Fc Receptors of Human Langerhans Cells

Didier A. Schmitt, M.Sc., Thomas Bieber, M.D., Jean-Pierre Cazenave, M.D., Ph.D., and Daniel Hanau, M.D., D.Sc.
Laboratoire d’Histocompatibility (DAS, DH), and INSERM U.311 (I-PC), Centre Régional de Transfusion Sanguine, Strasbourg, France; and Dermatologische Klinik und Poliklinik der Ludwig-Maximilians Universität (TB), Munich, F.R.G.

Receptors for the Fc fragment of immunoglobulins (Fc R) exhibit specificities for a wide variety of immunoglobulin classes and subclasses. In humans, at least three distinct classes of receptors for the Fc fragments of IgG (Fcγ RI, II, III) and two classes of receptors for the Fc fragments of IgE (Fcε RI, II) have been characterized. These classes were largely defined on the basis of their affinities for different immunoglobulin subclasses and their reactivities with monoclonal anti-receptor antibodies. Among these FcR, in healthy individuals, epidermal Langerhans cells (LC) express only the Fcγ RII/CDw32. This FcR — a member of the immunoglobulin superfamily — is only present on about 50% of freshly isolated CD1a positive cells, as determined by rosette assays. It has a Mr of 40 kDa, is trypsin resistant, binds polymeric human IgG and murine IgG1-coated erythrocytes, and reacts with anti-CDw32 monoclonal antibodies (MoAb). LC internalize Fcγ RII by receptor-mediated endocytosis. After 48 h of culture, human LC loose their Fcγ RII, as revealed by flow cytometry. While the function(s) of the Fcγ RII on human LC remain(s) unknown, this receptor may be primarily involved, like the Fcγ RII present on macrophages, in the clearance of extra-cellular immune complexes. In patients with atopic dermatitis having an elevated IgE serum level, beside an increased expression of the Fcγ RII by LC located on lesional skin, IgE-bearing epidermal and dermal LC are present, again essentially on lesional skin. Double immunolabeling on cryosections reveals that on lesional skin only about 50% of the epidermal CD1a positive cells bear IgE. This capacity of LC to bind IgE molecules appears to be due to the presence of a specific Fcε R. While the class of this Fcε R still remains unclear, it appears to have some particularities: i) an associated expression with the CD1a antigen, ii) an affinity for IgG, and iii) a trypsin resistance. In vitro, human recombinant interleukin (IL)-4 and/or interferon (IFN)-γ are able to induce the synthesis and expression of Fcε RII/CD23 on a percentage of normal human epidermal LC. This Fcγ RII seems to be functional since it binds IgE molecules, this binding being prevented by preincubation with anti-CD23 MoAb. The expression of Fcε RII/CD23 by LC in vitro is accompanied by the release of IgE-binding factors (BF). Addition of human recombinant IL-1, IL-3, and IL-6 decreases the IL-4/IFN-γ-induced CD23 expression on LC. These data suggest that T-cell derived cytokines (IL-4, IFN-γ) may induce the Fcε RII/CD23 expression on normal human LC, while keratinocyte-derived cytokines (IL-1,3,6) may have a regulatory role in this phenomenon. Fcε RII/CD23-positive LC may play a major part in the pathogenesis of atopic eczema and, by the release of IgE-BF, in the regulation of IgE synthesis. J Invest Dermatol 94:15S–21S, 1990

Monocytes, macrophages, granulocytes, platelets, many lymphocytes, and certain epithelial cells express surface Fc R. These Fc R comprise a heterogeneous group of molecules, whose different members exhibit specificities for a wide variety of immunoglobulin classes and subclasses and carry out different functions. Indeed, Fc R can mediate activities ranging from triggering the secretion of cytotoxic and inflammatory mediators by macrophages to the endocytosis and transcellular transport of immunoglobulins by epithelial cells. These functions depend both on the classes of Fc-R and on the cell type by which they are expressed. However, despite this heterogeneity, molecular cloning has recently revealed that the Fc R represent a series of closely related molecules, which belong either to the immunoglobulin superfamily or to the superfamily of lectins (reviewed in [1]).

HUMAN LEUKOCYTE AND PLATELET Fc RECEPTORS FOR IgG.* Fcγ R

Three distinct classes of Fcγ R (Fcγ RI, Fcγ RII, and Fcγ RIII) have recently been characterized by their molecular weight, affinity for human and mouse immunoglobulin subclasses, and reactivity with specific MoAb (reviewed in [5] and [6]). Molecular cloning has demonstrated that all three belong to the immunoglobulin super-

* We exclude from this review other Fcγ R. Indeed, there exist murine counterparts to the Fcγ R expressed by human leukocytes. Moreover, the

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Reprint requests to: Dr. D. Hanau, Laboratoire d’Histocompatibility, Centre Régional de Transfusion Sanguine, 10 rue Spielmann, 67085 Strasbourg Cedex, France.

Abbreviations:
AD: atopic dermatitis
BF: binding factors
EC: epithelial cells
FcR: receptors for the Fc fragment of immunoglobulin
Fcγ R: receptors for the Fc fragment of IgG
Fcε R: receptors for the Fc fragment of IgE
hr: human recombinant
IFN-γ: interferon-γ
IL: interleukin
LC: Langerhans cell(s)
MoAb: monoclonal antibodies
RBC: red blood cells

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family and are integral membrane proteins with an amino-terminal extracellular portion containing two or three immunoglobulin-related domains, a single transmembrane segment, and a carboxy-terminal cytoplasmic "tail" [7]. There is, however, one exception, the neutrophil FcyRII, which was found to be anchored in the membrane through a phosphatidylglycerol glycan linkage.

FcyRI has a M₀ of about 72 kDa, is trypsin sensitive, demonstrates high affinity (Ka = 10⁻⁸ to 10⁻⁹ M) for monomeric human IgG, binds human IgG-coated erythrocytes and murine IgG2a and IgG3, and is recognized by anti-CD64 MoAb such as 32. Its expression can be induced or increased by incubation with IFN-γ. Fcy RI mediates murine IgG2a and IgG3 anti-CD3-induced T-cell mitogenesis. FcyRII has a M₀ of 40 kDa, is trypsin resistant, shows low affinity for monomeric human IgG, binds polymeric human IgG (Ka = 10⁻⁴ M), and interacts with murine IgG1 and at low ionic strength with aggregated murine IgG2b. It binds murine IgG1-coated erythrocytes and reactivates anti-CD3 MoAb such as IV.3 and C1R K5 [8]. It mediates murine IgG1 anti-CD3-induced T-cell mitogenesis. Lastly, FcyRIII has a M₀ of 50–70 kDa, binds polymeric human IgG, murine IgG2a, and human IgG-coated erythrocytes [8] and is recognized by MoAb reacting with CD16 moieties. FcyRIII appears to be sensitive to elastase treatment [9].

These three receptors can also be distinguished on the basis of their distribution among various cell types [5]. Thus, FcyRI is expressed only on mononuclear phagocytes, whereas FcyRII is present on a much broader range of cells including not only the mononuclear phagocytes but also granulocytes, platelets, and B lymphocytes. FcyRIII is expressed on macrophages, granulocytes, and K/NK cells.

The three FcR are thus expressed on distinctive but overlapping populations of cells and various combinations can be noted. While macrophages bear all three FcR monococytes and neutrophils express only two of the three FcR: either FcyRI and FcyRII in the case of monocytes or FcyRII and FcyRIII on granulocytes. Platelets, B lymphocytes, and K/NK cells bear either FcyRII or FcyRIII.

**HUMAN Fc RECEPTORS FOR IgE: Fcε R**

Two distinct classes of Fcε R (FceRI and FceRII) have been characterized by their affinity for monomeric IgE.

FceRI (reviewed in [7]) is a tetrameric complex of one IgE binding α subunit, one β subunit, and two disulfide-linked γ subunits. Molecular cloning has revealed that the α subunit is structurally similar to the Fc γ R described above with, in particular, in the extracellular portion, two immunoglobulin-related domains. Fcε RI is found exclusively on the surface of mast cells and basophils. It is trypsin resistant and binds the Fc fragment of monomeric IgE with high affinity (Ka = 10⁻⁹ M). This attachment of monomeric IgE via its Fc region does not in itself stimulate the cells. However, when specific allergens react with the receptor-bound IgE, the cells explosively discharge their granular contents, synthesizes leukotrienes, and release mediators responsible for many of the symptoms of allergic diseases.

FceRII (reviewed in [10] and [11]) is a single-chain glycoprotein with a M₀ of about 45 kDa. Unlike the other FcR sequenced to date, FceRII is not a member of the immunoglobulin superfamily, but belongs to the superfAMILY of lectins. A common feature of this family of molecules is that, when membrane bound, they display the unusual orientation of a cytoplasmic amino terminus with the carboxy terminus being extracellular (Fig 1). The lectin homology region is located on the extracellular domain, near the carboxy terminus. The latter contains the Arg-Gly-Asp (RGD) triplet of amino acids in "reverse" orientation, i.e., DGR. This triplet is common to a number of molecules (such as fibronectin, fibrinogen, collagen, and so on) that bind to the integrin family of receptors, some of which are involved in cell-cell adhesion reactions. Because in the FceRII this "RGD" motif is located at the extracellular extremity, it could be available for binding to receptors on adjacent cells or it could even bind proximal structures within the cell membrane. The IgE-binding site is contained in the lectin homology region [11] but it is now clear that IgE binds to FceRII independently of any lectin-like activity that the receptor may possess [10]. FceRII is expressed by B lymphocytes, platelets, eosinophils, monocytes/macrophages, NK cells, follicular dendritic cells, and even on activated T lymphocytes. For many of the above-mentioned cells, FceRII expression is not a constitutive event but rather regulated by cytokines including human recombinant (r) interleukin-4, hr interferon-γ, and phorbol esters. FceRII is trypsin sensitive and binds monomeric IgE with low affinity (Ka = 10⁻⁷ M). However, this affinity is increased for IgE dimers or complexes (Ka = 10⁻⁸ M). Thus, by using methods involving multiple IgE-Fcε R interactions, such as rosette formation with IgE-coated erythrocytes, cells expressing the low affinity Fcε R can be detected relatively easily. Finally, the IgE-binding site on all the cells expressing FceRII reacts with the anti-CD23 MoAb. The function of FceRII depends on the cell type expressing it. On monocytes/macrophages for example, the FceRII mediates IgE-dependent cytotoxicity against parasites, promotes the phagocytosis of IgE-coated particles, and, when linked to IgE immune complexes, induces the release of inflammatory mediators. In addition, FceRII has been thought to be involved in the regulation of growth and differentiation of B cells and in the regulation of IgE synthesis. However, these two latter properties seem to be related to the release, by the Fce RI, of soluble fragments: the human IgE-BF.

**HUMAN IgE-BINDING FACTORS: IgE-BF**

Like certain members of the lectin superfamily, the membrane-bound cell surface FceRII undergoes autoproteolytic cleavage (reviewed in [10] and [11]). This process leads to the formation of several soluble fragments with molecular weights of 37 kDa, 33 kDa, 25–27 kDa, and 12 kDa. The 37 kDa and 33 kDa are short-lived intermediates that are cleaved into the more stable 25–27 kDa. The lectin homology region is found entirely within these three fragments which hence contain the IgE-binding site and are all three "IgE-BF" (Fig 1). Indeed, the three fragments are able to prevent the binding of IgE to FceRII-bearing cells, to bind IgE-immunoabsorbent, and to bind soluble IgE. But these fragments are also "soluble lectins." The latter are considered to be important in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Structural model of the membrane-bound FceRII. Note the unusual presence of a cytoplasmic amino terminus and of an extracellular carboxy terminus. In the extracellular domain are located the "RGD" triplet of amino acids, in reverse orientation, and the lectin homology region which contains the IgE-binding site. Auto- or proteolytic cleavage leads to the formation of several soluble fragments, the IgE-BF, containing beside the IgE-binding site the lectin homology region and the RGD motif (modified from Gordon et al [10] according to the data of Delespesse et al [11]).
regulating both cell migration and adhesion. Moreover, these fragments contain in reverse orientation the "RGD" motif which can inhibit binding of RGD-containing ligands to integrins. All these characteristics could explain the recent results of Flores-Romo et al (cited in [10]) who observed that IgE-BF/soluble CD23 inhibit the spontaneous migration of U 937 cells from glass capillaries.

All the FcR II/CD23 positive cells are potential sources of IgE-BF, because they are released by the autoproteolysis of the FcR II. Thus, the production of IgE-BF may be controlled at two levels: 1) the cellular expression of FcR II and 2) the cleavage of FcR II. The cellular expression of FcR II is, as we have seen in the previous section, essentially regulated by cytokines. The cleavage of FcR II into IgE-BF is inhibited by IgE. Therefore, IgE may bind at or near the site of cleavage and stabilize the FcR II molecule. Concerning the activities of the IgE-BF, observations strongly suggest that some IgE-BF regulate the B-cell proliferation induced by IL-4, serve as B-cell differentiation factors, and potentiate the synthesis of IgE on IL-4-stimulated PBMC and on B lymphocytes from allergic individuals.

Fcγ R ON HUMAN EPIDERMAL LANGERHANS CELLS
In 1977, Stingl et al [12] demonstrated, by a rosette technique, that human LC possess Fcγ R. At this time, however, the molecular and functional heterogeneity of the Fcγ R was unknown. Recently, using anti-Fcγ RI, anti-Fcγ RII, and anti-Fcγ RIII MoAb, we have investigated by immunolabeling techniques and rosette assays the classes of Fcγ R expressed at the surface of normal human LC [13].

Rosette Assays on Epidermal Cell Suspensions
For rosette assays, epidermal cells (EC) in suspension were incubated with untreated Rhesus positive human red blood cells (RBC), or with Rhesus positive RBC sensitized either by a human polyclonal antiserum against rhesus or by a mouse IgG1 MoAb anti-glycoporin A. Repeated experiments showed that only the EC exposed to the murine IgG1-coated RBC formed rosettes, i.e., bound three or more erythrocytes. Furthermore, preincubation of the EC with the MoAb anti-Fcγ RII/III C1KM5 prevented rosette formation. In order to reveal the nature of the rosette-forming EC, the rosetted cells were examined by fluorescence microscopy after incubation with FITC/OKT6 or by electron microscopy. Under the fluorescence microscope all the rosetted cells were CD1a positive. However, the percentage of rosetted cells was consistently lower than the percentage of CD1a positive cells (Fig 2), and in nine experiments only 49 ± 1.5% (mean ± SEM) of the CD1a positive cells were rosetted. The LC nature of the rosetted cells was confirmed under the electron microscope, where all the rosetted cells exhibited the ultrastructural marker of LC: the characteristic Birbeck granule. No other EC exhibited rosette formation. Thus, in our hands, human epidermal LC form rosettes only with murine IgG1-coated erythrocytes. Brzyzze-Koemen et al [14] found, however, that LC obtained from healthy volunteers form rosettes with human IgG-coated erythrocytes. These apparently contradictory results could be explained by differences in methodology. Indeed, the method followed by Brzyzze-Koemen et al [14] to prepare the EC suspension required a more vigorous trypsinization of LC. Now, recently, Van de Winkel et al [15] observed that proteolytic treatment by trypsin increased "dramatically" the interaction of monocyte Fcγ RII with human IgG-coated erythrocytes. Moreover, Brzyzze-Koemen et al [14] used sheep red blood cells treated by trypsin and pyruvic aldehyde and fixed by formaldehyde. This method could induce a coating of the erythrocytes by a large number of IgG molecules resulting in an affinity of the LC Fcγ RII for the coated erythrocytes.

Immunolabeling by MoAb anti-Fcγ R of Epidermal Cell Suspension
Double immunofluorescence staining of EC suspensions was performed by incubating the EC successively with 1) MoAb anti-Fcγ RII/III (C1KM5) or anti-Fcγ RIII (ION2), then with 2) rhodamine-conjugated rabbit-anti-mouse antibody, 3) with normal mouse serum, and finally with 4) OKT6/FITC. Enumeration of the Fcγ RII (C1KM5) and OKT6 positive cells gave an average percentage of 55 ± 1.5% (mean ± SEM, n = 9) Fcγ RII positive cells among the CD1a positive cells. However, attempts to detect Fcγ R1 and Fcγ RIII by the same technique remained negative. Flow-cytometry analysis of LC-enriched EC suspensions confirmed the results obtained by fluorescence microscopy. In three experiments the EC failed to display any reactivity with the MoAb anti-Fcγ R1 (32) or anti-Fcγ RII (ION2) but showed positive staining with the MoAb anti-CD1a BL6 (52 ± 7%) and anti-Fcγ RII C1KM5 (27 ± 4%). Such reactivity of LC for the different MoAb anti-Fcγ R was also recently observed, by flow cytometry, by Romani et al [16]. Immunoprecipitation confirmed these findings (Fig 3); while no specific immunoprecipitate was noted with either the MoAb anti-Fcγ R1 (32) or the MoAb anti-Fcγ RIII (anti-Leu-1b), two MoAb anti-Fcγ RII (C1KM5 and IV.3) immunoprecipitated a protein of M, 40 kDa. Anti-Leu-11b precipitated small amounts of different proteins (M, 68, 52 and 27 kDa) which differed from the characteristic heterodisperse band of 50-70 kDa [8]. Thus, by incubating EC suspensions with human IgG sensitized RBC and with the MoAb anti-Fcγ R1 (32) or anti-Fcγ RIII (ION2 and anti-Leu-11b) —using a double immunofluorescence technique, flow-cytometric analysis, and immunoprecipitation—we were unable to demonstrate the presence of Fcγ RI or Fcγ RIII moieties at the LC surface. As we know that Fcγ R1 and Fcγ RIII are protease sensitive [9,17], and as to obtain EC in suspension we have to incubate the skin specimens with trypsin, we attempted to reveal the two Fc R on epidermal sheets obtained by suction blisters, without enzymatic digestion, and to observe their eventual reappearance after 24-48 h in culture.

Single Immunolabeling by MoAb Anti-Fcγ R of Epidermal Sheets
Single immunolabeling was performed using the APAAP method. Again only Fcγ RII (C1KM5) positive cells could be dem-
onstrated on epidermal sheets, no cell displaying reactivity with the
MoAb anti-Fcε RI (32) or anti-Fcε RIll (ION2).

Fate of Fcε R on Epidermal Cell Cultures LC-enriched EC
were cultured for 24 and 48 h, harvested, and stained with different
anti-Fcε R MoAb for flow-cytometric analysis. After 24-48 h of
culture, no reactivity with the MoAb anti-Fcε RI (32) or anti-Fcε RIll
(ION2) could be detected. Moreover, after 48 h in culture, no
reactivity could be observed any longer with the MoAb anti-Fcε RIll
CK5. This last result is in accordance with the recent observation of
Romani et al in humans [16] but differs from the results obtained
with mouse LC. Indeed, cultured epidermal mouse LC retain small
but detectable levels of Fcε RIll, detected using 125I-anti-Fcε RIll MoAb in quantitative binding studies [18].

All these observations strongly suggest that CD1a positive EC
express only the Fcε RIll. However, the presence of Fcε RIll at the
surface of freshly isolated LC is not observed on all CD1a positive
cells. Indeed, enumeration of the CD1a positive cells forming rosettes
or labeled by the MoAb CK5 (in double immunofluorescence or in
flow-cytometric analysis) shows that only about 50% of the
CD1a positive cells express the Fcε RIll. These numerical data
may be compared with the results of Moretta et al [19] and Bjerkel
et al [20]. Moretta et al [19] found that if 3 - 5% of the EC in
suspension are OKT6/ FITC positive, only 2% of the EC are rosetted.
Bjerkel et al [20] incubated skin cryosections with soluble immu
mune complexes and found that in situ only 48 ± 12% (n = 8) of
the CD1a positive cells bind the immune complexes. In these two
reports, as in the previous work of Stingl et al [12] and Schmitt et al
[21], the authors used rabbit IgG to coat either bovine [12,19] or
sheep [21] RBC or to form complexes with the antigen horseradish
peroxidase [20]. These results suggest that the Fc fragments of rabbit
IgG interact with Fcε RIll. In fact, such an interaction has been
demonstrated at the cell surface of eosinophils and neutrophils [8].
Moreover, recently Jung et al [22] provided evidence that bovine
IgG1 and certain preparations of sheep, goat, and bovine IgG in
teract with Fcε RIll of human monocytes/macrophages and polymor
phonuclear leucocytes.

In one of the ten cases studied in rosette assays (Fig 2) only 3% of
the LC formed rosettes, i.e., bound at least three RBC per LC. Obviously, some individuals express—at least at a given time—
almost no Fcε RIll on their LC. Conversely, we have observed in
atopic patients and in psoriasis, in lesional skin, an increased expres
sion of Fcε RIll [23]; Bieber T et al, manuscript in preparation). In
the epidermis, there thus appears to exist a modulation of the expres
sion of Fcε RIll at the surface of LC. This modulation could be
due at least in part to the keratinocytes which are known to synthe
size diverse cytokines able to modulate and modify LC antigenicity.
What could be the functions of Fcε RIll at the LC surface? On
human monocytes and neutrophils the Fcε RIll mediate cell
activation by immune complexes or opsonized antigens. Activation
results in the endocytosis of the immune complexes, in antibody
dependent cell-mediated cytotoxicity and in the release of inflamma
ry mediators and superoxide production. As yet nothing is known as
to the activation of Fcε RIll and the consequences of such an
activation for LC. However, as a first approach to this problem we
conjugated gold particles either with F(ab) fragments of the
MoAb anti-Fcε RIll IV.3 or with non-immune mouse IgG1
(Schmitt DA et al, manuscript in preparation). After having incu
bated these conjugates—which are multivalent as are immune
complexes and opsonized antigens—with unfixed LC, we observed
internalization of the conjugates by receptor-mediated endocytosis.
This receptor-mediated endocytosis involved coated pits, coated
vesicles, and endosomes. It resulted in labeling of the lysosomes, as
could be observed with Fc R-multivalent IgG complexes in monocytes/macrophages [24]. Thus LC are probably capable of internaliz
ing immune complexes, via their Fcε RIll, like monocytes/macrophages.

Fce R on HUMAN EPIDERMAL LANGERHANS CELLS

Epidermal and Dermal Langerhans Cells Bind IgE Mole
cules in Patients with atopic Dermatitis In 1986, Bruynzeel
Koemen et al [25] demonstrated the presence of IgE molecules on
epidermal LC. This demonstration was achieved under the light
microscope by double immunolabeling techniques, using anti
CD1a MoAb and monoclonal or polyclonal anti-human IgE anti
bodies, and under the electron microscope, by immunogold labeling
techniques. The anti-IgE staining of the LC disappeared after re
moval of cell-bound IgE by acid elution, and could be reconstituted
by incubating the skin sections with serum containing high IgE
levels but not with serum from normal controls. However, this
property of LC to bind IgE molecules was only observed in patients
suffering from atopic dermatitis (AD), with elevated IgE serum
levels, in involved (in acute and chronic skin lesions) and, although
to a much lesser extent, in uninvolved skin.

In 1987 Leung et al [26] and in 1988 Barker et al [27] observed the
presence of IgE molecules on dermal CD1a positive cells, in patients
having AD. More recently, Bieber et al [28] confirmed the presence
of IgE-bearing epidermal and dermal LC, in patients with AD hav
ing an elevated IgE serum level (> 300 U/ml). However, while
IgE-bearing dermal LC were present in involved as well as in uninv
olved skin, Bieber et al [28] “could not demonstrate a clearly posi
tive pattern in uninvolved epidermis” in contrast to the findings of Bruynzeel-Koemen et al [25]. Moreover, in lesional skin, vertical
serial cryosections and double immunolabeling with anti-CD1a and
anti-IgE mAb revealed that only 47 ± 11% (mean ± SEM, n = 40)
of the CD1a-positive epidermal cells were IgE positive. The num
ber of epidermal LC bearing IgE molecules progressively decreased
after one week of topical glucocorticoid therapy [28] and disappeared completely after two weeks treatment [29]. Thus, from all these studies a conclusion emerges: epidermal LC from patients with AD can bind IgE molecules. How could this fixation take place?

**Epidermal Langerhans Cells from Patients with Atopic Dermatitis Bear an Fcε R** To demonstrate the presence of an Fcε R at the surface of LC in AD, i.e., to prove that the IgE molecules are bound to LC via their Fc-fragments, Bruynzeel-Koomen et al. [14] used a rosette assay and characterized by immunolabelling techniques the fragments of the IgE molecules remaining at the LC surface after pepsin-digestion.

Pepsin-digestion significantly decreased the number of cells stained with anti-light chain antibodies, whereas the number of cells stained with anti-ε heavy chain remained unchanged. Thus the light chains, which are an integral part of the Fab'γγ fragment, are accessible to the enzyme and the only remaining fragments at the surface of the LC would be the heavy-chain fragments of Fc.

Rosette assay showed that epidermal CD1a positive cells form rosettes with fixed sheep erythrocytes coated with purified human myeloma IgE. This rosette formation is specific because it is significantly inhibited by preincubating the LC with IgE or with the MoAb BB10. Because the MoAb BB10 is “directed against the Fc receptor for IgE on human eosinophils, platelets and macrophages” [14], i.e., against Fcε RII, the inhibition of the rosette formation by BB10 strongly suggests that the Fcε R on LC is of the Fcε RII class. However, if it is indeed an Fcε RII it may be distinguished from “classical” Fcε RII by some unique properties [14]: i) a resistance to trypsin, ii) an absence of reactivity with the anti-CD23 mAb, and iii) an affinity for human IgG. Thus in AD the LC would have two types of Fcε R, one Fcε RII with low affinity for monomeric human IgG but no affinity for human IgE, and one Fcε RII with low affinity for both human IgE and IgG. Lastly, inhibition of the rosette assay with the anti-CD1a MoAb OKT6, and capping experiments with rhodamine-conjugated anti-ε antibody and fluorescein-conjugated anti-CD1a antibody suggest that on epidermal LC from patients with AD CD1a antigens and the Fcε R are associated.

We have seen that in AD LC are able to bind IgE molecules via their Fc fragments. This finding indicated that, in AD, LC are induced by synthesize Fcε R. The question arises whether distinct mediators may induce an expression of Fcε R on normal human LC.

**In Vitro, the Induction of the Fcε RII on Normal Human Langerhans Cells is Accompanied by the Release of IgE-BF** Recently, Bieber et al. [30] were able to induce the expression of Fcε RII on normal human LC. Starting from the fact that in uninvolved skin of patients with AD the anti-IgE-staining is very weak, but increases in involved T-cell infiltrated areas, they suspected that T-cell derived cytokines may be involved in the induction of the Fcε RII expression. As the LC are buried among keratinocytes, which themselves produce diverse cytokines, Bruynzeel-Koomen et al. [29] envisaged for the latter a possible regulatory role in the induction of the Fcε RII expression. Consequently, LC-enriched human EC were cultured for 8–36 h in the presence or absence of various concentrations of T-cell derived cytokines: hr IL-2, hr IL-4, and hr IFN-γ; and in the presence or absence of phorbol myristate acetate or keratinocyte-derived cytokines: hr IL-1, hr IL-3, hr IL-6, and hr granulocyte/macrophage-colony stimulating factor. Fcε RII expression was controlled at various times of culture by double-marker analysis using phycoerythrin-labeled anti-CD1a MoAb and anti-CD23 MoAb-coated erythrocytes. While LC-enriched EC either freshly isolated or cultured in media alone or in the presence of hr IL-2 or phorbol myristate acetate failed to react with anti-CD23 reagents, hr IL-4 as well as hr IFN-γ led to a gradual, dose-dependent emergence of CD1a/CD23 reactive cells (Fig 4). A combined use of varying concentrations of hr IL-4/hr IFN-γ showed that these cytokines act synergistically on CD23 expression leading, at maximum, to the expression of CD23 in about 50% of LC. Addition of cycloheximide to the cultures entirely abrogated the inducive capacities of the cytokines, which demonstrated that the appearance of Fcε RII on LC was due to the de novo synthesis of this molecule. The synthesized Fcε RII seems to be functional as it binds IgE molecules, this binding being prevented by preincubation of the LC with anti-CD23 MoAb. Addition of hr IL-1, IL-3, and IL-6 decreased the hr IL-4/IFN-γ- induced CD23 expression on LC, whereas hr granulocyte/macrophage-colony stimulating factor had no effect. These data suggest that T-cell-derived cytokines (IL-4, IFN-γ) induce Fcε RII/CD23 expression on normal human LC, while keratinocyte-derived cytokines (IL-1,3,6) may have a regulatory role in this phenomenon.

Fcε RII-bearing cells are potential sources of IgE-BF, they are released by autoproteolysis of the Fcε RII. Therefore, Bieber and co-workers [29,31] tested if the culture supernatants of hr IL-4/IFN-γ-stimulated Fcε RII positive LC was able to release, in vitro, IgE-BF. Using a radio immunoassay, they observed that while the culture supernatants of unstimulated LC or of stimulated but LC-depleted EC failed to contain IgE-BF, the supernatant of hr IL-4/IFN-γ-stimulated LC exhibited significant amounts of IgE-BF. These results indicate that hr IL-4 and hr IFN-γ not only induce the expression of Fcε RII on normal LC but also the release of IgE-BF.

All these in vitro results support the concept that in atopic disease, under distinct conditions, the expression of Fcε RII is induced on LC. The latter, after binding an allergen, may be able to activate T cells and provoke the eczematous lesions of AD. Moreover, LC may leave the epidermal compartment and reach the afferent lymph nodes, where they probably present the antigen to T-cells. Therefore, we may speculate that the Fcε RII-positive LC can participate in the lymph nodes in the regulation of IgE synthesis by producing IgE-BF.

**PROSPECTS**

As shown in this review on Fc R of human LC, many questions still remain unsolved. Thus concerning the Fcε RII, as already indicated, nothing is known as to the activation of this receptor and the consequences of such an activation for LC. Moreover, we do not know what mechanism leads to the disappearance of this receptor when LC are cultured. Is there in culture release of Fcε RII from the surface of LC — eventually by proteolysis of their extracellular portion — and liberation of IgG-BF? Such IgG-BF have been de-

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**Figure 4.** Induction of Fcε RII/CD23 on normal human LC. LC-enriched epidermal cells were cultured for 24 h in the presence of increasing concentrations of hr IL-4 and/or hr IFN-γ. The expression of Fcε RII was controlled by double-marker analysis using anti-CD23 MoAb-coated ox erythrocytes and FITC-labeled anti-CD1a MoAb. Results of a representative experiment are shown here.
scribed in the mouse [32], in which they are produced by T cells, activated B cells, and some macrophage and T-cell lines.

Concerning the Fcε R, it is still unclear which class of receptor is expressed by LC in patients suffering from AD. In particular, it is unclear why freshly isolated LC from patients with AD fail to react with anti-CD23 reagents, and why CD23 is inducible in vitro by IL-4 and/or IFN-γ. What role do these Fcε R play in the pathogenesis of AD? Do they enter into the processing of allergens? Recently [33], it was shown that there exists a second intracellular pathway for the cleavage of Fcε RI and the generation of IgE-BF. Hence, one can ask the question, "What level are the IgE-BF formed in LC?" Do these IgE-BF—which are "soluble" lectins—contain the "RBD" motif—influence, in skin, the cellular migration of LC or other cells?

Recent cDNA cloning studies [1,7] have shown that the Fcε RI actually comprises a family of at least three highly related receptors, designated Fcε RI-A, Fcε RI-B, and Fcε RI-C. While these three receptors are almost identical in their extracellular domains—which explains why all three bind aggregated IgE and react with the same specific anti-Fcε RI MoAb—Fcε RI-A and B differ from Fcε RI-C in their cytoplasmic domain. Therefore, it is possible that the Fcε RI-A and B on the one hand and the Fcε RI-C on the other hand may be associated with a different function (or spectrum of functions) previously thought to reflect the activity of a single Fcε RI molecule. If this were the case, it would be interesting to determine which receptor(s) is expressed by LC. A heterogeneity of Fcε RI has also recently been demonstrated [7]. Two forms have been isolated: Fcε RIAs and Fcε RIIb, which differ only at seven residues in the amino-terminal cytoplasmic region and share identical carboxy-terminal extracellular domains. Fcε RIAs is expressed in resting B cells, whereas Fcε RIIb is found on a variety of cells such as B cells, monocytes, and eosinophils following IL-4 induction. Again, what are the form(s) expressed by LC?

In the epidermis, 50% of CD1a positive cells express Fcε RI on their surface. The same proportion of epidermal LC fixed IgE in the lesional skin of AD [28] and in vitro, under the combined effect of hr IL-4 and IFN-γ, 50% of epidermal CD1a positive cells synthesize FceRII [30]. Lastly, Longley et al [34] have recently shown that about 50% of epidermal CD1a positive cells contain CD1a mRNA. While it has not yet been demonstrated that a unique population of LC is Fcε RI, Fcε RII, and CD1a mRNA positive, nevertheless one can speculate that there could exist in the epidermis two sub-populations of CD1a positive cells. One would be Fcε RI, Fcε RII, and CD1a mRNA positive, whereas the second would be Fcε RI, Fcε RII, and CD1a mRNA negative and would be, as we have suggested [35], more mature and immunostimulatory for allogeneic lymphocytes. Finally, it has been shown [36] that antibody enhanced infection by HIV-1 takes place via Fc receptor-mediated entry. Thus, it is not excluded that LC internalize HIV via their Fcε RII.

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