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Expression, but lack of calcium mobilization by high-affinity IgE Fcε receptor I on human epidermal and dermal Langerhans cells

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Abstract: In atopic dermatitis (AD) patients, IgE molecules are demonstrated on the surface of Langerhans cells (LC). FcεRI molecules, which are present on the surface of LC in AD patients as well as normal individuals, are responsible for this binding. In this study, we have investigated phenotypic and functional characteristics of FcεRI on epidermal and dermal cell populations. Epidermal and dermal cell suspensions were prepared enzymatically with dispase followed by either trypsin or collagenase treatment, respectively. Peripheral blood basophils were negatively selected by excluding other leukocytes with surface marker staining. Consistent with previous reports, both peripheral blood basophils and epidermal LC were positively stained with anti FcεRI monoclonal antibody. In addition, an FcεRI positive population was demonstrated among dermal HLA-DR positive cells. These cells express significant amounts of HLA-DR molecules (DR^{Hi}) and co-express CD1a molecules, which identifies them as LC-like dendritic APC of the dermis. No other FcεRI positive population was found in the other dermal DR^{Mid} or DR^{-} populations, except for a minor DR^{Lo} population, presumably mast cells. To analyze whether these FcεRI molecules are signal transducing for LC, intracellular calcium mobilization after crosslinking of FcεRI was measured with flow cytometry. Following crosslinking, peripheral blood basophils clearly increased intracellular calcium. On the other hand, neither normal epidermal LC nor dermal $DR^{Hi}CD1a^{+}$ cells changed their intracellular calcium level after FcεRI crosslinking. These data indicate that normal epidermal and dermal LC, but not basophils, are resistant to calcium flux following FcεRI engagement.

A. Shibaki[1,2], A. Ohkawara[2], S. Shimada[3], C. Ra[4], S. Aiba[5] and K. D. Cooper[6]
[1]Immunodermatology Unit, University of Michigan, Ann Arbor, USA; [2]Department of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan; [3]Department of Dermatology, Yamanashi Medical University, Kofu, Japan; [4]Department of Immunology, Juntendo University, Tokyo, Japan; [5]Department of Dermatology, Tohoku University, Sendai, Japan; [6]Department of Dermatology, University Hospitals of Cleveland, Case Western Reserve University, and VA Hospital, Cleveland, USA

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Akihiko Shibaki, Department of Dermatology, Hokkaido University School of Medicine, Kita 15 Nishi 7, Kita-ku, Sapporo, 060 Japan.
Tel.: +81 11 716 1161, ext. 5962
Fax: +8111 706 7820
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Introduction

Epidermal Langerhans cells (LC) are bone-marrow-derived antigen-presenting cells (APC) (1) which are critical for the induction of T-cell mediated cutaneous immune responses, in particular contact hypersensitivity (2, 3). In addition, LC are thought to play an important rôle in the pathophysiology of T-cell-driven chronic inflammatory diseases such as atopic dermatitis (AD) (4, 5). The association of respiratory allergy, high serum IgE and the presence of IgE molecules on the surface of LC in AD patients (6) allowed the formation of a hypothesis which links respiratory allergen-directed IgE to cell-mediated responses in the skin of AD patients (5). High affinity receptors for IgE molecules, FcεRI, are present on the surface of LC in AD patients, as well as normal individuals, and are responsible for binding of IgE to LC (7, 8). However, the biological importance of this receptor in human skin still remains to be elucidated. One rôle could be antibody-facilitated antigen processing, whereby IgE on the surface of an APC can serve to capture an antigen for subsequent endocytosis (9), proteolytic cleavage, and binding to class II MHC molecules for surface display to antigen-reactive T cells (10, 11). Fc IgE receptors in the skin may play an even broader rôle, because in certain cell types, FcεRI crosslinking can cause signal transduction (12, 13). Therefore, we investigated FcεRI on epidermal LC, dermal $CD1a^{+}$

LC-like dendritic APC, and on non-LC FcεRI+ dermal cells in human skin, as compared to peripheral blood basophils.

Material and methods

Preparation of epidermal and dermal cell suspensions

Skin was obtained from normal adult volunteers by keratome biopsy after informed consent. Both epidermal and dermal cell suspensions from normal human skin keratomes were prepared by the method previously described (14). Briefly, epidermal sheets were separated from dermis after overnight incubation with dispase (50 U/ml) (Collaborative Research, Waltham, MA), trypsinized, teased into a cell suspension, and filtered through a nylon mesh to achieve a single cell suspension. Disaggregated cells were resuspended with appropriate media for the following procedures. In certain experiments, in order to obtain sufficient flow of LC for the experiments measuring intracellular calcium mobilization, LC were enriched by Ficoll centrifugation (15, 16). After separation from the epidermis, dermal sheets were digested in an enzyme solution containing RPMI 1640 (Whittaker Biomedicals, Walkersville, MD), supplemented with collagenase (200 U/ml), hyaluronidase (200 U/ml), 0.01% deoxyribonuclease (Sigma, St. Louis, MO), HEPES (10 nM), and sodium pyruvate (1 mM) (Irvine Scientific, Santa Ana, CA) for 3 to 4 h at 37°C. The disaggregated dermal cells (DC) were filtered through 112 and 50 μM sterile mesh nylon gauze to separate the non-digested tissue and hair follicles from the cell suspension (17).

Enrichment of peripheral blood basophils

Basophils (BPH) were prepared from normal human blood essentially as previously described (18). Briefly, peripheral blood was drawn from normal healthy volunteers and basophils from EDTA-anticoagulated blood were enriched by a discontinuous gradient (specific gravity: 1.070 and 1.079) centrifugation using Percoll (Pharmacia, Uppsala, Sweden).

Analysis of cell surface antigens

The expression of surface antigens was measured by direct and indirect immunofluorescence staining with appropriate monoclonal antibodies (MoAb), followed by quantitative analysis by flow cytometry. After the cell preparation, each sample was incubated with heat-aggregated (65°C×20 min) purified human IgG (Sigma) for 30 min at 4°C. For EC staining, the cells were stained with PE-conjugated anti HLA-DR (Becton Dickinson, Mountain View, CA, USA) and anti-FcεRIα MoAb, AER24 (19), followed by FITC conjugated goat anti-mouse IgG1 (Boehringer Mannheim, Indianapolis, IN). For DC staining, the cells were first stained with AER24 and PE-conjugated anti HLA-DR), followed by the FITC-conjugated goat anti mouse IgG1. For basophil staining, cells were stained with AER24 and a cocktail of FITC-conjugated MoAbs against CD2, CD14 (Becton Dickinson), CD16 (MEDAREX, INC. West Lebanon, NH) and B1 (Courter Immunology, Hialeah, FL), followed by PE-conjugated goat anti mouse IgG1 (Boehringer Mannheim). Normal human serum (10%) and normal goat serum (10%) were supplemented for each step to block nonspecific binding. After the complete staining steps, the cells were fixed in PBS with 1% paraformaldehyde.

Immunohistochemical staining

Normal human skin was obtained by punch biopsy from volunteers after informed consent and frozen immediately. The tissue sections were double stained with FITC-conjugated anti-HLA-DR (Becton Dickinson) and biotinylated anti-FcεRI monoclonal antibodies, followed by visualization with RITC-streptavidin.

Cell preparation for intracellular calcium measurement by flow cytometry

Cell suspensions were resuspended at 1×10^6cells/ml in PBS and 1% FBS containing 1 μM indo–1 AM (Molecular Probes, Eugene, OR), and incubated for 30 min at 30°C in the dark. After the incorporation of indo-1, epidermal LC and dermal APC were marked with PE-conjugated anti-HLA-DR or a cocktail of FITC-conjugated anti HLA-DR and PE-conjugated anti-CD1 a (Pharmingen, San Diego, CA), respectively, by incubating for 30 min at room temperature. For basophils, a cocktail of FITC conjugated anti-CD2, -CD14, -CD16 and–B1 was used in place of PE-conjugated anti-HLA-DR to negatively select basophils by removing T cells, monocytes, NK cells and B cells, respectively, from analysis. Simultaneously, MoAb for FcεRI were bound to the appropriate surface molecules for subsequent crosslinking. 1.0×10^6cells were used for each experiment.

Intracellular calcium measurement by flow cytometry

Intracellular calcium measurements were performed with an Epics Elite flow cytometer (Courter Cytometry, Hialeah, FL), equipped with an air-cooled ar-

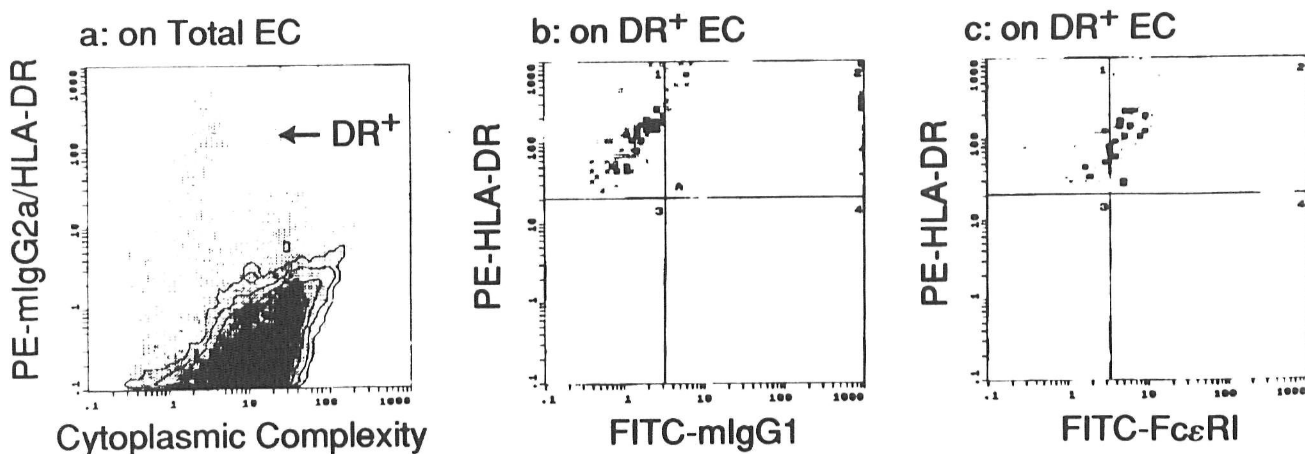


Figure 1. Human epidermal LC express high-affinity IgE Fc receptor, FcεRI. Human epidermal LC were selected by their HLA-DR expression (a - dot plot) relative to the isotype control staining (a - contour plot). DR$^+$ epidermal LC were positively stained with anti-FcεRI monoclonal antibody (panel c) compared with its isotype control staining (panel b).

gon laser for PE excitation at 488 nm, and an air cooled UV-laser for the excitation of indo-1 at 350 nm. Intracellular calcium levels were measured by calculating the ratio of fluorescence intensity at 2 different emission wave lengths of indo-1; calcium bound form (violet: 405 nm) and unbound form (blue: 485 nm). As intracellular calcium increases, the violet/blue ratio of indo-1 emission wave lengths (indo–1 ratio) increases. After selecting LC and dermal cell subsets by PE fluorescence of their HLA-DR or basophils by gating out lymphocytes and monocytes, the baseline indo-1 ratio was measured for 30 s before the addition of the stimuli and thereafter for up to 400 s. After establishing baseline indo-1 fluorescence, goat anti-mouse IgG1 (Caltag Laboratories Inc., South San Francisco, CA) was added to crosslink the anti-FcεRI which was allowed to bind to the high affinity IgE Fc receptor. Cells were kept at 37°C using a circulating water bath during the real-time data acquisition. All data was stored in Listmode, down-loaded onto optical disks (Mitsubishi), and analyzed using both Elite software version 3 (Courter) and Multi Time software (Phoenix flow Systems, Inc., San Diego, CA).

Results

Expression of FcεRI on cell subsets from normal human epidermis, dermis and peripheral blood

Epidermal Langerhans cells were selected on the basis of HLA-DR expression (dot plot) above isotype background (contour plot) (Fig. 1a); selected LC clearly exhibited FcεRI on their surface above background (Fig. 1c. versus b) (representative of findings in 3 subjects). To determine whether dermal FcεRI expression (7) might be consistent with the dermal LC-like dendritic APC population in human dermis as well as on mast cells (14), we costained normal human skin with anti-HLA-DR and anti-FcεRIα (AER24). Indeed, in 3/3 subjects

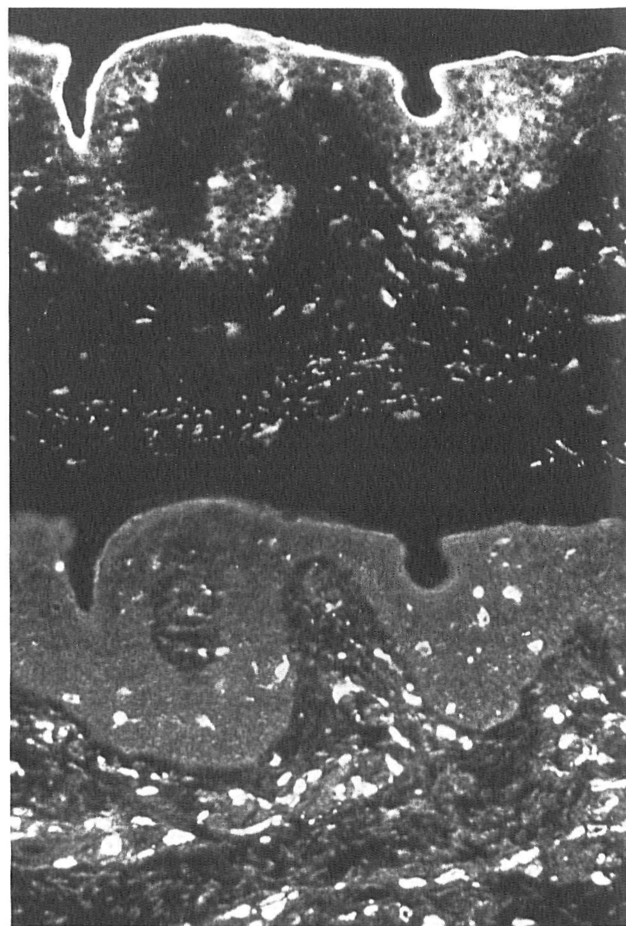
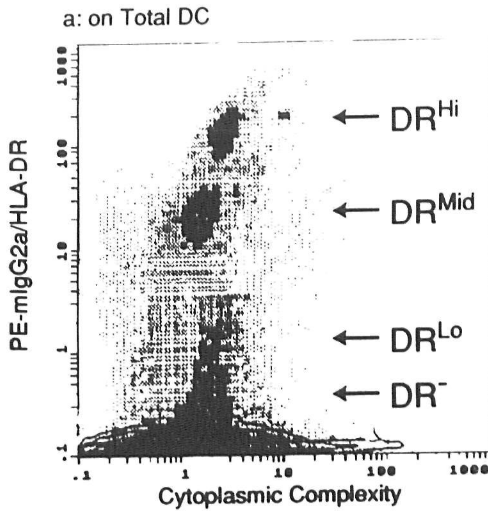


Figure 2. Immunofluorescence localization of FcεRI in normal human skin. Punch biopsied normal adult human skin was double stained with anti-HLA-DR (upper panel) and anti- FcεRI (lower panel) monoclonal antibodies.



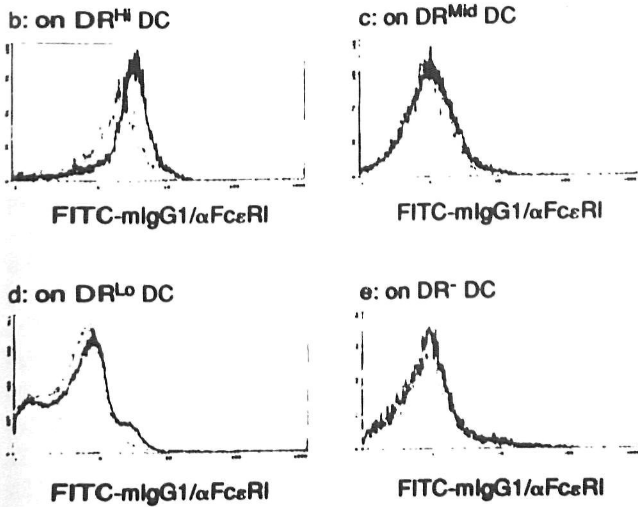


Figure 3. FcεRI expression by human dermal cell subsets. Normal human dermal cells were subdivided into 4 groups (DR^{Hi}, DR^{Mid}, DR^{Lo} and DR^{-}) by their HLA-DR expression (a - dot plot) compared with isotype control staining (a - contour plot). FcεRI expression in each cell subset (b to e - bold line) were compared with its isotype control (b to e - dotted line).

tested, non basophil (DR^{+}) dermal cells co-stained with both markers (Fig. 2). To more precisely define this subset, we utilized flow cytometry. DC were subdivided into DR^{Hi}, DR^{Mid}, DR^{Lo} and DR^{-} subsets by their HLA-DR expression (Fig. 3a). FcεRI expression relative to isotype control antibody binding was then compared for each DC subset (Fig. 3). The DR^{Hi} subset, which we have previously demonstrated to contain the dermal LC-like dendritic population with potent APC activity (14), stained positively for FcεRIα (Fig. 3b). By contrast, DR^{Mid} (monocytes/macrophage) (20) and DR^{-}(fibroblast-containing) (14) DC did not stain with AER24 (Fig. 3, *c*, e). However, a minor population of DR^{Lo}DC, which appears to contain mast cells, some endothe-

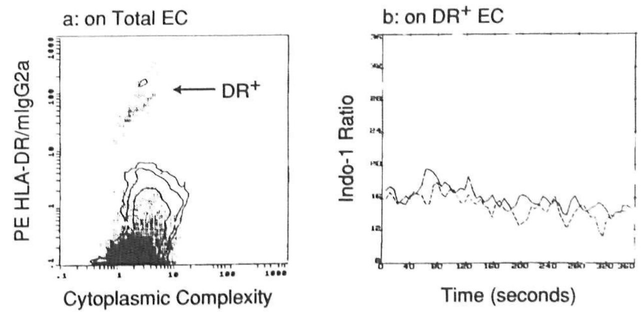


Figure 4. Normal human epidermal LC did not cause calcium flux after FcεRI crosslinking. Epidermal LC were pre-incubated with either monoclonal antibodies to the FcεRI (b - bold line) or its isotype control (b - dotted line), selected by their HLA-DR expression (a - dot plot) compared with its isotype control (a - contour plot) and stimulated by adding goat anti-mouse IgG1 to crosslink FcεRI. LC did not show significant calcium flux after crosslinking (panel b).

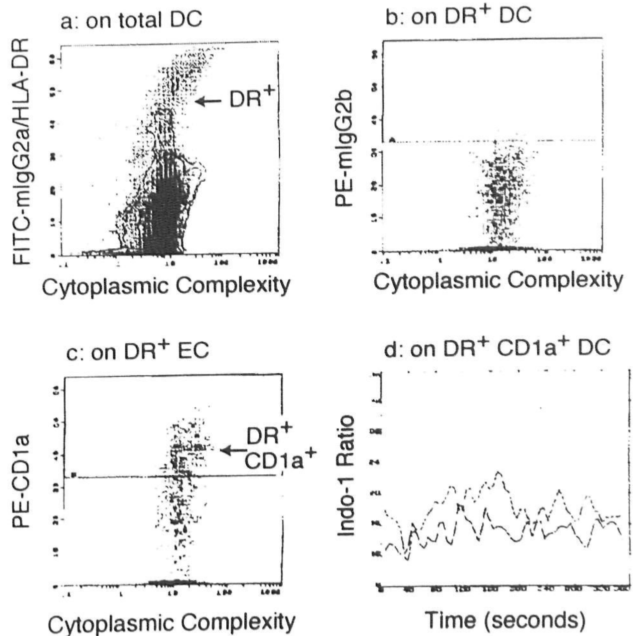


Figure 5. Dermal DR^{+} $CD1a^{+}$ LC-like dendritic APC failed to cause calcium flux after FcεRI crosslinking. Dermal cells were pre-incubated with either anti-FcεRI MoAb (d; bold line) or its isotype control (d: dotted line). LC-like dendritic APC were selected by their HLA-DR (a - dot plot) and CD1 a (panel c) expression compared with their isotype control (a - contour plot and panel b, respectively). No calcium flux was induced by FcεRI crosslinking in this cell population (panel d).

lial cells, and non-bone marrow-derived stromal dendrocytes (14), did stain with FcεRI (Fig. 3d).

Neither epidermal LC nor dermal LC-like dendritic APC exhibit calcium flux after FcεRI crosslinking

Triggering of FcεRI on mast cells and basophils is known to cause sequential signal transduction

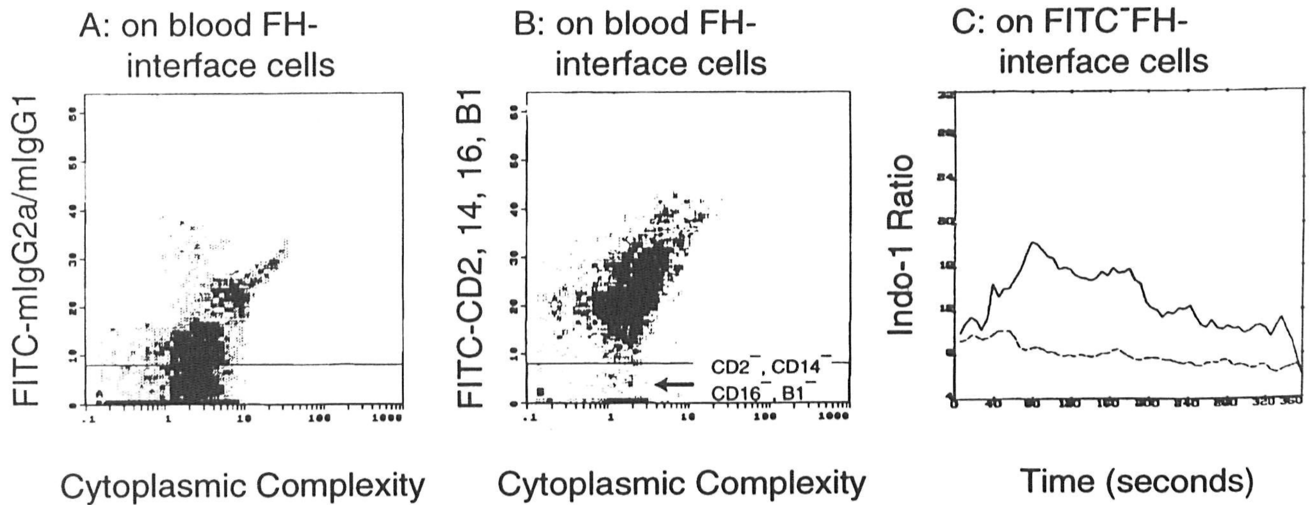


Figure 6. Human peripheral blood basophils cause calcium flux after crosslinking FcεRI. Peripheral blood basophils were negatively selected by staining basophil enriched peripheral blood cells with FITC-conjugated anti-CD2, CD14, -CD16 and B1 (panel B) relative to their isotype control (panel A). FcεRI pre-incubated basophils (C: bold line) increased intracellular calcium compared with isotype control antibody pre-incubated cells (C: dotted line), after the addition of goat anti-mouse IgG1 to crosslink FcεRI molecules.

events, including intracellular calcium mobilization and tyrosine phosphorylation, resulting in degranulation and release of chemical mediators such as histamine and serotonin. Because quantities of fresh LC for biochemical extraction are limited, functional properties of FcεRI on epidermal and dermal LC on signal transduction events after receptor crosslinking were accessed by flow cytometry. Epidermal LC were selected by their HLA-DR expression (Fig. 4a), and their FcεRI was crosslinked with goat anti mouse IgG1 30 s after initiation of flow cytometric analysis of intracellular calcium levels by the calcium indicator, indo-1. Although DR^+cells in both EC and DC flux calcium in response to calcium ionophore (21) (not shown), epidermal LC intracellular calcium was not significantly modified by the FcεRI crosslinking (Fig. 4b) in 5 of 5 subjects tested. To determine whether epidermal LC are uniquely unable to undergo calcium flux to FcεRI crosslinking, whereas dermal LC-like dendritic APC might be more responsive, dermal LC-like dendritic APC were selected as the DR^+CD1a^+DC subset (Figs. 5a-c) and were triggered by FcεRI crosslinking as described for epidermal LC. Again, in 3 of 3subjects tested, no significant calcium mobilization was induced by FcεRI crosslinking (Fig. 5d), despite that these cells could flux in response to ionophore (not shown). On the other hand, peripheral blood basophils, which were negatively selected as the $CD2^-$, $CD14^-$, $CD16^-$ and $B1^-$ cell population (Figs. 6a, b), quickly increased their intracellular calcium level and reached a peak at 60 s after FcεRI crosslinking (Fig. 6c). Insufficient numbers of DR^{Lo} dermal cells expressing FcεRI were available for analysis of crosslinking.

Discussion

2 types of IgE Fc receptors have so far been described in humans. These 2 receptors are structurally different and have distinct affinity for IgE molecules. The low-affinity Fc receptor, FcεRII (CD23), has more broad cell distribution than FcεRI (22). The high-affinity Fc receptor for IgE, FcεRI, has limited distribution to the surface of peripheral blood basophils, tissue mast cells (23) and epidermal Langerhans cells (7, 8).

In this study, we have confirmed the presence of FcεRI molecules on the surface of epidermal LC and have identified FcεRI expression on 2 subsets in human dermis, DR^{Hi} LC-like dendritic APC population and a small subset of the DR^{Lo} population which appears to contain the dermal mast cell population. Our data extend the finding of Wang et al. (7)in which FcεRI molecules were not only on epidermal LC but also on dermal cells with elongated morphology. It is not yet clear, however, whether dermal $FcεRI^+APC$ might be premature LC which have just extravasated from the dermal vasculature, activated LC which have emigrated from epidermis after antigen-stimulation, or long-lived dermal elements.

In mast cells and basophils, activation of FcεRI by receptor-ligand reaction or receptor crosslinking, causes degranulation and release of variable chemical mediators such as histamine and serotonin (23). These biological consequences are the products by

sequential signal transduction events, including activation of protein kinase C (PKC) and protein tyrosine kinase, following receptor aggregation (13, 24). We have investigated cytosolic calcium mobilization in epidermal and dermal LC after FcεRI crosslinking. Cytosolic calcium mobilization is one of the major signal transduction pathways related to activation of PKC, and known to be a crucial event after the FcεRI triggering (13, 24). Even though we could observe quick and vigorous responses by basophils after FcεRI crosslinking, no significant calcium mobilization was induced both in epidermal and dermal LC. Differences in local environmental requirements between basophils and dermal DR^+ $CD1a^+$ cells might influence the responsiveness of these cells. For instance, cell adhesion to fibronectin augments the tyrosine phosphorylation observed after the crosslinking of FcεRI in rat basophilic leukemia cell lines (25). Maurer et al. (21) reported that peripheral blood monocytes from atopic patients could cause calcium flux by FcεRI crosslinking only if they were allowed adherence prior to stimulation (11), although LC adhered to fibronectin still were FcjRII unresponsive.

Another possibility is the influence of the local inflammatory environment. Fc receptors on LC might function for calcium flux only when they are activated by specific inflammatory cytokines. Bieber et al. (26) have showed that only atopic LC exhibited calcium mobilization via FcεRI crosslinking, in contrast with the non-responsiveness of normal LC, and these LC from atopic patients demonstrated different CD45 tyrosine phosphatase isoform profile from LC of normal individuals. Recently, Jurgens et al. (9) demonstrated a rapid tyrosine phosphorylation of several proteins and receptor mediated endocytosis after FcεRI crosslinking on LC from both normal and atopic individuals, even though intracellular calcium flux was induced only in atopic individuals. Tyrosine phosphorylation after surface receptor triggering is a consequence of the balanced reactions by protein tyrosine kinase and protein phosphatase. Because it is well-known that the CD45 isoform on T cells can change after activation (27), the specific CD45 isoform seen on atopic LC may contribute to the activation of LC by the inflammatory cytokine environment, and it may also contribute to the distinct usage of signal transduction pathways such as calcium flux, leading to unique effector functions other than receptor endocytosis. Supporting this hypothesis, FcεRI crosslinkings cause more vigorous protein tyrosine phosphorylation in atopic LC with enhanced FcεRI expression than in normal LC (9). In addition, LC in normal individuals are unresponsive to a number of immunological stimuli, such as FcjRII or ß2 integrin crosslinking, which cause calcium flux in other types of cutaneous APC, including infiltrating macrophage in human sunburned skin (21).

In summary, we have investigated the phenotypic and functional properties of epidermal and dermal $FcεRI^+$ cells. Both normal human epidermal LC and dermal DR^{Hi} cells (LC-like dendritic APC subset), as well as a subset of dermal DR^{Lo} cells and peripheral blood basophils, express the high affinity IgE Fc receptor, FcεRI. FcεRI crosslinking caused a significant increase of intracellular calcium in peripheral blood basophils, but not in epidermal LC or dermal DR^{Hi}cells. However, FcεRI expression by both epidermal LC and dermal DR^{Hi} LC-like dendritic APC may be more important for IgeE-directed antigen capturing and presentation by these cutaneous APC.

Acknowledgments

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