"Dermal Dendritic Cells" Comprise Two Distinct Populations: CD1$^+$ Dendritic Cells and CD209$^+$ Macrophages

Maria Teresa Ochoa[1], Anya Loncaric[1], Stephan R. Krutzik[1], Todd C. Becker[1] and Robert L. Modlin[1,2,3]

A key cell type of the resident skin immune system is the dendritic cell (DC), which in normal skin is located in two distinct microanatomical compartments: Langerhans cells (LCs), mainly in the epidermis, and dermal DCs (DDCs), in the dermis. Here, the lineage of DDCs was investigated using monoclonal antibodies and immunohistology. We provide evidence that "DDC" comprise at least two major phenotypic populations of dendritic-appearing cells, immature DC expressing CD1, CD11c and CD208; and macrophages expressing CD209, CD206, CD163, and CD68. These data suggest that dermal dendritic-appearing macrophages comprise a novel part of the innate immune response in the resident skin immune system.

Journal of Investigative Dermatology (2008) **128,** 2225–2231; doi:10.1038/jid.2008.56; published online 13 March 2008

INTRODUCTION

The resident skin immune system functions to defend the host against external threats, including microbial pathogens and allergens, as well as physical assaults, including trauma and UV light. The innate immune response is rapid, involving cells expressing germline-encoded pattern-recognition receptors, and has a role in directly dealing with the threat by triggering microbicidal mechanisms, inflammation, and/or apoptosis. Cells of the innate immune response include keratinocytes, endothelial cells, Langerhans cells (LCs), and dermal dendritic cells (DDCs). In contrast, the adaptive immune response is slower in onset, involves receptors clonally selected after gene rearrangement, results in immunologic memory, and is mediated by T and B lymphocytes.

A major skin-resident innate immune cell subset is the "DDC". Initially, DDCs were identified using a polyclonal antibody against clotting factor XIIIa and thought to be a homogeneous population (Cerio *et al.*, 1988, 1989). As such, they have been isolated from skin using various markers and used in functional assays (Tse and Cooper, 1990; Meunier *et al.*, 1993; Nestle *et al.*, 1993a; Duraiswamy *et al.*, 1994). Since DDCs are classified as DCs, they are presumed to be professional antigen-presenting cells that have been recognized as crucial regulators of cutaneous adaptive immune response in humans and mice (Dupasquier *et al.*, 2004). However, the homogeneity and origin of these cells has also been questioned (Adany *et al.*, 1985, 1988; Muszbek *et al.*, 1985).

One of the problems in determining whether DDC comprise a single homogeneous population or represent two or more subsets has been the lack of specific markers. We recently demonstrated that activation of Toll-like receptors causes rapid differentiation of distinct precursors of human peripheral monocytes into CD1b$^+$ and CD209$^+$ (DC-SIGN) cells (Krutzik *et al.*, 2005). CD1b$^+$ cells had an immature DC phenotype, release proinflammatory cytokines, and function as efficient APCs. The CD209$^+$ (DC-SIGN) cells had a macrophage-like phenotype, were phagocytic, and utilized CD209 to facilitate uptake of bacteria. These data provide evidence that the dual host defense roles of the innate immune system are mediated by induction of two distinct phenotypic and functional populations, CD1b$^+$ DCs and CD209$^+$ (DC-SIGN) macrophages. The surprising finding was that CD209$^+$ (DC-SIGN), which was previously thought to be a marker for DCs (Geijtenbeek *et al.*, 2000; Engering *et al.*, 2002), was found on a population of macrophages in tonsil and inflamed skin. Using this observation, we sought to investigate the phenotype of various "DC" populations in the skin with monoclonal antibodies that discriminate against DCs and macrophages, using immunoperoxidase and confocal laser microscopy.

RESULTS

Identification of cells bearing DC markers in normal human skin

We investigated the *in situ* expression of different DC markers (Table S1) in normal human skin tissue to determine their

[1]*Division of Dermatology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA;* [2]*Department of Microbiology and Immunology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA and* [3]*Molecular Biology Institute, David Geffen School of Medicine at UCLA, Los Angeles, California, USA*

Correspondence: Dr Robert L. Modlin, Division of Dermatology, David Geffen School of Medicine at UCLA, 52-121 CHS, 10833 Le Conte Avenue, Los Angeles, California 90095, USA.
E-mail: rmodlin@mednet.ucla.edu

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; DC, dendritic cell; DDC, dermal dendritic cell

Received 4 September 2007; revised 10 January 2008; accepted 1 February 2008; published online 13 March 2008

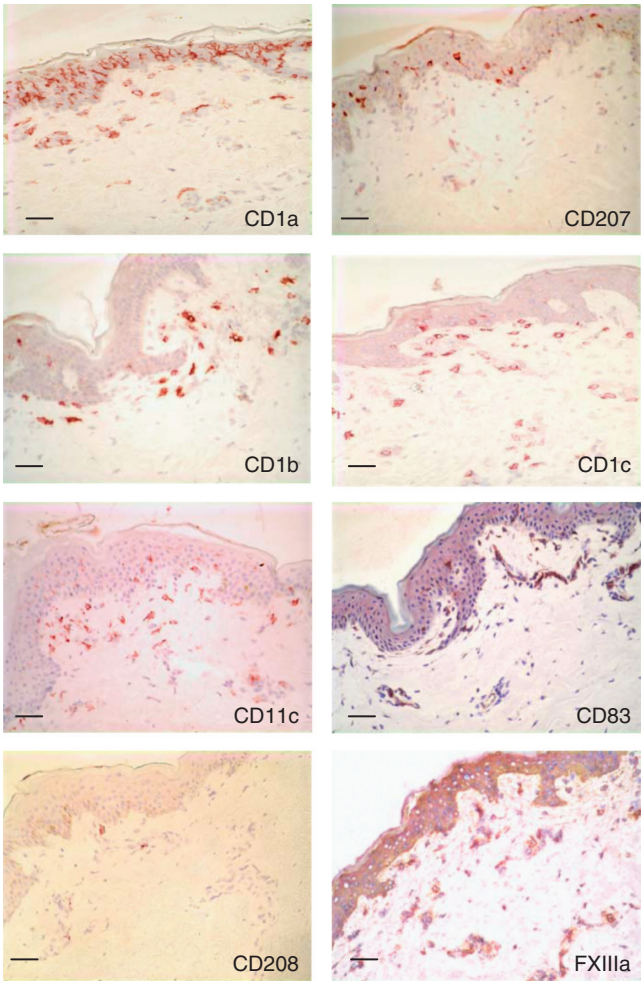

Figure 1. Identification of DCs in normal human skin. Normal human skin tissue was labeled with mAbs specific for CD207 (Langerin), CD1a, CD1b, CD1c, CD83, CD208 (DC-LAMP), and CD11c, using the immunoperoxidase method. Original magnification ×200. Bars = 50 μm.

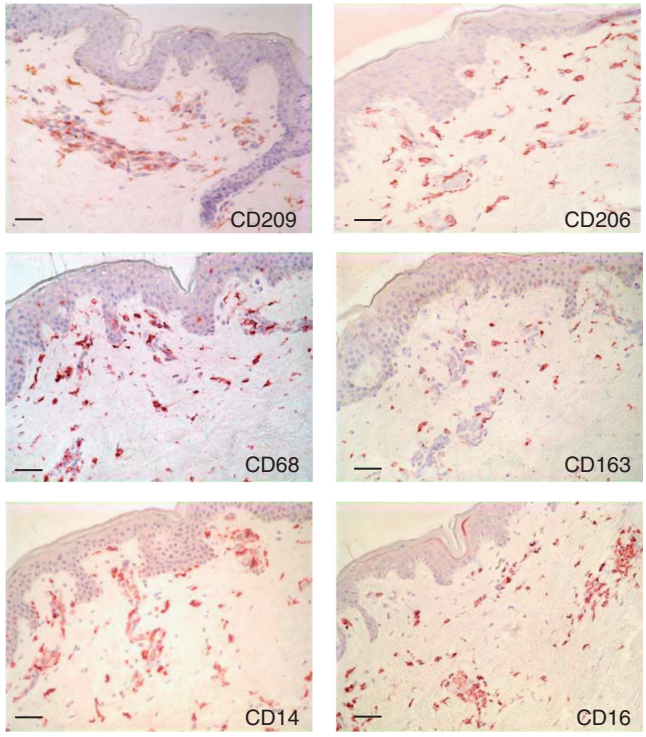

Figure 2. Identification of monocyte/macrophage cells in normal human skin. Normal human skin tissue was labeled with mAbs specific for CD209 (DC-SIGN), CD206 (mannose receptor), CD163, CD14, CD16, and CD68, using the immunoperoxidase method. Original magnification ×200. Bars = 50 μm.

morphology, phenotype, and distribution (Figure 1). Cells with a dendritic morphology were found in the epidermis and dermis. CD1a$^+$ and CD207$^+$ (Langerin) cells, presumably LCs, were located in the epidermis. Other DC markers, including CD1b, CD1c, and CD11c, were found on dendritic-appearing cells mainly in the superior dermis. We also used markers for mature DC such as CD83 and CD208 (DC-LAMP); however, very few DCs expressing these markers could be identified in normal skin.

Identification of cells expressing monocyte/macrophage markers in normal human skin

The highest level of expression (that is, greatest frequency of cells) using characteristic monocyte/macrophage markers such as CD14, CD68, and CD163 (Table S1) was detected in the dermis. Similar to DCs, cells bearing monocyte/macrophage markers were also characterized by dendritic morphology, and were located at the level of the capillaries and in the higher part of the reticular dermis. The distribution of CD209$^+$ cells was similar to that of cells expressing CD14, CD68, CD163, and CD206 (mannose receptor) (Figure 2).

DCs and macrophages in the dermis have similar morphology

Although DCs can be distinguished from monocytes/macrophages by their dendritic morphology, we found that cells expressing characteristic macrophage markers can also display a dendritic morphology (Figures 3 and 4a). The morphology of the macrophages in the papillary and reticular dermis ranged from elongated and spindled to compact and stellate shape (Figure 4b).

Macrophage and DC markers are expressed on distinct subsets of cells in normal human skin

To determine the phenotype of CD209$^+$ (DC-SIGN) cells in normal human skin tissue, frozen tissue sections were labelled with antibodies to CD209 (DC-SIGN) and paired with different DC and macrophage markers. CD209$^+$ (DC-SIGN) cells did not express CD207$^+$ (Langerin), CD1a, CD1b, CD1c, CD11c, CD83, and CD208 (DC-LAMP). These data indicate that CD209$^+$ (DC-SIGN) expression is not restricted to DCs in the skin (Figure 5a). In addition, CD1b$^+$ cells did not express macrophage markers (CD163, CD68, and CD14), suggesting that CD1 expression is restricted to DCs in the skin (Figure 5b).

We reasoned that CD209$^+$ (DC-SIGN) and other monocyte/macrophage markers might colocalize on cells in the

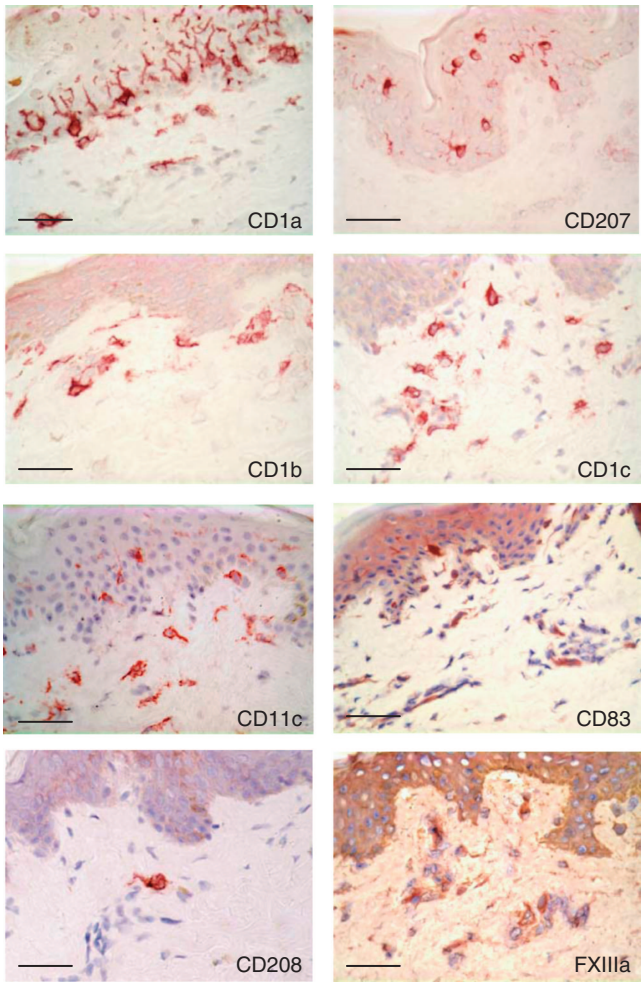

Figure 3. Morphology and distribution of DC markers in normal human skin. DC markers as CD207 (Langerin), CD1a, CD1b, CD1c, CD83, CD208 (DC-LAMP), and CD11c were located in the epidermis and the superior dermis. Original magnification ×400. Bars = 50 μm.

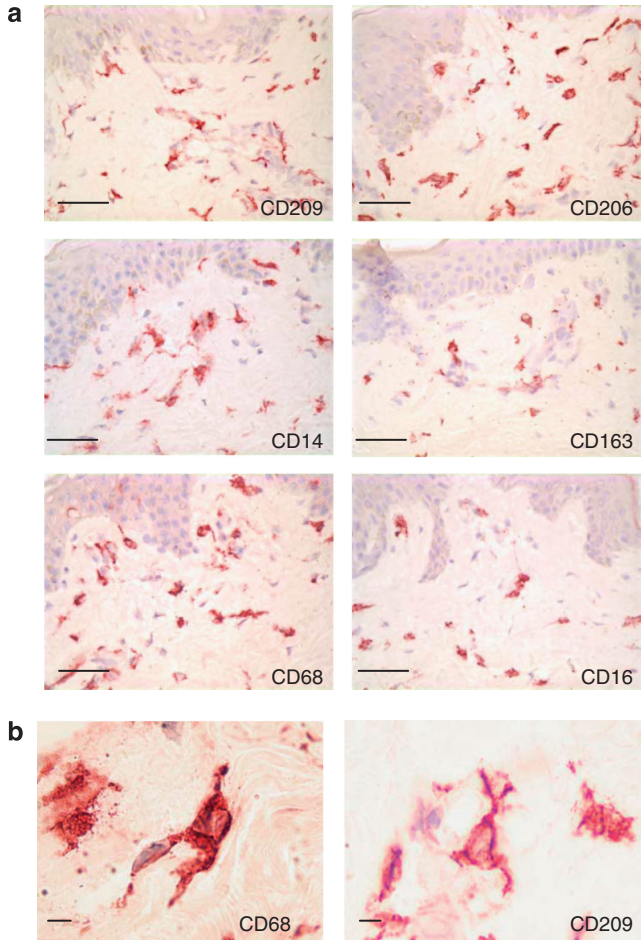

Figure 4. Morphology and distribution of monocyte/macrophage cell markers in normal human skin. (**a**) Monocyte/macrophage cell markers such as CD209 (DC-SIGN), CD206 (mannose receptor), CD163, CD14, CD16, and CD68 were only located in the superior papillary and reticular dermis, and not in the epidermis. Original magnification ×400. Bars = 50 μm. (**b**) Monocyte/macrophage cells display dendritic morphology. Original magnification ×1,000. Bars = 5 μm.

dermis of normal human skin. Therefore, expression of CD209$^+$ (DC-SIGN) and CD68, CD14, CD163, and CD206$^+$ (macrophage mannose receptor or MMR) was examined in normal human skin tissue by immunofluorescence. A major population of dendritic-appearing cells was located at the level of the capillaries and in the higher part of the reticular dermis, and coexpressed CD209$^+$ (DC-SIGN), CD206$^+$ (macrophage mannose receptor or MMR), and monocyte/macrophage markers CD14, CD163, CD68, but not DC markers (CD1a, CD1b, CD1c, CD11c, DC-LAMP, and Langerin) (Figure 6).

Therefore, we conclude that CD209$^+$ (DC-SIGN) macrophages, expressing CD68, CD14, and CD163, markers typically found on monocytes/macrophages, comprise a major subset of dendritic-appearing dermal cells.

Isolated dermal cells from normal human skin contain CD209$^+$CD11c^{lo} cells

To substantiate the immunohistological identification of distinct populations of macrophages and DCs, dermal cells were isolated from normal human skin and analyzed directly by flow cytometry. Distinct populations of CD209$^+$CD11c^{lo} and CD209$^-$CD11c^{hi+} cells were identified (Figure 7). These data were obtained by separating epidermis from dermis, and then dispersing cells in the dermis using a scalpel, which resulted in low yield of mononuclear cells. Treatment of the dermis with collagenase overnight, as described by Zaba *et al.* (2007), resulted in loss of CD209 expression as did treatment of peripheral blood-derived CD209$^+$ cells (data not shown). Loss of CD209 expression may be due to dedifferentiation of macrophages during collagenase treatment or due to proteolytic digestion of the CD209 epitope. Therefore, additional technological considerations will be required to obtain sufficient numbers of CD209$^+$ cells from normal skin for functional study.

DISCUSSION

The resident skin immune system stands guard to deal with attacks from the outside environment, including microbes,

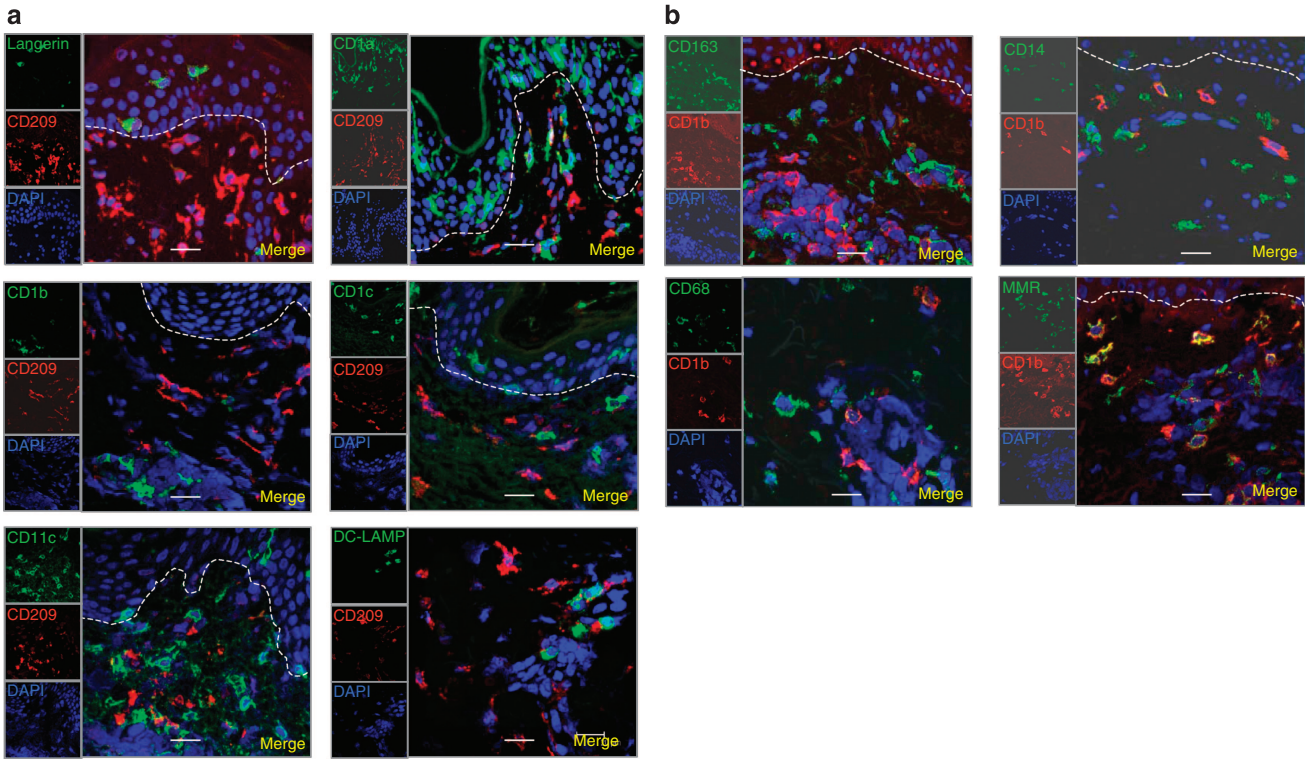

Figure 5. CD209$^+$ (DC-SIGN) cells and DC markers are expressed on distinct subsets of cells in normal human skin. (**a**) Normal human skin tissue sections were labeled with specific antibodies for DCs and visualized by confocal laser microscopy. CD209-positive cells (red images) did not colocalize with other DC markers (CD207 (Langerin), CD1a, CD1b, CD1c, CD11c, CD208 (DC lamp)) (green images). (**b**) CD1b$^+$ cells did not express monocyte/macrophage markers (CD163, CD68, CD14). Nuclei were labeled with DAPI (blue images). Original magnification $\times 400$. Bars = 20 μm.

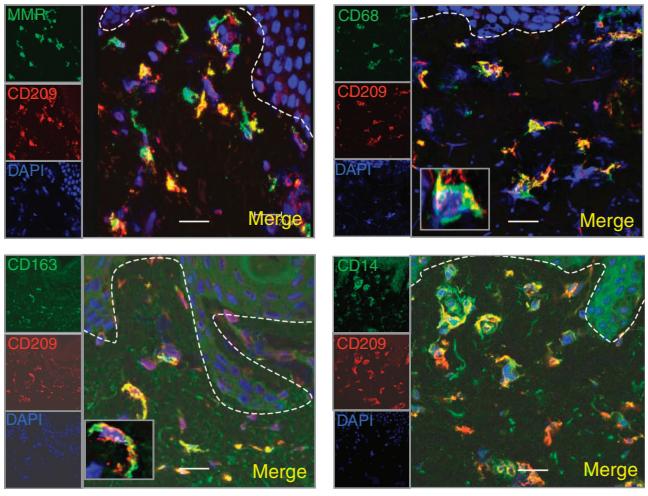

Figure 6. CD209$^+$ (DC-SIGN) cells and monocyte/macrophage cell markers are expressed on the same subsets of cells in normal human skin. Normal human skin tissue sections were labeled with specific antibodies for monocyte/macrophage markers and visualized by confocal laser microscopy. CD209 (DC-SIGN)-positive cells (red images) colocalize with other monocyte/macrophage markers (CD206 (mannose receptor), CD163, CD68, CD14) (green images). Nuclei were labeled with DAPI (blue images). Original magnification 400 $\times$. Bars = 20 μm. The box in panel 2 and 3 indicates a magnified positive cell.

allergens and UV light. The epidermis serves as the initial barrier; however, the resident immune cells in the dermis must serve to deal with host threats that either escape detection by the epidermis or enter via the cutaneous vasculature. The dermal immune system is known to not only involve circulating T cells that survey the skin for foreign antigens, but also a population of ''DDCs''. Here, we provide evidence that ''DDCs'' comprise at least two major phenotypic populations of dendritic-appearing cells, immature DCs that express CD1, CD11c, and CD208, and macrophages that express CD209, CD206, CD163, and CD68 (Figure 8). We conclude that dermal dendritic-appearing macrophages are a novel component of the skin immune system.

DDCs were initially identified and characterized using a polyclonal antibody that recognizes clotting factor XIIIa. This antibody was used to identify spindle- or stellate-shaped cells in human connective tissue (Fear *et al.*, 1984). Later, Reid *et al.* (1986) called them fibroblasts. But it was Headington (1986) who in 1986 named them ''dermal dendrocytes''—or uncommitted stem cells—using a polyclonal antibody for FXIIIa (Cerio *et al.*, 1989). Unfortunately, factor XIIIa is not a specific marker, as it can also be expressed on several cell types, including endothelial cells (Suvarna and Cotton, 1993). However, in 1987, using macrophage markers such as RFD7, RFDR2 (HLA-DR), and KiM7 (CD68), Adany and

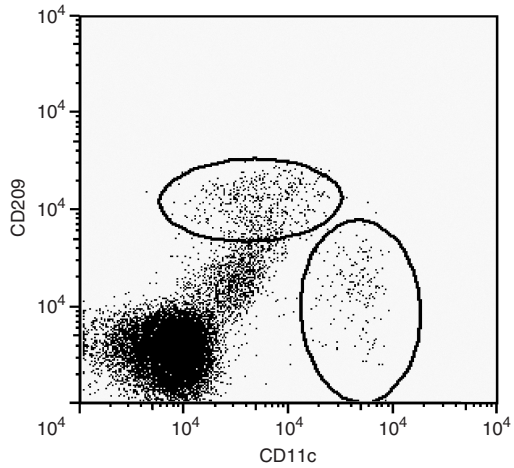

Figure 7. Dermal cells isolated from normal human skin contain CD209$^+$CD11c^{lo+} cells. Distinct populations of CD209$^+$CD11c^{lo} and CD209$^-$CD11c^{hi+} cells were identified in dermal single-cell suspensions from normal human skin.

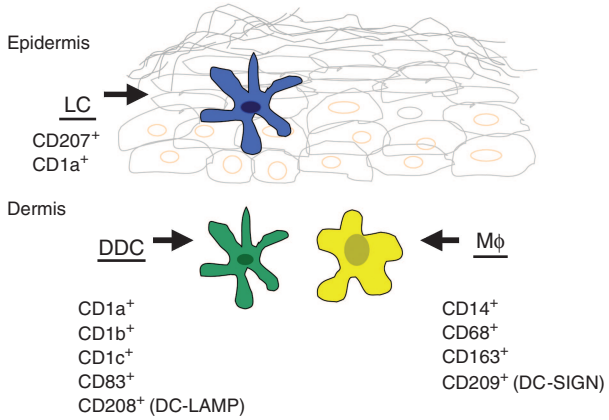

Figure 8. Subsets of DCs and macrophages in normal human skin. "DDCs" include at least two major populations of dendritic-appearing cells: immature DCs that express CD1and CD208, and macrophages that express CD209, CD163, CD14, and CD68.

Muszbek (1987) (1989) suggested that these cells were tissue macrophages. Functionally, DDCs demonstrate features of both macrophages and DCs. A key macrophage function of DDCs is their ability to efficiently phagocytose hemosiderin, melanin, and bacteria (Altman *et al.*, 1992; Filgueira *et al.*, 1996; Nestle *et al.*, 1998). A key DC function of DDCs is their ability to take up and process antigens, migrate to lymphatic organs, and activate naïve T cells (Nestle *et al.*, 1993b; Nestle and Nickoloff, 1995). Our data indicate that in fact resident DDCs in normal skin are comprised of at least two phenotypically distinct populations of CD209$^+$ macrophages and CD1$^+$ DCs.

The CD209$^+$ dermal dendritic-appearing macrophage population is also present in inflamed skin, as we reported in leprosy lesions (Krutzik *et al.*, 2005). The phenotype of these dermal dendritic-appearing macrophages includes several markers of phagocytic function, CD209 (DC-SIGN), CD206 (macrophage mannose receptor), and CD163 (Figure 5). Both CD209 and CD206 are pattern-recognition receptors; CD209 (DC-SIGN) is a C-type lectin that binds various glycoproteins and glycolipids (Geijtenbeek *et al.*, 2000; Ebner *et al.*, 2004). CD209 has been shown to facilitate the uptake of bacteria, including *Mycobacterium tuberculosis*, and viruses, including HIV (Pohlmann *et al.*, 2001). CD206 (macrophage mannose receptor) is known to facilitate uptake of microbial molecules containing a repeating mannose motif (Sallusto *et al.*, 1995; Wollenberg *et al.*, 2002). CD163 is the haptoglobin receptor involved in the transport of iron into cells (Kristiansen *et al.*, 2001). CD206 (mannose receptor) and CD11c (myeloid marker) can be expressed in the two populations, DCs and macrophages, as we previously reported (Krutzik *et al.*, 2005). Together, coexpression of these molecules indicates that dermal dendritic-appearing macrophages are resident in normal skin, and are likely to recognize microbial pathogens via multiple pattern recognition receptors and phagocytose them. This points the way to devise strategies to isolate these cells from normal skin and study their function *in vitro.*

The function of dermal CD1$^+$ DCs in terms of antigen presentation activity has been demonstrated in earlier studies (Meunier *et al.*, 1993; Nestle *et al.*, 1993a, b; Turville *et al.*, 2002). It has also been suggested that human LCs are replenished by precursors in the dermis and that CD14$^+$ dermal APCs are the main precursor population (Larregina *et al.*, 2001). Further examination of leprosy skin lesions using confocal laser microscopy revealed that distribution and frequency of these resident skin populations might correlate with disease status (Krutzik *et al.*, 2005). Within self-limited tuberculoid leprosy lesions, CD1a$^+$ cells were frequent in the epidermis, and CD209 (DC-SIGN) and CD1b are expressed on distinct, non-overlapping dermal cell populations. In contrast, within progressive lepromatous leprosy lesions, the frequency of CD1a$^+$ LCs and CD1b$^+$ dermal DCs was lesser. The CD209$^+$ cells in tuberculoid leprosy lesions were found to express monocyte/macrophage markers CD14, CD16, CD64, and CD68, but did not express DC markers CD1a, CD1b, CD63, CD83, or DC-LAMP. These data indicate that the majority of CD209$^+$ cells express cell-surface markers typically found on monocytes/macrophages, and reflect the phenotype found here in normal skin. Future studies should be directed toward identifying the phenotype of CD209$^+$ cells in other disease states.

The studies by Zaba *et al.* (2007) support the presence of distinct populations of macrophages and DCs in normal human dermis, but differ in identifying the specific phenotypic of the dermal macrophages. Our study clearly identifies a CD209$^+$CD163$^+$ macrophage population in normal skin that do not express characteristic DC markers CD1a, CD1b, CD1c, and DC-LAMP, in addition to CD11c. The restricted expression of CD209 is of particular interest because it mediates the phagocytosis of a range of microbial pathogens. Zaba *et al.* demonstrated CD209 expression on both macrophage and DC populations, but used fluorescence microscopy for their analysis. Our studies used confocal

microscopy, which has the capability to analyze thin (0.6–0.7 μm) optical sections that helps differentiate epitope colocalization from cell overlap.

The mechanism by which these dermal DCs and macrophages differentiate from monocyte precursors is fundamental to understanding the resident skin immune system. We previously demonstrated that human peripheral monocytes activated with a Toll-like receptor 2/1 ligand were differentiated into two distinct subsets: CD209$^+$ CD16$^+$ macrophages and CD1b$^+$ CD209$^-$ DCs. CD209$^+$ cells were expanded by Toll-like receptor-mediated upregulation of IL-15, had a macrophage-like phenotype, were phagocytic, and used CD209$^+$ to facilitate uptake of bacteria. In contrast, CD1b$^+$ DC were expanded by Toll-like receptor-mediated upregulation of GM-CSF, promoted T-cell activation and secreted proinflammatory cytokines. The mechanisms by which these DDC and dendritic-appearing macrophages accumulate in normal skin and disease lesions, as well as their functional role in disease pathogenesis, should provide new therapeutic targets for skin disease.

MATERIALS AND METHODS

Human normal skin tissue

Human normal skin tissue (obtained after breast reduction and abdominal plastic surgery) from healthy volunteers was obtained from the UCLA Tissue Procurement & Histology Core Laboratory (HTLPC) at the UCLA School of Medicine. Acquisition of all skin biopsy specimens from human donors was reviewed and approved by the committees on investigations involving human subjects of the University of California, Los Angeles, and adhered to the Declaration of Helsinki Principles.

Biopsy specimens were embedded in OCT medium (Ames Co., Elkhart, IN) and snap-frozen in liquid nitrogen. Sections (4-μm thick) were acetone-fixed and kept frozen (−80 °C) until use.

Immunohistochemical studies of DC and monocytes/macrophage expression in human normal skin tissue

Antibodies used for immunohistochemical studies are shown in Table S1. Frozen tissue sections were blocked with normal horse serum before incubation with the mAbs for 60 minutes, followed by incubation with biotinylated horse anti-mouse IgG for 30 minutes. Primary antibody was visualized using the ABC Elite system (Vector Laboratories, Burlingame, CA), which uses avidin and a biotin-peroxidase conjugate for signal amplification. The ABC reagent was incubated for 30 minutes followed by addition of substrate (3-amino-9-ethylcarbazole) for 10 minutes. Slides were counterstained with hematoxylin and mounted in crystal mounting medium (Biomeda, Foster City, CA).

Two-color immunofluorescence staining of cryostat sections

Double immunofluorescence was performed by serially incubating cryostat tissue sections with mouse anti-human mAbs of different isotypes (for example, O10 (anti-CD1a, IgG2a) and DCN46 (anti-CD209, IgG2b)), followed by incubation with isotype-specific, fluorochrome (A488) or (A568)-labelled goat anti-mouse immunoglobulin antibodies (Molecular Probes, Carlsbad, CA). Controls included staining with isotype-matched antibodies, as well as with CD1a or CD209, followed by secondary antibodies mismatched to the primary antibody isotype, to demonstrate the isotype specificity of the secondary labeled antibodies. Images were obtained using confocal laser microscopy. Nuclei was stained with 4′,6-diamidino-2-phenylindole (DAPI).

Confocal microscopy

Double immunofluorescence of normal skin sections was examined using a Leica-TCS-SP MP inverted single confocal laser-scanning and two-photon laser microscope (Heidelberg, Germany) fitted with DPSS-diode (561 nm), argon (488 nm), and two-photon lasers (Spectra-Physics Millenia, Mountain View, CA, X 532 diode pump laser and Tsunami picosecond Ti-sapphire laser), at the Carol Moss Spivak Cell Imaging Facility at the UCLA Brain Research Institute. Sections were illuminated with 488 and 561 nm lasers for red and green labels, and with Ti-sapphire laser set at 770 nm for DAPI excitation. Images of specimens with Alexa-488, or Alexa-568, (Molecular Probes), or DAPI were recorded sequentially through spectral emission filters set from 500 to 550 nm for Alexa-488, from 580 to 700 nm for Alexa-568, and from 410 to 490 nm for DAPI. Pairs of single images were superimposed for colocalization analysis. All micrographs are compiled serial *Z*-stack images 1 μm apart.

Isolation of dermal cells

Human normal skin was placed in cold RPMI and minced using two scalpels until single cells were obtained. Non-specific staining was blocked by incubating with non-specific mouse IgG for 10 minutes in fluorescence-activated cell sorting buffer (phosphate-buffered saline 0.1% sodium azide and 2% fetal bovine serum). Cells were then incubated with fluorescently labeled primary antibodies (CD209-FITC and CD11c-PE) (BD Biosciences, San Diego, CA). Cells were washed two times before fixing in 1% paraformaldehyde. Cells were then acquired using a FACSCalibur (BD Biosciences) in the UCLA Flow Cytometry Core Facility. Data analysis was performed using FlowJo software (Treestar, Ashland, OR).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr Matthew Schibler and the Carol Moss Spivak Cell Imaging Facility at the UCLA Brain Research Institute for use of the confocal laser microscope; Ping Fu, Christopher Creencia, and Delia Adefuin at the UCLA Tissue Procurement & Histology Core Laboratory for providing normal skin and preparation of tissue sections and; Dr Jenny Kim, Jenny Phan, and Dr Peter Sieling for helpful technical assistance. We acknowledge the financial support received from the National Institutes of Health (AI 22553, AI 056489, AI 47868, to RLM).

SUPPLEMENTARY MATERIAL

Table S1. Antibodies used to evaluate DC and macrophage (MΦ) expression in normal human skin.

REFERENCES

Adany R, Belkin A, Vasilevskaya T, Muszbek L (1985) Identification of blood coagulation factor XIII in human peritoneal macrophages. *Eur J Cell Biol* 38:171–3

Adany R, Muszbek L (1987) Cells containing factor XIII subunit a in benign and soft tissue tumours. *Histopathology* 11:1341–3

Adany R, Muszbek L (1989) Immunohistochemical detection of factor XIII subunit a in histiocytes of human uterus. *Histochemistry* 91:169–74

Adany R, Glukhova MA, Kabakov AY, Muszbek L (1988) Characterisation of connective tissue cells containing factor XIII subunit a. *J Clin Pathol* 41:49–56

Altman DA, Fivenson DP, Lee MW (1992) Minocycline hyperpigmentation: model for *in situ* phagocytic activity of factor XIIIa positive dermal dendrocytes. *J Cutan Pathol* 19:340–5

Cerio R, Griffiths CE, Cooper KD, Nickoloff BJ, Headington JT (1989) Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 121:421–31

Cerio R, Spaull J, Jones EW (1988) Identification of factor XIIIa in cutaneous tissue. *Histopathology* 13:362–4

Dupasquier M, Stoitzner P, van OA, Romani N, Leenen PJ (2004) Macrophages and dendritic cells constitute a major subpopulation of cells in the mouse dermis. *J Invest Dermatol* 123:876–9

Duraiswamy N, Tse Y, Hammerberg C, Kang S, Cooper KD (1994) Distinction of class II MHC+ Langerhans cell-like interstitial dendritic antigen-presenting cells in murine dermis from dermal macrophages. *J Invest Dermatol* 103:678–83

Ebner S, Ehammer Z, Holzmann S, Schwingshackl P, Forstner M, Stoitzner P *et al.* (2004) Expression of C-type lectin receptors by subsets of dendritic cells in human skin. *Int Immunol* 16:877–87

Engering A, Geijtenbeek TB, Van Vliet SJ, Wijers M, van Liempt E, Demaurex N *et al.* (2002) The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J Immunol* 168:2118–26

Fear JD, Jackson P, Gray C, Miloszewski KJ, Losowsky MS (1984) Localisation of factor XIII in human tissues using an immunoperoxidase technique. *J Clin Pathol* 37:560–3

Filgueira L, Nestle FO, Rittig M, Joller HI, Groscurth P (1996) Human dendritic cells phagocytose and process *Borrelia burgdorferi*. *J Immunol* 157:2998–3005

Geijtenbeek TB, Torensma R, Van Vliet SJ, van Duijnhoven GC, Adema GJ, Van Kooyk Y *et al.* (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 100:575–85

Headington JT (1986) The dermal dendrocyte. *Adv Dermatol* 1:159–71

Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK *et al.* (2001) Identification of the haemoglobin scavenger receptor. *Nature* 409:198–201

Krutzik SR, Tan B, Li H, Ochoa MT, Liu PT, Sharfstein SE *et al.* (2005) TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. *Nat Med* 11:653–60

Larregina AT, Morelli AE, Spencer LA, Logar AJ, Watkins SC, Thomson AW *et al.* (2001) Dermal-resident CD14+ cells differentiate into Langerhans cells. *Nat Immunol* 2:1151–8

Meunier L, Gonzalez-Ramos A, Cooper KD (1993) Heterogeneous populations of class II MHC+ cells in human dermal cell suspensions. Identification of a small subset responsible for potent dermal antigen-presenting cell activity with features analogous to Langerhans cells. *J Immunol* 151:4067–80

Muszbek L, Adany R, Szegedi G, Polgar J, Kavai M (1985) Factor XIII of blood coagulation in human monocytes. *Thromb Res* 37:401–10

Nestle FO, Filgueira L, Nickoloff BJ, Burg G (1998) Human dermal dendritic cells process and present soluble protein antigens. *J Invest Dermatol* 110:762–6

Nestle FO, Nickoloff BJ (1995) A fresh morphological and functional look at dermal dendritic cells. *J Cutan Pathol* 22:385–93

Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ (1993a) Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J Immunol* 151:6535–45

Nestle FO, Zheng XG, Thompson CB, Turka LA, Nickoloff BJ (1993b) Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets. *J Immunol* 151:6535–45

Pohlmann S, Baribaud F, Lee B, Leslie GJ, Sanchez MD, Hiebenthal-Millow K *et al.* (2001) DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J Virol* 75:4664–72

Reid MB, Gray C, Fear JD, Bird CC (1986) Immunohistological demonstration of factors XIIIa and XIIIs in reactive and neoplastic fibroblastic and fibrohistiocytic lesions. *Histopathology* 10:1171–8

Sallusto F, Cella M, Danieli C, Lanzavecchia A (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 182:389–400

Suvarna SK, Cotton DW (1993) Dermal dendrocytes and other factor XIIIa-positive cells. *J Pathol* 171:251–2

Tse Y, Cooper KD (1990) Cutaneous dermal Ia+ cells are capable of initiating delayed type hypersensitivity responses. *J Invest Dermatol* 94:267–72

Turville SG, Cameron PU, Handley A, Lin G, Pohlmann S, Doms RW *et al.* (2002) Diversity of receptors binding HIV on dendritic cell subsets. *Nat Immunol* 3:975–83

Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Gunther S, Moderer M (2002) Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J Invest Dermatol* 118:327–34

Zaba LC, Fuentes-Duculan J, Steinman RM, Krueger JG, Lowes MA (2007) Normal human dermis contains distinct populations of CD11c$^+$BDCA-1$^+$ dendritic cells and CD163$^+$FXIIIA$^+$ macrophages. *J Clin Invest* 117:2517–25