Human Keratinocytes Release the Endogenous β-Galactoside-binding Soluble Lectin Immunoglobulin E (IgE-Binding Protein) which Binds to Langerhans Cells Where It Modulates Their Binding Capacity for IgE Glycoforms

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Summary

A better understanding of the pathophysiological role of Langerhans cells (LC) in atopic diseases is dictated by the characterization of the structures involved in immunoglobulin (IgE)-binding on their cell surface. We previously reported that human LC express the high affinity receptor for IgE (FcεRI), as well as the low affinity receptor for IgE (FcεRII/CD23). In the present study, we document the presence of a third IgE-binding structure on human LC, the IgE-binding protein (εBP), an endogenous soluble β-galactoside binding lectin. Immunohistochemical studies performed on normal human skin revealed an anti-εBP reactivity in the cytoplasm of keratinocytes and in that of acinous cells of eccrine sweat glands. εBP was also found on the cell surface of LC, as shown by anti-εBP/anti-CD1a double labeling and flow cytometric analysis. Anti-εBP binding to the surface of LC was completely abolished by preincubation with lactose and restored by addition of recombinant human εBP, indicating that εBP binds to LC surface by virtue of its lectin property. Immunoblot analysis of anti-εBP-reactive material in keratinocytes and purified LC disclosed a protein with an apparent molecular weight of 33,000 consistent with εBP. Interestingly, mRNA transcripts for εBP were detected only in keratinocytes but not in purified LC isolated from normal skin. εBP was found to be released in culture supernatants of keratinocytes. Incubation of LC with these supernatants resulted in εBP-binding to LC surface via protein–carbohydrate interaction. Most importantly, we could show that binding of human myeloma IgE to LC was inhibited by εBP. In contrast, neuraminidase-treated human myeloma IgE binds to LC only in the presence of εBP. In situ binding studies revealed that keratinocytes, although containing εBP intracytoplasmatically, failed to exhibit any IgE-binding properties. Collectively, our results suggest that human keratinocytes produce the β-galactoside-binding lectin εBP, which subsequently binds to the surface of LC where it is functional in modulating their binding capacity for IgE glycoforms.

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IgE-binding structures play a pivotal role in many pathophysiological mechanisms of atopic diseases. Recently, increasing attention has been drawn to antigen presenting dendritic cells in the skin, the epidermal Langerhans cells (LC)[1], since they have been found to bind IgE molecules in situ in these diseases (1). Therefore we have focused our interest on structures involved in interactions between IgE molecules and LC. So far, we have demonstrated the presence of two IgE receptors on LC: the low affinity receptor for IgE (FcεRII/CD23) (2) and the high affinity receptor for IgE (FcεRI) (3, 4), the latter being initially described to be exclusively found on mast cells and basophils (5). Beside these well-described receptors, a third type of IgE-binding structure has been characterized in the human and rodent immune systems namely, the so-called IgE-binding protein, εBP, which was originally de-

[1] *Abbreviations used in this paper:* APAAP, alkaline phosphatase mouse anti–alkaline phosphatase; εBP, IgE binding protein; CM, conditioned media; EC, epidermal cell; FcεRI, high affinity receptor for IgE; FcεRII low affinity receptor for IgE; GaR, goat anti–rabbit; LC, Langerhans cell; Ma-h, mouse anti–human; MaR, mouse anti–rabbit; neur, neuramidase; RaM, rabbit anti–mouse; TBS, Tris-buffered saline.

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scribed on rat basophilic leukemia cells (RBL) (6, 7). cDNA coding for rat and human εBP revealed that this protein was distinct from FcεRI and FcεRII (8, 9). Subsequently, it was demonstrated that εBP is identical to: (*a*) the carbohydrate binding protein 35 (CBP 35) (10, 11), a mouse galactose–binding lectin found in fibroblasts; (*b*) Mac-2, a protein expressed mainly on the cell surface of murine thioglycollate–elicited peritoneal macrophages, macrophage cell lines, interdigitating dendritic cells, and also in the cytoplasm of some epithelial cells (12–14); and finally to (*c*) HL-29 or L34, both lectines with specificity for β-galactoside (15–17). All these structures share common characteristics (18) including a molecular weight of 29,000–35,000, the lack of both identifiable signal peptide and transmembrane segment. The carbohydrate recognition domain is located in the COOH-terminal portion whereas the NH_2-terminal portion consists of tandem repeats. Finally, they are expressed by various cell types, and localized on the cell surface and/or in the cytoplasm and/or in the nucleus. The functional role of εBP is, as yet, poorly understood. However, some studies suggest its involvement in a growth regulation (11, 19), as a nonintegrin laminin binding protein (20), and most interestingly, as an IgE-binding structure (9).

εBP/Mac-2-reactivity has been observed in murine skin (21), but studies of this protein in the skin of other species are still lacking. In particular, it would be desirable to establish the expression and function of this IgE-binding protein in human skin, which is the localization and/or the target of many IgE-mediated allergic reactions. Therefore, we investigated the expression of εBP in normal human skin and addressed the question of whether epidermal LC express this molecule. Furthermore, we studied the functional significance of εBP by analyzing its involvement in the IgE-binding capacity of human LC.

Materials and Methods

Reagents. Human myeloma IgE (hIgE) was purchased from Calbiochem Corp. (Bad Soden, FRG) and the FITC-labeled mouse anti–human-IgE (Ma-hIgE/FITC) antibody was obtained from Nordic (Tilburg, The Netherlands). Mouse IgE anti-β-lactoglobulin and rabbit anti–mouse IgE (RaM-IgE) were a kind gift from Dr. D. Granato (Nestec Research Center, Lausanne, Switzerland). Unlabeled mAb IOT6a (IgG1) (Immunotech, Marseille, France) and PE-labeled T6/RD1 (IgG1) (Coultertronics, Krefeld, Germany) are directed against CD1a which, in the skin, is present only on LC (22). The monospecific rabbit anti-εBP antibody and the recombinant human εBP (rhεBP) have been produced as described in detail elsewhere (23–25). Rabbit anti–mouse antibody (RaM-IgG), mouse anti-rabbit (MaR-IgG), and alkaline-phosphatase mouse anti–alkaline-phosphatase complexes (APAAP) were purchased from Dakopatts (Hamburg, Germany). FITC-labeled goat anti–rabbit (GaR/FITC) obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Lactose, neuraminidase, NP-40, and normal rabbit serum were from Sigma Chemical Co. (St. Louis, MO). Isotype controls were from Becton Dickinson & Co. (Mountain View, CA).

Cell Lines. The monoblastic cell line U937 was obtained from the American Type Tissue Culture Collection (Rockville, MD). The spontaneously transformed human keratinocyte cell line HaCaT (26) was generously provided by Dr. N. Fusenig (German Cancer Institute, Heidelberg, Germany).

In Situ Immunolabeling on Cryosections. For studies on normal skin, split-thickness specimens from cadaver skin (buttocks) were obtained by using a handdermatome (Weck & Co., Research Triangle, NC). For the preparation of vertical skin cryosections, 6-mm punch biopsies were taken from those specimens, snap-frozen in liquid nitrogen, and stored at −70°C. 6-μm cryosections were then prepared, air-dried, fixed for 10 min in pure acetone, and then processed for immunohistochemistry using the APAAP technique. Anti-εBP was diluted 1:500 in Tris-buffered saline (TBS) and incubated on the sections. After two washes in TBS, the sections were overlayed with MaR-IgG (1:20), followed, after two washes, by an incubation with the RaM-IgG antibody (diluted 1:10). After two more washes in TBS, the sections were incubated with the APAAP complexes (final dilution 1:10). The second and third step were repeated once. All incubations were performed for 1 h at room temperature. After two washes in TBS, the substrate was added. This substrate was prepared essentially as described (27). The color reaction was controlled under the light microscope. After ∼10 min, the sections were washed in distilled water and mounted in Kayser's gelatine (Merck, Darmstadt, FRG).

LC-enriched Epidermal Cell Suspensions. Crude epidermal cell (EC) suspensions were obtained by trypsinization of split-thickness cadaver skin specimens as described elsewhere in details (2). Resulting cells were subjected to Lymphoprep (Nyegaard, Oslo, Norway) centrifugation. The interface cell layer (viability >95% as determined by trypan blue exclusion) was then collected and the enrichment for LC (30–70%) was controlled by T6/RD1 immunolabeling. The whole procedure takes ∼10 h and the final cell preparation is now referred to as LC-enriched EC.

Keratinocyte-conditioned Medium. HaCaT cells or collagen-adherent human normal keratinocytes (HNK) were cultured for 38 h in DMEM supplemented with 10% FCS, 1% antibiotics/antimycotics (Gibco, Essenstein, FRG), 2 mM L-glutamine (Gibco), 25 mM Hepes buffer (Seromed, Berlin, FRG), 5 mM sodium-pyruvate (Gibco) and 1% nonessential amino acids (Gibco). These keratinocyte-conditioned media (HaCaT-CM or HNK-CM) were then collected, filtered through 0.2-μm filters (Millipore, Eschborn, FRG), concentrated, and stored frozen at −20°C.

Flow Cytometric Analysis. Freshly isolated LC-enriched EC (2×10^5) were washed twice in cold PBS containing 0.01% sodium azide and were incubated for 30 min with heat-inactivated human AB serum. Then cells were subjected to double labeling and flow cytometric analysis. LC-enriched EC were first incubated with anti-εBP (diluted 1:500 in PBS) for 30 min. After two washes with cold PBS, the cells were incubated for 30 min with GaR/FITC (diluted 1:500 in PBS). After another two washes, the cells were then incubated with the anti-CD1a mAb T6/RD1 (1 μg/ml) for 30 min. Controls were performed by omitting anti-εBP or using normal rabbit serum (diluted 1:500 in PBS). In experiments evaluating the εBP-binding capacity of various cells, suspensions were preincubated with rhεBP at 4 μg/ml or 0.2 M lactose, respectively, and then stained as described above. All incubations and washes were performed at 4°C. Cells were then washed twice in PBS plus 0.01% sodium azide and analyzed on a FACScan® (Becton Dickinson & Co.). Fluorescence parameters were collected using a built-in logarithmic amplifier after gating on the combination of forward light scatter and T6/RD1 labeling. Nonviable cultured cells were excluded by 7-amino-actinomycin-D labeling as described (28). In all experiments, fluorescence data of 10,000 cells were acquired with logarithmic scale, the instruments set up on 256 channels, and analyzed with Lysis II software (Becton Dickinson & Co.). All experiments were performed at least in triplicate.

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Western Blot Analysis. Crude EC were isolated by trypsinization, and LC were first enriched by gradient density configuration as described above. Then, purified LC or LC-depleted EC were prepared by using anti-CD1a antibody (IOT6a) bound to sheep anti-mouse coated 280 nm magnetic beads according to the manufacturer's protocols (Dynal, Oslo, Norway). The purity of the LC preparation was controlled after each application to the magnet by light microscopy, and the procedure was stopped when unbound cells, i.e., HNK and other cells, were completely removed. Usually, at least 8–10 applications and washes were necessary to yield highly purified LC (>99%) preparation. This preparation was also adequate for Northern blot analysis (see below). The cells were washed, lysed in NP-40–containing buffer, and 14 μg of protein were separated by 12% SDS-PAGE. The separated proteins were electroblotted onto nitrocellulose, treated with 5% dry milk, and then incubated for 1 h with anti-εBP (diluted 1:500 in PBS). Binding of the primary antibody was revealed by the incubation with a MaR-IgG and then a peroxidase-conjugated goat anti-mouse Ig antibody (Bio-Rad Laboratories, Richmond, CA) followed by an enhanced chemiluminescence Western blot detection system (ECL; Amersham Corp., Arlington Heights, IL) according to the manufacturer's protocol.

Northern Blot Analysis. Purified LC and LC-depleted EC were prepared as described for Western blot analysis. Total RNA was isolated from these cells and from εBP-negative U937 cells by standard procedure (29) and prepared for Northern blot after transfer to Hybond N+™ membrane (Amersham Corp.). EcoRI fragments of a plasmid containing the human εBP cDNA (9) were labeled with ^{32}P with a random primer labeling kit (Amersham Corp.). Nylon membranes were prehybridized and hybridized overnight according to the manufacturer's recommendations. Membranes were washed at room temperature for 30 min in 2× SSC/0.5% SDS, followed by 30 min in 1× SSC/0.1% SDS. Final washes were performed for 30 min at 68°C in 0.2× SSC/0.1% SDS. Hybridized membranes were exposed to Kodak X-AR film for 24 h at −80°C.

IgE-Binding and -Blocking Studies. IgE-binding on EC was analyzed in vitro on LC-enriched EC suspensions and in situ on cryosections of normal human skin. For these experiments, both untreated hIgE and a desialylated form of this IgE were used. The latter was prepared by treatment with neuraminidase (0.2 U for 5 μg hIgE at 37°C for 5 h) and will be referred to as neur-hIgE. IgE-binding capacity of LC-enriched EC suspensions was evaluated by first incubating the cells with 0.2 M lactose followed by staining with T6/RD1 at 1 μg/ml, and two washes in PBS/0.1% sodium azide. Then, the cells were either incubated with rhεBP and hIgE or they were directly incubated with either of the IgE forms without preincubation with rhεBP. Finally, cells were washed once, stained with Ma-hIgE/FITC or FITC-labeled isotype control (both at 5 μg/ml), washed again, and analyzed by flow cytometry.

Cryosections of normal human skin were incubated for 2 h with either hIgE or human polyclonal IgE or murine IgE (all 30 μg/ml), diluted in TBS followed by Ma-hIgE antibody or a RaM-IgE antibody (diluted 1:20 in TBS), respectively. After two washes in TBS, sections incubated with murine IgE were incubated with a mouse anti-rabbit Ig antibody (1:20 in TBS). Finally, all sections were incubated successively with RaM-IgG, and APAAP complexes and the enzyme substrate as described above.

Results

εBP Reactivity in Normal Human Skin. Immunohistochemical studies revealed εBP reactivity on various distinct cells in normal human skin. In the deep dermis, a strong and diffuse staining was observed in the cytoplasm, but not in the nucleus of acinous cells from eccrine sweat glands (Fig. 1 *a*). Only rare and scattered, large and sometimes dendritic cells with a membranous pattern were observed in the mid and upper dermis. Other cells failed to react with anti-εBP. In the epidermal compartment, two types of cells were reactive. First, nearly all HNK were stained. This pattern was, as for eccrine sweat glands, cytoplasmic and sparing the nucleus (Fig. 1 *b*). Second, between HNK, cells with dendritic shape exhibiting a membranous pattern were observed in the epidermis. The staining was generally weak but a more pronounced reactivity was detected on dendritic cells located in the follicular epidermis (Fig. 1 *b*). Control staining with normal rabbit serum as a first step remained negative. Similarly, preincubation of sections with 0.2 M lactose resulted in a negative anti-εBP staining of all cells. These results show that, in normal human skin, in addition to epithelial cells, εBP reactivity is found on epidermal dendritic cells.

Surface Expression of εBP on Human LC. Since, in other cell types, εBP has been shown to be present as an intracellular and/or cell surface protein, double staining experiments on freshly isolated LC-enriched EC were then performed in order to (*a*) determine whether the membranous pattern observed in immunohistochemistry is due to a surface expression of εBP on these epidermal dendritic cells, and (*b*) to identify this cell type. The staining was performed on unfixed cells, since fixation of EC would have potentially lead to the staining of intracytoplasmic εBP and/or to the release of intracellular εBP from keratinocytes with a subsequent possible absorption/binding of εBP on EC. Thus, flow cytometric analysis on LC-enriched EC confirmed that a CD1a-positive subpopulation of EC, i.e., LC, display anti-εBP reactivity (Fig. 2 *b*), whereas keratinocytes were found to be hardly stained (Fig. 2 *a*). Interestingly, there was a marked difference in anti-εBP reactivity on LC isolated from different individuals. Hence, from these experiments we concluded that, in human epidermis, εBP reactivity can be detected on cell surface of LC and, to a much lesser extent, keratinocytes.

Biochemical Characterization of εBP in Human Epidermis. To verify the specificity of the observed immunoreactivity in the above experiments, immunoblot analysis was performed on lysates from LC-depleted EC and from purified LC. Thus, after SDS-PAGE separation of cell lysates from LC-depleted EC, the monospecific antibody recognized a single protein with an apparent molecular weight of about 33,000 (Fig. 3, lane *2*). A similar protein was also found on lysates from purified LC (Fig. 3, lane *1*), whereas incubation of normal rabbit serum as the first step only show some few unspecific weak bands with higher molecular weights, most probably due to adsorption of serum proteins on the blot (Fig. 3, lane *3*). Lysates of U937 cells remained negative (data not shown). These results further established the presence of εBP in HNK and LC.

εBP Is Bound Via Carbohydrates onto LC Surface. Since εBP is found as a cell surface protein but lacks a hydrophobic transmembrane domain, this implies a distinct binding mechanism most likely involving its lectin property. To verify this

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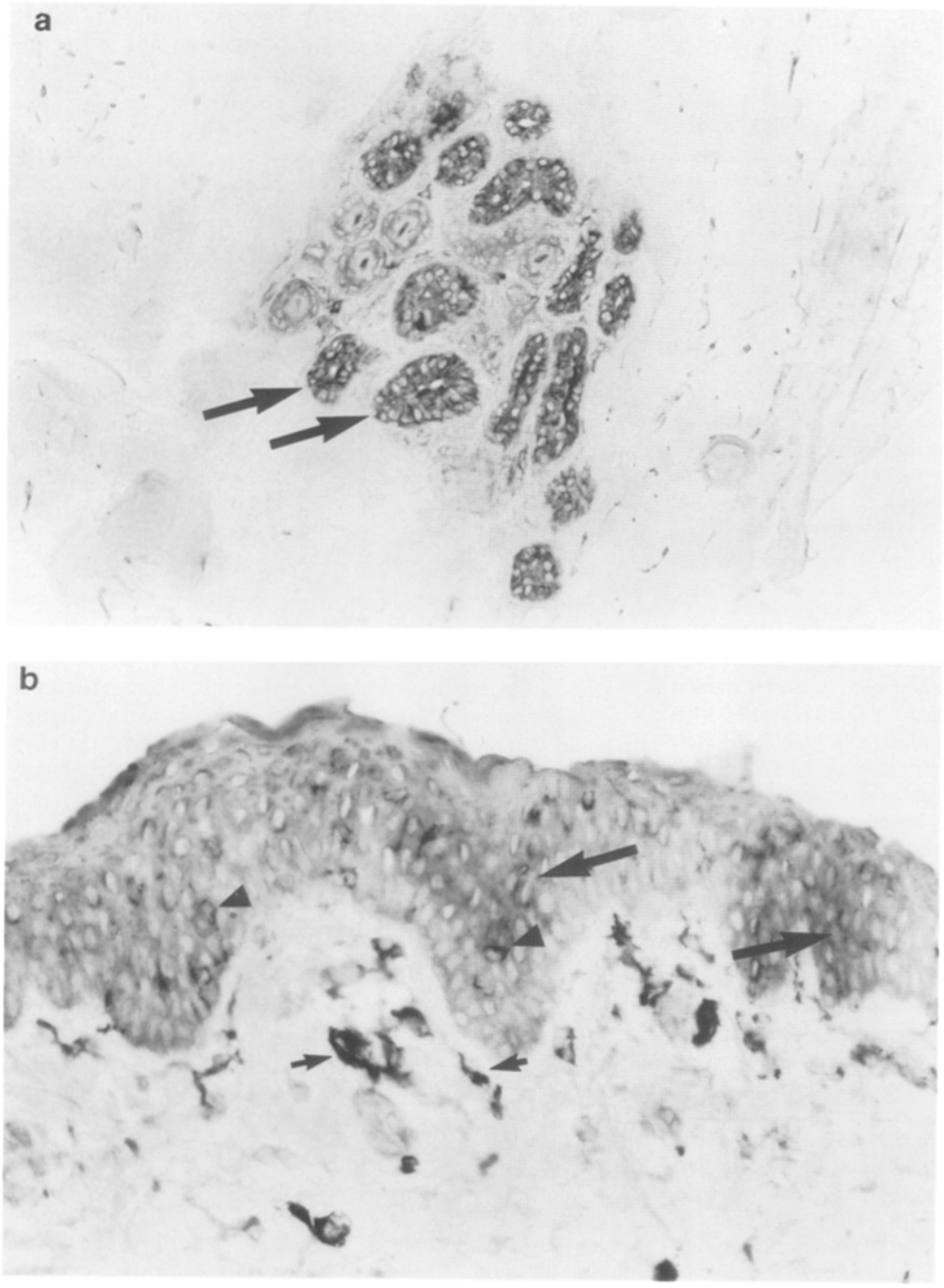

Figure 1. Presence of anti-εBP reactivity in skin epithelia. Cryosections of normal human skin were incubated with anti-εBP antibody and the binding revealed by alkaline-phosphatase mouse anti–alkaline-phosphatase technique. (*a*) Reactivity in eccrine sweat glands (*arrows*) or normal human skin. (*b*) Reactivity in keratinocytes (*long arrows*) and dendritic cells (*arrowheads*) in the epidermis and dermis (*short arrows*).

hypothesis on LC, LC-enriched EC were either left untreated or incubated with 0.2 M lactose or with PBS and then double stained with anti-εBP and anti-CD1a and finally subjected to flow cytometric analysis. Whereas PBS-treated LC displayed an anti-εBP reactivity similar to untreated LC, preincubation with lactose led to a complete disappearance of the anti-εBP reactivity on LC and HNK, suggesting that εBP has been eluted from the cell surface (Fig. 4*b*). This could be confirmed in experiments in which the staining was restored by incubating the cells with rhεBP after the lactose treatment. Most importantly, when LC either untreated, treated with PBS, or treated with lactose were incubated with increasing concentrations (0.1–10 μg/ml) of rhεBP, the staining intensity was much higher than that of untreated LC, saturation being reached with 4 μg/ml rhεBP (Fig. 4*c*). This indicated the presence of high amounts of potential εBP-binding

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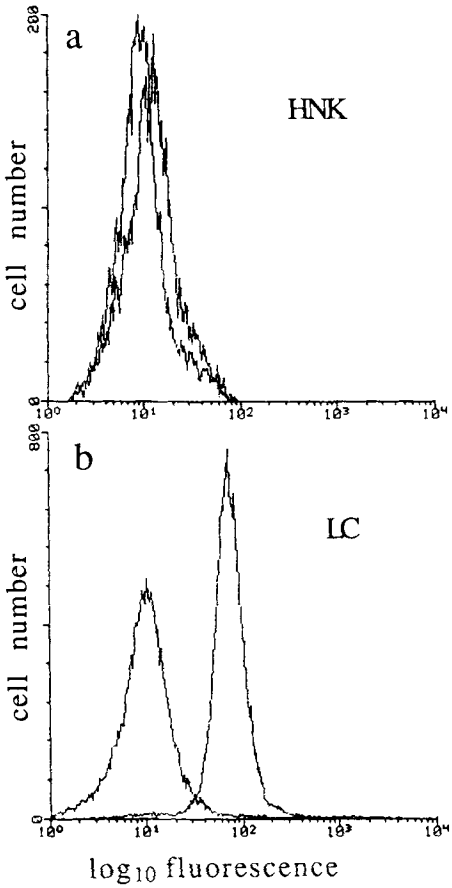

Figure 2. Analysis of εBP expression on freshly isolated HNK (*a*) and human LC (*b*). Epidermal cell suspensions were obtained by trypsinization and double labeling was performed on unfixed cells using anti-CD1a and anti-εBP followed by flow cytometric analysis. For overlay histograms, HNK (*a*) and LC (*b*) were gated based on the lack or presence of CD1a positivity, respectively.

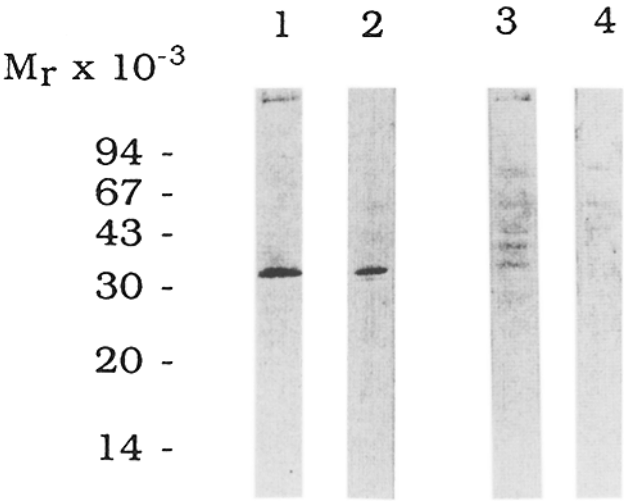

Figure 3. Immunoblot analysis of εBP on LC and HNK. Cell lysates from purified LC (lanes *1* and *3*), LC-depleted EC (lanes *2* and *4*) were subjected to SDS-PAGE separation, blotted to nitrocellulose, and incubated either with anti-εBP antibody (lanes *1* and *2*) or with normal rabbit serum (lanes *3* and *4*) followed by peroxidase-conjugated goat anti-mouse Ig antibody and revealed by chemiluminescence.

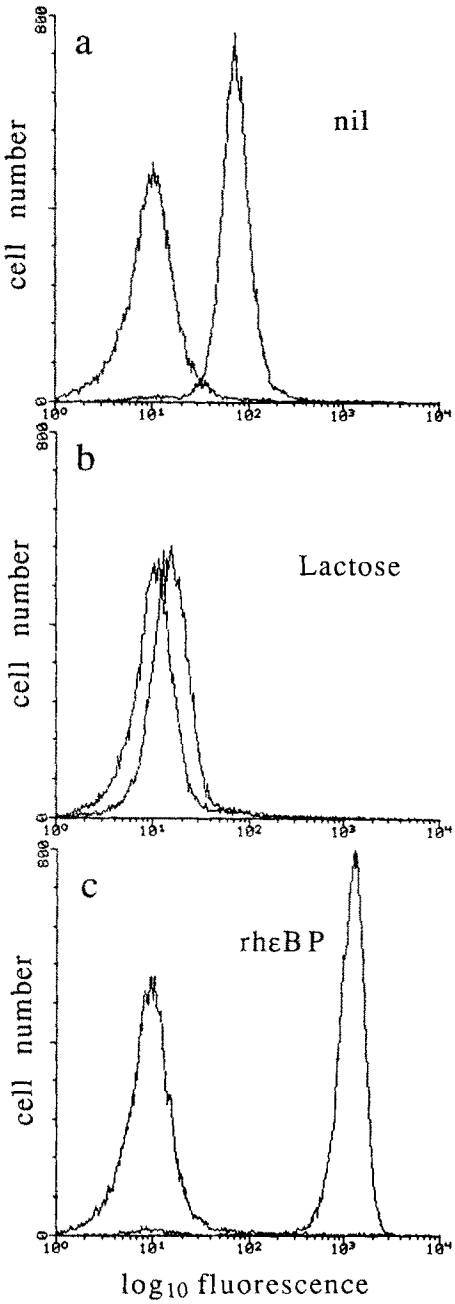

Figure 4. Carbohydrate-mediated εBP-binding onto LC surface. Epidermal cell suspensions were obtained by trypsinization of normal skin and LC were enriched by gradient density centrifugation. Then cells were either directly double stained with anti-CD1a and anti-εBP (*a*) or treated with 0.2 M lactose before staining (*b*) or treated with lactose and then incubated with rhεBP before staining (*c*). For overlay histograms, LC were gated based on the presence of CD1a positivity.

sites on these cells. In contrast, only very low amounts of such potential εBP-binding sites were observed on HNK. From these experiments, we can conclude that εBP binds to LC surface by the virtue of its lectin property.

Keratinocytes Are the Major Source of εBP Found on LC. To ascertain the origin of the εBP molecules found on LC surface, the presence of specific transcripts for εBP was inves-

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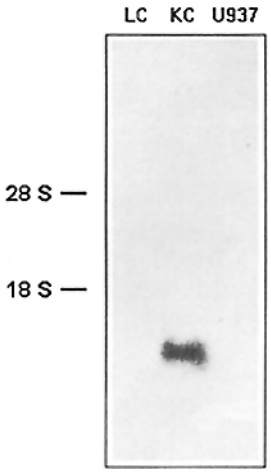

Figure 5. Detection of εBP mRNA on EC. RNA was extracted from cell lysates of purified LC (lane *1*), LC-depleted EC (lane *2*), and U937 cells (lane *3*) and subjected to agarose gel electrophoresis and transferred to nylon membrane. Then they were hybridized with EcoRI fragments of a plasmid containing the human εBP cDNA (9) which were labeled with ^{32}P, and the membrane exposed overnight.

tigated in LC-depleted EC, i.e., HNK, and in purified LC. Therefore, Northern blot analysis, using a specific probe for human εBP, was performed on total RNA isolated from either cell population and from U937 cells used as a control. As shown on Fig. 5 (lane *2*), a specific signal at 1.4 kb was obtained from lysates of HNK, but no transcript was found in purified LC (Fig. 5, lane *1*). Hybridization of lysates from U937 cells remained negative (Fig. 5, lane *3*). Thus, in contrast to HNK, LC extracted from normal human skin fail to exhibit detectable εBP gene transcription.

To verify the hypothesis that HNK release εBP, LC were incubated with HNK-CM, stained for surface anti-εBP-reactivity and analyzed by flow cytometry. Thereby, CM of both normal HNK and HaCaT cells led to an increase of the anti-εBP-reactivity of freshly isolated LC (Fig. 6). Similarly, when LC were first pretreated with lactose, leading to the loss of surface-bound εBP, the subsequent incubation with HNK- or HaCaT-CM restored and even enhanced the anti-εBP reactivity on these cells (data not shown). Since CM have been obtained from subconfluent cells with >98% viability, εBP was most probably produced by keratinocytes in these CM and not passively released during cell death. This suggests that, at least in normal skin, εBP on the LC surface is most likely derived from HNK.

Human LC Bind IgE Molecules via εBP. It has been demonstrated that soluble εBP binds to IgE molecules via their carbohydrates (8). In a first series of experiments, the contribution of surface εBP to the IgE-binding capacity of LC was evaluated by using either untreated highly sialylated hIgE or low sialylated neur-hIgE. As shown on Fig. 7, whereas freshly isolated LC were loadable with hIgE, only a faint binding of neur-hIgE was detectable in the absence of exogenous εBP. In contrast, the addition of rhεBP (4 μg/ml) enhanced the binding of neur-hIgE to LC. Furthermore, when the cells were loaded with neur-hIgE and high concentrations of rhεBP (50 μg/ml), a dramatic increase in the binding was noticed. In contrast, preincubation of untreated hIgE with rhεBP (50 μg/ml) led to a dramatic decrease in the IgE-binding capacity of LC. It should be noticed that, before the binding studies, the presence of IgE and the expression of FcεRI on freshly

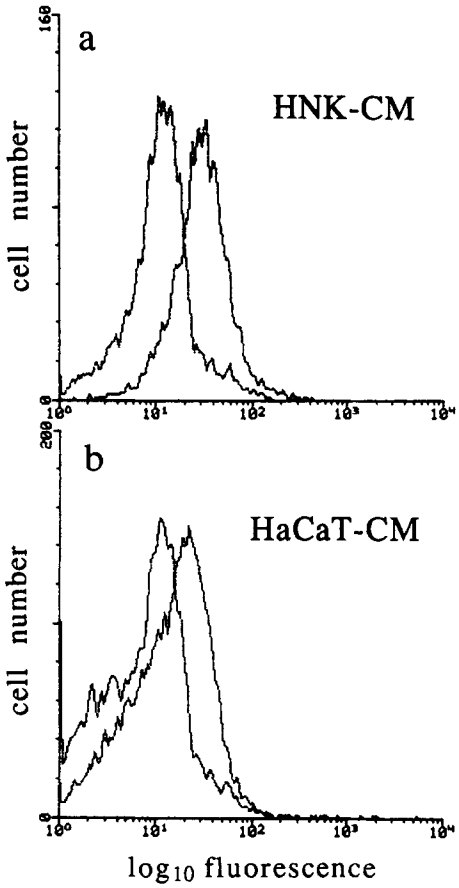

Figure 6. Binding of keratinocyte-derived εBP on LC. LC-enriched EC were obtained as described in Fig. 4 and treated with 0.2 M lactose. Then they were incubated with conditioned media of cultured HNK (*a*) or HaCat cells (*b*) and double labeling with anti-CD1a and anti-εBP was performed as described. For overlay histograms, LC were gated based on the presence of CD1a positivity.

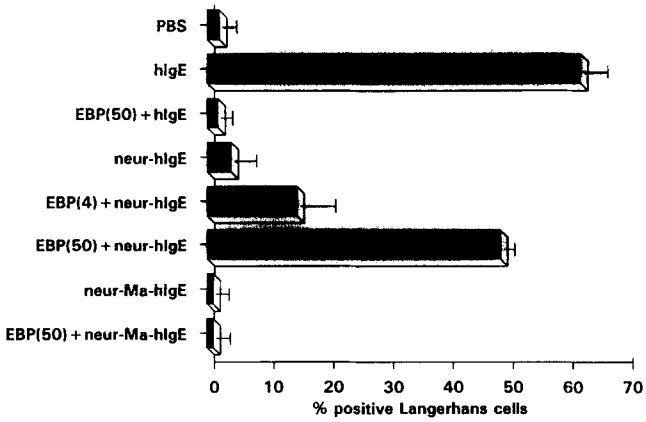

Figure 7. εBP-mediated binding of hypo-sialylated IgE on LC. Freshly isolated LC were incubated with PBS or various forms of IgE in the presence or absence of rhεBP. Cells were then double stained with anti-CD1aPE and Ma-hIgE, and analyzed by flow cytometry. Neuraminidase treatment of the Ma-hIgE (neur-Ma-hIgE) was included as a control. The results are given in percent positive cells (mean ± SEM of three independent experiments) relative to isotype control.

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isolated LC were loadable with hIgE, only a faint binding of neur-hIgE was detectable in the absence of exogenous εBP. In contrast, the addition of rhεBP (4 μg/ml) enhanced the binding of neur-hIgE to LC. Furthermore, when the cells were loaded with neur-hIgE and high concentrations of rhεBP (50 μg/ml), a dramatic increase in the binding was noticed. In contrast, preincubation of untreated hIgE with rhεBP (50 μg/ml) led to a dramatic decrease in the IgE-binding capacity of LC. It should be noticed that, before the binding studies, the presence of IgE and the expression of FcεRI on freshly isolated LC were first controlled by anti-IgE and anti-FcεRI staining, respectively. Whereas no IgE was detectable on LC, FcεRI expression was, as expected, consistently present in all experiments (data not shown). In control experiments, neuraminidase treatment of the anti-IgE antibody failed to induce any binding to εBP.

In a second series of experiments, in situ binding studies on cryosections from normal human skin were designed to obtain further clues to the IgE-binding capacity of εBP in situ. Cryosections were either preincubated with buffer or with lactose (0.2 M), followed by an incubation with hIgE and subsequently stained with mAb anti-IgE. Thereby, in either condition, hIgE-binding was detectable only on LC and not on HNK (data not shown), implying that lactose does not interfere with the binding site of myeloma IgE. This suggests that εBP is not involved in this binding but most probably, FcεRI is. Similar experiments were then performed using IgE which, in contrast to the highly sialylated hIgE, is constitutively low sialylated and is bound with low affinity to FcεRI. Thereby, binding of murineIgE was clearly demonstrated on LC in conditions without preincubation with lactose, whereas after lactose treatment of the sections, binding of mIgE was hardly detectable. Interestingly, IgE-binding was not detected on HNK or on eccrine sweat glands although these cells were shown to contain εBP. From these experiments, we concluded that only cell surface εBP is able to bind IgE molecules. Thus, εBP contributes to the surface binding capacity of LC toward hyposialylated IgE species.

Discussion

In the present study, we report for the first time on the presence of the endogenous soluble lectin, IgE-binding protein εBP, in normal human skin. This 33-kD protein was primarily found in high amounts in the cytoplasm of HNK and in eccrine sweat glands cells. The former were shown to represent the source of εBP found on the cell surface of epidermal LC, where εBP attaches to carbohydrates by virtue of its lectin property. Most importantly, εBP present on LC conferred on them the possibility to enhance their IgE-binding ability by selectively binding hyposialylated IgE.

In mice, εBP was shown to be quite ubiquitously localized (30). Although highly homologous lectins of the S-type family such as HL-29 have been reported in several tissues in humans (16), reports on human εBP were still missing. Our observation that HNK represent a source for the β-galactoside binding soluble lectin εBP sheds a new light on the biology of these cells and on εBP itself in the human system as well. We found εBP mainly in the cytoplasm of HNK, but it could be recovered from culture supernatants of viable cells, thereby demonstrating that they are releasing this lectin. Similar to other soluble lectins and a number of secreted proteins such as IL-1 and fibroblast growth factor (31, 32), εBP lacks a classical signal peptide or transmembrane domain (13) and is therefore transported to the cell surface by mechanisms that remain to be elucidated. As it has been reported for εBP/Mac-2 expression in mouse peritoneal macrophages (12), it is not excluded that mediators present in an inflammatory environment may induce LC to synthesize and secrete εBP themselves. Moreover, such mediators could stimulate HNK to release high amounts of εBP to the epidermal intercellular space. Thus, with regard to the skin surface (∼1.7 m^2), one may speculate the amounts of εBP molecules that could be released by the epidermis under appropriate stimulation, such as in widespread skin diseases or sunburn dermatitis. Whether the released εBP would be found systemically remains to be clarified, but there is clear evidence that proteins of similar or much higher molecular weights such as immunoglobulins can have access from the circulation to the epidermis and vice versa.

We have shown that keratinocyte-derived εBP binds to LC surface via carbohydrates. This was conclued from the fact that εBP could be eluted with lactose from the surface of freshly isolated LC. Incubation of lactose-treated LC with HNK-CM led to a reconstitution of the anti-εBP reactivity of the cells. Interestingly, however, when freshly isolated LC were incubated with increasing concentrations (up to 10 μg/ml) of recombinant human εBP, a dramatic increase of the εBP-binding on LC was observed and saturation was reached at 4 μg/ml. Hence, LC most likely express abundant potential binding sites for εBP which are far from being all occupied on freshly isolated LC. It is known that this lectin specifically binds to terminal β-galactose residues found in complex type N-linked oligosaccharide chains (33). Therefore only glycoproteins undergoing distinct posttranslational processing leading to this particular glycosylation pattern represent putative candidates as joining structures of εBP on the cell surface. Studies are now in progress for identifying these glycoproteins on LC.

Our results provide for the first time evidence for an εBP-mediated modulation of the IgE-binding capacity of LC. Significantly, a dichotomy in the function of εBP was observed: whereas εBP mediates binding of neuraminidase-treated myeloma IgE to LC, it inhibits the binding of untreated IgE. The results suggest that the function of εBP depends on the sialylation state of the IgE molecules. Previously, it was found that human εBP binds rodent IgE but not human myeloma IgE molecules, unless the latter have been pretreated with neuraminidase, thereby removing sialic acid residues from the oligosaccharide side chains (9). The observation that human εBP binds subpopulations of human polyclonal IgE (34) supports the concept that human IgE molecules are quite heterogeneous in terms of sialylation. The results presented herein can be best understood in the context of differential recognition of IgE glycoforms by εBP.

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The conditions required for binding hyposialylated human IgE to LC via εBP are noteworthy. IgE binding is observed in the presence of exogenous εBP at relatively high concentrations. Indeed, whereas maximal loading of the lectin on LC surface was reached with εBP concentration of 4 μg/ml, an increased binding of IgE through the lectin was observed with concentrations up to 50 μg/ml εBP. This is in line with a very recent demonstration that εBP molecules are monovalent in terms of galactose binding sites, but that they apparently form oligomers or polymers when high concentrations of εBP are used (25). Although it remains to be verified, it is conceivable that such high concentrations of keratinocyte-derived εBP are locally reached in the narrow epidermal intercellular space. Hence, we postulate that, under these conditions, hyposialylated human IgE glycoforms are bridged to yet-to-be-defined LC surface glycoproteins via εBP-oligomers.

The observation that εBP inhibits the binding of human myeloma IgE untreated with neuraminidase is intriguing. Since εBP binds untreated human myeloma IgE poorly, the observed inhibitory effect is likely not due to εBP binding to IgE. Rather, the results may be explained by binding to FcεRI and thereby exerting a steric hindrance for IgE binding to FcεRI. It is to be noted that, recently, εBP was found to bind to FcεRI on rodent mast cells, in addition to binding to IgE (Frigeri, L., R. Zuberi, and F.-T. Liu, manuscript in preparation). Therefore, it is quite possible that εBP binds FcεRI on LC.

The findings that neuraminidase treatment of IgE led to a decrease in its binding to LC also remains intriguing. It has been reported that removal of sialic acid from RBL cell surface resulted in an increase in the association constant of IgE binding to these cells (35). To our knowledge, no studies have been conducted to test the effect of desialylation of IgE on the binding of IgE to FcεRI or FcεRII. The removal of negatively charged sialic acid residues from human IgE may lead to conformational changes that are detrimental to its binding to FcεRI. Regardless of the explanation, the findings again underscore the differential behaviors of IgE glycoforms in either direct binding or εBP-mediated binding to LC.

Very recently, εBP has been implicated in the IgE binding to neutrophils and IgE-mediated activation of these cells (36). In that case too, preferential binding of neuraminidase-treated, desialylated IgE was noted. A picture is emerging that εBP is another IgE-binding structure on various cell types. The observations also highlight the functional variability of various IgE-glycoforms as a result of selectivity of εBP for distinct hyposialylated IgE molecules.

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References

1. Bruijnzeel-Koomen, C.A., W.J. Fokkens, G.C. Mudde, and P.L. Bruijnzeel. 1989. Role of Langerhans cells in atopic disease. *Int. Arch. Allergy. Appl. Immunol.* 90:51.
2. Bieber, T., A. Rieger, C. Neuchrist, J.C. Prinz, E.P. Rieber, G. Boltz-Nitulescu, O. Scheiner, D. Kraft, J. Ring, and G. Stingl. 1989. Induction of Fc epsilon R2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and gamma interferon. *J. Exp. Med.* 170:309.
3. Bieber, T., H. De la Salle, A. Wollenberg, R. Chizzonite, J. Hakimi, J. Ring, D. Hanau, and C. De la Salle. 1992. Human Langerhans cells express the high affinity receptor for IgE (FcεRI). *J. Exp. Med.* 175:1285.
4. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Födinger, J.-P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J. Exp. Med.* 175:1353.
5. Metzger, H., G. Alcaraz, R. Hohman, J.-P. Kinet, V. Pribluda, and R. Quarto. 1986. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* 4:419.
6. Liu, F.-T., and N. Orida. 1984. Synthesis and surface immunoglobulin E receptor in Xenopus oocytes by translation of mRNA from rat basophilic leukemia cells. *J. Biol. Chem.* 259:10649.
7. Liu, F.T., K. Albrandt, E. Mendel, A. Kulczycki, Jr., and N.K. Orida. 1985. Identification of an IgE-binding protein by molecular cloning. *Proc. Natl. Acad. Sci. USA.* 82:4100.
8. Albrandt, K., N.K. Orida, and F.-T. Liu. 1987. An IgE-binding protein with a distinctive repetitive sequence and homology with an IgG receptor. *Proc. Natl. Acad. Sci. USA.* 84:6859.
9. Robertson, M.W., K. Albrandt, D. Keller, and F.-T. Liu. 1990. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. *Biochemistry.* 29:8093.
10. Crittenden, S.L., C.F. Roff, and J.L. Wang. 1984. Carbohydrate-binding protein 35: identification of the galactose-specific lectin in various tissues of mice. *Mol. Cell. Biol.* 5:1252.

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11. Laing, J.G., M.W. Robertson, C.A. Gritzmacher, J.L. Wang, and F.-T. Liu. 1989. Biochemical and immunological comparisons of carbohydrate-binding protein 35 and an IgE-binding protein. *J. Biol. Chem.* 264:1097.
12. Ho, M., and T.A. Springer. 1982. Mac-2, a novel 32,000 Mr mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. *J. Immunol.* 128:1283.
13. Cherayil, B.J., S.J. Weiner, and S. Pillai. 1989. The Mac-2 antigen is a galactose-specific lectin that binds IgE. *J. Exp. Med.* 170:1959.
14. Cherayil, B.J., S. Chaitovitz, C. Wong, and S. Pillai. 1990. Molecular cloning of a human macrophage lectin specific for galactose. *Proc. Natl. Acad. Sci. USA.* 87:7324.
15. Leffler, H., and S.H. Barondes. 1986. Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian β-galactosides. *J. Biol. Chem.* 261:10119.
16. Sparrow, C.P., H. Leffler, and S.H. Barondes. 1987. Multiple soluble β-galactoside-binding lectins from human lung. *J. Biol. Chem.* 262:7383.
17. Raz, A., G. Pazerini, and P. Carmi. 1989. Identification of the metastasis-associated, galactoside-binding lectin as a chimeric gene product with homology to an IgE-binding protein. *Cancer Res.* 49:3489.
18. Liu, F.-T. 1990. Molecular biology of IgE-binding protein, IgE-binding factors, and IgE-receptors. *Crit. Rev. Immunol.* 10:289.
19. Moutsatsos, I.K., M. Wade, M. Schindler, and J.L. Wang. 1987. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc. Natl. Acad. Sci. USA.* 84:6452.
20. Woo, H.J., L.M. Shaw, J.M. Messier, and A.M. Mercurio. 1990. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). *J. Biol. Chem.* 265:7097.
21. Haines, K.A., T.J. Flotte, T.A. Springer, I. Gigli, and G.J. Thorbecke. 1983. Staining of Langerhans cells with monoclonal antibodies to macrophages and lymphoid cells. *Proc. Natl. Acad. Sci. USA.* 80:3448.
22. Fithian, E., P. Kung, G. Goldstein, M. Rubenfeld, C. Fenoglio, and R. Edelson. 1981. Reactivity of Langerhans cells with hybridoma antibody. *Proc. Natl. Acad. Sci. USA.* 78:2541.
23. Frigeri, L.G., and F.-T. Liu. 1992. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. *J. Immunol.* 148:861.
24. Frigeri, L.G., M.W. Robertson, and F.-T. Liu. 1990. Expression of biologically active recombinant rat IgE-binding protein in *Escherichia coli*. *J. Biol. Chem.* 265:20763.
25. Hsu, D.K., R.I. Zuberi, and F.-T. Liu. 1992. Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. *J. Biol. Chem.* 267:14167.
26. Boukamp, P., R.T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, and N.E. Fusenig. 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell. Biol.* 106:761.
27. Bieber, T., B. Dannenberg, J.C. Prinz, E.P. Rieber, W. Stolz, O. Braun-Falco, and J. Ring. 1989. Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. *J. Invest. Dermatol.* 93:215.
28. Schmid, I., W.J. Krall, C.H. Uittenbogaart, J. Braun, and J.V. Giorgi. 1992. Dead cell discrimination with 7-amino-actinomycin D in combination with dual color immunofluorescence in single laser flow cytometry. *Cytometry.* 13:204.
29. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294.
30. Gritzmacher, C.A., M.W. Robertson, and F.-T. Liu. 1988. IgE-binding protein. Subcellular location and gene expression in many murine tissues and cells. *J. Immunol.* 141:2801.
31. Abraham, J.A., A. Mergia, J.L. Whang, A. Tumolo, J. Friedman, K.A. Hjerrild, D. Gospodarowicz, and J.C. Fiddes. 1986. Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibroblast growth factor. *Science (Wash. DC).* 233:545.
32. March, C.J., B. Mosley, A. Larsen, D.P. Cerretti, G. Breadt, V. Price, S. Gillis, C.S. Henney, S.R. Kronheim, K. Grabstein, et al. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature (Lond.).* 315:641.
33. Barondes, S.H. 1984. Soluble lectins: a new class of extracellular proteins. *Science (Wash. DC).* 223:1259.
34. Robertson, M.W., K. Albrandt, D. Keller, and F.-T. Liu. 1991. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. *Biochemistry.* 29:8093.
35. Pecoud, A.R., S. Ruddy, and D.H. Conrad. 1981. Functional and partial chemical characterization of the carbohydrate moieties of the IgE receptor on rat basophilic leukemia cells and rat mast cells. *J. Immunol.* 126:1624.
36. Truong, M.J., V. Gruart, J.P. Kusnierz, J.P. Papin, S. Loiseau, A. Capron, and M. Capron. 1993. Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/ϵBP) of the S-type lectin family: role in IgE-dependent activation. *J. Exp. Med.* 177:243.

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