Antinuclear Antibodies Appearing during PUVA Therapy

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Antinuclear antibodies (ANA) were studied in patients receiving PUVA therapy. Ten patients out of 124, (8%), considered for PUVA had ANA prior to therapy. During PUVA treatment ANA appeared in 34 out of 100 patients. Eight patients with ANA initially were treated and in 4 of them a significant increase in ANA titre was noted. A statistically significant difference was noted, when the first and last ANA tests for each patient were compared. No such difference was seen in a control group consisting of 33 patients. All PUVA patients generating ANA were evaluated clinically and with a laboratory screening. This evaluation was negative in all patients except one who developed ANA of the nucleolar staining pattern together with symptoms consistent with a collagen vascular disease. The ANA titres were generally low and the staining pattern was of the homogenous type in all patients but one. *Key words: Auto-antibodies; Photochemotherapy.* (Received April 18, 1984).

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Photochemotherapy with 8-methoxypsoralen and long wave ultraviolet light (PUVA) was introduced in 1974 for the treatment of psoriasis, and has since then became an established and effective form of therapy (1). The indications have been extended to include also other diseases such as vitiligo, mycosis fungoides, urticaria pigmentosa and certain photodermatoses. Short term side effects are well known and usually harmless while long term risks still have to be evaluated. The appearance of antinuclear antibodies (ANA) during PUVA as reported from our department (2, 3) has been noted also by others (4, 5). Some studies, however, have failed to demonstrate ANA development during PUVA therapy (6, 7). The generation of ANA might indicate a connection with connective tissue disease, and there have been reports lately of a few such cases possibly related to PUVA (8, 9, 10). We report here on the presence of ANA in our PUVA treated patients. This is an extension of a previous preliminary report (2).

MATERIAL AND METHODS

Patients considered for PUVA treatment. 124 patients (70 men and 54 women) were considered for PUVA treatment. They were 16–83 years of age with a mean age of 52 years. Besides psoriasis (102 patients) this group consisted of patients with 8 other diagnoses as well.

Patients accepted for PUVA have been investigated and treated according to the protocol of the European Cooperative Clinical Trial (ECCT) (11). The laboratory investigation included serum liver enzymes, serum creatinine and haematologic screening. In order to avoid PUVA treatment of patients with undetected systemic lupus erythematosus (SLE) ANA was included in our test battery. Blood samples for ANA were drawn before as well as repeatedly during and after treatment. The first sample during treatment was obtained after one month and subsequent ones after varying time intervals. When positive the ANA test was repeated and if still positive a blood sedimentation rate, serum electrophoresis, urine analysis and the *Crithidia luciliae test* for antibodies to native DNA were also performed.

PUVA treatment was initially given 4 days a week. Patients were irradiated 2 hours after receiving an oral dose of 8-methoxypsoralen (AB Draco, Lund, Sweden) according to the ECCT dosage scheme. Source of the long wave ultraviolet radiation (UVA) was a PUVA 4000 equipment (Waldman

AG, Schwenningen, GFR). After remission PUVA was terminated and only restarted if the disease relapsed. A small number of patients, however, were given maintenance therapy.

Control patients. Thirty-three psoriasis patients, 26–76 years of age (mean 49 years), were followed up with ANA determinations prior to and 4–6 weeks after therapy with topical steroids, tar or dithranol. Most patients also received medium wave ultraviolet (UVB) irradiation. The blood samples were always drawn in close relation to therapy.

Method for demonstration of antinuclear antibodies (ANA). ANA were detected by the indirect immunofluorescence test using cryostat sections of snap-frozen rat kidney (6 μm) (12). Whenever a positive reaction was detected in sera diluted 1/5, duplicate serial dilutions were made. The fluorescein isothiocyanate conjugate, conforming to standard requirements, had an antibody content of 2.6 mg/ml and a F/P quotient of 4.8×10^{-3}. It was used in a dilution determined by performance test (usually 1/20). The preparations were read in a Leitz Dialux 20 EB immunofluorescence microscope for incident illumination equipped with a filter system and with a HBO 50 W mercury lamp. The magnification used was ×312.

Dilutions for determination of ANA start at 1/8. Because of the error of the semiquantitative method a titre of 8 is considered negative in this study, and consequently, 16 is the lowest ANA titre accepted as positive. A significant difference in ANA titre requires a change of at least 2 titre steps.

Crithidia luciliae test. The kinetoplast of this haemoflagellate was used as a source of DNA as previously described by Arden et al. (13). Flagellates were grown in bacto tryptose medium at 24°C (14), washed and resuspended in phosphate-buffered saline (PBS), pH 7.4. Ten μl drops of this suspension were applied to glass slides, air dried and fixed in 95% ethanol for 10 min and used either immediately or after storage at −20°C. The indirect immunofluorescence assay was performed as described above for ANA.

Statistical methods. Student's *t*-test and X^2-test were used for statistical calculations.

RESULTS

Ten (8%) out of the 124 patients considered for PUVA, had ANA (titre ≥16) before treatment. This group consisted of 9 psoriasis and one pompholyx patients.

Of the remaining 114 ANA-negative patients 100 were followed up with ANA tests during and after treatment. Thirty-four of them (34%) developed ANA in at least two consecutive samples. After discontinuation of PUVA ANA disappeared in 19 of these 34 patients. The ANA titres were generally low (Fig. 1). The staining pattern was homogenous in 33 patients and nucleolar in one. Eight of the patients with ANA initially were also PUVA-treated since further investigations showed no signs of collagen vascular disease. In 4 of them (50%) the ANA titre increased significantly during PUVA therapy. The ANA titres of the other 4 patients remained unchanged.

Thus, totally 108 patients were studied with tests for ANA prior to, during and after the PUVA treatment. Eight of the first tests (7%) and 23 of the last (21%) were positive. This difference is statistically significant ($X^2=7.4^{**}$) (Table I).

All patients developing ANA were evaluated clinically and with a laboratory screening including the *Crithidia luciliae test* in order to reveal collagen vascular disease. This evaluation was negative in all patients except one who developed ANA of the nucleolar staining type (10). This patient had psoriasis and was ANA negative before treatment. During PUVA therapy the ANA titre increased to 64 without other signs indicating a collagen vascular disease. Soon after PUVA treatment was terminated polyarthralgia followed by fatigue, dyspnea and dysphagia developed and the patient died two years later. This case is reported in detail elsewhere (10).

The number of PUVA treatments given to each patient ranged from 6 to 304. There was no correlation between the number of treatments given and ANA development. Nor was there an increasing probability of positive ANA tests with increasing amounts of UVA energy. These comparisons were based on the number of treatments and the amounts of energy given up to the time when a positive ANA test was first noted.

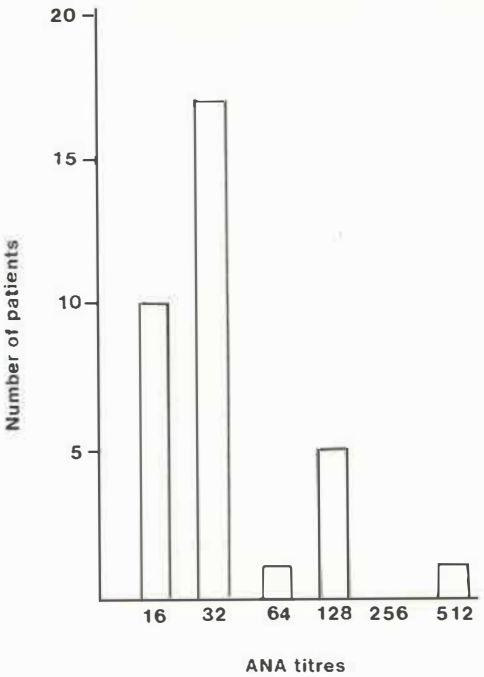

Fig. 1. ANA titers in the 34 patients developing ANA during PUVA therapy. Highest titer reached by each individual is indicated.

The mean age of the ANA-negative group was 47.8 (±17.9) years versus 58.0 (±15.6) years in the ANA-positive group. This difference is statistically significant (t-=2.8**).

The incidence of positive ANA tests was slightly higher in women than in men, but this difference was not statistically significant.

Patients with ANA did not react unfavourably to the PUVA therapy and treatment course and therapeutic efficacy did not differ between ANA-negative and -positive patients.

In this study repeated determinations of 8-methoxypsoralen in blood were performed in

Table I. *The distribution of PUVA patients and control patients with regard to the presence of antinuclear antibodies (ANA)*

	Number of patients		
	With ANA	Without ANA	Total
Patients considered for PUVA therapy			
ANA test before therapy	10	114	124
Patients accepted for PUVA therapy			
ANA test before therapy	8	100	108
Last ANA test	23	85	108
Patients developing ANA during therapy			34
Patients losing their ANA during therapy			0
Control patients			
ANA test before therapy	4	29	33
ANA test after therapy	4	29	33
Patients developing ANA during therapy			3
Patients losing their ANA during therapy			3

most patients (15). No association between 8-methoxypsoralen serum levels and ANA development was noted.

The results of the ANA determinations for the patients in the control group are given in Table I. Four patients had ANA prior to therapy but in three the ANA disappeared during treatment. Three patients developed ANA during therapy.

DISCUSSION

Antinuclear antibodies are present in the serum of a small proportion of normal subjects. Hooper et al. found a prevalence of 6% when more than 3000 individuals of all ages in a small town were examined (16). Age is a factor known to increase the number of individuals with ANA (17, 18). Similar prevalences have been reported prior to therapy in patients with psoriasis considered for PUVA. Bjellerup et al. (2, 3) noted 6% and Stern et al. (6) 8%. With wider indications for PUVA therapy including diseases other than psoriasis Kubba et al. (5) found a prevalence of 11%. This last figure was obtained in a group of PUVA patients where the ANA tests were sometimes performed only after the start of the PUVA therapy. We found 8% positive ANA tests, a figure well corresponding to the prementioned results. Levin et al. (7), on the other hand, did not find a single patient with a positive ANA test in a group of 22 psoriatics prior to PUVA therapy. Although this might be due to the low number of patients, it could also indicate too low a sensitivity of the method used.

The presence of ANA among a certain amount of normal subjects is thus a well established fact. The question if PUVA therapy promotes the development of ANA, however, is controversial. Stern et al. (6) did not find an increase in the number of patients with ANA from the initial to the final test. Furthermore they found no consistency in the reactivity as only 40% of patients with their first test positive had final tests that were also positive. In our group of patients we find statistically significant differences when the proportion of ANA-positive patients prior to PUVA therapy is compared to the proportion of patients positive when only the final test is included (Table I). The final ANA proportion may be assumed to be underestimated since there were no fixed time intervals for blood sampling. Thus a patient with a final negative ANA test was not followed with additional ANA determinations if the therapy was completed, while a patient with a positive ANA test during or after the therapy was followed up with frequent analysis. Kubba et al. (5) have not analysed the proportions of patients with ANA in the first test compared to the final test statistically, but if this is done a significant difference is noted indicating the probability of PUVA therapy as a promoting factor for the development of ANA.

Further support for the role of PUVA therapy in the development of ANA is obtained from the result in the control group (Table I). There were as many patients with ANA before therapy as after. Furthermore, in three out of four patients, ANA disappeared during therapy, compared to none out of eight in the PUVA-treated group (Table I).

The reason for the controversial results as reported in the literature is not clear. One possible explanation may be differences in methodology for demonstrating ANA. A number of techniques were used in the multi-center study of Stern et al. (6) all differing from that applied by Kubba et al. (5) and by ourselves using rat kidneys. Theoretically PUVA-induced ANA might differ from other types of ANA and therefore the sensitivity of the methods may vary. Another reason for the discrepancy may be the fact that the ANA determinations were performed at different time intervals in relation to the therapy. Most of our determinations were performed in close relation to therapy (during or directly after) and the same was true for the patients of Kubba et al. (5). For most patients of Stern et al. (6) the first ANA determination after the start of the PUVA therapy was not performed in

close connection to the initial treatment period. Furthermore, we have noticed a relationship between the ANA and the treatment course. In many patients the titres have a tendency to rise during the initial therapy and then be stationary during the period of maintenance treatment. When the PUVA therapy was completed most titres decreased and eventually normalized before changing to positivity again if PUVA was reinstituted.

No relation was found between the total amount of UVA energy delivered or the number of treatments given and the probability of developing ANA. To make this comparison valid the amount of energy or number of treatments should be calculated for the time period up to the moment, when the first positive ANA test is noted, and these figures should be compared with the total number of treatments that ANA-negative patients receive continuously. Otherwise a high sensitivity to PUVA (with regard to the tendency of developing ANA) would be hidden if the comparison is made a long time after the change from negativity to positivity. Kubba et al. (5) have found a correlation between the number of treatments and the tendency of developing ANA. This relationship might instead be based on the higher mean age of the PUVA-positive group, as increasing age is a factor of importance for development of ANA, a conclusion drawn by ourselves and Kubba et al. (5).

It is at present difficult to ascribe any pathogenetic importance to these antibodies. The relationship between the development of ANA during PUVA therapy and a collagen vascular disease for the patient reported by Eyanson et al. and for our own patient may be only coincidental (9, 10). For all other patients in our investigation nothing indicated the development of collagen vascular disease. No antibodies to native DNA were found, which argues against a more serious implication of the ANA findings (13). All patients with positive ANA except one showed a homogenous staining pattern. The patient with the highest titre (512) showed a nucleolar staining pattern and developed a collagen vascular disease (10).

ANA may be induced by other treatments than PUVA. Drugs most commonly associated with ANA include procainamide, hydralazine and isoniazid. These drugs may also induce an SLE-like syndrome (19). For procainamide the ANA titres usually are high even in the absence of the SLE-like syndrome (20). The pathophysiological background for PUVA-induced ANA is not known. UVB may alter the immunogenic properties of DNA (21). PUVA has also been shown to produce psoralen-DNA photoadducts that are immunogenic (22). Lately, UVA-induced DNA breaks have been reported in cultured human fibroblasts (23, 24) and this finding provides a possible theoretical explanation for psoralen and UVA-induced ANA. Mice exposed to the UVA component of PUVA have been shown to generate ANA (25).

Our results indicate that PUVA therapy has the capacity to induce ANA. The mechanism for this is not clear and the clinical importance is also obscure. Whether a nucleolar pattern implies a higher risk for developing a connective tissue during PUVA therapy is uncertain. We agree with Stern et al. (6) and Kubba et al. (5) in their recommendations concerning PUVA therapy in ANA-positive patients. If there are no clinical or laboratory signs of connective tissue disease, PUVA may be administered. As long as a possible relationship between PUVA-induced ANA and the development of a connective tissue disease has not been ruled out, monitoring ANA among PUVA patients is, however, advisable.

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