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DEMONSTRATION OF Ia-LIKE ANTIGENS ON T LYMPHOCYTES IN LESIONS OF PSORIASIS, LICHEN PLANUS AND DISCOID LUPUS ERYTHEMATOSUS

Jens Roar Bjerke and Roald Matre

Department of Dermatology and Broegelmann Research Laboratory for Microbiology, University of Bergen, Bergen, Norway

Abstract. A combined indirect immunofluorescence technique with a murine monoclonal antibody against human Ia-like antigens (OKIa1) and an IgG F(ab')$_2$ preparation of a rabbit anti-T lymphocyte serum was used to study Ia-like antigens on T lymphocytes in skin lesions of psoriasis, lichen planus and discoid lupus erythematosus. The majority of the T lymphocytes in the various skin lesions expressed Ia-like antigens. T lymphocytes expressing Ia-like antigens were also demonstrated in the dermis and the epidermis in sections of unaffected skin, although of a markedly lower proportion than in affected skin. The results may indicate that the T lymphocytes in these skin lesions are activated cells involved in cell-mediated immune reactions.

Key words: Psoriasis; Lichen planus; Discoid lupus erythematosus; Ia-like antigens; Activated T lymphocytes

A characteristic histological feature of psoriasis, lichen planus and discoid lupus erythematosus (DLE) is dermal infiltrates consisting mainly of T lymphocytes and macrophages (3, 5, 6, 7). Recently, using monoclonal antibodies specific for T lymphocyte subpopulations, we found that most cells in these dermal infiltrates stained with OKT4 (helper/inducer T cell) antibody and that fewer cells stained with OKT8 (suppressor/cytotoxic) T cell antibody (4).

Human Ia-like (HLA-DR) antigens are present on a variety of cell types, i.e. most B lymphocytes (18, 22), monocytes (18, 22), macrophages (18) and dendritic cells in the skin (19). Ia-like antigens have usually not been detected on non-activated human T lymphocytes (13, 18). However, activated T lymphocytes express Ia-like antigens, whether the lymphocytes are activated by mitogens, antigens, or in mixed lymphocyte reaction (13, 18). Accordingly, investigation of the presence of surface Ia-like antigens on T lymphocytes in skin lesions may help to determine whether the T lymphocytes are activated cells.

In this study, a murine monoclonal antibody against human Ia-like antigens (OKIa1) and an IgG F (ab')$_2$ preparation of a specific rabbit anti-T lymphocyte serum was used in combination to study the occurrence of Ia-like antigens on T lymphocytes in lesions of psoriasis, lichen planus and DLE.

MATERIALS AND METHODS

Patients and tissues

Skin biopsies, approximately 10×15 mm, were taken from fully developed lesions in 5 patients with psoriasis vulgaris, 3 with lichen planus and 3 with DLE. The lesions chosen for biopsy had not received any local treatment for at least 2 weeks. In some cases, a 4 mm punch biopsy was also taken from unaffected skin. The specimens were quick-frozen in isopentane which had been pre-cooled with liquid nitrogen and sectioned 4 μm thick on a cryostat. The sections were stored at −20°C until use.

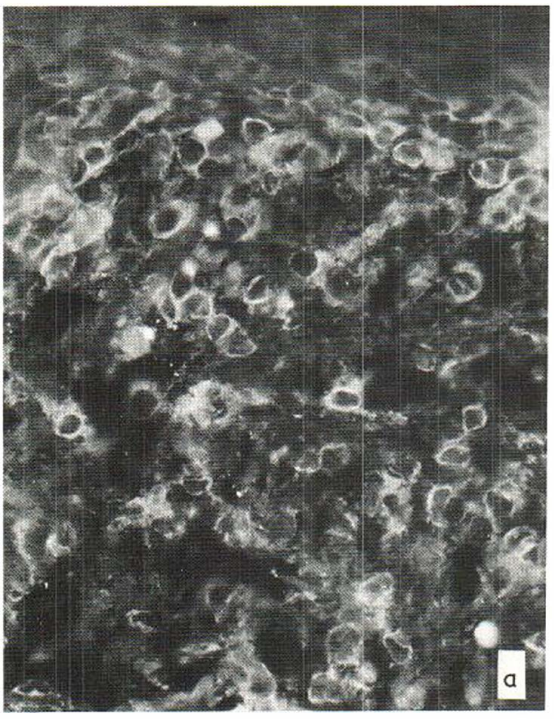
Immunoglobulins

A monoclonal antibody against human Ia-like (HLA-DR) antigens, OKIa1, was purchased from Ortho Pharmaceutical Corporation, Raritan, N. J., USA. Reinherz et al. (18), examining mononuclear cells isolated from peripheral blood, showed that OKIa1 reacts with more than 90% of B lymphocytes and monocytes, 20% of null cells, and with activated T lymphocytes, but no with non-activated T lymphocytes.

Immunoglobulin preparations

IgG F (ab')$_2$ preparation of a rabbit anti-T lymphocyte serum was obtained and rendered specific by extensive absorption, as described elsewhere (16).

Fluorescein isothiocyanate (FITC)-conjugated swine anti-mouse IgG was purchased from Nordic Immunological Laboratories, Tilburg, Holland (code no. 12–380, protein concentration 10 mg/ml, molar F/P ratio between 1 and 4), and tetramethylrhodamine isothiocyanate (TRITC)-conjugated swine anti-rabbit IgG from DAKO-immunoglobulins A/S, Copenhagen, Denmark (code no. Z-109, rhodamine/protein extinction ratio, E_{544nm}/E_{278nm},



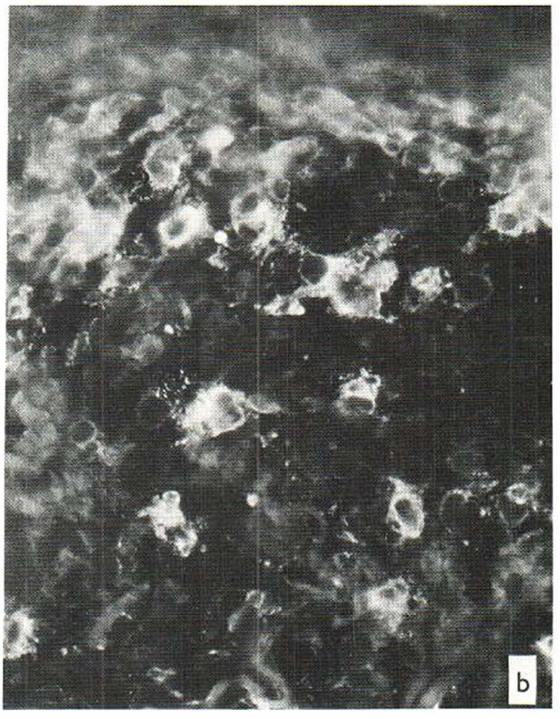


Fig. 1 a–b. Section of a stationary lesion of psoriasis vulgaris double-stained with IgG $F(ab')_2$ preparation of anti-T lymphocyte serum (Fig. 1*a*, red fluorescence) and with OKIal antibody (Fig. 1*b*, green fluorescence). Several of the T lymphocytes express Ia-like antigens. ×350.

is 0.40±0.05). Before use, the preparations were centrifuged at 100 000 g for 1 hour to remove aggregates.

Pooled native human IgG (Fraction II, 16.5% solution) was purchased from AB Kabi, Stockholm, Sweden. Aggregation was performed at 63°C for 15 min. Since the swine anti-mouse IgG gave one precipitation line in double diffusion in agar against human serum, this IgG preparation was absorbed with glutaraldehyde insolubilized human serum proteins prepared as described by Avrameas & Ternynck (1). Swine anti-rabbit IgG did not react with human serum proteins. There was no cross-reactivity between the other antibody preparations used, as demonstrated by double diffusion in agar.

Immunofluorescence technique

Sections for the demonstration of T lymphocytes were incubated at room temperature for 30 min with the IgG $F(ab')_2$ preparation of anti-T lymphocyte serum diluted in phosphate-buffered saline, pH 7.2 (PBS). The sections were then incubated for 30 min with TRITC-conjugated swine anti-rabbit IgG diluted in PBS, washed and mounted in PBS-glycerol. Sections for the demonstration of Ia-like antigens were similarly incubated with OKIal antibody diluted in PBS and then with FITC-conjugated swine anti-mouse IgG diluted in PBS. Titration experiments showed that the optimal working dilution was 1 in 4 for the preparation of anti-T lymphocyte serum, 1 in 16 for OKIal, and 1 in 40 for the fluorochrome-conjugated antibodies. In some experiments, the OKIal antibody was diluted in PBS containing 4 mg/ml aggregated human IgG.

Double staining was usually performed first by incubating the sections with the IgG $F(ab')_2$ preparation of anti-T lymphocyte serum, followed by incubation with OKIal antibody. In some experiments, OKIal was applied before the preparation of the anti-T lymphocyte serum. The sections were washed in PBS, and incubated with a mixture of the TRITC-conjugated swine anti-rabbit IgG and the FITC-conjugated swine anti-mouse IgG. The sections were then washed in PBS and mounted in PBS-glycerol.

Controls were incubated with PBS instead of either the preparation of anti-T lymphocyte serum of the monoclonal antibody.

The preparations were examined in a Zeiss fluorescence microscope with an Osram HBO-200 mercury lamp. The proportion of stained cells in double-stained preparations was evaluated on colour slides, obtained by subsequently photographing the preparations for green and red fluorescence on Ektachrome 400 daylight film with a Zeiss 40/1.0 immersion objective.

RESULTS

The IgG $F(ab')_2$ preparation of anti-T lymphocyte serum and the OKIal antibody both stained the majority of mononuclear cells in the dermal infil-

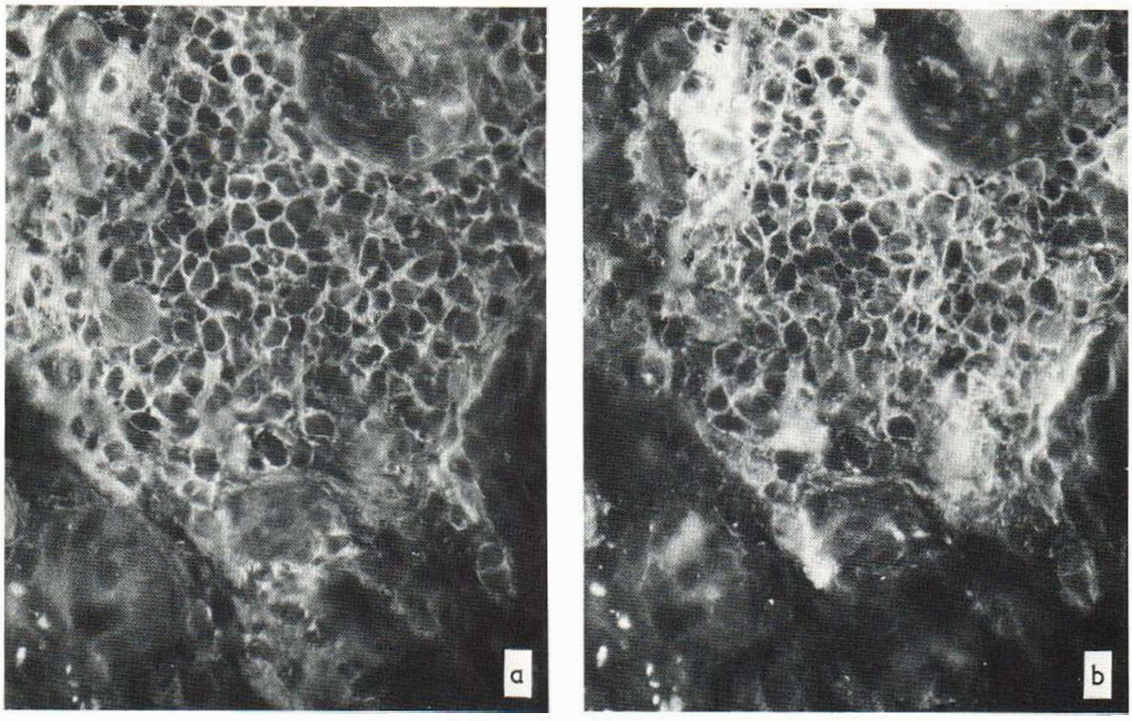


Fig. 2a–b. Section of a DLE lesion double-stained with IgG $F(ab')_2$ preparation of anti-T lymphocyte serum (Fig. 2*a*, red fluorescence) and with OKIal (Fig. 2*b*, green fluorescence). The majority of the T lymphocytes express Ia-like antigens. ×350.

trates of psoriasis, lichen planus and DLE. Applying the combined immunofluorescence technique, most cells were stained both with the preparation of anti-T lymphocyte serum and with OKIal (Figs. 1–2). Some cells were only stained with OKIal. In addition, some T lymphocytes were demonstrated in the epidermis. The epidermis in lesions of psoriasis contained more T lymphocytes than the epidermis in lesions of lichen planus and DLE. Double staining showed that most T lymphocytes in the epidermis were $OKIal^+$. OKIal also stained several dendritic epidermal cells with long cytoplasmatic extensions. These cells were not stained with the IgG $F(ab')_2$ preparation of anti-T lymphocyte serum. OKIal gave apparent intercellular staining in the basal part of the epidermis in the lichen planus lesions and in one of the DLE lesions (Fig. 3). OKIal did not stain corresponding areas in the psoriatic lesions.

In sections of unaffected skin the IgG $F(ab')_2$ preparation of anti-T lymphocyte serum regularly stained a few cells in the dermis, and occasionally also in the epidermis. In double staining, markedly fewer of these T lymphocytes expressed Ia-like antigens, as compared with the T lymphocytes in affected skin.

Sections incubated with PBS instead of either OKIal or the IgG $F(ab')_2$ preparation of anti-T lymphocyte serum, were not stained. Results of these control experiments showed that there was no cross-reactivity between the swine anti-mouse IgG and the preparation of anti-T lymphocyte serum, or between the swine anti-rabbit IgG and the monoclonal antibody, as was also indicated by the results of double diffusion in agar. Aggregated human IgG (4 mg/ml) did not inhibit the staining with OKIal. The results showed that the staining obtained could not be due to binding of OKIal antibody to receptors for the Fc-portion of IgG. These receptors have previously been shown to be present in sections of various skin lesions (8).

DISCUSSION

The results obtained demonstrated that the majority of mononuclear cells in lesions of psoriasis, lichen

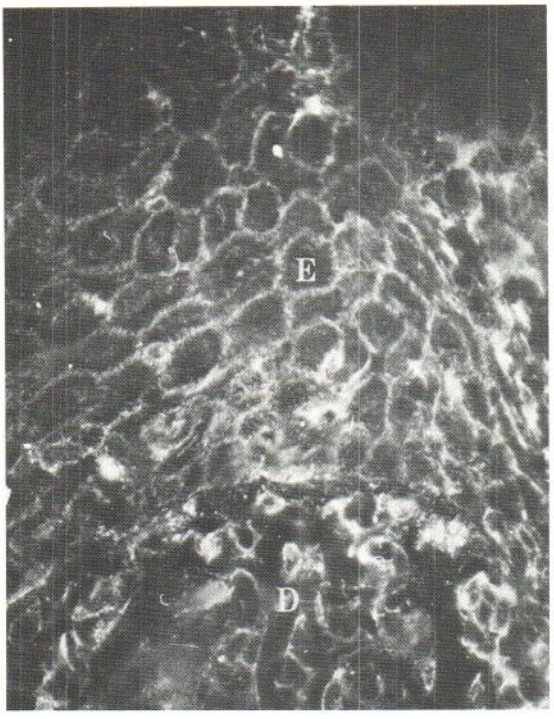


Fig. 3. Section of a lichen planus lesion stained with OKIal antibody. There is epidermal "intercellular" staining above the dermo-epidermal junction. *D*, dermis, *E*. epidermis. ×350.

planus and DLE express Ia-like antigens. The results obtained with sections of lichen planus lesions are consistent with data reported by Malmnäs-Tjernlund (15). Using a rabbit anti-Ia antiserum she claimed that virtually all cells infiltrating lesions of lichen planus were Ia^+. On the other hand, in a recent report, Bhan et al. (2) found that besides dendritic cells, only a small number of mononuclear cells in the dermal infiltrates in lesions of lichen planus were stained with a monoclonal anti-Ia antibody (probably identical with OKIal). The reason for this discrepancy is at present not known.

Using the double staining technique we directly demonstrated that the vast majority of the T lymphocytes both in the dermis and in the epidermis in the skin lesions express Ia-like antigens. These cells may be activated T lymphocytes. Due to the large number of cells stained with the anti-Ia serum, Malmnäs-Tjernlund (15) concluded that some of the mononuclear cells in lichen planus lesions could be activated T lymphocytes. However, she did not specifically identify the T lymphocytes.. Recently, Morhenn et al. (17), examining two adjacent sections from one biopsy of a psoriatic plaque with monoclonal antibodies against Ia-like (HLA-DR) antigens and T lymphocytes, respectively, interpreted the results to be consistent with the presence of activated T lymphocytes. Observations comparable to our results, have been reported by Burmester et al. (9), demonstrating markedly elevated levels of T lymphocytes expressing Ia-like antigens in the synovial membranes and synovial fluids of patients with rheumatoid arthritis.

In line with Malmnäs-Tjernlund (15), we too found "intercellular" immunofluorescence staining with OKIal in the basal part of the epidermis in lichen planus lesions and to a lesser degree in DLE lesions. The origin of these Ia-like antigens is at present not known. It is possible that Ia-like antigens have been acquired by the keratinocytes. It is of interest that Lampert et al. (14) showed that Ia antigens may appear on rat keratinocytes in graft–versus–host disease. In certain cases of human graft–versus–host disease, clinical and histological findings similar to those of lichen planus (20, 21) and DLE (10) have been described. Ia antigens are expressed on thymic epithelium (11), and may also be acquired by breast epithelial cells (12).

A characteristic histological finding of lichen planus and DLE is degeneration of basal keratinocytes. Recently, we demonstrated suppressor/cytotoxic ($OKT8^+$) T lymphocytes intra-epidermally among basal keratinocytes in these skin lesions (4). As a large proportion of these T lymphocytes possess Ia-like antigens, they may be involved in a cell-mediated immune reaction against basal keratinocytes.

Some T lymphocytes, in both the dermis and the epidermis in unaffected skin also expressed Ia-like antigens. It is therefore possible that the appearance of Ia-like antigens is a property of T lymphocytes accumulating in the skin.

In addition to the demonstration of Ia-like antigens, the demonstration of local production of lymphokines would provide further evidence for the presence of activated T lymphocytes in these skin lesions. Preliminary results do show significantly increased concentrations of lymphokines in blister fluid obtained from psoriatic lesions (data to be published).

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J. R. Bjerke, M.D.
Department of Dermatology
N-5016 Haukeland sykehus
Norway