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Fcγ-receptors on Langerhans' Cells and Keratinocytes in Suspension from Normal Skin Characterized using Soluble Immune Complexes and Monoclonal Antibodies

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Fc-receptors for IgG (FcR) on epidermal cells in suspension were studied using soluble immune complexes and monoclonal antibodies (MoAbs) against FcR I, FcR II and FcR III using an indirect immunofluorescence technique. The binding of immune complexes demonstrated that most Langerhans' cells (> 95%) and a proportion of keratinocytes (25 ± 6%) expressed functional FcR activity. The reactivity with MoAbs showed that epidermal cells possess different types of FcR. Langerhans' cells reacted only with IV.3 (anti-FcR II/-CDw32). A varying percentage of keratinocytes reacted with 32.2 (anti-FcR I/-CD64) (18 ± 10%), Leu 11b (anti-FcR III/-CD16) (19 ± 6%) and B1D6 (against a placental FcR) (35 ± 8%). Both with immune complexes and MoAbs the staining was strongest along the cell surface, but cytoplasmic staining was also regularly present. The data add further support to the contention that keratinocytes have an immune function. FcR on epidermal cells may play an immunoregulatory role interacting with isotypes and cytokines in the skin. Keratinocyte-produced soluble FcR could also be an immunological mediator. ***Key words: FcR I; FcR II; FcR III; Epidermal cells.***

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Receptors for the Fc-part of the IgG molecule (FcR) are present on a variety of human immunocompetent cells, viz. mononuclear phagocytes, lymphocytes and granulocytes (1). In addition, FcR are demonstrated on epithelial cells in different anatomical areas: human placenta (2), plexus chorioidalis (3), intestinal epithelium (4) and dermal endothelium (5). In the epidermis, Langerhans' cells (LC) were long considered to be the only cells expressing FcR (6). However, FcR was also detected recently in our laboratory on keratinocytes (KC) in tissue sections (7, 8). Three classes of FcR have been characterized using monoclonal antibodies: FcR I, FcR II and FcR III (1). FcR I (mol. weight 72 kD) is a high-affinity receptor with the capacity to bind both monomeric and complexed IgG. FcR II (mol. weight 40 kD) and FcR III (mol. weight 50–70 kD) are low-affinity receptors that preferentially bind complexed IgG (1). The individual roles of the different FcR are not yet fully understood.

Recently, we demonstrated reactivity of MoAbs against FcR I, FcR II and FcR III with epidermal cells (EC) *in situ* in cryosections of normal skin (9). The aim of the present study was to further characterize the FcR on human EC using cell suspension and staining with soluble immune complexes and monoclonal antibodies (MoAbs) against the various FcR.

MATERIAL AND METHODS

Tissues

Normal skin specimens from the retroauricular areas and upper eyelids of 6 healthy individuals undergoing surgical skin correction were kindly provided by the Department of Plastic Surgery. Normal placental tissue at term was provided by the Department of Gynecology and Obstetrics.

Epidermal cell suspension

EC suspension was prepared according to Czernielewski et al. (10) with minor modification. Excess dermal tissue was removed from the skin specimens with a keratotome and slices of skin 0.4 mm thick were incubated with the dermis side down at 37°C with 0.25% trypsin 1:250 (Difco Laboratories, Detroit, Michigan, USA) in phosphate buffered saline, pH 7.6 (PBS) for 45 min. Then the skin slices were washed in PBS and quickly transferred to 15% heat-inactivated fetal calf serum (FCS) (Gibco, Grand Island, NY, USA). The dermis and epidermis were gently agitated between two scalpel blades. The resulting EC suspension was filtered through sterile gauze and after repeated washings in RPMI-1840 medium (Gibco) the cells were resuspended as 1×10^6 cells/ml in RPMI-1640 containing 10% FCS. The viability of the EC was > 90% as determined by trypan blue exclusion.

Table I. *Epidermal cells from normal human skin stained with soluble immune complexes and monoclonal anti-FcR antibodies; percentage of cells stained and reactivity*.*

	Immune complexes	Monoclonal antibody against			
		FcR I CD64 (32.2)	FcR III CD16 (Leu 11b)	FcR II CDw32 (IV.3)	FcR placental (B1D6)
Langerhans cells					
Percentage pos.	> 95	0	0	> 90	0
Reactivity	2+	–	–	2+	–
Keratinocytes					
Percentage pos.	25 ± 6	18 ± 10	19 ± 6	0	35 ± 8
Reactivity	1+	2+	3+	–	3+

* Grading of reaction: 1+ = weak, 2+ = moderate, 3+ = strong, and – = no IF staining.

Immunoglobulins

Antiserum to HRP (Type IV, Sigma, St. Louis, Mo., USA) was raised in rabbits, and IgG antibody to HRP was purified as described elsewhere (2). Rabbit $F(ab')_2$ was prepared as described by Stewart et al. (11). The monoclonal antibodies (MoAbs) 32.2 (IgG1) against FcR I (CD64) and IV.3 (IgG2b) against FcR II (CDw32) were kind gifts from Dr Anderson, Ohio State University, USA. The MoAbs Leu 11b (IgM) against FcR III (CD16) and Leu 6 against LC antigens (CD1a) were purchased from Becton-Dickinson, Sunnyvale, Calif., USA. A murine MoAb (B1D6), isotyped as IgG1, against placental FcR was prepared as described previously (12). This antibody reacts with a 40 kD placental FcR with low affinity for IgG (13). Biotinylation of B1D6 was performed using the method described by Goding (14). Fluorescein isothiocyanate (FITC)-conjugated IgG $F(ab')_2$ preparation of goat anti-rabbit IgG was purchased from DAKO $A/_S$, Copenhagen, Denmark, and FITC-conjugated IgG $F(ab')_2$ of goat anti-mouse Ig was purchased from Behringwerke, Marburg-Lahn, FRG. Biotinylated horse anti-mouse Ig and tetramethylrhodamine isothiocyanate (TRITC)-conjugated streptavidin were purchased from Bethesda Research Laboratories, Gaithersburg, Md, USA. Pooled native human IgG (Fraction II, 16.5% solution) was purchased from AB Kabi, Stockholm, Sweden. To remove aggregates, the immunoglobulin preparations were centrifuged at 100 000 *g* for 1 h before use. Aggregation of IgG was performed by heating at 63°C for 15 min.

Immune complexes

Immune complexes were prepared by adding either dilutions of rabbit IgG anti-HRP or the preparation of $F(ab')_2$ fragments to HRP to equal amounts of four-fold dilutions of HRP from 1 mg/ml in PBS. The mixtures were incubated at 2 h at room temperature before use. Immune complexes prepared at slight antigen excess were used (2).

Immunofluorescence staining

10^6 EC were incubated with soluble immune complexes of HRP-anti-HRP at room temperature for 60 min, followed by FITC-conjugated IgG $F(ab')_2$ of goat-anti-rabbit IgG diluted 1:32 in PBS with 10% FCS.

The MoAb dilutions mostly used were determined by results of preliminary experiments: 32.2, 1:16; IV3, 1:16; Leu 6, 1:32; Leu 11b, 1:64; and B1D6, 1:64. EC were incubated with the MoAbs at 4°C for 45 min, followed by FITC-conjugated IgG of goat anti-mouse Ig (1:32) in PBS with 10% FCS at room temperature for 30 min.

In double-staining experiments EC were first incubated with soluble immune complexes, then with FITC-conjugated IgG $F(ab')_2$ of goat-anti-rabbit IgG, followed by MoAb; dilutions, temperature and time as described above. The EC were further incubated with biotinylated horse anti-mouse Ig (1:32), and finally with TRITC-conjugated streptavidin (1:200) at room temperature for 30 min.

EC double-stained with Leu 6 and B1D6 were first incubated with Leu 6 followed by FITC-conjugated goat-anti-mouse Ig, and then with biotinylated B1D6 (1:64) followed by TRITC-conjugated streptavidin (1:200).

Each incubation was followed by washing in RPMI-1640 at 4°C for 10 min.

The EC were finally mounted in PBS – glycerol with 0.1% paraphenylenediamine as anti-fading agent and examined in a Zeiss fluorescence microscope with an Osram HBO-200 mercury lamp and a 150 W xenon lamp.

Controls

EC were also incubated with immune complexes containing $F(ab')_2$ fragments of IgG anti-HRP, and further processed as described above. In other control experiments EC were incubated with PBS instead of either immune complexes of HRP-anti-HRP or MoAb. EC were also incubated with normal mouse serum (1:16) as an isotypic control. As positive control, the reactivity of immune complexes with cryosections of placental tissue was examined.

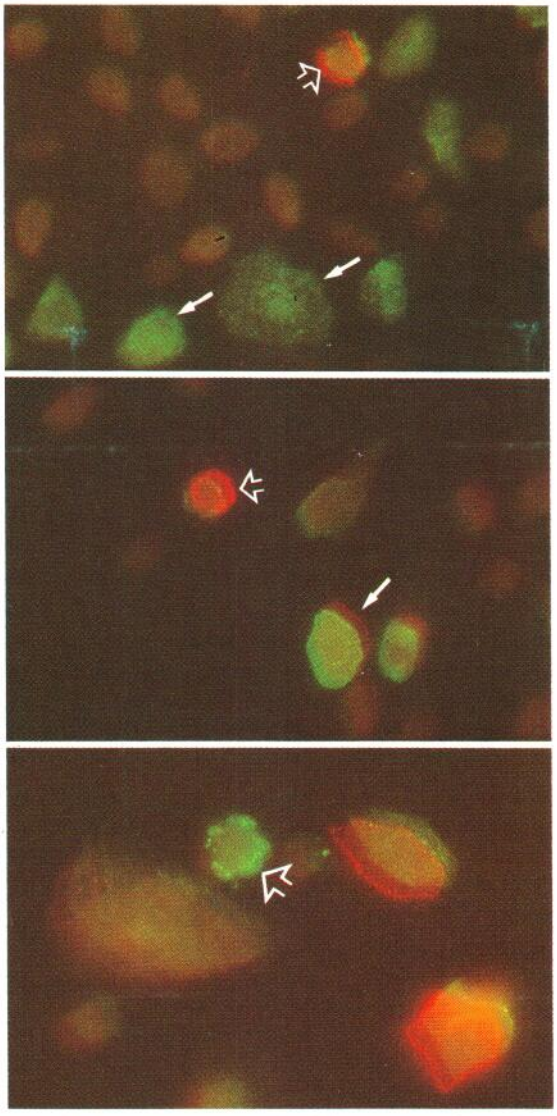

Fig. 1a–c. Double-staining of epidermal cells. Green fluorescence shows staining with soluble immune complexes of HRP-anti-HRP and red fluorescence staining with *a)* Leu 6, *b)* IV.3 (anti-FcR II), *c)* 32.2 (anti-FcR I). LC are stained with immune complexes, Leu 6 and IV.3 *(open arrows)*. There is staining of KC with both immune complexes (*small arrows*) and 32.2. × 630.

RESULTS

The results are summarized in Table I. Leu 6 gave strong granular membrane fluorescence of 0.8–1.5% of the EC. More than 95% of these CD1a+ cells bound HRP-anti-HRP, with a moderately strong granular membrane fluorescence (Fig. 1*a*). IV.3 gave a similar staining pattern of > 90% of the LC (Fig. 1*b*). Both the immune complex positive and the IV.3+ LC were round cells, sometimes with dendrites. There was no staining of immune complex positive LC by either 32.2, Leu 11b or B1D6. Neither gave B1D6 any staining of CD1a+ cells.

A varying proportion of KC were stained with immune complexes (25 ± 6%), 32.2 (18 ± 10%) (Fig. 1*c*), Leu 11b (19 ± 6%) and B1D6 (35 ± 8%) (Table I). The immune complex staining was granular and weak, strongest along the cell membrane (Fig. 1*a–c*). The MoAb staining varied in strength among KC within the same suspension, mostly strong granular staining, apparently along the cell membranes with strongest staining of large EC (Fig. 1*c*). In many cells there was apparent cytoplasmic granular staining with immune complexes (Figs. 1–2). Double-staining showed that most KC stained with anti-FcR MoAbs were also stained with immune complexes. Many of the FcR+ KC were relatively large and irregularly shaped cells (Fig. 2). IV.3 did not stain KC.

There was no staining of EC incubated with $F(ab')_2$ fragments of IgG anti-HRP or with PBS instead of immune complexes. Nor was there any staining of EC incubated with PBS or normal mouse serum instead of MoAb. In sections of human normal placenta, immune complexes bound to trophoblasts and to endothelial cells of fetal stem vessels, as previously described (2).

DISCUSSION

The results obtained in experiments with immune complexes clearly demonstrated that LC and KC in suspension express functionally active FcR. The specificity of the reactions was sustained by the lack

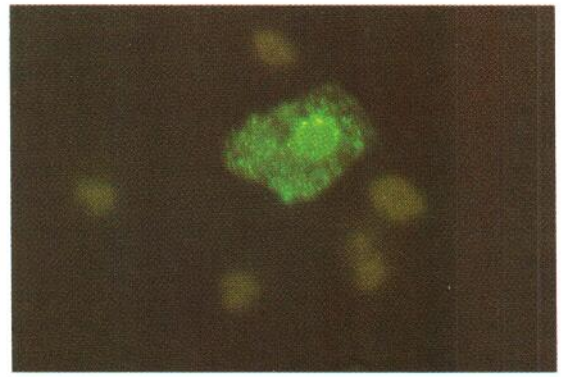

Fig. 2. Keratinocyte stained with soluble immune complexes. × 1000.

of binding of $F(ab')_2$ containing immune complexes. The data extend our previous observations that both LC and KC in cryostat sections of normal human skin express FcR (8, 9). The only anti-FcR MoAb reacting with LC was IV.3, indicating the presence of FcR II. The latter is in line with recent findings by Romani et al. (15), examining LC in suspension. On the other hand, applied on cryosections, the anti-FcR I MoAb, 32.2, also stained some dendritic EC, possibly LC (9). Enzymatic and other environmental effects may explain the differing results when LC is examined in suspension and in tissue sections. FcR I on human leukocytes is sensitive to and FcR II resistant to trypsin (1, 16). Jensen & Thestrup Pedersen (17) found that trypsinization of T cells affected the Fc-IgM receptors, but not Fc-IgG receptors. Kobayashi et al. (4) reported that IgG Fc binding to human intestinal epithelium is not affected by trypsin. Since the trypsin treatment may affect the number of FcR+ EC obtained, it would be of interest to study the FcR expression on EC during culture.

Evaluated by staining with immune complexes, the FcR activity on KC was weaker than on LC. 32.2 is directed against an epitope not directly associated with the IgG binding site of the receptor (18). Possibly the receptor detected on KC by 32.2 has lower binding affinity for IgG than the FcR I on mononuclear phagocytes. Cross-reactivity of 32.2 with an epidermal non-FcR epitope seems less probable, in view of the reactivity of KC with other anti-FcR MoAbs (Leu 11b, B1D6) and immune complexes. In addition to 32.2 (9), Leu 11b (9) and B1D6 (7) also react with KC in cryosections.

The surface and cytoplasmic staining for FcR of KC both with immune complexes and with MoAbs indicate that the cells may synthesize the receptors. Soluble FcR in serum are present in humans under both normal and pathological conditions. Recent data indicate that circulating FcR increase in various diseases. Both FcR II and FcR III (19) as well as the placental FcR are being measured (20).

FcR belong to the immunoglobulin superfamily, i.e. both the antibodies and their receptors are members of the same general family. The receptors connect the humoral and cellular immune responses. FcR on monocytes and macrophages are involved in phagocytosis and endocytosis of antibody-coated particles (9, 21), in the release of inflammatory mediators (22), in antibody-dependent cell-mediated cytotoxicity (21) and in the clearance of circulating immune complexes (16). Cross-linking of FcR can trigger the oxidative burst (16). FcR also have immunoregulatory functions. Most extensively studied is the murine FcR II, which is important in the activation and proliferation of T and B cells and in antibody secretion by B cells (16, 23). FcR on epithelial structures are probably involved in IgG transport across the cells, e.g. trophoblasts of placenta (2, 18) and plexus chorioidalis cells (3). The recent report by Kobayashi et al. (4) that human intestinal epithelium has receptors not only for aggregated but also for monomeric IgG is of particular interest in relation to the present data.

The FcR on KC could be of importance for EC interactions. The present findings add to other observations suggesting an immune function of KC, alone or in co-operation with LC or passenger T lymphocytes. There is recent data that epidermal FcR binding of IgG can down-regulate the LC response to cytokines (24). T lymphocyte traffic and retention in the epidermis, possibly regulated by γ-IFN and other cytokines, inducing intercellular adhesion molecules, might be influenced by FcR.

It is also possible that the receptors are internalized in the KC. In mouse macrophages the receptor and ligand can be internalized, delivered to endosomes and either recycled back to the plasma membrane or degraded in lysosomes (16). KC have endocytic and phagocytic capacity (25), whereas LC are poor phagocytes (26). Thus, an interesting possibility which merits further studies is that the FcR on KC can bind IgG, possibly in complex with harmful antigens, which subsequently are elliminated by phagocytosis.

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