

THE EFFECTS OF X-RAY, ULTRAVIOLET AND INFRARED IRRADIATION ON THE BASEMENT MEMBRANE ZONE ANTIBODY REACTION OF THE HUMAN SKIN IN VITRO

Wolfgang Remy, Hans Bockendahl and Günter Stüttgen

From the Department of Dermatology of the Free University, Rudolf-Virchow Hospital, Berlin

Abstract. Two patients with bullous pemphigoid were treated with radio cobalt or betatron irradiation. Thereafter bullous eruptions developed in the non-irradiated skin. It is known from the literature that ultraviolet light applied to the normal-appearing skin of patients with bullous pemphigoid produced fresh histologic and immunohistochemical changes in the irradiated skin. X-ray irradiation applied to human skin biopsy specimens using single doses of 7 000 and 8 000 R resulted in an increase of basement membrane zone antibody binding of 2-3 titre dilution steps. An increase of 3-4 titre dilution steps was achieved by irradiation with ultraviolet light. Infrared irradiation of skin biopsy specimens, on the contrary, caused the total loss of the basement membrane zone fluorescence. It merits further study, whether these contrary effects on the basement membrane zone antibody binding of human skin in vitro (activation of bound antigen after X-ray or ultraviolet irradiation and inactivation of antigen caused by infrared irradiation) are of clinical value concerning the bullous pemphigoid problem in vivo.

Key words: X-ray irradiation; Ultraviolet irradiation; Bullous pemphigoid; Activation of basement membrane zone antigens; Antigen-antibody reaction; Human skin

It has long been noted that many cutaneous diseases are aggravated by sunlight, and, at times, sunlight may initiate lesions of atypical bullous dermatoses (7). In 1965 Cram et al. (4) showed that fresh skin lesions can be produced in the uninvolved skin of pemphigus foliaceus patients within 24 hours after applying erythema doses of ultraviolet light, while infrared irradiation failed to produce a similar reaction. Later it could be demonstrated by Cram et al. (5) that ultraviolet light applied to the normal-appearing skin of patients with pemphigus vulgaris and bullous pemphigoid produced fresh histologic and immunohistochemical changes in the irradiated skin.

These clinical observations and results known from the literature (4, 5) led us to study whether

there was an increase or decrease in basement membrane zone antibody binding of the skin after irradiation with X-ray, ultraviolet, or infrared light in vitro.

In our clinic we observed 2 patients with bullous pemphigoid who developed fresh lesions after irradiation with radio cobalt and betatron.

CASE REPORTS

Case 1. A 74-year-old female patient developed lesions in the oral mucosa which were first misdiagnosed as a squamous-cell carcinoma. Treatment with radio cobalt was therefore begun. After the second irradiation with 400 R radio cobalt, the patient developed a generalized bullous eruption and thereafter the diagnosis of a benign mucus membrane pemphigoid could be stated clinically, histologically and by immunofluorescence.

Case 2. In another case, an 81-year-old woman complained of a unilateral localized bullous pemphigoid on the shin. Because of a gonarthrosis the patient was treated with betatron irradiation (three fields, 200 R each). The following day she developed bilateral blister formation on the shins.

MATERIALS AND METHODS

Six skin biopsy specimens were irradiated with single doses of 300-12 000 R (Dermopan, Siemens Company, West-Germany, type AEW 50/250) at a distance of 15 cm. These skin specimens were taken from 6 different healthy persons (Fig. 1). Further skin biopsy specimens from 3 other people were irradiated with ultraviolet light for 2-6 minutes at a distance of 40 cm using a standard hot quartz lamp (Elektro-Vakuum GmbH, Berlin; type UNV., 1500 W, 7 A) (Fig. 2). Infrared light was applied to 7 skin specimens for 1/2-5 minutes at a distance of 10 cm using a standard infrared lamp (type Original Hanau, Sollux 750, West-Germany) (Fig. 3).

The investigation of the basement membrane zone antibody binding of the irradiated skin was carried out using indirect immunofluorescent staining according to the procedure of Beutner et al. (1). Cryostat sections of non-irradiated

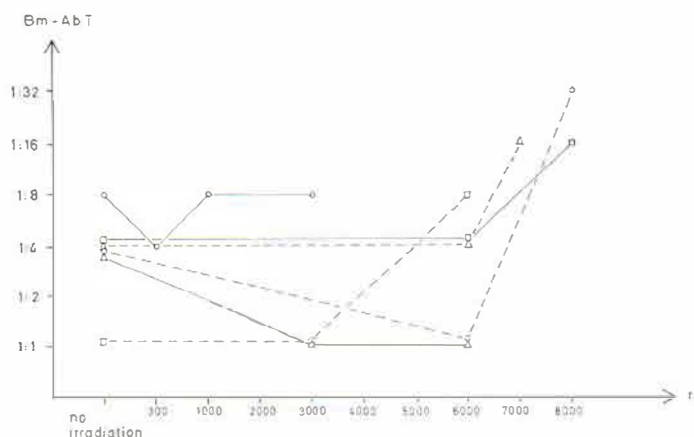


Fig. 1. Titres of basement membrane zone antibody binding of uninvolved human skin biopsy specimens before and after X-ray irradiation (Dermopan) with single doses of 300–12 000 R. Each mark represents a series of experiments using human skin biopsy specimens from 6 different patients. *Abscissa*: X-ray dose (R); *ordinate*: basement membrane zone antibody titre (Bm-AbT).

and irradiated skin biopsy specimens were incubated with the serum (diluted 1:1 – 1:64) of a patient with bullous pemphigoid (the titre of this serum for basement zone antibody was 256, using guinea pig tongue as antigenic substrate). Sections were washed with Coons buffer and incubated with diluted 1:10 commercially available fluorescein isothiocyanate-conjugated antihuman IgG (Behringwerke AG, West-Germany) with the following general characteristics: protein concentration of the FITC-conjugated gamma-globulin fraction: ca. 10 ± 3 mg/ml, total protein concentration (after addition of human albumin): ca. 40 ± 5 mg/ml, specific antibody content: ca. $10 \pm 5\%$ of the gamma-globulin concentration, molar F/P ratio: ca. 2.5 ± 1.5 . Details to this method have been described elsewhere (2, 3).

RESULTS

The findings after X-ray irradiation of human skin biopsy specimens are summarized in Fig. 1. After irradiation with doses between 300 and 6 000 R the titres of the basement membrane zone antibody remained constant in 3 of 6 experiments, decreased

in 2 skin specimens and showed an increase in one case. Irradiation with 7 000 and 8 000 R resulted in an increase of 2–3 titre dilution steps. Doses of 9 000 R and more (till 12 000 R) led to degeneration of the skin and unreproducible results.

Ultraviolet irradiation applied to the skin biopsy specimens for 4–6 minutes produced an increase of 3–4 titre dilution steps (Fig. 2).

The effects of infrared light are shown in Fig. 3. After irradiation for 1/2–2 minutes the titres of the basement membrane zone antibody decreased, while irradiation for 3–5 minutes caused the total loss of the basement zone fluorescence.

DISCUSSION

Interpreting our results, we agree with the assumption discussed by Cram et al. (6) that ultraviolet irradiation of the skin could result in the release or

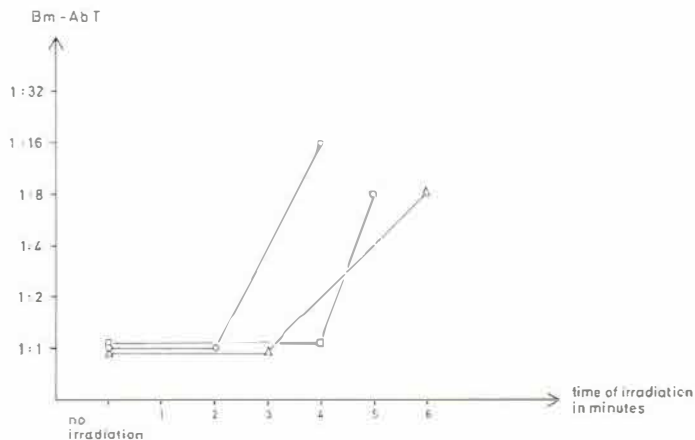


Fig. 2. Titres of basement membrane zone antibody binding of human skin biopsy specimens after ultraviolet irradiation (hot quartz lamp) for 2–5 minutes at a distance of 40 cm. Each mark represents a series of experiments using human skin biopsy specimens from 3 different patients. *Abscissa*: time of irradiation (minutes); *ordinate*: basement membrane zone antibody titre (Bm-AbT).

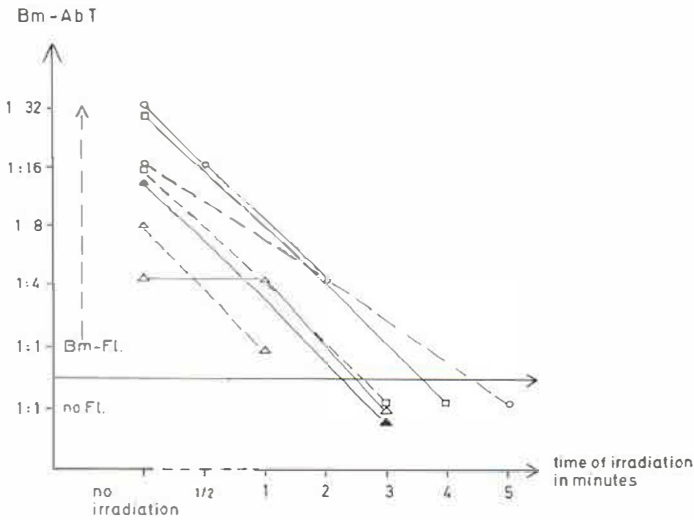


Fig. 3. Titres of basement membrane zone antibody binding of human skin biopsy specimens after infrared irradiation (standard infrared lamp) for 1/2-5 minutes at a distance of 10 cm. Each mark represents a series of experiments using human skin biopsy specimens from 7 different patients. *Abseissa*: time of irradiation (minutes); *ordinate*: Basement membrane zone antibody titre (Bm-AbT). *Bm-FL.*: basement membrane zone fluorescence. *no FL.*: no basement membrane zone fluorescence.

activation of bound antigen and/or promote antibody fixation. Our findings show that this effect could be produced by X-ray irradiation, too. If irradiation of the skin causes an increase in antibody binding sites of the basement membrane zone, the number of bound antibodies must show an increase too, following the law of chemical equilibrium. Therefore, a positive correlation exists between the number of the antibody binding sites and the titre dilution step still showing basement membrane fluorescence (lowest serum concentration). In comparison with irradiated and non-irradiated skin biopsy specimens and using a patient's serum of the same titre dilution step, the basement membrane zone with the greatest number of antibody binding sites actually shows the most intense fluorescence. For the activation of bound antigen, either by ultraviolet or by X-ray irradiation, a definite quantity of energy is necessary. Small doses showed no effect, a medium dose induced an increase of basement membrane zone antibody binding, and overdoses of applied energy destroyed the bound antigen.

Conceivably, the influence of such energy on the skin is combined with an effect of heat. Heat, however, as shown by our findings with infrared irradiation, caused the loss of antigen on the basement membrane zone.

The collaboration of these two contrary effects—the activation of bound antigen after X-ray or ultraviolet irradiation and the inactivation of antigen caused by infrared irradiation—could possibly explain the decrease in basement zone antibody

binding found in two experiments following X-ray irradiation with doses of 3 000-6 000 R (Fig. 1).

REFERENCES

1. Beutner, E. H., Rhodes, E. L. & Holborow, E. J.: Autoimmunity in chronic bullous skin diseases. *Clin Exp Immunol* 2: 141, 1967.
2. Bockendahl, H., Remy, W. & Peters, T.: Fraktionierung und Klassifizierung menschlicher Immunoglobuline und ihre Reaktion mit sessilen Antigenen beim bullösen Pemphigoid. *Z klin Chem klin Biochem* 10: 329, 1972.
3. Bockendahl, H. & Remy, W.: Der Einfluss von Nettoladung und pH-Milieu auf die Präparation von Antibasalmembran-Antikörpern und auf ihre Bindungskapazität beim bullösen Pemphigoid. *Res Exp Med* 163: 211, 1974.
4. Cram, D. L. & Winkelmann, R. K.: Ultraviolet induced acantholysis in pemphigus. *Arch Dermatol* 92: 7, 1965.
5. Cram, D. L. & Fukuyama, K.: Immunohistochemistry of ultraviolet induced pemphigus and pemphigoid lesions. *Arch Dermatol* 106: 819, 1972.
6. Cram, D. L.: Photo-induction of lesions in patients with pemphigus and bullous pemphigoid. In (ed. E. H. Beutner, T. P. Chorzelski, S. F. Bean & R. E. Jordon), p. 44. *Immunopathology of the Skin: Labeled Antibody Studies* Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania, 1973.
7. Jablonska, S. & Chorzelski, T.: Zur Anwendbarkeit der Immunfluoreszenz-Technik in der Dermatologie. 30. Tagung Deutsch Dermatolog Gesellschaft, Graz, 1974.

Received December 9, 1974

W. Remy, M.D.
Department of Dermatology
Free University
Rudolf-Virchow Hospital
D-1 Berlin 65
Augustenburger Platz 1