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Fcγ-receptors and HLA-DR Antigens on Endothelial Cells in Psoriatic Skin Lesions

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Receptors for the Fc-part of IgG (FcR) and HLA-DR antigens on endothelial cells in normal and lesional skin from patients with psoriasis were studied in cryostat sections, using soluble immune complexes and monoclonal antibodies. FcR and HLA-DR antigens were detected on endothelial cells of dermal vessels both in sections of normal and lesional skin. The expression of FcR varied from one vessel to another and on endothelial cells within one and the same vessel. The expression of FcR and HLA-DR antigens was enhanced in sections of lesional skin compared with normal skin and most pronounced in lesional skin from active psoriasis. The enhanced expression may be mediated by interferon produced in psoriatic lesions. The presence of FcR and HLA-DR antigens on endothelial cells adds further evidence of he involvement of these cells in immune processes in the skin. (Received November 5, 1987.)

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Receptors for the Fc-part of the IgG molecule (FcR) have been demonstrated on endothelial cells of fetal stem vessels in human placenta (1) and on endothelial cells of human umbilical cord (unpublished data). FcR have also been found on endothelial cells in pulmonary vessels, but usually only after viral infection (2) or other tissue injury (3, 4). Recently, data indicating FcR on endothelial cells in rat liver were presented (5). It has been claimed that FcR are topographically closely associated with HLA-DR antigens (6, 7). Cultured umbilical endothelial cells can activate T lymphocytes, and this activation is restricted by class II histocompatibility complex antigens (HLA-DR) (8, 9). FcR have also important immunoregulatory functions (10). To elucidate the role of endothelial cells in immune processes in the skin, in particular in psoriasis, we have studied the presence of FcR and HLA-DR antigens on endothelial cells using immune complexes and monoclonal antibodies.

MATERIALS AND METHODS

Tissues

Punch biopsies were taken from normal skin in 4 healthy individuals, and from lesional skin in 4 patients with psoriasis vulgaris, 2 patients with generalized pustular psoriasis and 2 patients with psoriatic erythroderma. Normal placental tissue at term was obtained from the Department of Gynaecology and Obstetrics. The specimens were quick-frozen in isopenthane which had been pre-cooled with liquid nitrogen and were sectioned 4–6 µm thick in a cryostat. Sections of normal placenta were included as control in each experiment. The sections were stored at −20°C until use.

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 (1). Rabbit $F(ab')_2$ was prepared as described by Stewart et al. (11). A monoclonal antibody (clone B1D6), isotyped as IgG1, against placental FcR was prepared *ad modum* Koehler & Milstein (12). This antibody reacts with an epitope on the placental FcR molecule, stains the apical aspects of the trophoblasts and the endothelium of the fetal stem vessels and inhibits the receptor activity (13). Recently, an approximately 40 kD protein with FcR ac-

tivity was isolated from placental extract, using immunosorbent chromatography with B1D6 (14). A monoclonal antibody against HLA-DR antigens (OKIa1) was purchased from Ortho Diagnostics, Raritan, N.J., USA. Fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG and IgG $F(ab')_2$ preparation of goat anti-rabbit IgG were purchased from DAKO-Immunoglobulins A/S, Copenhagen, Denmark. Before use, these antibody preparations were absorbed with glutaraldehyde insolubilized human serum proteins, prepared as described by Avrameas & Ternynck (15). Pooled native human IgG (Fraction II, 16.5% solution) was purchased from AB Kabi, Stockholm, Sweden. Aggregation of IgG was performed by heating at 63°C for 15 min.

Immune complexes

Immune complexes were prepared by adding either dilutions of the IgG or the preparation of $F(ab')_2$ fragments to HRP to equal amounts of four-fold dilutions of HRP from 1 mg/ml in phosphate-buffered saline, pH 7.2 (PBS). The mixtures were incubated for 2 h at room temperature before use. Complexes prepared at slight antigen excess were used (1).

Demonstration of FcR with immune complexes

Light microscopy. Cryostat sections were incubated with soluble immune complexes of HRP-anti-HRP at room temperature for 45 min and then washed in PBS at room temperature for 30 min. The peroxidase activity was then revealed by incubating the sections in 3.3-diaminobenzidine tetrahydrochloride as described by Graham & Karnovsky (16). Sections were finally stained with haematoxylin. Endogenous peroxidase activity was examined by incubating the sections with DAB solution alone.

Immunofluorescence. Cryostat sections were first incubated with complexes of HRP-anti-HRP and washed as above. The secions were then incubated at room temperature for 30 min with FITC-labelled IgG $F(ab')_2$ of goat anti-rabbit IgG (diluted 1 in 30 in PBS), washed in PBS and finally mounted in PBS–glycerol. The preparations were examined in a Zeiss fluorescence microscope with an Osram HBO-200 mercury lamp. Some sections were first incubated with twofold dilutions (16–0.5 mg/ml) of human heat-aggregated IgG at room temperature for 60 min and washed at room temperature in PBS for 30 min. These sections were then incubated with immune complexes of HRP-anti-HRP followed by FITC-labelled IgG $F(ab')_2$ preparations of goat anti-rabbit IgG. Sections were also incubated with immune complexes containing $F(ab')_2$ fragments of IgG anti-HRP, and further processed as described above. Other control sections were incubated with PBS followed by incubation with FITC-labelled rabbit anti-mouse IgG or IgG $F(ab')_2$ preparation of goat anti-rabbit IgG.

Demonstration of FcR and HLA-DR antigens with monoclonal antibodies

Cryostat sections were incubated with various dilutions of the monoclonal antibodies at room temperature for 30 min, washed in PBS, incubated with FITC-labelled rabbit anti-mouse IgG (diluted 1 : 30 in PBS), again washed in PBS and finally mounted in PBS–glycerol. In some experiments, sections were pre-incubated with twofold dilutions of anti-FcR antibody, before they were incubated with HRP-anti-HRP as described above.

RESULTS

Immune complexes of HRP-anti-HRP bound to endothelial cells in sections of lesional skin from patients with active, exudative psoriasis vulgaris, erythroderma and pustular psoriasis. HRP-anti-HRP did not bind to endothelial cells in sections from lesional skin in patients with stable psoriasis vulgaris or normal skin. However, when we used the more sensitive indirect immunofluorescence (IIF) technique, most endothelial cells in sections from lesional skin were stained. In addition, endothelial cells in some vessels in sections of normal skin were stained. Endothelial cells of both venules and arterioles appeared to be stained. The staining was linear and localized mainly to the apical aspect of the endothelial cells (Fig. 1 *a*). The intensity of the staining varied from one vessel to another in the same section, and was always weaker than the strong, granular staining of macrophages in the dermis (Fig. 1 *b*). Strongest staining of endothelial cells was obtained with sections of lesional skin from patients with very active psoriasis, especially psoriatic erythroderma and pustular psoriasis. Immune complexes of HRP-anti-HRP bound to endothelial cells of fetal stem vessels and to trophoblasts in sections of human normal placenta, as previously described (1).

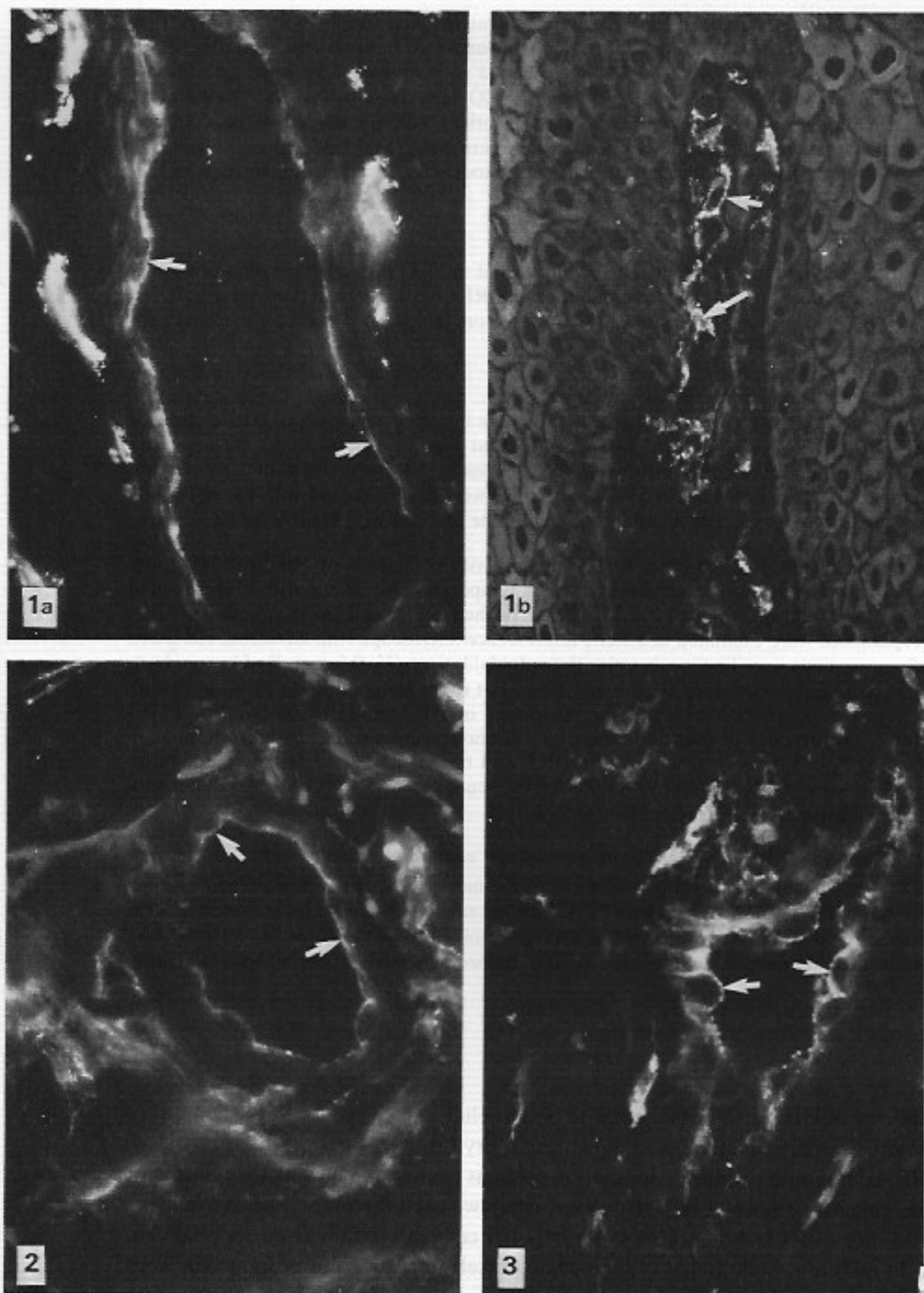

Fig. 1. Section of a stationary lesion of psoriasis vulgaris incubated with HRP-anti-HRP and FITC-labelled goat anti-rabbit IgG. (*a*) Moderate positive reaction on endothelial cells (*arrows*) (×355). (*b*) Strong positive reaction on dermal macrophages (*arrows*) (×355).

Fig. 2. Section of a stationary lesion of psoriasis vulgaris incubated with monoclonal anti-FcR antibody and FITC-labelled rabbit anti-mouse IgG, showing strong positive staining on endothelial cells (*arrows*) (×355).

Fig. 3. Section of stationary lesion of psoriasis vulgaris incubated with OKIa1 antibody and FITC-labelled rabbit anti-mouse IgG, showing a moderate positive staining of endothelial cells (*arrows*) (×355).

Sections incubated with immune complexes prepared with $F(ab')_2$ fragments of IgG anti-HRP were not stained. Heat-aggregated IgG (2 mg/ml) inhibited the binding of immune complexes to the sections. Evidently, the binding of HRP-anti-HRP was mediated by the Fc part of the IgG molecule.

Endogenous peroxidase activity was regularly observed in some dermal macrophages and epidermal dendritic cells, but never in endothelial cells.

In IIF test with monoclonal anti-FcR antibody a linear staining mainly along the apical aspect of endothelial cells was detected in all sections, from both normal and psoriatic skin (Fig. 2). The positive reactions on endothelial cells were recorded in sections of normal skin with the antibody up to a dilution of 1 : 256 and in psoriatic skin up to a dilution 1 : 2 048. The anti-FcR antibody inhibited the binding of immune complexes up to a dilution of 1 : 8.

In the IIF test with OKIa1 antibody, endothelial cells in most sections of psoriatic skin were stained, usually with a granular pattern (Fig. 3). Endothelial cells in sections of normal skin showed weak staining, or else they were unstained. With OKIa1 diluted 1 : 32, the staining intensity of dermal macrophages and epidermal Langerhans' cells was strong, while the staining intensity of endothelial cells in psoriatic skin was considerably weaker.

There was no immunofluorescence staining of sections pre-incubated with PBS instead of monoclonal antibody or immune complexes.

DISCUSSION

The results strongly indicate that endothelial cells of skin vessels express FcR. The FcR were detected on endothelial cells of both venules and arterioles, and mainly on the apical aspect of the cells. This was shown using two different methods: First, the binding of soluble immune complexes (which is a functional assay) and second, the use of a monoclonal anti-FcR antibody, which detects an epitope on the FcR (13). Using the sensitive IIF methods, we were able to demonstrate FcR on endothelial cells both in normal and lesional skin. The reaction with the monoclonal antibody shows that the endothelial cell FcR in the skin share an epitope with the FcR on endothelial cells in placental vessels.

The expression of both FcR and HLA-DR antigens was stronger on endothelial cells in sections of lesional skin than in normal skin and strongest in clinically active lesions. Furthermore, the strength of reaction varied from one vessel to another within the same section. This can be explained by variation in the number of molecules or affinity sites available.

Exact localization of the FcR is not possible using IF technique with tissue sections. This localization is important, since vimentin, actin, myosin and other intermediate filaments in animal endothelial cells can bind IgG (17). Preliminary data of electron microscopical study on fetal stem vessels in placental tissue indicate that FcR are localized at the surface of the endothelial cells (unpublished data). However, electron-microscopy studies on FcR are difficult since the sensitivity of the receptors to fixatives demands the use of frozen tissue which gives unsatisfactory morphology.

The origin of the dermal endothelial cell FcR is at present obscure. Using the monoclonal anti-FcR antibody, we have recently demonstrated an FcR-like molecule in serum from patients with psoriasis (unpublished data). The FcR could therefore be adsorbed from serum onto the endothelial cells in the skin. On the other hand, the FcR augmentation demonstrated in lesional skin could be the result of in situ induction equivalent to the HLA-DR expression in vitro by stimulated endothelial cells (18).

It has been shown that immune interferon (IFN-γ) can induce HLA-DR antigens on endothelial cells (8) and keratinocytes (19). IFN-γ can also enhance the FcR activity on immunocompetent cells (20, 21). In psoriatic lesions we have demonstrated local production of IFN (α and/or γ) (22). It is possible that the augmented FcR and HLA-DR expression on en-

dothelial cells in psoriatic and other skin lesions are due to IFN-γ produced at the inflammatory site by activated T lymphocytes (23) or by epidermal cells (24, 25).

In vivo deposits of immunoglobulins and complement (C) factors in and around vessels can be detected in several dermatoses (26), including psoriasis (27). The mechanism by which immune complexes are deposited in vascular tissue is not clear. One possibility is that immunoglobulins and/or immune complexes are bound to FcR. It has previously been reported that endothelial cells in the skin have receptors for C3b (28). However, we have not been able to detect endothelial complement receptors using complement-coated immune complexes or monoclonal antibodies (unpublished data).

The previously reported association of FcR and HLA-DR antigens (7) is sustained by the present observations. The FcR may be involved in several functions: binding and phagocytosis of antibody-coated particles, antibody dependent cytotoxicity, transport across epithelial cells and in the release of mediators of inflammation (29). The FcR could also be involved in the adherence of T lymphocytes to endothelial cells, recently described (30). In addition, FcR are probably involved in immunoregulation (10). Thus, the present demonstration of both FcR and HLA-DR antigens on endothelial cells strengthens the possibility that endothelial cells have a role mediating immune reactions in the skin.

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