

PEMPHIGUS ACANTHOLYSIS IN TISSUE CULTURE: STUDIES ON PHOTO-INDUCTION

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Abstract. It has previously been shown that UV irradiation can give rise to fresh skin lesions in the uninvolved skin of patients with pemphigus. In this study no increase of acantholytic activity was found in an organ culture model system for pemphigus in vitro, after in vitro and in vivo irradiation under various experimental conditions. By immunofluorescence no increase in IgG binding in irradiated specimens was observed. From these in vitro findings, which contrast the effect of UV light in pemphigus in vivo, it may be concluded that the circulatory system of the skin plays a major role in the photo-induction of acantholysis in pemphigus.

Key words: Pemphigus; Tissue culture; UV light; Acantholysis

Ultraviolet light has previously been shown to induce acantholysis in vivo in the uninvolved skin of pemphigus patients (1-3, 8) and to progressively increase the intercellular staining for bound IgG and complement (1, 2).

The present study deals with the effect of UV light in an in vitro organ culture model system for pemphigus (9). Contrary to in vivo conditions, UV light has no inducing effect on pemphigus skin in vitro.

MATERIAL AND METHODS

1. Organ culture

Organ culturing was carried out according to the method of Sarkany (10). Buttock skin of several normal human volunteers was cut to 0.5 mm thickness with a keratome, washed in medium 199 (Flow Laboratories), cut into approximately 4x4 mm pieces, placed on lens paper and immersed in the culture medium. The culture medium consisted of pemphigus vulgaris serum (undiluted or diluted with normal human serum) obtained from one single patient. Sera were stored in small aliquots at -80°C. The immunofluorescence titre (6) on monkey esophagus remained constant at 1/320.

2. Immunofluorescence (IF)

Tissue specimens were snap frozen in liquid nitrogen and stored at -80°C until examined, and subsequently cut to 4

µm in a cryostat and incubated with goat antihuman IgG conjugates following established methods for direct immunofluorescence (6).

Details of antiserum: Goat antihuman IgG conjugate (Hyland). Fluorescein/protein ratio: 7.3×10^{-3} (weight) or 3.0 (molar); protein bound FITC concentration 158.6 µg/ml; total protein concentration: 21.8 mg/ml; specific antibody concentration 2.1 mg/ml. The conjugate was used 1:16 diluted in phosphate-buffered saline (Flow Laboratories).

Normal human skin specimens grown on normal human serum under in vitro conditions served as controls and were found to be negative by direct immunofluorescence.

3. In vitro experiments

Specimens were grown in each organ culture experiment for 6, 24 and 48 hours in undiluted pemphigus serum and in pemphigus serum diluted 1/2, 1/4 and 1/8 with normal human serum, and were then subjected to hematoxylin and eosin staining and immunofluorescence. Experiments were performed in duplicate at least. Skin specimens grown under identical conditions on normal human serum, but treated with UV light as well, served as controls.

3.1. *Irradiation experiments.* An Osram XBF 6000 water-cooled Xenon arc lamp, emitting a continuous UVB+UVA spectrum, was used as light source. Prior to experimental irradiation the minimal erythema dose (MED) of the skin donor was determined.

3.2. *In vivo irradiation.* Buttock skin, irradiated with 1 and 3 MED was biopsied immediately after, and 24 hours after irradiation and subjected to tissue culturing.

3.3. *In vitro irradiation.* Buttock skin, subjected to tissue culturing was irradiated immediately after, and 15 hours after biopsy with 1 and 3 MED.

4. Clinical control experiments

Uninvolved skin of 2 patients with pemphigus vulgaris was irradiated with the Xenon arc lamp. (The diagnosis was confirmed by histopathology, direct and indirect immunofluorescence, titres ranging between 1/160 and 1/320.) The 1 and 3 MED test sites were biopsied 24 hours later and subjected to routine histology.

RESULTS

1. In Vitro Experiments

1.1. Unirradiated skin

The 6-hour specimens showed no changes by light microscopy. Suprabasal separation occurred 24

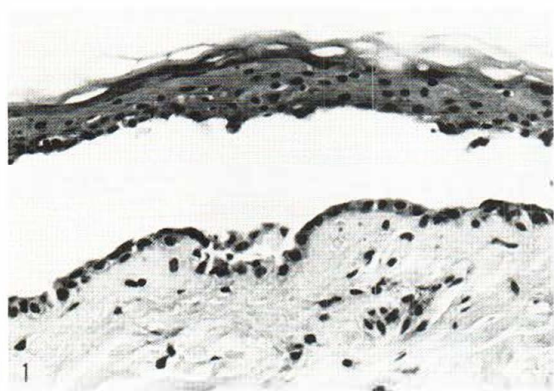


Fig. 1. Acantholysis in tissue culture. Suprabasal blister formation in normal, non-irradiated human skin, after 48 hours cultivation in undiluted pemphigus serum. HE. $\times 230$.

hours after treatment with undiluted, and with 1/2 diluted, pemphigus sera, and led to blister formation after 48 hours (Fig. 1). The controls as well as the specimens cultivated in 1/4 and 1/8 diluted pemphigus sera exhibited no specific alteration (10). 6 and 24 hours after incubation in undiluted (Fig. 2) and 1/2 diluted pemphigus sera, specimens disclosed typical IF staining of the inter-cellular space of the epidermis. Specimens grown on 1/4 or 1/8 diluted pemphigus sera exhibited only traces of fluorescence or were negative. No intercellular fluorescence could be observed in skin specimens at 48 hours after incubation.

1.2. Irradiated skin

1.2.1. Irradiation with 1 MED in vitro and in vivo.

The histologic changes as well as the immunofluorescence findings were identical with those observed in unirradiated skin, save for some slight epidermal changes (vacuolization and a few sunburn cells) due to the UV irradiation. In particular, there was no increase of the acantholytic activity or increase in the intensity of immunofluorescence in the irradiated specimens. Nor was there any gross difference between specimens irradiated in vivo and in vitro. Sometimes the UV-induced tissue alteration seemed to be more pronounced after in vitro irradiation.

1.2.1. Irradiation with 3 MED in vitro and in vivo.

Irradiation with 3 MED resulted in the typical sunburn reaction at 24 and 48 hours. Most specimens exhibited numerous sunburn cells and gross de-

struction of the epidermis with unspecific cleft formation. There was no significant difference between specimens on pemphigus serum and control specimens.

2. Clinical Control Experiments

The 1 MED as well as the 3 MED sites of both patients showed a positive Nikolski sign and pronounced acantholytic activity. Sunburn cells and vacuolization of keratinocytes, present in each specimen, were more pronounced in the 3 MED biopsies.

DISCUSSION

Ultraviolet light can be an aggravating factor in various vesiculo-bullous disorders, such as pemphigus (1-3, 8), pemphigoid, and Hailey-Hailey's disease (5), and it has been shown that fresh lesions can be produced in pemphigus patients by application of at least 1 MED of UV light to uninvolved skin (1-3). Immunofluorescent and immunohistochemical studies showed a progressive increase in the in vivo binding of IgG and complement in the irradiated areas, suggesting that the pathogenic mechanism involved in the production of skin lesions following UV light, is that of a progressive immunologic reaction (1, 2).

An organ culture model recently described by Michel & Ko (9) for the study of pemphigus acantholysis prompted our investigations on the mechanisms involved in photo-induction of pemphigus. The present study fully supports the histologic and immunofluorescence findings of

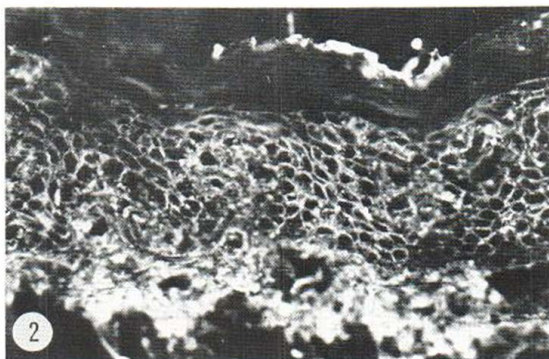


Fig. 2. Typical immunofluorescence of the epidermal intercellular space after 6 hours of cultivation of normal human skin in undiluted pemphigus serum. $\times 275$.

Michel & Ko (9). However, within the limits of the experimental design employed in this study, no increase in acantholytic activity was found in vitro after in vitro and in vivo irradiation under various experimental conditions. Furthermore, no increase in IgG binding in irradiated areas could be detected in vitro. Thus these findings contrast with the in vivo situation.

The mechanisms involved in the photo-induction of pemphigus lesions in vivo have been discussed in detail (1, 2) and may be summarized as follows:

1) Epidermal factors, including inactivation of enzymes, alteration of DNA synthesis, changes in sulfhydryl and disulphide content of keratinocytes and stimulation of immunogenicity by UV light.

2) Dermo-epidermal factors, i.e. a breakdown of the dermo-epidermal barrier by UV light (1, 2, 7).

Since, in tissue culture, epidermis behaves like epidermis in vivo even after UV irradiation (4), our results suggest that epidermal factors apparently do not play an important role in the photo-induction of pemphigus lesions in tissue culture.

Failure of UV light to induce intra-epidermal lesions in specimens of normal skin cultivated in pemphigus serum may therefore be attributable to the following.

1) No impairment by UV of the dermo-epidermal barrier is possible under experimental conditions of tissue culture.

2) diffusion of antibody into the specimens cannot be sufficiently enhanced by these means to proceed via other pathways so as to accelerate the development of intra-epidermal clefts (e.g. lateral access).

3) processing of specimens for, and keeping of tissue in culture media already sufficiently alters biological barriers (dermo-epidermal junction and possibly cell membranes); additional UV irradiation cannot thus increase this effect.

It has been questioned whether an increased blood flow plays a major role in the photo-induction of pemphigus lesions in vivo (3). Nevertheless, the most obvious difference between in vivo and in vitro conditions is the intact circulatory system which should account for the differing effect of UV

on pemphigus skin in vivo, and of UV on normal skin cultivated in media containing pemphigus antibodies.

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