Plasmacytoid Dendritic Cells: A New Cutaneous Dendritic Cell Subset with Distinct Role in Inflammatory Skin Diseases

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Epidermal dendritic cells found in inflamed skin include Langerhans cells and the recently identified population of inflammatory dendritic epidermal cells. Another subset of dendritic cells in humans is the plasmacytoid dendritic cell in peripheral blood, which is characterized by the production of large amounts of type I interferon (interferon-α and interferon-β) upon viral infection. We hypothesized that plasmacytoid dendritic cells might be involved in anti-viral defense mechanisms of the skin. Here we investigated plasmacytoid dendritic cells, inflammatory dendritic epidermal cells, and Langerhans cells in epidermal single cell suspensions of normal looking skin from healthy volunteers and of lesional skin from patients with different inflammatory skin diseases. Langerhans cells were found in normal and in inflamed skin samples. In normal skin, plasmacytoid dendritic cells and inflammatory dendritic epidermal cells were low or absent. Lesional skin samples from patients with psoriasis vulgaris and contact dermatitis contained relatively high numbers of both inflammatory dendritic epidermal cells and plasmacytoid dendritic cells. In contrast, many inflammatory dendritic epidermal cells but only very few plasmacytoid dendritic cells could be detected in atopic dermatitis lesions. Lupus erythematosus was characterized by high numbers of plasmacytoid dendritic cells but low numbers of inflammatory dendritic epidermal cells. These results demonstrate that in addition to resident Langerhans cells, plasmacytoid dendritic cells and inflammatory dendritic epidermal cells are selectively recruited to the skin lesions depending on the type of skin disease. The lack of plasmacytoid dendritic cells in atopic dermatitis may predispose atopic dermatitis patients to viral infections such as eczema herpeticum, a secondary infection of atopic dermatitis lesions with herpes simplex virus. The composition of dendritic cell subsets may help to clarify the etiology of inflammatory skin diseases and forms the basis for therapeutic intervention with selective microbial molecules such as immunostimulatory CpG oligonucleotides.

Following written informed consent, skin biopsies were obtained from human skin. DC are characterized by the lack of lineage and the Wilcoxon rank test. Differences were considered statistically significant of differences was determined by the Mann–Whitney U test.

4.51 software (Abacus Concepts Inc., Calabasas, CA).

(rH 5, 5, 0.0015% H2O2, 6% dimethylsulfoxide) was used as chromogen. After incubation with biotinylated horse anti-mouse IgG (1/300, Dako) for 1 h at room temperature after blocking endogenous peroxidase activity with 0.3% H2O2. An IgG1 isotype MoAb was used as negative control.

Flow cytometric analysis Flow cytometric data were acquired on a Becton Dickinson FACSCalibur equipped with two lasers (excitation at 488 and 635 nm wavelength). Spectral overlap was corrected by appropriate compensation. A light gate based on the physical properties of the cells FSC vs SSC (forward scatter vs side scatter) was set to exclude cell multiplets from acquisition. Data were analyzed using Cellquest software (Becton Dickinson).

 Immunohistochemical analysis of PDC in skin sections For immunohistochemical staining, tissue specimens were embedded in Tissue tek (Sakura Finetek, Torrance, CA), cryopreserved in N2 and stored at −20 °C. Acetone-fixed cryosections (4 μm) were prepared and incubated with the PDC-specific monoclonal antibody (MoAb) BDCA-2 (Dzionek et al, 2001) for 1 h at room temperature after blocking endogenous peroxidase activity with 0.3% H2O2. An IgGl isotype MoAb was used as negative control. After incubation with biotinylated horse anti-mouse IgG (1/300, Dako A/S, Glostrup, Denmark) for 1 h at room temperature, sections were rinsed and incubated with avidin–biotin peroxidase complex (1/200, Sigma, Deisenhofen, Germany) for 10 min at room temperature. As a next step, 0.01% 3-amino-9-ethyl-carbazol in 0.1 M Na-acetate buffer (pH 5.5, 0.0015% H2O2, 6% dimethylsulfoxide) was used as chromogen. With this procedure, PDC stained red. Other cellular elements were counterstained with Mayer’s hemalaun (blue).

Statistical analysis Data are expressed as mean ± SEM. Statistical significance of differences was determined by the Mann–Whitney U test and the Wilcoxon rank test. Differences were considered statistically significant for p<0.05. Statistical analyses were performed using StatView 4.5I software (Abacus Concepts Inc., Calabasas, CA).

RESULTS

Identification of Langerhans cells, IDEC, and PDC in human skin DC are characterized by the lack of lineage markers (CD3 for T cells, CD14 for monocytes, CD16 and CD56 for natural killer cells, CD19 and CD20 for B cells) and by the expression of MHC II. Within the CD1a+ population in skin biopsies of patients with inflammatory skin disease, two DC subsets can be distinguished based on the expression of CD11b: Langerhans cells (CD1a+; CD11b–) and IDEC (CD1a+; CD11b++). Normal skin contains only Langerhans cells but no IDEC (Wollenberg et al, 1996, 1999).

In peripheral blood, PDC can be identified by the expression of MHC II and CD123 within lineage-negative cells (Krug et al, 2002a). Additional staining with the pan-myeloid marker CD11c allowed a clear separation of PDC from myeloid DC (Fig 1A). When the same four-color staining was performed on cells derived from inflammatory skin lesions, a population of PDC could be clearly separated from other DC subsets (Fig 1C). The identity of PDC was confirmed by similar FSC and SSC characteristics and distinctive CD123 expression profile (Fig 1A); as well as by staining with a new PDC-specific antibody for BDCA-2 (blood DC antigen-2, recently identified C-type lectin) (Dzionek et al, 2001), which detected a similar frequency of PDC as the four-color staining protocol (Fig 2).

In order to identify the other lineage-negative/MHC II-positive cell subsets, CD123 was replaced by CD1a (Fig 1C, fourth panel from left). A large proportion of CD11c bright cells and of CD11c-intermediate or CD11c-low cells were positive for CD1a suggesting that these cell populations contain Langerhans cells and IDEC (Fig 1C, fourth panel from left). CD11b but not the pan-myeloid marker CD11c is an established marker for the distinction of Langerhans cells and IDEC, therefore, we compared the expression level of CD11b and CD11c on CD1a-positive cells (Fig 1B). Like CD11b, CD11c showed a bright staining of IDEC but was low on Langerhans cells. Thus, like CD11b, CD11c can be used to separate IDEC from Langerhans cells within a CD1a-positive population. As (besides a small proportion of CD11c bright cells) the expression of CD1a and CD123 was mutually exclusive (within MHC II+/lineage - cells of the skin), a four-color staining (lineage, MHC II, CD11c, CD123) was sufficient to simultaneously identify three subsets of DC in human skin: IDEC (CD11c++; CD123+), Langerhans cells (CD11c++; CD123−), and PDC (CD11c−; CD123++)

Distinct profile of DC subsets in skin from patients with different inflammatory skin diseases Cells positive for CD45 and CD123 have been found in skin lesions of patients with LE by using immunohistologic analysis (Farkas et al, 2001). To confirm the presence of PDC in LE, we performed four-color flow cytometry allowing simultaneous detection and quantification of PDC, Langerhans cells, and IDEC in single cell suspensions of skin biopsies. In two different LE patients, PDC represented 0.32% and 0.35% of all epidermal cell population. This number of PDC is relatively high considering the percentage of PDC in peripheral blood (0.2–0.4% of all PMBC), and considering the fact that this number reflects the percentage of PDC within all epidermal cells, including keratinocytes, melanocytes, and others. Besides PDC, Langerhans cells and only low numbers of IDEC were observed in LE (Fig 3A).

Next we studied the profile of DC subsets in lesional skin of a number of individual patients with AD (n = 9), psoriasis (n = 6), CD (n = 5), and in skin from healthy donors (n = 3) (Fig 3). PDC were absent in normal skin. The number of PDC was significantly lower in skin samples from AD patients as compared with skin samples from patients with psoriasis (p = 0.02) and contact eczema (p = 0.02) (Fig 3C, left panel). In contrast, high numbers of IDEC were found in all three inflammatory skin diseases, including AD. IDEC were low or absent in normal skin (p < 0.05) (Fig 3C, middle panel). Relatively high numbers of Langerhans cells were found in all samples examined, including normal skin (Fig 3C, right panel). The percentage of Langerhans cells among all lineage+MHC II+ cells, however, was significantly higher in normal skin as compared with skin from patients with AD (p = 0.04).
Comparing the number of PDC, IDEC, and Langerhans cells within the different diagnoses (horizontal comparison), the following significant differences were found: in normal skin, the percentage of Langerhans cells was higher than the percentage of PDC ($p=0.04$) or IDEC ($p=0.05$). In samples from patients with AD, the numbers of IDEC were higher than the number of Langerhans cells ($p=0.02$), and there were much more Langerhans cells than PDC ($p<0.001$). In patients with psoriasis, PDC were always much lower than IDEC ($p=0.01$) or Langerhans cells ($p=0.04$). Furthermore, in skin samples from patients with CD, the numbers of IDEC were higher than PDC ($p=0.03$). Together these results revealed a distinct profile of DC subsets in lesions of different skin diseases. One of the most intriguing observations was the relative absence of PDC in AD, and the absence of PDC and IDEC in normal skin.

**Localization of PDC in lesional skin** BDCA-2 has been reported to be selectively expressed on PDC (Dzionek et al., 2001). We used the BDCA-2 specific MoAb to confirm the presence of PDC in the basal epidermis and papillary dermis of inflamed skin. We evaluated the use of this BDCA-2 antibody to stain specifically PDC in cryosections of human tonsils that contain a relatively high number of PDC. As demonstrated in Fig 4(A), staining of tonsillar cryosections with BDCA-2 antibodies revealed a considerable number of PDC in the area of high endothelial venules and in the perifollicular T cell areas.

In agreement with our flow cytometry studies, BDCA-2-positive cells were absent in both epidermis and dermis of healthy volunteers (not shown), supporting the PDC specificity of the BDCA-2 antibodies (Dzionek et al., 2001).

In lesional skin of patients with psoriasis, BDCA-2-positive cells were detected in the basal layer of the epidermis and the papillary dermis (Fig 4B,C). In addition to providing information on localization of PDC, immunohistochemistry with BDCA-2 also confirmed our results of the presence of PDC in inflammatory skin lesions obtained by flow cytometry.

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**Figure 1. Identification of DC subsets in human skin.** (A) Detection of myeloid DC and PDC in PBMC. PBMC isolated from peripheral blood of healthy volunteers were stained with HLA-DR (PerCP), lineage markers (FITC), CD123 (PE), and CD11c (APC). A morphologic gate (FSC vs. SSC) was set to exclude cell multiples from acquisition (not in figure). HLA-DR$^+$ and lineage$^-$ cells were examined for the expression of CD11c (myeloid DC) and of CD123 (PDC) by flow cytometry. The FSC and SSC characteristics of PDC are indicated. (B) Detection of Langerhans cells and IDEC in normal and inflammatory skin and comparison of CD11b and CD11c expression. Epidermal single cell suspensions of biopsies of normal skin and of skin lesions of a patient with AD were prepared and stained with CD1a (PE) in combination with either CD11b (FITC) or CD11c (FITC) (no additional staining with lineage markers and HLA-DR). Langerhans cells both in normal and inflamed skin are identified by high expression of CD1a and the lack of CD11b. IDEC identified by coexpression of CD1a and CD11b are detected in inflamed skin but not in normal skin. For both CD11b and CD11c, expression on IDEC is higher than on Langerhans cells. (C) Simultaneous detection of PDC, IDEC, and Langerhans cells in inflamed skin: Epidermal single cell suspensions of biopsies of skin lesions of a patient with psoriasis were prepared and stained with HLA-DR (PerCP), lineage markers (FITC), CD11c (APC), and either CD123 (PE) or CD1a (PE). HLA-DR$^+$ and lineage$^-$ cells were gated and examined for the expression of CD123 (PDC) and CD11c (bright: IDEC; intermediate/low: Langerhans cells). IDEC show a lower CD1a staining than Langerhans cells. The identity of PDC is confirmed by FSC and SSC characteristics (cf. A).
DISCUSSION

Langerhans cells play an established role as sentinels in normal skin. Recent observations provide evidence that humans have different subsets of DC, which are specialized for the detection of various pathogen-derived microbial molecules (Enk et al., 1993; Krug et al., 2002b; Wollenberg et al., 2002). The immune system seems to employ different DC subsets to trigger different sets of immune responses appropriate for the defense against the corresponding pathogens. In this study, we use a technique that allows the simultaneous detection of PDC, IDEC, and Langerhans cells in skin samples of different inflammatory skin diseases. Our studies showed that PDC were as frequent in lesional skin of psoriasis, CD, and LE as in peripheral blood, whereas PDC were reduced or absent in AD and in normal skin. PDC were localized in the basal layer of the epidermis and the papillary dermis. High numbers of IDEC were found in AD, psoriasis, and CD, whereas IDEC were low or absent in LE and in normal skin. Langerhans cells were found in all skin samples, but were highest in normal skin.

Without clinical information such as the history and distribution pattern of the lesions, it is often difficult to establish the correct diagnosis of inflammatory skin diseases on histology alone. Epidermal DC phenotyping has been reported to contribute to the accurate diagnosis of inflammatory skin diseases (Wollenberg et al., 1995, 1999). In this study we demonstrate an improved technique that allows a quantitative assessment of Langerhans cells, IDEC, and PDC in skin biopsies. Quantification of DC subsets may help to distinguish lesional skin from AD, psoriasis, CD, and normal skin.

PDC have been named DC2 based on studies demonstrating that CD40 ligand-activated PDC promote T helper (Th)2 responses (Rissoan et al., 1999; Liu and Blom, 2000; Liu et al., 2000). Others have questioned this view of PDC as DC2 by showing that PDC stimulated by a virus can induce a Th1 response [IFN-γ, interleukin (IL)-4] or Th0 response (T cells that produce both IFN-γ and IL-10) (Cella et al., 2000; Kadokawa et al., 2000). PDC express a limited toll like receptor (TLR) profile, including TLR9 (Krug et al., 2002b), which is known to be involved in the recognition of CpG motifs within microbial DNA (Hartmann and Krieg, 2000; Hemmi et al., 2000; Krug et al., 2001; Hornung et al., 2002). There are hints that PDC may be involved in the pathogenesis of autoimmune diseases (Ronnblom and Alm, 2001), allergy (Jahnsen et al., 2000), viral infections (Donaghy et al., 2001; Feldman et al., 2001; Patterson et al., 2001), and cancer (Zou et al., 2001).

It has been proposed that the phenotype of the epidermal DC subsets may reflect the disease-specific microenvironment associated with different disease entities and thus may provide additional information about the etiology of the disease (Wollenberg et al., 1999). For example, the microenvironment of inflamed AD skin, which is characterized by an increased production of Th2 cytokines (IL-4 and IL-13) in the acute skin lesions and is frequently associated with an increased total serum IgE (Leung, 2000; Wollenberg et al., 2000), may account for the selective lack of PDC in AD lesions. Th2 cytokines are thought to play...
a part in the pathogenesis of AD by enhancing IgE synthesis, eosinophilia, and induction of molecules that are involved in the migration of inflammatory cells into the skin lesions (Schleimer et al., 1992; Akdis et al., 1997). Exposure of PDC to the Th2 cytokine IL-4 leads to rapid cell death of PDC, an effect that is potentiated by IL-10, but blocked by CD40 ligand and IFN-\(\gamma\) (Rissoan et al., 1999). The Th2 bias with increased IL-4 and decreased IFN-\(\gamma\) in the microenvironment of early AD lesions may induce cell death in those PDC that infiltrate these areas. Despite the low numbers of PDC found within the skin lesions, the number of circulating PDC seems to be increased in patients with AD (Uchida et al., 2001). One possible explanation for these seemingly contradictory findings is that the increased number of circulating PDC may compensate for the PDC loss in AD skin.

As PDC are regarded the key sensors of viral infection and play an important part in the initiation of anti-viral immune responses by producing large amounts of the anti-viral cytokine type I IFN (Cella et al., 1999), the lack of PDC in lesional skin of AD patients is in good accordance with the clinically known predisposition of atopic individuals to cutaneous infections. Whereas nonatopics may easily clear human papillomavirus or molluscum contagiosum virus infection from their skin by mounting anti-viral immune responses employing their type I IFN producing PDC, the impaired PDC recruitment of atopic individuals may help to explain the long known susceptibility of atopic individuals to cutaneous infections. This concept fits with our finding that disseminated skin infections with the herpes simplex virus known as eczema herpeticum occurs almost exclusively in AD patients (Bork and Brauninger, 1988; Wollenberg et al., 1997). The impaired ability of AD patients to recruit PDC to their skin lesions may be the immunologic basis for eczema herpeticum, which still represents the most severe and feared complication of AD (Bork and Brauninger, 1988). Eczema herpeticum is uncommon in patients with CD and psoriasis (Morganroth et al., 1992; Wollenberg et al., 1997), both of whom contain considerable numbers of PDC in their skin lesions as demonstrated in this study.

PDC are thought to play a specific role in the pathogenesis of LE, in which high systemic IFN-\(\gamma\) levels are associated with disease activity (Ronnblom and Alm, 2001). We found a higher

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**Figure 3.** PDC, Langerhans cells, and IDEC in normal skin and in lesional skin of patients with AD, psoriasis vulgaris, CD, and LE. Epidermal single cell suspensions of biopsies of normal skin and of lesional skin of patients with AD, psoriasis vulgaris, CD, and LE were prepared. (A) DC subsets (PDC, IDEC, and Langerhans cells) were quantified by four-color-staining with HLA-DR (PerCP), lineage markers (FITC), CD11c (APC), and CD123 (PE) as indicated. (B) The identity of PDC is confirmed by FSC and SSC characteristics as compared with PBMC (see Fig 1A). (C) The mean frequencies of PDC, IDEC, and Langerhans cells (±SEM) are compared between normal skin (n = 3), AD (n = 9), psoriasis vulgaris (n = 6, patients not identical with the six patients in Fig 2), and CD (n = 5). Note different scale of the x axis. Statistical significance was determined by Mann–Whitney U test (*p<0.05).
frequency of PDC in the skin lesions of LE as compared with peripheral blood. These results are in agreement with another study, in which PDC were identified by immunohistochemical staining (CD123 and CD45) in skin lesions of LE patients but not in normal skin (Farkas et al., 2001). LE is an autoimmune disorder associated with anti-DNA antibodies and increased IFN-α/β production. Anti-double-stranded DNA antibodies in combination with immunostimulatory plasmid DNA mimic the endogenous IFN-α inducer in systemic LE (Vallin et al., 1999). PDC have been found to produce IFN-α in response to plasmid DNA/anti-DNA antibody complexes (Dzionek et al., 2001).

With regard to the pathogenic role of PDC in different situations, besides viral infection, so far CpG DNA is the only defined microbial stimulus that is recognized by PDC (Kadowaki et al., 2001; Krug et al., 2002b). CpG DNA mimics the presence of microbial DNA, promoting survival and maturation of PDC (Hartmann et al., 1999), stimulates the production of IFN-α (Krug et al., 2002a) and IL-12 (Krug et al., 2002b), and promotes an IL-12-dependent Th1 response. In the absence of the appropriate stimulation, PDC are known to support a Th2 response (DC2) (Kadowaki et al., 1999). Little is known about the properties of PDC at effector sites with direct antigen exposure, such as the skin and mucosa. Synthetic CpG ODN might be useful to protect against viral infections such as eczema herpeticum by supporting PDC survival and by inducing the production of Th1 cytokines IFN-α and IL-12 by PDC.

The regular presence of PDC in LE, psoriasis, and CD but striking rareness of PDC in AD suggests a distinctive pathogenetic role of this cell type in inflammatory skin diseases, which provides a rationale why patients with AD show a predisposition to viral skin infections, such as eczema herpeticum. The function of the PDC with its specific properties is likely to fill some of the gaps in our understanding of the pathophysiology of inflammatory skin diseases. PDC may also be a novel target for the immunotherapy of such disorders.


