INDUCTION OF FceR2/CD23 ON HUMAN EPIDERMAL LANGERHANS CELLS BY HUMAN RECOMBINANT INTERLEUKIN 4 AND γ INTERFERON

BY THOMAS BIEBER,*§ ARMIN RIEGER,§ CSILLA NEUCHRIEST, JÖRG C. PRINZ,‡ ERNST P. RIEBER,‡ GEORGES BOLTZ-NITULESCU,‡ OTTO SCHEINER,‡ DIETRICH KRAFT,‡ JOHANNES RING,* AND GEORG STINGL§

From the *Department of Dermatology and the ‡Institute of Immunology, University of Munich, Federal Republic of Germany; and the §Department of Dermatology I, Division of Cutaneous Immunobiology and †Institute of Experimental Pathology, University of Vienna, Austria

Recently, IgE molecules have been demonstrated on Langerhans cells (LC) from involved and, to a much lesser extent, in uninvolved skin of patients with atopic dermatitis, but not on LC from nonatopic individuals (1, 2). This finding indicated that, in atopic dermatitis, LC are induced to either synthesize receptors for IgE and/or to acquire IgE-binding FcεR2 split products. As opposed to the selective expression of FcεR1 by basophils and mast cells, low affinity Fc-IgE receptors (FcεR2/CD23) have been described on numerous cell types including B and T cells, eosinophils, platelets, and monocytes (3, 4). The most recent finding that an anti-FcεR2 mAb can inhibit the binding of IgE-coated ox erythrocytes to LC from atopic dermatitis patients (5) supports the contention that the presence of IgE on LC surfaces in this disease results from the binding of IgE to FcεR2. For many of the above cells, FcεR2/CD23 expression is not a constitutive event but rather regulated by soluble mediators including human rIL-4 (hrIL-4; 6, 7), human rIFN-γ (hrIFN-γ; 6, 8), and PMA (8).

To establish an in vitro model for studying putative T lymphocyte-LC interactions in atopic dermatitis, we asked in this study whether these substances can induce FcεR2/CD23 expression on LC isolated from the epidermis of nonatopic individuals and, if so, whether such LC are capable of IgE binding.

Materials and Methods

Reagents. mAbs against CD1a antigen (phycoerythrin [PE]-labeled T6/RDI, IgG1, Coultertronics, Krefeld, FRG; FITC-labeled OKT6, IgG1, Orthopharmaceuticals, Raritan, NJ) were used for LC labeling (9). M-L25 (IgG1; Institute for Immunology, Munich, FRG) (10) and 3-5 (IgG1; kindly provided by T. Kishimoto, Institute of Molecular and Cellular Biology, University of Osaka, Japan) (11) are both anti-CD23 mAbs. BIP-1 (IgG1; Institute of General and Experimental Pathology, University of Vienna, Austria) (12), anti-IgM mAb AF-6 (IgG1; Immunotech, Marseille, France), and anti-IgG mAb 8a4 (10 μg/ml) (IgG1, Immunotech,

This work was supported by grant Bi 334/1-1 from the “Deutsche Forschungsgemeinschaft” (DFG) and by a grant from the Burgermeisterfonds der Stadt Wien. Address correspondence to Dr. Thomas Bieber, Dermatologische Klinik und Poliklinik der Ludwig-Maximilians Universität, Frauenlobstrasse, 9-11, 8000 München 2, Federal Republic of Germany.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/89/07/0309/06 $2.00

Volume 170 July 1989 309-314
Marseille, France) were used for control purposes. The IgE myeloma protein was purified by DEAE chromatography and used at a concentration of 10 μg/ml. FITC-labeled goat anti-mouse (GAM/FITC) was obtained from Grub Laboratories, Scandic, Vienna, Austria. FITC-labeled goat anti-human IgE (GAHuIgE/FITC) was purchased from Dakopatts, Hamburg, FRG.

**LC-enriched Human Epidermal Cells.** Cells were prepared as described elsewhere (Yokozeki, H., A. Rieger, C. Forster, A. Binder, V. Groh, and G. Stingl, submitted for publication). Briefly, split-thickness specimens of cadaver skin were subjected to 0.5% trypsin/PBS for 90 min at 37°C. The loosened epidermis was then peeled off and vigorously agitated in FCS (Gibco Laboratories, Berlin, FRG)-supplemented RPMI 1640 containing 0.01% DNase (Sigma Chemical Co., St. Louis, MO). After several washes, resulting single cell suspensions were incubated on collagen-coated petri dishes, which resulted in a preferential attachment of basal keratinocytes. Based on our previous observation that differentiating keratinocytes are more susceptible to osmotic shock treatment than other epidermal cells, nonadherent cells were then collected by vigorous pipetting and subjected to hypotonic PBS/Aqua destillata mixture for 1 min under mechanical agitation. Resulting cells (viability ~20%) were freed of dead cells by Ficoll-Hypaque density gradient centrifugation; interface cells recovered (viability >95% by trypan blue exclusion) were collected and the quality of LC-enrichment was controlled by FITC-OKT6. The possibility that other hematopoietic cells were substantially coenriched for by this negative selection procedure can be excluded by our previous finding that >98% of CD45+ cells in the final cell preparation coexpressed CD1a antigens (Yokozeki, H., et al., submitted).

**The FceR2/CD23+ Human Monoblastic Cell Line U937.** The U937 cell line was donated by H. L. Spiegelberg (Dept. of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA). This cell line reacts with M-L25 and 3-5 (Bieber, T., unpublished data).

**Cell Cultures.** 10^5 LC-enriched epidermal cells cultured for 8–48 h either in U-shaped bottom 96-well microtiter plates or in 24-well plates (10^6/ml) in RPMI 1640 (Flow Laboratories, Meckenheim, FRG) containing 10% FCS (Gibco Laboratories), 1% antibiotics/antimycotics (Gibco), 2 mM L-glutamine (Gibco), 25 mM Hepes buffer (Seromed, Berlin, FRG), 5 mM sodium-pyruvate ( Gibco), and 1% nonessential amino acids (Gibco). U937 cells were grown in RPMI 1640 supplemented with 10% FCS, 1% antibiotics/antimycotics, and 2 mM L-glutamine (all from Gibco Laboratories).

For the FceR2/CD23 induction assay, cells were cultured in either absence or the presence of one or several of the following substances: hrIL-2, hrIL-4, 1-1,000 IU/ml (Genzyme Corp., Boston, MA); hrIFN-γ, 1-1,000 IU/ml (Bender, Vienna, Austria); 2 ng/ml PMA (Sigma Chemical Co.); and 5 μM/ml cycloheximide (Sigma). As negative control experiments, the cells were cultured in medium without exogenous cytokines.

**LC Immunolabeling by Double-Marker Analysis.** LC-enriched epidermal cells were first stained with T6/RD1, then washed and reacted with ox erythrocytes coated with either M-L25 or 3-5, or as a control, with BIP-1 as described previously (13). Finally, the cells were resuspended and analyzed for viability, surface staining, and rosette formation using a Leitz Aristoplan fluorescence microscope. To verify the specificity of the rosette formation, an inhibition study was carried out by incubation of the epidermal cell cultures with unlabeled M-L25 or 3-5 (20 μg/ml) or isotype control at 4°C for 30 min. The cells were then washed twice in supplemented RPMI 1640 and incubated with anti-CD23 mAb–coated erythrocytes as described above.

**Determination of IgE Binding and FceR2/CD23 Expression on Cultured Cells.** After being cultured in the presence of cytokines, LC-enriched epidermal cells (5 × 10^6/ml) or U937 cells (5 × 10^6/ml) were washed twice in supplemented RPMI 1640 and were then incubated for 30 min with heat-inactivated AB serum. A small aliquot of this cell suspension was used to monitor by immunofluorescence CD23 expression by LC using M-L25 (20 μg/ml) and GAM/FITC in a consecutive order. A larger portion of the cultured cells was incubated with 10 μg/ml myeloma IgE for 45 min on ice, washed twice in PBS supplemented with 1% FCS and 0.1% sodium azide at 4°C and then incubated with GAHuIgE/FITC (10 μg/ml) for 45 min on ice. Controls included the use of GAHuIgE/FITC alone as well as F(ab')2 fragments of GAHuIgM/FITC and GAHuIgG/FITC (all obtained from Immunotech), respectively. In other experi-
ments, the cells were first incubated with M-L25 (20 μg/ml), or for control purposes with BIP-1, and then subsequently with myeloma IgE and GAHuIgE/FITC. After washing twice in PBS, the cells were analyzed by a FACScan (Becton Dickinson & Co., Sunnyvale, CA). The background was determined by the percentage of cells stained by the IgG1 isotype and GAM/FITC, or by GAHuIgE/FITC.

Results

LC Enrichment. Using the negative selection procedure for LC enrichment (see Materials and Methods), a starting population of 10⁹ epidermal cells containing 0.5–2% CD1a⁺ cells results in the generation of 5–10 × 10⁶ epidermal cells containing 50–70% CD1a⁺ cells (viability ≥95% by trypan blue exclusion).

Induction of Anti-FceR2/CD23 Reactivity of LC by hrIL-4 and hrIFN-γ. While LC-enriched epidermal cells either freshly isolated or cultured in media alone did not react with anti-CD23 reagents by either immunofluorescence or rosetting, hrIL-4 as well as hrIFN-γ led to a gradual, dose-dependent emergence of anti-CD23-reactive cells. Double-marker analysis revealed that CD23 expression was confined to the CD1a⁺ cells, i.e., to LC. Reactivity of LC with M-L25-coated ox erythrocytes occurred in a dose-dependent fashion, whereas 10 U/ml of each cytokine resulted in anti-CD23 reactivity of only 3 ± 1% (hrIL-4) and 2 ± 2% (hrIFN-γ) of LC, respectively, 1,000 U/ml of hrIL-4 and hrIFN-γ led to a specific anti-CD23 immunolabeling of 15 ± 7 and 8 ± 3% of LC, respectively (Table I). Similar results were obtained with 3-5-coated erythrocytes under the same conditions. A combined use of varying hrIL-4/hrIFN-γ concentrations showed that, at each combination chosen, these cytokines act synergistically on CD23 expression by LC (Table I) but only in an additive fashion on that of U937 cells (data not shown); maximal CD23 induction on LC was regularly seen with 1,000 U/ml of both cytokines. In this context, it should be noted that the cytokine concentrations needed for the induction of CD23 antigen on LC are either within the same range or only slightly higher than those needed for CD23 induction of B cells and U937 cells, respectively (6, 14). Kinetic studies revealed that, when using each cytokine (1,000 U/ml) either alone or in combina-

| Percent of FceR2/CD23⁺ LC using hrIFN-γ (U/ml) | 0 | 1 | 10 | 100 | 1,000 |
|-----------------------------------------------|---|---|----|-----|------|
| **hrIL-4**                                   |   |   |    |     |      |
| 0 U/ml                                       | 0 | 0 | 2 ± 2 | 6 ± 2 | 8 ± 3 |
| 1 U/ml                                       | 0 | 0 | ND | ND | ND |
| 10 U/ml                                      | 3 ± 1 | ND | 7 ± 3 | 10 ± 4 | 12 ± 3 |
| 100 U/ml                                     | 11 ± 3 | ND | 22 ± 5 | 32 ± 6 | 32 ± 5 |
| 1,000 U/ml                                   | 15 ± 7 | ND | 25 ± 6 | 35 ± 7 | 47 ± 6 |

LC enriched (50%) epidermal cell suspensions were stimulated with various combined concentrations of hrIL-4 and hrIFN-γ. After 24 h, the cultures were assessed by double-marker analysis with T6/RD1 and M-L25-coated ox erythrocytes (mean ± SE of three experiments).
tion, substantial CD23 expression by LC was first detectable after 16 h and peaked after 24 h. Time course studies beyond this point were hardly feasible because of the rapidly declining viability of the cells (<20% after 48 h of culture). Our further observation that the addition of cycloheximide to the cultures entirely abrogated the inductive capacities of the cytokines used either alone or in combination, shows that the cytokine-induced CD23 expression on LC is due to the synthesis of this antigen rather than due to unhiding of preformed moieties.

As opposed to hrIL-4 and hrIFN-γ, hrIL-2 (100 and 200 U/ml) and PMA failed to induce CD23 antigens on any epidermal cell. Comparative studies with U937 cells which are known to constitutively exhibit FceR2/CD23 revealed a mean baseline expression of CD23 expression on 17 ± 4% of the cells and an increase of the expression after 24 h of culture with either hrIL-4 (58 ± 10%) or hrIFN-γ (49 ± 8%). While IL-2 was again ineffective in CD23 induction, PMA, in contrast to LC, greatly upregulated CD23 expression (90 ± 5% of cells).

FceR2/CD23+ LC Bind IgE Molecules. To see if the FceR2/CD23+ LC were able to bind IgE molecules, LC-enriched epidermal cells that had been stimulated with 1,000 U/ml of both hrIL-4 and hrIFN-γ were successively incubated with myeloma IgE and anti-IgE/FITC and then analyzed by flow cytometry. These experiments revealed a positive staining of 23 ± 4% of the LC (Fig. 1). This percentage was less than that obtained by flow cytometry with the anti-CD23 reagent (40 ± 6%) and probably due to the low affinity of the receptor. When preincubation with M-L25 was followed by myeloma IgE, no IgE binding could be demonstrated on cultured LC-enriched epidermal cells or U937 cells.

Discussion

In this study, we have shown that (a) freshly isolated human epidermal cell from nonatopic donors greatly enriched for CD1a+ LC fail to react with anti-CD23 mAb as assessed by indirect immunofluorescence and rosette assays, respectively; (b) a percentage of the LC exhibit anti-FceR2/CD23 reactivity when cultured for 24 h in the presence of hrIL-4 and/or hrIFN-γ, but not when cultured in the presence of hrIL-2, PMA, or culture medium alone, and that this hrIL-4/hrIFN-γ-induced emergence of anti-FceR2/CD23 immunolabeling of LC can be prevented by adding cycloheximide to the culture media; (c) a considerable percentage of LC when cul-
tured in the presence of hrIL-4/hrIFN-γ, but not when cultured in media alone, bind IgE molecules and that this binding can be prevented by preincubation of the cells with anti-FcεR2/CD23 mAb.

The key observation made in this study, i.e., the triggering of anti-FcεR2/CD23 moieties on LC by hrIL-4/hrIFN-γ, can be either explained by the induction of biosynthesis of these structures, or alternatively, by the unmasking or exocytosis of preformed proteins. The prevention of the cytokine-induced appearance of anti-FcεR2/CD23-reactive LC by cycloheximide proved the validity of the former assumption. Epidermal cell suspensions used in our study were greatly enriched for LC, but still contained a large number of keratinocytes, a putative source of cytokines (15). The cytokine-driven synthesis of anti-FcεR2/CD23-reactive structures by LC may therefore either be due to a direct effect of hrIL-4/hrIFN-γ on LC, or alternatively, due to keratinocyte activation by these cytokines leading to the production/release of other mediators responsible for the observed phenomenon.

In many cell types investigated, pronounced differences exist between hrIL-4 and hrIFN-γ in their capacity to modulate FcεR2/CD23 expression (6-8). One of the few cell types exhibiting reaction patterns to these cytokines similar to LC, i.e., upregulation of anti-FcεR2/CD23-reactive structures by both hrIL-4 and hrIFN-γ, is the monoblastic cell line U937 (14; this study). In view of the limited availability of purified human LC, this cell line may thus serve as a useful model to study the molecular mechanisms underlying this phenomenon. Limitations in cell number are also the reason why we were not able to characterize the exact molecular configuration of anti-FcεR2/CD23-reactive moieties (16) on hrIL-4/hrIFN-γ-stimulated LC. However, we strongly feel that these antibody-reactive structures actually represent functional FcεR2, as evidenced by the IgE-binding capacity of hrIL-4/hrIFN-γ-stimulated LC and its inhibition by the M·L25 or 3·5 reagent.

Although data presented in this study suggest that secretory products of either circulating or infiltrating T cells are responsible for the in vivo induction of FcεR2 in atopic dermatitis patients, the possibility still exists that functional alterations of resident skin cells contribute to the occurrence of this event. In this context it should be noted that anti-IgE+ LC have not been detected in noneczematous atopic patients (1, 2). This may indicate that the presence of FcεR2-bound IgE molecules on LC surfaces in atopic dermatitis patients is not simply a functionally irrelevant bystander phenomenon of atopy itself but is rather involved in the pathogenesis of the eczematous process.

The experimental model presented in this study will allow us to investigate the molecular aspects of native IgE or IgE-antigen complexes binding to LC-FcεR2, as well as the effects of this interaction on the function of this cell type.

Summary

Human rIL-4 and human rIFN-γ are able to induce the expression of the low affinity receptor for IgE (FcεR2/CD23) on normal human epidermal Langerhans cells, whereas IL-2 and PMA have no effect. A synergistic effect is observed when both cytokines are combined. These receptors are synthesized de novo by the LC since cycloheximide completely inhibits the appearance of FcεR2/CD23. FcεR2/CD23+ LC may have a major role in the pathogenesis of atopic eczema, as well as in the regulation of IgE synthesis.

Received for publication 7 February 1989 and in revised form 24 April 1989.
References

1. Bruynzeel-Koomen, C., D. F. van Wichen, J. Toonstra, L. Berrens, and P. L. B. Bruynzeel. 1986. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. Arch. Dermatol. Res. 278:199.

2. 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. In press.

3. Spiegelberg, H. L. 1984. Structure and function of Fc receptors for IgE on lymphocytes, monocytes and macrophages. Adv. Immunol. 35:61.

4. Capron, A., J. P. Dessaint, M. Capron, M. Joseph, J. C. Ameison, and A. B. Tonnel. 1986. From parasites to allergy: a second receptor for IgE. Immunol. Today. 7:15.

5. Bruynzeel-Koomen, C., E. M. van der Donk, P. L. B. Bruynzeel, M. Capron, G. C. de Gast, and G. C. Mudde. 1988. Associated expression of T6 antigens and Fc-receptors for IgE on epidermal Langerhans cells from patients with atopic dermatitis. Clin. Exp. Immunol. 74:137.

6. Defrance, T., J. P. Aubry, F. Rousset, B. Vanbervliet, J. Y. Bonnefoy, N. Arai, Y. Takebe, T. Yokota, F. Lee, K. Arai, J. de Vries, and J. Blanchereau. 1987. Human recombinant interleukin-4 induces Fce receptors (CD23) on normal human B lymphocytes. J. Exp. Med. 165:1459.

7. Vercelli, D., H. H. Jabara, B. W. Lee, N. Woodland, R. S. Geha, and D. Y. M. Leung. 1988. Human recombinant interleukin 4 induces FceR2/CD23 on normal human monocytes. J. Exp. Med. 167:1406.

8. Mayumi, M., T. Kawabe, K. M. Kim, T. Heike, K. Katamura, J. Yodoi, and H. Mikawa. 1988. Regulation of Fce receptor expression on a human monoblast cell line U 937. Clin. Exp. Immunol. 71:202.

9. 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. 78:2544.

10. Prinz, J. C., and E. P. Rieber. 1987. Fc-Receptors for IgE on human lymphocytes. Detection with a rosetting assay using a recombinant human/mouse IgE antibody and characterization with monoclonal antibodies. Hybridoma. 6:115.

11. Suemura, M., H. Kikutani, E. L. Barsumian, Y. Hattori, S. Kishimoto, R. Sato, A. Maeda, H. Nakamura, H. Ohaki, R. Hardy, and T. Kishimoto. 1986. Monoclonal anti-Fc epsilon receptor antibodies with different specificities and studies on the expression of Fc epsilon receptors on human B and T cells. J. Immunol. 137:1214.

12. Jarolim, E., H. Rumpold, A. T. Endler, G. Schierka, H. Ebner, O. Scheiner, and D. Kraft. 1989. Specificities of IgE and IgG antibodies in patients with birch pollen allergy. Int. Arch. Allergy Appl. Immunol. In press.

13. Wilhelm, M., H. Pechumer, G. Rank, E. Kopp, G. Riethmüller, and E. P. Rieber. 1986. Direct monoclonal antibody rosetting. An effective method for weak antigen detection and large scale separation of human mononuclear cells. J. Immunol. Methods. 90:89.

14. Kawabe T., M. Takami, M. Hosoda, Y. Maeda, S. Sato, M. Mayumi, H. Mikawa, K. I. Arai, and J. Yodoi. 1988. Regulation of FcR2/CD23 gene expression by cytokines and specific ligands (IgE and anti-FcR2 monoclonal antibody). J. Immunol. 141:1376.

15. Kupper, T. S. 1988. Interleukin 1 and other human keratinocyte cytokines: Molecular and functional characterization. Adv. Dermatol. 3:293.

16. Yokota, A., H. Kikutani, T. Tanaka, R. Sato, E. L. Barsumian, M. Suemura, and T. Kishimoto. 1988. Two species of human Fc receptor II (FcεR2/CD23): tissue-specific and IL-4-specific regulation of gene expression. Cell. 55:611.