Human Epidermal Langerhans Cells Express the High Affinity Receptor for Immunoglobulin E (FcεRI)

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Summary

It has been suggested that epidermal Langerhans cells (LC) bearing immunoglobulin E (IgE) may be involved in the genesis of atopic disease. The identity of the IgE receptor(s) on LC remained unclear, although it represents a crucial point in understanding cellular events linked to the binding of allergens to LC via IgE. In this report, we demonstrate that epidermal LC express the high affinity receptor for the Fc fragment of IgE (FcεRI) which has, so far, only been described on mast cells and basophils. Epidermal LC react with antibodies specific for the α subunit of the tetrameric (α,β,2γ) FcεRI. Specific transcripts for FcεRIα and FcεRIγ were detected in LC and correspond to those of human basophils and of the human basophil cell line KU812. Furthermore, human basophils, KU812 cells, and LC express the putative β subunit. Thus human LC express the complete structure of FcεRI. This finding opens new perspectives in the putative functional role of this structure on antigen-presenting cells.

The demonstration of IgE molecules on epidermal Langerhans cells (LC)[1] in patients with atopic dermatitis has implied that these cells should perform a major function in the pathophysiology of atopic disease (1, 2). Although initially, only receptors for the Fc fragment of IgG were identified on epidermal LC (3, 4), the low affinity receptor for IgE FcεRII/CD23 (5) and the human IgE binding protein (εBP) (6) have now also been found on these cells in lesional, as well as in normal skin. However, attempts to completely block IgE binding on LC by a variety of anti-FcεRII/CD23, anti-εBP, and/or anti-FcγR reagents remained unsuccessful, suggesting the presence of a third IgE-binding structure actually responsible for a part of the IgE-binding capacity of LC. We report here, that normal human LC also express the high affinity receptor for IgE, FcεRI, demonstrating that the presence of this structure is not restricted to mast cells and basophils. Our results also document the presence of the putative β chain on both human basophils and LC.

[1] *Abbreviations used in this paper:* BP, binding protein; ECL, enhanced chemiluminescence; LC, Langerhans cells.

Materials and Methods

Individuals and Biopsy Specimens. Fresh human skin specimens were obtained during plastic abdominal surgery or reduction mammoplasty. 6-mm punch biopsies were taken from the buttocks of healthy volunteers. All samples were immediately processed.

Basophils and Basophilic Cell Line. Human basophils were obtained by standard isolation technique from the peripheral blood of healthy volunteers (7). The human basophilic KU812 cell line was a kind gift from Dr. Becker (Forschungsinstitut, Borstel, Germany). These cells, which constitutively express FcεRI (8), were cultured in RPMI 1640 (Gibco, Berlin, Germany) supplemented with 5% FCS (Gibco), antibiotics, and antimycotics (Gibco).

Reagents. Unlabeled mAb IOT6 (IgG1; Immunotech, Marseille, France), and PE-labeled T6/RD1 (IgG1, Coulter Corp., Krefeld, Germany) react with CD1a which is, in the skin, exclusively expressed on LC (9). Murine mAb 29C6 and 6F7 (both IgG1) were raised from mice immunized with a purified chimeric α subunit of FcεRI (FcεRIα) expressed in Chinese hamster ovarian cells (10). FITC-labeled goat anti-mouse was obtained from Jackson ImmunoResearch Labs, Inc. (West Grove, PA) and unlabeled IgG1 and PE-labeled IgG1 from Becton Dickinson & Co. (Mountain View, CA). For immunohistologic purposes, we also used rabbit anti-mouse Ig antibody and alkaline-phosphatase mouse anti-alkaline-phosphatase complexes (Dakopatts, Hamburg, Ger-

many). A peroxidase-conjugated goat anti-mouse Ig antibody (Bio-Rad Laboratories, Richmond, CA) was used for immunoblot analysis. Oligonucleotides were synthesized by Appligène (Illkirch, France).

In Situ Immunolabeling on Cryosections. 6-μm cryosections were prepared from the punch biopsies, air-dried, fixed for 10 min in pure acetone, and then processed for immunohistochemistry using the mAb IOT6a, 29C6, 6F7, or the IgG1 isotype control (all at 10 μg/ml) and the alkaline-phosphatase mouse anti-alkaline-phosphatase technique as described previously in detail (2).

Preparation of Epidermal Cell Suspensions and Enrichment of LC. Isolation and enrichment of LC from fresh human skin specimens was performed as described in detail elsewhere (5).

Flow Cytometric Analysis of FcεRI Expression on Epidermal Cells. 10^5 LC-enriched EC were incubated for 30 min with heat-inactivated AB serum. After several washes in PBS supplemented with 1% FCS and 0.1% sodium azide, doublestaining experiments were performed by first incubating (1 h, 4°C) the cells with either 29C6 or 6F7 (both at 10 μg/ml). After washing twice in 1% FCS-supplemented PBS, the cells were incubated (1 h, 4°C) with FITC-labeled goat anti-mouse antibody. Then, after washing twice, cells were incubated (30 min, 4°C) with normal mouse serum (final dilution 1:10) to saturate the second step antibody. The cells were washed twice and incubated (30 min, 4°C) with the PE-labeled mAb T6/RD1 (1 μg/ml). Isotype controls were performed with unlabeled IgG1 and PE-labeled mouse IgG1. The cells were then washed twice in 1% FCS-supplemented PBS at 4°C and were analyzed by flow cytometry using a FACScan® (Becton Dickinson & Co.). Fluorescence parameters were collected using a built-in logarithmic amplifier, and the data of 10,000 cells obtained with Consort 30 software were analyzed with the Lysis-I program (Becton Dickinson & Co.).

Immunomagnetic Depletion/Purification of CD1a⁺ Cells. The separation of CD1a⁺ LC for the preparation of LC-depleted epidermal cells (EC), or for purification of LC was achieved according to the manufacturer's protocol. The purity of the LC preparation was controlled after each application on the magnet by light microscopy, and the procedure was stopped when unbound cells, i.e., keratinocytes and other cells, were completely removed. Addition of the beads and depletion procedure were repeated three to four times. The LC depletion of EC was controlled by anti-CD1a staining and flow cytometric analysis.

Immunobiochemical Analysis of FcεRI on Epidermal Cells. Purified LC or LC-depleted EC were prepared by positive selection as described above. The cells were washed, lysed in NP-40 (Sigma Chemical Co.)-containing buffer and 14 μg of protein were separated by 12% SDS-PAGE. Then, separated proteins from purified LC or from LC-depleted EC were electroblotted onto nitrocellulose (Hybond C; Amersham Corp., Arlington Heights, IL). The strips were blocked with dry milk, and incubated for 1 h with either the anti-FcεRIα 29C6 or the isotype control (both at 10 μg/ml). Binding of the primary antibody was revealed by incubation with a peroxidase-conjugated goat anti-mouse Ig antibody followed by an enhanced chemiluminescence (ECL) Western blot detection system, according to the manufacturer's protocol (Amersham Corp.).

Amplification of mRNA Transcripts. Total RNA was isolated from purified LC, LC-depleted EC, basophils, and KU812 cells by standard procedure (11), and cDNA was synthesized by extension of 3 pmol of reverse primers specific for tryptase, FcεRIα, or FcεRIγ on 100 ng of total RNA by Moloney murine leukemia virus (MoMLV) reverse transcriptase (Gibco). The second-strand synthesis and 30 cycles of amplification were performed directly after the reverse transcription step by adding 10 pmol of direct primers, 10 pmol of the reverse primers, 1.25 mM of each dNTP, and 1 U of Taq DNA Polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT). For FcεRIβ, cDNA was synthesized by extension of 10 pmol of reverse primer on 100 ng of total LC-RNA, or 1 μg of total RNA from basophils. The second-strand synthesis and 35 cycles of amplification were performed by adding 20 pmol of direct primer and 50 pmol of the reverse primer, 1.25 mM of each dNTP, and 1 U of Taq polymerase. Temperatures were 94°C for denaturation, 55°C for annealing of FcεRIα and FcεRIγ, 58°C for annealing of FcεRIβ, and 72°C for polymerizations. Sequences of PCR primers were as follows: for tryptase based on (12): reverse primer 5′-GGATCCAGTCCAAGTAGTAG-3′, and direct primer 5′-CTCCCTCATCCACCCCCAGT-3′; for FcεRIα based on (13): reverse primer 5′-CTTAGGATGTGGGTTCAGAAGT-3′, and direct primer 5′-GACAGTGGAGAATACAAATGTCA-3′; for FcεRIβ based on (14): reverse primer 5′-TAATTCTTCATAAAGACGAT-CATC(A,T,G,orC)GG-3′ and direct primer 5′-ATATGCCTTTGTT-TTGGAACAATIGTITG-3′; for FcεRIγ based on (15): reverse primer 5′-TAGGGCCAGCTGGTGTTAATGGCA-3′, and direct primer 5′-GATGATTCCAGCAGTGGTCTTGCT-3′. PCR products were then electrophoresed on a 2% agarose gel.

Cloning and Sequencing of PCR Amplified Products. After RT-PCR, the fragments were digested with 10 U S1 nuclease (Appligène) for 10 min, at room temperature to increase the efficiency of cloning. Then, they were treated with 5 U Kleenow DNA polymerase (Appligène), and the resulting DNA products were cloned in the EcoRV restriction site of pKS⁺ (Stratagene Inc., La Jolla, CA). Three independent clones were sequenced using Sequenase 2.0 sequencing kit (US Biochemical, Cleveland, OH) and Hydrolink sequencing gel matrix (AT Biochem, Malvern, PA). RT-PCR artifacts were excluded by deduction of the nucleotide sequence from at least two identical results.

Southern Blot Analysis. Alkaline blotting and Southern hybridizations were performed on Hybond N⁺™ membranes (Amersham Corp.) according to the manufacturer's conditions. Oligonucleotide probes were labeled with T4 polynucleotide kinase and γ-[^{32}P]ATP, and then hybridized to the blot at 50°C. Sequences of the probes for Southern blot are 5′-GAGAGTGAACCTGTGTAC-3′, 5′-AGAATGGTGAGAAACAGC-3′, 5′-AATTGTCCTCACCC-TCC-3′, and 5′-CCTCCCACCGCCATTTC-3′, specific for FcεRα, FcεRβ, FcεRγ and tryptase respectively.

Results and Discussion

Immunodetection of FcεRI on Epidermal LC. FcεRI is a tetrameric structure composed of one 50–55-kD α chain (FcεRIα), one 33-kD β chain (FcεRIβ), and two disulphide-linked identical 7–9-kD γ chains (FcεRIγ) (16). Recently, a series of anti-FcεRIα mAbs were raised from mice immunized with a purified chimeric FcεRIα expressed in Chinese hamster ovarian cells (10). Using two of these reagents, the anti-FcεRIα antibodies 6F7 and 29C6, we investigated the expression of FcεRIα on cryosections from normal human skin by immunohistochemistry. Positive staining was found not only on dermal mast cells but also on some dermal dendritic cells and, most important, on dendritic cells in a suprabasal localization in the epidermis (Fig. 1 *A*) where mast cells have never been observed. Preliminary experiments on cryosections revealed that, as expected from the characterization of these antibodies (10), 6F7 blocks the IgE binding on epidermal dendritic cells, as well as on dermal mast cells, while 29C6 is ineffective.

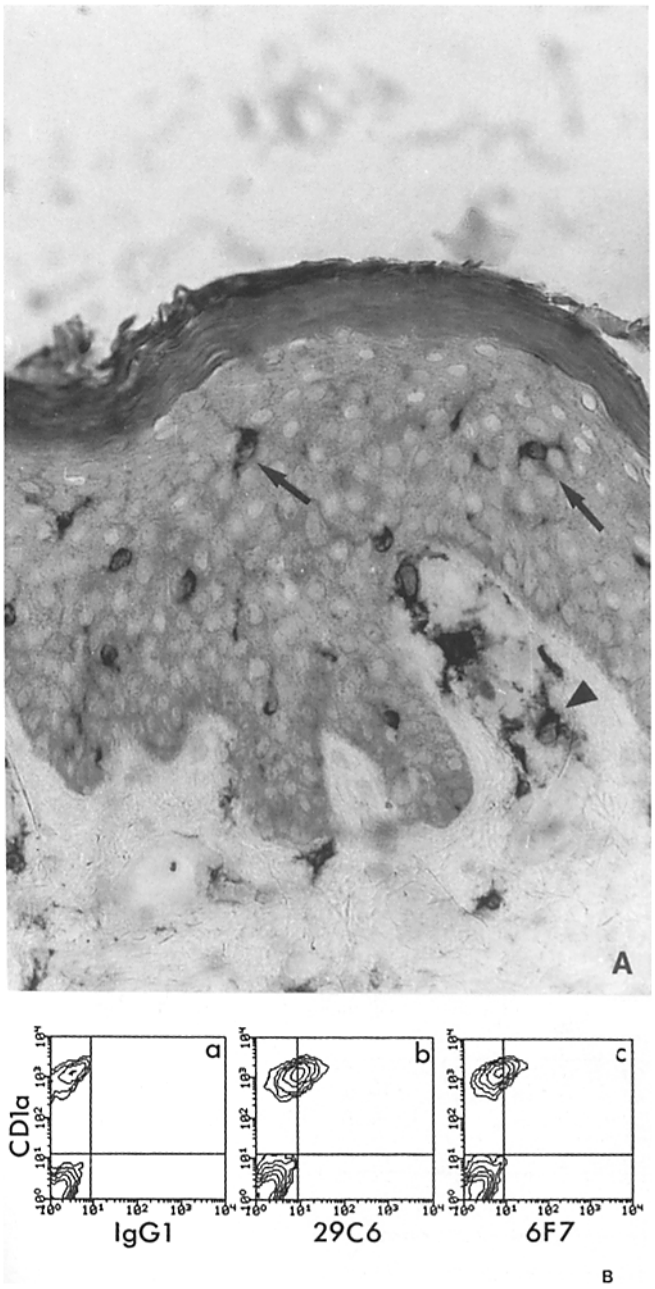

Figure 1. Immunocytochemical staining of FcεRIα on human LC. (*A*) Anti-FcεRIα antibody stains resident human LC. Positive staining is observed on epidermal dendritic cells (←). Positive control is obtained by the reactivity of dermal mast cells (◀) on the very same sections. (*B*) Flow cytometric analysis of the surface expression of the α chain of FcεRI on freshly isolated human LC. Double labeling was performed with the PE-labeled anti-CD1a mAb T6/RD1 and the anti-FcεRIα antibodies 29C6 (*b*) or 6F7 (*c*). Irrelevant mouse IgG1 was used for control purposes (*a*).

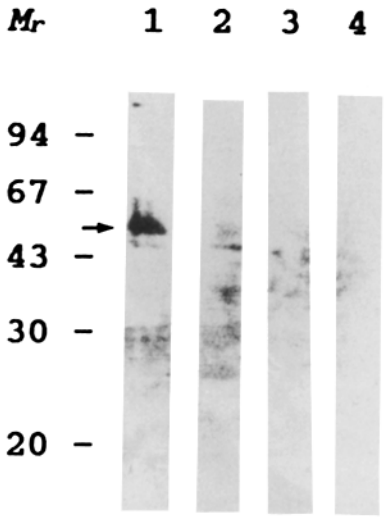

Figure 2. Immunoblot analysis of FcεRIα on human LC. LC depleted EC and purified LC were prepared by the means of immunomagnetic separation. Cell lysates from purified LC (lanes *1* and *3*) and LC-depleted EC (lanes *2* and *4*) were separated by 12% SDS-PAGE, electroblotted onto nitrocellulose, and incubated with either the anti-FcεRIα mAb 29C6 (lanes *1* and *2*), or the isotype control (lanes *3* and *4*).

To ascertain that FcεRI-reactive epidermal dendritic cells were LC, double-labeling experiments and flow cytometric analysis were performed on freshly isolated LC-enriched EC using anti-FcεRIα and anti-CD1a reagents. As expected, anti-FcεRIα antibodies only reacted with CD1a-expressing EC, i.e., with LC (Fig. 1 *B*, panels *b* and *c*), thus excluding a contamination of isolated EC with other FcεRIα-expressing cells, e.g., mast cells.

Biochemical characterization of the anti-FcεRIα reactive structure by immunoblot analysis on lysates from purified LC revealed a structure of an apparent molecular mass of 50 kD (Fig. 2, lane *1*), which is consistent with the α subunit of FcεRI (16). The fact that lysates from LC-depleted EC failed to react with anti-FcεRIα antibodies (Fig. 2, lane *2*) strongly indicates that, in the epidermal compartment, FcεRIα expression is restricted to LC.

Identification of the FcεRI α and γ Subunits mRNA in LC. A molecular genetic approach based on RT-PCR with specific primers for human FcεRIα and FCεRIγ was chosen to further characterize the structure of FcεRI on human LC. Agarose gel electrophoresis analysis of the amplification products obtained from purified LC using the primers for FcεRI subunits revealed the predicted 495- and 364-bp fragments for FcεRIα and FcεRIγ, respectively (Fig. 3 *b*, lanes *1* and *3*). These fragments correlated exactly with the results obtained with peripheral blood basophils (Fig. 3 *b*, lanes *5* and 7) and KU812 cells (not shown) which were used as a positive control. The specificity of the fragments was confirmed by restriction enzyme analysis of the RT-PCR products of purified LC and basophils (with HindII, PvuII, and SalI for the γ chain, and with StuI, PvuII for the α chain, data not shown), and by Southern analysis with oligonucleotidic probes specific for FcεRIα (Fig. 3 *c*, lanes *1* and *5*) and FcεRIγ (Fig. 3 *c*, lanes *3* and 7). RT-PCR experiments using the primers for FcεRIα and FcεRIγ on RNA from LC-depleted EC remained negative, while weakly positive in crude EC RNA (data not shown). It should be mentioned that the possibility of a contamination by dermal mast cells or basophils was formally excluded in either condition, since RT-PCR experiments using

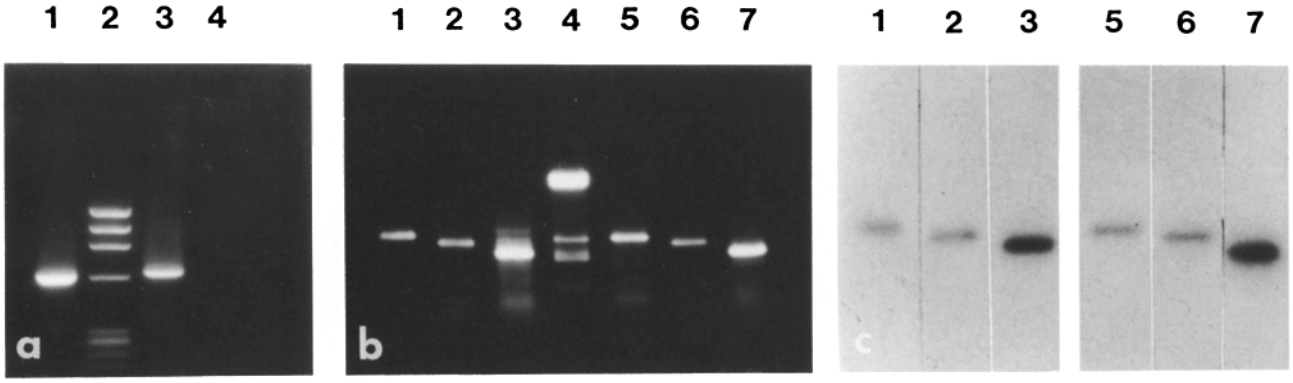

Figure 3. Analysis of RT-PCR–amplified transcripts of tryptase (*a*) and of the α, β, and γ subunits of FcεRI (*b* and *c*) from human purified LC (*a*, lane *4*; *b* and *c*, lanes *1–3*), from human basophils (*a*, lane *1*; *b* and *c*, lanes *5–7*), and the cell line KU812 (*a*, lane *3*). Purified LC were prepared by immunomagnetic separation, and basophils were obtained by gradient density centrifugation of peripheral blood. (*a* and *b*) Ethidium bromide staining of the electrophoretic gels. (*c*) Autoradiogram of the Southern blot hybridized with oligonucleotides specific for the three subunits of FcεRI. FcεRIα: *b* and *c*, lanes *1* and *5*. FcεRIβ: *b* and *c*, lanes *2* and *6*. FcεRIγ: *b* and *c*, lanes *3* and *7*. Sizes of HaeIII-digested φ χ 174 (1,358, 1,078, 872, 603, and 310-bp DNA fragments): *a*, lane *2*, and of Taq 1-digested pBR322 (1,444, 1,305, 475, 368, 315, 313, and 141-bp DNA fragments): *b*, lane *4*.

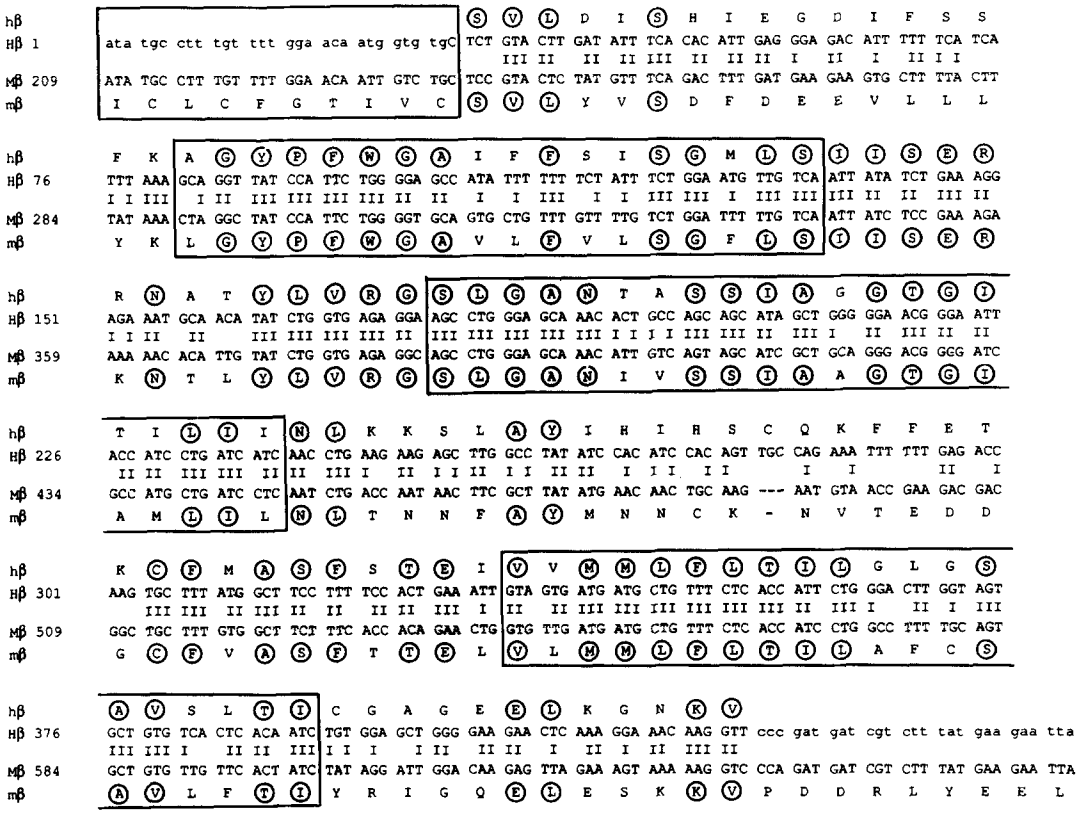

Figure 4. Analysis of the human basophil cDNA fragment amplified with murine FcεRIβ-derived oligonucleotides. Nucleotidic sequence of the human cDNA fragment (Hβ) is compared with the murine FcεRIβ cDNA (Mβ). Conserved bases are linked by (*I*). The numbers in the left margin refer to the nucleotide position within the human cDNA fragment or the published murine cDNA sequence (14). Sequences of the primers are noted in small letters. The deduced amino acid sequence encoded by the human cDNA is given using the single-letter code (hβ), and is compared with the murine FcεRIβ amino acid sequence (mβ); (*circles*) conserved residues. (*Boxes*) transmembrane domains (14). Note that the intracytoplasmic domain (IISER . . . . YLVRG) is well conserved in human and mouse.

the primers for the tryptase remained negative on purified LC RNA (Fig. 3 *a*, lane *4*), while a fragment of ~620 bp was easily amplified from peripheral blood basophils RNA and from the control cell line KU812 RNA (Fig. 3 *a*, lanes *1* and *3*, respectively). Identity of this fragment was checked by restriction enzyme analysis (with PvuII and PstI) and by Southern analysis (data not shown). Thus LC express at least the α and γ chains of FcεRI.

Characterization of a Human β Chain cDNA Fragment and Detection of the FcεRIβ mRNA Expressed in LC. Transfection experiments have revealed that the IgE binding capacity of FcεRI is confined to the α subunit (18) and that, in humans, the cotransfection of FcεRIγ appears necessary and sufficient for the expression of FcεRIα at the cell surface (19). Thus the question remained whether human basophils and LC express a putative β chain. Since cloning and sequencing of the human FcεRIβ have not yet been published, RT-PCR of the human FcεRIβ was attempted using oligonucleotides designed on the basis of two observations: (*a*) the murine FcεRIβ and Ly 44, the murine equivalent of CD20, are structurally homologous and include four transmembrane domains; (*b*) the amino acid sequences of the murine Ly 44 and the human CD20 are quite well conserved, especially within their transmembrane domains (20). Moreover, given that cysteines should be very probably conserved in the transmembrane domains, one primer corresponding to nucleotide numbers 209–238 of the mouse FcεRIβ cDNA (14) was synthesized, whereas the other primer was deduced from the COOH-terminal intracytoplasmic domain. To increase the probability of success of the RT-PCR, two inosines and one codon degeneracy were introduced in the first and second primers, respectively. We thereby isolated a 457-bp product from total RNA of peripheral basophils (Fig. 3 *b*, lane *6*). Cloning and sequencing of this fragment revealed 71% homology to the corresponding fragment of the FcεRIβ in the mouse (Fig. 4). This fragment encodes a polypeptide that presents 54% identity with a portion of the mouse FcεRIβ, the most homologous region (68% identity) being the transmembrane domains. By screening EMBL and SWISS-PROT databanks using the PC-Gene program, significant homologies were found only with the mouse and rat FcεRIβ. Moreover, the same fragment could be amplified by RT-PCR from KU812 RNA (data not shown). Given these data, we assume that we have isolated a cDNA fragment of the human β chain. Using these primers, we then amplified transcripts from total RNA of purified LC and obtained the expected fragment of 457 bp (Fig. 3 *b*, lane *2*). The identity of this fragment was confirmed by Southern analysis (Fig. 3 *c*, lane *2*) using an oligonucleotide deduced from the sequence of the above described cDNA cloned from the basophils (Fig. 3 *c*, lane *6*).

Taken together, our results clearly demonstrate that resident or freshly isolated human LC express the three components of FcεRI. To our knowledge, this is the first report on the expression of FcεRI on cells distinct from mast cells and basophils. It remains to be determined whether, as for mast cells or bone marrow-derived precursors of basophils (21), cross-linking of IgE via FcεRI on LC may activate the cells or induce the secretion of cytokines, which in turn possibly initiate a series of events leading to inflammatory reactions in the skin or other tissues where these cells are localized.

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