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In situ localization of interferons in psoriatic lesions

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Summary. An indirect immunofluorescence technique, using murine monoclonal antibodies (MoAbs) against human IFN-α and human IFN-γ was used to study IFNs in cryostat sections from psoriatic skin lesions. The IFNs were more pronounced in sections from highly active psoriasis than in sections from stationary psoriasis. In highly active psoriatic lesions IFN-α was localized to keratinocytes is stratum basale, to some epidermal dendritic cells, probably Langerhans cells, and to some mononuclear cells in dermis. IFN-α was usually not detected in sections from stationary psoriasis. IFN-γ was localized to stratum corneum, to keratinocytes around microabcesses and to mononuclear cells in the dermal cell infiltrates, predominantly in highly active psoriatic lesions. Both IFN-α and IFN-γ were localized to some endothelial cells in the papillary dermis. The MoAbs did not stain sections from unaffected skin from patients with psoriasis or sections from healthy individuals. The findings indicate that the IFN system in the skin may be of significance in the pathophysiology of psoriasis.

Key words: Psoriasis – Interferons

Previously, we demonstrated interferon (IFN) in serum and suction blister fluid from patients with psoriasis using an infectivity inhibition micromethod [4]. Sera from patients with psoriasis had higher levels of IFN than sera from healthy individuals. Higher IFN levels were detected in suction blister fluid from psoriatic skin lesions than in serum and in suction blister fluid from unaffected skin, indicating IFN production in the skin lesions. Characterization experiments indicated the presence of IFN-γ and both acid labile and acid stable IFN-α in blister fluids and sera. Extended studies using an ELISA assay indicate increased levels of IFN-γ in sera from patients with active psoriasis compared with sera from healthy individuals, while sera from patients with stationary psoriasis have decreased levels of IFN-γ (unpublished data). Recently, Kapp et al. [11] found decreased IFN production by peripheral leukocytes from patients with psoriasis.

IFN-γ is mainly produced by activated T lymphocytes, whereas IFN-α is produced by several cell types after various viral and nonviral stimuli. Previously, we demonstrated retrovirus-like particles in suction blister fluids from psoriatic skin lesions [5]. The presence of IFN-α and retrovirus-like particles in blister fluids from psoriatic lesions indicated that virus may be of significance in the pathogenesis of psoriasis [23].

Further information on IFN in psoriasis is of particular interest in relation to disease activity and cellular localization. Therefore, we examined sections of psoriatic and normal skin using an immunofluorescence technique with monoclonal antibodies to human IFN-α and IFN-γ.

Materials and methods

Patients and tissue

Skin biopsy specimens were obtained from 5 patients with stationary psoriasis, from 5 patients with highly active peripherally spreading psoriasis (average age, 46 years) and from 10 healthy individuals (average age, 32 years). Punch biopsy specimens (6 mm) were taken from margins of fully developed lesions and from unaffected skin at least 10 cm from visible lesions. Local treatment was interrupted for at least 1 week before the biopsy specimens were taken and none of the patients received systemic treatment. Punch biopsies (3 mm) from healthy individuals were obtained from the same areas as biopsies from the psoriatics. All tissue samples were washed for 10 min in phosphate buffered saline (PBS), pH 7.2, snap-frozen in isopentane prechilled to −140°C in liquid nitrogen, and stored at −70°C. Cryostat sections were cut at 4–6 μm.

Antibodies and sera

A murine monoclonal antibody (MoAb; IgG1) against human IFN-α was purchased from Hybritech, San Diego, Calif., USA,

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and a murine MoAb (IgG2) against human IFN-γ was purchased from Chemicon, Los Angeles, Calif., USA. MoAb Leu6 (anti-CD1a), reacting with epitopes on Langerhans cells, was purchased from Becton Dickinson Laboratory Systems, Mountain View, Calif., USA. Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (RAMIgG) and FITC-conjugated goat anti-rabbit IgG (GARIgG), purchased from DAKO Immunoglobulins, Copenhagen, Denmark, were used for detection of the reaction of the primary antibodies. Pooled native human IgG (Fraction II, 16.5% solution) was purchased from Kabi, Stockholm, Sweden. Normal mouse serum was provided from the local animal unit. Before use the preparations were centrifuged at 100,000 *g* for 1 h to remove aggregates.

Immunofluoresence technique

Unfixed cryostat sections were incubated with MoAbs at 4°C overnight (24 h) in a moist chamber. In some experiments the MoAbs were diluted in PBS-containing human IgG (8 mg/ml) to prevent binding to receptors for the Fc part of IgG [13]. Preliminary experiments showed that the anti-IFN-α antibody stained sections of active psoriasis up to a dilution of 1 in 4096, and the anti IFN-γ antibody up to a dilution of 1 in 1024. In further experiments the anti-IFN-α was used diluted 1 in 1024 and the anti IFN-γ diluted 1 in 256 if not otherwise stated. The anti-CD1a MoAb was used diluted 1 in 2048. The sections were then washed in PBS, incubated for 45 min with FITC-conjugated RAMIgG diluted 1 in 32 in PBS containing 25% pooled human serum. Finally, the sections were washed in PBS and incubated for 45 min with FITC-conjugated GARIgG diluted 1 in 32 in PBS containing 25% pooled human serum. The sections were then washed in PBS and mounted in PBS and glycerol. Control sections were incubated with PBS or mouse serum diluted 1 in 16 in PBS in the first step and further incubated as described.

The sections were examined in a Leitz Orthoplane microscope equipped with a Ploemopak for incident light fluorescence, using a 150 W Xenon lamp and filters L2 or K2. Photomicrographs were taken with a Leitz Varioorthomat camera and Kodak Tri X (400 ASA black and white) film.

Results

The MoAb against IFN-α stained cells both in epidermis and dermis in highly active psoriatic lesions characterized by spongiosis and microabscesses in stratum corneum. In the epidermis the staining was localized to keratinocytes in the basal layer. The staining was granular and showed two main patterns, one mainly localized to the outer aspect of the keratinocytes, the other mainly localized to the nuclei and diffusely in the cytoplasm (Fig. 1a). The keratinocytes in stratum spinosum and in stratum corneum were not stained. The intensity and staining pattern varied from one area to another in the same section, and in sections from different patients. The keratinocytes in sections from stationary psoriasis were not stained. The MoAb also gave a strong intracellular staining of some epidermal dendritic cells (Fig. 1b). The morphology of the dendritic cells corresponded to and had similar distribution as the CD1a+ cells. The number of cells stained by the MoAb against IFN-α was, however, lower than the number of CD1a+ cells. In the dermis the IFN-α antibody gave a granular staining of the cell membranes of some mononuclear cells (Fig.1b) and of the cytoplasm of some endothelial cells in the papillary dermis (Fig. 1c). In sections from stationary psoriasis the staining of dendritic cells and mononuclear cells was weak to negative.

Sections from unaffected skin from patients with highly active psoriasis, sections from unaffected skin from patients with psoriasis vulgaris, and sections from normal skin were not stained with the MoAb against IFN-α even at a dilution of 1 in 16 (Fig. 1d).

The MoAb against IFN-γ gave a strong laminar staining of stratum corneum in sections from highly active and a weaker staining in sections from stationary psoriasis (Fig. 2a). Stratum corneum in sections from unaffected skin from patients with psoriasis and in sections from normal skin were only occasionally stained. The cell membranes of keratinocytes around microabscesses were also stained, whereas cells in stratum basale and stratum spinosum were not. In the dermis the IFN-γ antibody gave a strong granular staining of cells in the mononuclear cell infiltrates in sections from highly active psoriasis (Fig. 2b) and a weaker staining in sections from stationary psoriasis apparently localized to the cell membranes. Staining was also seen in the cytoplasm of some endothelial cells in the papillary dermis. The MoAb did not stain dermal cells in sections from unaffected skin from patients with psoriasis nor dermal cells in sections from normal skin.

Control sections incubated with PBS or normal mouse serum diluted 1 in 16 were all negative (Fig. 2c).

Discussion

In the present study, IFN-α and IFN-γ were demonstrated on various cell types in highly active psoriasis lesions. In lesions from stationary psoriasis IFN-γ could be detected whereas IFN-α were only occasionally present. The demonstration of IFN mainly in highly active psoriasis accords with the results recently reported by Traugott and Lebon [29]. They found IFN on various cell types in active multiple sclerosis (MS) lesions, but not on cells in inactive chronic MS lesions. Furthermore, Jilbert et al. [10] demonstrated IFN on cells in chronic active hepatitis but only occasionally on cells in chronic persistent hepatitis. The present data obtained could be due to a possible cross-reaction of the MoAbs with epitopes on other molecules than IFNs. This is, however, unlikely since the MoAbs did not react with sections from unaffected skin from patients with psoriasis or sections from normal skin. Recently, Yaar et al. [33] could not detect IFN-α in sections from normal epidermis using three different MoAbs.

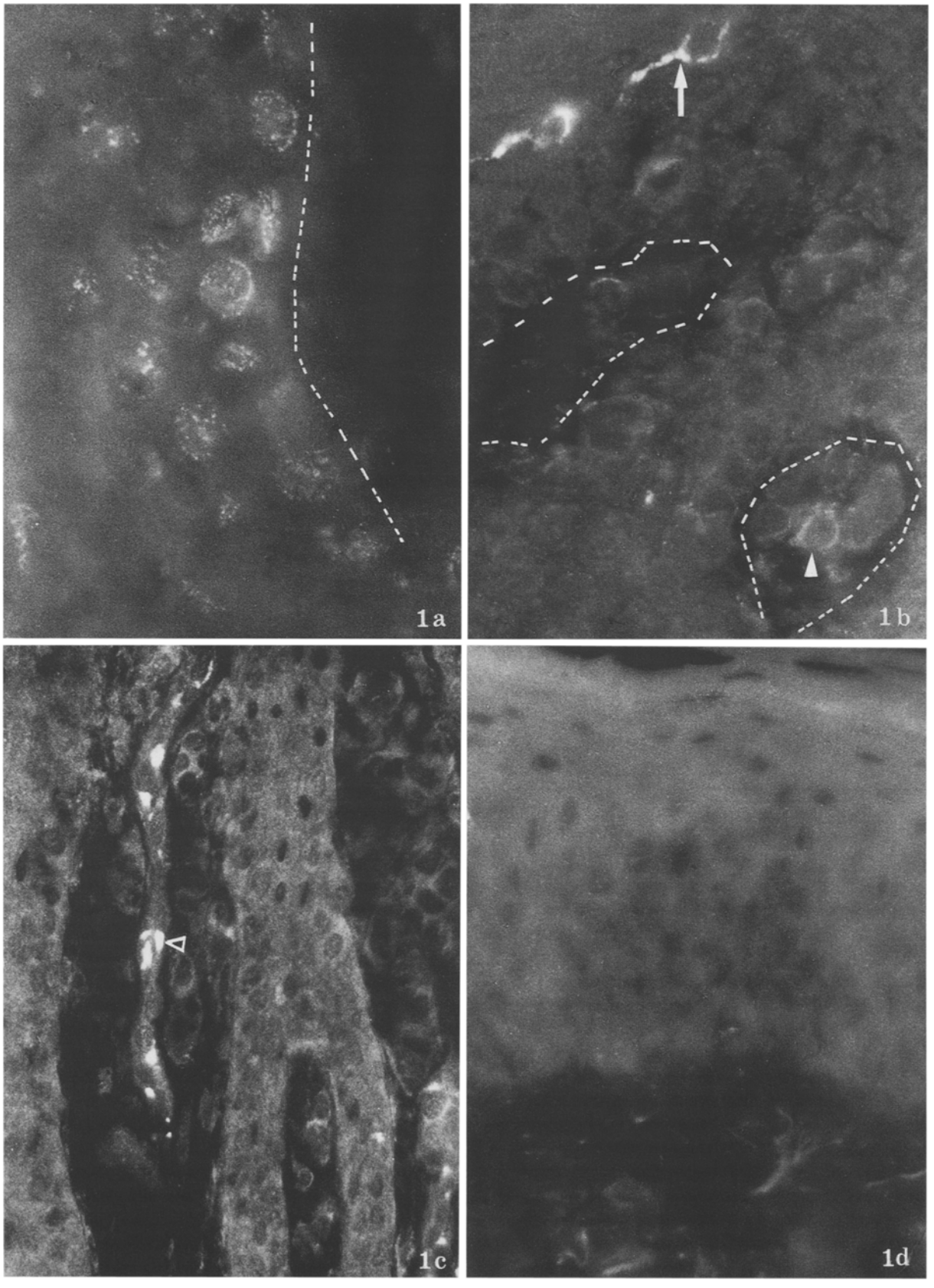

Fig. 1a–d. Sections of highly active psoriasis stained with the MoAb against IFN-α. **a** Staining mainly localized to the nuclei and cytoplasm of keratinocytes in the basal layer (*dotted line*, basement membrane zone; ×530); **b** staining localized to dendritic cells in stratum spinosum (*arrow*) and to mononuclear cells in the papillary dermis (*arrowhead*; dotted line, basement membrane zone; ×470); **c** staining localized to endothelial cells in the papillary dermis (*open arrow*; ×300); **d** section from unaffected skin from a patient with highly active psoriasis. No staining (×250)

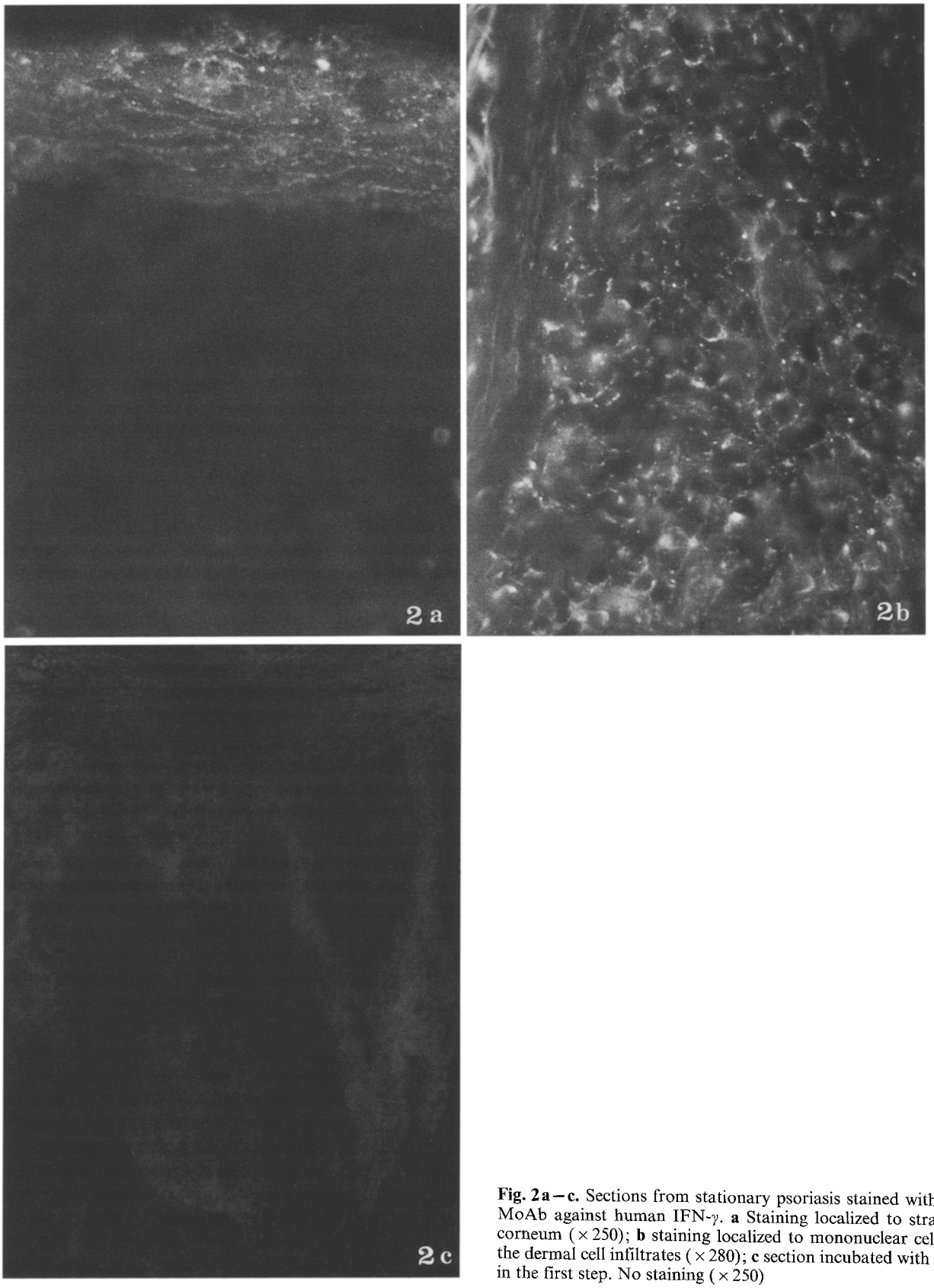

Fig. 2a–c. Sections from stationary psoriasis stained with the MoAb against human IFN-γ. **a** Staining localized to stratum corneum ($\times 250$); **b** staining localized to mononuclear cells in the dermal cell infiltrates ($\times 280$); **c** section incubated with PBS in the first step. No staining ($\times 250$)

The expression of IFN-α by basal keratinocytes, epidermal dendritic cells, mononuclear cells and by endothelial cells may indicate that the cells have been stimulated to produce IFN. This may be due to a virus infection [7, 21]. Whether these cells are virus-infected is at present unknown, but our demonstration of virus-like particles [5] in suction blister fluid from psoriatic lesions is intriguing. Alternatively, IFN-α in these cells may be due to IFN-inducing molecules present as a result of the mononuclear cell infiltrate. IFN-γ has been shown to trigger the production of IFN-α [12]. IFN-α may therefore be induced by IFN-γ diffusing from the IFN-γ-positive mononuclear cells. The marked expression of IFN-γ in stratum corneum does in fact support the assumption that IFN-γ may diffuse through the epidermis. The data do not, however, exclude the possibility that the presence of IFN-α in these cells is due to receptor-mediated endocytosis of IFN-α produced by other cell types. According to Grossberg et al. [9] membrane-bound IFN are rapidly processed by receptor-mediated endocytosis into coated vesicles and receptosomes which then empty into the perinuclear space. IFN molecules are then seen adjacent to or in nuclear pores and within the nucleus, primarily in dense chromatin. The staining pattern of the basal keratinocytes may therefore indicate that IFN-α present in these cells is due to endocytosis. In contrast, the diffuse staining within the cytoplasm of epidermal dendritic cells, mononuclear and endothelial cells makes it unlikely that IFN-α detected was due to endocytosis [10]. Whether the IFNs detected in psoriatic lesions are produced locally is unclear. However, our previous data obtained studying IFNs in serum and suction blister fluid indicated a local production of IFN [4].

The strong expression of IFN-γ by the mononuclear cells sustained our previous assumption that these cells are activated [3]. IFN-γ-positive mononuclear cells have also been demonstrated in lesions of active chronic MS [29]. The expression of IFN in some of the endothelial cells in highly active psoriasis also accords with the data reported by Traugott and Lebon [29] when studying IFNs in MS. They found that endothelial cells in active MS lesions were occasionally positive for all three IFN types. According to Pober et al. [19], HLA-DR expression by vascular endothelium is inducible by human IFN-γ. The expression of IFN-γ on endothelial cells is therefore of interest in relation to our previous demonstration of HLA-DR antigen and receptors for the Fc part of IgG (FcR) on endothelial cells in psoriatic skin lesions [6].

Recently, we demonstrated FcR on keratinocytes in normal and psoriatic skin [13]. Enhanced FcR activity by keratinocytes was found in psoriatic skin lesions compared with normal skin and strongest above areas with mononuclear cell infiltrates. This was of interest in relation to our previous demonstration of IFN-γ in suction blister fluid from psoriatic lesions [4], as IFN-γ can induce or enhance the expression of FcR [30]. We assumed that the increased FcR expression by keratinocytes was mediated by IFN locally produced. The present demonstration of IFN-γ positive cells is in favor of this assumption.

In several cutaneous disorders such as lichen planus [27], cutaneous T-cell lymphoma [28], allergic contact dermatitis [15], and lupus erythematosus [1] keratinocytes have been shown to express HLA-DR antigens. The keratinocyte cell-surface HLA-DR expression is particularly seen overlying a dermal inflammatory infiltrate consisting largely of T lymphocytes. Regulatory interactions beween IFNs and interleukins are of importance in skin diseases involving T lymphocytes [18]. It has been shown that interleukin-1 (IL-1), produced by keratinocytes, induces T lymphocytes to produce IL-2 which then induces the production of IFN-γ by T lymphocytes [24]. IFN-γ produced by activated T lymphocytes, is capable of stimulating HLA-DR synthesis by keratinocytes [2, 16]. It has therefore been postulated that IFN-γ produced by the dermal T cells and diffusing into epidermis is responsible for the cell-surface expression of HLA-DR in these disorders. Accordingly, the demonstration of IFN-γ on cells in the dermal cell infiltrate and in stratum corneum is of particular interest. However, HLA-DR expression on keratinocytes is more infrequently found in psoriatic lesions than in other inflammatory skin lesions [26]. The reason for this is presently unknown. A number of substances, however, have been shown to antagonize the induction of HLA-DR expression by IFN-γ. These include prostaglandins [22], cyclic AMP [35], LPS [35], immune complexes [31], α-fetoprotein [14], serotonium [25], and glucocorticosteroids [32]. In addition IFN-α has been shown to decrease the level of HLA-DR specific mRNA [8]. The presence of IFN-α in the basal keratinocytes and in epidermal dendritic cells may therefore influence the HLA-DR expression induced by IFN-γ. According to Yasutaka et al. [34] cross-linking of FcR on various cell lines in vitro inhibited the ability of IFN-γ to induce HLA-DR. An in vivo cross linking of FcR on keratinocytes [13] may therefore be another explanation for the reduced expression of HLA-DR on keratinocytes in psoriatic lesions.

The significance of IFNs in the pathogenesis of psoriasis is unclear. However, due to their multiple biological effects the IFNs may be of importance in the pathophysiology of psoriasis. The strong expression of IFNs by different cell types in highly active psoriasis, the observation that treatment with recombinant-DNA-derived IFN-α ("Roferon-A") [20] caused ex-

acerbation or induced onset of psoriasis as well as the beneficial effect of cyclosporin A, which may be due to its inhibitory effect on IFN-γ production [17], sustain such a hypothesis. Furthermore, the demonstration of IFN-α in psoriatic lesions may support the hypothesis of a virus etiology in psoriasis. A more detailed study of the skin IFN system will undoubtedly increase our understanding of the pathophysiology of psoriasis.

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