

IL-4 induces the intracellular expression of the α chain of the high-affinity receptor for IgE in in vitro-generated dendritic cells

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Background: Recent findings have shown that the surface expression of the high-affinity receptor for IgE (Fc ϵ RI) on human CD1a⁺ Langerhans cells (LC) and related dendritic cells (DC) in the skin, despite a constant intracellular expression of its α chain (Fc ϵ RI α), is highly up-regulated in atopic dermatitis. Moreover, this surface expression correlates with the IgE serum level, strongly suggesting yet-to-be-defined common signals in the regulation of Fc ϵ RI display on LC/DC and IgE synthesis.

Objectives: In this study we examined the influence of different cytokines on the expression of Fc ϵ RI on in vitro-generated CD1a⁺ LC/DC.

Methods: CD34⁺ precursor cells were isolated from cord blood with use of high-gradient magnetic cell sorting, cultured with GM-CSF, TNF- α , IL-4, or IFN- γ , and surface and cytoplasmic staining for flow cytometry were performed.

Results: IL-4 strongly enhanced the generation of CD1a⁺ LC/DC and also up-regulated the expression of the skin-homing structures E-cadherin and cutaneous lymphocyte antigen. In contrast, IFN- γ was found to suppress the E-cadherin expression and to be a strong antagonist of IL-4 by inhibiting the production of CD1a⁺ cells. Most important, IL-4 induced the cytoplasmic expression of Fc ϵ RI α in CD1a⁺ LC/DC but not its surface expression. This up-regulation was antagonized by IFN- γ .

Conclusion: IL-4 is not only a key cytokine in the regulation of IgE but also induces the expression of its receptor binding chain as well as up-regulation of skin homing molecules on LC/DC. Expression of these structures during generation of LC/DC reflects the in vivo situation encountered in LC. (J Allergy Clin Immunol 2000;105:150-6.)

Key words: Dendritic cells, Langerhans cells, Fc receptors, allergy, stem cells, IL-4, homing receptors

Recently, much progress has been made in understanding the ontogenesis of professional antigen-presenting dendritic cells (DCs).¹ Several in vitro models were developed that contributed largely to dissect the generation of these highly specialized leukocytes.²⁻⁶ Thus in vitro production of DCs can lead to cells displaying some of the characteristics typical of CD1a⁺ epidermal Langerhans cells (LCs) (eg, expression of Birbeck granules or the skin-homing molecules cutaneous lymphocyte antigen (CLA) and E-cadherin⁷⁻¹⁰ and other related DCs. This has provided a new source for the generation of sufficient cell amounts to overcome the problems inherent to the handling of very limited numbers of freshly isolated LC/DCs obtained from human skin. Furthermore, these culture systems now allow for the exploration of biologic phenomena that may take place before LC/DCs have entered the skin. Similarly, they also enable simulation of pathologic conditions to which LC/DCs are subjected. This is of particular interest in the context of atopic diseases (ie, atopic dermatitis [AD]), where LCs and related DCs are suspected to play a crucial role because they express the high-affinity receptor for IgE (Fc ϵ RI)¹¹⁻¹⁴ and may use this structure for antigen focusing.^{15,16} Interestingly, in contrast to basophils and mast cells, Fc ϵ RI surface expression on LCs is highly variable, the strongest expression being specifically displayed in AD,^{17,18} whereas an intracellular pool of its α chain can be found constantly.¹⁹ Furthermore, Fc ϵ RI on CD1a⁺ LCs and related DCs in inflammatory skin strongly correlates to the serum IgE level of each individual, suggesting common regulatory pathways between the IgE synthesis and receptor display on the LC surface.²⁰ In the current report we generated CD1a⁺ LC/DCs from CD34⁺ stem cells isolated from cord blood and explored the regulatory mechanisms underlying the expression of skin homing receptors and Fc ϵ RI expression.

MATERIAL AND METHODS

Reagents

Phycoerythrin (PE)-labeled T6/RD1 (IgG1, Coultertronics, Krefeld, Germany) and unlabeled IOT6 mAbs (IgG1, Immunotech, Marseilles, France) were directed against CD1a, which is, in the

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Abbreviations used

AD:	Atopic dermatitis
CLA:	Cutaneous lymphocyte antigen
DC:	Dendritic cells
EC:	Epidermal cells
FcεRI:	High-affinity IgE receptor
FcεRIα:	α-Chain of the high-affinity IgE receptor
FcεRIβ:	β-Chain of the high-affinity IgE receptor
FcεRIγ:	γ-Chain of the high-affinity IgE receptor
GAPDH:	Reduced glyceraldehyde phosphate dehydrogenase
LC:	Langerhans cells
PE:	Phycoerythrin
rFI:	Relative fluorescence index

epidermis, present only on LCs.²¹ Murine mAb 22E7 (IgG1) was raised from mice immunized with a purified chimeric α-subunit of FcεRI (FcεRIα) expressed in Chinese hamster ovarian cells²² and murine mAb 4D8 (IgG2b) against the cytoplasmic domain of the γ-subunit (FcεRIγ)²³ (kind gifts from Dr J. Kochan, Department of Autoimmune Diseases, Hoffman-LaRoche, Nutley, NJ); mAb Lag reacts specifically with Birbeck granules in human LCs (IgG1, generous gift of Dr S. Imamura, Department of Dermatology, Kyoto University, Japan). Unlabeled mAb CD14 (IgG2a) was obtained from Immunotech and monoclonal mouse anti-E-cadherin antibody (IgG1) from Zymed Laboratories (San Francisco, Calif), and mAb HECA-452 against human CLA antigen (rat IgM)²⁴ was a generous gift of Dr L. Picker (Department of Pathology, University of Texas, Southwestern Medical Center). FITC-labeled and FITC-unlabeled F(ab)2 fragments of goat antimouse antibody were purchased from Jackson ImmunoResearch Laboratories (West Grove, Pa) and FITC-conjugated mouse antirat IgM antibody from Pharmingen (San Diego, Calif). Unlabeled IgG1, PE-labeled IgG1 and PE-conjugated CD14 were from Becton Dickinson (Mountain View, Calif) and unlabeled IgM (rat) was from Immunotech. Normal mouse serum for blocking purposes and 7-amino-actinomycin-D were purchased from Sigma (St Louis, Mo). Sheep antimouse-coated magnetic beads (M-280) were from Dynal (Oslo, Norway). Digitonin (D-1407) and saponin (S-7900) were from Sigma. Oligonucleotides were synthesized by MWG-Biotech (Munich, Germany). All cytokines were from Genzyme (Boston, Mass). Human myeloma IgE was obtained from Calbiochem (Bad Soden, Germany).

Purification of CD34⁺ stem cells

Cord blood was obtained from vaginal or cesarean deliveries (Departments of Gynaecology, Universities of Munich and Bonn). The samples contained 300 to 500 × 10⁶ PBMCs in 30 to 80 mL of blood. After the blood was diluted 3 times with RPMI 1640 (Seromed, Berlin, Germany), 25 mL of suspended cells was overlaid on 15 mL of Lymphoprep (1.077 g/mL, Nycomed, Oslo, Norway). PBMCs were isolated as interface cells after density gradient centrifugation (20 minutes at 900g at room temperature). To reduce residual platelets, the recovered cells were washed twice in PBS and recovered in PBS supplemented with 1% BSA and 5 mmol EDTA. Isolation of CD34⁺ cells was performed with use of anti-CD34 mAb (QBEND/10) conjugated to magnetic microbeads and the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The enrichment of CD34⁺ cells was determined in each experiment by staining with the non-cross-reacting PE-conjugated anti-CD34 mAb HPAC-2 (Becton Dickinson). Typically, 2 × 10⁵ cells with more than 90% CD34⁺ cells were obtained from a 40-mL blood sample. The eluate was analyzed by flow cytometry with use of PE-conjugated anti-CD14 and FcεRIα antibody (see reagents).

Culture conditions

Purified CD34⁺ cells were cultured (1 × 10⁵ cells/mL) in 24-well plates (Costar, Cambridge, Mass) with 1 mL of Iscove's modified Dulbecco's medium (Seromed) supplemented with 10% FCS (Life Technologies, Eggenstein, Germany), 1% antibiotics/antimycotics (Life Technologies), and 1% L-glutamine (Life Technologies). For the generation of CD1a⁺ cells, GM-CSF (300 IU/mL) and TNF-α (50 IU/mL) were added as described.^{2,25} Cultures were maintained by replacing one half the culture medium with fresh cytokine-supplemented medium every 4 days for a maximum of 3 weeks. In some experiments IL-4 (5, 50, or 500 IU/mL) or IFN-γ (5, 50, or 500 IU/mL) were added at the beginning of the culture time. In some experiments human myeloma IgE was added to the culture medium at a concentration of 2 μg/mL.

Immunoelectron microscopy

Cell suspensions were cooled at 4°C and incubated for 45 minutes with anti-CD1a MoAB IOT6a gold-labeled with 5-nm particles. The samples were washed in Iscove's medium and further processed as described in detail elsewhere.²⁶

Flow cytometric analysis of surface and intracellular expression of FcεRI subunits

A total of 1 × 10⁵ in vitro generated cells were subjected to double-staining experiments for surface or intracellular staining with saponin or digitonin as described in detail elsewhere.^{19,20} As controls, freshly isolated epidermal cells (ECs) were used as negative controls (LC-depleted keratinocytes) and positive controls (LC). For quantitative evaluation, dead cells were excluded by 7-amino-actinomycin D staining and the CD1a⁺ populations were gated out manually and the results expressed in percent of positive cells and mean fluorescence index (MFI). Relative fluorescence index values (rFI) of the different structures were determined according to the following formula:

$$rFI = (MFI[receptor] - MFI[control])/MFI[control].$$

For statistical evaluation of significance, the Mann-Whitney *U* test was performed. Results are given in mean percent of positive cells ± SEM.

Amplification of messenger RNA transcripts

Highly purified in vitro generated LC/DCs (>98%; the remaining CD1a⁺ cells contained no basophils, no mast cells as verified by tryptase PCR, not shown) were obtained by positive selection with use of anti-CD1a antibody (IOT6a) bound to sheep antimouse-coated 280-nm magnetic beads, as described.¹¹ Total RNA extraction was extracted from cultured cells by an improved guanidinium isothiocyanate single-step procedure with Trizol (Life Technologies) according to the manufacturer's instructions. Reverse transcription and amplification were performed as described in detail elsewhere.¹¹ Specific primer sequences for each gene were as follows: for reduced glyceraldehyde phosphate dehydrogenase (GAPDH), sense 5'-CCA CCC ATG GCA AAT TCC ATG GCA-3', antisense 5'-TCT AGA CGG CAG GTC AGG TCC ACC-3', yielding a fragment of 598 bp; FcεRIα, sense 5'-CTG TTC TTC GCT CCA GAT GGC GT-3', antisense 5'-TAC AGT AAT GTT GAG GGG CTC AG-3' (fragment size 536 bp); FcεRIβ, sense 5'-GGA CAC AGA AAG TAA TAG GAG AG-3', antisense 5'-GAT CAG GAT GGT AAT TCC CGT T-3' (fragment size 446 bp); FcεRIγ, sense 5'-CCA GCA GTG GTC TTG CTC TTA C-3', antisense 5'-GCA TGC AGG CAT ATG TGA TGC C-3' (fragment size 338 bp); tryptase, sense 5'-CTC CCT CAT CCA CCC CCA GT-3', antisense 5'-GGA TCC AGT CCA AGT AGT AG-3' (fragment size 616 bp).

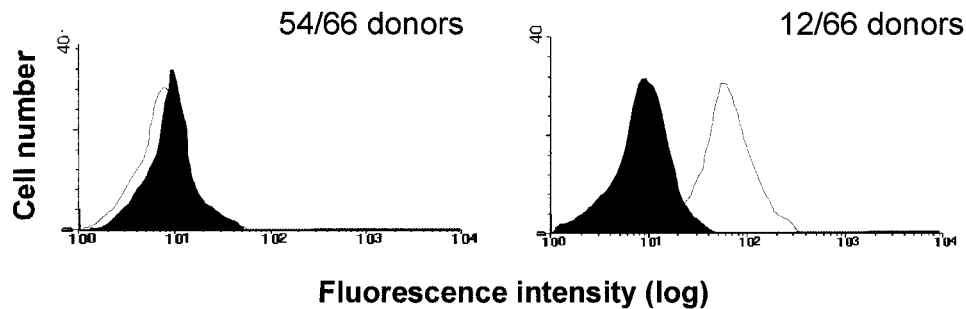


FIG 1. Spontaneous surface expression of FcεRI on in vitro-generated CD34⁺-derived CD1a⁺ LC/DCs. After 12 days of culture, CD1a⁺ cells were analyzed by double labeling and flow cytometry as described in Material and methods. Positive expression was found in a limited number of samples (right), whereas the majority of samples failed to display spontaneous surface expression of FcεRI (left). Expression is shown in one representative histogram, respectively. Filled curve shows the isotype control whereas the other one represents FcεRI.

Amplification was performed on a Perkin-Elmer Cetus GeneAmp PCR System 9600 thermocycler (Applied Biosystems, Weiterstadt, Germany). The PCR cycle numbers for amplification of the respective complementary DNAs were 32 for GAPDH and 40 for FcεRIα, FcεRIβ, and FcεRIγ. Specific PCR fragments were separated on 0.8% agarose gels and visualized with use of ethidium bromide staining. RNA from peripheral blood leukocytes and LC-depleted keratinocytes were used as positive and negative controls, respectively.¹⁹

RESULTS

Spontaneous surface expression of FcεRI on in vitro generated CD1a⁺ cells

In a first approach, we asked whether in vitro generated CD34-derived CD1a⁺ LC/DCs spontaneously express FcεRI. Therefore cord blood samples were examined at day 12 after CD34⁺ cells were cultured in the presence of GM-CSF and TNF-α. Under these conditions, 15% to 65% of total cells were CD1a⁺, the rFI varying from 5 to 56. These cells exhibited dendritic morphologic characteristics and the cell yields achieved about 5- to 15-fold of the initial cell number. Immunoelectronmicroscopic investigations demonstrated that a low but significant proportion (5%-10%) of the CD1a⁺ DCs contained Birbeck granules. This was confirmed by permeabilization experiments with an mAb specific for antigen associated with Birbeck granules (data not shown). Further phenotypic characterization revealed that these CD1a⁺ LC/DCs expressed low amounts of E-cadherin (10% ± 4% positive cells, rFI 1.9 ± 0.7) and a variable but consistent CLA expression (30% ± 12% positive cells, rFI 11.7 ± 5.2). Surface FcεRI expression was detected on CD1a⁺ cells in only 12 of 66 samples (Fig 1) with a range of 8% to 50%, and rFI were 1.05 to 3.60. Similar to experiments with LC¹⁹ and in contrast to what has been reported in mast cells,^{27,28} the addition of human myeloma IgE in the culture conditions did not have any influence on the receptor expression (data not shown). In contrast to our observation with surface expression of FcεRI on epidermal LC/DCs from lesional skin, the presence of FcεRI on in vitro-generated CD1a⁺ LC/DCs did not correlate with the IgE serum level of the cord blood samples (data not

shown). Because no information about the atopic status of the donors was available, attempts to establish other correlations were unsuccessful. Significant intracellular staining for FcεRIα (ie, >15%) was observed only in those conditions where surface staining was already present. In further experiments (n = 6) we were unable to detect any surface expression of FcεRI on monocytes isolated from the same cord blood samples (data not shown).

IL-4 induces messenger RNA and protein synthesis of FcεRIα in in vitro-generated CD1a⁺ cells

IL-4 is known as a key cytokine in the biologic functions of IgE and induces the low-affinity receptor for IgE FcεRIIb/CD23 on several hematopoietic cells.²⁹ Therefore we questioned whether this cytokine may exert a similar effect on FcεRI. For this purpose, IL-4 was added to the combination of GM-CSF and TNF-α to CD34⁺ cells (n = 4). After 12 days of culture, PCR analysis was performed on highly purified CD1a⁺ cells with use of primers specific for all 3 subunits of FcεRI. The presence of mast cells was excluded with use of tryptase-specific primers (data not shown). Low but constitutive expression of transcripts for FcεRIγ was detected in either condition. Transcripts for FcεRIα were found in all conditions with exogenous IL-4 but were only hardly detectable in 1 of 4 samples with GM-CSF and TNF-α alone (Fig 2, A). However, transcripts for FcεRIβ were never detected under these conditions, which makes a contamination with basophils very unlikely. Because it has been reported that, on one hand, IL-4 induces transcripts for FcεRIα but not its translation³⁰ in eosinophils and, on the other hand, it provokes the surface expression of FcεRI on cord blood-derived mast cells,³¹ in vitro generated CD1a⁺ LC/DCs were subjected to surface and intracellular staining for FcεRIα and FcεRIγ. Surface expression of FcεRI was found in 3 of 10 samples under GM-CSF and TNF-α but was never detectable when IL-4 was added. In contrast, significant intracellular FcεRIα expression (>15%, rFI >1.3) was found only in culture

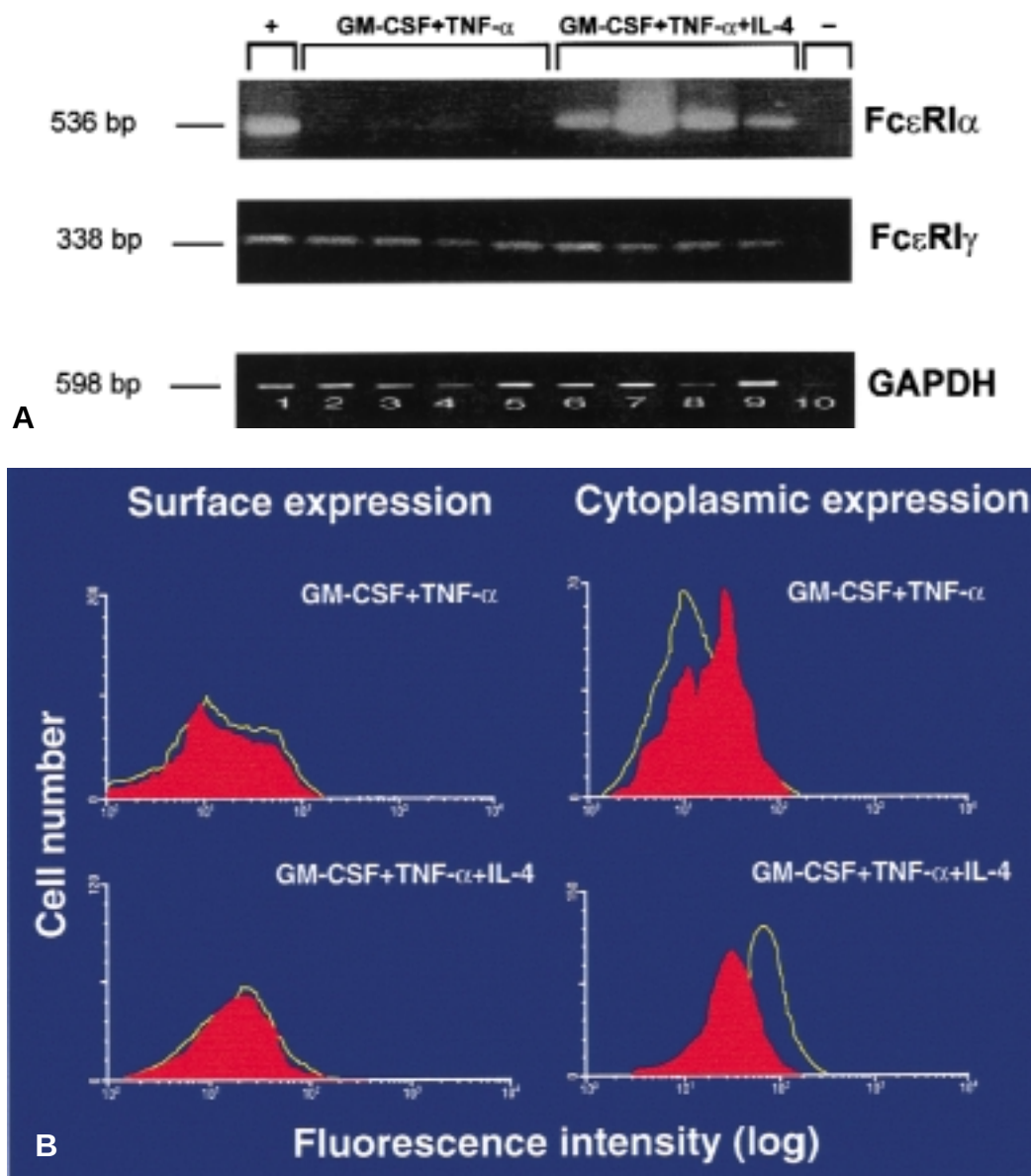


FIG 2. IL-4 induces transcripts and intracellular protein expression of FcεRIα in in vitro-generated CD34⁺-derived CD1a⁺ LC/DCs. CD1a⁺ LC/DCs were generated with GM-CSF and TNF-α and in absence (lanes 2-5) or presence of IL-4 (lanes 6-9). After 12 days of culture, the presence of transcripts for FcεRIα (**A**) and the expression of the protein on the cell surface or in the cytoplasm (**B**) were monitored by PCR and flow cytometry, respectively. Lanes 1 and 10, Positive and negative controls, respectively. **B**, Red curve represents staining of control antibody whereas yellow curve shows FcεRIα.

conditions with IL-4 (Fig 2, *B*) (6/10 experiments). In the remaining 4 experiments, expression of the α chain was not changed under IL-4. Expression of FcεRIγ was consistently detectable under all culture conditions (data not shown) (42.7% ± 5.8% positive cells with rFI of 4.9 ± 1.3 under GM-CSF + TNF-α and 35.4% ± 4.9 % positive cells and rFI of 3.9 ± 0.6 under GM-CSF + TNF-α + IL-4).

IFN-γ inhibits the generation of CD1a⁺ cells

It is well accepted that IFN-γ is one of the most potent antagonists of IL-4, especially with regard to IgE biolog-

ic functions. Thus, in a series of experiments, we first evaluated the effect of IFN-γ on the in vitro generation of CD34-derived CD1a⁺ cells in our culture system (n = 3). We found that IL-4 increased the number of CD1a⁺ cells in a dose-dependent manner. At the same time IFN-γ not only inhibited their generation with GM-CSF and TNF-α (Fig 3) but appeared to be a strong antagonist of IL-4. Comparing the absolute CD1a⁺ cell yields and considering the proliferation of the cells, down-regulation of CD1a⁺ cells by 500 U/mL IFN-γ was 12-fold, whereas 500 U/mL IL-4-induced up-regulation was about 7- to

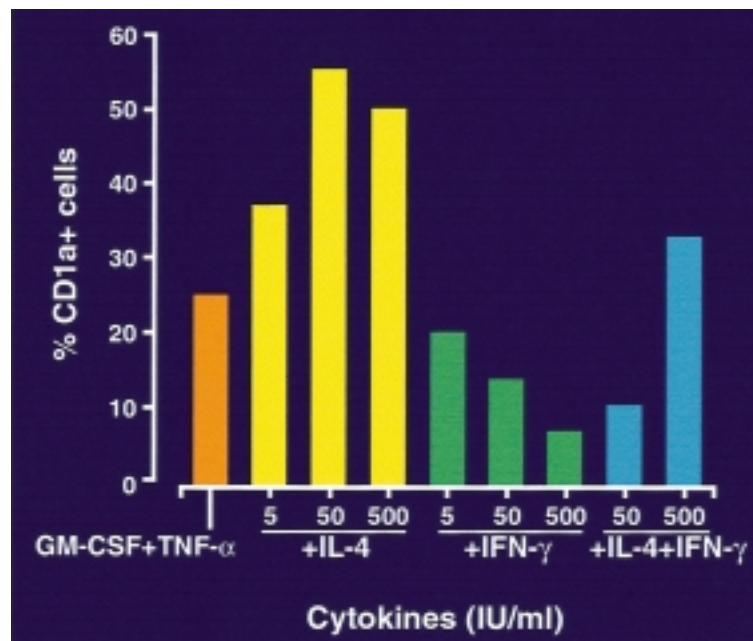


FIG 3. Antagonistic effects of IL-4 and IFN- γ on in vitro generation of CD34⁺-derived CD1a⁺ LC/DCs. CD1a⁺ LC/DCs were generated as described above and in absence or presence of increasing concentrations of IL-4 or IFN- γ . The percentage of CD1a⁺ cells among total cell population was then monitored by flow cytometry. This figure illustrates one representative set of 3 experiments.

14-fold, respectively. In this respect, it should be considered that IFN- γ affects the viability of cells as controlled by flow cytometry with 7-amino-actinomycin D staining. Furthermore, while decreasing CD14 expression, 500 U/ml IL-4 up-regulated the expression of the skin-homing molecules E-cadherin ($P < .01$, $n = 6$) and CLA (not significant) (Fig 4). In contrast, further experiments with the addition of 500 U/mL IFN- γ resulted in a low expression of E-cadherin while CLA was also up-regulated, although not significantly (see also Fig 4). Finally, addition of 500 U/mL IFN- γ suppressed the IL-4-induced transcripts for Fc ϵ RI α as well as the cytoplasmic expression of this receptor subunit (not shown).

DISCUSSION

Recently, much progress has been made in the in vitro generation of CD1a⁺ DCs from blood progenitors.²⁻⁶ In such an in vitro model DCs can be generated that display some of the characteristics of CD1a⁺ epidermal LCs. Thereby a sufficient amount of cells may be gained to simulate pathologic conditions of the skin such as AD, where LCs and related DCs are suspected to play a crucial role because they express the high-affinity receptor for IgE (Fc ϵ RI)¹¹⁻¹⁴ and may use this structure for antigen focusing.^{15,16} The expression of Fc ϵ RI is highly variable on LCs from normal skin, whereas the displayed expression is the strongest in lesional skin of AD.^{17,18} In contrast, an intracellular pool of the α chain of the high-affinity receptor for IgE is constantly found.¹⁹ In this study we also found a varying spontaneous surface

expression of Fc ϵ RI on in vitro-generated CD34-derived DC. Because it cannot be excluded (there was no information available about the atopic status of the donors and the further status of the children) that the ability to express Fc ϵ RI on these cells is linked to the propensity of newborn infants for the development of atopic diseases, further investigations are currently in progress to explore this possibility.

Furthermore, IL-4 has a crucial role in the induction of the low-affinity receptor for IgE on different hematopoietic cells.²⁹ Here, we found that this cytokine also induces intracellular expression of Fc ϵ RI α in most cases. But there was a certain heterogeneity with a constant expression of Fc ϵ RI α in some experiments. This heterogeneity may be either the result of alterations of the IL-4 receptor, as it has been suggested recently,³² or to other yet-to-be-defined mechanisms involved in Fc ϵ RI α gene regulation.

In contrast to cord blood-derived mast cells, IL-4 is not sufficient to induce surface expression of the Fc ϵ RI complex on CD34⁺-derived CD1a⁺ LC/DCs. Indeed, as determined by flow cytometric analysis, Fc ϵ RI γ was weakly but significantly expressed in all conditions. The lack of surface expression may be because mechanisms regulating the assembly of both receptor subunits (eg, posttranslational modifications of these molecules or yet-to-be-defined structures) that initiate the coupling of the subunits may not be operative in these cells. Furthermore, Fc ϵ RI γ is known to be part of other Fc receptors as Fc γ RIII (CD16). It could be assumed that Fc ϵ RI γ is preferentially associated with these receptors and conse-

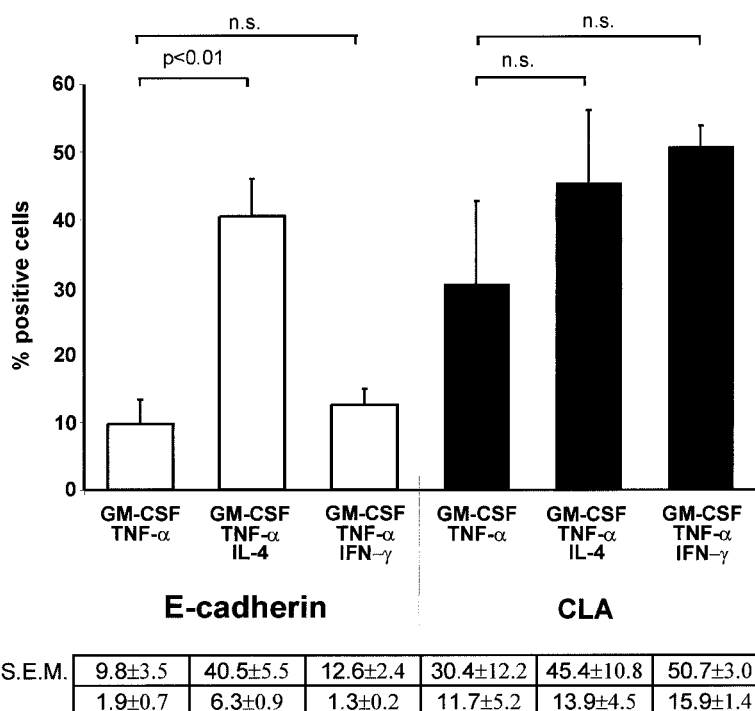


FIG 4. E-cadherin and CLA expression on CD34⁺-derived CD1a⁺ LC/DCs. After 12 to 14 days of culture cells were collected, double stained for E-cadherin/CLA and CD1a, and analyzed by flow cytometry. Note the dramatic up-regulation of E-cadherin on addition of 500 U/mL IL-4 to the culture conditions, whereas 500 U/mL IFN- γ did not significantly alter E-cadherin expression. Bars, Mean of percent of positive cells $\pm$ SEM from 6 experiments. Table below figure shows percent of positive cells and rFI $\pm$ SEM value.

quently lacking the assembly of Fc ϵ RI. Finally, besides a competitive use for other receptors, Fc ϵ RI γ expression may be that low in these CD1a⁺ DC/LCs that it acts as the limiting factor for the surface expression of Fc ϵ RI α , as we already found in freshly isolated epidermal LCs.¹⁹ In these cells the lack of surface expression of Fc ϵ RI α is linked to the absence or very low expression of Fc ϵ RI γ .

Furthermore, IL-4 up-regulated the expression of the skin-homing molecules E-cadherin and CLA, whereas IFN- γ led to low expression of E-cadherin but also to an increase of CLA. CLA, a molecule involved in migration of skin-homing T cells across the endothelium,³³ has been reported to be expressed *in vivo* on freshly isolated LCs and to be down-regulated on maturation of LCs, whereas it is not expressed on dermal DCs.³⁴ E-cadherin, known to be essential for adhesion between LCs and keratinocytes,⁷ is also more abundant on LCs compared with mature lymph node DCs.³⁵ Taken together, generation of DCs with additional IL-4 leads to DCs expressing the above skin-homing structures typical of immature DCs, like LCs, and potentially enabling them to transmigrate vessel walls and adhere to keratinocytes. Thus these cells may be of potential use in skin organ culture models.

Not only IL-4 but also IFN- γ significantly alters not only the generation of CD1a⁺ cells but also affects the profile of adhesion molecules, which have been proposed to play an important role in the immunobiologic features of DCs.^{26,34,36,37} Recently, it has been reported that the

culture system used here leads to the generation of 2 distinct populations of CD1a⁺ DCs (ie, authentic LCs and other DCs).²⁵ Therefore we suspect that IL-4 and IFN- γ modulate the emergence of each respective population (eg, IL-4 may promote the differentiation of CD1a⁺ DCs, probably from monocytic cells in this system).

Because it has been reported that IL-4 and IFN- γ share a common responsive element in the promotor region of some genes, including Fc ϵ RIIb/CD23,³⁸ our results (eg, the suppression of Fc ϵ RI α by IFN- γ) suggest that this could also be the case for the gene encoding for Fc ϵ RI α . Interestingly, IFN- γ has been shown to be effective in the treatment of AD, although not influencing the IgE serum level of the patients.³⁹ Therefore, it may be speculated that this cytokine exerts some of its therapeutic effects in down-regulating the generation of Fc ϵ RI expressing LCs or other DCs.

Taken together, our results provide evidence that IL-4 and IFN- γ play a crucial role (1) in the generation of CD34⁺ cells derived from DCs, (2) in the regulation of skin-homing structures, and (3) in Fc ϵ RI α in these cells. Thus IL-4 is not only crucial for the regulation of IgE synthesis but it may also play a key role in the induction of its receptor-binding site. However, because, in contrast to bone marrow-derived mast cells, IL-4 is not sufficient for efficient surface display of the receptor complex, studies aiming to unravel the regulation of Fc ϵ RI γ expression and other mechanisms directing the assembly

of the receptor subunits are now in progress and should contribute to a better understanding of the regulation of FcεRI expression in LCs and DCs.

REFERENCES

1. Cella M, Sallusto F, Lanzavecchia A. Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol* 1997;9:10-6.
2. Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature* 1992;360:258-61.
3. Sallusto F, Lanzavecchia A. Efficient presentation of antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J Exp Med* 1994;179:1109-18.
4. Mackensen A, Herbst B, Kohler G, Wolffvorbeck G, Rosenthal FM, Veelen H, et al. Delineation of the dendritic cell lineage by generating large numbers of Birbeck granule-positive Langerhans cells from human peripheral blood progenitor cells in vitro. *Blood* 1995;86:2699-707.
5. Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, et al. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 1994;180:83-93.
6. Strunk D, Rappersberger K, Egger C, Strobl H, Kromer E, Elbe A, et al. Generation of human dendritic cells Langerhans cells from circulating CD34(+) hematopoietic progenitor cells. *Blood* 1996;87:1292-302.
7. Tang A, Masayuki A, Granger LG, Stanley JR, Udey MC. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature* 1993;361:82-5.
8. Ross EL, Barker JN, Allen MH, Chu AC, Groves RW, MacDonald DM. Langerhans' cell expression of the selectin ligand, sialyl Lewis x. *Immunology* 1994;81:303-8.
9. Yasaka N, Furue M, Tamaki K. Expression of cutaneous lymphocyte-associated antigen defined by monoclonal antibody HECA-452 on human Langerhans cells. *J Dermatol Sci* 1996;11:19-27.
10. Birbeck M, Breathnach A, Everall J. An electron microscopy study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. *J Invest Dermatol* 1961;37:51-63.
11. Bieber T, De la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, et al. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc εRI). *J Exp Med* 1992;175:1285-90.
12. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Fodinger D, et al. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc εRI. *J Exp Med* 1992;175:1353-65.
13. Bieber T. Fc εRI on human antigen presenting cells. *Curr Opin Immunol* 1996;8:773-7.
14. Maurer D, Fiebiger E, Reininger B, Wolffwinski B, Jouvin MH, Kilgus O, et al. Expression of functional high affinity immunoglobulin E receptors (Fc εRI) on monocytes of atopic individuals. *J Exp Med* 1994;179:745-50.
15. Maurer D, Fiebiger E, Ebner C, Reininger B, Fischer GF, Wichlas S, et al. Peripheral blood dendritic cells express Fc εRI as a complex composed of Fc εRI α- and Fc εRI γ-chains and can use this receptor for IgE-mediated allergen presentation. *J Immunol* 1996;157:607-16.
16. Bieber T. Fc εRI on human Langerhans cells: an old receptor with new structure and functions? *Int Arch Allergy Appl Immunol* 1997;113:30-4.
17. Wollenberg A, Wen S-P, Bieber T. Langerhans cells phenotyping: a new tool for the differential diagnosis of eczematous skin diseases. *Lancet* 1995;346:1626-7.
18. Jurgens M, Wollenberg A, Hanau D, Delasalle H, Bieber T. Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, Fc εRI. *J Immunol* 1995;155:5184-9.
19. Kraft S, Weßendorf JHM, Hanau D, Bieber T. Regulation of the high affinity receptor for IgE on human epidermal Langerhans cells. *J Immunol* 1998;161:1000-6.
20. Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996;106:446-53.
21. Fithian E, Kung P, Goldstein G, Rubinfeld M, Fenoglio C, Edelson R. Reactivity of Langerhans cells with hybridoma antibody. *Proc Natl Acad Sci U S A* 1981;78:2541-4.
22. Riske F, Hakimi J, Mallamaci M, Griffin M, Pilson B, Tobkes N, et al. High affinity human IgE receptor (FcεRI): analysis of functional domains of the α-subunit with monoclonal antibodies. *J Biol Chem* 1991;266:11245-51.
23. Schoneich JT, Wilkinson VL, Kado-Fong H, Presky DH, Kochan JP. Association of the human Fc εRI γ subunit with novel cell surface polypeptides. *J Immunol* 1992;148:2181-5.
24. Picker LJ, Terstappen LW, Rott LS, Streeter PR, Stein H, Butcher EC. Differential expression of homing-associated adhesion molecules by T cell subsets in man. *J Immunol* 1990;145:3247-55.
25. Caux C, Vanbervliet B, Massacrier C, Dezutterdambuyant C, deSaintVis B, Jacquet C, et al. CD34(+) hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF α. *J Exp Med* 1996;184:695-706.
26. Strunk D, Egger C, Leitner G, Hanau D, Stingl G. A skin homing molecule defines the Langerhans cell progenitor in human peripheral blood. *J Exp Med* 1997;185:1131-6.
27. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc(ε)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
28. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell Fc(ε)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* 1997;185:663-72.
29. Bonnefoy JY, Platerzyberk C, Lecoanethenchoz S, Gauchat JF, Aubry JP, Graber P. A new role for CD23 in inflammation. *Immunol Today* 1996;17:418-20.
30. Terada N, Konno A, Terada Y, Fukuda S, Yamashita T, Abe T, et al. IL-4 upregulates Fc εRI α-chain messenger RNA in eosinophils. *J Allergy Clin Immunol* 1995;96:1161-9.
31. Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. Induction of the high-affinity IgE receptor (Fc εRI) on human mast cells by IL-4. *Int Immunol* 1996;8:1367-73.
32. Hershey GKK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4-receptor. *N Engl J Med* 1997;337:1720-5.
33. Santamaria-Babi LF, Moser R, Perez-Soler LJ, Blaser K, Hauser C. Migration of skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte associated antigen (CLA), the very late antigen-4 (VLA-4), and the lymphocyte function-associated antigen-1 (LFA-1). *J Immunol* 1995;154:1543-50.
34. Ebner S, Lenz A, Reider D, Fritsch P, Schuler G, Romani N. Expression of maturation-/migration-related molecules on human dendritic cells from blood and skin. *Immunobiology* 1998;198:568-87.
35. Cumberbatch M, Dearman RJ, Kimber I. Adhesion molecule expression by epidermal Langerhans cells and lymph node dendritic cells: a comparison. *Arch Dermatol Res* 1996;288:739-44.
36. Lardon F, Snoeck HW, Berneman ZN, Van Tendeloo VFI, Nijs G, Lenjou M, et al. Generation of dendritic cells from bone marrow progenitors using GM-CSF, TNF-α, and additional cytokines: antagonistic effects of IL-4 and IFN-γ and selective involvement of TNF-α receptor-1. *Immunology* 1997;91:553-9.
37. Borkowski TA, Vandyke BJ, Schwarzenberger K, McFarland VW, Farr AG, Udey MC. Expression of E-cadherin by murine dendritic cells: E-cadherin as a dendritic cell differentiation antigen characteristic of epidermal Langerhans cells and related cells. *Eur J Immunol* 1994;24:2767-74.
38. Kohler I, Alliger P, Holl-Neugebauer B, Bieber EP. The nuclear factor IL-4 (NF-IL4). *Immunobiology* 1995;193:273-80.
39. Hanifin JM, Schneider LC, Leung DY, Ellis CN, Jaffe HS, Izu AE, et al. Recombinant interferon gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189-97.