Acta Derm Venereol (Stockh) 1986; 66: 398–403

Distribution of Circulating Mononuclear Cells in Short-term PUVA-treated Psoriatic Patients and Healthy Subjects

F. M. LARUSSA,[1] L. M. LAROCCA,[2] A. VENIER,[1] M. ZOLLINO,[2] L. RUSCIANI,[1] and F. SERRI[1]

[1]*Department of Dermatology and* [2]*Department of Hematology, Catholic University of the Sacred Heart, Rome, Italy*

Larussa FM, Larocca LM, Venier A, Zollino M, Rusciani L, Serri F. Distribution of circulating mononuclear cells in short-term PUVA treated psoriatic patients and healthy subjects. Acta Derm Venereol (Stockh) 1986; 66: 398–403.

Peripheral blood mononuclear cells (PBMC), as defined by monoclonal antibodies (OKT3, OKT4, OKT8, OK 1, Leu 7, Leu 11b) were determined in 10 psoriatic patients and in 10 healthy subjects before and after administration of short-term PUVA therapy. A comparison of the mean baseline percentages of the two groups showed a statistically significant increase in Leu 7+ cells ($p<0.001$) as well as a slight increase in OKM1 and OKT8 positive cells in the psoriatic subjects. After 21 exposures, these subsets showed a reduction towards control values, while a significant increase in OKT3 and OKT4 positive cells ($p<0.01$) could be observed only in the control group. These results indicate that short-term PUVA therapy is associated with changes in PBMC subpopulations. This modification, however, does not necessarily imply a disturbance of immune system function, including natural killer activity. *Key words: Monoclonal antibodies: Photochemotherapy.*
(Received December 16, 1985.)

F. M. Larussa, Department of Dermatology, Catholic University of the Sacred Heart, Largo Gemelli, 8, 00168 Rome, Italy.

The recent availability of monoclonal antibodies (MoAb) has provided a powerful tool for the study of certain cutaneous disorders, such as psoriasis, in which disturbances of cell-mediated immunity is commonly believed to play an important role in the pathogenesis (1, 2, 3). On the other hand, although the in vitro action of UV radiation upon the immunocompetent cells is well-established (4), the effects of photochemotherapy (PUVA) on circulating lymphocytes are not yet completely elucidated.

The purpose of the present study was to determine whether a consistent relationship exists between PUVA exposure and the distribution of peripheral blood T-cell subsets by investigating psoriatic patients and healthy subjects, both receiving short-term PUVA therapy.

MATERIALS AND METHODS

Ten patients (6 males, 4 females, mean age 37±17 years) suffering from psoriasis vulgaris with durations from 3 to 34 years were studied. All patients had active, extensive (more than 30% of the body surface) large-plaque psoriasis, widely and bilaterally distributed. None of the patients had received systemic anti-psoriatic remedies, photochemotherapy or local corticosteroids for at least 4 weeks prior to the beginning of the study. All ten were submitted to PUVA therapy according to the revised protocol of the European Cooperative Clinical Trial on Photochemotherapy of Psoriasis (5). Controls consisted of ten healthy subjects (staff members), all of whom voluntarily underwent PUVA treatment.

Peripheral blood samples were collected and hemochromocytometric analysis was performed in both groups before starting PUVA therapy and following 21 exposures, which proved to be the average time required for clearing of the lesions in the psoriatic subjects. The average cumulative dose for the psoriatic group was 104 J/cm^2 as compared to 96 J/cm^2 for the control group.

Analysis of the Peripheral Blood Mononuclear Cells (PBMC) was performed by means of MoAb. PBMC were separated from heparinized venous blood samples by Ficoll/Hypaque density gradient centrifugation (Pharm Fine Chem, Piscataway, N.J.). Cells were washed three times in Hanks' Balanced Salt solution (HBSS) and were adjusted to a concentration of 6×10^6 cells/ml.

Cell surface membrane determinants reactive with the monoclonal antibodies were demonstrated in cell suspension by an indirectr immunofluorescence technique as previously described (6). Fluorescein-conjugated F(ab')2 antibody fragments of affinity-purified goat anti-mouse IgG were employed as the second antiserum (Cappel Laboratories, Cochranville, MD). The MoAb panel employed in this study included OKT3, OKT4, OKT8, OKM1 (produced by Ortho Diagnostics, Raritan, N.Y.) and Leu 7, Leu 11b (obtained from Becton Dickinson, Sunnyvale, CA). OKT3 reacts with ≥95 % of the peripheral T cells (7). OKT4 recognizes antigens expressed on approximately 55–75 % of peripheral T cells, and OKT8, the determinants present on 20–30 %. These two subpopulations include the inducer and the suppressor/cytotoxic subsets, respectively (8). OKM1 recognizes cell surface determinants on most monocytes/macrophages and granulocytes (9). Leu 7 reacts with large granular lymphocytes that are believed to possess K/NK function (10). Leu 11b reacts with the Fc receptor on NK cells, granulocytes and basophils (11).

Group comparisons for determination of significance were done using Student's two-sample *t*-test. Statistical analysis of the results before and after therapy was performed using Student's *t*-test for paired observations. As a level of significance, $p<0.05$ was adapted.

RESULTS

In Table I, total peripheral blood mononuclear cells, T lymphocytes and monocytes are expressed in absolute numbers for the following groups: normal controls before PUVA, normal controls after PUVA, psoriatic patients before PUVA and psoriatic patients after PUVA. The numbers of mononuclear cells and T lymphocytes did not differ significantly among these four groups. The absolute number of monocytes, however, was significantly higher in the psoriatic group ($p<0.05$) than in the control group both before and after PUVA therapy.

Table II shows the basal conditions of the two groups. A statistically significant ($p<0.001$) increase is evident in the Leu 7+ cells in the psoriatic with respect to the control

Table I. *Absolute numbers of mononuclear cells, T lymphocytes and monocytes in psoriatic patients and healthy subjects before and after PUVA*

Subjects	No.	Mononuclear cells (mean ± SD)	T Lymphocytes (mean ± SD)	Monocytes (mean ± SD)
Normal controls before PUVA	10	1680±473	1158±261	260±138
Psoriatic patients before PUVA	10	1731±516	1128±244	426±207*
Normal controls after PUVA	10	1740±415	1294±246	220±118
Psoriatic patients after PUVA	10	1768±396	1118±198	325±117*

* $p<0.05$.

Table II. *Percentages (mean ± SD) of peripheral blood mononuclear cells before photochemotherapy*

Population	No.	OKT3	OKT4	OKT8	OKM1	Leu 7	Leu 11b
Controls	10	68±5	45±4	25±2	15±6	16±4	15±3
Psoriatics	10	66±6	44±5	32±6	25±3	25±4*	16±3

* $p<0.001$.

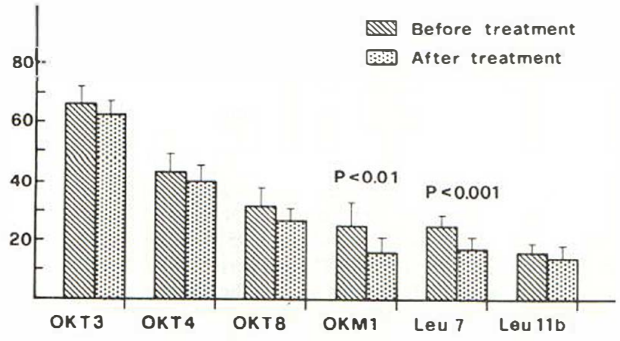

Fig. 1. Percentages of peripheral blood mononuclear cells in psoriatic patients before and after short-term PUVA therapy. The bars represent the mean value and 1 SD is indicated.

group. There are also increases, though not significant, in the OKM1 positive cells and OKT8 positive cells.

In Fig. 1, percentages of mononuclear cell subsets in psoriatic subjects before and after PUVA are represented. Markedly decreased percentages of OKM1 positive ($p<0.01$) and Leu 7 positive ($p<0.001$) cells were seen. Histograms from the control group (Fig. 2) reveal a significant increase in both the OKT3 and OKT4 positive cell subsets following photochemotherapy. Finally a comparison of mononuclear cell percentages in both groups after PUVA treatment (Table III) shows that, following therapy, Leu 7 and OKM1 positive cells in psoriatic patients begin to approach the values encountered in the controls. A significant increase in T3 and T4 positive cells is present only in the control group ($p<0.01$).

DISCUSSION

Previous studies of the distribution of T cell subpopulations in psoriatic patients are characterized by some discrepancies. Numerous studies have been performed by means of the MoAb technique. Normal percentages of OKT4 positive and OKT8 positive cells have been reported by several investigators (12, 13, 14, 15). A decrease of T suppressor lymphocytes as defined by OKT8 has been noted by Kokelj et al. (16). A significant reduction in the number of T cells reactive with the MoAb OKT3 and OKT4, however, has been demonstrated by Baker et al. (17) in 14 patients with extensive lesions. Contradictory data have also been obtained by investigators, who used differential expression of Fc receptor binding to define T cell subpopulations. Using this method, Gu et al. (18) were able to demonstrate elevated numbers of Tγ cells and increased suppressor activity as defined by lowered pokeweed mitogen Ig synthesis in mononuclear cell suspension. While

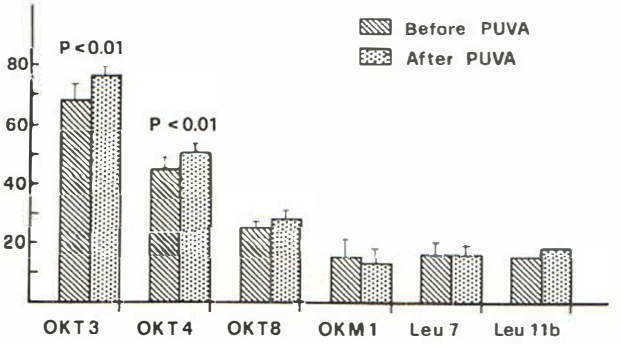

Fig. 2. Percentages of peripheral mononuclear cells in normal controls before and after short-term PUVA therapy. The bars represent the mean value and 1 SD is indicated.

Clot et al. (19) found no abnormality in the distribution of T cell subsets, a decrease in the percentages of T cells has been noted in two other studies (15, 20). These authors also demonstrated a direct correlation between the Tγ deficit and the extent of cutaneous involvement. In addition, Ligresti et al. (20) showed increased Tμ values.

These conflicting results could depend primarily on the use of different methods for the quantitation of peripheral blood T cell subsets. Moreover, the dishomogeneity of parameters considered (extent and activity of the disease, clinical type of skin lesions, number and ages of patients) could provide another conceivable explanation for such divergent data.

In the present study, a larger panel of MoAb was employed. We found a markedly increased number of Leu 7 positive cells and a less evident augmentation of mononuclear cells reactive with OKM1 and OKT8. This is consistent with the results showed by Glinski (21) in active untreated disease, and by Baker (17) in chronic plaque psoriasis by means of the count of null cells.

In the past few years, many investigators have directed their attention to the effect of PUVA therapy on circulating lymphocytes with a similar discrepancy in the results. A significant decrease in the number of E-rosette forming cells has beeen reported by Ortonne et al. (22) in PUVA treated psoriatics. This decrease has been found to be transient (23), appearing after 4 PUVA exposures, with a return to baseline values after 8 sessions. In contrast, Fraki et al. (24) showed that the proportion of circulating E-rosette forming cells rose in a group of patients with psoriasis during a 12-week course of PUVA therapy. Finally Morison et al. (25) reported no alteration in the percentage of circulating B and T lymphocytes in 11 psoriatic patients investigated before, during and after an intensive course of PUVA (18–37 sessions).

Our study suggests that short-term PUVA therapy can influence the distribution of different mononuclear cells in peripheral blood. In normal subjects, we found increased numbers of OKT3 and OKT4 positive cells. In psoriatic patients, a reduction of Leu 7 and OKM1 positive cells has been noted after PUVA without any quantitative variation of Leu 11b positive cells. Moreover, the OKT8 positive cell level was slightly elevated.

These data are not in agreement with findings reported by Moscicki et al. (12) who found decreased percentages of OKT3 and OKT4 positive cells in psoriatic subjects on maintenance PUVA therapy for more than 4 years as compared with non-irradiated psoriatic and healthy subjects. The dishomogeneity of respective study groups, the more restricted MoAb panel employed and the cumulative effect of PUVA in long-term treatment can all explain these discrepancies.

It is interesting that in our psoriatic patients, we found after PUVA therapy a marked decrease in Leu 7 and OKM1 positive cells without any variation in the number of Leu 11b positive cells.

Recent data (11) showed that MoAb defined three subsets of NK cells, each expressing NK functional capability, but different levels of cytotoxic efficiency (Leu 7+Leu

Table III. *Percentages (mean ± SD) of peripheral blood mononuclear cells after photochemotherapy*

Population	No.	OKT3	OKT4	OKT8	OKM1	Leu 7	Leu 11b
Controls	10	76±3	51±3	28±3	13±5	16±3	19±4
Psoriatics	10	63±5*	40±6*	27±4	16±6	17±4	14±4

* $p<0.001$.

11b−<Leu 7+Leu 11b+<Leu 7−Leu 11b+). Recent immunoelectronmicroscopy studies (26) showed different ultrastructural features, clearly suggesting both morphological and functional heterogenity of these three subsets. One might speculate that PUVA therapy can produce a selective action on the weaker NK cell subset and/or pre-NK cells. Two-colour immunofluorescence studies, which are in progress, could confirm this hypothesis.

Notably, data recently presented by Jansen et al. (27) showed proper function of the NK cell system as defined by means of responsiveness to α and γ interferon (IFN) in psoriatics. IFN is commonly reported to exert stimulating effects on NK activity. Therefore it is of interest that a significantly increased IFN production in psoriatic patients after PUVA therapy could be detected by Diezel et al. (28). These findings lend support to the hypothesis that the effect of PUVA therapy on the NK cell system in psoriatics is not one of depression. This may also be important in relation to the suggested viral etiology of psoriasis (29).

ACKNOWLEDGEMENT

This work was partially financed by a grant from the Foundation for Research in Dermatology of Rome, Italy.

REFERENCES

1. Guilhou JJ, Meynadier J, Clot J. New concepts in the pathogenesis of psoriasis. Br J Dermatol 1979; 98: 585–592.
2. Cormane RH. Immunopathology of psoriasis. Arch Dermatol Res 1981; 270: 201–215.
3. Bergstrasser PR, Gilliam JN. The immunology of psoriasis. Pharmacol Ther 1981; 14: 345–354.
4. Krüger JP, Christophers E, Schlaak M. Dose effect of 8-methoxypsoralen and UVA in cultured human lymphocytes. Br J Dermatol 1978; 98: 141–144.
5. Wolff K, Fitzpatrik TB, Parrish JA et al. Photochemotherapy for psoriasis with orally administered methoxsalen. Arch Dermatol 1976; 112: 943–950.
6. Nagel JE, Chrest FJ, Adler WH. Enumeration of T lymphocyte subsets by monoclonal antibodies in young and aged humans. J Immunol 1981; 127: 2086–2088.
7. Kung PC, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T-cell surface antigens. Science 1979; 206: 347–349.
8. Reinherz EL, Schlossman SF. The differentiation and function of human lymphocytes. Cell 1980; 19: 821–827.
9. Breard J, Reinherz EL, Kung PC, Goldstein J, Schlossman SF. A monoclonal antibody reactive with human peripheral blood monocytes. J Immunol 1980; 124: 1943–1948.
10. Abo T, Balch CM. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 1981; 127: 1024–1029.
11. Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF. Subpopulation of human natural killers defined by expression of Leu 7 (HNK-1) and Leu 11 (NKP-15) antigens. J Immunol 1983; 131: 1789–1796.
12. Moscicki RA, Morison WL, Parrish JA, Bloch KJ, Colvin RB. Reduction of the fraction of circulating helper-inducer T cell identified by monoclonal antibodies in psoriatic patients treated with long-term psoralen/ultraviolet A radiation (PUVA). J Invest Dermatol 1982; 79: 205–208.
13. De Pietro WP, Berger CL, Harber LC, Edelson RL. Normal numbers of phenotypic helper, suppressor and total T-cell populations in psoriasis vulgaris: quantitation by monoclonal antibodies. J Am Acad Dermatol 1981; 5: 304–307.
14. Fulton R, Thivolet J, Garcier F, Gaucherand M. Les sous-populations de lymphocytes T-helper et suppressor etudiées par les anticorps monoclonaux dans diverses dermatoses. Ann Dermatol Venereol 1981; 108: 243–250.
15. Willemze R, Daamsteg WJM, Meijer CJLM. Distribution of T-cell subpopulations in the peripheral blood of patients with erythrodermic psoriasis. Arch Dermatol Res 1985; 277: 19–23.
16. Kokelj F, Perticarari S, Presani G, Trevisan G. Sottopopolazione di linfociti T "helper" e T "suppressor" nella psoriasi volgare. G Ital Dermatol Venereol 1983; 118: 9–11.
17. Baker BS, Swain AF, Valdimarsson H, Fry L. T-cell subpopulations in the blood and skin patients with psoriasis. Br J Dermatol 1984; 110: 37–44.
18. Gu SQ, Ros AM, von Stedingk LV, Thyresson N, Wasserman J. T-lymphocyte subpopulations

and pokeweed mitogen induced immunoglobulin synthesis in vitro by mononuclear cells from psoriatic patients. Int Archs Allergy Appl Immunol 1981; 66: 372–381.
19. Clot J, Guilhou JJ, Andary M. Immunological aspects of psoriasis. V. T cell subsets and suppressor cell functions regulating immune response in peripheral blood. J Invest Dermatol 1982; 78: 313–315.
20. Ligresti DJ, Neff JC, Lowney ED. Increased helper-suppressor T cell ratio in psoriasis. Arch Dermatol 1982; 118: 966–970.
21. Glinski W, Obalek S, Langner A, Jablonska S, Haftek M. Defective function of T lymphocytes in psoriasis. J Invest Dermatol 1978; 70: 105–110.
22. Ortonne JP, Claudy A, Alario A, Thivolet J. Impairment of thymus derived rosette forming cells during photochemotherapy (Psoralen-UVA). Arch Dermatol REs 1978; 262: 143–151.
23. Cormane RH, Hamerlinck F, Siddiqui AH. Immunological implications of PUVA therapy in psoriasis vulgaris. Arch Dermatol Res 1979; 245–267.
24. Fraki JE, Eskola J, Hopsu-Havu VK. Effect of 8-methoxypsoralen plus UVA (PUVA) on lymphocyte transformation and T cells in psoriatic patients. Br J Dermatol 1979; 100: 543–550.
25. Morison WL, Parrish JA, Bloch KJ, Krugler JI. Transient impairment of peripheral lymphocyte function during PUVA therapy. Br J Dermatol 1979; 101: 391–397.
26. De Panfilis C, Ferrari C, Manara GC. Morphological characterization of natural killer subpopulations expressing Leu 7 and/or Leu 11 antigens. J Invest Dermatol 1985; 84: 449A.
27. Jansen CT, Viander M. Basic and interferon-augmented natural killer (NK) cell activity in psoriasis. Acta Derm Venereol (Stockh) 1983; 63: 384–387.
28. Diezel W, Waschke SR, Sönnichsen N. Detection of interferon in the sera of patients with psoriasis and its enhancement by PUVA treatment. Br J Dermatol 1983; 109: 549–552.
29. Bjerke JR, Haukenes G, Karsten Lividen J, Matre R. Activated T lymphocytes, interferon and retrovirus-like particles in psoriatic lesions. Letter to the Editor Arch Dermatol 1983; 119: 955–956.