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Regulation of the High Affinity Receptor for IgE on Human Epidermal Langerhans Cells

Stefan Kraft,* Jörg H. M. Weßendorf,* Daniel Hanau,† and Thomas Bieber2‡

Human epidermal Langerhans cells (LC) express variable amounts of the high affinity receptor for IgE (FceRI); the strongest expression is characteristic of atopic dermatitis. The receptor is suggested to take part in the pathophysiology of this disease by acting as a link between aeroallergens and Ag-specific T cells in an IgE-mediated, delayed-type hypersensitivity reaction. In the present study we show that even in the absence of surface expression, normal LC maintain an intracellular pool of the α-chain of FceRI (FceRIα) of the same m.w. as the surface-bound FceRIα that is able to bind significant amounts of IgE. The lack of surface expression is linked to the absence or very low expression of the γ-chain (FceRIγ). Moreover, the amount of FceRIα expressed at the cell surface significantly correlates with the amount of FceRIγ. LC differentiation toward lymphoid dendritic cells is accompanied by the disappearance of expression for FceRIα, but not for FceRIγ. This leads to a rapid decrease in the intracellular and surface levels of FceRIα, which cannot be influenced by IL-4, IgE, or other agents. Overall, our findings suggest that these mechanisms enable LC to be highly versatile APCs by rapidly adapting the surface level of FceRI to distinct inflammatory environments. The Journal of Immunology, 1998, 161: 1000–1006.

The high affinity receptor for IgE (FceRI) belongs to the family of so-called multichain immune recognition receptors (1) that includes the TCR, the B cell receptor, and receptors for the Fc fragments of IgG (FcγRI, FcγRIII). FcεRI has been shown to be expressed on two distinct groups of cells: 1) on effector cells of anaphylaxis and allergy, i.e., on mast cells, basophils, and eosinophils (2–4); and 2) on professional APCs, including Langerhans cells (LC) (5, 6), monocytes (7), circulating dendritic cells (DC) (8), as well as inflammatory dendritic epidermal cells (EC) (9). LC serve as an outpost of the immune system in the skin (and other interface epithelia in the lung, gastrointestinal tract, and nasal mucosa), continuously informing about the invasion of pathogens or allergens (10). We previously reported that FcεRI is not constitutively expressed on human epidermal LC, unlike on effector cells of anaphylaxis, but displays a large variation of expression density (9, 11–13). Most interestingly, the strongest up-regulation of FcεRI expression is specifically observed on LC and related DC in lesional skin of patients with atopic dermatitis (AD) and correlates to the IgE serum level (9, 14). There is also evidence that LC from atopic skin use IgE for Ag presentation (15), and that FcεRI is the structure used for allergen uptake by monocytes (16) and peripheral blood DC (8). Furthermore, only LC from atopic individuals expressing high amounts of FcεRI are fully activated upon receptor ligation (12, 13). These and other findings suggest a role for this structure on LC as the crucial link between aeroallergens and Ag-specific T cells in a delayed-type hypersensitivity reaction (reviewed in Refs. 17–19). With regard to these profound functional consequences, the characterization of the mechanisms regulating FcεRI expression on human LC is of major interest.

Materials and Methods

Reagents

Anti-CD16 mAb 3G8 (IgG1) was obtained from Immunotech (Marseille, France). Phycoerythrin (PE)-labeled T6/RD1 (IgG1; Coultertronics, Krefeld, Germany) and unlabeled IOT6 mAb (IgG1; Immunotech) are directed against CD1a, which is present in the epidermis only on LC (20). The mAbs 22E7 (IgG1) and 4D8 (IgG2b; gifts from Dr. J. Kochan, Department of Autoimmune Diseases, Hoffmann-La Roche, Nutley, NJ) react with the α and γ subunits of FceRI, respectively (21, 22). RAB1 was a gift from Dr. Torbjörn Bjerke (Aarhus, Denmark) and is a polyclonal Ab specific for human FceRIα generated in rabbits by injecting purified soluble FceRIα expressed in Chinese hamster ovarian cells. Human myeloma IgE (PS) was obtained from Calbiochem (Bad Soden, Germany). FITC-labeled anti-human IgE mAb was purchased from Nordic Immunology (Tilburg, The Netherlands). FITC-labeled F(ab′)2 of goat anti-mouse Ab (GaM/FITC) was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Unlabeled and PE-labeled IgG1 were obtained from Becton Dickinson (Mountain View, CA). Oligonucleotides were synthesized by MWG Biotech (Ebersberg, Germany). Sheep anti-mouse coated magnetic beads (M-280) were obtained from Dynal (Oslo, Norway). Peroxidase-conjugated goat anti-mouse Ig Ab was obtained from Bio-Rad (Richmond, CA). Peroxidase-conjugated goat anti-rabbit Ig Ab, DNase (D-4263), digitonin (D-1407), and saponin (S-7900) were purchased from Sigma (St. Louis, MO). RPMI 1640 medium was obtained from Biochrom (Berlin, Germany). FCS, L-glutamine, and antibiotics/antimycotics were purchased from Life Technologies (Eggenstein, Germany).

Cell lines

The cell lines U937 and Jurkat E6.1 were obtained from the American Tissue Culture Collection (Rockville, MD). The endothelial cell line ECV304 was obtained from Dr. Thomas Maciag (American Red Cross,

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3. Abbreviations used in this paper: FcγRI, FcγRIII, FceRI, α-chain of high affinity receptor for immunoglobulin E; GM-CSF, granulocyte macrophage colony-stimulating factor; FcεRIγ, γ-chain of high affinity receptor for immunoglobulin E; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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Rockville, MD). RBL-48 cells were obtained by transfecting the gene coding for human FcεRIa into RBL-2H3 cells (25) and were a gift from Dr. J. Kochan (Nutmeg, NJ).

Preparation and culture of LC-enriched EC suspensions

Split-thickness skin specimens from normal appearing skin were obtained from our surgery department and from chronic, untreated (at least 3 wk) lesional skin of patients with AD. Written informed consent was obtained from all of the patients and volunteers. Crude EC suspensions were obtained as follows. The skin samples were cut into pieces of about 3 to 4 mm × 10 to 20 mm and incubated with 0.5% trypsin in PBS at 37°C for 90 to 120 min. Then the epidermal sheets were removed with a fine forceps and stirred for 10 min in washing medium (containing RPMI 1640, 10% FCS, 1% l-glutamine, and 1% antibiotics/antimycotics) with additional DNase in a final concentration of 0.01% to yield an EC suspension, which was filtered through a 100-μm nylon filter. Enrollment of LC was performed in detail previously (26). Briefly, EC suspensions were subjected to density gradient centrifugation (density = 1.077; Lymphoprep, Nye-gaard, Oslo, Norway). The interface cell layer was then collected, washed, and resuspended in washing medium, and the enrichment for LC (20–50%) was controlled by T6/RD1 immunolabeling. The entire procedure takes about 6 h, and the final cell preparation is referred to as LC-enriched EC. Spontaneously emigrating LC were prepared as described previously (25). A possible contamination of the cell samples by CD1a-negative cells expressing FcεRI, i.e., basophils, mast cells, eosinophils, or monocytes, was excluded by double immunolabeling and flow cytometry as previously described (5).

Preparation of purified LC and LC-depleted EC

Crude EC suspensions were prepared as described above. Then, EC were purified by positive selection with an anti-CD1a mAb bound to magnetic beads according to the manufacturer’s protocol. Briefly, Dynabeads M-280 precoupled with sheep anti-mouse Ab were incubated with the anti-CD1a mAb IOT6. After a washing step, crude EC suspensions were added to the beads and incubated for 1 h at 4°C. CD1a-positive cells bound to the beads were then purified by performing several washing steps with the magnet. The purity of the LC preparation was controlled after each application to the magnet by light microscopy, and the procedure was stopped when under 1% of cells were completely removed. Usually, at least 10 applications and washes were necessary to yield a highly purified LC preparation. These cells are referred to as purified LC. LC-dependent EC was performed in the same method.

Flow cytometric analysis of surface and intracellular expression of FcεRI subunits and IgE-binding studies on epidermal Langerhans cells

EC (5 × 10^6) were chilled on ice and washed several times with cold PBS supplemented with 1% FCS and 0.1% sodium azide. Double-staining experiments for the detection of surface or intracellular distribution of FcεRI subunits were performed as described in detail previously (5, 9, 26). Briefly, for determination of intracellular FcεRI expression, up to 500,000 EC were washed twice in PBS, fixed in PBS and 4% formaldehyde for 20 min, washed in PBS, incubated in PBS and 0.1 M glycine for 10 min, washed in PBS twice, and permeabilized in PBS, 0.5% saponin, 0.5% BSA, and 0.01% sodium azide (saponin buffer), and 10% goat serum for 30 min. 221E7 mAb or an IgG1 isotype control was added for 20 min, and the cells were washed twice in saponin buffer. The GaM/FITC secondary Ab 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 again were washed twice in saponin buffer. After washing twice with PBS, 0.5% BSA, and 0.01% sodium azide, T6/RD1 Ab was added for 10 min, and the cells were finally washed twice in PBS, 0.5% BSA, and 0.01% sodium azide. For intracellular expression of surface or intracellular IgE binding, the unpermeabilized or permeabilized cells were incubated with human myeloma IgE for 90 min at room temperature followed by counterstaining with anti-human IgE/FITC or an isotype control.

For investigation of FcεRI expression, a permeabilization protocol using digitonin as a detergent was necessary. All of the following steps were performed at 4°C. EC were washed twice with PBS, fixed with PBS and 0.5% formaldehyde for 20 min, and washed with PBS four times. After addition of digitonin at a final concentration of 10 μg/ml for 5 min, the cells were washed once with PBS, 0.5% BSA, 0.01% sodium azide, and 10% goat serum, and mAb 4G8 or an IgG2b isotype control was added for 30 min. Then, the cells were washed three times with PBS, 0.5% BSA, 0.01% sodium azide, and 0.05% Tween-20, followed by an incubation step with GaM/FITC Ab for 30 min. The latter washing step was repeated, and the cells were incubated with normal mouse serum for 20 min. After washing the cells twice in PBS, 0.5% BSA, and 0.01% sodium azide, and 0.05% Tween-20 and then using this buffer without Tween-20, surface labeling with the anti-CD1a mAb T6/RD1 was performed for 30 min, followed by two final washing steps with PBS, 0.5% BSA, 0.01% sodium azide, and 0.05% Tween-20. Control experiments without fixation and detergent for determination of surface-expressed structures or background fluorescence were performed with both permeabilization protocols.

For quantitative evaluation, the CD1a-positive population was gated out manually, and the percentage of FcεRI- or FcεRI-positive or IgE-binding cells was determined using LYSIS II software (Becton Dickinson). Gating on CD1a-negative cells, i.e., keratinocytes, was used as a negative control. For statistical evaluation of significances, the Mann-Whitney U test was performed with SPSS for Windows. Results are given as the mean percentage of positive cells ± SEM.

Amplification of mRNA and analysis of transcripts

Total RNA was extracted from highly purified LC, RBL-48, U937, Jurkat E6.1, or ECV304 cell lines using Trizol (Life Technologies, Eggenstein, Germany) following the manufacturer’s instructions. RT reactions were performed as previously described (5) 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. A final extension phase of 5 min was added. Specific primer sequences for each gene were as follows: human glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense, 5′-CCA CCC ATG GCA AAT TCC ATG GCA-3′; antisense, 5′-TCT AGA CGG CAG GTC GTC ACC-3′; yielding a fragment of 598 bp; rat GAPDH: sense, 5′-TGC CAC TCA GAA GAC TGT GG-3′; antisense, 5′-GCA CAG GGA GAT GCT CAG TG-3′ (fragment of 575 bp); human FcεRIα: sense, 5′-CTG TTC TCC GCT CCA GAT GGC GT-3′; antisense, 5′-TAC AGT GAT GAG GGG CTC AG-3′ (fragment size 536 bp); human FcεRIγ: sense, 5′-GCA GCA GTG GTG TTG TCT TTA C3′; antisense, 5′-GCA TGC AGG CAT ATG TGC TAC C3′ (fragment of 338 bp); and trypstatin: sense, 5′-CTC CCT CAT CCA CCC CCA GT-3′; antisense, 5′-GGA TCC AGT CCA AGT AG-3′ (fragment of 616 bp).

Amplification was performed on a Perkin-Elmer GeneAmp PCR System 9600 thermocycler (Applied Biosystems, Weiterstadt, Germany). The PCR cycle numbers for the amplification of the respective CDNAs were 30 for GAPDH and 37 for FcεRIα and FcεRIγ. Specific PCR fragments were separated on 0.8% agarose gels and visualized using ethidium bromide staining. The PCR products were evaluated semiquantitatively by comparing the ratio of the specific products vs the GAPDH band by digital image analysis using the WinCam system (Cybertech, Berlin, Germany).

Biochemical analysis of FcεRI

LC-enriched EC were prepared as described above. After lysis in the presence of protease inhibitors, the proteins (14 μg/lane) were fractionated by electrophoresis on 10% SDS-PAGE for FcεRIα and 18% for FcεRIγ and electro transferred to nitrocellulose membranes. Lysates from RBL-48 cells, normal human PBMC, ECV304, and LC-depleted EC were used as controls. After blocking, proteins were identified using the Abs RAB1 for FcεRIα and 4D8 for FcεRIγ (both at a final dilution of 1/1000). The bands were visualized with a peroxidase-conjugated goat anti-mouse or goat anti-rabbit Ig Ab followed by an enhanced chemiluminescence Western blot detection system (ECL, Amersham, Arlington Heights, IL) according to the manufacturer’s protocol.

Results

Evidence for the maintenance of an intracellular pool of FcεRIα in freshly isolated LC lacking surface receptor expression

We and others have previously reported that FcεRI surface expression on freshly isolated LC is highly variable (6, 9, 12). Indeed, the highest FcεRI surface expression was found in inflammatory skin diseases, specifically in lesional skin of AD, whereas in 10% of the examined samples of normal skin (n = 60) LC failed to express any detectable receptor moieties (referred to as FcεRIwaLC) even when using several reagents reacting with distinct epitopes of FcεRIα (not shown). Since we could not exclude that LC express at least one of the receptor components, we questioned whether
Cytoplasmic expression | Surface expression

Cell number

Cell number

FceRI expression

FIGURE 1. Surface and intracellular expression of FceRIα in LC. Freshly isolated EC containing FceRI\textsuperscript{pos} or FceRI\textsuperscript{neg} LC (upper four histograms) were obtained from normal skin (NS) or lesional skin of atopic dermatitis (AD), respectively, as described in Materials and Methods, and then subjected to double labeling with anti-CD1a/PE and either surface staining (right panels) or intracellular staining (left panels) after permeabilization with saponin, using the anti-FcεRIα mAb 22E7. LC were gated on their CD1a expression, and the stainings were shown as overlays with the respective isotype control. Keratinocytes (lower two histograms) were used as negative controls.

FceRI\textsuperscript{neg} LC may contain intracellular receptor moieties. Therefore, as shown in Figure 1, surface (light panels) and intracellular (left panels) anti-FceRIα stainings were performed with FceRI\textsuperscript{neg} (upper panels) and FceRI\textsuperscript{pos} (middle panels) LC obtained from normal and atopic skin, respectively. While permeabilization with low (10 μg/ml) concentrations of digitonin failed to reveal any staining in FceRI\textsuperscript{neg} cells, permeabilization with high (5 mg/ml) concentrations of saponin disclosed a strong intracellular anti-FceRIα reactivity (84 ± 5%; n = 11) regardless of the surface expression (17 ± 10%). Unspecific labeling has been ruled out by analyzing FceRI\textsuperscript{neg} EC cells, i.e., keratinocytes (Fig. 1, lower panels). This strong intracellular signal was not due to the unmasking of hidden epitopes by saponin, since similar results were obtained with several reagents (21) reacting with distinct epitopes of FceRIα (data not shown). When using FceRI\textsuperscript{pos} LC obtained from biopsies of lesional skin of AD (n = 4) containing LC with high FceRIα surface expression (93 ± 8%), no significant variation in the intracellular staining could be noted (83 ± 9%). Thus, LC constitutively express an intracellular pool of FceRIα-moieties regardless of FceRI surface levels.

Characterization of intracellular FceRIα

In previous reports, amplification of total RNA from FceRI\textsuperscript{neg} LC using primers spanning the transmembrane domain of FceRIα yielded a single PCR product (5, 6), excluding the possibility of a splicing variant lacking the transmembrane domain.

Since intracellular sequestration of FceRIα could be due to variations in N-linked glycosylation of the N-terminal portion of FceRI, which seems to be critical to the folding machinery in the endoplasmic reticulum and subsequently to the surface expression (27), we addressed this possibility by performing immunoblot analyses of lysates from FceRI\textsuperscript{neg} LC and FceRI\textsuperscript{pos} LC. However, as shown in Figure 2A, under both conditions (lanes 3 and 4), a reactive band of the expected molecular mass of 45 to 66 kDa, but not in the range of 24 kDa, which would have been compatible with the unglycosylated protein backbone, was detectable. The band at 30 kDa was identified as galectin-3 by immunoblotting using a recombinant protein produced in our laboratory (data not shown). Lysates from RBL-48 cells transfected with the gene of the human FceRIα revealed the same reactivity (lane 1).

We then questioned whether intracellular FceRIα was able to bind IgE. Using intracellular staining in flow cytometry, we were able to detect a significant (Fig. 2B), but weaker than expected, intracellular binding of IgE in permeabilized FceRI\textsuperscript{pos} LC compared with the amount of intracellular FceRIα. Whether this is due to steric hindrance by yet to be defined associated structures remains to be determined.

Thus, from this series of experiments we conclude that LC express an intracellular form of FceRIα that is able to bind IgE.

The presence of FceRIγ correlates with the surface expression of the intracellularly preformed FceRIα on LC

It has been suggested that FceRIγ is ubiquitously expressed on various types of hemopoietic cells as part of multimeric surface receptors (28). Furthermore, mouse mast cells lacking FceRIγ do not express surface FceRIα (29). Finally, it has been reported that in the human system, the presence of FceRIγ is necessary and
A. PCR analysis of the transcripts for human FcεRIα and related structures and the function of the receptor complexes (30–32). Therefore, we investigated the presence of FcεRIγ in LC with different levels of surface receptor expression.

In the first approach, we performed semiquantitative analysis of PCR products for human FcεRIγ (Fig. 3A). The presence of the transcripts correlated to the receptor surface expression (n = 5; r = 0.91; p < 0.03). To verify whether FcεRIγ transcripts also lead to protein synthesis, LC were analyzed for their FcεRIα surface expression and were permeabilized by a low digitonin concentration, which is known to leave multimeric surface structures such as the TCR undamaged (26), for the detection of FcεRIγ protein levels. Permeabilization with high concentrations of saponin did not lead to staining of FcεRIγ regardless of FcεRI surface levels, suggesting a solubilization of surface-expressed multimeric structures such as FcεRI. While no or very low amounts of FcεRIγ were detected in FcεRIα pos LC, which have been demonstrated to contain a high level of intracellular FcεRIα protein (Figs. 1 and 2A) despite the absence of receptor surface expression, clear-cut reactivity was observed in LC expressing surface receptor, and most importantly, there was a significant correlation between the expression of surface FcεRIα and FcεRIγ at the protein level (n = 8; r = 0.89; p < 0.005; Fig. 3, B and C). CD16, which could associate with FcεRIγ, has never been detected on these cells (data not shown). In addition, another structure that could be associated with FcεRIα, the ζ-chain of the TCR, was not found to be expressed in LC after performing RT-PCR, intracellular staining in flow cytometry, and immunoblotting (data not shown).

Finally, to verify the specificity of our PCR and flow cytometric analyses, immunoblots were performed with lysates from FcεRIα pos and FcεRIα neg LC using the anti-FcεRIα mAb 4D8. As shown in Figure 3D, a positive band at 9 kDa was found in FcεRIα pos LC (lane 3), but not in FcεRIα neg LC (lane 4). This excludes the possibility that surface FcεRIα is masked on FcεRIα neg LC and unmasked by the permeabilization technique herein. Thus, our experiments strongly indicate that the presence of FcεRIγ correlates with the surface expression of the intracellular pool of preformed FcεRIα on LC.

Lack of detectable transcripts for FcεRIα, but not for FcεRIγ, upon in vitro maturation of LC

It has been demonstrated that resident or freshly isolated LC are immature DC that, after Ag capture, undergo profound phenotypical and functional alterations during their migration to the regional lymph nodes (33). This maturation into potent stimulatory cells is reproduced in vitro by short term culture and supported by GM-CSF (34). Therefore, we asked whether this maturation is accompanied by changes in the expression of FcεRI and its subunits.

Freshly isolated LC (n = 6) were cultured in the presence of GM-CSF for 36 h, and their FcεRI expression was analyzed. We observed a dramatic decrease in the surface expression of FcεRIα on LC leading to nearly negative cells by 36 h (0 h, 27 ± 15%; 36 h, 2 ± 7%; p < 0.05). This down-regulation was also detected on spontaneously emigrating LC (data not shown) prepared without a trypsinization procedure. Similarly, the intracellular pool of

FIGURE 3. FcεRIγ modulates the surface expression of FcεRIα in LC. A. PCR analysis of the transcripts for human FcεRIα, FcεRIγ, tryptase, and GAPDH in LC with various surface expressions, RBL-48 cells (RBL cells transfected with human FcεRIα, which associates with rat FcεRIγ), T cells, Jurkat cells, and U937 cells as controls. B. Correlation between the surface expression of FcεRIα and the presence of FcεRIγ on LC determined by flow cytometry. The percentage of positive cells for FcεRIγ was plotted for LC with various FcεRIα surface expressions. C. LC expressing high (upper histograms) and low (lower histograms) amounts of surface FcεRIα were tested for the presence of FcεRIγ. EC were subjected to double labeling with anti-CD1a/PE and surface staining using the anti-FcεRIα mAb 22E7 (right panels) or to intracellular staining using the anti-FcεRIγ mAb 4D8 after permeabilization (left panels). LC were gated on their CD1a expression, and the stainings were shown as overlays with the respective isotype control. D. Western blot of FcεRIγ from FcεRIα pos and FcεRIα neg LC. FcεRIα pos LC (lane 4) and FcεRIα neg LC (lane 3) were isolated from normal skin and subjected to immunoblot as described in Figure 2. Normal human PBMC (lane 1) were used as a positive control. ECV304 (lane 2) and LC-depleted EC (lane 5) were used as negative controls.
FceRIα and transcripts rapidly disappeared after a short culture, suggesting that differentiating LC dramatically down-regulate their FceRIα protein synthesis (0 h, 85 ± 5%; 36 h, 3 ± 7%; p < 0.0001; Fig. 4A) and probably gene transcription (Fig. 4B). In contrast, FceRIγ (Fig. 4A) and its transcripts (Fig. 4B) were still found in cultured LC, indicating a possible additional role of a shedding process in the down-regulation of surface FceRIα.

A second point of interest was the search for agents regulating surface levels of FceRI during short term culture. Recent reports showed a major regulatory role of the IgE level on the surface level of FceRI of monocytes in vitro (35). An amplifying effect of the IgE concentration on FceRI expression has also been demonstrated for basophils and mast cells (36–38). However, in our experiments the presence of human myeloma IgE (10 μg/ml) failed to sustain or enhance the receptor expression (data not shown). IL-4 has recently been reported to induce FceRI on human mast cells (39), but several attempts to overcome this down-regulation by culturing LC in the presence of the cytokines IL-4, IFN-γ, TNF-α, and TGF-β remained unsuccessful. Thus, FceRI expression on LC is probably restricted to their differentiation stage of immature DC, i.e., to their epidermal localization, and is rapidly down-regulated upon maturation into lymphoid DC.

Discussion

We and others have previously reported on the great variation of FceRI surface expression on human APCs, including LC, monocytes, and inflammatory dendritic EC (5–7, 9). Since this phenomenon seems to be important for the receptor function (12), understanding the mechanisms underlying the regulation of the FceRI surface expression in vivo is of crucial importance. In the present study we provide the first insight into the mechanisms involved in the regulation of the surface expression of human FceRI in freshly isolated LC.

Analysis of PCR products and biochemical characterization of the preformed intracellular FceRIα moieties indicate that the presence of transmembrane-deleted splice variants is unlikely and disclose a mature form of this FceRI subunit. Thus, based on these observations, we speculate that these preformed FceRIα-chains are most likely stored in the endoplasmic reticulum. This is further supported by the fact that permeabilization of LC with 5 mg/ml saponin, which is known to permeabilize not only the plasma membrane but also intracellular organelles such as rough endoplasmic reticulum when used at high concentrations (40), showed strong reactivity for FceRIα. In contrast, permeabilization with 10 μg/ml digitonin, which at low concentrations has been reported to selectively permeabilize the cholesterol-rich plasma membrane while leaving the membranes of intracellular organelles, which are poor in cholesterol (41), intact (42–45), did not lead to staining of intracellular FceRIα (for a summary of the properties of digitonin and saponin, see Ref. 46). It is unlikely that this massive amount of α-chains is due to cryptic receptors hidden in the plasma membrane approached only by specific procedures (e.g., the effect of saponin or cell lysis for subsequent immunoblotting), because in the investigated FceRIαC′′′ LC, the FceRI γ-chains were absent or expressed in very low amounts. To our current knowledge, there are no reports of surface-expressed FceRI containing only an α-chain but no β- or γ-chains.

FcyRIIα has been reported to contain a sequence in its transmembrane domain that promotes its rapid degradation in the endoplasmic reticulum when not associated with FceRIγ (47). The transmembrane domain of FcyRIIα shows 62% sequence homology to that of FceRIα and contains a sequence of eight conserved amino acids near the cytoplasmic domain of FceRIα (48, 49) with an aspartic acid residue whose mutation reduced association of FcyRIIα with FceRIα as well as its susceptibility to degradation (50). In the same report, similar regulatory mechanisms for FceRIα have been proposed. However, to our knowledge, its intracellular degradation by such a mechanism has not been documented to date. Moreover, the exact localization of this sequence within the transmembrane domain of FcyRIIα has not been determined exactly. In TCR regulation (51) only a short sequence of nine amino acids within the transmembrane domain promotes rapid degradation of TCR-α in the absence of CD3-δ. In the case of FcyRIIα, there is no evidence that such a sequence lies within the sequence conserved between FcyRIIIα and FceRIα. It also has been reported that Tac-I/IL2-Rα, which shows relative sequence homology to TCR-α even within the short sequence responsible for degradation (five of nine amino acids), is not degraded but is transported to the cell surface (51). This indicates that relative sequence homology may not lead to same consequences in terms of degradation, e.g., since protein conformation, as an additional determinant of protein degradation, could be different. Other events that could prevent FceRIα from degradation in LC may be envisaged, e.g., post-translational modifications such as glycosylation, which could confer some protective properties preventing intracellular proteolysis of FceRIα. In addition, structures other than FceRIγ that have not yet been detected may associate with FceRIα, thus inhibiting its degradation.

Based on the current knowledge, several not mutually exclusive possibilities may explain the lack of surface expression of FceRIα.
First, FcεRIα is associated with a yet to be defined structure(s), as mentioned above, that precludes its coupling to FcεRIγ. Second, the coupling mechanisms leading to the active association of FcεRIα and FcεRIγ are deficient or themselves are subject to distinct regulatory signals in LC. Third, and most likely in the case of normal resident epidermal LC, FcεRIγ, the partner of FcεRIα, is lacking or present only in low concentrations, thereby precluding its surface expression under nonpathologic situations. This is suggested by our data showing that LC negative for surface FcεRI exhibit high amounts of intracellular FcεRIα, as demonstrated by intracellular staining in flow cytometry and Western blotting, but no or very low amounts of FcεRIγ at the protein as well as the mRNA levels. Since human LC express neither FcγRIII or FcγRIIα and FcγRIIID, a competition between FcεRIα and FcγRIIID for the γ-chain, as recently reported in mast cells (52), seems unlikely. Most importantly, our observations are in line with transfection experiments that have revealed that unlike in the rodent system, FcεRIγ, but not FcεRIβ, is mandatory for the surface expression of the human IgE-binding FcεRIα (53, 54).

Besides the fact that the genes coding for FcεRIα and FcεRIγ are regulated differently, our observations also strongly imply that in contrast to cells of the monocyte/macrophage lineage, FcεRIγ is not constitutively expressed in resident epidermal LC. Instead, based on our previous findings of a receptor up-regulation in various inflammatory skin conditions (9, 12, 13), this subunit seems to be induced by distinct signals provided by an inflammatory microenvironment in the skin. However, it is important to note that, on the one hand, the highest FcεRIα surface expression, i.e., the highest FcεRIγ expression, is characteristic of atopic dermatitis (14), and, on the other hand, this high receptor expression strongly correlates with the IgE serum level (9). The fact that various cytokines thought to be involved in the pathophysiology of inflammatory skin diseases such as IL-4, IFN-γ, or TNF-α had no effect on FcεRI surface levels implicates a complex model of FcεRIγ regulation, perhaps, besides a complex combination of cytokines, involving intercellular contacts mediated through adhesion molecules. Clearly, more information on the regulation of the gene encoding for FcεRIγ is needed for a better understanding of the regulation of FcεRI expression in LC.

Finally, based on the observation that one of the main functions of FcεRI on monocytes, LC, and DC may be to allow IgE-mediated Ag capture (8, 15, 16), our finding of the loss of surface and intracellular expression of FcεRIα on LC during their maturation into lymphoid DC strongly suggests that the receptor expression and function on LC are confined to their physiologic stage of resident DC. Interestingly, this loss is the result of 1) the disappearance of FcεRIα transcripts, as witnessed in our PCR experiments, possibly combined with 2) a rapid degradation/shedding of the surface moieties, since the down-regulation of FcεRIγ expression is much less pronounced. The concept that these regulatory principles for FcεRI observed are restricted to immature DC, e.g., freshly isolated LC, is further substantiated by the absence of surface expression of FcεRIα when culturing LC in the presence of IL-4, which has been reported to induce FcεRIα gene transcription in eosinophils (55), in vitro generated CD1a+ DC (R. Magerstaedt, J.W., E. Geiger, S.K., D.H., and T.B., manuscript in preparation), and in bone marrow-derived mast cells (39). The IgE level, which is another factor known to up-regulate the expression of FcεRI on monocytes, basophils, and mast cells (35–38), also shows no effect on FcεRI on LC during maturation. Binding of IgE to surface FcεRI is thought to stabilize the receptor complexes, inhibiting receptor internalization and thereby leading to an accumulation of newly synthesized FcεRI on the cell surface. From that point of view it is obvious that in cultured LC, incubation with IgE shows no effect, since FcεRIα synthesis is rapidly down-regulated. This supports the concept of a differential regulation of FcεRI on LC.

Taken together, we provide evidence for the first time that LC use a unique and to date unreported mechanism involving a constitutive pool of preformed intracellular FcεRIα. The surface expression of FcεRIα from this pool may be regulated by the variable presence of FcεRIγ. This mechanism would allow the cells to rapidly and finely tune their FcεRI expression and confers upon them a high versatility in a tissue constantly exposed to rapid changes in allergic and environmental challenges. Whether these mechanisms are restricted to APC or whether FcεRI expression in effector cells of anaphylaxis, i.e., mast cells and basophils, is subjected to similar mechanisms is not yet known.

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References

REGULATION OF FcεRI ON LANGERHANS CELLS


