Acta Dermatovener (Stockholm) 56: 15–18, 1976

IMMUNOLOGICAL PHENOMENA INDUCED BY UV RAYS

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Abstract. Antiserum against UV-irradiated DNA was prepared and used as a specific reagent in an indirect immunofluorescence (IF) technique for the detection of photochemically damaged DNA in the epidermis of hairless mice. Fluorescence of cell nuclei was found in sections of dorsal epidermis of mice immediately after the irradiation. It persisted for 24 hours. The IF reaction became negative after 48 hours, irrespective of the duration of UV exposure to which the mice were subjected. This may indicate: either (*a*) the occurrence of DNA repair processes, or (*b*) the relation of DNA repair to the life cycle of the epidermal cell. Mice exposed to UV radiation similar to the erythema spectrum of sunlight do not show any changes in the cellular DNA, even after a dose of 40 MED.

Key words: UV irradiation; Hairless mice; DNA repair, UV denaturated DNA; Immunofluorescent studies; UV-DNA antigen

UV radiation of wavelength 320 nm causes photochemical damage in DNA (mainly by producing thymine dimers), leading to partial denaturation of DNA and changes in its antigenic properties. The altered DNA becomes a strong antigen which can be used to immunize experimental animals (4, 9). Specific antibodies are directed against DNA altered by UV (UV-DNA) and give precipitation and complement fixation with irradiated DNA, but not with native DNA (8, 4, 5).

Immune serum directed against UV-DNA can be used in the IF method as a specific reagent for detecting in vivo altered DNA in cells of animals irradiated with UV in the range 254–290 nm.

Changes in cellular DNA in vivo under the influence of UV have been demonstrated by the IF method in the epidermis of hairless mice and humans (10, 11).

There seems to be a parallel between the curve of the erythema action spectrum and the action spectrum of photochemicaly induced DNA lesions in cell nuclei. Dimerization of thymine under the influence of UV is a reversible process. Two mechanisms for the repair of damaged DNA are known. One depends on reproduction of the normal structure of the nucleotide chain by enzymes which excise damaged fragments of the chain and substitute the correct bases in their place, synthesizing lacking fragments (dark repair, repair replication). The other repair mechanism depends on the monomerization of the pyrimidine dimers by visible light and longwave UV radiation (360 nm) which seems to activate a special repairing enzyme, the so-called PR-Enzyme (phenomenon of photoreactivation).

The purposes of this study were:

(*a*) To determine by means of the IF method the time of repair of UV-DNA in the epidermis of hairless mice following UV irradiation,

(*b*) to determine whether the UV dose is correlated with the rate of disappearance of the phenomena revealed by IF,

(*c*) to ascertain whether photoreactivation plays some role in the disappearance of IF reaction,

(*d*) to examine the influence on the cellular DNA of various wavelengths in the range 290–400 nm present in natural sunlight.

MATERIAL AND METHODS

DNA irradiation

A British Thermal Syndicate 40-watt mercury resonance lamp was used as source of 254 nm radiation. Radiation below 240 nm was filtered out by a 5-mm layer of concentrated sodium acetate. DNA solutions, 500 μg/ml, were irradiated at 20° in 2 mm path length spectrophotometric cells, 0.5 ml volume, optical density $A_{260} = 2.0$, thus fully absorbing the incident radiation.

DNA from two different calf thymus preparations was investigated: a commercial preparation of Miles-Seravac, and another received from Dr H. Panusz (Medical Academy, Łódź, Poland). Rabbits were immunized with the Miles preparations only. For each injection, 3 ml of irradiated DNA was dissolved in 0.15 M sodium salt and 0.015 M phosphate buffer, pH 7.4. During irradiation, samples were removed at 15 min intervals for investigation of: (i) extent of denaturation of DNA as shown by its melting profiles (14);

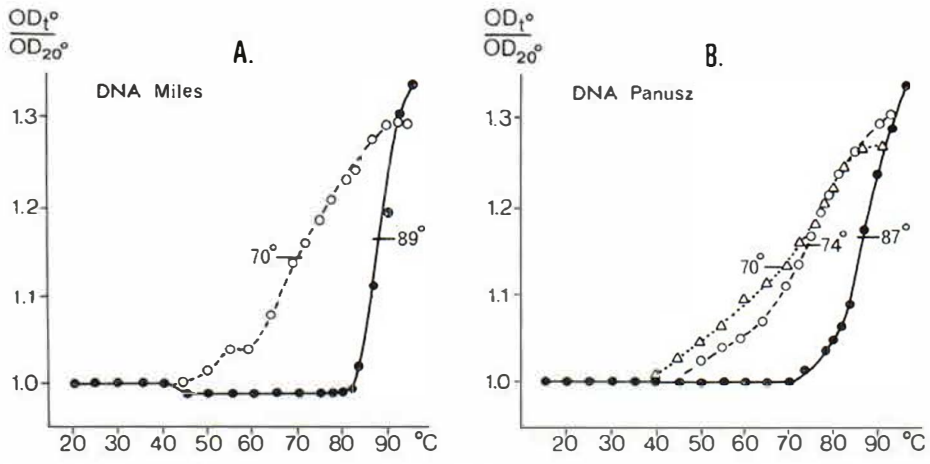

Fig. 1. Melting profiles of the DNA from calf thymus in the medium 0.12 M NaCl, 0.02 M buffer phosphate, pH 7.4. (A) DNA of Miles preparation; ●—●, native; ○---○, UV-254 fully denatured with a dose 2.16×10^6 ergs/mm². (B) DNA of Panusz preparation; ●—●, native; ○---○, UV-254 partially denatured with a dose 2.16×10^6 ergs/mm²; △···△, UV-254 fully denatured with a dose 4.4×10^6 ergs/mm².

(ii) ability to complex with methylated bovine serum albumin (MBSA) as carrier (6).

Melting profiles are shown in Fig. 1. Some striking differences between the two preparations were noted. While the native DNA complexed with MBSA gave a thread-like precipitate, the extensively UV denatured DNA gave fully soluble, turbidity-free complexes with MBSA (dashed line profile in Fig. 1A). Samples of DNA irradiated with smaller doses and then complexed with MBSA gave turbid solutions, although less turbid than the complex of native DNA. It will be seen from Fig. 1B that the Panusz DNA required two-fold higher doses of irradiation than Miles DNA in order to obtain fully UV denatured DNA (second dotted line in Fig. 1B).

The two samples of DNA differ only in that the Panusz DNA preparation consisted of longer chains. There is some evidence (1) that DNA irradiated with doses as large as 10^6 ergs/mm² shows single chain breakage (sedimentation in alkaline sucrose gradient). Increasing complement fixation inhibition, described by Natali & Tan (6), is another indication of the role of chain length. Hence the antigenic properties of DNA may be induced by some structural changes in DNA other than local disturbances following pyrimidine dimer formation.

Conditions for irradiating of animals

Experiments were carried out with hairless mice aged 4 months.

Irradiations were performed with: (i) a germicidal lamp which emits predominantly UV light with a wavelength of 254 nm, (ii) a 150-W xenon lamp, type Solar Simulator, using a Schott WG-320 filter (emission of UV radiation in the range 290–400 nm). In addition, irradiations were performed with a xenon lamp without WG-5 filter (emission 240–400 nm).

In the experiments on disappearance of the IF reaction and on the influence of the radiation dose on time of disappearance, 60 hairless mice were used. Groups of 10 mice were irradiated with the germicidal lamp at a distance of 20 cm (radiation energy 700 μW/cm²); times: 10, 30, and 60 min, and 3, 5 and 7 hours. Two mice from each group were killed immediately after the end of irradiation, and 12, 24, 48 and 72 hours after completion of irradiation, and skin sections were examined by the indirect IF method.

For studies on the photoreactivation phenomenon, 18 mice were irradiated with the germicidal lamp for 30 min. Two mice were killed immediately after irradiation, 8 were kept in darkness, and 8 under natural (day–night) conditions. Two mice from each group were killed after 12, 24, 48 and 72 hours, and skin sections examined by the indirect IF method.

For studies on the influence of UV radiation of wavelength 290–400 nm on cellular DNA, 20 mice irradiated with the Solar Simulator lamp were used. Groups of 2 mice were irradiated 2 to 80 min (2 min is equal to 1 minimal erythema dose, 1 MED, i.e. length of irradiation required to cause the appearance of macroscopic changes in the skin). In addition, 10 mice were irradiated with the xenon lamp without the Schott WG-5 filter (which eliminates UV radiation below 299 nm). Groups of 2 mice were irradiated for periods from 5 sec to 5 min.

Immunologic tests

Four rabbits were immunized by the method described by Tan & Stoughton (11). The immune sera were checked by the immunodiffusion test in agarose. Irradiated and native DNA were used as antigens. Precipitin lines were obtained with immune sera from all the immunized animals. The immune sera gave no cross reactions with native DNA. The sera were tested also by the indirect IF method on mouse kidney sections irradiated with the germicidal lamp (UV 254) from a distance of 10 cm, time 1 min and 5 min, or not irradiated. For IF studies, labelled goat immune serum against rabbit gamma-globulin was used (anti-rahbit γ-glob., F/P = 3.5, Ab = 16 units, using 1/4 unit/ml). Positive results (fluorescence of cell nuclei) were obtained only in sections irradiated with antiserum diluted 1:10 to 1:640. In the experiments, antiserum diluted 1:40 was used.

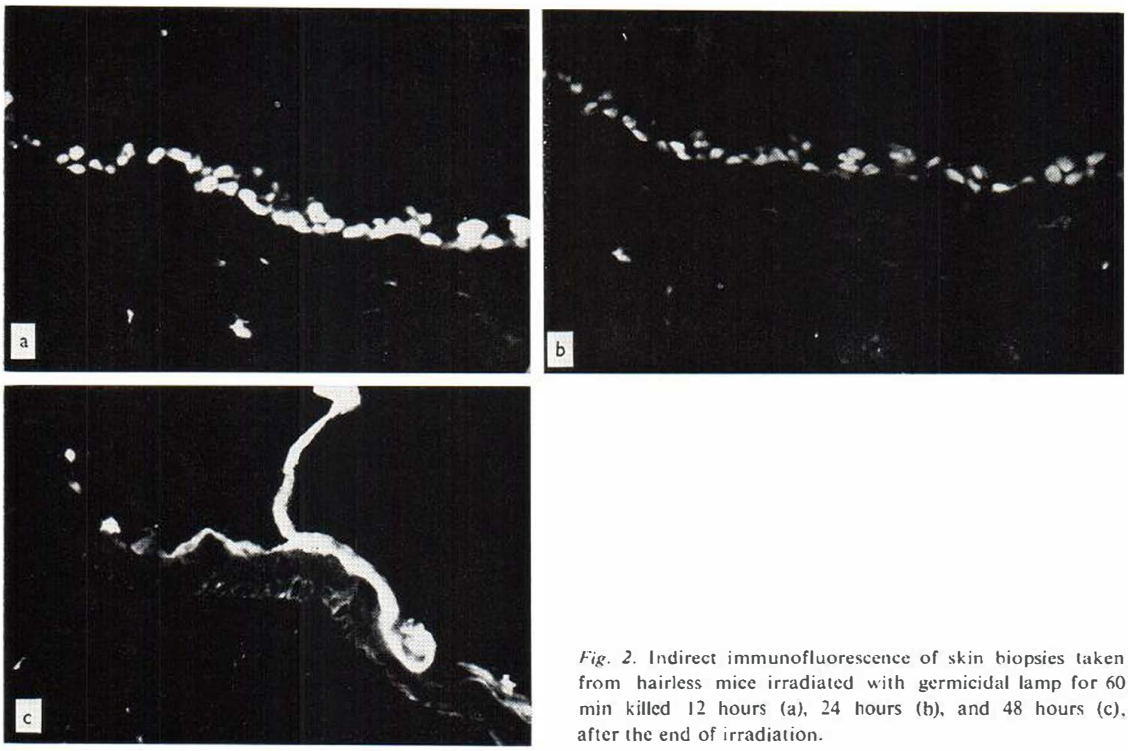

Fig. 2. Indirect immunofluorescence of skin biopsies taken from hairless mice irradiated with germicidal lamp for 60 min killed 12 hours (a), 24 hours (b), and 48 hours (c), after the end of irradiation.

RESULTS

The antiserum against UV-DNA detected changes in cellular DNA caused by UV irradiation (with a wavelength predominantly of 254 nm). Time of repair of damaged DNA was equal to time of disappearance of antigen specific for the immune serum used, i.e. time of disappearance of the phenomena observed by IF. Fluorescence of cell nuclei was found by using the indirect IF technique in mice killed immediately after the end of irradiation. A positive result of the IF reaction persisted in the skin of mice killed after 12 and 24 hours regardless of duration of irradiation, from 10 min to 7 hours (Table 1) (Fig. 2). The IF reaction became negative after 48 hours in mice irradiated for 10 min, 30 min, 60 min, 3, 5, and 7 hours, as well as in mice kept in darkness after irradiation, or under natural (day–night) conditions.

In the skin sections from mice exposed to solar simulating radiation, 290–400 nm, no changes in cellular DNA were detected by the IF method, even after 80 min irradiation, i.e. 40 MED.

In the skin of mice irradiated with the xenon lamp (emission 240–400 nm), fluorescence of cell nuclei was apparent after 50 sec irradiation, and in mice irradiated for 1 to 5 min, fluorescent cell nuclei were also observed in the upper layers of the dermis.

DISCUSSION OF RESULTS

The antiserum prepared by us is a reagent by means of which early specific chemical changes in the epidermal cells can be detected following UV irradiation. The IF method visualizes fluorescent nuclei in the epidermis of irradiated animals, but

Table 1. *Results of indirect immunofluorescence studies in the epidermal sections of hairless mice irradiated with UV light*

Expt no.	Mice *n*	Time of irradiation	IF findings in mouse epidermis killed after 0	12	24	48	72 hours
1	10	5 min	±	±	±	–	–
2	10	10 min	+	+	+	–	–
3	10	30 min	+	+	+	–	–
4	10	60 min	+	+	+	–	–
5	10	3 hrs	+	+	+	–	–
6	10	5 hrs	+	+	+	–	–
7	10	7 hrs	+	+	+	–	–

gives completely negative results in the epidermis of control animals. Intensity and extent of fluorescence parallel the time of exposure (10).

Our results showing the disappearance of the IF reaction in the epidermis of mice irradiated with UV confirm the existence of a process of repair of damaged DNA. According to Cleaver (3), repair in cultures of human fibroblasts irradiated with UV begins immediately after irradiation and is essentially complete after 24 hours. In our experiments, repair of damaged DNA in the skin of hairless mice was complete after 48 hours. Our results are consistent with those of Natali & Tan (7). In their experiment, hairy mice were immunized with UV-irradiated DNA to produce high titres of ciculating antibodies to a UV-DNA and then whole body irradiated with UV for 7 hours. They found by the IF method almost complete repair of photochemically damaged DNA in the biopsy of mouse ear taken 48 hours after completion of UV irradiation. The staining related to the presence of thymine dimers was observed only in some areas of the dermal-epidermal junction.

Time of repair is independent of UV exposure of mice (10 min to 7 hours), but is evidently related to the cell life cycle of the dorsal epidermis of mice. This is supported by findings indicating that the duration of DNA synthesis in cells of the hair follicles and ear epidermis of mice lasts from 6 to 30 hours (2).

Views on photoreactivation phenomena in mammalian cells are divergent. Our results do not confirm those of Ten Veen (13), who observed an influence of daylight on UV-DNA repair. In our experiments, the IF reaction became negative at the same time in mice kept in darkness and those exposed to daylight.

Tan & Stoughton (12), using monochromatic light, obtained positive IF results in the skin of hairless mice by irradiating with UV from 254 to 320 nm. However, at wavelengths from 305 to 320 nm, the same effect required many times higher dose. The xenon lamp (SSR) used in our experiments emitted UV radiation between 290 and 400 nm, i.e. including the erythema spectrum (290–320 nm) arriving at the earth's surface. Negative results of the IF reaction in hairless mice irradiated with the xenon lamp indicate a lack of radiation energy sufficient to produce photochemical damage of DNA in light emitted by the xenon lamp.

Regarding changes in epidermal DNA as an index of processes taking place under the influence of life, a protective effect of 5% alcoholic solutions of PABA (*p*-aminobenzoic acid) was demonstrated. In mice irradiated with the germicidal lamp for 5 min, following application of PABA solution in an amount of 60 μl/cm^2, no fluorescence of cell nuclei was observed in the epidermis, whereas cell nuclei in the control group showed fluorescence.

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Received March 6, 1975

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