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Distinctive dendritic cell subsets expressing factor XIIIa, CD1a, CD1b and CD1c in mycosis fungoides and psoriasis

The papillary dermis of psoriasis and mycosis fungoides (MF) lesions is characterized by prominent collections of cells with dendritic morphology. Immunophenotypically distinct populations of cutaneous dendritic cells have been identified as CD1a+, FXIIIa-Langerhans cells (LC) and CD1a-, FXIIIa+ dermal dendritic cells (DDC). In this study, antibodies against the human CD1 cluster of antigens (i.e. CD1a, CD1b and CD1c) and the DDC marker (FXIIIa) were used to further characterize the subsets of dendritic cells in normal skin as compared to neonatal foreskin, psoriasis and MF by both immunoperoxidase and double immunofluorescence techniques. Normal skin and foreskin epidermis and dermis contained few CD1b+ or CD1c+ cells along with normal numbers of CD1a+ LC and FXIIIa+ DDC. Both MF and psoriasis were characterized by CD1a+ cells in the epidermis and dermis. FXIIIa+ cells were greatly expanded in the upper dermis of MF lesions and to a lesser degree in psoriasis as has been previously described by our group. MF contained significantly increased epidermal and dermal CD1b+ (15.7/5 high power fields [HPF] and 59.7/5 HPF respectively) and CD1c+ dendritic cells (33.8/5 HPF and 95.9/5 HPF respectively), while in psoriasis these cells were not statistically different from normal skin. Double immunofluorescence studies revealed that some (<25%) FXIIIa+ cells co-expressed CD1b and CD1c in MF>psoriasis> foreskin, while FXIIIa+ DDC never co-expressed CD1a. Thus, in contrast to normal skin in which epidermal or dermal dendritic cells rarely express CD1b and CD1c antigens, these members of the CD1 family are upregulated on both LC and DDC in benign and malignant inflammatory states. Upregulation of CD1b and CD1c on MF epidermal and dermal dendritic cells, as compared to psoriasis, foreskin and normal skin, may be useful in the immunophenotypic recognition of MF, as well as in helping to understand its immunobiology.

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A growing body of evidence is available regarding the role of dermal dendritic cells (DDC) (1). We have described prominent collections these cells (defined by factor XIIIa expression) in lesional skin from mycosis fungoides (MF), psoriasis and allergic contact dermatitis (2–5). Data from several laboratories have shown that DDC express a variety of surface antigens including: Thy-1, FXIIIa, CD14,

EBM-11, CD36, CD11b, and CD11c, suggesting they are related to blood-derived monocytes and/or tissue macrophages (6–10). Despite this immunophenotyping data, the exact relationship between the various epidermal and dermal dendritic cells remains to be elucidated (1, 2). Recently, protocols for purification of DDC have revealed that there are several subsets identifiable and that these cells are efficient antigen presenting cells which selectively activate Th1-type T cells (11, 12).

CD1a, CD1b and CD1c antigens have been identified as markers for several of these cutaneous antigen presenting cells in benign lichenoid dermatoses such as lichen planus, allergic contact dermatitis and atopic dermatitis (3, 14). CD1c has also been shown to be expressed by dermal endothelial cells (13). In this report, we have studied the distribution of antibodies which differentiate CD1 cluster of antigens (CD1a vs CD1b vs CD1c) in relationship to FXIIIa in both the epidermal and dermis by immunostaining a series of specimens from normal adult skin, neonatal foreskin and untreated MF and psoriasis. We report that normal skin and foreskin contains rare CD1b+ or CD1c+ dendritic cells similar to previous reports (13, 14). In contrast, epidermal and dermal CD1b+ and CD1c+ dendritic cells were regularly seen in both psoriasis and MF, FXIIIa+ DDC were found to be increased in both MF and psoriasis as we have previously shown. A prominent minority of these cells are shown to co-express CD1c and CD1b, but not CD1a, in diseased skin only.

Material and methods

Patients – Punch biopsies from 16 patients with untreated MF (6 patch and 10 plaque-stage patients) and 9 patients with untreated plaque-type psoriasis were obtained after informed consent at the time of diagnostic biopsy procedure. Normal skin (non-sun exposed, n = 7) specimens were obtained from buttock skin of healthy volunteers. Neonatal foreskins (n = 11) were obtained within 4 h of circumcision from the newborn nursery. All specimens were obtained under IRB approved protocols at Henry Ford Hospital and the University of Michigan Medical Center.

Immunohistochemistry – Immunoperoxidase staining was performed on air-dried, acetone-fixed, 4-micron thick cryostat sections of all specimens. Serial sections were exposed to primary antibodies for one hour. Antibodies were visualized using an avidin-biotin immunoperoxidase staining technique (Vectastain ABC Kit, Vector Labs Inc., Burlingame, CA) as previously described (4). The chromagen used was 3-amino, 9-ethylcarbazole (AEC-Sigma Co., St. Louis, MO). All sections were counterstained with 1% hematoxylin. An irrelevant, isotype-matched antibody (anti-murine IaP or anti-human FXIIIs) was included with each set of patient slides as a control.

The antibodies were diluted in phosphate buffered saline (PBS) with 0.5% bovine serum albumin (BSA-Sigma) and included: anti-human CD1a (1:200), CD1b (1:25), CD1c (1:50) (Biodesign, Kennebunkport, ME), anti-factor XIIIa (1:400), anti-factor XIIIs (1:400) (Calbiochem, LaJolla, CA). Preliminary experiments on reactive lymph node and normal skin specimens were performed to determine the optimal dilution for each antibody. Anti-murine IaP-class II (1:50) was used as an isotype control (Accurate Chemicals, Inc., Westbury, New York).

Double immunofluorescence – Four-micron thick cryostat sections were fixed in cold acetone for ten minutes and air dried. Primary antibodies (diluted using PBS with 0.5% BSA) were mouse anti-human CD1a (1:100), CD1b (1:10), CD1c (1:25) and rabbit anti-human factor XIIIa (1:200). These were applied and incubated at room temperature for 45 min. The slides were washed for 10 min in PBS, and labeled secondary antibodies (goat anti-mouse-FITC) (Dako-1:40) and goat anti-rabbit-rhodamine (Calbiochem-1:20) were applied for 45 min. Controls included deletion of one or both primary antibodies, and/or one or both secondary antibodies.

All sections were examined by light microscopy, and the numbers of reactive cells for each monoclonal antibody recorded. Quantitation was limited to cells of dendritic morphology in MF, psoriasis, normal skin and foreskin in all staining studies and was performed by counting all cells reactive with the antibody in at least five consecutive 40x high power fields (HPF) of contiguous epidermis and dermis and expressed as mean ± SEM/5 HPF.

Statistical analysis – Pairwise comparisons (Wilcoxon tests)were made of the epidermal and dermal expression of CD1a vs CD1b vs CD1c between MF (patch and plaque stage samples were evaluated separately and as one group), psoriasis, normal skin and neonatal foreskin. Descriptive statistics were performed using the Kruskal-Wallis test. Statistically significant differences between the 6 pairwise comparison tests were defined as $p < 0.0083$ after Bonferroni's adjustment for the comparison of multiple groups.

Table 1. Differential distribution of CD1 antigens in normal skin, neonatal foreskin, psoriasis and mycosis fungoides*

	MF-epidermis n = 16	MF-dermis n = 16	PS-epidermis n = 9	PS-dermis n = 9	NS-epidermis n = 7	NS-dermis n = 7	FS-epidermis n = 11	FS-dermis n = 11
CD1a	59.4 ± 9.5	46.1 ± 10.7#	35.5 ± 6.3	9.9 ± 2.5	42.8 ± 10.0	4.3 ± 1.6	30.6 ± 3.5	0.8 ± 0.50
CD1b	15.7 ± 5.9**	59.7 ± 14.3	2.6 ± 1.4	6.4 ± 2.0	0.8 ± 0.5	4.5 ± 2.1	0	3.1 ± 2.1
CD1c	33.8 ± 9.6***	95.9 ± 20.8#	8.3 ± 3.1	21.6 ± 4.7	1.1 ± 0.5	8.4 ± 4.0	4.5 ± 1.5	26.4 ± 5.1

MF = Mycosis fungoides, PS=Psoriasis, NS=Normal adult skin, FS=Neonatal foreskin

* = mean ± SEM of 5-consecutive high power fields in the epidermis or dermis

** = statistical significance increase in MF vs foreskin, $p < 0.0083$ after Bonferroni's adjustment on the rejection level for the pairwise comparison of 6 Wilcoxon tests

*** = statistical significance increase in MF vs NS and foreskin but not PS, $p < 0.0083$ after Bonferroni's adjustment on the rejection level for the pairwise comparison of 6 Wilcoxon tests

\# = statistical significance increase in MF vs PS, NS and foreskin, $p < 0.0083$ after Bonferroni's adjustment on the rejection level for the pairwise comparison of 6 Wilcoxon tests

Results

Immunoperoxidase staining – Immunostaining results for the expression of CD1a, CD1b and CD1c antigens on dendritic cells in MF, psoriasis and normal skin are summarized in Table I. Normal skin was found to contain rare epidermal and dermal CD1b+ or CD1c+ dendritic cells, while CD1a+ epidermal and dermal cells were found in proportions comparable to prior reports (14–16). Epidermal CD1b+ and CD1c+ cells were rarely seen in normal skin and foreskin specimens. Those observed were found in a suprabasal location and had prominent dendritic processes. Foreskins tended to have greater numbers of CD1c+ cells than normal skin, but these numbers were not statistically significant (Fig. 1a).

CD1b+ dendritic cells were significantly increased in the epidermis and dermis of MF lesions only in comparison to foreskin ($p < 0.083$) but were marginally increased in comparison to psoriasis and normal skin ($p = 0.021$ and $p = 0.023$ respectively) (see Table I and Fig. 1b). CD1c+ dermal dendritic cells were statistically increased ($p < 0.0083$) in MF as compared to psoriasis, normal skin and/or foreskin specimens (Fig. 1c). CD1c+ epidermal cells were also statistically increased in MF as compared to normal skin and foreskin ($p < 0.0083$) but only marginally increased in comparison to psoriasis ($p = 0.02$) (see Table I and Fig. 1d). When analyzed separately, there were no appreciable differences in the mean numbers of CD1b+ or CD1c+ dendritic cells between patch and plaque stage MF specimens, therefore all statistical results represent the composite findings from the entire group of 16 MF patients.

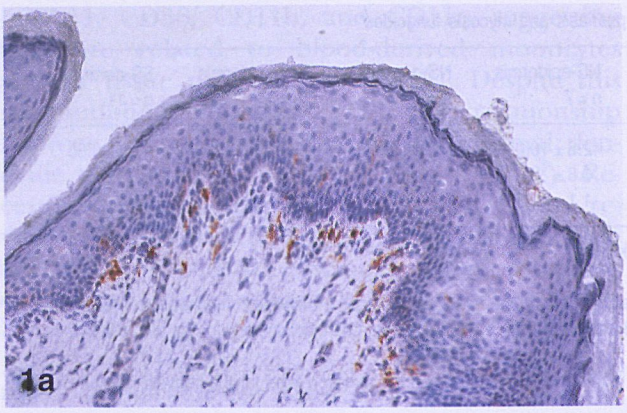
LC were regularly identified in all specimens as CD1a+ dendritic cells in a suprabasal epidermal location, but were not significantly increased in MF as compared to normal skin, as previously observed (15,16). CD1b+ and CD1c+ dendritic cells were also seen in both compartments in psoriasis (Fig. 1d) but were not statistically increased in comparison to foreskin or normal skin numbers of cells. It could not be determined if CD1b+ or CD1c+ epidermal cells appreciably co-localized with CD1a+ cells in serial sections. CD1a+ dendritic cells in the dermis of MF were significantly increased over those in psoriasis, normal skin and foreskin dermis ($p < 0.0083$, see Table I). CD1a+ epidermal and dermal cells in psoriasis were not significantly different from normal skin.

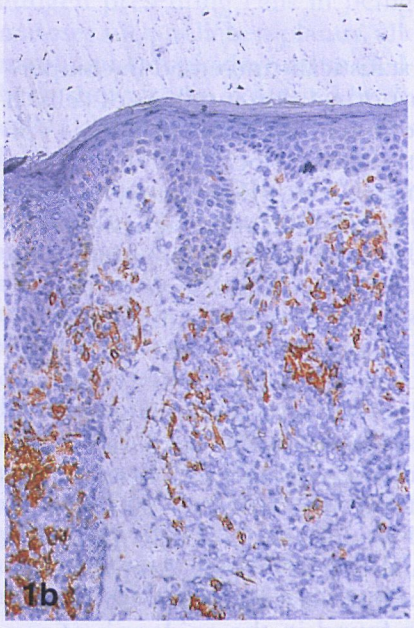
As compared to the numbers of FXIIIa+ dermal dendritic cells observed in normal skin (20–40/5 HPF) or neonatal foreskin (30–60/5 HPF), FXIIIa+ dendritic cells were greatly expanded in the upper dermis of MF lesions (100–300/5 high power fields[HPF]) and in psoriasis (50–70/5 HPF). The IaP and FXIIIs isotype controls were negative in all experiments (results not shown). No FXIIIa+ cells were found in the epidermis of any specimen.

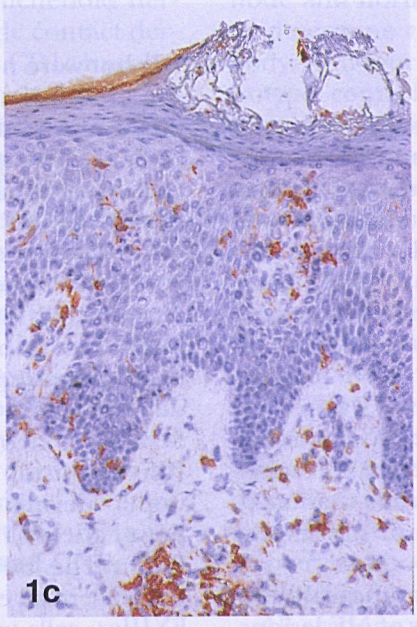
Double immunofluorescence staining – Double IF studies were performed on all patient specimens. 10–25% of the FXIIIa+ DDC were found to co-express CD1c+ in both MF and psoriasis, while few (< 10%) FXIIIa+ DDC co-expressed CD1b in both MF and psoriasis. Precise quantification was not possible due to the extensive dendritic arborizations present throughout the inflammatory lesions of MF and psoriasis (Fig. 2a, 2b). No FXIIIa+ cells were found to co-express CD1a in normal skin, foreskin, MF or psoriasis.

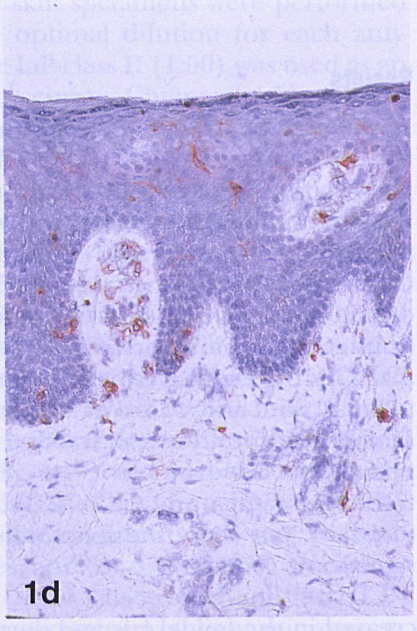
Discussion

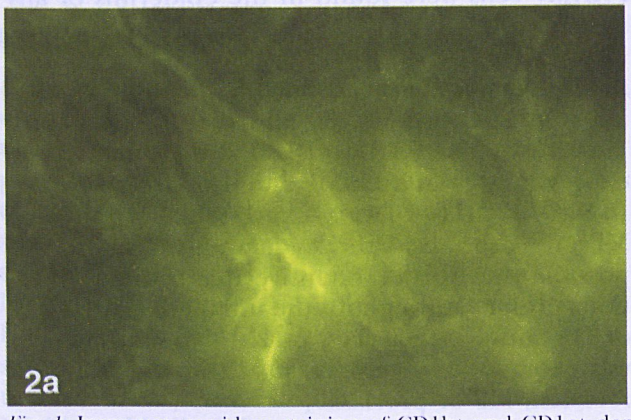
This study demonstrates that dendritic cell populations in both epidermal and dermal skin compartments can express of CD1b and CD1c antigens in inflammatory states. We have shown that epidermal CD1b+ and CD1c+ dendritic cells were rarely found in normal adult skin but were regularly recognized in MF and psoriasis. CD1a+ LC and FX-











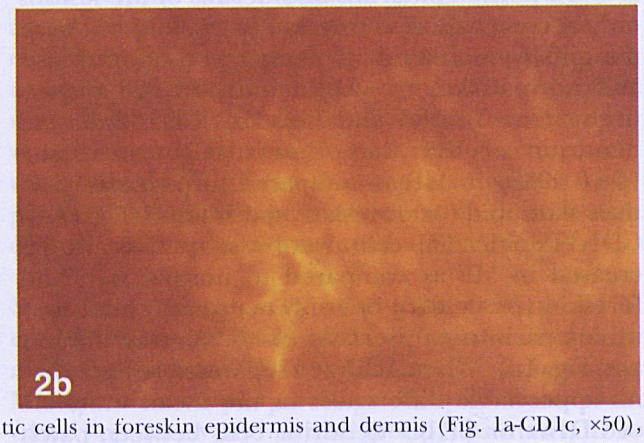


Fig. 1. Immunoperoxidase staining of CD1b+ and CD1c+ dendritic cells in foreskin epidermis and dermis (Fig. 1a-CD1c, ×50), plaque stage mycosis fungoides (Fig. 1b-CD1b, ×50; Fig, 1c-CD1c, ×50) dendritic cells and in lesional psoriasis (Fig. 1d-CD1c, ×50). There are several prominently stained dendritic cells in the dermis as well as in the epidermis in both Figs. 1b and 1c. Mycosis fungoides was found to have the greatest increase in CD1c+ epidermal and dermal dendritic cells compared to normal skin (see text).

Fig. 2. Double immunofluoresence labelling of dermal dendritic cells in plaque stage mycosis fungoides. Fig. 2a- A CD1c+ dendritic cell is stained green with the fluoresceinated secondary antibody (100). Fig. 2b-The same cell is also FXIIIa+ and stains red with the rhodamine labeled secondary antibody (100X). Note the prominent dendritic processes are present in both panels demonstrating co-expression by the identical cell.

IIIa+ DDC were also found to be increased in MF, as previously reported (4,6). These increases coincided with statistically increased numbers of CD1b+ and CD1c+ dendritic cells in both the epidermis and dermis as compared to normal skin and/or neonatal foreskin.

A subset of FXIIIa+ dermal dendritic cells in all cases of MF, psoriasis and foreskin were found to co-express CD1c+ and to a lesser degree CD1b+, yet never expressed CD1a. CD1b+ and CD1c+ FXIIIa+ DDC were rarely seen beyond their routine perivascular location in normal adult skin. Dermal co-expression of FXIIIa and CD1 cluster antigens beyond CD1a (i.e. CD1b and/or CD1c) by dendritic cells in psoriasis and MF suggests that there are distinct subsets of dermal dendritic cells that become apparent under certain inflammatory and neoplastic conditions (14). Unique immunophenotypes appear to exist in epidermal vs dermal compartments, as co-expression of CD1a and FXIIIa was not shown. Thus, although similar in morphology, DDC remain as distinct from LC. Recently, subpopulations of dermal dendritic cells have been isolated from psoriasis and normal skin and a CD14+, FXIIIa+, CD1a+ subset was found in vitro (11, 12).

These data give no direct insights into the functional properties of DDC vs. LC. However, CD1 antigens have been shown to resemble HLA- Class Ib molecules which are selectively involved in antigen presentation to CD8+ cells (15). Physiologic upregulation of these CD1 antigens in fetal and neonatal skin could help in generation of self-tolerant clones of CD8+ cells. Aberrant CD1b or CD1c expression by dendritic cells in MF and psoriasis might be a mechanism through which autoreactive signals are generated which allow disease progression or a mechanism through which cytotoxic T cell are generated locally. Taylor et al. recently reported CD1b and CD36 "aberrantly" expressed by epidermal LC as well as CD1a+, CD1b+, CD36+, HLA-DR+ dermal cells in association with increased autoreactivity in atopic dermatitis (9). CD1a-, CD36+ macrophages in man and Thy-1+ dendritic epidermal T cells in mice have also been reported to possess this type of suppressor-inducer function in vitro (9, 17–20).

While the function of FXIIIa+ DDC is unknown , this study demonstrates that certain subsets of epidermal and dermal dendritic cells are interrelated immunophenotypically. CD1b and CD1c expression by FXIIIa+ DDC, while rare in normal skin, is shown to be more common in inflammatory skin conditions. We speculate that, in T cell-mediated skin disease (e.g. psoriasis and MF), some unknown stimulus is functioning to regulate CD1b and CD1c expression on both DDC and LC. The mediators which regulate dendritic cell expression of CD1 cluster antigens are currently under investigation. The upregulation of CD1b and CD1c expression in all lesional MF specimens on epidermal and dermal dendritic cells demonstrated here as compared to psoriasis, foreskin and normal skin, may be useful in the immunophenotypic recognition of MF, as well as in helping to understand its immunobiology.

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