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Impaired Immune Function in Patients with Xeroderma Pigmentosum[1]

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ABSTRACT

The development of contact allergy in sun-exposed skin is markedly impaired in patients with xeroderma pigmentosum as compared to the responses in healthy control subjects. The degree of this immunological impairment is directly related to the severity of the cutaneous disease. These findings raise the possibility that sunlight-induced alterations of immune function may be involved in the marked susceptibility of these patients to the development of nonmelanoma skin cancer.

INTRODUCTION

XP[7] is a rare genetic disease characterized by abnormal sensitivity to sunlight and the development of multiple skin cancers early in life. In most cases, these abnormalities are associated with an inability of the cells to remove and repair lesions in their DNA resulting from exposure to UV radiation (1). Recent studies with laboratory animals showed that UV radiation has 2 effects that contribute to the formation of skin cancers. In addition to producing neoplastic transformation of cells following a direct interaction with DNA, UV radiation also suppresses an immune surveillance mechanism that otherwise would destroy developing skin cancers (2). It is not known whether such immunosuppressive effects of UV radiation contribute to the formation of skin cancers in humans. However, because cells from XP patients, including their lymphoid cells, are extremely sensitive to the damaging effects of UV rays, it has been postulated that these persons are especially susceptible to immunosuppressive effects of UV radiation and that immunosuppression contributes to the development of their skin cancers (3).

To test this hypothesis, we tested patients with XP for the development of contact allergy to DNCB as a measure of immunological competence. This reaction is suppressed in mice exposed to UV radiation, and it has been proposed that suppression of immunity to UV-induced skin cancers occurs by means of a similar mechanism (4). Because XP is such a rare disease, this study was conducted in Cairo, Egypt, where there is a relatively large group of affected persons living within a limited geographical area. In addition, because of the high level of insolation in this region, it is difficult for these individuals to avoid exposure to sunlight, and consequently, most have evidence of skin damage resulting from sunlight exposure (5).

In animals, suppression of contact allergy following application of a sensitizer to UV-irradiated skin is sometimes associated with a functional alteration or inactivation of cutaneous Langerhans' cells, which leads to induction of suppressor lymphocytes (4). Langerhans' cells are derived from the bone marrow and serve as antigen-presenting macrophages in the epidermis (6). Following exposure of both human and mouse skin to UV radiation, Langerhans' cells are reduced in number, and the remaining cells exhibit altered morphology (7, 8). Therefore, a second question addressed in this study was whether Langerhans' cells are morphologically normal in sun-exposed skin of patients with XP.

MATERIALS AND METHODS

Patients. Twelve patients, 6 females and 6 males, were studied; the mean age of the group was 15 years, and the range of ages was 7 to 27 years. The exposed skin of all patients showed changes consistent with a diagnosis of XP. Two patients had neurological deficits consistent with De Sanctis-Cacchione syndrome, but apart from this and the cutaneous changes, all the patients were well. All patients had a family history of XP, and at least one and as many as 6 close relatives had the disease. The repair of UV-induced damage to DNA in fibroblasts from 9 of the patients had been evaluated and found to be defective. The patients were all residents of upper Egypt.

The severity of the disease was evaluated in each patient using a scale of: minimal, changes in pigmentation only; moderate, changes in pigmentation plus few solar keratoses and disease restricted to areas maximally exposed to sun; and marked, changes in pigmentation plus numerous solar keratoses and disease affecting areas maximally and minimally exposed to sun.

Control Subjects. Fourteen apparently healthy control subjects, 8 females and 6 males, were studied; the mean age of the group was 20 years, and the range of ages was 9 to 48 years. The control subjects all lived in Cairo and had frequent exposure to intense sunlight.

Evaluation of Contact Sensitization to DNCB. Testing for contact sensitization was performed according to the method of Catalona (9). Briefly, 1000 and 50 μg of DNCB in acetone were applied to the left dorsal forearm using a stainless steel ring, 2 cm in diameter, to confine the solution. The sites were covered with Band-Aids for 24 h and examined 14 days later. A spontaneous flare (erythema with or without crusting and scaling) at both sites was read as 4+ response, and a flare at the 1000-μg site only was read as a 3+ response. If there was no reaction, 50 μg of DNCB were applied to a third site, and the response was evaluated 24 h later. Erythema of the whole test site was read as a 2+ response. Erythema confined to the margin of the site (exposed to a higher concentration of DNCB due to run-off of the acetone solution) was read as a 1+ response. No reaction at the site was read as a zero response.

The test sites in most of the patients included macules of hyperpigmentation, but care was taken to avoid the depigmented somewhat atrophic areas and areas showing epidermal hyperplasia.

Examination of Langerhans' Cells. Biopsies of skin were obtained under local anesthetic using a 4-mm punch. Right dorsal forearm skin

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[7] The abbreviations used are: XP, xeroderma pigmentosum; DNCB, dinitrochlorobenzene.

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was biopsied in all patients and 4 control subjects. Biopsies from 2 XP patients were also obtained from the volar aspect of the forearm, which is relatively protected from sun exposure and showed no evidence of disease. The specimens were fixed and divided. One half was processed for 1-μm sections, stained with Lee's methylene blue-basic fuchsin stain, and examined in the light microscope. Langerhans' cells were identified by the characteristic shape and staining of the nuclei as well as by the light staining of the cytoplasm. The other half of the specimen was examined by electron microscopy; Langerhans' cells were identified by the presence of Birbeck granules, electron lucent cytoplasm, and absence of desmosomes.

RESULTS

Contact Sensitization to DNCB. The majority of the XP patients failed to develop an allergic reaction to the contact sensitizing agent, and only 3 of the 12 exhibited a 3+ or 4+ response (Chart 1*a*). In contrast, 13 of 14 normal subjects exhibited 3+ or 4+ responses, and only one was unresponsive. Thus, as a group, the XP patients exhibited decreased immune responsiveness to DCNB when compared to normal subjects. When the group of XP patients was subdivided according to the extent of their cutaneous disease, there appeared to be an inverse relation between disease severity and the development of contact allergy (Chart 1*b*).

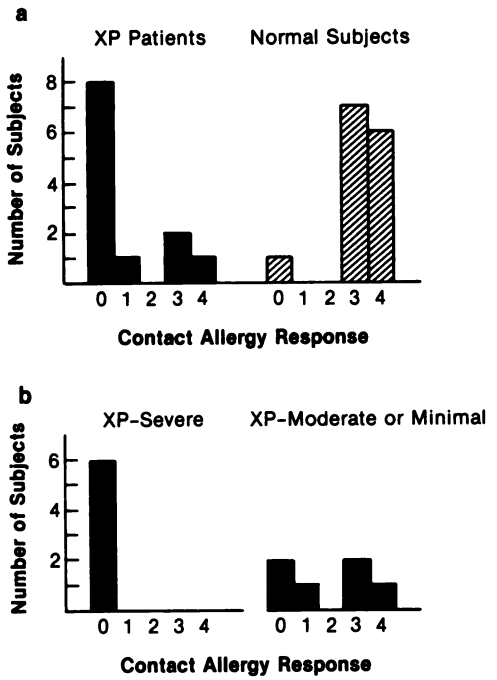

Chart 1. Contact allergy exposure of XP patients to DNCB. DNCB (1000 μg and 50 μg) in acetone was applied to the dorsal forearm within a steel ring, and the sites were examined 4 days later. Response was graded as: 4+, spontaneous flare at both sites; 3+, flare at 1000-μg site only; 2+, diffuse erythema 24 h after challenge with 50 μg; 1+, erythema only at margin of challenge site; 0, no response. *a*, contact allergy response in patients with XP and control subjects; *b*, response in patients as a function of severity of disease.

Table 1

Number of epidermal Langerhans' cells in dorsal forearm skin of patients with XP and control subjects

	No. of Langerhans' cells/100 basal cells[a]	Median	Range	P^b
XP patients	2.2, 2.6, 3.3, 4.0, 4.4, 4.8, 5.3, 5.8, 6.4, 6.5, 8.7	4.8	2.2–8.7	>0.20
Control subjects	3.4, 3.5, 4.5, 6.9	4.0	3.4–6.9	

[a] Biopsies obtained under local anesthesia were fixed and processed for electron microscopic examination. Langerhans' cells were identified by the presence of Birbeck granules, translucent cytoplasm, and absence of desmosomes.

[b] Significance of difference in number of Langerhans' cells in patients *versus* control subjects as determined by the Mann-Whitney test.

Examination of Langerhans' Cells. Enumeration of Langerhans' cells on light-microscopic examination of thin sections and electron microscopic examination of ultrathin sections yielded concordant results, but the latter method had the advantage of permitting detailed examination of the morphology of the cells. There was no detectable difference between the numbers of Langerhans' cells in XP patients and control subjects (Table 1), and the morphology of the cells was normal in both groups. Furthermore, there were no correlations between disease severity and Langerhans' cell number or between contact allergy response and Langerhans' cell number. The number of Langerhans' cells was not diminished in the dorsal skin biopsies relative to that in the volar skin biopsies from the 2 XP patients tested, and all Langerhans' cells exhibited a normal morphological appearance.

DISCUSSION

Chronic exposure of mice to UV radiation produces several specific alterations of immune function. One alteration is the generation of suppressor T-lymphocytes that prevent the normal immunological rejection of UV-induced tumors, and this change plays a central role in the pathogenesis of these lesions (2). Another alteration is suppression of contact allergy, which also involves generation of suppressor T-lymphocytes, and thus it has been proposed that these 2 alterations occur by a similar mechanism (4). It is not known whether sunlight produces similar immunosuppression in humans. An increased incidence of sunlight-associated skin cancer has been observed in immunosuppressed renal transplant recipients (10–12), but this is only indirect evidence of an involvement of the immune system in human photocarcinogenesis.

In this study, we have found that patients with XP have a marked deficiency of their capacity to mount a normal immune response to a contact sensitizer that is applied to sun-exposed skin. Furthermore, the degree of immunological impairment was directly related to the severity of the cutaneous disease. Because the severity of the disease is directly related to the amount of sunlight exposure, this result suggests that the immunosuppression may also be related to sunlight-induced injury.

One mechanism whereby contact allergy could be suppressed in patients with XP would be by way of an alteration of the processing and presentation of the antigen by Langerhans' cells in the skin. These cells are sensitive to damage by UV radiation (7, 8), but in our study, we found Langerhans' cells to be normal in both exposed and relatively nonexposed skin of patients with XP. Thus, the marked deficiency in immunological function in the XP patients does not correlate with an alteration in the number or morphology of epidermal Langerhans' cells. Other possible mechanisms for the immune suppression could be: (*a*) an alteration in the function of Langerhans' cells without a concomitant alteration in morphology; (*b*) a systemic impairment of the allergic response caused by the accumulation of UV-induced lesions in circulating leukocytes; or (*c*) an alteration in the absorption

properties of the skin, leading to poor penetration of the antigen. Irrespective of the mechanism by which it occurs, the finding of impaired cutaneous immunological function in XP patients is consistent with the hypothesis that immunosuppression may play a role in the pathogenesis of skin cancer in these individuals.

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