Immunomorphological and Ultrastructural Characterization of Langerhans Cells and a Novel, Inflammatory Dendritic Epidermal Cell (IDEC) Population in Lesional Skin of Atopic Eczema

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We investigated epidermal cell suspensions prepared from lesional and nonlesional atopic eczema skin, other inflammatory skin conditions, and normal human skin for high-affinity IgE receptor (FcεRI) expression on dendritic CD1a cells by quantitative flow cytometric analysis. A single CD1a^{bright}/CD1b^{neg}/FcεRI^{dim}/CD23^{neg}/CD32^{dim}/HLA-DR^{bright}/CD36^{neg} population was found in normal skin. In contrast, lesional skin of atopic eczema and other inflammatory skin diseases harbored variable proportions of two distinct CD1a populations. Both populations exhibited typical ultrastructural features of Langerhans cells, but the second one lacked Birbeck granules and was unreactive to the Birbeck granule-specific LAG antibody. Both populations differed phenotypically: classical Langerhans cells were CD1a^{bright}/CD1b^{neg}/FcεRI^{dim}/CD23^{neg}/CD32^{dim}/HLA-DR^{bright}/CD36^{dim}, while the second population was CD1a^{dim}/CD1b^{dim}/FcεRI^{bright}/CD23^{dim}/CD32^{dim}/HLA-DR^{bright}/CD36^{bright}. The highest FcεRI expression was found on the second CD1a population in lesional atopic eczema skin. Furthermore, FcεRI expression on CD1a cells correlated significantly with the serum IgE level of the patients. Thus, a distinct population of CD1a inflammatory dendritic epidermal cells different from classical Langerhans cells appears in the epidermis of lesional skin and is subjected to specific signals leading to the upregulation of FcεRI in atopic eczema skin. ***Key words: IgE receptor/langerhans cells/atopy/psoriasis. J Invest Dermatol 106:446–453, 1996***

Langerhans cells are bone marrow–derived cells which belong to the family of dendritic antigen-presenting cells (Steinman, 1991). In the epidermis, they are located in the basal and suprabasal layers, where they form a close network that may be regarded as a "first barrier" of the immune system against the environment. Therefore, it has been assumed that Langerhans cells play a critical role in the pathogenesis of several skin diseases, including allergic contact eczema (Silberberg *et al*, 1989; Toews *et al*, 1980), psoriasis vulgaris (Bieber *et al*, 1989a; Bos *et al*, 1983; Demidem *et al*, 1991), mycosis fungoides (McMillan *et al*, 1982; MacKie, 1981), and, more recently, atopic eczema (AE) (Bruynzeel-Koomen *et al*, 1986; Taylor, 1991).

Great interest in AE, in particular, has evolved since the presence of IgE molecules has been reported on CD1a-positive Langerhans cells in this disease, and we and others have demonstrated the presence of the high-affinity receptor for IgE, FcεRI, on these cells (Bieber *et al*, 1992b; Grabbe *et al*, 1993; Wang *et al*, 1992). It has also become clear that IgE-bearing Langerhans cells are not specific for AE but are found in various inflammatory skin conditions (Bieber *et al*, 1991). Interestingly, however, some reports emphasize the presence of Langerhans cell–like cells in the inflammatory infiltrate of AE (Barker *et al*, 1988; Bos *et al*, 1986; Leung *et al*, 1987). Our previous immunohistochemical studies suggested that increased IgE binding on Langerhans cells may be correlated with an upregulation of FcεRI expression (Bieber *et al*, 1989b); however, there is little information regarding the *in vivo* regulation of FcεRI under different physiological and pathological conditions. In the present study, we therefore analyzed in detail the epidermal CD1a-positive population by a flow cytometric approach. We were able to delineate two distinct CD1a/FcεRI-positive cell populations in AE and other skin conditions.

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Abbreviations: AE, atopic eczema; FcεRI, high-affinity IgE receptor; FcεRII/CD23, low-affinity IgE receptor; FcγRII/CD32, low-affinity IgG receptor; LAG, monoclonal antibody specific for Birbeck granules; rFI, relative fluorescence index.

MATERIALS AND METHODS

Reagents FcεRI was detected by monoclonal antibody (MoAb) 29C6 (IgG1, generous gift of Drs. J. Hakimi and R. Chizzonnite, Hoffmann LaRoche Co., Nutley, NJ), which is directed against the α-chain of FcεRI but does not interfere with the binding site for IgE on this molecule (Riske *et al*, 1991); MoAb IV.3 (IgG2b, Medarex, W Lebanon, NH) is directed against the human low-affinity IgG receptor, FcγRII/CD32; MoAb IOT2b (IgG1, Immunotech, Marseille, France) reacts with HLA-DR; IOB8 (IgG1, Immunotech) reacts with the low-affinity receptor for IgE, FcεRII/CD23; IOP36 (IgG2b, Immunotech) is raised against CD36, which is the thrombospondin receptor and represents a putative collagen-binding structure; MoAb LAG (IgG1, generous gift of F. Furukawa and S. Imamura, Department of Dermatology, Kyoto University, Japan) reacts specifically with Birbeck granules in human Langerhans cells (Kashihara *et al*, 1986);

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phycoerythrin-labeled T6RD1 (IgG1, Coulter, Krefeld, Germany) recognizes CD1a, which in the skin has been shown to be expressed only by Langerhans and related cells (Fithian *et al*, 1981). MoAb IOT6b (IgG1, Immunotech) is directed against CD1b. MOPC (IgG1) and UPC10 (IgG2b) (Sigma, Deisenhofen, Germany) and IgG1RD1 (Coulter) were used as appropriate isotype controls. Fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse (GaM/FITC) antibody was from Jackson Laboratories (West Grove, PA). Normal mouse serum for blocking purposes and 7-amino-actinomycin-D were from Sigma.

Preparation of Epidermal Cell Suspensions Skin biopsies (punch or shave biopsies) were obtained from patients of the Department of Dermatology, University of Munich, with written informed consent. After local anesthesia, biopsies were taken from at least 2 wk untreated, chronic skin lesions of classic AE (n = 8) and from corresponding nonlesional, untreated skin of identical patients (n = 4). Normal skin (n = 10) of patients undergoing cosmetic or melanoma surgery and lesional skin from patients with various inflammatory skin conditions, such as mycosis fungoides (n = 3), allergic contact eczema (n = 5), psoriasis vulgaris (n = 4), were used for comparative purposes. AE was diagnosed according to the established criteria (Hanifin and Rajka, 1980), and all atopic patients had elevated IgE serum levels. Epidermal single cell suspensions were prepared from skin biopsies by trypsinization as described (Bieber *et al*, 1992c), washed with phosphate-buffered saline (PBS) containing 1% fetal bovine serum and 0.1% sodium azide, filtered through a 50-μm nylon mesh, and subjected to immunolabeling. Total serum IgE levels were determined by an enzyme-linked immunosorbent assay technique (Enzygnost, Behringwerke, Marburg, Germany).

Immunolabeling of Epidermal Cell Suspensions An indirect triple-staining for unfixed, vital Langerhans cells was performed as follows: up to 500,000 epidermal cells were first incubated with the primary MoAb (all at 2 μg/ml) for 30 min. Then the cells were washed in PBS + 1% fetal bovine serum + 0.1% sodium azide and further incubated with GaM/FITC for 30 min. After washing, GaM/FITC was blocked with normal mouse serum (final dilution 1:10) for 15 min, washed, and counterstained with T6/RD1 and 7-amino-actinomycin D (both at 1 μg/ml) for 30 min. Finally, the cells were washed and analyzed by flow cytometry. All incubations and washes were performed at 4°C.

For intracytoplasmic staining of Birbeck granules, up to 500,000 epidermal cells were washed twice in PBS, fixed in PBS + 4% formaldehyde for 20 min, washed in PBS, incubated in PBS + 0.1 M glycine for 10 min, washed in PBS twice, and permeabilized in PBS + 0.5% saponin + 0.5% bovine serum albumin + 0.01% sodium azide (saponin buffer) + 10% goat serum for 30 min. LAG MoAb was added for 20 min, and the cells were washed twice in saponin buffer. The GaM/FITC antibody was added for 20 min, and the cells were washed twice in saponin buffer. Normal mouse serum was added for 20 min, and the cells were washed twice in saponin buffer. After washing twice with PBS + 0.5% bovine serum albumin + 0.01% sodium azide, T6RD1 MoAb was added for 10 min, and the cells were finally washed twice in PBS + 0.5% bovine serum albumin + 0.01% sodium azide.

Flow Cytometric Analysis The cells were analyzed on a FACScan (Becton Dickinson, Mountain View, CA). The vital Langerhans cell population was gated out by a combination of forward and side scatter (FSC/SSC) and CD1a/7-amino-actinomycin D gate sets (Schmid *et al*, 1992). Fluorescence parameters were collected using a built-in logarithmic amplifier and the data of about 10,000 cells were obtained and analyzed with the Lysis-II program (BD). For quantitative evaluation, the CD1a populations were gated out manually and the mean fluorescence intensity (MFI) was determined for each population of interest using Lysis II software. Relative fluorescence indices (rFI) of all surface receptors were determined as follows:

$$rFI = [MFI(Receptor) - MFI(Control)]/MFI(Control).$$

Immunoelectron Microscopy Freshly isolated crude epidermal cell suspensions were prepared as described above. Then, the cells were washed and resuspended in Iscoves medium (10^6 cells/ml) with 1% fetal bovine serum, cooled at 4°C and incubated for 45 min with F(ab')2 fragments of anti-FcεRIα MoAb 29C6 and anti-CD1a MoAb IOT6a gold-labeled with 5- and 10-nm particles, respectively. The samples were washed in Iscoves medium and further processed as described in detail elsewhere (Hanau *et al*, 1987).

Statistics For statistical evaluation of significances, the Mann-Whitney U Test was performed. Correlations were calculated by Pearson's linear regression analysis. Results are shown as arithmetic mean ± SEM.

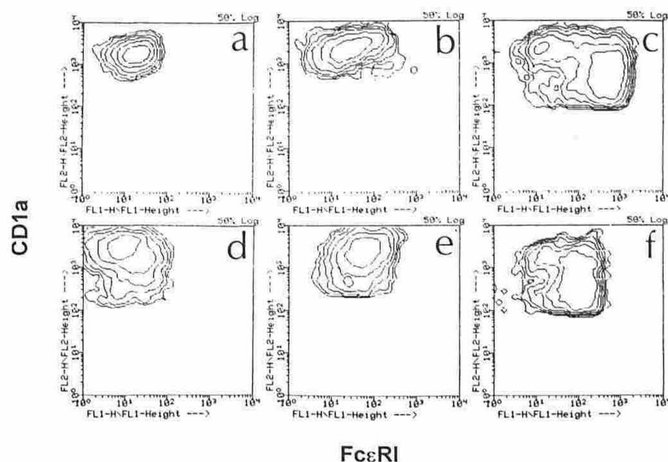


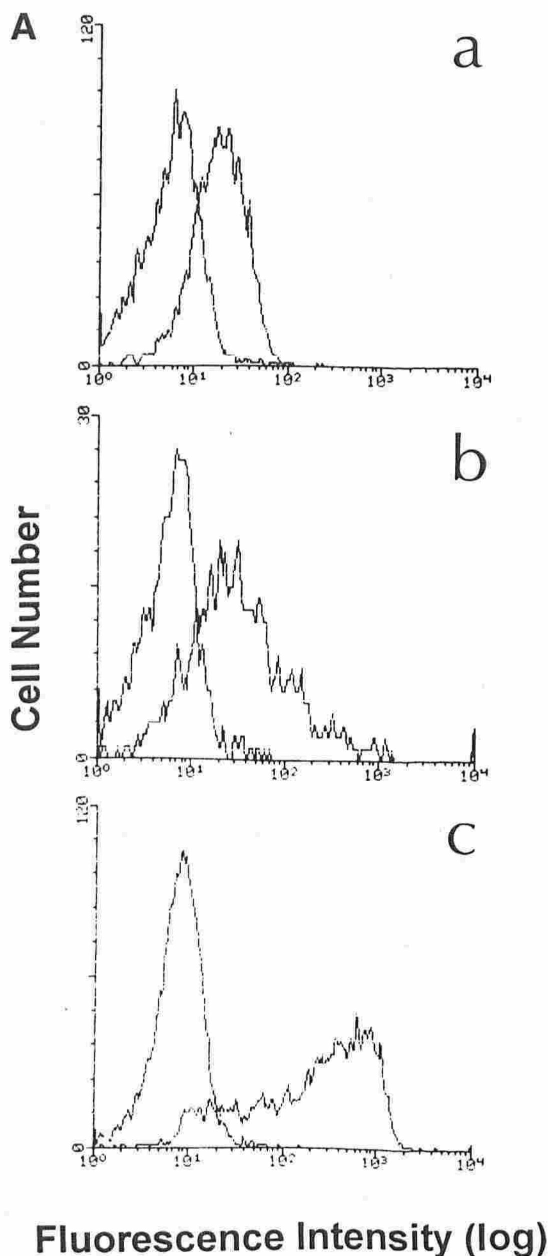
Figure 1. Two subpopulations of CD1a cells in inflammatory lesions. Epidermal cells were isolated by trypsinization and subjected to double labeling as described in *Materials and Methods*. Dead cells were excluded by 7-amino-actinomycin D staining, and CD1a cells were gated. Contour plot analysis of all CD1a/FcεRI cells isolated from a representative skin sample is shown. *a*, normal skin; *b*, nonlesional skin of atopic eczema; *c*, lesional skin of atopic eczema; *d*, allergic contact eczema; *e*, mycosis fungoides; *f*, psoriasis.

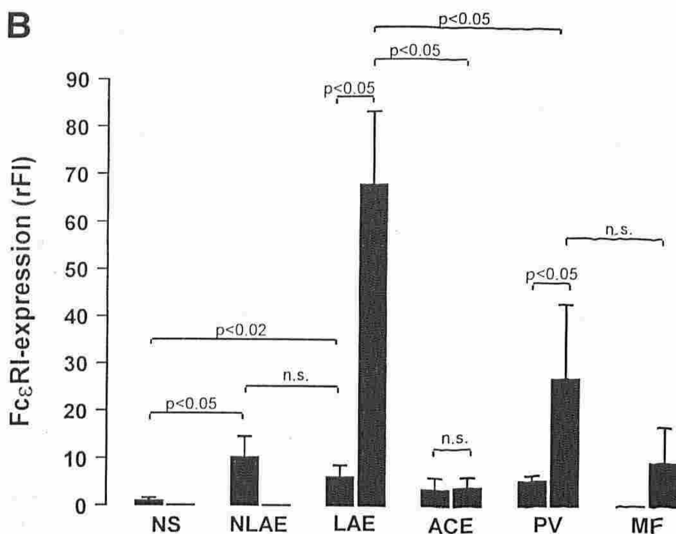
RESULTS

Two Cellular Components in the Epidermal CD1a/FcεRI Population in Atopic Eczema Since it has been postulated that some heterogeneity in the Langerhans cell population may be present in AE in phenotype (Taylor *et al*, 1991) and IgE binding (Bieber *et al*, 1989b), we first performed detailed contour plot analysis of the FcεRI/CD1a cells in normal skin and AE, and extended the studies by studying CD1b, CD23, CD36, and HLA-DR expression. As can be seen in **Fig 1*a***, the CD1a population in normal skin was homogeneous and $CD1a^{bright}/CD1b^{neg}/Fc\epsilon RI^{dim}/CD23^{neg}/CD32^{dim}/HLA\text{-}DR^{bright}/CD36^{neg}$. In some samples, a small $Fc\epsilon RI^{bright}$ population of less than 2% of the total CD1a cells was detected (not shown). In contrast, CD1a cells from lesional AE showed a more heterogeneous CD1a expression resulting in a broad "smear" **(Fig 1*c*)**. This was due to two CD1a cellular components: (i) a $CD1a^{bright}/CD1b^{neg}/Fc\epsilon RI^{dim}/CD23^{neg}/CD32^{dim}/HLA\text{-}DR^{bright}/CD36^{dim}$ population (now referred to as the first population) and (ii) a $CD1a^{dim}/CD1b^{dim}/Fc\epsilon RI^{bright}/CD23^{dim}/CD32^{dim}/HLA\text{-}DR^{bright}/CD36^{bright}$ population (now referred to as the second population). The relative percentages of the second population in lesional AE, however, varied substantially, from 28 to 91%, with a mean of 65%. Nonlesional skin taken from one allergic contact eczema (not shown) and from three AE **(Fig 1*b*)** patients showed only the first but not the second cell population. In most of the other inflammatory skin samples studied here, a second population proved to be present as well, with mean percentages of 17% in allergic contact eczema, 77% in mycosis fungoides, and 49% in psoriasis.

Interestingly, FcεRII/CD23 expression on CD1a cells was present almost only in lesional AE and psoriasis but not in the other conditions tested (not shown). Since FcεRII/CD23 expression on Langerhans cells in lesional AE is hardly detectable by immunohistochemical methods, this suggests that either immunohistochemistry is too insensitive for its demonstration and/or the epitope is masked. Due to the limited number of samples tested with this marker, the specificity of this finding warrants further confirmation.

In contrast, CD36 expression was consistently found in both CD1a cell populations of all lesional inflammatory skin samples, but the second stained brighter for CD36 than the first (not shown). CD36 expression was not detected on CD1a cells of normal skin.





FcεRI Expression Is Highly Upregulated on CD1a Cells in Atopic Eczema and Correlates with the Serum IgE Level Variations in IgE receptor expression on Langerhans cells in AE have been previously suspected in immunohistochemical analysis (Bieber *et al*, 1989b; Bieber and Ring, 1992a; Preesman *et al*, 1991). Therefore, we next investigated more precisely the receptor expression on the CD1a cells, and additionally on both epidermal CD1a cell populations separately. Multicolor flow cytometric analysis was performed on single cell suspensions obtained from skin biopsies of normal skin and nonlesional and lesional AE. As shown by histogram analysis, CD1a cells prepared from nonlesional and lesional AE (**Fig 2*A***, *Panels b and c*) stained much more brightly for FcεRI than did CD1a cells from normal skin (**Fig 2*A***, *Panel a*), confirming an increase of FcεRI on CD1a cells in AE. The marked upregulation observed in lesional skin was due primarily to the second, CD1a^{dim} population (**Fig 1*C***).

Determination of the mean fluorescence levels of FcεRI confirmed marked upregulation of the receptor in the single first (rFI = 10.17 ± 4.3) population of nonlesional AE (n = 4) and the first (rFI = 6.17 ± 2.4) as well as the second population (rFI = 67.96 ± 15.27) of lesional AE (n = 8), compared with the single first population (rFI = 0.99 ± 0.56) of the normal skin (n = 10), which all proved to be highly significant (**Fig 2*B***).

For comparative purposes, similar experiments were then performed with cell suspensions obtained from other inflammatory skin diseases not related to atopy with T-cell infiltrates (**Fig 2*B***). Under these conditions, FcεRI expression on CD1a cells varied substantially depending on the clinical diagnosis but was consistently upregulated compared with normal skin. For example, in allergic contact eczema the FcεRI expression of the respective first (rFI = 3.53 ± 2.49) and second populations (rFI = 3.98 ± 2.15) were, as well as in psoriasis (rFI = 5.46 ± 1.06 and 26.72 ± 15.7), significantly upregulated ($p < 0.05$) compared with normal skin. Furthermore, FcεRI expression of the second CD1a cell population in lesional AE (rFI = 67.96 ± 15.27) was significantly higher than in allergic contact eczema (rFI = 3.98 ± 2.15; $p < 0.05$), psoriasis (rFI = 26.72 ± 15.7; $p < 0.05$), and mycosis fungoides (rFI = 9.25 ± 7.28; $p < 0.02$).

Most interestingly, linear regression analysis revealed a significant correlation of FcεRI expression on CD1a cells from lesional skin with the serum IgE level of the patients included in this study ($r = 0.637$; $p < 0.01$) (**Fig 3**). The correlation was even stronger when only patients with AE were considered ($r = 0.8$; $p < 0.001$). This strongly suggests a link between the mechanisms involved in the regulation of IgE synthesis and those regulating the FcεRI expression on CD1a cells in lesional skin.

Differential Regulation of FcεRI and FcγRII Expression on Epidermal CD1a Cells Langerhans cells express the low-affinity receptor for IgG FcγRII/CD32 (Schmitt *et al*, 1990; Stingl *et al*, 1977). In order to determine whether this Fc receptor undergoes similar regulatory mechanisms as FcεRI, we next studied the possible variations of FcγRII on CD1a cells in our cell samples. FcγRII/CD32 fluorescence showed a relatively sharp peak with a small coefficient of variation, reflecting a homogeneous distribution of FcγRII in the CD1a population (**Fig 4*A***, *panels d–f*). In

Figure 2. FcεRI is highly expressed on epidermal CD1a cells. Receptor expression of FcεRI was quantitated by flow cytometry on both CD1a populations isolated from normal skin and nonlesional and lesional skin of inflammatory skin conditions as described in *Materials and Methods*. *A*) Flow cytometric analysis of the surface expression of FcεRI on CD1a-positive cells from nonatopic normal human skin (*a*), nonlesional skin of an atopic eczema patient (*b*), and lesional atopic eczema skin (*c*). *B*) Mean ± SEM of rFI is given for both CD1a cell populations separately, as isolated from various inflammatory skin diseases. *NS*, normal skin (n = 10); *LAE*, lesional skin of atopic eczema (n = 8); *NLAE*, nonlesional skin of atopic eczema (n = 4); *ACE*, allergic contact eczema (n = 5); *MF*, mycosis fungoides (n = 3); *PV*, psoriasis vulgaris (n = 4).

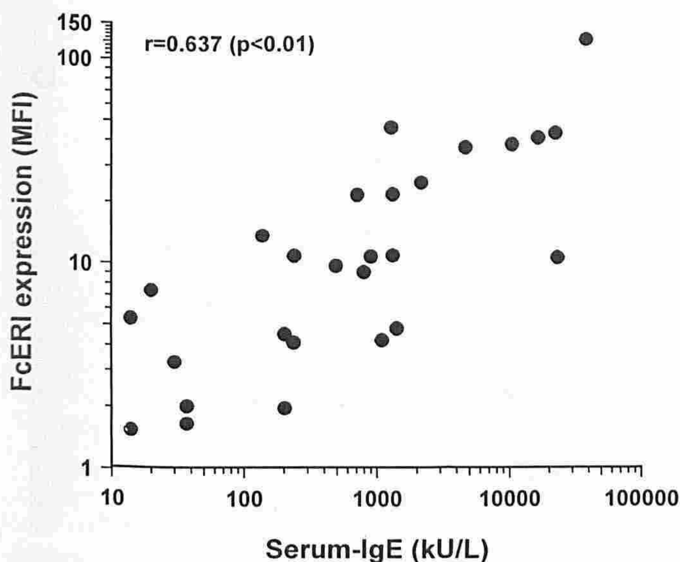


Figure 3. FcεRI expression on CD1a cells correlates to serum IgE level. Epidermal cells were isolated by trypsinization and subjected to double labeling. rFI values for FcεRI expression of all CD1a epidermal cells were determined and plotted with the respective serum IgE level ($n = 27$). The correlation, as calculated by Pearson's linear regression analysis, was found significant.

nonlesional AE, allergic contact eczema, and psoriasis, but not in lesional AE, the rFI values for FcγRII/CD32 of the first CD1a population were significantly increased compared with normal skin ($p < 0.05$). Among all conditions under investigation, patients with psoriasis had the highest FcγRII/CD32 expression on their first (rFI $= 12.23 \pm 3.84$) as well as second (rFI $= 20.14 \pm 4.11$) lesional CD1a cell population **(Fig 4*B*)**. For the first population, the differences in FcγRII/CD32 expression from psoriasis compared with lesional AE were highly significant ($p < 0.01$).

Ultrastructural Characterization of Epidermal CD1a Cells in Lesional Skin of AD In order to gain more information about both populations at the ultrastructural level, epidermal cells obtained from normal skin and lesional AE were subjected to double labeling and immunoelectron microscopy. For this approach, FcεRI and anti-CD1a antibodies labeled with gold particles of different sizes were used. In normal skin, double positive Langerhans cells with Birbeck granules were found, as expected. In lesional AE, in addition to some Langerhans cells **(Fig 5*A*)**, double-labeled FcεRI/CD1a cells with typical features of Langerhans cells (clear cytoplasm devoid of keratin filaments and melanosomes), but without Birbeck granules, were observed **(Fig 5*B*)**.

To formally correlate the immunomorphological and ultrastructural findings, permeabilized epidermal cells were stained with a Birbeck granule specific MoAb, LAG, and counterstained with T6RD1 MoAb reactive to the CD1a surface molecule. By this means, the heterogeneous staining intensity of the CD1a population was confirmed as expected from the surface molecule double staining experiments. Most interestingly, the first, CD1a^{bright} population was strongly reactive to LAG, whereas the second, CD1a^{dim} population was completely negative **(Fig 6)**. Therefore, lesional CD1a cells do not represent a homogeneous group but are composed of two distinct subpopulations defined on both immunophenotypic and ultrastructural criteria.

DISCUSSION

The high-affinity receptor for IgE, FcεRI, was demonstrated on resident CD1a Langerhans cells only recently (Bieber *et al*, 1992b; Grabbe *et al*, 1993; Wang *et al*, 1992). With regard to its putative pathophysiological role in AE, a better characterization of the

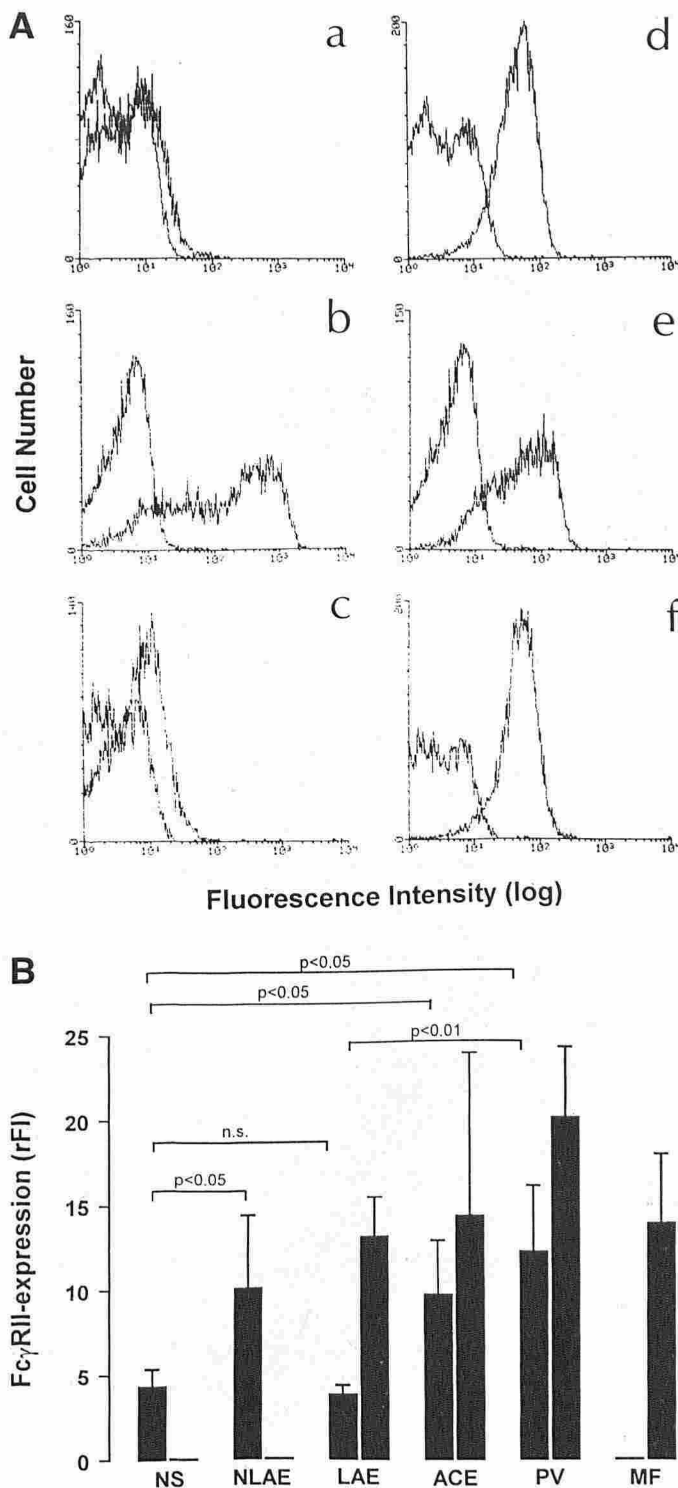
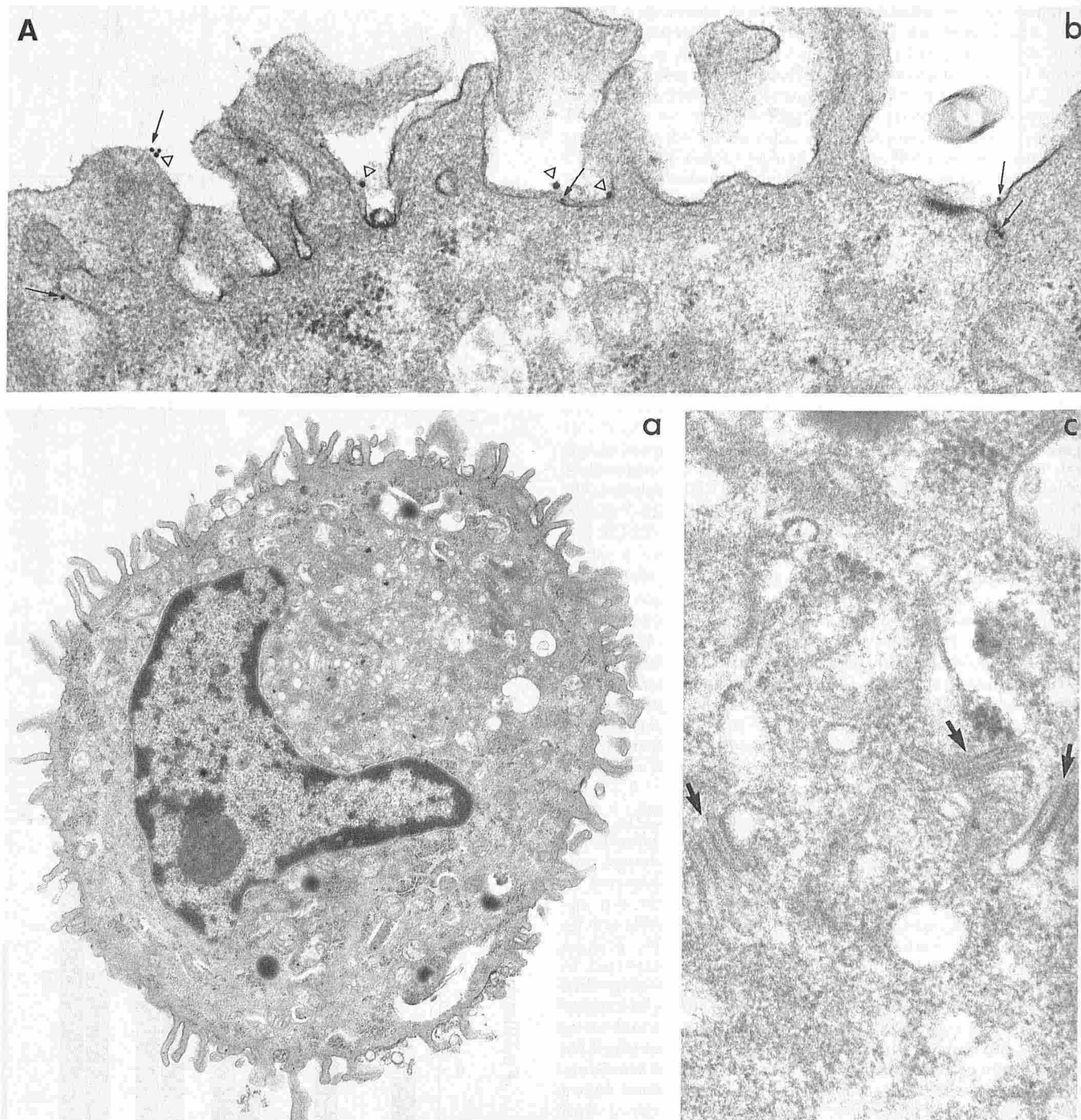


Figure 4. FcεRI and FcγRII/CD32 are differentially regulated on CD1a cells. Receptor expression of FcεRI and FcγRII/CD32 was quantitated by flow cytometry on both CD1a populations isolated from normal skin and lesional skin of inflammatory skin conditions. *A*) Histogram data showing comparative expression of FcεRI (*a*, *b* and *c*) and FcγRII/CD32 (*d*, *e* and *f*) on CD1a positive cells isolated from normal skin (*a* and *d*), lesional skin of atopic eczema (*b* and *e*), and allergic contact eczema (*c* and *f*). *B*) Mean ± SEM of rFI of FcγRII/CD32 is given for both populations of CD1a cells isolated from various inflammatory skin diseases. (NS = normal skin ($n = 10$); LAE = lesional skin of atopic eczema ($n = 8$); NLAE = non-lesional skin of atopic eczema, ($n = 4$); ACE = allergic contact eczema, ($n = 5$); MF = Mycosis fungoides, ($n = 3$); PV = Psoriasis vulgaris, ($n = 4$).



FcεRI-bearing epidermal Langerhans cell population *in vivo* is of great interest. In this study, we show high FcεRI expression on CD1a cells in lesional skin of AE, confirming previous observations in which FcεRI expression on Langerhans cells in various skin diseases was roughly estimated by immunohistochemical staining (Bieber and Ring, 1992a). The more quantitative approach by comparative flow cytometric analysis used in the present study clearly indicates that the highest level of FcεRI-expression is restricted to AE. Moreover, even in normal-appearing skin of these patients, expression was significantly higher than in normal skin. This may be due to the mild inflammatory environment found in uninvolved skin of patients with AE (Mihm *et al*, 1976). Since there is a lack of data concerning the regulation of the FcεRI expression on mast cells and basophils, one may only speculate about the mechanisms leading to the upregulation of the receptor on CD1a cells. Several mechanisms which are not mutually exclusive may be considered. First, the receptor may be stabilized by the binding of IgE molecules, as has been suggested to be the case for FcεRI on RBL cells or for the low-affinity receptor FcεRII/CD23. The significant correlation between the expression and the serum IgE level would further support this assumption. On the other hand, some systemically or locally released mediators involved in the regulation of the IgE-synthesis may also exert signals upregulating the FcεR expression on Langerhans cells and possibly on monocytes (Maurer *et al*, 1994). For example, interleukin-4, known to induce the expression of FcεRII/CD23 in many cell systems, represents a good candidate for a key cytokine involved in the regulation of FcεRI expression on Langerhans cells (Bieber *et al*,

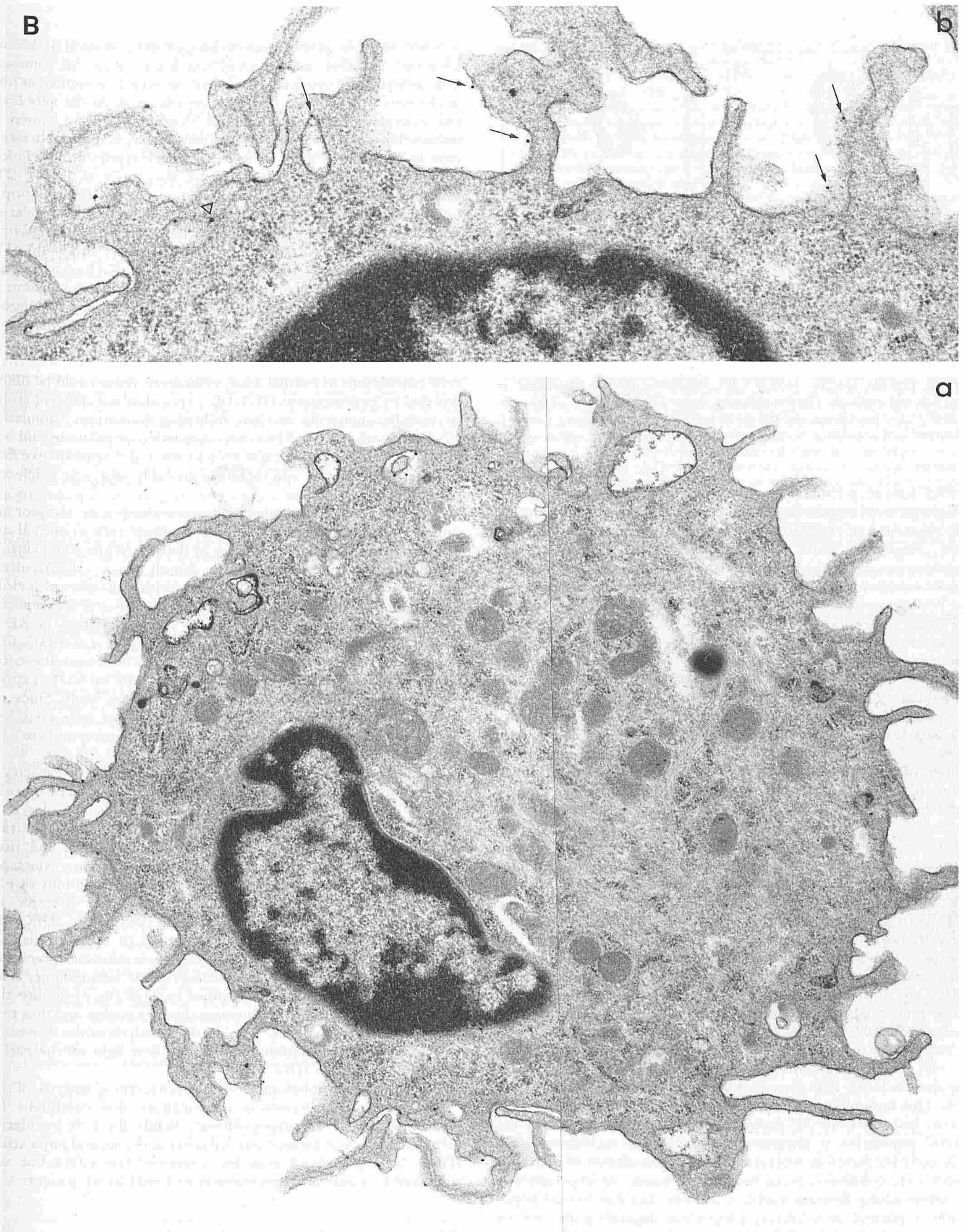


Figure 5. Ultrastructural charaterization of two distinct CD1a/FcεRI cell types in atopic eczema. Epidermal cell suspensions were prepared from lesional atopic eczema skin and subjected to double immunogold labeling. Transmission electron microscopy revealed two ultrastructural similar, but clearly distinct CD1a/FcεRI cell types. *A*) A higher magnification of a classical LC (a) with cell surface colabeling by the anti-CD1a (10 nm, *arrowheads*) and the anti–FcεRI (5 nm, *arrows*) MoAb (b) and many cytoplasmic Birbeck granules (*arrows*) (c). *B*) A higher magnification of an inflammatory dendritic epidermal cell (a) without any visible Birbeck granules and a higher intensity of the cell surface labeling by the anti-FcεRI (5 nm, *arrows*) mAB (b).

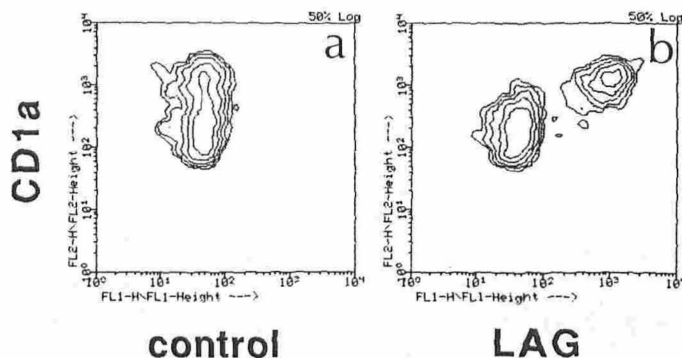


Figure 6. Identity of the phenotypically defined CD1a/FcεRI subpopulations with the two ultrastructurally distinct cell types. Epidermal cells from lesional atopic eczema were isolated by trypsinization and, after permeablization, were subjected to double labeling with the Birbeck granule specific MoAb LAB and anti-CD1a as described in materials and methods. The contour plot shows LAG-reactivity of the first, CD1a^{bright} LC population and the second, CD1a^{dim} inflammatory dendritic epidermal cell population versus the isotype control.

1989c). Indeed, preliminary results obtained on CD34$^+$ Langerhans cell progenitors suggest that interleukin-4 upregulates the cytoplasmic expression of FcεRI (Magerstaedt *et al*, manuscript in preparation). Finally, in atopic skin, endothelial cells, fibroblasts, and keratinocytes may be subjected to yet-to-be-defined inflammatory signals, which in turn lead them to release mediators upregulating the FcεR expression on epidermal CD1a cells. A *de novo* invasion of the epidermis by dermal CD1a cells already expressing higher levels of FcεRI (Osterhoff *et al*, 1994) might be a cause for the observed upregulation. Our finding that not only the second population of CD1a^{dim} cells, but also the CD1a^{bright} first population, exhibits an upregulated FcεRI, favors the hypothesis that local factors cause this upregulation. Whatever the exact regulatory mechanisms are, this very high expression may be of diagnostic value. If analysis of larger series of skin samples confirms the specificity of this phenomenon, the method would be a helpful diagnostic tool in the differential diagnosis of eczematous lesions.

In the epidermis, CD1a is exclusively expressed on Langerhans and related cells (i.e., the so-called indeterminate cells). The latter differ ultrastructurally from Langerhans cells in their lack of Birbeck granules and usually account for less than 2% of the total CD1a population in the normal skin (Czernielewski *et al*, 1983; Rowden *et al*, 1979; Vaigot *et al*, 1985). Langerhans cells completely devoid of Birbeck granules have recently been described in an otherwise perfectly normal, healthy individual (Mommaas *et al*, 1994). This illustrates that Birbeck granule formation is not just a nonspecific phenomenon, easily inducible in other cell types (Hanau *et al*, 1991), but furthermore is not an obligate requisite of immunologically functional Langerhans cells. Since, in our contour plot analysis of LAG reactivity from lesional skin, a clearcut separation of both CD1a subpopulations from one another was possible **(Fig 6)**, we may postulate that they represent entirely different cell populations rather than discrete differentiation stages of Langerhans cells. Our immunoelectron microscopic study of the CD1a populations isolated from AE supports this hypothesis insofar as the second population is ultrastructurally identical to indeterminate cells and may therefore represent a local accumulation of such cells under these conditions. Since we could demonstrate its presence in all inflammatory diseases tested, it is likely that this second population is present in a varying proportion depending on one or several yet-to-be-defined factors secreted in most inflammatory skin conditions. Moreover, the demonstration of such inflammatory dendritic epidermal cells (IDEC) above the basement membrane strongly suggests that distinct chemotactic factors are produced in the epidermis in order to recruit these IDEC in the upper skin compartment, where they may fullfil distinct regulatory functions.

Since variable proportions of Langerhans cells and IDEC are observed in other inflammatory conditions than AE, one can conclude that the cytokine profile may be somehow similar, at least at the time at which the samples were obtained. At the very least, the emergence of CD36 indicates that interferon-γ, known to induce this molecule (Johnson *et al*, 1993), may be present in either case and especially in AE, where it has been reported to be linked to the clinical course of the disease (Grewe *et al*, 1994). The observation of FcεRII/CD23 expression on both CD1a cell populations in lesional AE and psoriasis provides an *in situ* correlation with the reported induction of this receptor on normal Langerhans cells by interleukin-4 and interferon-γ (Bieber *et al*, 1989b). The dramatic upregulation of FcεRI in lesional skin of AE on a level not reached in other skin conditions and the differential regulation of FcεRI and FcγRII/CD32 in AE and psoriasis strongly suggest, however, that qualitatively and/or quantitatively distinct local signals are present in these diseases.

Very recently, Shibaki *et al* reported two different Langerhans cells populations in normal adult volunteers; these could be differentiated by heterogenous HLA-DR expression and different ability to mobilize cytosolic calcium following ionomycin stimulation (Shibaki *et al*, 1995). Their two respective populations did not differ, however, in CD1a expression as assessed by quantitative flow cytometric evaluation and therefore probably represent a different phenomenon.

In recent years, the presence of the thrombospondin receptor and putative collagen receptor CD36 on dendritic cells in normal and diseased skin has become a matter of debate. While some authors found these cells in normal skin (Smolle *et al*, 1985), others observed the immigration of CD36 dendritic cells after ultraviolet irradiation (Cooper *et al*, 1986). More recently, Foster *et al* reported the presence of two CD36 dendritic cell populations in AE as detected by immunohistochemical analysis (Foster *et al*, 1991). One population lacked CD45, HLA-DR, and CD1a, and the other expressed at least HLA-DR and CD45, but data on CD1a expression in this population were not included in this study. Since we investigated only epidermal cells and gated our cells on CD1a expression, both our populations should correspond to the HLA-DR population shown by these authors.

Recently, Taylor *et al* reported enhanced stimulatory capacity of Langerhans cells isolated from AE to autologous peripheral blood T cells in the absence of exogenously added antigen (Taylor *et al*, 1991). These CD1a, CD1b, and CD36 cells ranged ultrastructurally from typical Langerhans cells to indeterminate cells and must probably correspond to a mixture of both populations, which we describe in the present study. Whether both populations display distinct functional properties is unclear, however, since the reported enhanced stimulatory capacity was confined to IDEC. On one hand, IDEC may exert actions similar to those of classical Langerhans cells, in that they reinforce their stimulatory activity, thereby contributing to an amplification of the inflammatory reactions. On the other hand, we cannot exclude the possibility that IDEC rather exhibit a more downregulating function and thus tend to exert negative feedback. The detailed analysis of the functional abilities of both populations should shed new light on the pathophysiological role of IDEC.

The immunomorphological and ultrastructural analysis of the CD1a epidermal cell pool in inflammatory skin conditions has identified two CD1a subpopulations. While the first population fulfilled all classical Langerhans cell criteria, the second population, IDEC, lacking Birbeck granules, expressed less CD1a but was subjected to a dramatic upregulation of FcεRI in AE patients.

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