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THE IN VITRO EFFECT OF PIDOTIMOD ON SOME IMMUNE FUNCTIONS IN CANCER PATIENTS.

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ABSTRACT.

There are several reports concerning an impairment of cellular immune response in patients affected by malignant disease. The aim of this study was to evaluate the in vitro effect of Pidotimod, a synthetic biological response modifier, on some immune functions in 14 cancer patients. In particular, we showed that these subjects had a significantly reduced peripheral blood mononuclear cell (PBMC) proliferation both in response to PHA and to Con A in comparison with a group of healthy subjects. Besides, they showed a significantly reduced PBMC IL2 production, which was evaluated both through an ELISA method and a biological assay. The in vitro addition of increasing concentrations of Pidotimod (10, 25 and 50 ug/ml) was able to enhance PBMC proliferation and IL2 production significantly. However, in spite of the addition of Pidotimod, both immune functions in our neoplastic patients did not reach normal values.

INTRODUCTION.

Interest in an immunologic basis for the etiology, progression and treatment of malignant disease has prompted considerable investigation into the status of the humoral and cellular immune system in cancer patients. In fact, there are several reports concerning an impairment of cellular immune response in patients affected by malignant disease and this impairment may correlate with the stage and clinical cause of the disease (1-3). In details, patients affected by cancer often have depressed reactivity to in vivo skin testing and they often show a reduced mixed cell lymphocyte reaction, a reduced mitogen-induced lymphoproliferation, a reduced cytokine production, a reduced NK and LAK-activity as well as a reduced T-cell mediated cytotoxicity (1-9).

Pidotimod is a synthetic biological response modifier which has been shown to enhance several immune parameters both in animals and in humans, both in vitro and in vivo, affecting polymorphonuclear and lymphocyte functions (10-15).

In our study, we considered the in vitro effect of Pidotimod on some cellular immune responses in neoplastic patients, in order to see

a) whether these patients showed an impairment of the immune system and in particular of lymphomonocyte proliferation and IL2 production and

b) whether Pidotimod was able to correct such impairment.

MATERIALS AND METHODS.

Patient Population.

14 cancer patients entered our study (Tab.1). All of them were affected by solid neoplasms; none of them had undergone chemotherapy or radiotherapy or surgical treatment. 14 healthy subjects were used as control group.

TABLE 1.
Patient Population.

BI	female	breast cancer
GT	male	colorectal cancer
SF	female	gastric cancer
SF	female	colorectal cancer
LM	male	colorectal cancer
PT	male	lung cancer
SS	male	prostate cancer
GL	male	lung cancer
AD	female	lung cancer
CS	male	lung cancer
RG	male	lung cancer
DR	male	lung cancer
AC	male	lung cancer
DR	male	kidney cancer

Cell Purification.

Heparinized peripheral blood samples were obtained from patients and healthy donors. Informed consent was obtained from each subject. PBMC were isolated by Ficoll-Hypaque (Sigma Chemical CO., St.Louis, MO) gradient centrifugation, spun down at low speed to remove platelets 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 ug/ml streptomicin (all purchased by Life Technologies, Gaithersburg, MD), at the final concentration of $1x10^6$ viable cells/ml. Cell viability was assayed by trypan blue exclusion technique and light microscopy observation.

Cell Proliferation.

1 ml of the cell suspension was added in duplicate to flat-bottom 24-well culture plates (Costar, Cambridge, MA) and

stimulated either with PHA (5 ug/ml) or with ConA (25 ug/ml) (Sigma Chemical CO., St.Louis, MO). Pidotimod (POLI, Milano, Italy), which had been reconstituted in RPMI 1640 and filtered, was added together with mitogens at the following concentrations: 10, 25 and 50 ug/ml. After 48 hr of incubation at 37°C with 5% CO_2 in air and 100% humidity, the cell proliferative response was evaluated through a chemiluminescent method (Bio-Orbit Oy, Turku, Finland). This chemiluminescence method substitutes tritiated thymidine uptake and is based upon the bioluminescent measurement of ATP which is present in all metabolically active cells and it is measured by using the luciferin-luciferase reaction. Briefly, 1 ml of lysing reagent is added to each well and after 5 min, 180 ul of the extract together with 20 ul of ATP monitoring reagent are loaded into the luminometer (Biocounter model M2010 Lumak B.K.) and the ATP concentration was measured by extrapolation from a curve obtained with an ATP standard. We obtained the proliferative index (P.I.) by calculating the ratio between PHA- and ConA-stimulated cells' ATP and non stimulated cells' ATP.

Quantitation of IL2 Production.

On the supernatants of the same cultures we evaluated both IL2 concentration through an ELISA method (Biosource Int., Camarillo, CA) and IL2 activity on IL2 dependent murine T cell line (CTLL), that is a T cell line which needs IL2 in order to proliferate. In details, we considered the ability of the supernatants to support the proliferation of CTLL, whose proliferation was measured by evaluating their ATP intracellular concentration through the above mentioned chemiluminescence method. In this case, we plotted a curve of IL-2 concentration versus chemiluminescence by using a

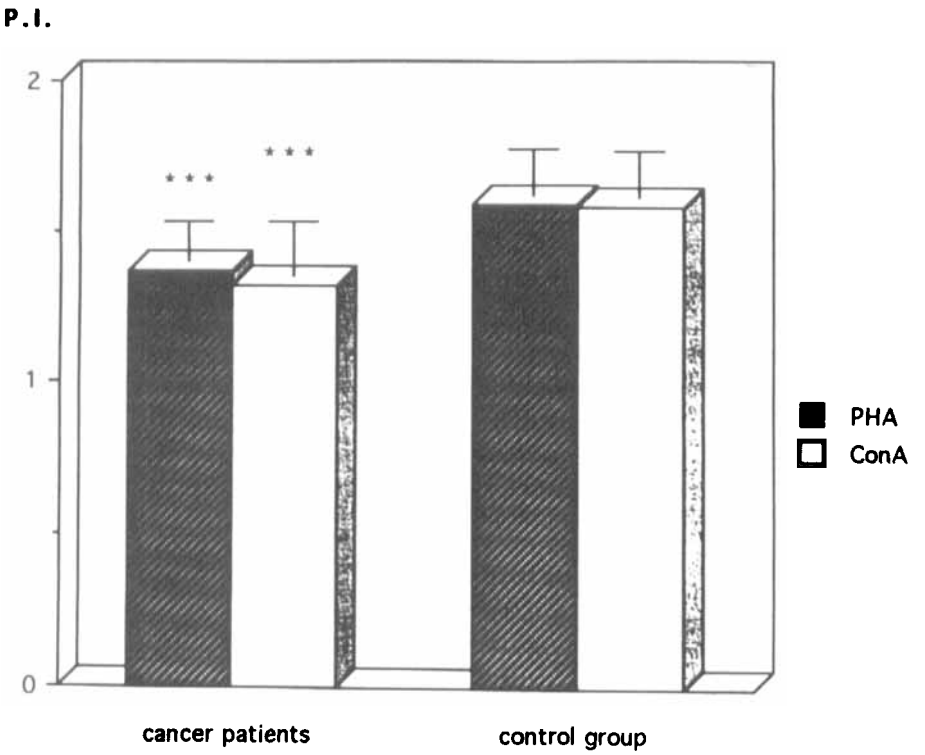

FIG.1: PBMC proliferative response expressed as P.I. (proliferative index) in cancer patients and in the control group (*** $p<0.001$).

standard preparation of IL2 and we considered chemiluminescence equivalent to cell proliferation. We used this curve to determine our samples' concentration by interpolation.

Statistical Analysis.

Statistical analysis was performed using the Student's "t" test for paired data and $p<0.05$ was considered significant.

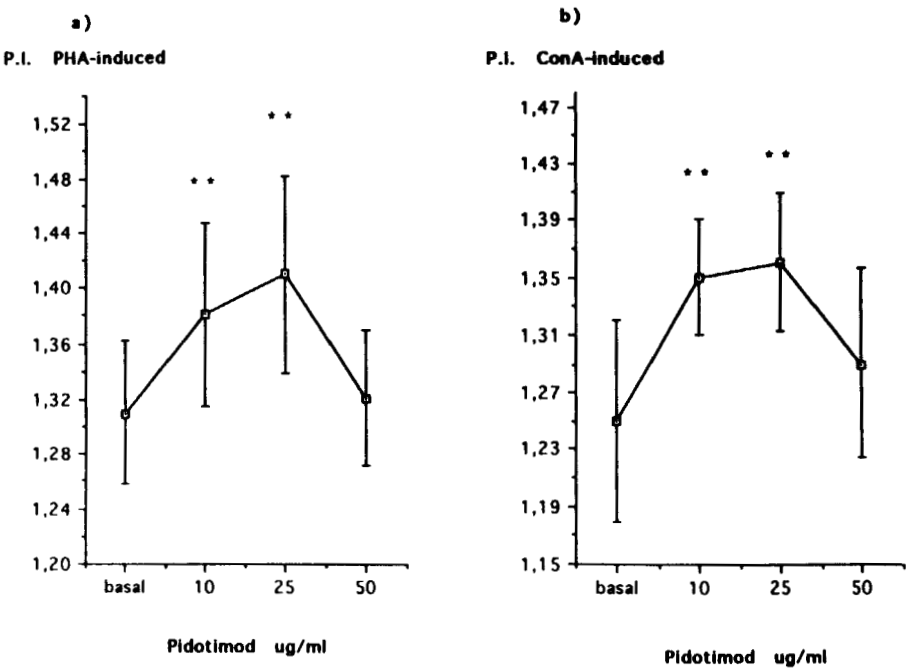

FIG.2: PBMC P.I. a) in response to PHA and b) in response to ConA in cancer patients after in vitro addition of Pidotimod (** $p<0.01$).

RESULTS.

In our patients, as shown in Fig.1, PBMC proliferation was significantly lower than the one obtained in the group of healthy subjects. This reduction was detectable both in response to PHA and to ConA. The in vitro addition of Pidotimod to PBMC obtained from the neoplastic patients induced a significant increase of the proliferative index at 10 and 25 ug/ml in comparison with basal values, whereas no effect was shown at the highest concentration, that is at 50 ug/ml (Fig.2). The in vitro addition of the drug to PBMC obtained from the healthy subjects did not have any effect (data not shown).

As regards PBMC IL2 production, evaluated through the ELISA method and through its biological activity, in the group

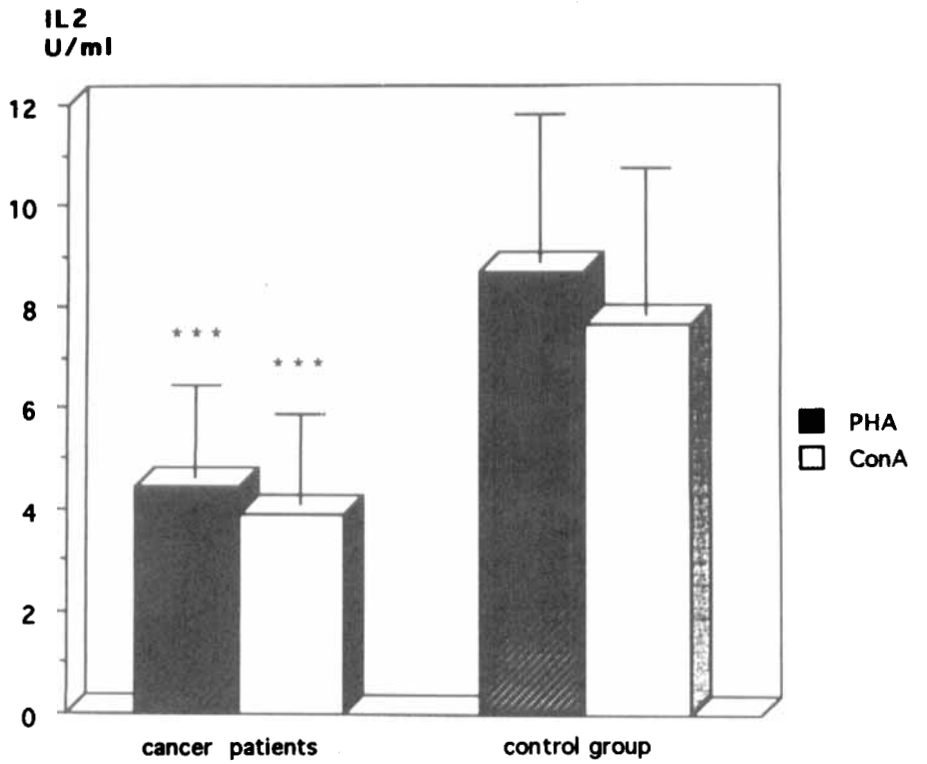

FIG.3: PBMC IL2 production evaluated through its biological activity in cancer patients and in the control group (*** p<0.001).

of neoplastic patients IL2 production was significantly lower than in the control group, both in response to PHA and to ConA (Fig.3-4). The in vitro addition of Pidotimod to PBMC obtained from the neoplastic patients induced a significant increase of IL2 production at all the tested concentration (10, 25 and 50 ug/ml) in comparison with basal values (Fig.5-6), whereas no effect was detectable in the healthy subjects (data not shown).

DISCUSSION.

Our study confirms previous findings which showed an impairment of cellular immune response in patients with

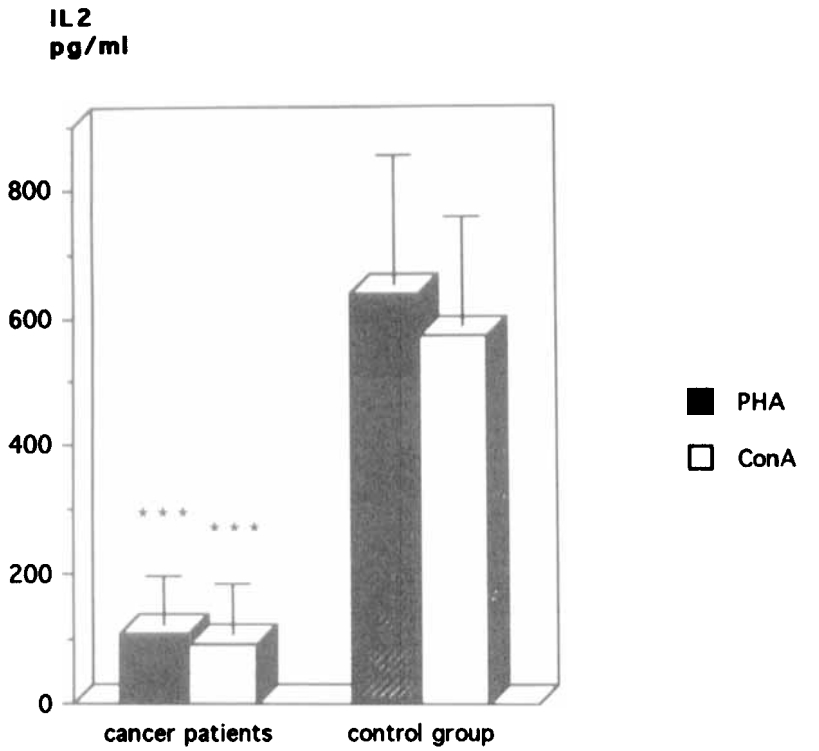

FIG.4: PBMC IL2 production evaluated through ELISA method in cancer patients and in the control group (*** p<0.001).

malignant disease. Such impairment involves several cell types and cell functions and it has been demonstrated in many different types of malignant disease including breast, renal, pancreas, gastrointestinal, urological and lung cancer (1-3, 16-21); besides it seems to be correlated with the stage of the disease and it is even stronger in patients with a second malignancy (1-3, 22). However, it is not clear whether this defect may contribute to the development of the cancer or whether it is a cancer result, as well as the exact mechanism of this immune impairment has not been established yet, even if recent data suggest abnormalities in signal transduction events, in particular at the level of protein tyrosine kinases and CD3

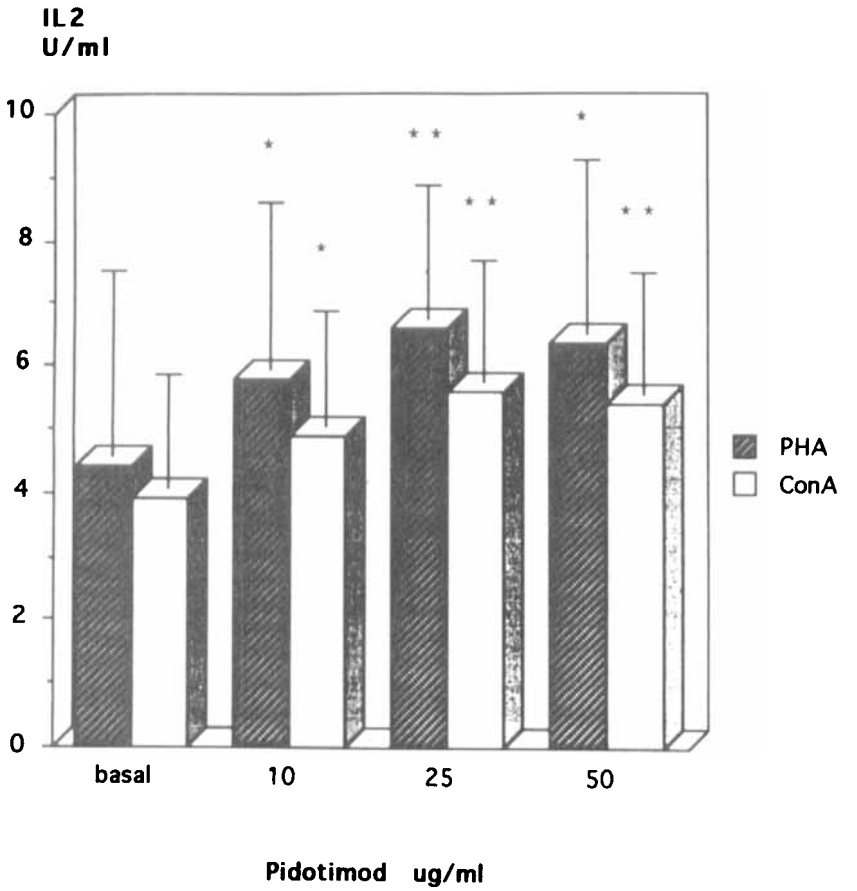

FIG.5: PBMC IL2 production evaluated through its biological activity in cancer patients after in vitro addition of Pidotimod (* $p<0.05$; ** $p<0.01$).

receptor (23, 24, 25). In our patients, the lymphomonocyte proliferative response was significantly reduced in comparison with healthy subjects in response to PHA and ConA, that is in response to two different stimuli which seem to act with a different mechanism: whereas PHA stimulates T-cell proliferation being perceived by the T cells on an appropriate presenting cell in the context of MCH antigens, ConA seems to be able both to mimick the nominal antigen presentation like PHA and to act on T cell surface directly, perhaps by binding to the T cell receptor complex (26).

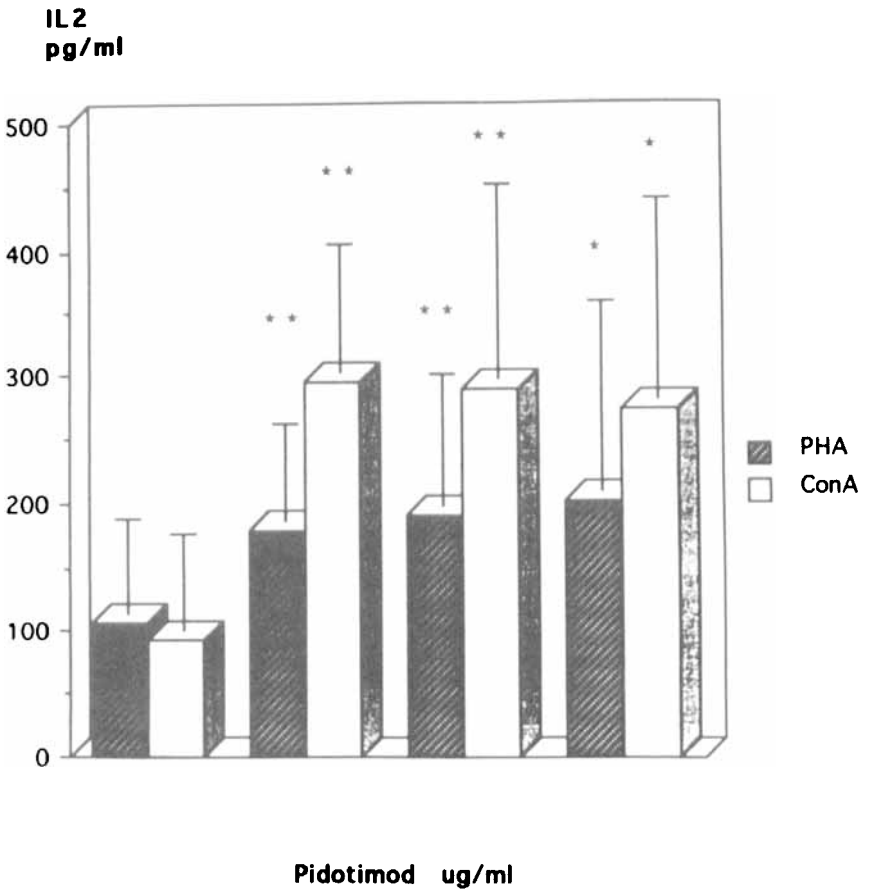

FIG.6: PBMC IL2 production evaluated through ELISA method in cancer patients after in vitro addition of Pidotimod (** p<0.01; *** p<0.001).

The in vitro addition of Pidotimod was able to increase this proliferation significantly, even if it did not reach normal values. It is not easy to explain the exact mechanism of action of Pidotimod, even if the most probable hypothesis is that, Pidotimod being a synthetic dipeptide, it might be presented by antigen presenting cells in the context of MHC antigens to the T cells and this might increase the stimulating activity of policlonal mitogens.

Besides, our cancer patients showed an impairment of lymphomonocyte IL2 production in response to the same mitogens, in agreement with other authors' data (1, 21, 27). This reduced IL2 production might be the cause of the reduced proliferation, because it is well-known that IL2 plays a key role in cellular interactions of immunocompetent cells: IL2 promotes the proliferation and enhances the secretory capacity of all of the major types of lymphocytes, including T cells, B cells and NK cells (28, 29). Therefore, the reduced IL2 production detectable in our cancer patients might be important because it might contribute to the cancer spreading, especially affecting LAK and NK cells which are involved in the immune response against tumors (30, 31). The in vitro addition of Pidotimod increased IL2 production, probably through the same mechanism as in the case of the proliferative response, that is by being presented to T cells in the context of MHC antigens by antigen presenting cells. The fact that Pidotimod increased IL2 production at all the tested concentrations, whereas it did not increase lymphomonocyte proliferation at the highest one, is probably due to the higher sensibility of IL2 production in evaluating immune functions in comparison with lymphomonocyte proliferation: for example, in HIV-infected individuals, a reduced IL2 production is one of the first laboratory signs of immunodeficiency and it arises before a reduced lymphoproliferation (32). However, in our patients, even if the addition of Pidotimod increased both lymphomonocyte proliferative response and IL2 production significantly, both parameters did not reach the normal values obtained in our laboratory. This might be explained with the inability of T cells from cancer-bearing animals and patients to be activated in response to the appropriate stimulation, because of a transduction defect (33, 23, 24, 25). However, our in vitro data suggest that Pidotimod might be used as an adjunctive immunotherapy in cancer patients.

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