

Dendritic Cells in Atopic Dermatitis: Expression of FcεRI on Two Distinct Inflammation-Associated Subsets

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Key Words

Dendritic cells · Atopic dermatitis · Fcε receptor

Abstract

Background: Dendritic cells (DCs) represent a major portion within the infiltrate of atopic dermatitis (AD) lesions. As antigen-presenting cells they have the ability to regulate both the quantity and quality of T-cell responses and, thus, are likely to play a key role in the pathogenesis of T-cell-dominated skin diseases such as AD. Thus we sought to identify the DC repertoire occurring in AD patients. **Methods:** For this purpose, we phenotypically analyzed various defined DC subsets of AD patients and healthy controls in skin biopsies and peripheral blood by immunofluorescence staining. **Results:** In AD lesions, two inflammation-associated DC subsets with varying expression of costimulatory molecules occurred besides epidermal Langerhans cells (LCs) and dermal myeloid DCs (dmDCs) indigenously residing in normal skin: (1) CD1a+/CD1c+/FcεRI+/IgE+/CD207– myeloid DCs (mDCs) in the epidermis and dermis and (2) CD123+/BDCA-2+/CD45RA+/CD68+ plasmacytoid DCs (pDCs) in the dermis. In the peripheral blood of the patients, these

cells exhibited an immature phenotype. Interestingly, we found FcεRI and cell-bound IgE to be expressed not only on myeloid, but also on plasmacytoid DCs from both the skin and peripheral blood of AD patients. **Conclusions:** It is tempting to speculate that the disease-regulating role of inflammatory DCs in AD is influenced by both FcεRI occupancy and their degree of maturity.

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Introduction

Atopic dermatitis (AD) is the cutaneous manifestation of atopy (AD, allergic rhinitis, allergic asthma) occurring in 5–20% of children and 1–3% of adults [1, 2]. It is generally assumed that the eczematous reaction results from a T-cell-mediated delayed-type hypersensitivity reaction against certain environmental and, perhaps, self-proteins. Dendritic cells (DCs), because of their potent antigen presentation function, are critical for this process to occur [3]. These cells have the potential to decide about the initiation and the type of the immune response emerging. In AD skin they are apparently triggered by epithelial-cell-derived thymic stromal lymphopoietin to migrate to draining lymph nodes and to prime naïve T cells to preferentially produce IL-4, IL-5 and IL-13, while downregulating IL-10 and IFN-γ [4].

G.S. and C.B. contributed equally to this study.

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Distinct subsets of cutaneous DCs have been described. Normal human skin (NS) harbors two types of resident DCs, i.e., epidermal Langerhans cells (LCs) and dermal myeloid DCs (dmDCs) [5]. In the epidermis of AD skin, the emergence of other myeloid DCs (mDCs), termed inflammatory dendritic epidermal cells (IDECs), has been well documented [6].

Recently, another DC subset, namely plasmacytoid dendritic cells (pDCs) has been detected in various human diseases including viral infections [7], cancer [8], neuroborreliosis [9], systemic lupus erythematosus [10], allergic rhinitis [11], psoriasis [12] and allergic contact dermatitis [13]. They were shown to contribute to innate and adaptive immune responses by the production of type I interferons and the induction of antiviral T cell responses [14, 15], but their full functional spectrum has yet to be elucidated.

In this study, we immunophenotypically characterized DC subsets in atopic skin lesions and correlated their number and phenotype to that of DCs in peripheral blood. By doing so, we sought to build a basis for future investigations aimed at defining the exact contribution of these cells to the pathogenesis of AD.

Materials and Methods

Patients and Tissue Material

The criteria of Hanifin and Rajka [16] were used for the diagnosis of AD. Healthy individuals were selected based on the following criteria: no current AD and/or allergic rhinitis/asthma, no history of AD and/or allergic rhinitis/asthma, total serum IgE within the normal range, no other skin disease, no clinically relevant other diseases, such as malignomas, infections (e.g. HIV, hepatitis B or C) or inflammatory diseases (e.g. Crohn's disease, rheumatoid arthritis). Ten AD patients (5 females, 5 males with a mean age of 36 ± 18 years) and 8 healthy controls (4 females, 4 males with a mean age of 32 ± 8 years) were enrolled in the study after giving their informed consent. The investigational protocol was approved by the Ethics Committee of the Medical University of Vienna (EK 408/2003). AD patients ceased atopy-specific therapy, such as steroids and topical calcineurin inhibitors as well as antihistamines, at least 2 weeks before enrollment. All AD patients had a chronic recurrent course, elevated serum IgE levels ($1,310 \pm 564.9$ KU/l) and a moderate to severe exacerbation of the disease (Investigators' Global Assessment 3–4) at the time of blood and skin sampling. Blood samples for flow cytometric analysis (approximately 50 ml peripheral venous blood) were obtained from all AD patients ($n = 10$) and all healthy individuals ($n = 8$). For immunohistological analysis, 4-mm punch biopsies were taken under local anesthesia from 6 out of 10 AD patients and 5 out of 8 healthy individuals. The biopsies were embedded in OCT Tissue-Tek (Sakura Finetek Europe B.V., Zoeterwoude, The Netherlands), snap-frozen in liquid nitrogen and stored at -80°C until further processing.

Immunofluorescence Staining

Frozen tissue was cut into 5- μm -thick sections and mounted on capillary gap microscope slides (Dako, Glostrup, Denmark). The cryostat sections were air-dried for 20 min, fixed in ice-cold acetone for 10 min and either stained immediately or stored at -20°C . To determine phenotype, distribution and number of the different DC subsets in lesional and healthy skin, single or multicolor immunostaining was performed. The monoclonal antibodies used in the study and their sources are shown in table 1. Stainings with antibodies against the high-affinity Fc ϵ receptor (anti-Fc ϵ RI) and the low-affinity IgE receptor (anti-Fc ϵ RII) were performed with and without elution of tissue-bound IgE by incubating frozen sections with glycine hydrochloride buffer (0.05 M glycine, 0.085 M NaCl, 0.005 M KCl, 0.01 M ethylenediamine tetra-acetic acid, pH 2.5) for 30 min at 4°C [17].

Single staining was performed as follows: purified/biotinylated antibodies were mixed with 2% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, Mo., USA) in PBS (Gibco, Invitrogen GmbH, Lofer, Austria) and applied overnight to frozen sections in a humid chamber at 4°C . After washing with PBS, primary antibody binding was visualized by incubating the sections with either streptavidin-fluorescein (1/100; Amersham Pharmacia Biotech UK Limited, Little Chalfont, UK) or rhodamine (TRITC)-conjugated AffiniPure F(ab')₂ fragment goat anti-mouse IgG (H+L) (1/200; Jackson ImmunoResearch Laboratories, West Baltimore, Pa., USA) for 45 min at room temperature. After washing, slides were placed in 96% ethanol, mounted in Vectashield Mounting Medium (Vector Laboratories, Burlingame, Calif., USA) and coverslipped.

In order to further characterize the cells of interest, we performed double and triple stainings. For these multicolor-staining procedures the first step was always performed as described above. Thereafter, the slides were washed and incubated for 20 min with normal mouse serum (3%, Dako) to diminish background staining. A second antibody, either biotinylated or FITC-labeled, was then applied to the sections for at least 6 h at 4°C . To prevent FITC-labeled antibodies from early fading, another staining step was performed with Oregon Green-labeled goat anti-FITC (1/300; Molecular Probes, Eugene, Oreg., USA) for 30 min at room temperature. Sections stained with biotinylated antibodies were further incubated for 45 min with streptavidin-FITC (1/200, Jackson ImmunoResearch Laboratories) to visualize antibody binding. For triple stainings, sections stained with the primary antibody were exposed to two additional monoclonal antibodies (biotinylated and FITC-labeled) and incubated for 1 h at room temperature. These were then visualized by simultaneous incubation with streptavidin-Cy5 (1/250, Jackson ImmunoResearch Laboratories) and Oregon Green-labeled goat IgG anti-FITC fraction for 45 min at 20°C . Negative controls were obtained in all staining experiments by substituting isotype-matched IgG or IgM (purified, biotinylated or FITC-labeled) for the primary antibody.

Evaluation of Immunofluorescence Results

Biopsy specimens were read in a blinded fashion at $400\times$ magnification by two independent investigators using a fluorescence microscope (ocular $10\times/25$; Axiophot, Zeiss, Oberkochen, Germany) equipped with a filter for double stainings. The mean observer coefficient was under 10%.

Skin-infiltrating DCs were quantified separately in the epidermis and dermis. Labeled cells were enumerated per visual field and

Table 1. List of monoclonal antibodies used in this study

Antibody specificity	Clone and source of antibodies	Isotype
CD1a (pur.)	WM35; PeliCluster, CLB, The Netherlands	mIgG2b
CD1b (pur.)	4.A7.6; Immunotech, Coulter, Marseille, France	mIgG2a
CD1c (pur., biot.)	M241; Ancell, Bayport, Minn., USA	mIgG1
CD45RA (pur.)	L48; BD, San Jose, Calif., USA	mIgG1
CD68 (pur.)	KP1; Dako, Glostrup, Denmark	mIgG1
CD14 (pur., PerCP)	MΦP9; BD	mIgG2b
CD19 (PerCP)	SJ25C1; BD	mIgG1
CD207 (pur.)	DCGM4; Immunotech	mIgG1
MHCII (pur., FITC)	L243; BD	mIgG2a
CDw123 (biot.)	9F5; BD PharMingen	mIgG1
CLA (FITC)	HECA-452; BD PharMingen	rlgM
CD80 (FITC)	L304.4; BD PharMingen	mIgG1
CD83 (pur.)	HB15e; BD PharMingen	mIgG1
CD83 (FITC)	HB15a; Immunotech	mIgG2b
CD86 (pur., FITC)	BU63; Dako	mIgG1
CD40 (pur., FITC)	LOB7/6; Serotec, Oxford, UK	mIgG1
IgE (pur., biot.)	B3102E8; SouthernBiotech, Ala., USA	mIgG1
CD23 (FITC) (FcεRII)	9P25; Immunotech	mIgG1
FcεRI (pur., biot.)	15.1 [20]	mIgG1
Ki-67 (pur.)	Ki-S5; Dako	mIgG1
BDCA-1 (PE)	AD5-8E7; Miltenyi Biotech GmbH, Bergisch Gladbach, Germany	mIgG2b
BDCA-2 (FITC, PE)	AC144; Miltenyi Biotech	mIgG1
BDCA-3 (APC)	AD5-14H12; Miltenyi Biotech	mIgG1
CD11b (FITC)	BEAR1; Immunotech	mIgG1
CD203c (PE)	97A6; Immunotech	mIgG1
MxA	2c12; gift of J. Pavlovic, PhD, Department of Virology, Zurich University Hospital, Switzerland	mIgG1

expressed as the number of cells per millimeter basement membrane epidermis and square millimeter dermis $\pm$ standard error of the mean (SEM). Only cells clearly positive for the antigen of interest were counted. Statistical significance between DC subsets in NS and AD lesions was determined by the Mann-Whitney U test. Confocal immunofluorescence microscopy (LSM 510, Zeiss) was used to evaluate triple stainings and to document the sites of preferential expression.

Immunohistochemical Staining

Frozen tissue sections were prepared and stored as described above for the immunofluorescence staining procedure. To determine the distribution and the number of CD1c+ and BDCA-2+ DCs in lesional skin, single immunostainings were performed. Type I interferon- α/β -inducible intracellular protein MxA (a gift of J. Pavlovic, PhD, Department of Virology, Zurich University Hospital) [18, 19]. The monoclonal antibodies used in the study and their sources are shown in table 1. To prevent nonspecific protein binding, antibodies were mixed with 1% normal horse serum and 2% BSA in PBS (Gibco) and applied overnight to frozen sections in a humid chamber at 4°C. An IgG1 isotype monoclonal antibody was used as negative control. After incubation of the slides with biotinylated horse anti-mouse IgG (Vector Laboratories) at room temperature for 45 min, the sections were rinsed and incubated with avidin-biotin peroxi-

dase complex (Vector Laboratories) at room temperature for 45 min. For visualization of the cells, 0.01% 3-amino-9-ethyl-carbazol in 50 mM acetate buffer (pH 5, 0.015% H₂O₂, 5% dimethylformamide) was used as chromogen. Sections were counterstained with hematoxylin (Merck, Darmstadt, Germany) and mounted in Aquamount (BDH Laboratory Supplies, Poole, UK).

Flow Cytometry

To characterize blood-derived DCs, peripheral blood mononuclear cells from 10 AD patients and 8 healthy donors were obtained by Ficoll-Paque density gradient centrifugation (Histopaque-1077, Sigma). For all immunostainings, cells were washed in ice-cold PBS and preincubated in blocking reagent (Octagam 5 mg/ml; Octapharma, Vienna, Austria) at 4°C for 20 min. Cells were then incubated on ice for 20 min with anti-CD1c (BDCA-1) PE, anti-BDCA-3 APC (both Miltenyi Biotech GmbH, Bergisch Gladbach, Germany), anti-CD14 PerCP, anti-CD19 PerCP (both Becton Dickinson, San Jose, Calif., USA) and anti-BDCA-2 FITC (Miltenyi Biotech GmbH). To phenotypically analyze the designated DC subsets in greater detail, we performed triple or quadruple stainings with the indicated subset-defining antibodies (anti-CD1c PE, anti-BDCA-2 PE, anti-CD14 PerCP, anti-CD19 PerCP or anti-BDCA-3 APC), several FITC-labeled antibodies [anti-CD80 FITC (Immunotech, Coulter, Marseille, France), anti-CD83 FITC (Immunotech), anti-CD86 FITC (Dako), anti-CD40 FITC (Immu-

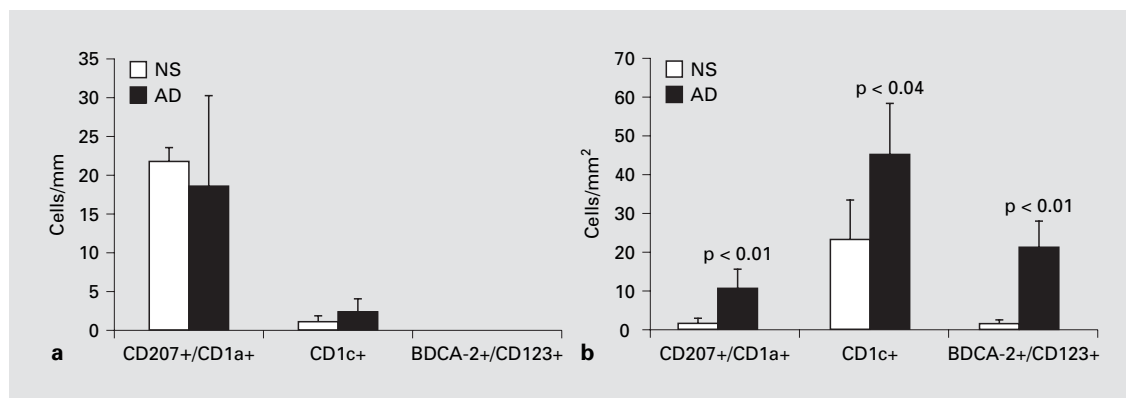


Fig. 1. Detection of LCs, dmDCs and pDCs in the epidermis (**a**) and dermis (**b**) of AD patients versus healthy controls. Frozen skin sections of 6 AD patients and 5 healthy individuals were subjected to immunofluorescence double labeling (CD207 TRITC/CD1a FITC; CD1c TRITC; CD123 TRITC/BDCA-2 FITC). Clearly positive cells were enumerated and recorded separately. Data represent the mean number of cells $\pm$ SEM. Statistical significance between DC populations of NS and AD lesions was determined using the Mann-Whitney U test.

tech), anti-CLA FITC (PharMingen), anti-CD23 FITC (Immuno-
tech)] as well as the biotinylated anti-IgE (SouthernBiotech, Bir-
mingham, Ala., USA) and anti-Fc ϵ RI α -chain antibodies [20]. The
anti-CD14 and anti-CD19 antibodies described above were includ-
ed in the staining to allow separation of monocytes and B cells from
the selected DC gates [21]. Following incubation with these mono-
clonal antibodies, PBMCs were washed twice with ice-cold PBS
and subjected to flow cytometric analysis using a FACSCalibur flow
cytometer (Becton Dickinson). Before staining with anti-Fc ϵ RI or
anti-Fc ϵ RII, samples were treated with lactic acid to dissociate IgE
from its receptor. To this end, cells were incubated with lactic acid
on ice (pH 3.9) for 7 min and washed twice with RPMI/FCS [22].
PBMCs stained with biotinylated anti-Fc ϵ RI and anti-IgE were
visualized by incubation with streptavidin-fluorescein (1/200; Am-
ersham Pharmacia Biotech UK Limited) and analyzed thereafter.

Results

Phenotypic Characterization of Epidermal and Dermal DC Subsets in AD and Healthy Control Skin

For the identification of LCs, both Langerin (CD207) and CD1a were used as markers. A single staining with CD1a for their characterization is not sufficient due to the presence of IDECs in the epidermis of AD skin, which are known to express CD1a, but not CD207. CD1c was applied as a marker for dermal DCs of myeloid origin and BDCA-2 and CD123 for pDCs.

In situ Staining of LCs in NS and AD Lesions

As in a previous study [13] and similarly to other teams [23, 24], we detected large numbers of CD207+/CD1a+

LCs in the epidermis of NS (fig. 1). Only few of these cells were found to be located in the dermis (fig. 1). Interestingly, we observed a notable increase in CD207+/CD1a+ cells in the dermis of AD lesions, while their density in the epidermis was not significantly different from that of NS (fig. 1).

Occurrence of Increased Numbers of CD1c+ DCs in AD Lesions

We found CD1c+ DCs in the dermis of NS in numbers reported previously [13] (fig. 1). This population was almost doubled in AD lesions, where CD1c+ cells were present not only in the dermal, but also in the epidermal compartment (fig. 1, 2a). Further phenotyping of CD1c+ DCs revealed coexpression of CD11b and CD1a on the majority of DCs, whereas CD1b was expressed only on a small subpopulation of these cells in NS (fig. 2b).

To obtain information on whether the CD1c+/CD1a+ dermal DC subset belongs to the LC versus the dmDC population, double and triple stainings of these cells with anti-CD207 were performed. Due to the lack of CD1c+/CD1a+ cells coexpressing the LC-specific marker CD207, these cells are likely to represent dmDCs or IDECs rather than LCs (fig. 2b, c).

Concerning their level of maturity, approximately 20% of the CD1c+ DCs were found to express CD80 and CD40 in both NS and AD skin, whereas CD83 and CD86 were almost absent (fig. 2b).

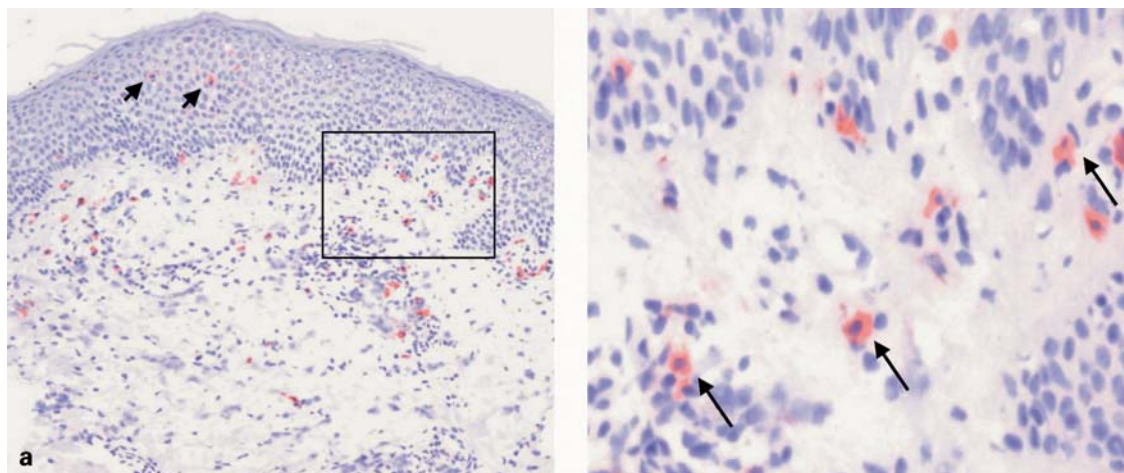
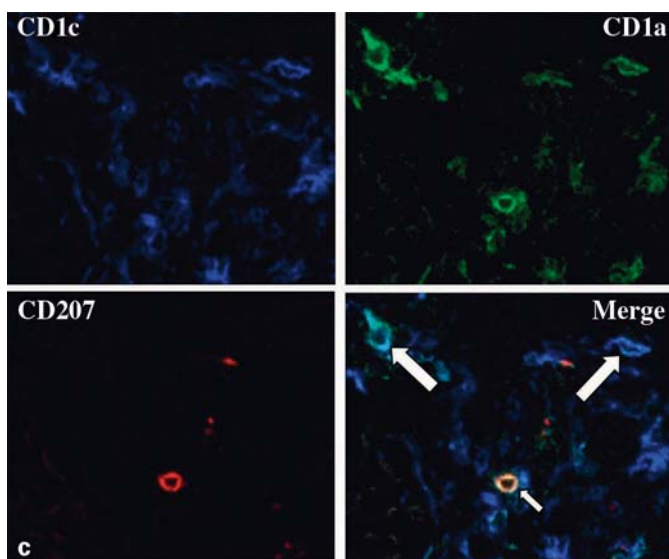
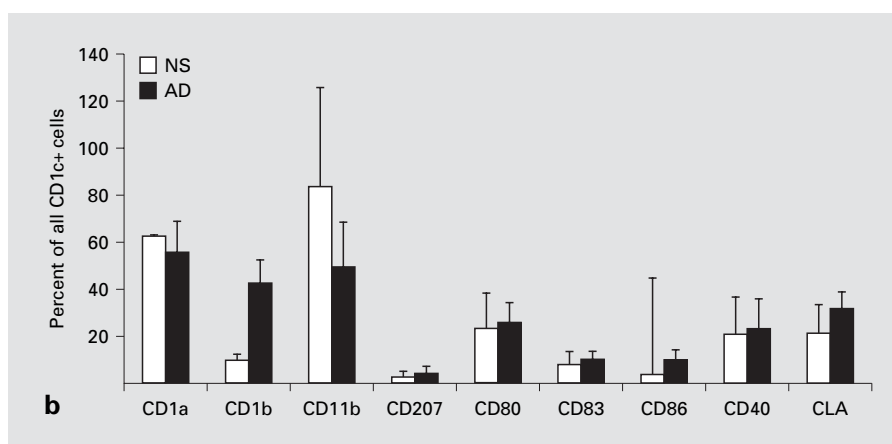


Fig. 2. Distribution and phenotypic characterization of dmDCs in AD lesions. **a** In situ immunohistochemical stainings of atopic lesions with an anti-CD1c monoclonal antibody revealed considerable numbers of dmDCs in the dermis (arrows, inset) and, to a much lesser extent, in the epidermis (arrowheads). Original magnification: $\times 100$. The picture is representative of all evaluated biopsies. **b** A detailed analysis of the phenotypic profile of CD1c+ dmDCs was performed by immunofluorescence double stainings with an anti-CD1c antibody and the indicated antibodies of interest. The data are given as mean percentages $\pm$ SEM of the individual markers of the total CD1c+ DC population (bar chart). The absolute number $\pm$ SEM of the entire CD1c+ cell population occurring in the dermis of either NS (23.3 ± 9.9 cells/mm²) or AD skin (44.7 ± 13.2 cells/mm²) served as reference value. **c** Lack of CD207 expression by CD1c+CD1a+ DCs occurring in the dermis of lesional AD skin. Triple stainings were performed using antibodies against CD207 (TRITC), CD1a (FITC) and CD1c (Cy5). The big arrows indicate CD1c+CD1a+CD207- cells. Please also note the presence of a single CD1c-CD1a+CD207+ LC (small arrow). Original magnification: $\times 400$.



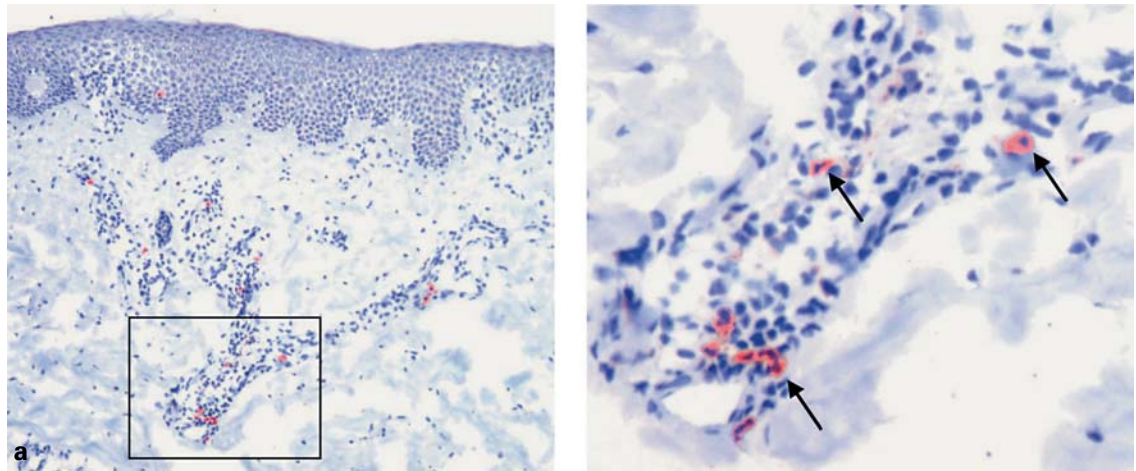


Table 2. Phenotypic characterization of pDCs in NS and AD lesions

	NS	AD
BDCA-2/CD123		
Epidermis, cells/mm	0	0
Dermis, cells/mm ²	1.6 ± 0.9	21.2 ± 6.1
CD45RA/CD123		
Epidermis, cells/mm	0	0.4 ± 0.7
Dermis, cells/mm ²	0.4 ± 0.3	19.2 ± 4
MHCII/CD123		
Epidermis, cells/mm	0	0.2 ± 0.3
Dermis, cells/mm ²	3.2 ± 1.1	20.4 ± 3.7
CD68/CD123		
Epidermis, cells/mm	0	0.5 ± 0.3
Dermis, cells/mm ²	4.6 ± 1.1	21.2 ± 2.8
CD80/CD123		
Epidermis, cells/mm	0	0
Dermis, cells/mm ²	0.1 ± 0.2	4.8 ± 1.2
CD83/CD123		
Epidermis, cells/mm	0	0
Dermis, cells/mm ²	1.8 ± 0.5	6.6 ± 2.6
CD86/CD123		
Epidermis, cells/mm	0	0
Dermis, cells/mm ²	0.3 ± 0.4	8.6 ± 2.2
CD40/CD123		
Epidermis, cells/mm	0	0.2 ± 0.1
Dermis, cells/mm ²	0.6 ± 0.8	11.4 ± 5.3
CLA/CD123		
Epidermis, cells/mm	0	0.2 ± 0.4
Dermis, cells/mm ²	0.4 ± 0.5	9.6 ± 3.2

A detailed evaluation of the phenotypic profile of pDCs in the epidermis and dermis of NS and AD lesions was performed by immunofluorescence double stainings with anti-CD123 and further subset-defining antibodies as well as antibodies against costimulatory and skin-homing molecules. Data represent the absolute number of cells ± SEM.

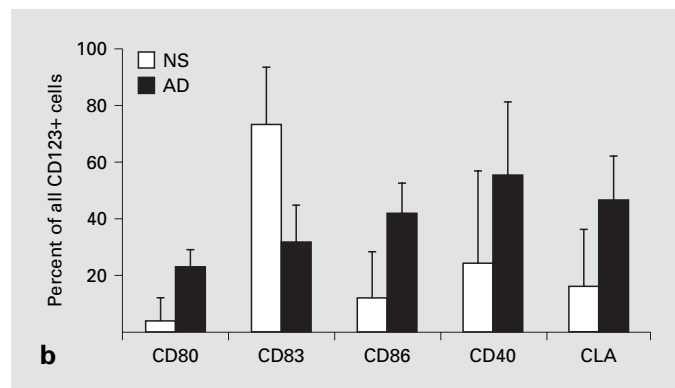


Fig. 3. Detection and phenotypic characterization of pDCs in AD skin lesions. **a** BDCA-2+ pDCs (arrows) were mainly observed in the dermis of AD lesions. Original magnification: ×100. The picture is representative of all evaluated biopsies. **b** A detailed analysis of the phenotypic profile of pDCs was performed by immunofluorescence double stainings with anti-CD123 and the indicated antibodies of interest. The data are given as mean percentage ± SEM of the individual markers of the total CD123+ pDC population (bar chart). The absolute number ± SEM of the entire CD123+ pDC population occurring in the dermis of either NS (1.6 ± 0.9 cells/mm²) or AD skin (21.2 ± 6.1 cells/mm²) served as reference value.

Lesional AD Skin Is Characterized by the Presence of Substantial Numbers of pDCs within the Dermis

Immunoperoxidase single staining disclosed substantial numbers of BDCA-2+ cells in the dermis of AD lesions (fig. 3a) and barely in NS. Double-staining experiments revealed that these cells are CD123+, but lack the basophil marker CD203c [25], and, thus, presumably represent pDCs (fig. 1 and data not shown). Indeed, these cells exhibited anti-MHC II, anti-CD45RA and CD68 reactivity (table 2). In AD skin, proportions thereof ex-

Table 3. Presence of IgE and FcεRI on CD1c+ mDCs and CD123+ pDCs

	Dermis		Epidermis	
	NS	AD	NS	AD
CD1c (total population)	14.3 ± 8.6 (100)	35.1 ± 5.5 (100)	0	1.5 ± 0.7 (100)
FcεRI/CD1c (unstripped)	11.9 ± 1.7 (83.2)	27.6 ± 5.6 (78.6)	0	1.1 ± 0.9 (73.3)
FcεRI/CD1c (stripped)	13.8 ± 3.2 (96.5)	33.7 ± 6.1 (96)	0	1.4 ± 0.7 (93.3)
FcεRII/CD1c (unstripped)	0	0	0	0
FcεRII/CD1c (stripped)	0	0	0	0
IgE/CD1c (unstripped)	0.7 ± 0.6 (4.9)	28.5 ± 4.3 (81.2)	0	1.2 ± 0.7 (80)
IgE/CD1c (stripped)	0	0	0	0
CD123 (total population)	1.3 ± 0.6 (100)	20.2 ± 1.9 (100)	0	0
FcεRI/CD123 (unstripped)	0.6 ± 0.7 (46.2)	16.1 ± 1.4 (79.7)	0	0
FcεRI/CD123 (stripped)	0.9 ± 0.8 (69.2)	18.5 ± 2.8 (91.6)	0	0
FcεRII/CD123 (unstripped)	0	0	0	0
FcεRII/CD123 (stripped)	0	0	0	0
IgE/CD123 (unstripped)	0.6 ± 0.7 (46.2)	12.9 ± 3.9 (63.9)	0	0
IgE/CD123 (stripped)	0	0	0	0

The presence of IgE and its receptors on CD1c+ DCs and CD123+ DCs in the epidermis and dermis of NS and AD lesions was evaluated by immunofluorescence double stainings before and after elution of cell-bound IgE. Data represent the absolute number of cells ± SEM. The mean percentages of the individual markers of the total CD1c+ and CD123+ cell population are indicated in parentheses.

pressed the costimulatory molecules CD40, CD86, CD83 and CD80 with decreasing frequency (fig. 3b, table 2). It thus appears that, in AD lesions, pDCs exhibit activation markers more frequently than mDCs (fig. 2b, 3b).

Double staining with the proliferation marker Ki-67 and anti-CD123 yielded negative results (data not shown), indicating that the CD123+ infiltrate is not generated by precursors proliferating in situ.

Presence of FcεRI and Cell-Bound IgE on DC Subsets in AD Biopsies

FcεRI may play an important role in the pathogenesis of AD, as it mediates IgE-facilitated antigen presentation by DCs [26]. To search for these cells in NS and AD, we used the anti-FcεRIα antibody 15.1. Because this antibody defines the IgE-binding site of the receptor [20], we performed experiments with and without elution of cell-bound IgE by acid treatment. In untreated AD tissue sections, the proportion of CD1c+ DCs and CD123+ pDCs co-expressing FcεRI was approximately 80% for each subset (table 3, fig. 4a, c). These percentages were barely increased upon elution of IgE (table 3). In contrast to FcεRI, FcεRII was not detectable on either DC type within the investigated tissues, either before or after elution of IgE (table 3).

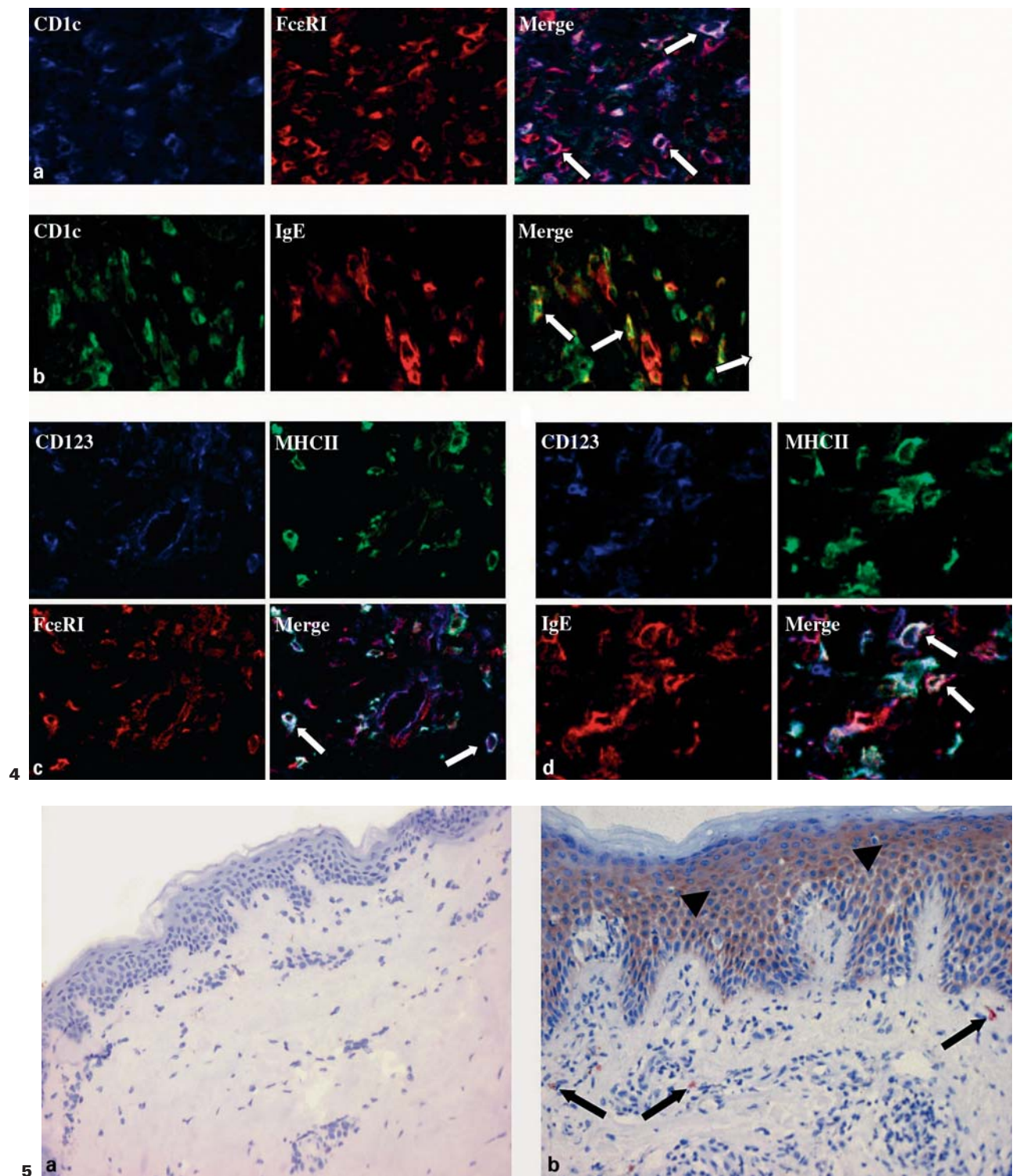
The lack of a significant increase in FcεRIα+ DCs upon acid treatment can be explained by intracellular FcεRIα expression in the majority of cells.

Cell-bound IgE was present on more than 80% of CD1c+ DCs (table 3, fig. 4b) and more than 60% of pDCs in AD lesions (table 3, fig. 4d). As expected, it disappeared upon elution.

Expression of MxA in Atopic Skin Lesions

To obtain an indirect measure of the type I interferon-producing capacity of pDCs occurring in AD lesions, we examined the cellular expression of the interferon-α/β-inducible protein marker MxA. Whereas NS was devoid of MxA+ cells (fig. 5a), AD samples consistently contained MxA+ cells. These cells represented the vast majority of keratinocytes within the epidermis and scattered infiltrating cells in the dermis (fig. 5b).

Fig. 4. Detection of FcεRI and IgE on dmDCs and pDCs in the dermis of AD lesions. CD1c+ DCs in the dermis of AD skin express FcεRI [**a**; CD1c (blue)/FcεRI (red)] and bear IgE [**b**; CD1c (green)/IgE, (red)] as shown by double stainings. Experiments were performed without prior elution of tissue-bound IgE. Arrows denote examples of clearly double-positive cells. PDCs display FcεRI and IgE as shown by immunofluorescence triple stainings of the dermis of AD lesions for CD123 (blue)/MHCII (green)/FcεRI (red) (**c**) and CD123 (blue)/MHCII (green)/IgE (red) (**d**). Experiments were per



formed without prior elution of tissue-bound IgE. Arrows denote examples of clearly triple-positive cells. Original magnification: $\times 400$. Pictures are representative of all evaluated biopsies.

Fig. 5. Detection of MxA+ cells in AD lesions. In situ immunohistochemical stainings of NS (a) and atopic lesions (b) were per-

formed with an anti-MxA monoclonal antibody. NS showed no anti-MxA reactivity (a), stainings of AD lesions (b) revealed expression of MxA in infiltrating cells in the dermis (arrows) and in keratinocytes (arrowheads). Original magnification: $\times 100$. Pictures are representative of all evaluated biopsies.

Table 4. Cell-bound IgE and IgE-binding receptors on DC subsets in peripheral blood

	CD1c+ DC		BDCA-2+ DC		BDCA-3+ DC	
	Hco	AD	Hco	AD	Hco	AD
IgE, %	35.4 ± 11.9	90.4 ± 1.5*	23.6 ± 11.5	81 ± 3.5*	0.3 ± 0.3	28.3 ± 7.3*
FcεRI, %	45.6 ± 9.5	72 ± 6.6**	21.8 ± 9.3	65 ± 6.9*	0.3 ± 0.3	14.6 ± 5.4
FcεRII, %	4.8 ± 1.4	5.9 ± 1.9	5.9 ± 2.5	10.4 ± 3.7	0	1 ± 1

CD1c+, BDCA-2+ and BDCA-3+ DC subsets of 10 AD patients and 8 healthy controls were analyzed for cell-bound IgE and surface expression of FcεRI and FcεRII. Results (means ± SEM) show the percentages of cells positive for the indicated molecules. Statistical significance of difference between DC subsets in atopic and healthy individuals was determined by the Mann-Whitney U test. * $p \leq 0.01$; ** $p \leq 0.05$.

Phenotypic Characterization of DCs in Peripheral Blood of AD Patients and Healthy Controls

Flow Cytometric Analysis of DC Subsets

In a next series of experiments, we assessed CD1c+ mDCs, BDCA-2+ pDCs and BDCA-3+ DCs in the peripheral blood of AD patients and healthy individuals by flow cytometry. BDCA-3 identifies a CD11c+CD123-CD1c- myeloid DC subset [21], which is not yet characterized in detail.

As opposed to the situation in skin, where AD lesions are marked by increased numbers of mDCs and pDCs, we were not able to detect any significant differences in the absolute numbers of such cells in peripheral blood of AD patients and healthy individuals. Comparing their relative numbers, certain AD patients exhibited a higher ratio of pDCs to mDCs, but this finding was not consistent and statistically not significant (data not shown). The BDCA-3+ DC subset was found to represent only a small part of all blood-derived DCs in atopic and non-atopic individuals, and the number of such cells in AD biopsies was negligible (data not shown).

Cell-Bound IgE and IgE-Binding Receptors on CD1c+ mDCs and BDCA-2+ pDCs in the Peripheral Blood of AD Patients

In healthy controls, approximately 45% of CD1c+ mDCs and 20% of pDCs displayed very weak reactivity with the anti-FcεRIα reagent 15.1. Similarly, cell-bound IgE was barely detectable on either CD1c+ mDCs or pDCs (table 4, fig. 6a, b). Compared to healthy individuals, a substantially higher percentage of CD1c+ mDCs and pDCs bore cell-bound IgE (90.4 and 81%) and expressed FcεRI (72 and 65%) in AD patients (table 4).

Moreover, atopic patients exhibited a far higher surface density of these molecules on both DC populations (fig. 6a, b). Peripheral-blood-derived BDCA-3+ cells from healthy individuals were essentially devoid of cell-bound IgE and FcεRI, and the situation in AD patients was not much different (table 4, fig. 6c).

FcεRII was barely displayed on either DC subset of atopic and healthy individuals (table 4, fig. 6a–c).

Surface Expression of Maturation and Adhesion Molecules on CD1c+ mDCs, BDCA-2+ pDCs and BDCA-3+ DCs

When investigating the maturation status of DC subsets in the peripheral blood of AD patients, we were somewhat surprised not to find significant amounts of CD40, CD80, CD83 and CD86 on either CD1c+ mDCs, pDCs or BDCA-3+ mDCs (fig. 6a–c). We also found that, in both healthy and atopic individuals, these DC subsets were reactive, to a varying extent, with antibodies against the cutaneous lymphocyte-associated antigen (CLA), i.e. the E- and P-selectin ligand on endothelial cells (fig. 6d) [27].

Discussion

Our knowledge about the potential influence of DCs on the extent of the allergen-specific T-cell-mediated cutaneous inflammation in AD is incomplete. We therefore attempted to characterize and quantify these cells in the skin and blood of AD patients and, for control purposes, in healthy individuals.

We observed comparable numbers of CD1a+/CD207+ LCs in the epidermis of atopic as well as normal individuals and an increase in LCs in the dermis of AD pa-

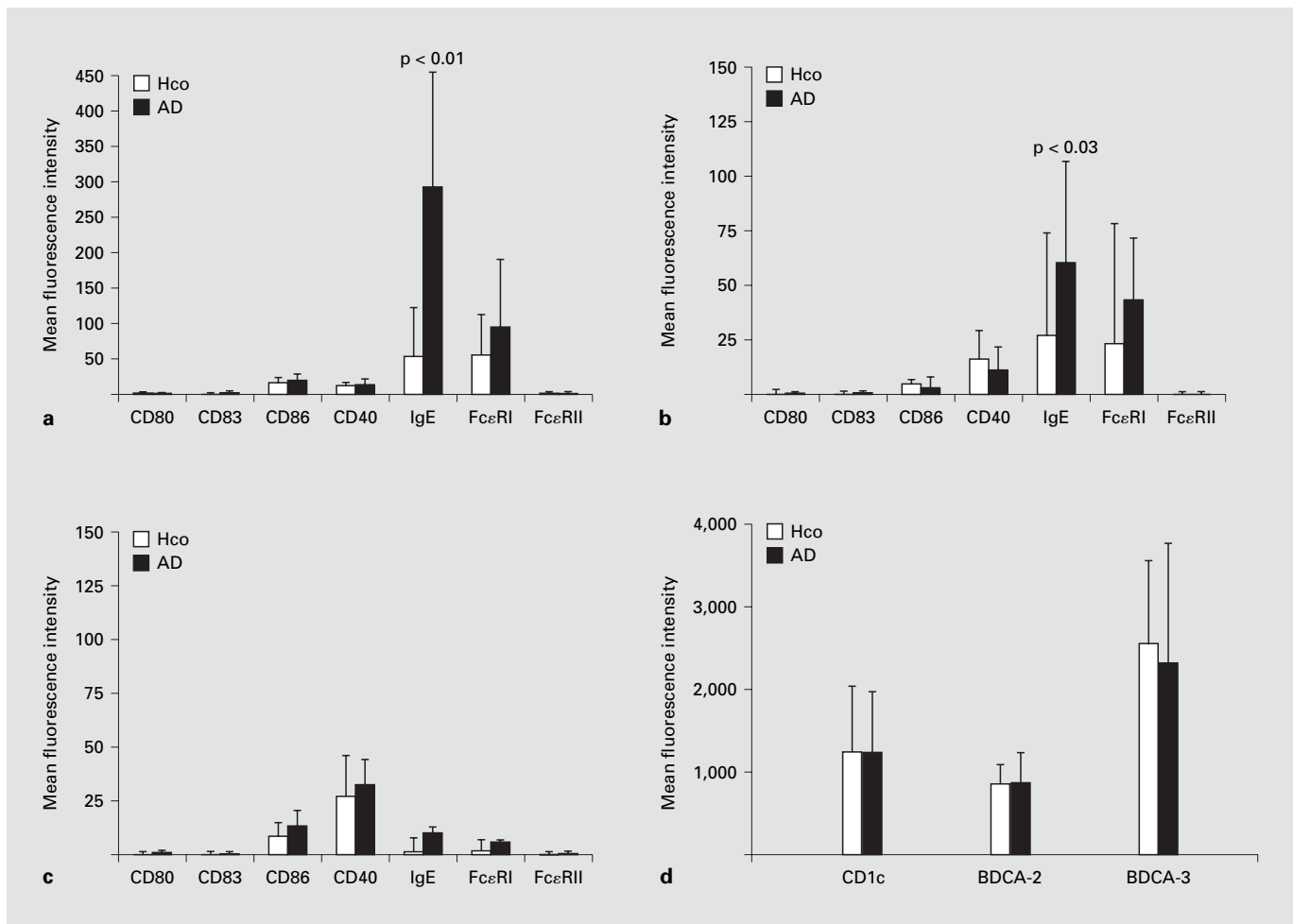


Fig. 6. Phenotypic characterization of DC subsets in peripheral blood of AD patients and healthy controls. To phenotypically analyze the designated DC subsets, we performed triple or quadruple stainings with subset-defining antibodies and several FITC-labeled antibodies by flow cytometry. Cell-bound IgE was evaluated in samples not exposed to acid stripping. For the detection of IgE-binding receptors (FcεRI and FcεRII), cells were exposed to lactic acid before immunolabeling. CD1c+ mDCs (**a**) and BDCA-2+ pDCs (**b**) show increased expression levels of cell-bound IgE and FcεRI in peripheral blood of AD patients as compared to healthy

controls (Hco). BDCA-3+ mDCs only display low reactivity to anti-FcεRIα and anti-IgE (**c**). CLA is expressed to a varying extent on DC subsets in healthy individuals and AD patients (**d**). **a–d** Data represent the specific geometric mean fluorescence intensity (geometric mean fluorescence intensity of antibodies of interest minus geometric mean fluorescence intensity of isotype controls) ± SEM of each population. Statistical significance between the marker expression of DCs of AD patients and healthy controls was determined by the Mann-Whitney U test.

tients only. This increase may reflect a dynamic process which keeps the number of epidermal LCs stable under inflammatory conditions. Dermal LCs may either represent LCs recruited from peripheral blood precursors on their way to the epidermis [28–31], and/or LCs migrating to the draining lymph nodes after antigen capture. Alternatively, it is also conceivable that these cells arise from CD1a-/Langerin- dermal precursors, i.e. CD14 LC precursors [32]. Besides CD1a+/CD207+ LCs, we found in-

creased numbers of CD1c+/CD207- DCs mainly located in the dermal and, to a lesser extent, in the epidermal compartment of lesional skin. Due to their lack of CD207 expression, these cells are distinct from immature LCs [33]. We can however not rule out that these cells are LC progenitors. Alternatively, they could be matured LCs, but this is unlikely, because CD1c is downregulated upon LC maturation [34]. Double stainings revealed coexpression of CD11b, CD1a and CD1b on a significant part of

these CD1c+ dmDCs. As a substantial proportion thereof were found to bear cell-bound IgE and FcεRI, their phenotypic marker profile corresponds to that of IDECs [6, 35].

A comparative phenotypic analysis revealed that, with the exception of CD1b and cell-bound IgE expression, dmDCs of normal healthy skin are essentially indistinguishable from CD1c+ DCs/IDECs (CD1a+/CD11b+/CD1b+/FcεRI+) occurring in the inflammatory infiltrate of AD skin. This observation can be interpreted in different ways. First, it is conceivable that IDECs of inflamed and dmDCs of normal skin originate from different blood precursor cells and use perhaps different stimuli for their migration into the tissue. Alternatively, one could argue that both populations derive from the same precursor cell either in the blood or in the skin [32] and, dependent on the cellular and molecular composition of the environment, differentiate into low IgE-bearing dmDCs under physiological conditions and into high IgE-bearing inflammatory DCs occurring in lesional skin [6]. The first hypothesis gains support from our finding that, both in peripheral blood and skin, the marker profile of mDCs in healthy individuals differs from that in AD patients concerning surface expression of FcεRI and cell-bound IgE.

While the epidermis of both AD and healthy skin was almost devoid of pDCs [12], we observed sizeable numbers of such cells in the dermis of AD lesions. In fact, they accounted for approximately one third of the entire DC population in the dermis.

As opposed to the situation in skin, which showed a dramatic increase of mDCs and pDCs in atopic individuals when compared to healthy controls, no significant differences in the absolute numbers of these cells were noted in peripheral blood. However, some AD patients had a polarization of the mDCs:pDCs ratio towards pDCs confirming previous results in patients with atopic disorders [36–38].

The precise role of pDCs in AD is not completely understood. Similar to LCs and dmDCs, these cells are able to stimulate antigen-specific T cell proliferation [39] [Bangert, unpubl. obs.] and to prime naïve T cells to produce allergy-promoting Th2 cytokines [40]. Besides these functions in the adaptive immune system, pDCs are well known to produce large amounts of type I interferons in response to viruses and bacteria and may thus play a role in innate immunity [41]. In cutaneous lupus erythematosus their presence is accompanied by the induction of lesional type I interferon activity, as detected by the expression of the interferon-α/β-inducible protein MxA [10]. In this study, we clearly identified MxA+ cells in AD lesions

indicating local type I interferon production. At first glance this finding was unexpected. However, AD skin is frequently colonized by *Staphylococcus aureus*. It is thus conceivable that MxA is produced as a consequence of type I interferon secreted by bacterially stimulated pDCs [41]. If so, pDCs may transmit signals from innate to adaptive immunity. Given the potent Th1 polarization elicited by virus-activated pDCs [42], in AD these cells may contribute to the switch from a Th2-mediated acute disease to a Th1-driven chronic disease [43, 44].

We also found that pDCs and inflammatory mDCs of AD skin display anti-FcεRIα as well as anti-IgE reactivity. It is likely that both DC subsets use these receptor sites for more efficient allergen presentation [26, 45], thereby contributing to the exacerbations typical of this disease. These results are in accordance with recent studies demonstrating increased expression of FcεRI by pDCs from patients with asthma [46] and AD [47].

Depending on their state of activation and maturation [48], both subsets of DC can promote Th-1 [15, 42], Th-2 [40, 49] and T-regulatory responses [50, 51]. Slightly different from what was reported by Novak et al. [47], we found that, in the peripheral blood, both mDCs and pDCs exhibit only low levels of surface-bound maturation markers. In sharp contrast, more than 50% of skin-infiltrating pDCs had upregulated expression of CD40 and, to a lesser extent, CD86, CD83, and CD80. This phenomenon was less pronounced in the case of mDCs. Thus, it is conceivable that the ratio immature:mature pDCs as well as the ratio mDCs:pDCs in lesional skin critically influences the extent of the allergen-specific inflammation and, consequently, the course of the disease. Future therapeutic strategies should thus be aimed at altering the pDC phenotype in a way that endows these cells with immunoinhibitory properties.

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