Gamma-Interferon Promotes the Release of IgE-Binding Factors (Soluble CD23) by Human Epidermal Langerhans Cells

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In a previous study, we have demonstrated that human epidermal Langerhans cells (LC) are induced to express FcεR2/CD23 by stimulation with IL-4 and/or IFN-γ. In this study, using LC-enriched and LC-depleted epidermal cell (EC) cultures, we have shown that stimulation with IL-4 and/or IFN-γ not only led to FcεR2/CD23 expression on normal human LC but also to the release of significant amounts of IgE-BF (soluble CD23). Furthermore, stimulation with IL-4 was more effective in induction of FcεR2/CD23 on LC when compared to IFN-γ, which, in contrast, strongly promoted the release of IgE-BF. These results indicate that FcεR2/CD23-positive LC represent a potential source of IgE-BF and that, as is observed in monocytes or U937 cells, IFN-γ and IL-4 differently regulate the FcεR2/CD23 expression and release on LC. J Invest Dermatol 97:600–603, 1991

The low-affinity receptor for the Fc fragment of IgE (FcεR2) is found on several subsets of lymphocytes, on monocytes, on eosinophils, and on platelets [1,2]. Recently, detailed analysis from the cDNA of FcεR2 indicated that two distinct subtypes, namely, FcεR2α (constitutively expressed by B cells) and FcεR2β (inducible by IL-4 on B cells and other hemopoietic cell types), may be distinguished by a few amino acid differences in the intracytoplasmic domain [3]. The extracellular domain of both receptor subtypes is identical and is cleaved probably by an autoproteolytic mechanism [4] into soluble 37-, 33-, and 25-kDa fragments named sCD23 or IgE-BF because they are still capable of binding IgE [5]. Thus, FcεR2α or FcεR2β expressing cells represent potential sources of IgE-BF.

Recently, we have shown that normal human epidermal Langerhans cells (LC), which do not express any FcεR, are induced to express FcεR2/CD23 upon stimulation with IL-4 and/or IFN-γ [6]. These findings prompted us to investigate whether LC may produce soluble CD23 in vitro. In the present study, it is shown that IL-4 and/or IFN-γ not only leads to the FcεR2/CD23-expression on LC but also to the release of significant amounts of IgE-BF.

Furthermore, although IL-4 induces the surface expression of FcεR2/CD23, IFN-γ preferentially promotes the release of IgE-BF by LC.

MATERIALS AND METHODS

Lymphokines Human recombinant interleukin 4 (hrIL-4) and human recombinant interferon-gamma (hrIFN-γ) were obtained from Genzyme Corporation (Boston, MA). Cycloheximide (CHX) was purchased from Sigma Chemical Co. (St Louis, MO).

Reagents Unlabelled monoclonal antibody (MoAb) BL6 (IgG1) from Immunotech (Marseille, France) and phycoerythrin (PE)-labeled T6/KD1 (IgG1) from Coulter (Krefeld, West Germany) are directed against CD1a, which is present in the epidermis only on LC. MoAb M-L25 (IgG1, Institute for Immunology, Munich) has been shown to react with CD23 as described previously [7]. Isotype controls were performed with mouse IgG1 from Sigma. Sheep anti-mouse IgG coated magnetic beads (SHAM/Dynal) (Dynal M-450) were obtained from Dynal (Oslo, Norway).

Preparation of Single Epidermal Cell Suspensions, Enrichment and Depletion of Langerhans Cells Epidermal cell (EC) suspensions were obtained by trypsinization of split-thickness specimens from normal human skin. LC enrichment by negative selection was performed as previously described [6]. To avoid heterogeneity in the results of independent experiments due to variations in LC enrichment (50–75%), uniformity between different culture conditions was reached by adding, after LC enrichment, autologous keratinocytes depleted in LC in order to adjust each culture condition to 50% LC enrichment. LC-depletion procedure was performed on freshly isolated EC by an indirect method using magnetic beads exactly as described in details elsewhere [8].

Culture of LC-Enriched and LC-Depleted EC One hundred fifty microliters of either 1 X 10^6/ml LC-enriched EC or 1 X 10^6/ml LC-depleted EC were cultured for 24 h in U-shaped bottom 96-well plates (Costar, Cambridge, MA) in Dulbecco’s modified Eagle’s medium (DMEM) (Flow Laboratories, Meckenheim, FRG) supplemented with 10% FCS, 1% antibiotics/antimycotics (Gibco, Berlin, FRG), 2 mM L-glutamine (Gibco), 25 mM Hepes buffer.

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Abbreviations:
CHX: cycloheximide
CSN: culture supernatant
DMEM: Dulbecco’s modified Eagle’s medium
FcεR2: type 2 Fc receptor for IgE
hrIFN-γ: human recombinant interferon gamma
hrIL-4: human recombinant interleukin 4
IgE-BF: IgE-binding factor
LC: Langerhans cells
MoAb: monoclonal antibody
SHAM/Dynal: sheep anti-mouse IgG coated magnetic beads

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**Table I.** Induction of FceR2/CD23 and Release of Soluble CD23 by hrIL-4- and/or hrIFN-γ-Stimulated EC and U937 Cells

<table>
<thead>
<tr>
<th>Cytokines (U/ml)</th>
<th>LC-Depleted EC</th>
<th>LC-Enriched EC</th>
<th>U937 Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%CD23 + LC</td>
<td>IgE-BF</td>
<td>%CD23 + LC</td>
</tr>
<tr>
<td>Nilh</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>IL-4 (10)</td>
<td>NT</td>
<td>NT</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>IL-4 (100)</td>
<td>0</td>
<td>ND</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>IL-4 (1000)</td>
<td>0</td>
<td>ND</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>IFN-γ (10)</td>
<td>NT</td>
<td>NT</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>IFN-γ (100)</td>
<td>0</td>
<td>ND</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>IFN-γ (1000)</td>
<td>0</td>
<td>ND</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>IL-4 (10) + IFN-γ (10)</td>
<td>NT</td>
<td>NT</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>IL-4 (100) + IFN-γ (100)</td>
<td>0</td>
<td>ND</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>IL-4 (1000) + IFN-γ (1000)</td>
<td>0</td>
<td>ND</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>IL-4 (1000) + IFN-γ (1000) + CHX</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

* U937 cells or EC either enriched (50%) or depleted in LC were cultured for 24 h with or without hrIL-4 and/or hrIFN-γ and cycloheximide (CHX) as indicated. Then the presence of FceR2/CD23 was assessed by double immunolabeling and culture supernatants were tested in triplicate by RIA for their content in IgE-BF (expressed in pg/ml). The results illustrate the data of three experiments (mean ± SD). (NT, not tested; ND, not detectable.)

(Received, Berlin, FRG), 5 mM sodium-pyruvate (Gibco), and 1% non-essential amino acids (Gibco) and in the presence or absence of hrIL-4 (10, 100, or 1,000 U/ml) and/or hrIFN-γ (10, 100, or 1,000 U/ml) and/or CHX (5 μM). According to previous results [6], the maximal concentrations of the cytokines are saturating doses with regard to the induction of FceR2/CD23 and release of IgE-BF on LC.

**Human Cell Line U937** The monoblast cell line U937 was kindly provided by Dr. H. L. Spiegelberg (Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA). This cell line constitutively expresses FceR2/CD23. The cells were maintained in culture with RPMI 1640 (Gibco) supplemented with 10% FCS, 1% antibiotics/antimycotics (Gibco), and 2 mM L-glutamine (Gibco).

**Immunolabeling by Double Marker Analysis** FceR2/CD23 expression on LC-enriched or LC-depleted EC was determined by double marker analysis with T6/RD1/PE and M-L25 coated ox-erthrocytes as previously described [6]. For U937 cells, CD23 expression was determined with M-L25 coated ox-erythrocytes alone. In each case, 2 X 10^6 CD1a-positive LC or U937 cells were counted and the percentage was calculated.

**Assessment of IgE-BF by RIA** IgE-BF in the EC or U937 culture supernatants (CSN) was measured exactly as previously described [9]. In all experiments, results are expressed in pg protein/ml. In order to compare the capacity of the cells to release IgE-BF under various culture conditions, we calculated the amount of IgE-BF produced on a per cell basis.

**RESULTS**

The culture supernatant (CSN) obtained under EC culture conditions described above were investigated for the presence of IgE-BF by a specific RIA and compared to the FceR2/CD23 expression obtained by double marker analysis after 24-h culture. CSN of hrIL-4- and/or hrIFN-γ-stimulated U937 cells (5 X 10^6 cells/ml) were used as a control.

Thus, although no FceR2/CD23-positive cells were detected in unstimulated LC-enriched EC cultures, only traces of IgE-BF (6.5 ± 2 ml) were found in these CSN. In contrast, significant amounts of IgE-BF were found in the CSN of LC-enriched EC stimulated with hrIL-4, where CD23/CD1a-positive cells were observed by double marker analysis. As for the presence of FceR2/CD23 reactive cells, the release of IgE-BF by hrIL-4-stimulated LC-enriched EC was dose dependent (Table I). In contrast, LC-depleted EC cultured in medium alone or stimulated with hrIL-4 and/or hrIFN-γ failed to display any FceR2/CD23-positive cells; the CSN of these conditions did not contain IgE-BF. This confirmed that the presence of soluble CD23 in CSN of EC is strictly related to the presence of LC.

Significant amounts of IgE-BF were also found in the CSN of hrIFN-γ-stimulated LC-enriched EC. However, compared to the data obtained with hrIL-4, the CSN of hrIFN-γ-stimulated LC-enriched displayed higher amounts of IgE-BF than expected by the analysis of FceR2/CD23 expression of LC in the very same conditions (Table I). Thus, the capacity to release IgE-BF calculated on a per-cell basis clearly indicates that the IgE-BF release by CD23-positive LC is significantly higher with IFN-γ than with hrIL-4 (Fig. 1). One should mention that the addition of hrIL-4 or hrIFN-γ did not alter the viability of LC when compared to medium alone. Because we did not add factors such as TNF-α or GM-CSF that sustain the viability of LC in vitro [10], the latter was decreased to 60% at 24 h. Consequently, studies after this time point were not feasible under such conditions and this fact has to be taken in account for the relative low amount of IgE-BF yielded from EC cultures.

The combined addition of hrIL-4 and hrIFN-γ was more effective in inducing IgE-BF release than either cytokine added alone. However, the additional effect of both mediators observed in the induction of FceR2/CD23 could not be reproduced at the level of

![Figure 1](#)

**Figure 1.** hrIFN-γ promotes the release of IgE-BF by LC and U937 cells. The release of IgE-BF was calculated on a per-cell basis (pg/CD23+ cell) for LC and U937 cells and comparatively presented here. The results illustrate the mean ± SD of three independent experiments.
the IgE-BF production (Fig 1). In all experiments, the addition of CHX completely inhibited the presence of FcεR2/CD23-positive cells and the production of IgE-BF.

Similar results were obtained on U937 cells in that the amounts of IgE-BF in the cultures with hrIFN-γ-stimulated cells were superior to that obtained with hrIL-4 for a comparable percentage of FcεR2/CD23-positive cells (Table I). Thus, the release on a per-cell basis obtained with U937 cells cultured in the presence of IFN-γ alone was, as with LC, clearly increased when compared to the addition of IL-4 alone (Fig 1). Furthermore, the combined use of both cytokines was less effective in the release of IgE-BF than expected by the additive induction of FcεR2/CD23 on U937 cells.

These findings strongly suggest that, on LC as well as on U937 cells, IL-4 and IFN-γ regulate the expression of CD23 and the release of soluble CD23 by different pathways.

DISCUSSION

Several studies of cell populations isolated from allergic individuals reported the existence of cytolytic IgE on large proportions of monocytes, alveolar macrophages, peripheral blood or tissue cosinophils, and platelets [2]. Similarly, although normal human LC do not bind IgE, recent findings showed that, in patients with atopic eczema, a subpopulation of epidermal LC express a specific receptor for IgE [11,12]. These findings indicate that IgE-bearing LC may play a pivotal role in the pathophysiology of this disease.

We have recently shown that normal human LC isolated from the skin of non-atopic patients are induced to express FcεR2/CD23 upon stimulation with IL-4 and/or IFN-γ [6].

In the present study, we extended the observations by examining the release of IgE-BF by unstimulated or IL-4 and/or IFN-γ-stimulated EC-cultures either enriched or depleted of LC. The results indicate that i) although after 24 h no FcεR2/CD23-positive cells were detectable in unstimulated LC-enriched EC, traces of IgE-BF are found in the CSM of these conditions; ii) in IL-4- and/or IFN-γ-stimulated LC-enriched EC, the FcεR2/CD23 expression on LC is accompanied by the release of IgE-BF in the CSM; and iii) IFN-γ preferentially enhances the release of soluble CD23 rather than the expression of surface FcεR2/CD23 on LC as well as on U937 cells that were used as a control.

The finding that CSM of unstimulated LC-enriched EC contain, at least, trace amounts of IgE-BF was surprising because no CD23/ CD1a-positive cells were detectable in either freshly isolated or in cultured EC. Furthermore, our data strongly imply that the presence of IgE-BF is strictly related to the presence of LC in the culture conditions. Hence, this would suggest that i) either unstimulated cultured LC have a spontaneous and limited capacity of releasing IgE-BF during their maturation in culture, ii) or resident LC “constitutively” express low amounts of FcεR2/CD23 (not detectable by immunohistochemistry) that are first altered due to the proteolytic treatment needed for the isolation procedure of LC and then transiently reexpressed during the recovery of the cells and finally released in vitro. Highly sensitive in situ immunoelectron-microscopic techniques will hopefully clarify this issue.

Most important, the further data clearly show that the CSM of IL−4- and IFN−γ-stimulated LC-enriched EC contain significant amounts of IgE-BF after 24-h culture. For each lymphokine, the effect is dose dependent and related to the presence of FcεR2/CD23-positive LC. Because we did not work with purified LC, it cannot be excluded that all or at least a part of the effect observed in LC-enriched EC is due to the induction of other mediators by the remaining keratinocytes, contributing in the FcεR2/CD23 expression and/or in the release of IgE-BF by LC. However, such an indirect effect seems unlikely because preliminary results of other experiments where we tested a series of cytokines known to be produced by keratinocytes, i.e., IL−1, IL−3, IL−6, TNF−α, and GM−CSF, show that none of these mediators was able to induce FcεR2/CD23 on LC or to up-regulate the release of IgE-BF (Bieber et al., manuscript in preparation). In contrast, all these mediators had more or less a down-regulating activity on the FcεR2/CD23 expression and on IgE-BF release by LC.

Although IL-4 is known to up-regulate FcεR2/CD23 on several types of cells, the effect of IFN-γ varies according to the cell type. On normal B cells [13], IFN-γ inhibits FcεR2/CD23 expression, whereas it has an enhancing effect on platelets [2] and, according to some studies, on U937 cells [6,14,15]. On monocytes as well as on U937 cells, IFN-γ was shown to increase the release of IgE-BF [15−17], this study) without necessarily increasing the expression of surface FcεR2/CD23 [17,18]. The dissociation between the enhancing effect of IFN-γ on the secretion of IgE-BF and its poor ability to up-regulate FcεR2/CD23 expression could be explained by an augmenting effect on the cleavage of FcεR2/CD23. Hence IFN-γ might accelerate the cleavage of surface FcεR2/CD23 or might increase the intracellular breakdown of this molecule into its secreted form, i.e., IgE-BF. In this study we show that the effect of IFN-γ on LC is similar to that on monocytes or U937 cells, i.e., little effect on FcεR2/CD23 expression but clear augmentation of the release of IgE-BF. On the other hand, our results suggest that the synergistic effect of IL-4 and IFN-γ on the FcεR2/CD23 expression may be correlated to an antagonistic activity of both cytokines with respect to the IgE-BF release. Indeed, with regard to the potent effect of IFN-γ on the IgE-BF on LC as well as on U937 cells, the addition of IL-4 to IFN-γ clearly inhibits the release of soluble CD23 (Fig 1). Hence, our results suggest a sensitive balance between expression and release of CD23 moieties regulated by IL-4 and IFN-γ.

The function of FcεR2/CD23 depends on the cell type on which it is expressed. Because LC are known to play a critical role in the generation of epidermal cell-driven T-cell responses and especially as antigen-presenting cells in allergic contact dermatitis, it has been proposed that in atopic eczema, FcεR2-positive LC may function similarly by fixing and presumably processing allergens via Fc-bound IgE [19]. As for monocyte-macrophages, IgE-dependent triggering may lead to the release of lysosomal enzymes, IL-1, or mediators of anaphylaxis as leukotrienes, prostaglandins, and platelet-activating factor (PAF). Due to the presence of a DGR sequence in the extracellular domain of FcεR2 [20], this structure may even play a role in cell adhesion of LC with keratinocytes. On the other hand, IgE-BF was recently shown to be a multifunctional molecule capable of regulating IgE-synthesis, of inducing, together with IL-1, the maturation of early thymocytes and myelocytes, and of modulating the proliferation of T cells and the migration of monocytes (reviewed in [20,21]). It is therefore most likely that the local production of IgE-BF by LC may play a role in the inflammatory response associated with atopic eczema and/or is involved in the regulation of LC-migration.

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