

## IMMUNOHISTOPATOLOGY OF CUTANEOUS T-CELL LYMPHOMAS TREATED WITH TOPIC IOR t1 (ANTI CD6) MONOCLONAL ANTIBODY.

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### SUMMARY

In this study we have considered the possibility of topic immunotherapy of cutaneous malignant T-cell lymphoma (CTCL). Four CTCL patients were treated topically for 30 days with an anti CD6 monoclonal antibody (ior t1) and the effect was monitored clinically and in sequential biopsies by histopathology and immunohistochemistry with lineage specific monoclonal antibodies (Mabs). Clinical remission and variable histopathological regression of lesions were seen in all four patients which correlated with a marked reduction of tumor cells in the treated lesions. One remained in complete remission for 1 year (patient 1) and another for 2 years (patient 2), showing both patients a marked regression of the immunohistopathologic picture of the lesions. The other two patients had partial remission for 3 and 2 months respectively and survived 4-5 months after start of the topic treatment. Our observations suggest that the topic application of ior t1 (anti CD6) Mab should be considered as an immunotherapeutic option for inducing regression of the dermic lesions in CTCL.

### RESUMEN

En este trabajo analizamos la posibilidad de la inmunoterapia tópica como opción terapéutica en el caso de los linfomas cutáneos de células T (CTCL). Cuatro pacientes con CTCL fueron tratados tópicamente durante 30 días con un anticuerpo monoclonal (AcM) anti CD6 (ior t1) y el efecto del tratamiento fue monitoreado clínicamente y mediante biopsias consecutivas por histopatología e inmunohistoquímica con AcMs linaje específicos. Una regresión clínica y una variable regresión histopatológica de las lesiones fueron observadas en los cuatro pacientes, lo cual se correlaciona con la marcada reducción de las células tumorales observada en las lesiones tratadas. Un paciente se mantuvo en remisión completa por un año (paciente 1) y otro por dos años (paciente 2), mostrando ambos pacientes una regresión marcada del cuadro inmunohistopatológico de las lesiones. Los otros dos pacientes mostraron una regresión parcial durante 3 y 2 meses respectivamente y sobrevivieron 4-5 meses después de iniciado el tratamiento tópico. Nuestros resultados sugieren que la aplicación tópica del AcM ior t1 (anti CD6) debe considerarse como una opción terapéutica para inducir la regresión de las lesiones dérmicas en los CTCL.

### INTRODUCTION

Immunotherapy protocols using Mabs to tumor associated antigens (TAA) are currently studied as potential alternative treatment modalities against certain tumors (Chatterjee, 1985; Dimaggio *et al.*, 1990).

In several studies complete and partial remissions have been documented after systemic administration of Mabs to both epithelial tumors and lymphomas/leukemias (Bertram *et al.*, 1986; Houghton *et al.*, 1985; Miller *et al.*, 1987). The effect of systemic administration of Mab on the local growth of solid tumors has been studied in colorectal cancer by needle biopsies (Shetye *et al.*, 1988). We considered topic treatment of readily accessible cutaneous lymphomas with an anti CD6 Mab (ior t1). In previous studies (García *et al.*, 1990; Sagaró *et al.*, 1993) we reported on the clinical improvement with no side effects of CTCL after topic treatment with the Mab ior t1 to the CD6 differentiation T cell antigen (Cardenas *et al.*, 1990; García *et al.*, 1992; Rieber 1989; Faxas *et al.*, 1993). Here we report the histopathologic and immunohistochemical changes observed in the cutaneous lesions of these lymphomas during treatment with ior t1 Mab.

### MATERIALS AND METHODS

#### Patient treatment and tissues.

Four clinically and histopathologically diagnosed CTCL cases of the Dermatology Department of the "Hermanos Ameijeiras" Hospital (Havana) were evaluated in this study. These patients had not responded satisfactorily to the conventional therapeutic regimens and all patients were without onco-specific treatment 3 months prior to trial. With the patient's consent, skin biopsies were taken prior to and after 30 days topic treatment of the lesions with ior t1 Mab cream (containing 3 mg ior t1 Mab/g neutral hydrophilic cream) applied to the whole body surface three times a day during three

days followed by two times a day only on lesions until day 30. One part of the biopsy was formalin fixed, and processed for routine histopathologic evaluation on H&E stained paraffin sections. The rest of the material was snap frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$ . Five microns thick cryostat sections were cut for immunostaining and fixed in cold acetone (10 minutes).

### Immunostaining.

Mab binding to cryosections was demonstrated by the avidin-biotin-peroxidase-complex method, as previously described (Hsu, et al., 1981). Briefly, acetone fixed cryostat sections were incubated with respective Mabs, followed by biotin-conjugated sheep anti mouse Ig and biotin-avidin-peroxidase-complex, each for 30 minutes at room temperature. Between incubations the sections were washed with phosphate buffer saline (PBS). Bound peroxidase was revealed using 3-amino-9-ethyl-carbazole (AEC, Sigma Lab., St.Louis, M.O.) as substrate. Biopsy sections were analysed independently by two pathologists, before and after counterstaining with Mayer's Hematoxiline. The following panel of Mabs: ior t1 (CD6), ior t3 (CD3), ior t4 (CD4), ior t8 (CD8) and ior L3 (CD45) was used. Sections of normal tonsils were used for evaluating specificity and sensitivity of immunostaining with the respective Mabs. Lymphoma sections incubated with PBS substituting for Mab was used as negative control.

The percent of immunoreactive lymphoid cells was estimated from counts of immunostained cells in relation to total number of lymphoid cells in 10 high power fields (HPF= $\times 40$ ) across the lesions. The criteria for clinical response was: complete response (CR) if all clinical signs and symptoms disappeared for more than four weeks; partial remission (PR) if 50% of the clinical signs and symptoms disappeared without the appearance of new lesions, for more than four weeks, stabilization (S) for a 25% decrease or progression of disease signs and symptoms for more than four weeks; and progression (P) if 50% of signs and symptoms increased or appearance of new lesions.

### RESULTS

Immunostaining of the cutaneous lesions showed that three of the lymphomas had the classical  $\text{CD4}^{+}$  tumor cell phenotype and one presented the  $\text{CD8}^{+}$  phenotype (table 1).

After topic treatment for one month with ior t1 (CD6) Mab, patients 1 and 2 with early stage disease

Table 1

Effect of topic treatment immunotherapy in skin lymphomas.

PAT	Clinical Response <sup>1</sup>	Degree of DLI before/after <sup>2</sup>	Immunophenotype of cells <sup>3</sup> (%) before & after treatment				
			ior t1 CD6	ior t3 CD3	ior t4 CD4	ior t8 CD8	ior L3 CD45
1	CR	+++ / +	60/30	70/70	5/20	95/70	90/95
2	CR	+/+ -	95/5	95/90	95/60	10/30	95/95
3	PR	++ / +	80/80	95/90	90/70	10/30	95/95
4	PR	++++ / +++++	60/50	50/50	90/95	10/30	95/95

1) PR: partial remission; CR: complete remission

2) DLI: Subjective evaluation of dermal lymphoid infiltration; ++++ 80-100 %; +++ 50-80 %; ++ 30-50 %; + 10-30 %; + less than 10 %

3) Number of immunostained cells in percent of total lymphoid cells.

(IA), showed macroscopically regression of all skin lesions consistent with complete clinical remission. Histological examination on H&E and immunostained sections showed marked decrease of the lymphocyte

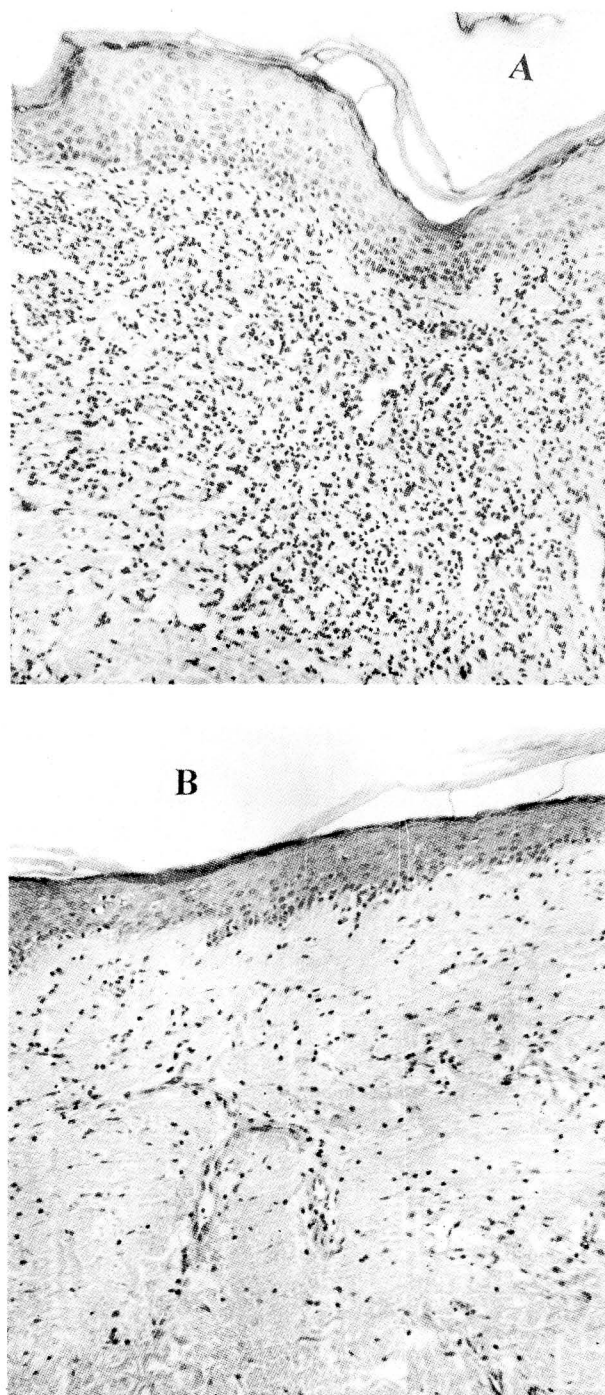
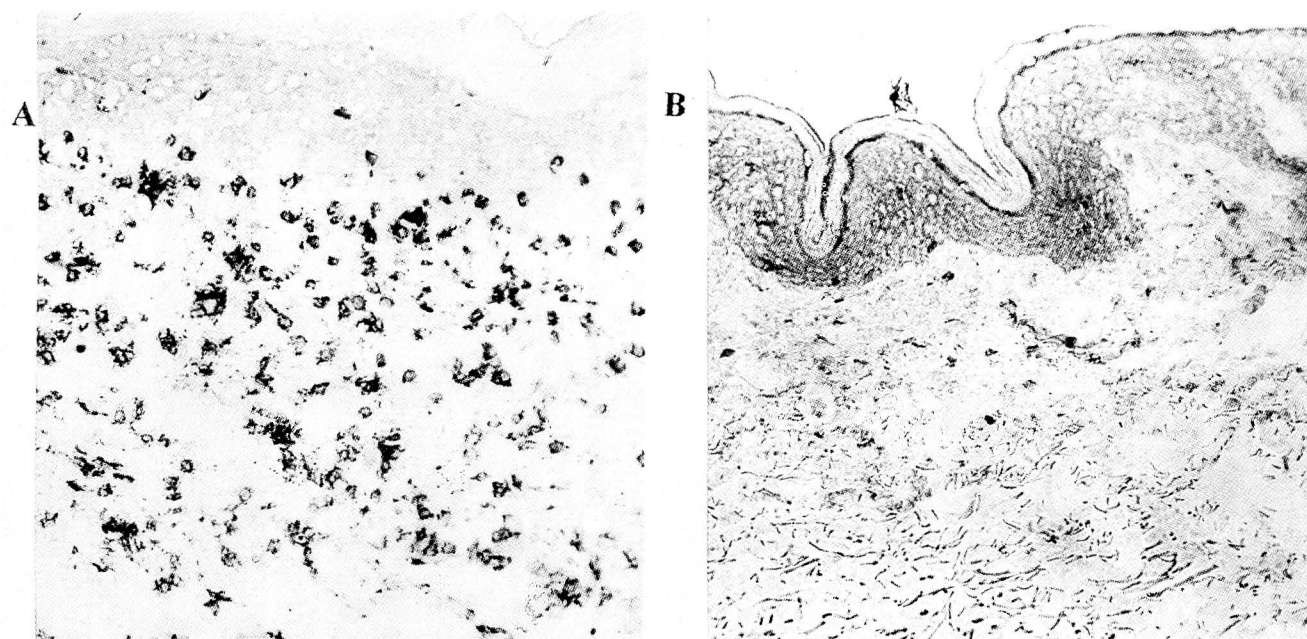


Fig. 1. Skin biopsy of a CTCL patient (No.1): a) pretreatment showing dense lymphoid infiltration band in dermis and epidermis with diffuse epidermotropism and b) after topic treatment, showing evident decrease of lymphoid infiltration with scattered cellular epidermotropism ( $\times 100$ ).



**FIG. 2.** Immunoperoxidase staining of: a) pretreatment skin biopsy (patient No.2) for CD6 antigen showing a strong staining of a high percentage (95%) of tumoral cells with ior t1 Mab (X 250) and b) post-treatment biopsy of the same patient for CD6 antigen. Very scarce ior t1 positive cells are observed (X 160)

infiltration in the dermis and epidermis (figure 1). The relative frequency of lymphocytes expressing the immunophenotype of lymphoma cells was also clearly decreased in the post treatment biopsies of cases 1 and 2 (table 1). Thus, patient 1 had a decrease of ior t8<sup>+</sup> (CD8), and a relative increase in ior t4<sup>+</sup> (CD4) cells. While patient 2 showed a decrease in ior t4<sup>+</sup> cells and an increase of ior t8<sup>+</sup> cells. Both patients also showed a relative decrease of ior t1<sup>+</sup> cells (Figure 2). Patients 3 and 4 were in advanced clinical stage (III) of the disease before treatment and achieved partial dermatological remission (50%) of the lesions. They showed however, only partial decrease in the number of infiltrating cells of the dermis and epidermis, and minor changes in the relative proportion of the lymphocyte subpopulations (table 1) during the topic treatment with ior t1 Mab. Thus, in patient 3 no decrease in the relative number of ior t1<sup>+</sup> cells was detected after treatment, but a relative decrease in the ior t4<sup>+</sup> cells and an increase in ior t8<sup>+</sup> cells was observed.

Patient 4 showed an increase in ior t8<sup>+</sup> cells, with no considerable changes in the other lymphoid surface markers evaluated.

## DISCUSSION

CTCL constitutes a category of malignant lymphoma with dermal and epidermal tropism which express terminally differentiated T lymphocyte markers

mainly of the CD4<sup>+</sup> phenotype (Edelson, 1983; García *et al.*, 1990; Norris and William, 1982). The most frequent types of these lymphomas are *Mycosis Fungoides* and the Sezary syndrome, the cells of which express the leucocyte differentiation antigens of "mature" T lymphocytes (Kung *et al.*, 1981; Norris and William, 1982). Treatment of such lymphomas with polyclonal (Barnett *et al.*, 1976; Edelson *et al.*, 1979) or monoclonal antibodies (Dillman *et al.*, 1986; Miller *et al.*, 1987; Miller and Levy, 1982) against various mature T lymphocyte markers has been attempted by endovenous infusion of patients, with variable clinical results.

In previous reports (García *et al.*, 1990; Sagaró *et al.*, 1993) we described the beneficial clinical and dermatological effect of a topically applied Mab ior t1 (CD6) to patients with CTCL. Here we report on the histopathological changes of the lesions as well as the expression of various lymphoid markers before and after topic application of ior t1 Mab to the lesions.

Two patients (1 and 2) showed clear reduction and two showed partial histopathological regression of treated lesions (3 and 4). This was corroborated by immunohistochemical evidence of a reduction of lymphocytes expressing individual lymphoma phenotype in patients 1, 2 and 3. This decrease in CD8<sup>+</sup> cells in patient 1 and in CD4<sup>+</sup> cells in patients 2 and 3 suggests a tendency to the normalization of the

CD4/CD8 relation corresponding to the phenotype of a non malignant, reactive lymphocyte subpopulation. Nevertheless, Genotypic studies (gene rearrangement) are required to confirm the non-malignant phenotype of the post treatment residual lymphocyte infiltration.

Antigenic modulation has been claimed to be an important mechanism responsible for decreased expression of tumor associated surface markers during Mab immunotherapy (Estabrook *et al.*, 1983; Faxas and García, 1990). We have shown (Faxas and García, 1990) by *in vitro* studies that the ior t1 Mab does not have a modulating effect on normal lymphoid cells. Nevertheless we can not completely exclude the possibility of a selective modulating effect on the malignant lymphoid cell population as suggested by the decrease in the ior t1<sup>+</sup> cells observed in patients 1 and 2. Moreover we can not exclude a cytotoxic effect of the Mab treatment. The persistent T cell population (CD3<sup>+</sup>) not expressing the CD6 marker could also be due to a selection of malignant or benign cells with a CD6<sup>-</sup> phenotype after treatment with ior t1 Mab.

Less marked changes were observed in the lymphoid surface markers and in the quantity of lymphoid infiltrating cells in patient 3 and 4 consistent with a more advanced stage of disease and a less favorable response to treatment. It seems that these patients have an immunological imbalance. The percent of ior t4<sup>+</sup> and ior t8<sup>+</sup> cells in the post treatment biopsy in patient 4 suggests the coexpression of both markers in part of the cell population. This phenotype, associated with a more immature T cell type usually correlated with a more aggressive tumor growth was probably the natural course of the disease in this patient in which the treatment was non effective. These results correlate with the clinical response and the histopathological picture of this patient.

Clinical results (Sagaró *et al.*, 1993) demonstrated an objective response in all the patients topically treated with ior t1 (anti CD6) Mab. Similar results are documented in a recently concluded phase I clinical trial of the endovenous administration of ior t1 Mab in 10 CTCL patients.

In consequence immunotherapy with ior t1 Mab could be considered as a potential alternative treatment modality for inducing regression of the dermic lesions in CTCL patients.

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