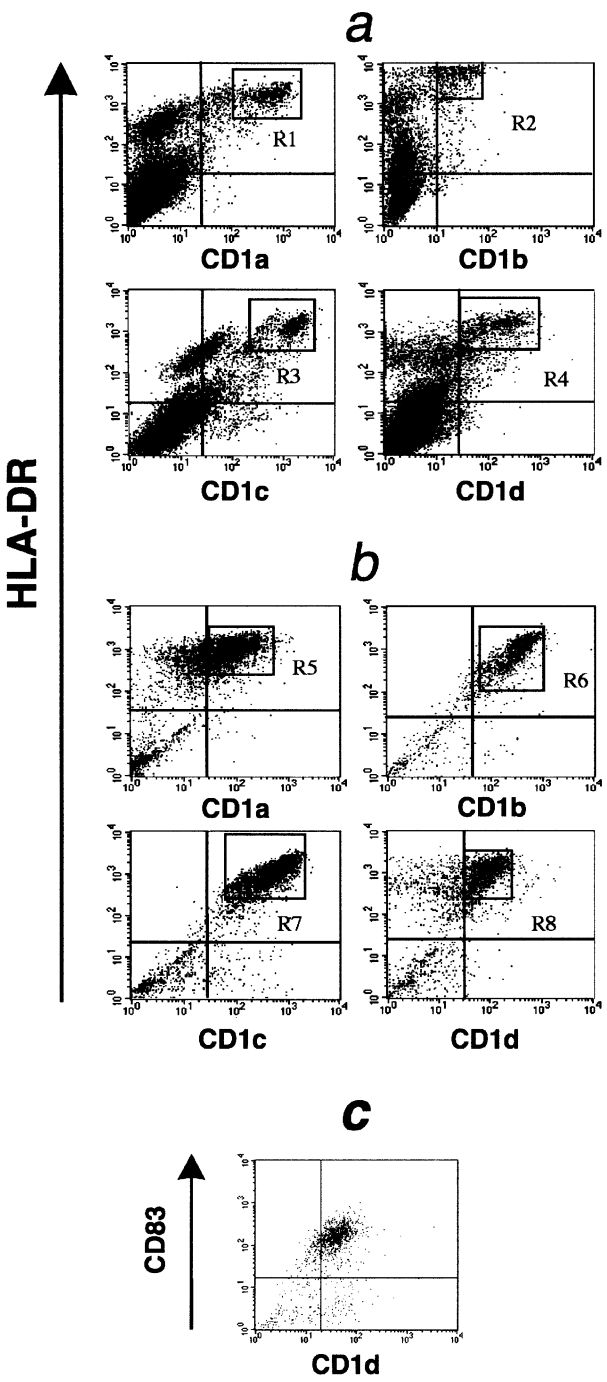

Figure 2. CD1d expression on fresh and migrated DDC. (*a*) HLA-DR^{bright} cells express CD1d in fresh dermal cell suspension. Two-color staining of dermal cell suspension analyzed by flow cytometry shows different HLA-DR$^+$ cell subsets: CD1a (25% ± 3.6% of the total HLA-DR$^+$ population), CD1b (16.3% ± 4.0%), CD1c (39.3% ± 3.0%), and CD1d (16% ± 3.6%). (*b*) Migrated DDC express CD1d. Two-color staining of migrated DDC analyzed by flow cytometry: 60.5% ± 8.0% of DDC were positive for CD1d. (*c*) Migrated mature CD83 positive DDC express CD1d.

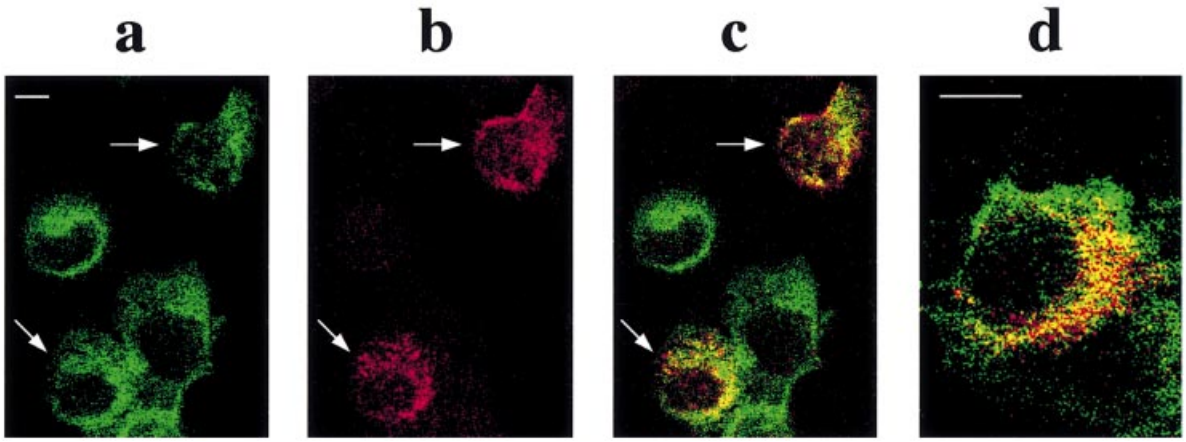

Figure 3. HLA-DR and CD1d molecules are colocalized in the same cellular compartments. Immunofluorescence staining of DDC analyzed by confocal laser scanning microscopy: (*a*) HLA-DR-FITC; (*b*) CD1d-Texas Red (42.1); and (*c*, *d*) double staining. *Arrows* show double positive cells (*c*, yellow/orange). Note at higher magnification the colocalization of HLA-DR and CD1d represented by yellow/orange spots (*d*). *Scale bars*: 10 μm.

high forward and side scatter properties. Three different HLA-DR^{+} subsets based on their fluorescence intensity (bright, medium, dim) were identified (**Fig 2*a***). Expression of CD1d was observed in the HLA-DR^{bright} population. Preferential expression of CD1d on the HLA-DR^{bright} population indicates that CD1d expression in human dermis is mainly confined to activated dendritic cells. Within HLA-DR positive cells, the mean percentage of CD1 expression was calculated from three independent experiments: CD1a, 25% ± 3.6%; CD1b, 16.3% ± 4.0%; CD1c, 39.3% ± 3.0%; and CD1d, 16% ± 3.6%. A 2 d culture of fresh cell suspensions in complete medium with GM-CSF and IL-4 to induce maturation of dendritic cells did not change the percentages of CD1 positive cells (data not shown).

To investigate CD1d expression on highly enriched dermal APC, cells migrating out of dermal fragments after 2 d culture were analyzed. DDC represented 70%–80% of the total population after metrizamide enrichment. These cells were highly HLA-DR positive and expressed CD83 (**Fig 2*c***), a marker of mature dendritic cells, as well as high levels of CD80 and CD86 (Nestle *et al*, 1993).

HLA-DR^{bright} cells coexpressed CD1b and CD1c. CD1d expression was found on 60.5% ± 8.0% of HLA-DR^{bright} cells (**Fig 2*b***). Therefore, mature DDC express both classical MHC and nonclassical CD1 antigen-presenting molecules.

CD1d colocalizes with HLA-DR containing cellular compartments To get an insight into the cellular distribution of CD1d molecules two-color immunofluoresence was performed on highly enriched DDC, obtained after migration out of dermal fragments. Cells were double stained with MoAb against HLA-DR and CD1d (42.1 MoAb) and were analyzed by confocal laser scanning microscopy. Strong expression of HLA-DR was observed on DDC, with HLA-DR containing compartments mostly localized close to or on the cell surface (**Fig 3*a***). Staining with anti-CD1d MoAb revealed a subset of cells that were CD1d positive (**Fig 3*b***). Colocalization of CD1d and HLA-DR (orange/yellow spots) was observed in compartments close to as well as on the cell surface (**Fig 3*c*, *d***).

Microdissected dermis contains a high molecular weight CD1d variant To further investigate the biochemical nature of CD1d expressed on dermal APC, Western blotting was performed on microdissected normal human dermis. CD1d protein was detected with antibody D5. CD1d in dermis was detected at a molecular weight of 50–55 kDa. This might be indicative of a highly glycosylated variant present in human dermis in the absence of a low or unglycosylated form as found in intestinal epithelium (Somnay Wadgaonkar *et al*, 1999). The corresponding band of the CD1d positive control cell line C1R CD1d was detected at 37 kDa, and appeared unglycosylated (**Fig 4**).

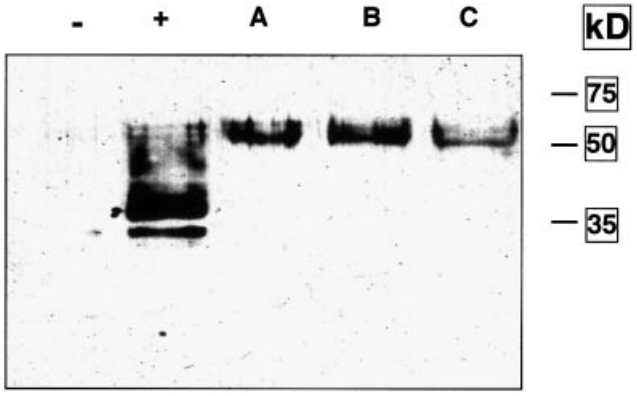

Figure 4. A high molecular variant of CD1d is present in human dermis. Western blot analysis of microdissected dermal sections using CD1d antibody (D5). C1R mock (–) and C1R CD1d transfectants (+) were used as negative and positive controls. *A*, *B*, *C* indicate different dermal samples. Note the presence of a CD1d molecule with apparent molecular weight 50–55 kDa.

CD1d is expressed on immature and mature mo-DC As a next step we investigated the expression of CD1 molecules on mo-DC. These cells correspond to DDC *in vivo* (Grassi *et al*, 1998) and are currently the favored *in vitro* study system that allows a discrimination between immature and mature dendritic cells (Banchereau *et al*, 2000). Furthermore they are increasingly used for immunotherapy of cancer. Therefore, CD1 expression in these cells might be of potential significance for basic research as well as clinical applications. Cells were cultured under FBS-free conditions using 1% AP as used in current clinical trials of dendritic cell vaccination. Under these conditions CD1a expression on immature and mature dendritic cells was weak to absent (**Fig 5**). CD1b and CD1c expression was upregulated during *in vitro* differentiation from monocytes (**Fig 5**). There was stable and equivalent expression of these molecules on immature (D6) and mature dendritic cells (D7 after addition of the maturation cocktail). Only few data are available on CD1d expression in human APC. Expression of CD1d was observed on monocytes as well as on mo-DC (**Fig 5**). The mean fluorescence of mo-DC reached the highest level around day 4. Immature as well as mature dendritic cells

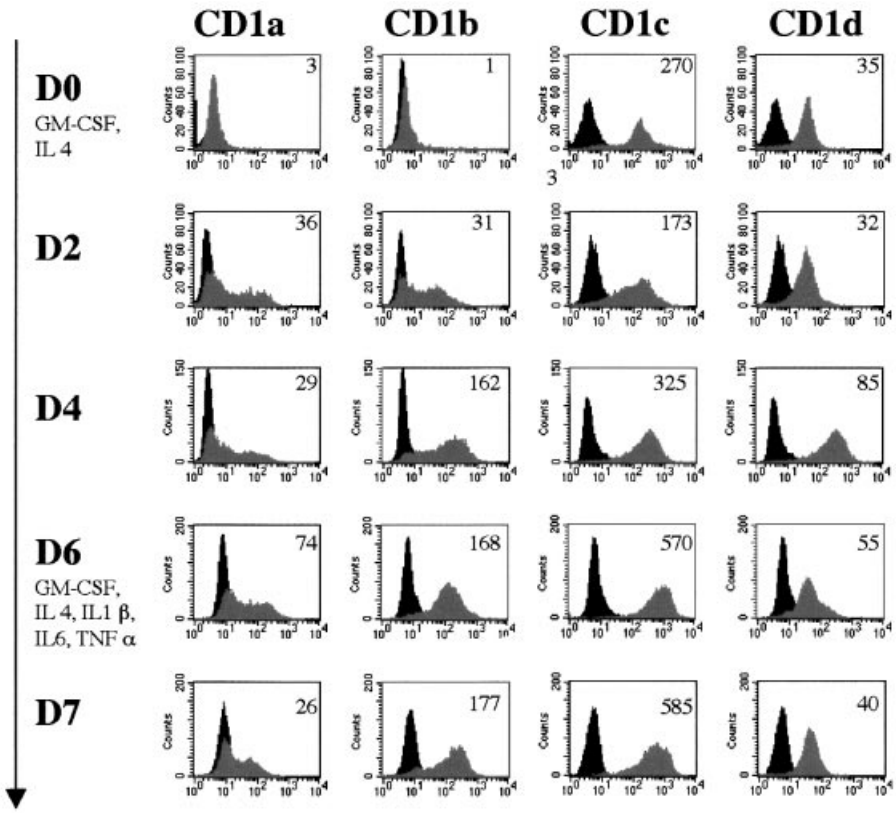

Figure 5. CD1 expression on mo-DC generated in the presence of AP. Monocytes were cultured in the presence of IL-4 and GM-CSF for 6 d in 1% AP. At day 6 they were stimulated with a maturation cocktail containing IL-6, TNF-α, and IL-1β for 24 h. At days 0, 2, 4, 6, and 7 cells were analyzed for CD1 molecule expression. Values represent the mean fluorescence. Mo-DC generated in AP display a low level of CD1a expression, whereas CD1b and CD1c molecules are strongly upregulated during maturation. CD1d is constantly expressed during culture.

expressed CD1d molecules indicating that the expression was not maturation dependent.

CD1d expression is downregulated in the presence of FBS Earlier studies on mo-DC including dendritic cell vaccination for patient treatment were performed in the presence of 10% FBS (Cella *et al*, 1997; Nestle *et al*, 1998). Recently, FBS-free culture conditions using 1% AP were introduced (Thurner *et al*, 1999). Mo-DC coming from the same patient were generated using AP or FBS as addition to the culture medium. CD1b and CD1c were expressed with the same pattern in both AP- and FBS-treated mo-DC (data not shown). In certain donors expression of CD1a was observed already in the starting population (day 0 monocytes). In contrast to mo-DC generated in AP, FBS-derived mo-DC expressed high levels of CD1a. The reverse was true for CD1d expression. Whereas mo-DC cultured in AP expressed high levels of CD1d, there was an important downregulation of CD1d during culture in FBS and in some cases it was completely absent on mature CD83-positive dendritic cells (**Fig 6**). In conclusion there is a strong dependence of CD1 expression on dendritic cell culture conditions.

DISCUSSION

The CD1 family of antigen-presenting molecules plays an important role in lipid antigen presentation as well as immune response to mycobacteria and tumors (Porcelli *et al*, 1998). In this study we focused on CD1d, a recently described member of this family, and show that CD1d expression in skin is largely restricted to dendritic appearing APC in dermis (DDC) apart from expression on keratinocytes in epidermis (Bonish *et al*, 2000). For comparison, we confirmed previous studies using an expanded panel of antibodies, demonstrating CD1a expression mostly on epidermal Langerhans cells, whereas CD1c was found on DDC (**Figs 1, 2**) (Lenz *et al*, 1993; Meunier *et al*, 1993; Nestle *et al*, 1993; Larregina *et al*, 1997). Expression of CD1b on DDC was detectable with two different antibodies. CD1d was found to be mainly expressed on 16% ± 3.6% of HLA-DR^{bright} cells in dermal cell suspensions, but was clearly absent on HLA-DR^{bright} cells in epidermal cell suspensions. 60.5% ± 8.0% of cells expressed CD1d in highly enriched DDC, whereas CD1d expression was absent on Langerhans cells (**Fig 2**). The fact that a distinct subpopulation of DDC expresses CD1d is in strong support of the hypothesis that there are phenotypically distinct subsets of DDC with otherwise homogeneous microscopic appearance and expression of adhesion, costimulatory, and HLA-DR molecules (Meunier *et al*, 1993; Nestle *et al*, 1993; Nestle and Burg, 1999). Interestingly, expression of all known members of the CD1 family was observed on DDC. This may be an advantage in the generation of immune response against tumor antigens or infectious agents such as mycobacteria. Mycobacterial antigens and glycolipids thereof have been shown to access distinct CD1 molecules at different intracellular sites (Schaible *et al*, 2000). DDC subsets, as defined by differential expression of CD1 molecules, might play a distinct role in skin immune response.

To get an insight into the cellular and subcellular distribution on DDC, CD1d was analyzed by confocal laser scanning microscopy. CD1d mostly colocalized to compartments that contained HLA-DR molecules (**Fig 3**). These findings might suggest a common pathway for classical MHC-related and nonclassical CD1-related antigen presentation. Microdissected dermis contained CD1d with a molecular weight of 50–55 kDa (**Fig 4**), indicating the presence of a glycosylated form of CD1d. Glycosylation is often considered to stabilize proteins and might allow for sustained expression of CD1d and prolonged presentation of CD1d-related antigens in human skin.

CD1d expression in skin is of potential interest for skin biology as it is involved in the presentation of glycolipids to natural killer T cells. It has been shown that α-galactosylceramide, a synthetic acylphytosphingolipid originally isolated from a marine sponge, is presented by CD1d to the clonotypic vα24/vβ11 T cell receptor of

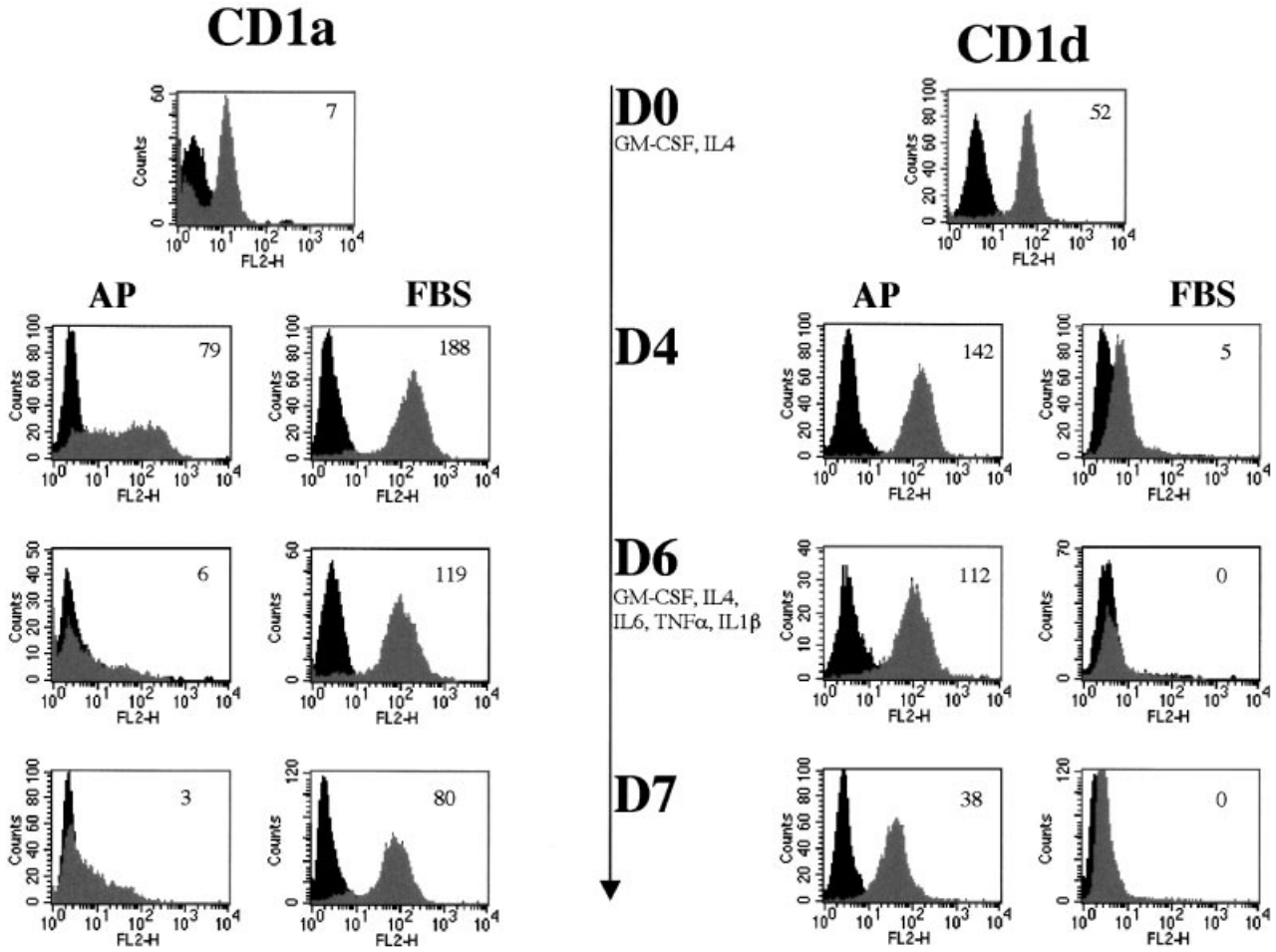

Figure 6. CD1d expression is downregulated in the presence of FBS. Effects of FBS *versus* AP on CD1a and CD1d expression were evaluated. Mo-DC cultured in FBS upregulate CD1a expression whereas CD1d expression is downregulated. Addition of AP instead of FBS to the culture medium leads to low or undetectable CD1a expression on mo-DC, whereas CD1d expression is detectable throughout the culture period.

natural killer T cells. Such cells were shown to have potent antitumor activity (Kawano *et al*, 1999) and also to induce strong TH-1 bias in autoimmune diseases such as diabetes mellitus (Wilson *et al*, 1998). Self glycolipids might represent potential autoantigens (Shamshiev *et al*, 1999). Human epidermis is rich in lipids and glycolipids (Wertz, 1992). A disturbance in lipid production might lead to aberrant presentation of lipids and glycolipids by CD1d molecules in inflammatory and neoplastic skin conditions. CD1d expression has been demonstrated on psoriatic KC using the anti-CD1d antibody NOR3.2 (Nickoloff *et al*, 1999; Bonish *et al*, 2000). This antibody might not always meet stringent staining criteria, however (Yong-Jun Liu, Stanford University, personal communication), or might recognize different CD1d epitopes compared to other available anti-CD1d antibodies. Here we have shown with a panel of anti-CD1d antibodies unequivocal expression on DDC. Therefore, expression of CD1d by keratinocytes and professional APC in human dermis might have implications for autoimmune type chronic inflammatory skin diseases, such as psoriasis.

Mo-DC are currently the preferred *in vitro* study system for dendritic cells. They resemble DDC in phenotype and function (Grassi *et al*, 1998). Mo-DC may be generated in large numbers from peripheral blood. They allow the investigation of the impact of maturation stimuli on dendritic cell biology. Furthermore, these cells are increasingly used for the immunotherapy of cancers such as melanoma (Nestle *et al*, 1999). We detected CD1d expression on monocytes and both mature and immature mo-DC cultivated in the presence of AP (**Fig 5**). In contrast, when AP was replaced by FBS a downregulation of CD1d during the differentiation and maturation process was observed, whereas CD1a was strongly expressed (**Fig 6**). Differential expression of CD1 molecules on dendritic cell preparations used for immunotherapy of cancer might have implications for the repertoire of T cells expanded during the vaccination process.

In conclusion we have shown that CD1d reactivity in human dermis is confined to dermal dendritic APC *in vivo* and *in vitro*. CD1d in dermis is highly glycosylated and colocalizes with HLA-DR containing cellular compartments. Expression of CD1d on mo-DC used for immunotherapy depends on culture conditions but not on maturation stage.

Expression of CD1d on dermal APC might have implications for presentation of lipids and glycolipids to effector T cells with potential relevance for autoimmune and anticancer immune response in skin.

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