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The Distribution of FcγR I, FcγR II and FcγR III on Langerhans' Cells and Keratinocytes in Normal Skin*

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The distribution of Fc-receptors for IgG (FcR) on human epidermal cells (EC) was characterized *in situ* using monoclonal antibodies (MoAbs) by indirect immunofluorescence staining of cryosections. The results showed heterogeneity of FcR expression on Langerhans' cells (LC) and keratinocytes (KC). The MoAb IV.3 against FcR II (CDw32) gave granular staining of most LC whereas the MoAb 32.2 against FcR I (CD64) occasionally stained a few dendritic cells. 32.2 demonstrated weak granular staining along the outer aspect of KC in stratum spinosum and stratum basale and intense staining of stratum corneum and stratum granulosum. The MoAbs Leu 11b against FcR III (CD16) and B1D6 reacting with a placental FcR with low affinity for IgG gave intense linear membrane staining of KC. Leu 11b produced strongest staining of stratum granulosum and B1D6 the strongest staining of stratum spinosum and basale. The results confirm our previous observations of FcR *in situ* on LC and KC in normal skin using functional assays and demonstrate that these EC possess different types of low affinity FcR. The data support the contention of an immune function of KC. FcR may be mediators for interaction between KC and LC. The FcR activity in stratum granulosum may have an immune function as a barrier against microorganisms and other antigens. *Key words: Epidermis; Fcγ-receptors; Monoclonal antibodies.*

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Receptors for the Fc-part of the IgG molecule (FcR) are present on most immunocompetent cells and are believed to be important in endocytosis, phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC) as well as in immune induction and regulation (1–3). In addition, FcR may be involved in active transcytosis of IgG across epithelia, e.g. in placenta (4) and in plexus choroideus (5). Recently three different FcR have been characterized using monoclonal antibodies (MoAb). Human FcR I is a 72 kD molecule binding both complexed and monomeric IgG (6). This high affinity receptor has been detected at high density on cells of the macrophage/monocytic lineage (3). FcR II is a 40 kD molecule and FcR III a 50–70 kD molecule. FcR II and III are low-affinity receptors, preferentially binding complexed IgG, and with a broad range of cellular expression (1). The functional significance of the different FcR is not fully understood, and little is known about their distribution in normal and inflamed skin.

The *in situ* expression of functionally active FcR both on epidermal Langerhans' cells (LC) (7) and keratinocytes (KC) (7, 8) was recently detected in our laboratory when using immune complexes. Both on LC and KC the receptor expression was weak and not detectable on all cells. In the present study we used MoAbs against the various FcR to further characterize the FcR on LC and KC *in situ* in normal human skin.

MATERIAL AND METHODS

Tissues

Normal skin specimens from the retroauricular areas and upper eyelids of 8 healthy individuals undergoing surgical skin correction were kindly provided by the Department of Plastic Surgery. 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 prechilled with liquid nitrogen. Cryostat sections were cut at 4–6 μm and stored unfixed at −20°C until used.

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Table I. *Staining of epidermal cells in unfixed cryosections of normal human skin with anti-FcR monoclonal antibodies*

	Monoclonal antibody against			
	FcR I CD64 (32.2)	FcR II CDw32 (IV.3)	FcR III CD16 (Leu 11b)	FcR placental (B1D6)
Langerhans' cells	±[a]	++	–	–
Keratinocytes				
str. corneum	++	–	+	±
str. granulosum	+++	–	+++	+
str. spinosum	+	–	++	+++
str. basale	+	–	++	+++

[a] Grading of staining: ± = occasional; + = weak; ++ = moderate; +++ = intense.

Antibodies

The monoclonal antibodies (MoAbs) 32.2 (IgG1) against FcR I (CD64), and IV.3 (IgG2b) against FcR II (CDw32), were kindly donated by Dr Anderson, Ohio State University, USA. The MoAb Leu 11b (IgM) against FcR III (CD16) and Leu 6 against LC antigens (CD1a) were purchased from Becton and Dickinson, Sunnyvale, Calif., USA. A murine MoAb (B1D6), isotyped as IgG1, against placental FcR was prepared *ad modum* Köhler & Milstein (9). This antibody reacts with a 40 kD placental FcR with low affinity for IgG (10). Fluorescein isothiocyanate (FITC)-conjugated Ig $F(ab')_2$ preparation of goat anti-mouse Ig was purchased from Behringwerke, Marburg-Lahn, FRG. To remove aggregates the immunoglobulin preparations were centrifugated at $100\,000 \times g$ for 1 hour before use.

Fig. 1. Section of normal skin stained with the MoAb against FcR II (IV.3). Staining localized to epidermal Langerhans' cells and to macrophages in the papillary dermis. Acetone-fixed, ×250.

Immunofluorescence staining

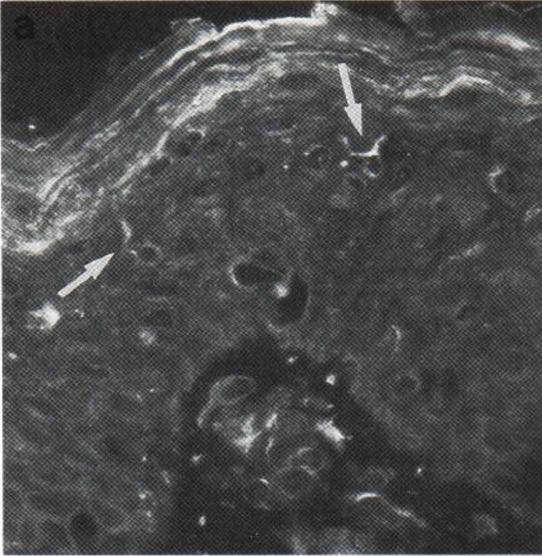
Unfixed as well as acetone-fixed (4°C, 5 min) cryostat sections were incubated overnight at 4°C with the MoAbs diluted in PBS. The dilutions mostly used were 32.2 : 16, IV.3 1 : 16, Leu 6 1 : 32, Leu 11b 1 : 64 and B1D6 1 : 64. The sections were then gently washed, twice, in PBS at room temperature for 10 min, further incubated with FITC-conjugated IgG $(Fab')_2$ of goat anti-mouse IgG diluted 1 : 32 in PBS for 45 min and again washed in PBS. The sections were finally mounted in PBS-glycerol with 0.1% paraphenylenediamine, pH 8.6, (11) and examined in a Zeiss fluorescence microscope with an 150 W Xenon lamp. Control sections were either incubated with normal mouse serum instead of MoAb, or the primary antibody was omitted.

RESULTS

The results obtained with unfixed cryosections are summarized in Table I.

Langerhans' cells

The anti-FcR II MoAb (IV.3) stained dendritic EC morphologically characteristic for LC in sections from all skin specimens up to a dilution of 1 : 128. The stained cells were regularly distributed in the supra-basal layer as well as higher up in the epidermis. Cell membranes and dendrites were both stained (Fig. 1). Examination of adjacent sections showed that IV.3 stained 50–70% of all CD1a+ dendritic EC. The anti-FcR I antibody (32.2) gave occasional staining of a few dendritic EC, comprising less than 10% of all CD1a+ cells (Fig. 2 *a*). The



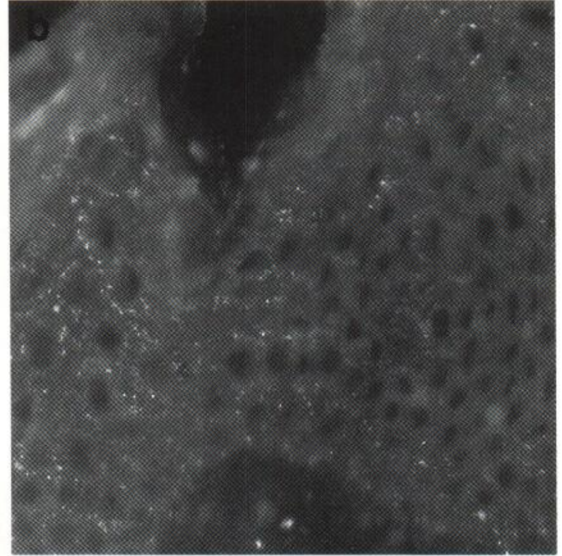


Fig. 2 a–b. Sections of normal skin stained with the MoAb agains FcR I (32.2). (*a*) Staining localized to dendritic epidermal cells (*arrows*) and to stratum corneum and granulosum. Acetone-fixed, ×250. (*b*) Granular staining localized along the outer aspect of keratinocytes. Unfixed, ×350.

MoAbs against FcR III (Leu 11b) and placental FcR (B1D6) did not stain dendritic EC.

IV.3 gave stronger and 32.2 weaker staining with acetone-fixed than with unfixed sections.

Keratinocytes

Leu 11b and B1D6 gave linear staining located along the outer aspect of KC (Figs. 3–4). Neither of these MoAbs gave cytoplasmic staining. The staining was intense and present up to a dilution of 1 : 2048 of

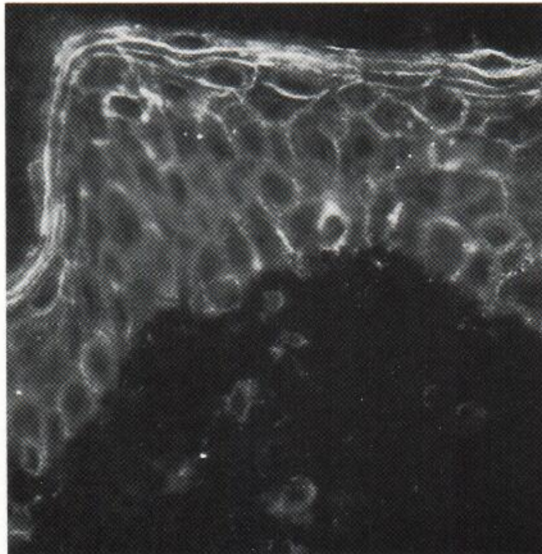


Fig. 3. Section of normal skin stained with the MoAb against FcR III (Leu 11b). Staining localized to stratum granulosum and to stratum spinosum. Acetone-fixed, ×250.

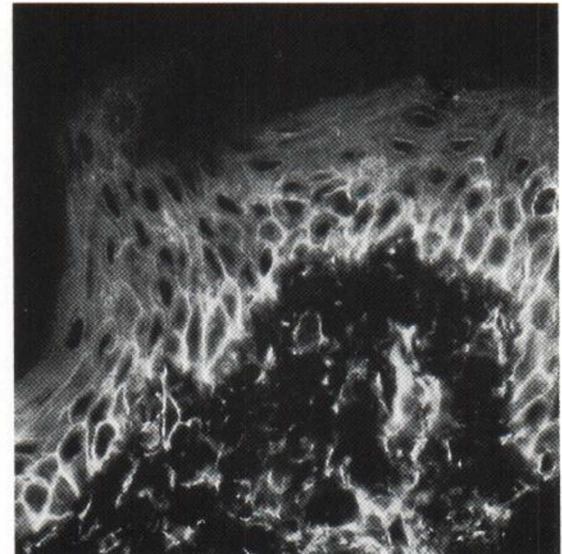


Fig. 4. Section of normal skin stained with the MoAb against placental FcR (B1D6). Staining localized to the lower part of stratum spinosum and to stratum basale. Acetone-fixed, ×250.

these MoAbs in unfixed sections and 1:512 in acetone-fixed sections. There were some variations in the staining pattern of several biopsies and in sections from the same biopsy. Leu 11b gave strongest staining in the upper part of epidermis, particularly in stratum granulosum. B1D6 gave the most pronounced staining in the lower part of epidermis, and only occasionally stained stratum corneum. IV.3 did not stain KC (Fig. 1).

The MoAb 32.2 gave a granular staining corresponding to the outer aspect of the KC in all unfixed sections, up to a dilution of 1:128 (Fig. 2*b*). The staining varied from biopsy to biopsy and within sections from the same biopsy both with respect to staining pattern, intensity and proportion of stained KC. In most biopsies there was a weak fine granular staining of the majority of KC. In two of the biopsies, a stronger and more coarse granular staining occurred in the stratum spinosum as well as in the stratum basale. Strongest staining was always found in the stratum granulosum and stratum corneum (Fig. 2*a*). There was no cytoplasmic staining with 32.2.

Using acetone-fixed sections, 32.2 gave weaker staining of stratum corneum and granulosum cells and usually no staining of KC in stratum spinosum and basale.

Controls

None of the sections showed any staining when mouse serum was used instead of MoAb or when the primary antibody was omitted.

DISCUSSION

The findings presented extend our recent observation that LC *in situ* in normal human skin express FcR activity with binding capacity for IgG containing soluble immune complexes (7). Most LC were stained by the anti-FcR II antibody, IV.3, indicating expression of low affinity FcR. This is supported by the previous finding that complexed IgG has a much higher capacity than native IgG to inhibit the binding of soluble immune complexes to LC (7). Some dendritic EC reacted weakly with the FcR I antibody. Expression of FcR I is characteristic for macrophages/monocytes (1–3). Since LC belong to the mononuclear phagocyte system, it would not be entirely unexpected if they were also to express FcR I. However, it has recently been reported that LC in suspension (12, 13) express only FcR II. FcR I is sensitive to and FcR II is resistent to trypsin (1). The differing results when examining FcR, *in situ* and in suspension, may be due to the processing of skin specimens to prepare cell suspensions and to fixation procedures.

FcR expression on KC has been previously detected using both soluble immune complexes (7, 8) and IgG-coated bovine erythrocytes (8). KC reacted similarly with Leu 11b and B1D6, indicating the presence of low affinity types of FcR on these cells. A reactivity of KC in normal skin with Leu 11b was recently also described by Hunyadi & Simon (14) and Niedecken et al. (15), but neither of them discussed this finding in the perspective of FcR III. Since the high affinity FcR I expression has been considered restricted to mononuclear phagocytes (1–3), the reactivity of KC with the anti-FcR I MoAb 32.2 was unexpected. Earlier studies using functional assays gave results indicating weak FcR activity on KC (7, 8). Possible explanations could be lower IgG affinity of FcR I on KC than on mononuclear phagocytes, or a low number of receptor molecules available on KC.

Another possibility is *in vivo* occupation of the receptors by IgG and immune complexes, affecting the results obtained with immune complex binding assay, but not with 32.2 which binds to an epitope outside the ligand-binding site of FcR I (1). Using indirect IF assay with MoAbs against IgG subclasses, we recently found IgG in normal human epidermis (unpublished data). The possibility that the three different MoAbs, 32.2, Leu 11b and B1D6, cross-react with epidermal non-FcR antigens seems remote.

A striking finding was the intense staining of stratum granulosum with the MoAbs against FcR I and FcR III. This is of particular interest in view of stratum granulosum being the primary permeability barrier of normal skin (16). The FcR activity in stratum granulosum may have an immune function in protection against micro-organisms and other antigens. Further studies are in progress to elucidate this new aspect of the skin immune system.

A peculiarity of IF testing of skin specimens is the affinity of the stratum granulosum and stratum corneum for fluorochrome-conjugated antibodies (17). The nature of this antigen non-specific binding has been obscure. The basic protein or filaggrin of granulosum cells has been excluded as the binding substance (17). The demonstrated FcR activity in these epidermal layers may easily give unwanted non-spe-

cific staining. This problem is avoided when using acetone-fixed sections, since the FcR activity is sensitive to fixatives (18, 19). However, as shown in this study, acetone fixation may modulate the antigens and give a different staining result. For unfixed sections the use of isotypic controls or $F(ab')_2$ fragments is important.

The functional role of the different types of FcR on various cells and in different organs is not fully understood. The intracellular events initiated by the triggering of FcR are just in the early stages of analysis. The presence of FcR on KC and LC suggests the ability to respond to immunoglobulins and immune complexes. The recently described FcR-mediated antibody-enhanced entry of HIV-1 by monocytic cells (20) is of particular interest in relation to the mechanism for virus uptake by LC (21).

The FcR expression on KC is a further support for an immunological role for KC in normal skin. FcR might be mediators for interaction between KC and LC. FcR binding influences the functional activity of phospholipase A which results in production of precursors for prostaglandins and leukotrienes (22) with subsequent effects on the cytokines. Recently, Epstein et al. (23) found that IgG with an intact Fc-part can inhibit cytokine-induced up-regulation of Ia antigens on murine LC. This observation favours the presence of FcR I on epidermal cells. The complexity of interacting cytokines in the epidermis is illustrated by the observations that γ-IFN induced expression of FcR I on leukocytes can be suppressed by interleukin-1 (24). The FcR could also mediate KC influence on mononuclear cells trafficking the skin, analogous to intercellular adhesion molecules (25, 26). Interestingly, there is FcR expression also on dermal endothelial cells (27), another cell-type involved in mononuclear cell retention and migration into the skin. The synthesis and possible shedding of FcR by KC in inflammatory skin disorders, triggered by γ-IFN from activated dermal T lymphocytes may well have pathophysiological implications.

Further investigations are needed to clarify the functional significance of the FcR in epidermis under normal and pathological conditions. It would appear that FcR plays an important role in the complex network of receptors and cytokines in the skin.

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