

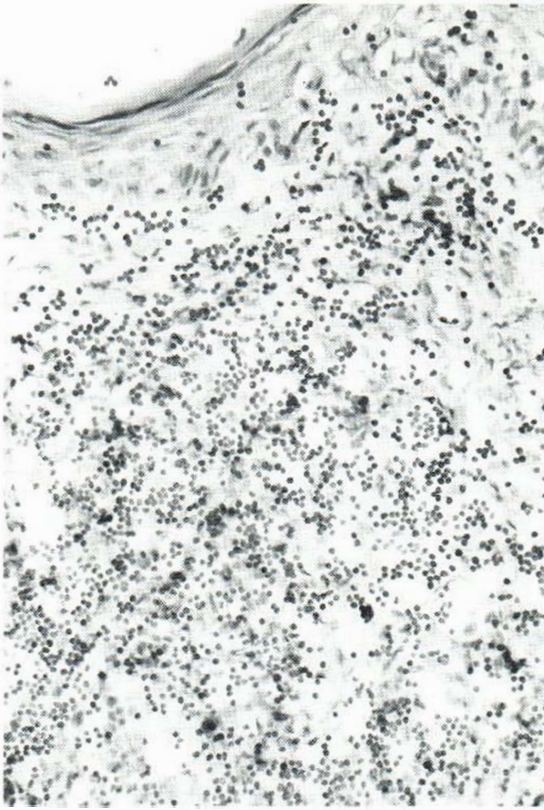


Fig. 5. Skin section showing adherence of EAC3b indicating macrophages and B-lymphocytes. Stained with benzidine and hematoxylin-eosin,  $\times 250$ .

data are in line with the results reported by Braylan et al. (1).

In the skin, the lymphocytic infiltration showed a focal distribution in the upper dermis in contrast to the usual, common band-like involvement. The majority of the mononuclear cells in the dermis lacked receptors for C3, Fc $\gamma$  and sheep erythrocytes. Furthermore, electron microscopy showed these cells to be similar to the atypical mononuclear cells seen in the peripheral blood. These results suggest that the Sézary cells in the peripheral blood and in the dermis are of the same cell type. Although most investigators have presented evidence for the T-lymphocytic nature of the Sézary cell, findings made with our patient strongly suggest that Sézary cells have dissimilar characteristics from patient to patient. Presumably the Sézary cells proliferate from cell populations at various stages of cell maturation, resulting in Sézary cells with differing

characteristics, dependent on the stage of maturation.

## REFERENCES

1. Braylan, R., Variakojis, D. & Yachnin, S.: The Sézary syndrome lymphoid cell: Abnormal mitogenic responsiveness and surface properties. *Blood* 42: 1024, 1973.
2. Crossen, P. E., Mellor, J. E. L., Finley, A. G., Ravich, R. B. M., Vincent, P. C. & Gunz, F. W.: The Sézary syndrome. Cytogenetic studies and identification of the Sézary cell as an abnormal lymphocyte. *Am J Med* 50: 24, 1971.
3. Hallberg, T., Guerner, B. W. & Coombs, R. R. A.: Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int Arch Allergy Appl Immunol* 44: 500, 1973.
4. Kaplan, M. E. & Clark, C.: An improved rosetting assay for detection of human T-lymphocytes. *J Immunol Methods* 5: 131, 1974.
5. Lutzner, M. A. & Jordan, H. W.: The ultrastructure of an abnormal cell in Sézary syndrome. *Blood* 31: 719, 1968.
6. Matre, R., Talstad, I. & Haugen, Å.: Surface markers in non-phagocytic hairy cell leukemia. *Acta Pathol Microbiol Scand [C]* 85: 406, 1977.
7. Matre, R. & Tønder, O.: Complement receptors in human renal glomeruli. *Scand J Immunol* 5: 437, 1976.
8. Steel, C. M. & Creasy, G. H.: A micromethod for the activation of human blood lymphocytes *in vitro*. *Immunol Commun* 5: 559, 1976.
9. Zucker-Franklin, D.: Thymus-dependent lymphocytes in lymphoproliferative disorders of the skin (Sézary syndrome and mycosis fungoides). *J Invest Dermatol* 67: 412, 1976.
10. Winkelmann, R. K.: Clinical studies of T-cell erythroderma in the Sézary syndrome. *Mayo Clin Proc* 49: 519, 1974.

## Antinuclear Antibodies during Puva Therapy

Mars Bjellerup, Magnus Bruze, Arne Forsgren,<sup>1</sup>  
Gösta Krook and Bo Ljunggren

Departments of Dermatology and <sup>1</sup>Clinical Bacteriology,  
University of Lund, General Hospital,  
S-214 01 Malmö, Sweden

Received July 25, 1978

**Abstract.** During PUVA therapy 7 patients out of 34 with severe psoriasis developed circulating antinuclear antibodies (ANA) (21%). Before treatment only 3 patients of 50 (6%) considered for PUVA had detectable ANA.

Table 1. Treatment course and ANA titres in the 9 PUVA patients with detectable ANA

—, treatment 4 days a week; ···, maintenance treatment 2 times a week or less. Patients 1–3 have psoriasis vulgaris, nos. 4–5 generalized pustular psoriasis, and nos. 6–9 palmar psoriasis

| Pat. no.   | Sex | Age | ANA titre before treatment | Treatment course |      |       |      |      |   |   |   |   |   |      |    |    |
|------------|-----|-----|----------------------------|------------------|------|-------|------|------|---|---|---|---|---|------|----|----|
| 1          | F   | 38  | <1/8                       | —                | 1/16 |       | 1/32 | 1/32 |   |   |   |   |   |      |    |    |
| 2          | F   | 73  | <1/8                       | —                | 1/32 | 1/16  | 1/16 | 1/16 |   |   |   |   |   |      |    |    |
| 3          | M   | 50  | <1/8                       | —                | 1/32 | 1/8   | 1/32 |      |   |   |   |   |   |      |    |    |
| 4          | F   | 75  | <1/8                       | —                | 1/16 |       | <1/8 | <1/8 |   |   |   |   |   | 1/16 |    |    |
| 5          | F   | 74  | 1/256                      | —                | 1/16 | 1/512 |      |      |   |   |   |   |   |      |    |    |
| 6          | F   | 62  | <1/8                       | —                |      | 1/64  | 1/32 |      |   |   |   |   |   |      |    |    |
| 7          | F   | 57  | 1/16                       | —                | 1/8  | 1/16  | 1/64 | 1/32 |   |   |   |   |   |      |    |    |
| 8          | F   | 28  | <1/8                       | —                | 1/16 | 1/16  | <1/8 |      |   |   |   |   |   |      |    |    |
| 9          | M   | 55  | <1/8                       | —                | 1/32 |       | <1/8 | <1/8 |   |   |   |   |   |      |    |    |
| Months ... |     |     |                            | 0                | 1    | 2     | 3    | 4    | 5 | 6 | 7 | 8 | 9 | 10   | 11 | 12 |

The ANA titres were usually low. Antibodies against native DNA as studied with the *Crithidia luciliae* test, were not found, and blood and urinary screening for collagenosis was negative. All 7 patients responded well to the PUVA treatment. The significance of these findings remains to be determined.

**Key words:** Antinuclear antibodies; PUVA; 8-MOP; UVA; Psoriasis

Photochemotherapy with 8-methoxypsoralen and longwave ultraviolet light (PUVA) was introduced for psoriasis in the early 'seventies and is now an established treatment for severe cases. This treatment was started in Malmö early in 1977. In order to eliminate the risk of admitting patients with connective tissue diseases such as systemic lupus erythematosus to PUVA, we included, as a screening procedure, antinuclear antibodies (ANA) in our test battery. As a substantial number of patients developed ANA during PUVA, we report here our preliminary results in the first 50 patients considered for this treatment.

## MATERIAL AND METHODS

### PUVA treatment

50 patients, 16–82 years of age, with severe psoriasis, refractory to conventional therapy, were considered for PUVA therapy. The patients which were accepted have been investigated and treated according to the principles of the European Cooperative Clinical Trial (ECCT) described by Wolff et al. (6). The laboratory investigation

included liver enzymes, serum creatinine, haematologic screening, ANA and, in the patients with detected ANA, the *Crithidia luciliae* test for antibodies to native DNA. Blood samples for ANA were withdrawn before the treatment was started and repeatedly during and after treatment.

In patients with detectable ANA a blood sedimentation rate, serum electrophoresis and urinalysis were also performed. One patient (No. 5) was accepted for PUVA despite the fact that she had positive ANA. She had widespread, severe, pustular psoriasis and she had been investigated for collagenosis on several occasions during recent years, with negative results.

Treatment was given 4 days a week for an initial period of about 6 weeks. Patients were irradiated 2 h after receiving an oral dose of 8-methoxypsoralen (AB Draco, Lund, Sweden) according to the ECCT dosage scheme. Source of the longwave ultraviolet radiation was a PUVA 4000 equipment (Waldmann AG, Schwenningen, GFR). The initial dose of UVA was decided after phototesting. Patients with solely palmar psoriasis were treated only on the hands with radiation from a dysprosium lamp (Osram HQI, 400 W) filtered through 3 mm window glass.

After the initial treatment period, maintenance therapy (2 treatments a week or less) was given in some of the cases.

### Method for demonstration of antinuclear antibodies (ANA)

ANA were detected by the indirect immunofluorescence test as adopted in this laboratory using cryostat sections of snap-frozen rat-kidney (6 µm) (2). Doubling serial dilutions of sera were made whenever a positive reaction was detected in sera diluted 1/5. The fluorescein–isothiocyanate conjugate conforming to standard requirements had an antibody content of 2.6 mg/ml and an F/P quotient of  $4.8 \times 10^{-3}$ . It was used in a dilution determined by

performance tests (usually 1/20). The preparations were read in a Leitz Dialux 20 EB immunofluorescence microscope for incident illumination equipped with filter system i and with an HBO 50 W mercury lamp. The magnification used was  $\times 312$ .

#### *Crithidia luciliae* (C.I.) test

The kinetoplast of this haemoflagellate was used as a source of DNA as previously described by Aarden et al. (1). Flagellates were grown in bacto tryptose medium at 24°C (3), washed and resuspended in phosphate-buffered saline (PBS), pH 7.4. 10  $\mu$ l drops of this suspension were applied to glass-slides, airdried 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.

### RESULTS

Of the 50 patients considered for PUVA, 3 (6%) had ANA (titre  $>1/8$ ) before treatment. Of the patients who were accepted for PUVA, 34 (18 males and 16 females) with no ANA initially have been observed for at least 6 weeks. 7 of these 34 patients (21%), 2 males and 5 females, developed ANA (titre increase  $\geq 2$  titre steps) during PUVA treatment. The 2 patients (Nos. 5 and 7) with ANA already before treatment showed a titre increase during PUVA. Details of the treatment course and the ANA titres are given for each of the 9 patients with detectable titres (Table I). ANA could not be correlated to total dose of either UVA or 8-MOP, nor to the skin type of the patient. Antibodies to native DNA, studied in 8 of the 9 patients were negative, as were the other tests for collagenosis performed. The patients with ANA did not react unfavourably to the PUVA therapy.

### DISCUSSION

Side effects during PUVA therapy have been remarkably few and not serious (5, 6), although the laboratory and physical examinations of these patients usually have been very thorough. In earlier reports, however, studies on antinuclear antibodies have not been included.

In our group of severe psoriatics we found a high frequency of ANA developing during PUVA treatment, compared with a low incidence of antibodies before treatment. The titres, however, were rather low and the changes were rarely more than three titre steps. Our patients had no other signs of colla-

genosis reflected by serum protein pattern, urinary, or clinical findings. This is in contrast to drug-induced systemic lupus erythematosus caused by hydralazine or procain amide (4) for instance. The fact that no antibodies to native DNA were found argues against a more serious implication of the ANA findings (1).

We have not observed the patients for a sufficiently long period yet to be able to detect whether the ANA titres disappear or not after treatment is stopped.

We have the impression that patients with pustular psoriasis or palmar lesions develop ANA more readily than the vulgaris patients, but the number of patients is too small to permit any conclusions. The mechanism of the ANA titre rise as well as the significance of these findings remain to be determined and studies in these directions are now in progress.

### REFERENCES

1. Aarden, L. A., de Groot, E. R. & Feltkamp, T. E. W.: Immunology of DNA. III. *Crithidia luciliae*, a simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. *Ann NY Acad Sci* 254: 505, 1975.
2. Bergquist, R.: Immunofluorescence. Evaluation of technique and reagents with special reference to methodological standardization. Thesis, Karolinska Institutet, Stockholm 1974.
3. Boné, G. J. & Steinert, M.: Isotopes incorporated in the nucleic acids of *Trypanosoma mega*. *Nature* 178: 308, 1956.
4. Henningsen, N. C., Cederberg, Å., Hanson, A. & Johansson, B. W.: Effects of long-term treatment with procaine amide. *Acta Med Scand* 198: 475, 1975.
5. Melski, J. W., Tanenbaum, L., Parrish, J. A., Fitzpatrick, T. B., Bleich, H. L. & 28 participating investigators: Oral methoxsalen photochemotherapy for the treatment of psoriasis: a cooperative clinical trial. *J Invest Dermatol* 68: 328, 1977.
6. Wolff, K., Fitzpatrick, T. B., Parrish, J. A., Gschnait, F., Gilchrist, B., Hönigsmann, H., Pathak, M. A. & Tanenbaum, L.: Photochemotherapy for psoriasis with orally administered methoxsalen. *Arch Dermatol* 112: 943, 1976.