Immunology 1997 **92** 99–103

Extracorporeal photochemotherapy restores Th1/Th2 imbalance in patients with early stage cutaneous T-cell lymphoma

M. DI RENZO,* P. RUBEGNI,† G. DE ALOE,† L. PAULESU,‡ A. L. PASQUI,* L. ANDREASSI,† A. AUTERI* & M. FIMIANI† **Department of Immunology, †Department of Dermatology, and ‡Institute of General Physiology, University of Siena, Italy*

SUMMARY

Extracorporeal photochemotherapy (ECP) has been shown to be a potent activator of peripheral blood macrophages because it causes a marked release of macrophage-dependent proinflammatory cytokines, and it is therefore currently considered to be a safe and non-toxic immunomodulatory treatment. On this basis we studied the function of peripheral blood mononuclear cells (PBMC) in eight patients with early stage (Ib) cutaneous T-cell lymphoma (CTCL), before and 1 year after ECP, together with their clinical and histological responses. In particular we evaluated *in vitro* phytohaemagglutinin (PHA)-stimulated proliferation and production of interleukin-4 (IL-4) and interferon-γ (IFN-γ) as well as lipopolysaccharide (LPS)-induced production of IL-12. Before treatment we observed that PBMC of patients produced significantly higher levels of IL-4 and lower levels of IFN-γ and IL-12 than those of healthy control subjects. After 1 year of ECP, IL-4, IFN-γ and IL-12 production no longer differed from that of control subjects. Moreover, we observed a good clinical result matched by histological response. Our data confirm that early-stage CTCL patients show a predominantly type-2 immune response that might be responsible for several immunological abnormalities found in this disease. We have demonstrated that ECP reverses the T-helper type 1/T-helper type 2 (Th1/Th2) imbalance and may therefore be considered an efficient biological response modifier.

INTRODUCTION

Extracorporeal photochemotherapy (ECP) is a relatively new treatment successfully used in cutaneous T-cell lymphoma (CTCL), and today it is considered standard therapy for Sézary syndrome.[1–4] Its mechanism of action is not entirely clear. Data obtained in animal models seem to indicate that ECP induces a specific immune response against circulating expanded clones of pathogenic T cells.[5,6] In CTCL, ECP seems to affect the antigenicity and immunogenicity of photo-irradiated neoplastic cells.[7] Reinfusion of these antigenically modified T cells induces a specific immune reaction, mediated by cytotoxic $CD8^+$ lymphocytes, that targets the malignant clones.[8] Recently, however, it has also been shown that ECP causes a marked release of macrophage-dependent pro-inflammatory cytokines[9] such as tumour necrosis factor-α (TNF-α) interleukin-6 (IL-6), as well as a significant activation of a specific subset of macrophages ($CD36^+$ cells).[10] This suggests that different mechanisms co-operate to determine the therapeutic response of ECP in CTCL.

In patients with advanced stages CTCL (III and IV) a typical set of immunological abnormalities is usually observed.[11] This includes decreased T-cell responses to antigens and mitogens, decreased natural killer cell activity, decreased lymphokine-activated killer cell activity, eosinophilia, and increased levels of IgE and IgA.[12–15] These abnormalities have been attributed to the malignant clone that expresses a T-helper type-2 (Th2) cytokine secretion pattern (IL-4–IL-5) in peripheral blood.[16–18] Increased production of IL-4 and IL-5 is known to underlie immunological alterations of these kinds. However, it is not yet certain whether these alterations are always present, and if so to what extent in the early stages. In some patients, altered cytokine production with a preferential Th2-like cytokine pattern qualitatively similar to that of advanced CTCL has been reported.[19] However, in these cases few, if any, of the circulating peripheral blood mononuclear cells (PBMC) represent the malignant clone.[20,21] This is why the relation between neoplastic cells, interleukin production and immune alterations needs to be clarified, especially in this stage of the disease.

In the present study, we evaluated the cytokine secretion

Received 26 February 1997; revised 20 May 1997; accepted 22 May 1997.

Abbreviations: CTCL, cutaneous T-cell lymphoma; ECP, extracorporeal photochemotherapy; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PUVA, psoralen-ultraviolet-A.

Correspondence: Dr P. Rubegni, Istituto di Clinica Dermatologica, Università degli Studi di Siena, Policlinico 'Le Scotte' 53 100 Siena, Italy.

pattern of PBMC in eight patients with stage Ib CTCL, before and after 1 year of ECP. These patients received no other type of treatment for CTCL.

MATERIALS AND METHODS

Patients

Eight stage Ib CTCL patients, no longer responsive to/or with contraindications for psoralen–ultraviolet-A (PUVA) therapy and/or topical chemotherapy, were treated with ECP (Table 1). They had not received any treatment for a period of 3 months before starting ECP.

Clinical and histological evaluation

The patients were examined for evidence of clinical response at each ECP session. A complete response (CR) was defined as the disappearance of all lesions for at least a month. A partial response (PR) was defined as disappearance of more than 50% of all measurable lesions for at least a month. Stabilization (St) was defined as less improvement than for a partial response but no evidence of new lesions. Progressive disease (PD) was defined as the appearance of new skin lesions. Skin biopsies were taken from the centre of large lesions (diameter >10 cm) before ECP, and in the same areas (0·5 cm from the first biopsy) 12 months later, for histological comparison. Following a scheme for CTCL diagnosis on the basis of European Organization for Research and Treatment of Cancer (EORTC) criteria,[22] we studied histology and immunophenotype in each case. For histology, 4-μm thick haematoxylin & eosin (H&E) stained sections were obtained from paraffin-embedded fragments and fixed in buffered 4% aqueous formaldehyde solution (pH 7·4). Immunohistochemistry was performed on frozen sections using antibodies to CD1a, CD2, CD3, CD4, CD5, CD7, CD8 (Dako), Leu-8 (Bekton Dickinson, Mountain View, CA) and Ki67 antigens (Immunotech, Marseille, France) by the avidin–biotin complex method. Before treatment, PBMC were checked for clonal T-cell populations by T-cell receptor β-gene rearrangement analysis in all patients following the steps reported by Bakels *et al.*[21]

ECP was performed with a UVAR^R apparatus (Therakos, Westchester, PA) as described previously[3] except that we used the liquid form of 8-methoxypsoralen (200 μg) directly in the buffy coat to avoid absorption problems leading to non-therapeutic concentrations in the blood.

Cell purification

Before and after 1 year of ECP, heparinized peripheral blood samples were obtained from CTCL patients and eight healthy donors matched for age and sex (six females and two males, mean age 58 years). Informed consent was obtained from each subject. PBMC were isolated by Ficoll–Hypaque (Sigma Chemical Co., St Louis, MO) gradient centrifugation, washed twice in RPMI-1640 medium supplemented with 20 mM HEPES, spun down at low speed to remove platelets, and suspended in RPMI-1640 medium supplemented with 2 mM HEPES, 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Life Technologies, Gaithersburg, MD) at the final concentration of 1×10^6 viable cells/ml. Cell viability was assayed by the trypan blue exclusion technique and light microscope observation.

PBMC proliferation

Aliquots (0·1 ml) of PBMC suspension were added per well in triplicate wells to 96-well flat-bottomed tissue culture plates (Costar, Cambridge, MA). PBMC were cultured without stimulation or stimulated with PHA at a final concentration of 5 μg/ml (Sigma Chemical Co.). Cell proliferation was evaluated by a colorimetric immunoassay (Boehriger Mannheim, Mannheim, Germany) based on BrdU incorporation. Briefly, after 48 hr of incubation at 37° with 5% CO_2 in air and 100% humidity, the cells were labelled with BrdU for 24 hr (10 UI/well). The cells were then fixed, anti-BrdU-POD antibody added and the immune complexes detected by the subsequent substrate reaction. The proliferative index (PI) was obtained, calculating the ratio between PHA-stimulated cells and unstimulated ones, after subtraction of the corresponding blanks.

Cytokine production

One millilitre of the cell suspension was added in duplicate to flat-bottomed 24-well culture plates (Costar) and cultured without stimulation or with PHA at a final concentration of 5 μg/ml (Sigma Chemical Co.). After 48 hr of incubation at 37° with 5% CO_2 in air and 100% humidity, the supernatants of the stimulated and unstimulated cultures were harvested and frozen at −20°C. IL-4 concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) as recommended by the manufacturer (Biosource Int., Camarillo, CA; detection limit 2 pg/ml).

Table 1. Medical history of CTCL patients

Patient	Gender	Age	Duration of disease	Previous therapies	*Stage at start of ECP
1 CB	F	65	6 months	None	Ib ($T_2N_0M_0$)
2 CG	M	48	18 years	PUVA, IFN-α, TSEB†	Ib ($T_2N_0M_0$)
3 MG	F	67	7 years	PUVA, IFN-α	Ib ($T_2N_0M_0$)
4 FG	M	48	6 years	PUVA TSEB	Ib ($T_2N_0M_0$)
5 BN	F	78	3 years	PUVA, IFN-α	Ib ($T_2N_0M_0$)
6 RR	F	65	6 months	None	Ib ($T_2N_0M_0$)
7 BF	F	63	9 months	PUVA	Ib ($T_2N_0M_0$)
8 KE	F	61	12 months	PUVA	Ib ($T_2N_0M_0$)

*According to metastasis node (TMN) classification.
†TSEB, total skin electron beam.

Interferon-γ (IFN-γ) activity was titrated with an antiviral assay using human amniotic cells (Wish) and vesicular stomatitis virus (VSV, Indiana strain) as challenge virus.[23] Titrations were performed using the international reference preparation for human IFN-α (Ga 23-902-530), human IFN-β (GO 23-902-527) and human IFN-γ (Gg 23-901-530) (NIAID, Bethesda, MD). Titres are expressed as international units (IU) per ml. Neutralization of IFN was carried out by incubating IFN samples (100 IU/ml) and the international standards (100 IU/ml) for 60 min at 37° in the presence of individual and pooled anti-IFN-α, anti-IFN-β and anti-IFN-γ antisera (NIAID). The residual viral activity was then assayed. The amounts of the antisera used in each experiment were sufficient to completely neutralize the corresponding IFN.

For IL-12 assay, PBMC were obtained as previously described and suspended in RPMI-1640 medium supplemented with 20 mM HEPES, 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Life Technologies) to a final concentration of 5×10^6 viable cells/ml. Aliquots (0·5 ml) of the cell suspension were added in duplicate to flat-bottomed 24-well culture plates (Costar) and cultured without stimulation or with LPS at a final concentration of 10 μg/ml (Sigma Chemical Co.). After 24 hr of incubation at 24° with 5% CO_2 in air and 100% humidity, the supernatants of the stimulated and non-stimulated cultures were harvested and frozen at −20°. IL-12 concentrations were quantified by ELISA as recommended by the manufacturer (R&D Systems, Minneapolis, MN; detection limit 5 pg/ml).

Statistical analysis

The Wilcoxon rank test was used to compare paired cytokine production data. A significance level greater than 95% was chosen, i.e. a probability of error $P<0·05$.

RESULTS

Clinical and histological results

A complete response was observed in one case, a partial response was obtained in six cases and stabilization of the disease was observed in one case after 12 months of therapy (Table 1). All biopsies examined before therapy showed typical epidermotropic band-like infiltration of lymphoid cells intermingled with medium-to-large cerebriform mononuclear cells (CMC) and other inflammatory cells in the upper dermis. Rare mitotic figures were identified in three cases. After 12 months of therapy, a noticeable reduction in infiltrate was observed in six cases but some atypical lymphocytes were still evident in the epidermis and papillary dermis, together with foci of $CD4^+$ lymphoid cells. In these patients, the minimum reliable histological criteria established by the EORTC[22] for the diagnosis of CTCL were therefore still fulfilled. Extensive fibrotic acellular areas were also observed in the dermis. In patient number 1 there was an almost complete absence of infiltrate. Most of the lymphoid cells were $CD4^+$ T-helper/inducer with a loss of CD7 and Leu-8 antigens. No modifications of lymphoid cell immunophenotype were observed after therapy. The TCR β-gene rearrangement analysis, performed before treatment, was negative in all patients.

Cytokine production

As regards PBMC PHA-induced proliferation, the PI of CTCL patients (mean ± SD, 50·77 ± 30·55) was not significantly different from that of controls (mean ± SD, 62·43 ± 23·52). After 1 year of ECP the PI of patients was similar to the basal one (mean ± SD, 58·1 ± 25·24) and still within the normal range.

The production of IL-4 by cultured PBMC from controls and CTCL patients in response to PHA is shown in Fig. 1. Statistical analysis revealed that IL-4 production was significantly higher in CTCL patients than in the healthy subjects ($P<0·05$), decreasing significantly after 1 year of ECP ($P<0·05$) and losing statistical significance with respect to the control group (Table 2).

IFN-γ production by PHA-stimulated PBMC of controls and CTCL patients, expressed in IU/ml, is shown in Fig. 2. IFN-γ production of CTCL patients was significantly lower than that of healthy subjects ($P<0·05$). After 1 year of ECP it was significantly higher, overlapping that of normal subjects ($P<0·05$) (Table 2).

Figure 3 shows IL-12 production by LPS-stimulated PBMC

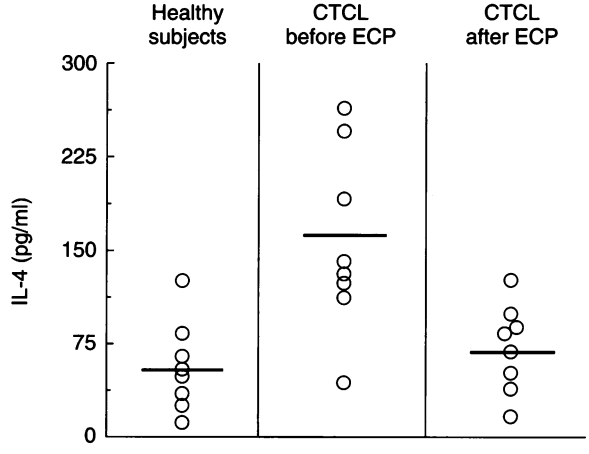

Figure 1. IL-4 production by control and patient PBMC cultures stimulated with PHA (eight CTCL patients + eight healthy controls). Horizontal bars indicate mean values.

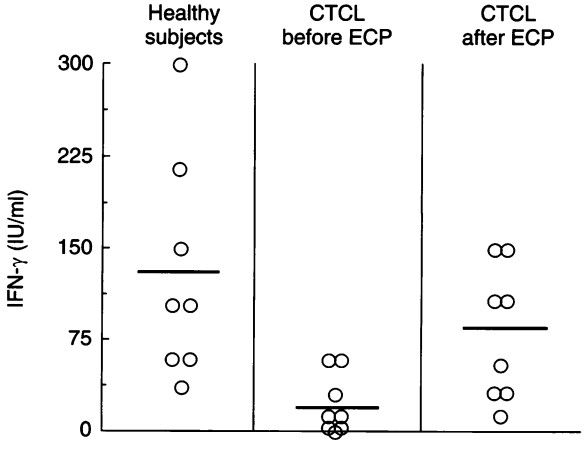

Figure 2. IFN-γ production by control and patient PBMC cultures stimulated with PHA. Horizontal bars indicate the mean values.

Table 2. PBMC cytokine production in eight stage Ib CTCL patients, before and after 1 year of ECP

Patients no.	IL-4 (pg/ml)		IFN-γ (IU/ml)		IL-12 (pg/ml)		Clinical response
	Before	After	Before	After	Before	After	
1	250·2	81·8	50	100	15·3	19·3	CR
2	143·1	20·4	3	10	17·2	19·4	PR
3	129·1	100·4	10	30	21·3	23·4	St
4	193·1	70·2	3	50	5·0	15·6	PR
5	51·8	39·1	10	150	ND	12·5	PR
6	115·0	45·9	ND	30	4·0	17·8	PR
7	262·3	123·4	30	150	12·5	19·3	PR
8	147·8	80·2	50	100	ND	12·5	PR
Mean	161·5	70·2	19·5	77·5	9·75	17·47	
SD	±70·5	±33·8	±21	±55·2	±17·44	±3·75	
*Statistical analysis	$P<0·05$		$P<0·05$		$P<0·05$		

*Wilcoxon rank test.

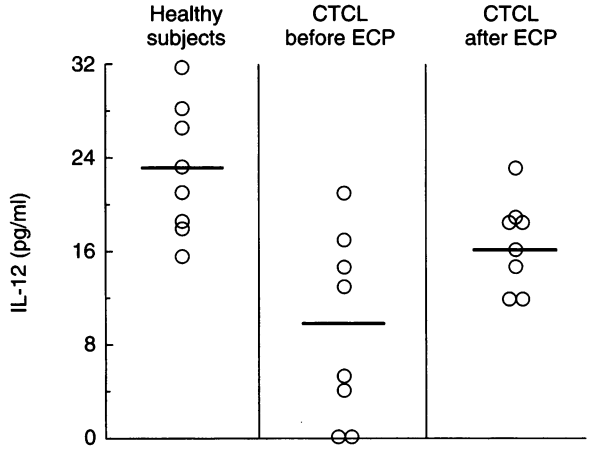

Figure 3. IL-12 production by control and patient PBMC cultures stimulated with LPS. Horizontal bars indicate the mean values.

of controls and CTCL patients. IL-12 production was not detectable in two CTCL patients. On the whole IL-12 production was significantly lower in CTCL patients than in controls ($P<0·05$). Curiously, after 1 year of ECP, IL-12 production increased significantly ($P<0·05$) and was no longer significantly different from normal subjects (Table 2).

DISCUSSION

Our data confirm that PBMC function is altered even in early stage CTCL. Before treatment, PHA-stimulated PBMC of patients produced higher levels of IL-4 than PBMC from healthy subjects. IFN-γ production was significantly lower than in the control group. Since IL-4 and IFN-γ are specific markers of type-2 and type-1 immune responses, respectively,[24] these findings suggest a predominantly type-2 immune response in early stage CTCL, as in advanced stages (III and IV). Before treatment we also observed reduced LPS-induced IL-12 production by PBMC, which has never been demonstrated in early stage CTCL, unlike in advanced CTCL.[25] After 1 year of ECP the predominant type-2 immune response was no longer detectable: PHA-induced IL-4 and IFN-γ production by PBMC was similar to that of control subjects and PBMC secreted normal amounts of IL-12 in response to LPS.

These results show that before treatment our patients had a Th2 type cytokine secretion pattern. Hence a Th1/Th2 imbalance is detectable not only in advanced but also in early stages of CTCL. In the advanced stages of CTCL this Th1/Th2 imbalance has been referred to the presence of circulating tumour cells that bear a strong similarity to the Th2 cells in mice.[16] In the early stages this hypothesis seems unlikely since tumour cells are not usually detectable in peripheral blood, even by the most sophisticated and sensitive molecular biology techniques.[20,21] Therefore, it is likely that the Th1/Th2 imbalance in the early stages of CTCL is mainly caused by an overproduction of IL-4 and IL-5 by neoplastic lymphocytes in the skin lesions. Due to the fact that IL-4 is the main inducer of Th2 development,[24] IL-4 overproduction by these neoplastic lymphocytes could promote a progressive differentiation of Th0 cells into Th2. Besides, IL-4 is able to suppress certain cellular immune functions, including natural killer and lymphokine-activated killer cell activities,[26] which are involved in the surveillance mechanisms against the dissemination of neoplastic cells. Because the Th2 cytokine IL-10 negatively regulates the expression of IL-12,[27] the reduced IL-12 production detectable in early stage of CTCL might be due to an increase of IL-10 production by the malignant T lymphocytes. Moreover, it is well known that IL-12 increases natural killer cytotoxicity and cytotoxic T-cell proliferation and function.[28,29] Therefore, the reduced IL-12 production could further contribute to the impairment of cell-mediated immunity. In agreement with this hypothesis, it has been demonstrated that the Th1/Th2 imbalance increases as the disease progresses through the stages. This suggests that the imbalance is directly implicated in the mechanisms that allow tumour progression in the host.

The normalization of the Th1/Th2 imbalance observed after 1 year of ECP is of great interest, especially in view of the clinical improvement confirmed by histological data. It is not easy to speculate on these data; in our opinion the mechanism of ECP-induced improvement may be dual. As in

Sézary syndrome, ECP may determine a reduction in tumour burden by inducing a specific immune response mediated by $CD8^+$ lymphocytes against the malignant clone.[3] The consequently fewer neoplastic cells would produce a reduced amount of Th2 cytokines, especially IL-4. On the other hand, it has recently been shown that ECP causes a marked release of macrophage-dependent proinflammatory cytokines[9] such as TNF-α and IL-6, as well as significant activation of a specific subset of macrophages ($CD36^+$ cells).[10] In the present study we have shown that after 1 year of ECP, monocytes release higher amounts of IL-12, which exerts significant inhibitory effects on the *in vitro* growth of Th2 clones.[30] Moreover, IL-12 is a powerful direct inducer of IFN-γ production[31] and it exerts potent Th1-inducing effects.[32] Normalization of the Th1/Th2 imbalance could therefore be the combined effect of two mechanisms of ECP: reduction of the number of tumour cells and hence of the production of IL-4 on one hand, and enhanced release of IL-12 on the other. Reduction of tumour burden and release of IL-12 and consequently IFN-γ may act synergistically to normalize the Th1/Th2 imbalance.

These results suggest that different mechanisms of action co-operate to determine the therapeutic response of CTCL patients to ECP.

REFERENCES

1. Holloway B.H., Floweres P.F. & Ramos-Caro F.A. (1992) Therapeutic alternatives in cutaneous T-cell lymphoma. *J Am Acad Dermatol* **27,** 367.
2. Heald P., Rook A. & Perez M. (1992) Treatment of erythrodermic cutaneous T-cell lymphoma with extracorporeal photochemotherapy. *J Am Acad Dermatol* **127,** 427.
3. Edelson R., Berger C. & Gasparro F. (1987) Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. *N Engl J Med* **316,** 297.
4. Fimiani M., Rubegni P., D'Ascenzo G. & Andreassi L. (1994) Extracorporeal photochemotherapy in the early treatment of cutaneous T-cell lymphoma. *J Am Acad Dermatol* **31,** 828.
5. Khavari P.A., Edelson R.L. & Lider O. (1988) Specific vaccination against photoinactivated cloned T cells. *Clin Res* **36,** 662.
6. Perez M., Edelson R.L. & Laroche L. (1989) Specific suppression of antiallograft immunity by immunization with syngenic photoinactivated effector lymphocytes. *J Invest Dermatol* **92,** 669.
7. Edelson R.L. (1990) Photopheresis. *J Clin Apheresis* **5,** 77.
8. Edelson R.L. (1991) Photopheresis: a clinically relevant immunobiologic modifier. *Ann NY Acad Sci* **636,** 154.
9. Vowels B.R., Cassin M., Boufal M.H. *et al.* (1992) Extracorporeal photochemotherapy induces the production of tumor necrosis factor-α by monocytes: implication for the treatment of cutaneous T-cell lymphoma and systemic sclerosis. *J Invest Dermatol* **122,** 686.
10. Fimiani M., Rubegni P., Pimpinelli N. *et al.* (1997) Extracorporeal photochemotherapy induces a significant increase in $CD36^+$ circulating monocytes in patients with mycosis fungoides. *Dermatology* **194,** 107.
11. Rook A.H., Vowels B.R., Jaworsky C. *et al.* (1993) The immunopathogenesis of cutaneous T-cell lymphoma. *Arch Dermatol* **129,** 486.
12. Laroche L. & Kaiserlian D. (1983) Decreased natural-killer activity in cutaneous T-cell lymphomas. *N Engl J Med* **308,** 101.
13. Levy S., Tempe J.-L., Caussade P. *et al.* (1984) Stage-related decrease in natural killer activity in untreated patients with mycosis fungoides. *Cancer Immunol Immunother* **18,** 138.
14. Du Vivier A., Harper R., Vonderheid E.C. & Scott E.V. (1978) Lymphocyte transformation in patients with staged mycosis fungoides and Sèzary syndrome. *Cancer* **42,** 209.
15. Heald P.W. & Edelson R.L. (1993) Lymphoma, pseudolymphoma and related conditions,. In: *Dermatology in General Medicin* (ed. T.B. Fitzpatrick), 4th edn, p. 1285. McGraw-Hill, New York.
16. Vowels B.R., Cassin M., Vonderheid E.C. & Rook A.H. (1992) Aberrant cytokine production by Sèzary syndrome patients: cytokine secretion pattern resembles murine Th2 cells. *J Invest Dermatol* **9,** 90.
17. Vowels B.R., Lessin S.R., Cassin M. *et al.* (1994) Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. *J Invest Dermatol* **103,** 669.
18. Lessin S.R., Vowels B.R. & Rook A.H. (1995) Th2 cytokine profile in cutaneous T-cell lymphoma. *J Invest Dermatol* **105,** 855.
19. Dummer R., Kohl O., Gillessien J. *et al.* (1993) Peripheral blood mononuclear cells in patients with nonleukemic cutaneous T-cell lymphoma. *Arch Dermatol* **129,** 433.
20. Wood G.S., Tung R.M., Haeffner A.C. *et al.* (1994) Detection of clonal T-cell receptor γ gene rearrangements in early mycosis fungoides/Sézary Syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). *J Invest Dermatol* **103,** 34.
21. Bakels V., Van Oostveen J.W., Gordijn R.L.J. *et al.* (1992) Frequency and prognostic significance of clonal T-cell receptor β-gene rearrangements in the peripheral blood of patients with mycosis fungoides. *Arch Dermatol* **92,** 1602.
22. EORTC/BMFT Cutaneous lymphoma Project Group (1987) *Classification and Staging of Cutaneous Lymphoma. A European Concept.*
23. Lanford M.P., Weigent D.A., Stanton G.J. & Baron S. (1981) Virus plaque reduction assay for interferon: microplaque and regular macrophage reduction assay. In: *Methods in Enzymology* (ed. S. Pestka), Vol. 78A, p. 339. Academic Press, New York.
24. Mossman T.R. & Coffman R.L. (1989) TH1 and TH2 cells: different pattern of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* **7,** 145.
25. Rook A.H., Kubin M., Cassin M. *et al.* (1995) IL-12 reverses cytokine and immune abnormalities in Sèzary syndrome. *J Immunol* **154,** 1491.
26. Kawakami Y., Custer M.C., Rosenberg S.A. & Lotze M.T. (1989) IL-4 regulates IL-2 induction of lymphokine-activated killer activity from human lymphocytes. *J Immunol* **142,** 3452.
27. D'Andrea A., Aste-Amezaga M., Valiante N.M., Ma X., Kubin M. & Trinchieri G. (1993) Interleukin-10 inhibits human lymphocyte IFN-γ production by suppressing natural killer cell stimulatory factor/interleukin 12 synthesis in accessory cells. *J Exp Med* **178,** 1041.
28. Kobayashi M., Fitz L., Hewick M. *et al.* (1989) Identification and purification of suppressing natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* **170,** 827.
29. Mehrotra P.T., Wu D., Crim J.A., Mostowski H.S. & Siegal J.P. (1993) Effects of IL-12 on the generation of cytotoxic activity in human $CD8^+$ T lymphocytes. *J Immunol* **151,** 2444.
30. Manetti R., Parronchi P., Giudizi M.G. *et al.* (1993) Natural killer cell stimulatory factor (IL 12) induces Th1-type specific immune responses and inhibits the development of IL4-producing Th cells. *J Exp Med* **177,** 1199.
31. Chan S.H., Perussia B., Gupta J.W. *et al.* (1991) Induction of INF-γ production by NK cell stimulatory factor (NKSF): characterisation of the responder cells and synergy with other inductors. *J Exp Med* **173,** 869.
32. Sypek J.P., Chung C.L., Mayor S.E.H. *et al.* (1993) Resolution of cutaneous leishmaniasis: IL12 initiates a protective T helper type 1 immune response. *J Exp Med* **177,** 1797.