

A Reducing Microenvironment Leads to the Generation of FcεRI^{high} Inflammatory Dendritic Epidermal Cells (IDEC)

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Inflammatory dendritic epidermal cells present in skin lesions of the atopic eczema/dermatitis syndrome display the highest expression of the high-affinity receptor for IgE (FcεRI), ever detected on human antigen-presenting cells. Owing to the instability of the FcεRI (αγ) complex and fast cleavage from the cell surface during the interleukin-4/granulocyte-macrophage colony-stimulating factor driven *in vitro* differentiation of monocytes, a method to generate inflammatory dendritic epidermal cells was not at our disposal in the past and the amount of *ex vivo* isolated inflammatory dendritic epidermal cells available for functional assays was limited. Therefore, information about the role of inflammatory dendritic epidermal cells and FcεRI on this dendritic cell subtype in atopic and inflammatory skin diseases is completely missing. In this study, we were able to: (i) increase the expression of a functional FcεRI complex on the cell surface of immature monocyte-derived dendritic cells from

atopic donors by creating a reducing microenvironment; (ii) enhance significantly the intracellular pool of the FcεRIγ chains, which is the limiting parameter for the FcεRI surface expression; and (iii) generate monocyte-derived dendritic cells displaying the phenotypic characteristics of inflammatory dendritic epidermal cells, producing high amounts of proinflammatory cytokines and chemokines similar to the cytokines found in lesional skin of the atopic eczema/dermatitis syndrome. Altogether the high expression of functional FcεRI on these cells enables us for the first time to study inflammatory dendritic epidermal cells and FcεRI-mediated mechanisms of inflammatory dendritic epidermal cells *in vitro*, in order to shed light on the putative role of this important cell type in the atopic eczema/dermatitis syndrome. **Key words:** atopic eczema/dermatitis syndrome/Fc receptors/FcεRI/cysteine/redox state/human dendritic cells/inflammatory dendritic epidermal cells. *J Invest Dermatol* 119:842–849, 2002

The high-affinity receptor for IgE (FcεRI) represents a key element on antigen-presenting cells, such as Langerhans cells and dendritic cells (DC) (Bieber *et al*, 1992; Wang *et al*, 1992; Maurer *et al*, 1995, 1998; Bieber, 1997; Novak *et al*, 2001b), mediating cellular mechanisms in the blood and skin of patients suffering from atopic diseases, such as allergic rhinitis, allergic asthma, or atopic eczema/dermatitis syndrome (AEDS) (Johansson *et al*, 2001). One of the hallmarks of these cells is the expression of the high-affinity receptor for IgE, FcεRI. The highest expression of FcεRI on antigen-presenting cells could be detected on the so-called inflammatory dendritic epidermal cells (IDEC) in lesional skin of AEDS patients (Wollenberg *et al*, 1995, 1996). Recent concepts support the hypothesis that IDEC are of monocytic origin and can be recruited into the epidermis

by signals released from cells of the inflammatory microenvironment in the acute phase of AEDS. Beside the high- and low-affinity receptor for IgE and the low-affinity receptor for IgG (FcγRII), IDEC express CD1a, CD1b, CD1c, CD11a, CD11b, CD11c, the thrombospondin receptor (CD36), the mannose receptor (CD206), the costimulatory molecules CD80, CD86, and CD40, major histocompatibility complex (MHC) class I, and MHC class II; however, in contrast to epidermal Langerhans cells, IDEC lack the typical Birbeck granules and are Langerin and Lag negative (Wollenberg and Bieber, 1998; Schuller *et al*, 2001). Interestingly, after successful topical treatment, the number of IDEC in epidermal skin lesions decreases below the detection level, further implying that this cell type is closely related to the state of inflammation of the skin (Wollenberg *et al*, 2001). Owing to the instability of the FcεRI receptor complex of *ex vivo* isolated and *in vitro* generated DC (Kraft *et al*, 1998), IDEC could not be generated *in vitro* so far and a reliable *in vitro* model has not been developed until now, limiting our knowledge in this field of research. A mandatory parameter for the regulation of the FcεRI surface expression on human antigen-presenting cells is the amount of the intracellular, disulfide-linked γ-chain dimers, which associate with the mature, terminally glycosylated α-chain in the Golgi compartment and are transported as a complete trimeric FcεRI (αγγ) complex to the cell surface (Kraft *et al*, 1998; Kinet, 1999; Albrecht *et al*, 2000). In the absence of a sufficient amount of

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Abbreviations: FcεRI, high-affinity IgE receptor; hIgE, monomeric human myeloma IgE; GaM-IgG, F(ab')₂ goat-anti-mouse IgG; IDEC, inflammatory dendritic epidermal cells; AEDS, atopic eczema/dermatitis syndrome; RFI, relative fluorescence intensity; MFI, mean fluorescence intensity; 2-ME, 2-mercaptoethanol; MoDC, monocyte-derived dendritic cells.

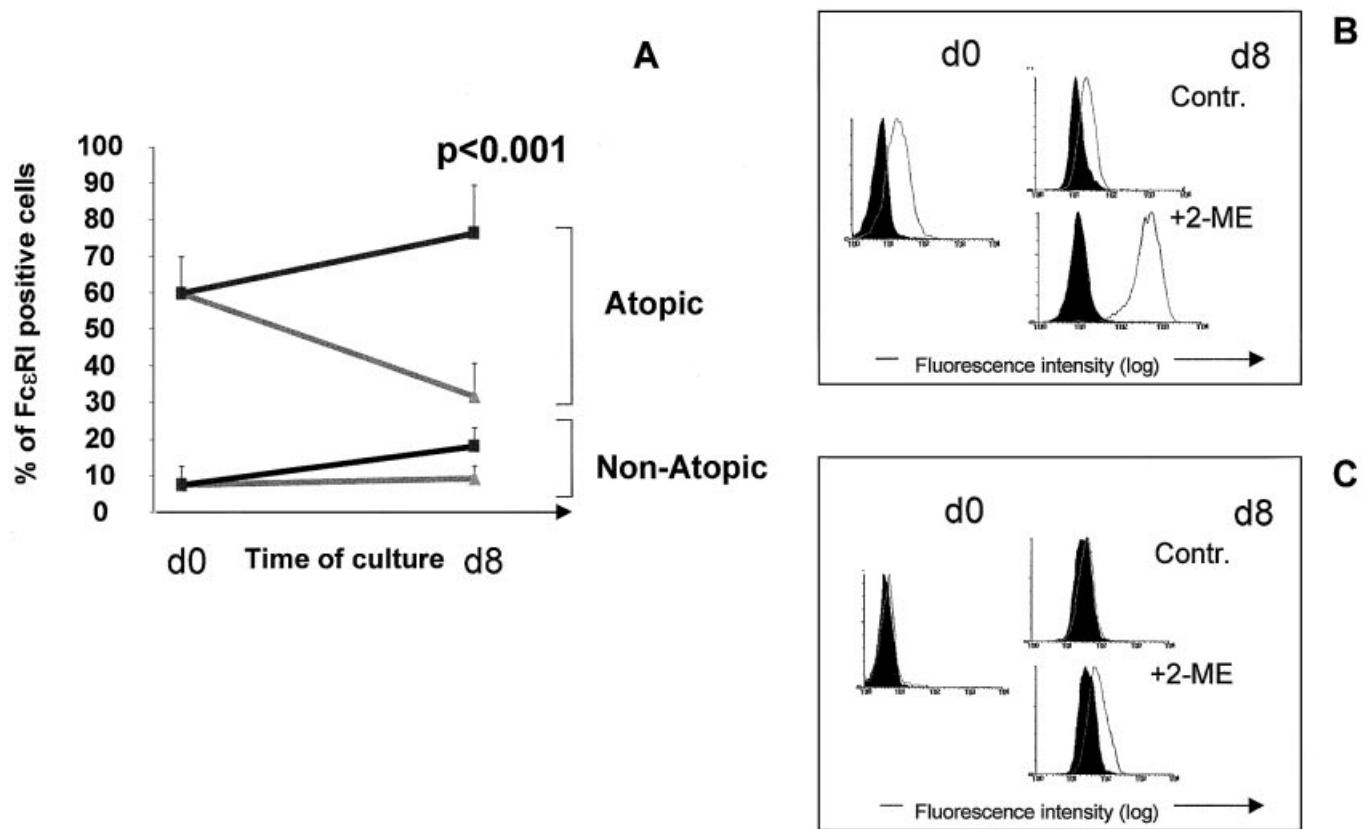


Figure 1. Enhanced FcεRI surface expression of dendritic cells *in vitro* could be achieved by incubation with 50 μM 2-ME. (A) The surface expression of FcεRI of monocytes (day 0) and dendritic cells on day 8 of the IL-4/GM-CSF driven culture of atopic and nonatopic donors generated in the presence of 50 μM 2-ME (black boxes) or in the absence of 50 μM 2-ME (gray triangles) is shown. The percentage of FcεRI positive CD14 (on day 0 of culture in the presence of 2-ME) or CD1a positive cells (on day 8 of culture) is indicated on the y-axis, the time of culture (days) is indicated on the x-axis. Taken together, dendritic cells generated from monocytes of atopic donors display a higher surface expression of the high-affinity receptor for IgE (FcεRI) on day 8 of culture in the presence of 2-ME, than dendritic cells generated from monocytes of nonatopic donors; $n = 20$ atopic donors and $n = 15$ nonatopic donors; $**p < 0.001$. (B/C) The FcεRI surface expression of monocytes on day 0 and day 8 of culture without (Contr.) and with 2-ME of an atopic (B) and nonatopic (C) donor is shown as overlay histogram; this is one representative experiment of $n = 20$ for atopic and $n = 15$ for nonatopic donors.

γ -chains, the immature α -chain remains mainly localized in the endoplasmic reticulum. In contrast to the α -chain, the γ -chain is not a unique feature of FcεRI and is required for other Fc receptor complexes, such as FcγRI (CD16) or the γ -chain of the T cell receptor complex (Kinet, 1999). Recently, Meuer *et al* (Sido *et al*, 2000) could show that reducing agents are able to stabilize the redox-sensitive disulfide dimers of the T cell receptor complex of T lymphocytes and protect it from rapid degradation in an oxidative milieu. In view of these findings, we assumed similar redox-sensitive mechanisms for the maintenance of FcεRI on the cell surface of DC *in vitro* and investigated, whether reducing agents could stabilize FcεRI and prevent the rapid cleavage of the receptor complex during the *in vitro* culture. Furthermore, we developed an *in vitro* system to generate IDEC from FcεRI^{pos} human monocytes of atopic donors, which were similar to the IDEC found in lesional skin of AEDS. This *in vitro* model enables us to study this important cell type in functional assays and get new insights into the putative role of FcεRI on these cells in AEDS.

MATERIALS AND METHODS

Patients and diagnostic criteria A total of 30 patients with atopic diseases such as allergic rhinitis or the allergic form of AEDS with a moderate to high IgE serum level (> 150 kU per liter) and nonatopic healthy volunteers with low serum IgE levels (< 100 kU per liter) were recruited. The protocol was approved by the local ethical committee.

AEDS was diagnosed according to the criteria of Hanifin and Rajka (1980) and the criteria of the European Task Force (Johansson *et al*, 2001). Patients on ultraviolet therapy, on systemic or local glucocorticosteroid treatment or taking anti-histamines were excluded from this study.

Reagents Phycoerythrin (PE)-labeled T6/RD1 (mIgG1) monoclonal antibody (MoAb) (Coultertronics, Krefeld, Germany) is directed against CD1a.

MoAb 22E7 (mIgG1) (a kind gift from J. Kochan, (Roche Inc., Nutley, NJ)) reacts with the α -subunit of FcεRI (Riske *et al*, 1991).

MoAb against FcεRI α (3G6) was from UBI (Lake Placid, NY).

The polyclonal rabbit anti-serum against FcεRI γ was from UBI.

MoAb 4D8 (mIgG2b) against FcεRI γ was kindly provided by J. Kochan (Schoneich *et al*, 1992).

Blocking antibody against FcεRI (15-5) competitive with IgE was a kind gift from J. Hakimi and J. Kochan, (Roche Inc., Nutley, NJ) (Riske *et al*, 1991).

The MoAb against CD40 (5C3) (mIgG1), CD86 (IT2.2) (mIgG2b), CD83 (HB15a) (mIgG2b), the mannose receptor (CD206) (mIgG1) (19.2), and cutaneous lymphocyte antigen (IgM) were from Pharmingen (Hamburg, Germany).

The MoAb against CD1b (4A7.6) (mIgG2a), CD1c (L161) (mIgG1), CD11a (25.3.) (mIgG1), CD11b (BEAR1) (mIgG1), CD11c (BU15) (mIgG1), CD16 (3G8) (mIgG1), FcεRII/CD23 (9P.25) (mIgG1), FcγRII/CD32 2E1 (mIgG2a), CD36 (FA6-152) (mIgG1), CD54 (84H10) (mIgG1), CD58 (AICD58) (mIgG2a), CD80 (MAB104)

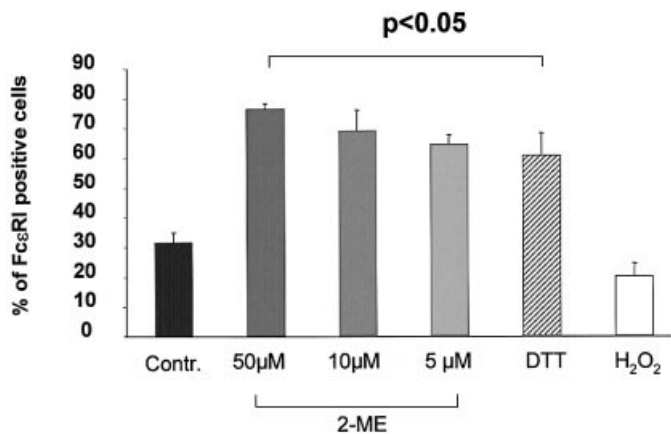


Figure 2. Reducing and oxidative agents influence exclusively FcεRI expression of MoDC from atopic donors *in vitro*. MoDC from atopic donors cultured for 8 d with IL-4 and GM-CSF (black bar; Contr.) or additionally with 50 μM, 10 μM or 5 μM 2-ME (gray bars), 0.5 mM DTT (hatched bar) or H₂O₂ (white bar). The percentage of the FcεRI surface expression of CD1a-positive cells is indicated on the y-axis; n = 5.

(mIgG1), human IgE (Dε2) (E124.2.8) (mIgG1), Langerin (CD207) (DCGM4) (mIgG1), E-cadherin (67A4) (mIgG1), and HLA-DR (G46-6) (mIgG2a) were from Immunotech (Marseille, France).

The hybridoma cell line that produces MoAb W6/32 against MHC class I (Barnstable *et al*, 1978) was kindly provided by Dr N. Koch (Institute of Zoophysiology, University of Bonn).

Fluorescein isothiocyanate (FITC)-labeled (F(ab')₂ fragments of goat anti-mouse antibody and donkey anti-rabbit antibody were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Unlabeled and PE-labeled IgG1 and IgG2 was from Becton Dickinson (Mountain View, CA).

PE-labeled antibody against interleukin (IL)-1α, tumor necrosis factor-α, monocyte chemoattractant protein 1, monocyte chemoattractant protein 3, macrophage inflammatory protein α, and RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) were from Pharmingen.

PE-labeled MoAb against IL-1β was from R&D systems (Wiesbaden, Germany). Rabbit anti-human IgE was purchased from Dako (Glostrup, Denmark).

Human myeloma IgE was obtained from Calbiochem (Bad Soden, Germany).

PE-labeled anti-CD14 antibody and FITC-labeled anti-CD1a antibody were from Becton Dickinson.

2-Mercaptoethanol (2-ME) and H₂O₂ were from Sigma-Aldrich, St. Louis, MO.

Dithiothreitol (DTT) was from Gibco (Life Technologies, Paisley, U.K.).

Flow cytometric analysis Double staining experiments with saponin or digitonin for the detection of the extracellular or intracellular distribution of FcεRI subunits were performed as described in detail elsewhere (Kraft *et al*, 1998). Saponin permeabilization was used for the detection of intracellular FcεRIα, whereas digitonin permeabilization was used for the detection of FcεRIγ expression. Cells were acquired using a FACSCalibur flow cytometer (Becton Dickinson). For quantitative evaluation, the CD1a-positive population was gated manually and the percentage of FcεRIα or FcεRIγ-positive cells was determined using Lysis II software (Becton Dickinson).

Immunolabeling for phenotyping was performed as reported before (Novak *et al*, 2001c). Intracellular staining for cytokines and chemokines was carried out according to the manufacturer's instructions. Briefly, cells cultured in the presence of 1 μM per ml Brefeldin A (Pharmingen) were fixed with 4% paraformaldehyde for 10 min, washed in phosphate-buffered saline supplemented with 1% fetal bovine serum and 0.1% sodium azide, and incubated for 30 min with 1 μg per ml FITC-conjugated CD1a. Then the cells were washed with phosphate-buffered saline containing 1% fetal bovine serum, 0.1% sodium azide, and 0.1%

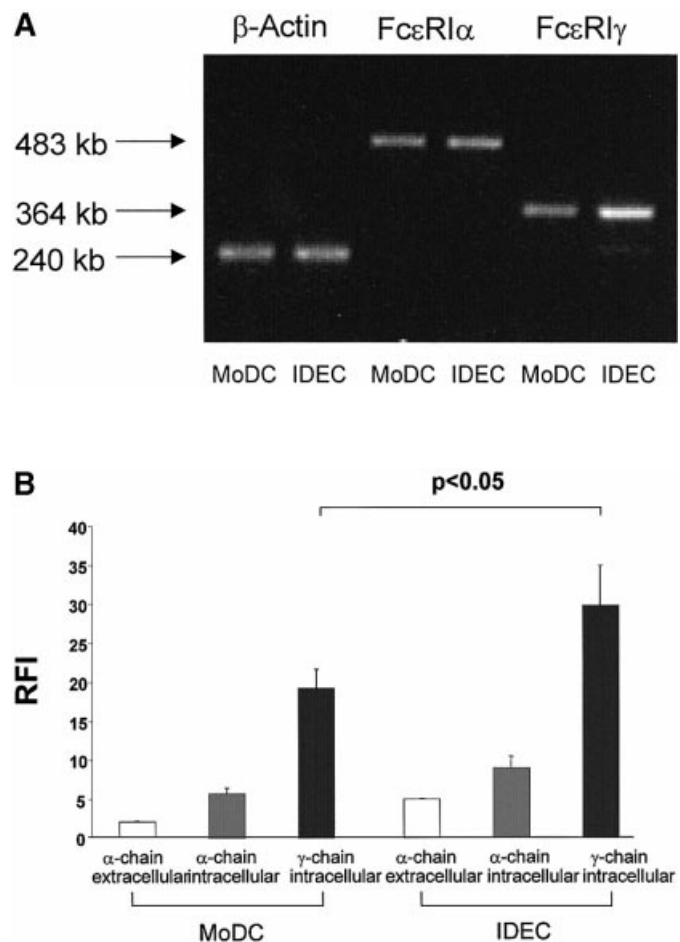


Figure 3. The amount of FcεRIγ is higher in IDEC. (A) Semiquantitative reverse transcription-PCR revealed an increased transcription of the FcεRIγ mRNA detectable in lysates obtained from IDEC, whereas no differences in the signal for the FcεRIα mRNA transcripts of MoDC and IDEC could be detected. This is one representative experiment of n = 4. (B) The extracellular expression of the FcεRI α-chain (white bars), the intracellular amount of FcεRIα (gray bars), and the amount of the FcεRI γ-chains (black bars) of MoDC and IDEC on day 8 of culture is indicated as relative fluorescence index (RFI) on the y-axis. These data demonstrate that the amount of FcεRIγ is significantly enhanced in IDEC, n = 6.

saponin twice. The cells were incubated with PE-labeled MoAb against cytokines or chemokines. After washing with phosphate-buffered saline, cells were analyzed on a FACSCalibur[®] (Becton Dickinson) as described in detail elsewhere (Novak *et al*, 2001c). As a control, cells were also stained with corresponding PE-labeled isotype-matched control immunoglobulin. All incubations and washes were performed at 4°C. Results are expressed as percentage positive cells and relative fluorescence intensity (RFI) calculated from the mean fluorescence intensity (MFI) as follows:

$$RFI = [MFI (\text{cytokine/chemokine}) - MFI (\text{control})] / MFI (\text{control}).$$

Isolation of epidermal cells and enrichment of Langerhans cells Shave biopsies were taken from patients after obtaining signed informed consent, according to the approval of the local ethics committee. After local anesthesia, biopsies were taken from patients with AEDS; chronic skin lesions, which had not been treated for 2 weeks were sampled. AEDS was diagnosed according to the criteria of Hanifin and Rajka (1980) and the new classification of the European Task Force (Johansson *et al*, 2001). Single cell suspensions were prepared from skin biopsies by trypsinization as described previously (Wollenberg *et al*, 1999), washed with phosphate-buffered saline containing 1% fetal bovine serum and 0.1% sodium azide, filtered through a 50 μm nylon mesh and subjected to immunolabeling. Resistance of all receptor molecules to the

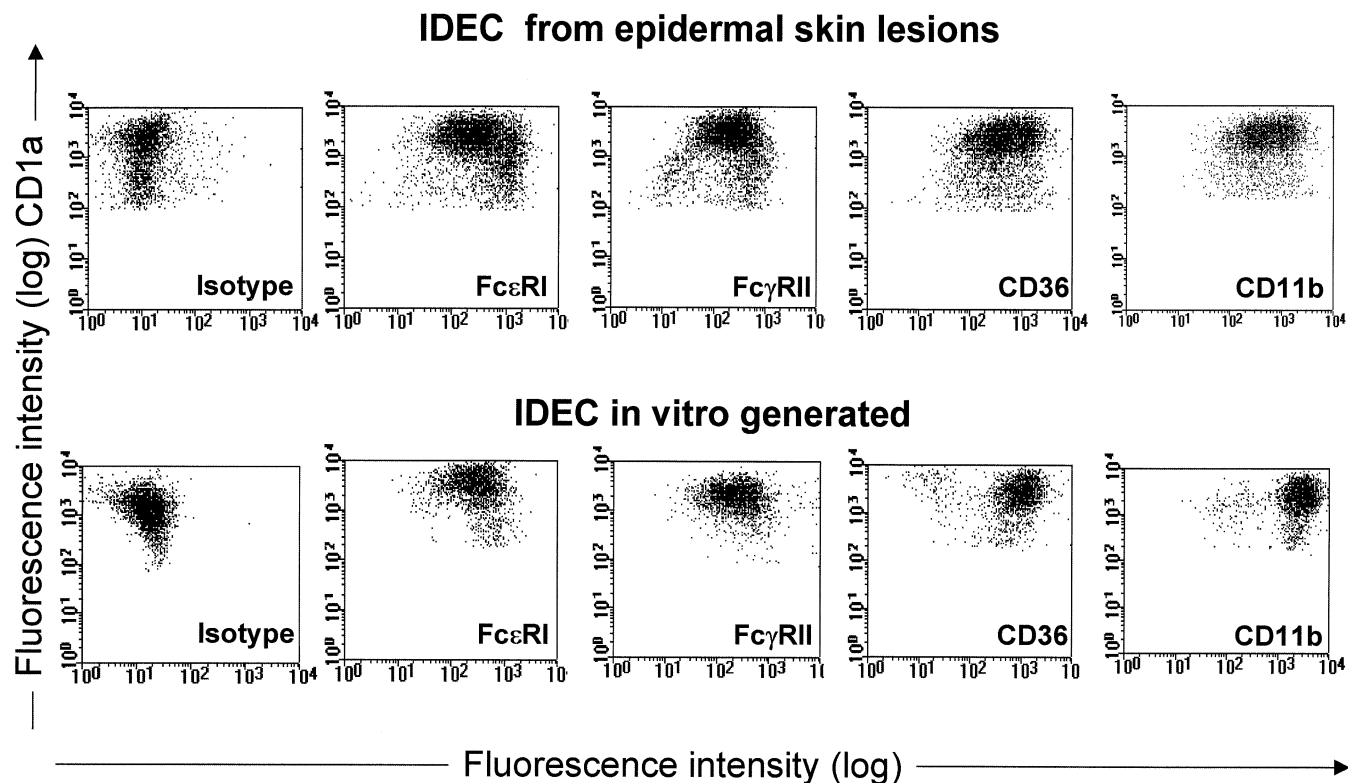


Figure 4. IDEC from lesional skin of AEDS and IDEC generated from monocytes of atopic donors are phenotypically similar. The expression of surface markers of IDEC isolated from lesional skin of AEDS is indicated as fluorescence intensity (log) on the x-axis and CD1a expression as fluorescence intensity on the y-axis (upper panel). In comparison, the expression of these surface molecules detectable on IDEC generated *in vitro* are shown (lower panel). This is a representative experiment of $n = 10$.

limited trypsin digestion procedure had been established in preliminary experiments.

Preparations and culture Whole blood was taken after obtaining signed informed consent from patients with allergic forms of AEDS ($n = 30$) according to the criteria of Hanifin and Rajka (Hanifin and Rajka, 1980). Blood samples were obtained in accordance with local ethics committee.

Monocytes from atopic donors were isolated from peripheral blood with a modified density gradient protocol after using Nycoprep® (Nycomed, Oslo, Norway). Briefly, red blood cells were separated from plasma by sedimentation from whole ethylenediamine tetraacetic acid blood with 1 : 10 (w/v) 6% Dextran 500 in 0.9% NaCl. Plasma was gently layered over Nycoprep® and centrifuged for 20 min at $600 \times g$. After separation, the interphase and all of the Nycoprep® were collected and washed four times with 0.9% NaCl + 0.13% ethylenediamine tetraacetic acid + 1% bovine serum albumin. Then CD14 expression was assessed by flow cytometry and was $>95\%$. Monocytes were cultured for 8 d in medium containing 500 IU per ml human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) (Leucomax, Novartis Pharma AG, Basel, Switzerland) and 500 IU per ml human recombinant IL-4 (Gibco). On the other hand, monocytes from the same donor were cultured with GM-CSF 500 IU per ml, recombinant IL-4 500 IU per ml, and reducing agents such as 5 μM , 10 μM , or 50 μM 2-ME or the anti-oxidant DTT 0.5 mM to obtain IDEC in 24-well plastic plates. On day 2, the cultures were fed with fresh medium, 500 IU per ml GM-CSF and 500 IU per ml IL-4 and the respective amount of 2-ME for the generation of IDEC on days 4, 6, and 8.

Amplification of mRNA and analysis of transcripts Total RNA was extracted after 8 d of culture from highly purified monocyte-derived dendritic cells (MoDC) or IDEC using Trizol (Life Technologies, Eggenstein, Germany) following the manufacturer's instructions. Reverse transcription reactions were performed using 1 μg of total RNA. Denaturation at 94°C for 40 s was followed by annealing of the primers at 55°C for 30 s and extension at 72°C for 30 s. A final extension phase of 5 min was added. Specific primer sequences for each gene were as follows:

Human β -actin: sense, 5'-GAG CGG GAA ATC GTG CGT GAC ATT-3'; anti-sense, 5'-GAT GGA GTT GAA GGT AGT TTC GTG-3', yielding a fragment of 240 bp.

Human FcεRI α : sense, 5'-CTG TTC TTC GCT CCA GAT GGC GT-3'; anti-sense, 5'-TAC AGT AAT GTT GAG GGG CTC AG-3' (fragment of 536 bp).

Human FcεRI γ : sense, 5'-CCA GCA GTG GTC TTG CTC TTA C-3' and anti-sense: 5'-GCA TGC AGG CAT ATG TGA TGC C-3' (fragment of 338 bp).

Amplification was performed on a Perkin-Elmer Gene Amp PCR System 9600 (Applied Biosystems, Weiterstadt, Germany) polymerase chain reaction (PCR) System 9600 thermocycler (Applied Biosystems, Weiterstadt, Germany). The PCR cycle numbers for the amplification of the respective cDNA were 25 for β -actin and 30 for FcεRI α and FcεRI γ . Specific PCR fragments were separated on a 1% agarose gel and visualized using ethidium bromide staining. The PCR products were evaluated semiquantitatively by comparing the ratio of the specific products *vs* the β -actin band by digital image analysis using the WinCam system (Cybertech, Berlin, Germany).

FcεRI-dependent activation of IDEC On day 8, IDEC were washed and incubated at 2.5×10^5 cells per ml in 24-well flat-bottomed culture plates in medium containing 10 μg per ml anti-FcεRI MoAb (F(ab')₂ 22E7 or isotype-matched control mIgG1 for 1 h at 37°C . Excessive IgE was removed, and surface-bound IgE was cross-linked by addition of medium containing goat anti-mouse IgG (GaM-IgG) alternative Ig cells were loaded with 1 mg/1 ml human myeloma IgE for 1 hour. As previously described (Jurgens *et al.*, 1995). In blocking experiments, cells were preincubated with anti-CD23 (9P.25; 5 μg per ml) and lactose (0.2 M, 15 min) in order to block ϵ BP binding and CD23 before loading with IgE or with blocking antibody to FcεRI (15-5; 1 μg per ml) and lactose (0.2 M, 15 min). As a control, the cells were incubated with goat anti-mouse IgG (GaM-IgG) MoAb alone.

Statistical analysis Statistical differences were determined by paired Student's *t* test using SPSS for Windows 8.5. $p < 0.05$ was considered statistically significant.

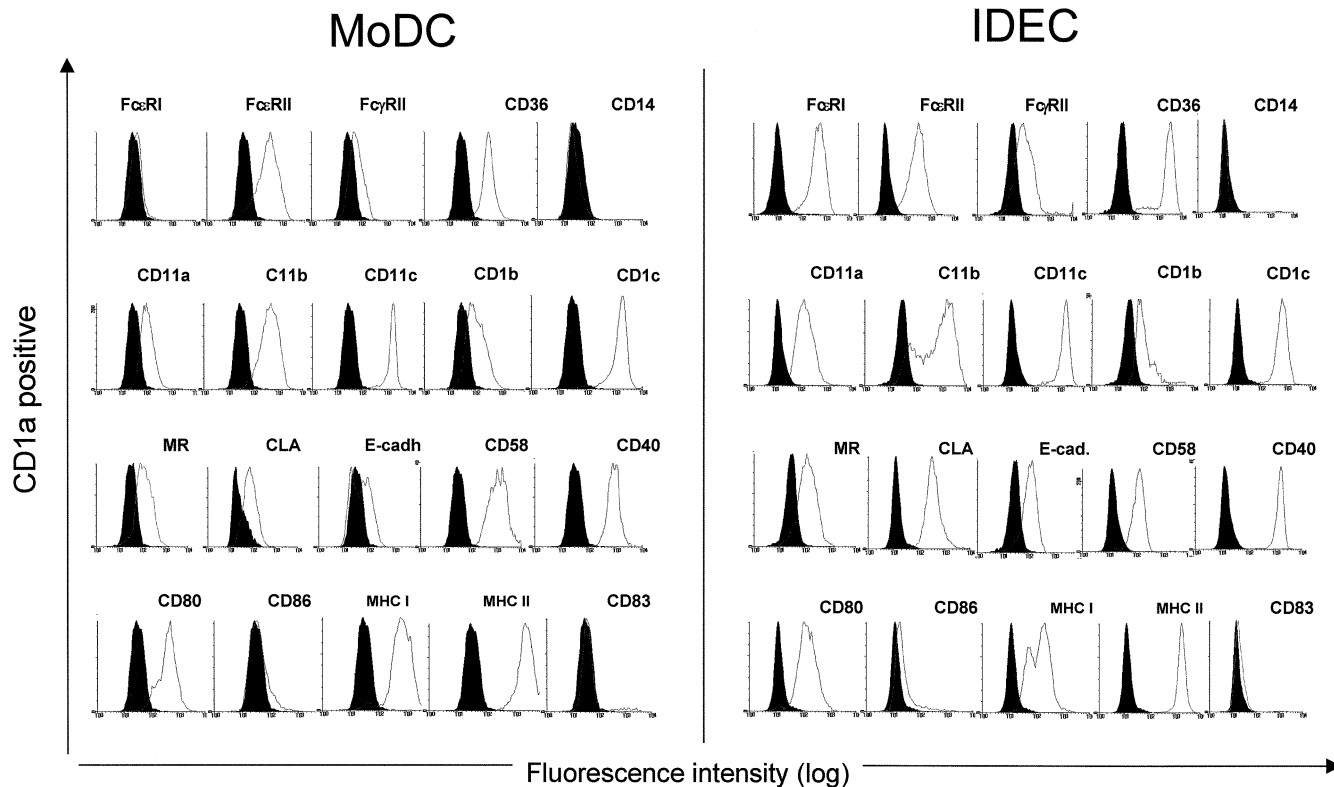


Figure 5. The most striking difference of MoDC and IDEC generated *in vitro* is the surface expression of FcεRI. MoDC and IDEC did not differ in the surface expression of the costimulatory molecules CD80, CD86, MHC class I, MHC class II, or CD83. The most striking difference of MoDC and IDEC generated *in vitro* of monocytes of the same atopic donor is the elevated FcεRI surface expression of IDEC. The surface marker profile of MoDC and IDEC on day 8 of culture is shown as overlay histogram (gray curve) in correlation to the respective isotype control (black curve). The fluorescence intensity (log) is indicated on the x-axis, the number of CD1a positive MoDC or IDEC is indicated on the y-axis. CLA, cutaneous lymphocyte antigen; MR, mannose receptor; E-cadh., E-cadherin. This is a representative experiment of $n = 15$.

RESULTS

FcεRI surface expression of DC can be influenced by reducing agents exclusively in atopic donors Addition of reducing agents, such as 2-ME 5 μ M, 10 μ M, and 50 μ M significantly enhanced the FcεRI surface expression of IDEC of atopic donors obtained on day 8 of the IL-4/GM-CSF driven *in vitro* culture, with the highest FcεRI expression achieved by incubation with 50 μ M 2-ME (**Fig 1 A,B**). In contrast, no significant increase of the FcεRI surface expression could be achieved in nonatopic donors (**Fig 1 A,C**). Neither the viability ($81.72 \pm 2.78\%$ viable cells on day 8 of culture without and $86.67 \pm 1.69\%$ viable cells on day 8 of culture with 50 μ M 2-ME) nor the CD1a surface expression of IDEC generated in the presence of 2-ME changed ($78.53 \pm 5.22\%$ CD1a-positive cells on day 8 of culture) in comparison with the control condition without 2-ME ($71.86 \pm 8.26\%$ CD1a-positive cells on day 8 of culture). To determine, whether this effect could be induced by reducing agents in general and reversed by an oxidative milieu, the reducing agent DTT 0.5 mM or the pro-oxidative H_2O_2 were added to the culture. In accordance with the previous observations, addition of 0.5 mM DTT led to a significant upregulation of the FcεRI surface expression on day 8 of culture, whereas a rapid downregulation of the FcεRI surface expression was induced by the addition of H_2O_2 (**Fig 2**).

Upregulation of the FcεRI complex by reducing agents results from an enhanced FcεRIγ expression For the human system, it has been reported that the presence of FcεRIγ is necessary for the surface expression of FcεRIα and the function of the receptor complex.

To determine, whether the source of FcεRIγ of IDEC in the presence of 2-ME was in part the result of an enhanced mRNA transcription, we performed semiquantitative analyses of PCR products from human FcεRIα and FcεRIγ of MoDC and IDEC. Whereas MoDC and IDEC displayed the same amount of FcεRIα transcripts, the level of FcεRIγ transcripts was 50% higher in IDEC than in MoDC, as evaluated by densitometry (**Fig 3A**). Transcripts for the FcεRI β-chain could neither be detected in MoDC nor in IDEC (data not shown). To confirm these results, we evaluated the FcεRIα surface expression in parallel to the intracellular pool of FcεRIα and the intracellular amount of FcεRIγ by flow cytometric staining. First, we analyzed the intracellular amount of the α-chain and the FcεRIα surface expression and permeabilized the cells with low digitonin concentrations to evaluate the intracellular content of FcεRIγ. Whereas the intracellular levels of FcεRIα were high in both conditions, with and without 2-ME, the amount of FcεRIγ was significantly higher in IDEC generated in the presence of 50 μ M 2-ME (**Fig 3B**). The extracellular and intracellular expression of CD16, which is known to associate with FcεRIγ was below 5% in MoDC and IDEC cultured with reducing agents (data not shown), excluding the fact that the upregulation of the γ-chain by 2-ME leads to a general enhancement of structures containing γ-chains.

***In vitro* generated FcεRI^{high} IDEC display a phenotype highly similar to IDEC isolated from lesional skin of atopic eczematodermatitis syndrome** In the next series of experiments, we compared the typical phenotypical characteristics of IDEC isolated from lesional skin of AEDS with IDEC generated *in vitro* (**Fig 4**). Detailed phenotypical analyses of IDEC generated in the presence of 50 μ M 2-ME evaluated by flow cytometry revealed that FcεRI^{high} IDEC from atopic donors were Langerin and Lag

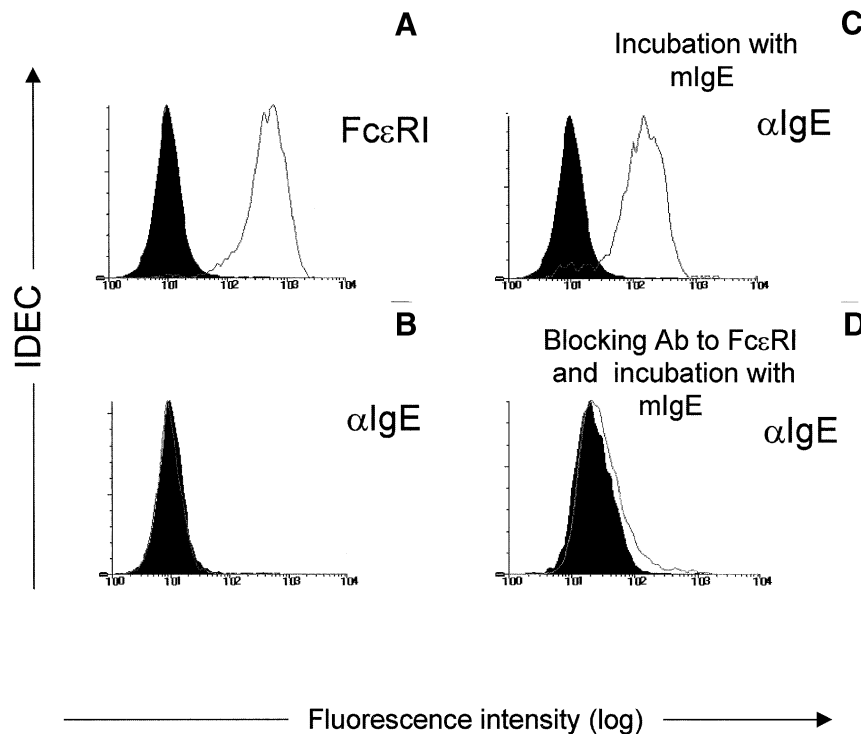


Figure 6. FcεRI on IDEC binds monomeric IgE in high amounts. The FcεRI surface expression of IDEC on day 8 of culture is shown as overlay histogram (A). On day 8 of culture, no constitutive IgE binding of IDEC could be detected by staining with FITC-labeled anti-IgE MoAb (B). In blocking experiments, IDEC were preincubated with blocking antibody to CD23 (9P.25; 5 μg per ml) and 0.5 lactose. Then IDEC were loaded with 0.5 lactose μg per ml human myeloma IgE and stained with FITC-labeled anti-IgE MoAb (C). Additionally, preincubation of IDEC with blocking antibody to FcεRI and lactose completely abrogates IgE binding (D). Taken together, IDEC bind IgE via FcεRI in high amounts on their cell surface.

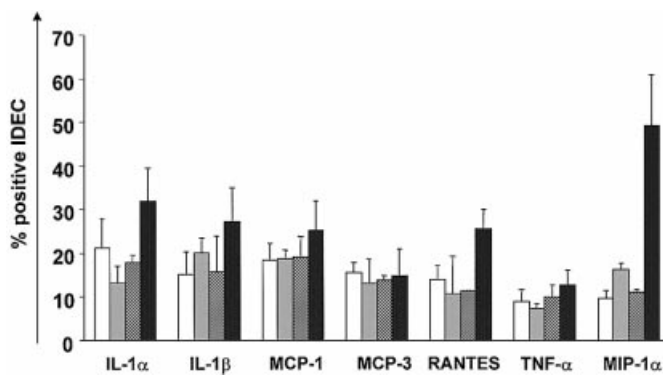


Figure 7. FcεRI stimulation of IDEC induces the production of pro-inflammatory cytokines. Stimulation of FcεRI with MoAb against FcεRI induces an enhanced production of pro-inflammatory cytokines and chemokines. The percentage of the intracellular cytokine production of CD1a⁺ IDEC is indicated on the y-axis. White bars indicate unstimulated IDEC on day 8 of culture, black bars indicate IDEC, which have been stimulated via FcεRI for a time period of 18 h, gray bars indicate IDEC stimulated via mIgG and GaM-IgG, hatched bars indicate IDEC stimulated with GaM-IgG alone; n = 8. From these data we conclude that FcεRI on IDEC generated *in vitro* is functional as cross-linking of FcεRI induces an increased cytokine production.

negative and lack the Langerhans cell specific Birbeck granules. This was evaluated additionally by electron microscopic staining (data not shown). The typical surface marker profile displayed by MoDC and *in vitro* generated FcεRI^{high} IDEC from the same atopic donor is shown in Fig 5. As characteristics of cells localized in the epithelial tissue, IDEC express the epithelial antigen E-

cadherin and skin-homing antigen cutaneous lymphocyte antigen on their cell surface. In summary, IDEC generated *in vitro* from atopic donors expressed FcεRI in high amounts and were therefore phenotypically similar to IDEC from lesional skin of AEDS.

FcεRI of IDEC generated *in vitro* is functional In order to investigate, whether FcεRI on IDEC generated *in vitro* could be efficiently loaded with IgE by incubation with human myeloma IgE for 1 h, blocking studies were performed.

IDEC were preincubated with lactose and MoAb 9P.25 to prevent the binding of IgE to the ε-binding proteins (εBP) and CD23, respectively. The IgE binding capacity was correlated with the surface expression of FcεRI. As a proof of concept, preincubation of IDEC with a blocking antibody to FcεRI (15-5) and lactose abrogates IgE binding to these cells. These experiments identified FcεRI as the main IgE binding structure on IDEC generated *in vitro* (Fig 6).

Engagement of FcεRI on IDEC on day 8 of culture induces the production of proinflammatory cytokines and chemokines As data about the cytokine and chemokine production induced by FcεRI activation of IDEC on day 8 of culture are not available due to the rapid downregulation expression FcεRI from the surface during the *in vitro* culture, we analyzed the quality of the cytokine and chemokine production of FcεRI-activated IDEC. We could clearly show that FcεRI activation enhanced the production of proinflammatory cytokines and chemokines such as IL-1α, IL-1β, MCP-1, MCP-3, RANTES, TNF-α, and MIP-1α.

DISCUSSION

In this study, we provide a reliable *in vitro* model to generate MoDC in a reducing micromilieu displaying a functional FcεRI receptor complex and a phenotype highly similar to IDEC, which

have been detected in the skin lesions of AIDS patients (Wollenberg *et al*, 1996). In addition, IDEC generated *in vitro* display typical characteristics of cells located in the epithelial tissue, such as E-cadherin and cutaneous lymphocyte antigen, which is required for the homing of cells within the epithelia (Ebner *et al*, 1998; Nakamura *et al*, 1998).

Most interestingly, engagement of Fc ϵ RI on IDEC induces the production of proinflammatory cytokines and chemokines, which are typically found in high amounts in the epidermal skin lesions of inflammatory skin diseases, such as AIDS (Pastore *et al*, 2000). The observation that IDEC could be generated exclusively from monocytes of atopic donors with a high Fc ϵ RI surface expression implies that a preformed pool of all components of the Fc ϵ RI complex is necessary for the Fc ϵ RI upregulation and enhanced transcription of the Fc ϵ RI γ chains induced by reducing agents, such as 2-ME. Research work over the last years has focused on a better understanding of the mechanisms regulating Fc ϵ RI surface expression on human effector and antigen-presenting cells. Borkowski *et al* (2001) designed an *in vitro* model to establish the minimal cellular requirements for the regulation of Fc ϵ RI by IgE on the monocytic cell line U937 and NIH3T3 transfectants. IgE binding to the α -chain is the minimal requirement for the induction of Fc ϵ RI upregulation in this model and the mechanism of the upregulation is the result of a combination of the stabilization of the receptor at the cell surface, the use of a preformed receptor pool, and a continued basal level of protein synthesis.

In this context the role of thiolic anti-oxidants as modulators of nuclear factor- κ B activation and inhibitors of the DC maturation should be addressed (Ardeschna *et al*, 2000). DC generated in the presence or absence of 2-ME did not differ significantly in the CD83 expression, the expression of costimulatory molecules, and in their stimulatory capacity toward autologous and allogeneic T cells assessed in mixed lymphocyte experiments (data not shown). Therefore, we conclude that the enhanced Fc ϵ RI surface expression on IDEC achieved by incubation with 2-ME does not result from an inhibition of the maturation of these cells by anti-oxidative agents; however, the relevance of a putative influence of thiolic anti-oxidants on nuclear factor- κ B mediated signal transduction events of IDEC generated with 2-ME has to be elucidated in more detail in studies of the future.

The effect of reducing agents, such as DTT, on living cells have been studied in depth by Braakman *et al* (1992). They could show that changes in the cellular redox state induced by agents, such as 2-ME or DTT interfere with the folding and export of proteins located in the endoplasmic reticulum. In addition, molecular chaperones of the cytosol are known to be redox regulated and their activity accelerates in a reducing environment (Angelini *et al*, 2002). This could lead to an enhancement of the intracellular transport mechanisms of $\alpha\gamma$ -chain associates to the cell surface in our model.

As another aspect, a limiting role of cysteine for the cell cycle progression of human peripheral blood lymphocytes has been described by different research groups (Sido *et al*, 2000). Cell culture media used for the generation of MoDC usually contain only cystin and the addition of 2-ME leads to the formation of mixed disulfides of cystin derived from cystin (Sido *et al*, 2000). In this way, the amount of cysteine available throughout the *in vitro* culture for IDEC is enhanced and could be used for the *de novo* synthesis of Fc ϵ RI γ , which consists of two cysteine residues per γ -chain (Kinet, 1999). *In vivo*, the reducing extracellular micro-environment could be created by antigen-presenting DC, which produce anti-oxidants such as thioredoxin in high amounts after contact with antigen-specific T cells (Jakob *et al*, 1999).

In conclusion, the modulation of the Fc ϵ RI surface expression by the redox state of the microenvironment could contribute to the upregulation of Fc ϵ RI on IDEC in favor of a higher antigen-presenting capacity of these cells, which might lead to the amplification of immune responses in allergic inflammatory skin diseases, such as AIDS.

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