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Fcγ-receptor as a Functional Marker on Epidermal Langerhans' Cells *in situ***

M. TIGALONOWA,[1,2,*] J. R. BJERKE[1] and R. MATRE[2,3]

[1]*Department of Dermatology,* [2]*Broegelmann Research Laboratory for Microbiology and* [3]*Department of Microbiology and Immunology, The Gade Institute, University of Bergen, Norway*

Fcγ-receptors (FcR) in cryostat sections of normal human skin were detected with soluble immune complexes of horseradish peroxidase (HRP) and rabbit IgG anti-HRP (HRP–anti-HRP). The binding of HRP–anti-HRP to Langerhans' cells (LC) was demonstrated using a double immunofluorescence staining in which LC were identified with a CD1a specific monoclonal antibody (Leu 6). The immune complexes gave granular staining of $CD1a^+$ epidermal cells in sections of all specimens from normal skin. The mean percentage of $CD1a^+$ cells that were FcR^+ was 49 ± 11 ($n = 8$). The $FcR^+/CD1a^+$ cells had a clearly defined dendritic pattern. The staining intensity of LC with HRP–anti-HRP was weaker than the intense staining of $CD1a^-$ macrophages in the dermis. Results of inhibition experiments indicate that human epidermal LC express low affinity FcR, but the presence of high affinity FcR as well cannot be excluded. The demonstration of FcR expression on normal LC clarifies previous uncertainty on LC membrane receptors, though the functional significance of these receptors is still not well understood.

Key words: Normal skin; Cryostat sections; Immune complex binding.

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M. Tigalonowa, Broegelmann Research Laboratory for Microbiology, MFH-bygget, Haukeland sykehus, N-5021 Bergen, Norway.

In 1977, Stingl et al. (1) reported that human epidermal Langerhans' cells (LC) in suspension express receptors for the Fc-part of the IgG molecule (FcR) and the complement component C3. They found that all epidermal LC in suspension of normal skin bound IgG coated bovine erythrocytes. Others, also examining LC in suspension, but from other species, have found that LC either express weak FcR as in murine skin, or not at all (2). However, the preparation and use of epidermal cell (EC) suspension affect the cells mechanically and chemically with possibilities for interference with the receptor activity. The receptors may be inactivated, unmasked or activated. Therefore, it is also important to study the receptors directly in the skin. So far, the detection of functionally active FcR on LC *in situ* has not been reported.

In the present study we have used the binding of soluble immune complexes (IC) to cryostat sections of normal skin in an indirect immunofluorescence (IIF) assay in order to demonstrate functionally active FcR on LC *in situ*.

MATERIAL AND METHODS

Tissues

Normal skin specimens from 8 healthy individuals undergoing surgical skin correction in the retroauricular areas and upper eyelids were kindly provided by the Department of Plastic Surgery. Normal placental tissue at term was provided by the Department of Gynecology and Obstetrics. The specimens were washed for 10 min in phosphate-buffered saline, pH 7.2 (PBS), embedded in Tissue-Tek II O.C.T. compound (Lab-Tek Products, Naperville, Ill., USA), quick-frozen in isopenthane pre-chilled with liquid nitrogen and stored at −70°C. Cryostat sections were cut at 4–6 μm and stored unfixed at −20°C until use. Some sections were washed in PBS for 1 h at room temperature before use. Sections of normal placenta which is a FcR rich tissue, were included as control in each experiment.

Immunoglobulins

Antiserum to horseradish peroxidase (HRP) (Type IV, Sigma, St. Louis, Mo., USA) was raised in rabbits, and IgG antibody to HRP was purified as described elsewhere (3). Rabbit $F(ab')_2$ was prepared as described by Stewart et al. (4). A monoclonal antibody against CD1a antigen (Leu 6) was purchased from Becton-Dickinson, Sunnyvale, Ca., USA. Fluorescein isothiocyanate (FITC)-conjugated IgG $F(ab')_2$ preparation of goat anti-rabbit IgG and FITC-conjugated IgG $F(ab')_2$ of rabbit anti-human IgG were purchased from DAKO A/S, Copenhagen, Denmark. Biotinylated horse anti-mouse IgG and tetramethylrhodamine isothiocyanate (TRITC)-conjugated streptavidin were purchased from Bethesda Research Laboratories (Gaithersburg, Mo., USA).

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* Dr. Tigalonowa is a guest researcher from Department of Dermatology, Academy of Medicine, Warsaw, Poland.

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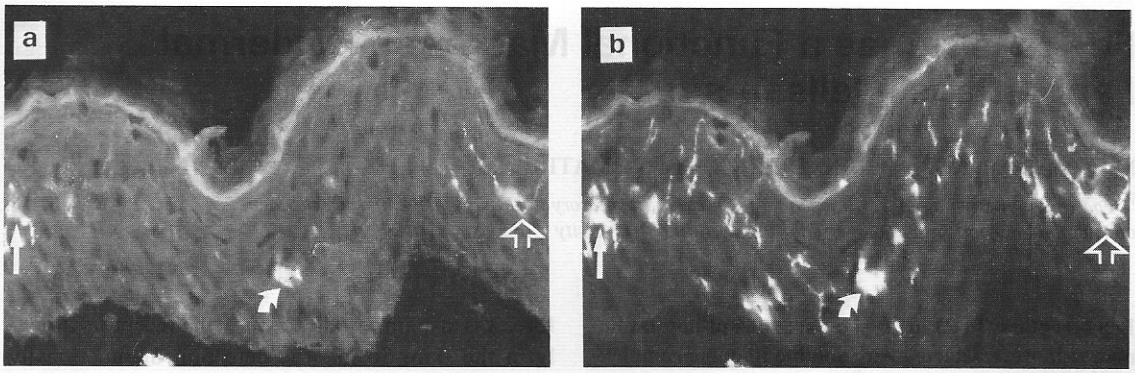

Fig. 1 a–b. Section of normal skin double-stained with immune complexes of HRP–anti-HRP (*a*, green fluorescence) and with Leu 6 (*b*, red fluorescence). The epidermal dendritic cells stained with immune complexes can be identified as $CD1a^+$ cells. Identical cells indicated by arrows (×220).

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

IC were prepared by adding either dilutions of the IgG or $F(ab')_2$ fragments to HRP to equal amounts of four-fold dilutions of HRP from 1 mg/ml in PBS. The mixtures were incubated for 2 h at room temperature before use. Complexes prepared at slight antigen excess were used (5).

Immunofluorescence methods

The sections were double-stained with the soluble immune complexes and Leu 6 as follows:

1. soluble immune complexes of HRP–anti-HRP for 45 min,
2. FITC-conjugated IgG $F(ab')_2$ of goat anti-rabbit IgG diluted 1:30 in PBS with 10% pooled human serum (PHS),
3. Leu 6 diluted 1:30 for 45 min,
4. biotinylated horse anti-mouse IgG diluted 1:30 in PBS,
5. TRITC-conjugated streptavidin diluted 1:200 in PBS with 10% PHS.

Each incubation was followed by twice gentle washings for 10 min in PBS at room temperature. The sections were finally mounted in PBS–glycerol with 0.1% paraphenylenediamine (PPD), pH 8.6, and examined in a Zeiss fluorescence microscope with an Osram 150 W Xenon lamp. From each biopsy, 5–8 sections were studied and at least 300 $CD1a^+$ cells counted and examined for binding of IC.

Sections were also incubated with IC containing $F(ab')_2$ fragments of IgG anti-HRP or HRP alone, and further processed as described above. Some sections were stained with FITC-labelled rabbit IgG $(Fab')_2$ against human IgG. Other control sections were incubated with PBS instead of either IC of HRP–anti-HRP or Leu 6.

Inhibition experiments

Cryostat sections were first incubated with two-fold dilutions (8–0.5 mg/ml) of either heat-aggregated or native IgG at room temperature for 60 min and washed in PBS at room temperature for 30 min. Then the sections were double stained with IC of HRP–anti-HRP and Leu 6.

Cryostat sections were also pre-treated with 0.25, 0.5, 1 and 2 mM periodic acid in PBS for 30 min at room temperature, or with 0.25, 0.5, 1 and 2% formaldehyde in PBS for 10 min. Control sections were treated with PBS. The treated sections were washed in PBS for 20 min before being examined for FcR activity.

RESULTS

IC of HRP–anti-HRP gave granular staining of $CD1a^+$ cells in sections from all specimens of normal skin, indicating the presence of FcR on LC. Sections incubated with IC prepared with $F(ab')_2$ fragments of IgG anti-HRP were not stained. The staining with HRP–anti-HRP showed a clearly defined dendritic pattern of the cells (Fig. 1). The mean percentage of $CD1a^+$ cells expressing detectable FcR was 49±11% (range 27–62%; 8 biopsies). The pattern of FcR expression of $CD1a^+$ cells and dendrites was the same in all parts of the epidermis, irrespective of whether the cells were located high or low in the epidermis, in the epidermal papillae, or above dermal papillae. Usually, not all dendrites of a $CD1a^+$ cell were stained with IC. The FcR activity on $CD1a^+$ cells could easily be differentiated from a weak granular IC staining of keratinocyte membranes found in sections from two of the normal skin biopsies (Fig. 2).

It was important to incubate the sections first with the soluble IC and later with Leu 6. If Leu 6 was applied first, a much weaker IC staining of $CD1a^+$ cells was obtained. The staining of $CD1a^-/FcR^+$ dermal cells was not affected by this order of the incuba-

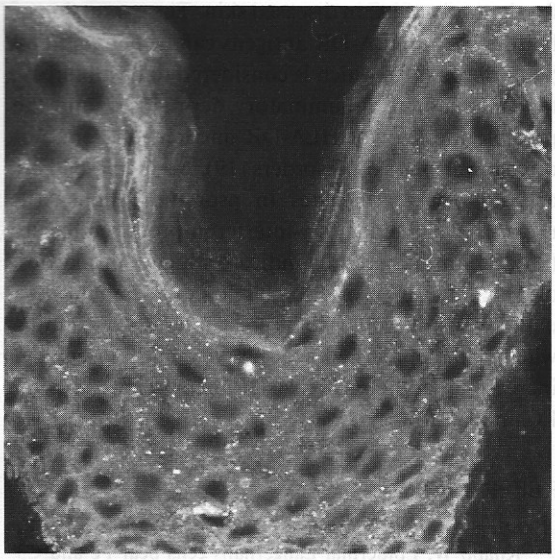

Fig. 2. Section of normal skin stained with immune complexes of HRP–anti-HRP. There is granular staining of the outer aspect of keratinocytes (×350).

tion. The FcR staining intensity of $CD1a^-$/FcR^+ dermal cells, which mainly represent macrophages (3), was always stronger than the staining of dendritic $CD1a^+$ epidermal cells.

Heat-aggregated IgG at a concentration of 2 mg/ml inhibited completely the binding of IC to the sections, whereas native IgG at a concentration of 8 mg/ml gave partial inhibition. Treatment with 1 mM periodic acid or 1% formaldehyde abolished the binding of IC to the sections (Table I).

Table I. *Binding of immune complexes of HRP–anti-HRP to cryostat sections of normal skin: inhibition with human IgG (mg/ml), periodic acid (mM) and formaldehyde (%)*

Inhibition with	Concentration					
	0.25	0.5	1	2	4	8
IgG						
heat-aggregated	2+[a]	1+	1+	–	–	–
native	3+	3+	3+	3+	2+	1+
Periodic acid	2+	1+	–	–	n.t.	n.t.
Formaldehyde	2+	1+	–	–	n.t.	n.t.

[a] Grading of reaction:
3+ = 30–50% of LC stained with immune complexes
2+ = 10–30% of LC stained with immune complexes
1+ = < 10% of LC stained with immune complexes
– = negative
n.t. = not tested.

Sections incubated with PBS instead of either HRP–anti-HRP or Leu 6 did not stain. No staining with rabbit anti-human IgG was detected in the epidermis, except from staining in stratum corneum in some sections. In sections of human normal placenta HRP–anti-HRP bound to trophoblasts and to endothelial cells of fetal stem vessels, as previously described (5).

DISCUSSION

The results showed that LC *in situ* express functionally active FcR. This conclusion was further supported by the lack of binding of IgG $F(ab')_2$ containing IC to the sections. The receptor expression was weak and only detectable on about 50% of the $CD1a^+$ EC. On the other hand, Stingl et al. (1) examining EC suspension, found that all LC expressed FcR. These differing results could be due to lower sensitivity of the *in situ* method or environmental effect on the FcR of LC in suspension. Since not all dendrites were stained with the IC, a greater proportion of $CD1a^+$ cells might have been obtained, using thicker sections. The present findings are probably similar to previous observations that only 50–80% of $CD1a^+$ EC in normal skin express HLA-DR antigen *in situ* (6–8). De Jong et al. (7) concluded that these varying results were influenced by the thickness of the sections.

Recently, Bieber et al. (9) reported that in normal human skin, receptors for mouse IgG1 on LC were only detectable by a rosette assay with EC suspension, but not immunohistochemically on cryosections. Even on EC in suspension it has been difficult to detect LC FcR. Thorbecke et al. (2) found that murine LC in EC suspension needed prolonged exposure to IgG sensitized sheep erythrocytes in order to form rosettes. A weak receptor activity, a low density of receptor molecules available, or *in vivo* IgG blocking could explain why FcR activity has been difficult to demonstrate on LC. The latter explanation is unlikely, however, since no *in vivo* bound IgG could be detected in the Malphigian or basal layer. Neither did pre-washing of the sections with PBS increase the binding of the HRP–anti-HRP IC.

The method used in the present study is sensitive and precise for the detection of weak FcR in tissue sections. Thus, we were able to detect weak binding of IC to keratinocytes, in line with the observations of

Livden (10). Recently, we have also demonstrated the presence of FcR on endothelial cells in the skin (11). We found that the combined use of PPD in the mounting medium to reduce the IF fading and the Xenon lamp to increase the fluorescence gave significant improvement in the method sensitivity.

Since Leu 6 partially blocked the FcR, the IC should be applied to the sections first. This blocking effect of Leu 6 could be due to steric inhibition by a topographic vicinity between FcR and CD1a antigens. Binding of Leu 6 to the FcR is unlikely, since the monoclonal antibody does not bind to other FcR^+ cells in the skin.

The FcR in tissue sections is easily destroyed by fixatives (12, 13). The demonstrated sensitivity of LC FcR to periodic acid and formaldehyde is similar to results of *in situ* studies with FcR on dermal cells (12) and keratinocytes (10). In addition, slight fixation with glutaraldehyde, acetone or methanol abolishes the receptor activity (unpublished data). Therefore, the method used in the present study can only be applied to unfixed cryostat sections. However, the method is well suited to double-marking with a monoclonal antibody, minimizing the problem of possible cross-reactions.

There are at least three types of FcR. The high affinity FcR (FcR I) binding native and complexed IgG are present mainly on mononuclear phagocytes, whereas the low affinity FcR (FcR II and FcR III) have a much broader range of cellular expression (14, 15). The low affinity FcR preferentially bind complexed IgG (14). We found that heat-aggregated IgG were better equipped than native IgG to block the binding of immune complexes to $CD1a^+$ epidermal cells. This indicates the presence of low affinity FcR on LC, but does not exclude the possibility that LC may express FcR I as well. Further studies using monoclonal anti-FcR antibodies to characterize the LC FcR are currently in progress.

The functional significance of human FcR is only partially understood. Studies with monoclonal antibodies have revealed a previously unrecognized molecular and functional heterogeneity of human FcR (14, 15). FcR can mediate endocytosis and phagocytosis of IgG. The receptors can also trigger effector cell functions like cytotoxic response and immunosuppression (15). The recent report that FcR can mediate an antibody enhanced binding of HIV-1 to monocytic cells (16) is of interest in relation to the virus uptake by LC (17).

As an activation marker the FcR may be valuable for the study of LC in diseased skin. The expression of both FcR and HLA-DR antigens can be enhanced by γ-interferon (18), which is considered to be an important mediator in inflammatory dermatoses, and the possible inductor of HLA-DR antigens on keratinocytes in various skin disorders (19). An increased FcR activity on keratinocytes in psoriatic lesions (10) might as well be due to γ-interferon produced locally (20). Preliminary results indicate a higher proportion of FcR^+ LC in psoriatic skin lesions (unpublished data). Thus, it would be of great interest to examine whether the epidermal FcR activity in diseased skin increases, paralleling HLA-DR expression both on LC and keratinocytes.

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