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The Role of Complement in UVB-Induced Inflammation

W. TORINUKI[1] and H. TAGAMI[2]

[1]*INSERM U.209, Pavillon R. Hôpital E. Herriot, Lyon, France, and* [2]*Department of Dermatology, Tohoku University School of Medicine, Sendai, Japan*

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To evaluate the role of the complement system in skin inflammation accompanying leukocyte infiltration induced by UVB irradiation, the response of guinea pigs decomplemented with cobra venom factor was compared with that in saline-treated animals. Complement-depleted animals showed a weaker clinical response in the late phase (12–48 h) of UVB-induced inflammation ($p<0.05$). However, complement activation, detectable as a decrease in CH50 and generation of chemotactic anaphylatoxin, did not occur after in vitro irradiation of guinea pig serum. These results suggest that although the complement system does not play a key role in initiating leukocyte chemotaxis to the UVB-induced inflammatory site, it contributes to the amplification of the late inflammatory reaction. *Key words: UVB; Chemotaxis.* (Received March 11, 1986.)

W. Torinuki, INSERM Unité 209, Pavillon R, Hôpital Edouard-Herriot, Lyon, France.

The role of various mediators of UV-induced inflammation is only partially understood. Several mediators that are believed to be important in the pathogenesis of UV-induced inflammation have been identified (1). Prostaglandins, histamine, bradykinin, plasmin and lysosomal enzymes have all been shown to be mediators in the development of UV inflammation. Other well-known plasma-derived mediators produced in many types of inflammation are those from the complement system. Although photoactivation of the complement system has been shown to take place in serum from guinea pigs with phototoxic substance-induced UVA phototoxicity (2, 3, 4), little has been done to study the role of the complement system in UVB-induced inflammation.

In this study, we have evaluated the role of complement system in UVB inflammation, by comparing the response of guinea pigs decomplemented by treatment with cobra venom factor with the response of saline-treated animals.

MATERIALS AND METHODS

Light source

Five tubes of fluorescent sunlamp (Toshiba FL 20SE30) were used as a radiation source of middle-wave ultraviolet radiation (UVB). This lamp emits wavelengths mainly between 280–320 nm, peaking at 310–315 nm. Total energy output was 2.0 mW/cm^2 at a distance of 25 cm, as measured with the aid of a UV-radiometer (UVR-305/365; Eisai Co., Tokyo), with spectral sensitivity in the range of 280–320 nm.

Animals

Female albino guinea pigs of the Hartley strain, weighing 300–400 g, were used throughout the experiments. The dorsal area was depilated 4 days before irradiation. The left side of the back of animals was exposed to UVB, while the right side was covered with black tape so that it could serve as a non-irradiated control side.

Decomplementation was performed in 10 guinea pigs by i.p. injection of 300 U/kg of cobra venom factor (CVF: Cordis Laboratories, Inc., USA) 16 h before irradiation (3). Such a treatment resulted in a decrease in the total complement hemolytic activity of about 97 %. Ten animals that were injected with saline served as controls.

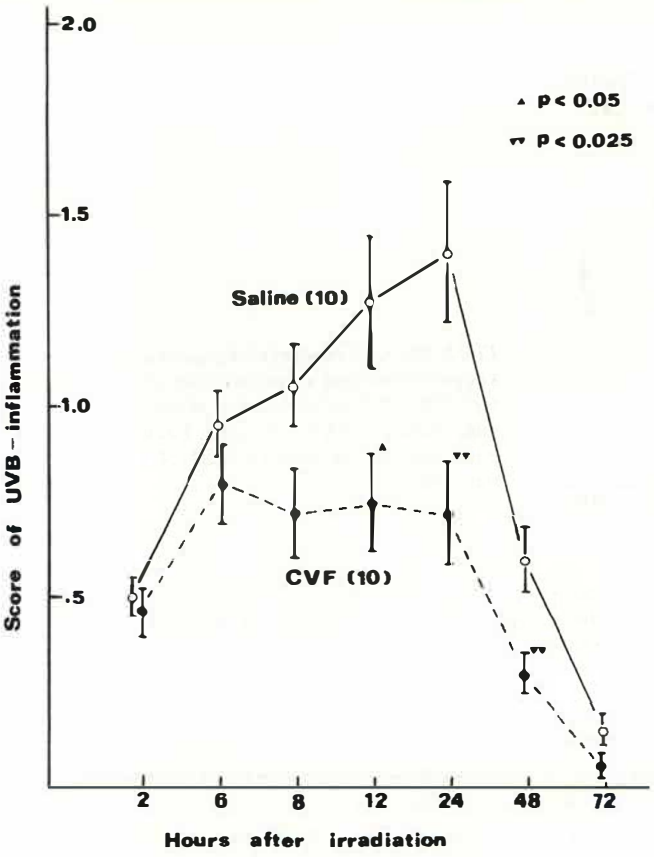

Fig. 1. UVB (1.8 J/cm²)-induced inflammation in decomplemented and control animals. Each point represents the mean score (± SEM) of ten animals.

Clinical observations in the development of UVB inflammation

Clinical changes were evaluated by 2 independent observers at the following times after the completion of UVB irradiation: 0, 2, 6, 8, 12, 24, 48 and 72 h. 1.8 J/cm² which corresponds to about 3 times the average MED was used. Skin changes were graded as follows: 0 = no reaction, 0.25 = barely perceptible erythema, 0.5 = easily perceptible erythema with ill-defined borders, 1.0 = distinct erythema with sharp borders, 1.5 = erythema more intense than a 1.0 response but not maximal and with slight edema, 2.0 = maximal erythema with edema. The results were expressed as the mean of clinical response score ± SEM. Statistical difference in clinical response between two groups at the various time points was assessed by Wilcoxon's rank-sum test.

Method of testing for leukocytes in the dermis following UVB irradiation

Before and at 4, 8, 16, 24 and 48 h after 1.8 J/cm² irradiation of UVB, 4-mm punch biopsies were taken and fixed in formalin for routine hematoxylin and eosin preparations. The total number of leukocytes in the dermis adjacent to the epidermis was counted by means of an ocular square grid covering, under 400 × magnification, 0.04 mm² of skin section surface. Five grid fields that were randomly selected were examined for every skin specimen. Then, we calculated the average number of cells in the dermis (0.04 mm²) of each skin specimen. Statistical analysis was accomplished by Student's *t*-test.

In vitro irradiation of guinea pig serum

In this experiment, we evaluated the effect of UVB irradiation on the animal serum complement. The normal sera, which were separated and pooled from 3 guinea pigs on the day of the experiment, were transferred to Petri dishes that were placed on ice 25 cm away from the light source. After UVB irradiation, total complement hemolytic activity (CH50) in the sera was determined using Mayer's method (5). In vitro chemotaxis of the sera was measured with a modified Boyden chamber (blind-

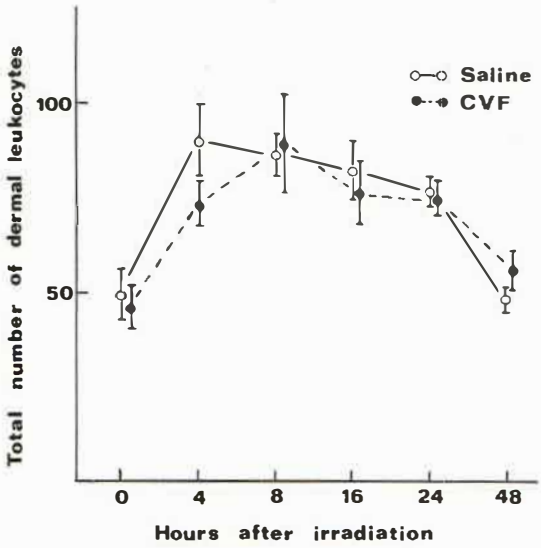

Fig. 2. The total number of dermal leukocytes before and at various times after UVB (1.8 J/cm^2) irradiation of animals treated with CVF or saline. Each point indicates the mean (± SEM) of three animals.

well chamber; Bio-rad, USA) assayed with a 3.0 μm polycarbonate filter (Bio-rad, USA), as has previously been reported (6). Guinea pig neutrophils as target cells were obtained 3 h after i.p. injection of 10 ml 0.1% oyster glycogen in isotonic saline.

RESULTS

Intensity and duration of UVB-induced inflammation

An average minimal erythema dose (MED) for UVB determined by the MED's of 8 untreated animals, 24 h after irradiation, was 540 mJ/cm^2. When 1.8 J/cm^2, i.e. about 3 MED, of UVB was given, the depilated backs of saline-treated animals developed erythema and edema, which peaked at about 24 h after irradiation. In the case of CVF-treated animals, there was a mild suppression of clinical response only between 12 and 48 h after irradiation of UVB (Fig. 1).

Leukocytes in the dermis after UVB irradiation

The total number of leukocytes before and after irradiation are seen in Fig. 2. Each point on the curves represents biopsies taken from 3 animals. The total number of leukocytes in the dermis of the CVF-treated and saline-treated animals did not differ significantly during the 48 h evaluation after irradiation. The increased number of cells in the dermis reached a maximum at 4–8 h after irradiation and persisted for about 24 h.

In vitro effect of UVB irradiation on animal-complement system

No alteration of hemolytic complement activity (CH50) was observed in 1.8 J/cm^2-irradiated normal guinea pig sera (data not shown). The irradiation of animal serum by UVB light resulted in no generation of highly potent chemotactic activity as compared to the non-irradiated serum (Table I).

Table I. *Chemotactic activity in normal guinea pig serum before and after UVB irradiation.*

Each datum indicates the mean (± SEM) of quadruplicate chambers

	Chemotaxis (cells/high power field)
Nonirradiated serum	3±2
1.8 J/cm^2-irradiated serum	3±1
Hanks solution (random migration)	4±1

DISCUSSION

In the present study, we found that decomplemented animals showed a weaker erythemic response to UVB irradiation, but the suppression was evident only during the late stage of UVB inflammation.

Several mediators that are believed to play a role in the pathogenesis of UV inflammation have been identified. Even in the plasma exsudate, that appears during UVB inflammation, mediators may be formed. Complement activation has been shown to occur in sera from patients with porphyrias (6, 7, 8). UVA irradiation of guinea pig serum containing exogenous phototoxic substances, i.e. hematoporphyrin and demethylchlortetracycline, also resulted in complement activation in vitro (3, 4). Moreover, the clinical response of phototoxic lesions, which were induced in guinea pigs by these phototoxic substances plus UVA, was suppressed in the animals decomplemented by the treatment with CVF. Therefore, we have taken some mechanisms, possibly involving photoactivation of the complement system in the plasma exudate, into our consideration.

The present study showed that direct UVB irradiation of guinea pig serum failed to induce either complement activation or generation of chemotactic anaphylatoxin. However, the in vivo observation that the late phase of UVB-induced erythema is suppressed in decomplemented animals indicates that the complement system participates in the augmentation of UVB inflammation. The negative results of in vitro study eliminate the possibility that the complement system plays a role as an initiator of inflammation by increasing vasopermeability or by inducing leukocyte chemotaxis. One possible explanation for the discrepancy between the in vitro and the in vivo result is that the leukocytes infiltrating the dermis in the case of UV inflammation activate the complement system in the plasma exudate during the later stages of UVB inflammation. Their lysosomes contain materials which interact with the complement system in at least two ways to generate C5a (9). Firstly, a lysosomal protease is capable of cleaving purified C5 at a neutral pH. Secondly, lysosomal lysates generate the active form of factor B in fresh serum, presumably by activation of the alternative pathway. However, the precise mechanisms involved in the complement activation in the late stage of UVB inflammation await further investigation.

We suppose that complement-derived mediators are responsible for the full development of UVB-induced inflammation, acting synergistically with products of other biological events triggered by such as arachidonic acid metabolites, histamine, bradykinin, and lysosomal enzymes released from leukocytes.

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