Abstract. Background: At present, it is known that cancer-related immunosuppression would mainly depend on an immunosuppressive action mediated by a subtype of CD4⁺ lymphocytes, the so-called regulatory T lymphocytes (T-reg), which are identified as CD4⁺CD25⁺ cells. Moreover, it has been shown that anticancer immunity is under psychoneuroendocrine regulation, mainly mediated by the pineal hormone melatonin (MLT). This study was performed to investigate the in vivo and in vitro effects of MLT on T-reg generation. Materials and Methods: We evaluated the in vivo effects of MLT (20 mg/daily orally in the evening) in 20 patients with untreatable metastatic solid tumor and the in vitro effects of MLT incubation (at 10 and 100 pg/ml) of pure lymphocyte cultures on T-reg cell count. Results: MLT induced a statistically significant decline in mean T-reg cell numbers in patients who achieved disease control, whereas no effect was seen in those who had progressed. In contrast, no in vitro effect of MLT incubation was apparent. Conclusion: This preliminary study would suggest that MLT may exert in vivo an inhibitory action on T-reg cell generation in cancer patients which is associated with a control of the neoplastic progression, whereas no direct effect was seen in vitro on lymphocyte differentiation. This finding would suggest that MLT may counteract T-reg cell generation in vivo by inhibiting macrophage activity which is involved in stimulating T-reg cell production.

Psychoneuroendocrine Modulation of Regulatory T Lymphocyte System: In Vivo and In Vitro Effects of the Pineal Immunomodulating Hormone Melatonin

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It is known that cancer progression is due at least in part to a lack of an effective anticancer immune reaction (1-3). However, recent discoveries in the immunobiology of tumors have suggested that cancer-related failure of the anticancer immune response would namely depend on the activation of an abnormally enhanced immunosuppressive response, which is mainly mediated by a subtype of CD4⁺ lymphocytes, the so-called regulatory T lymphocytes (T-reg), which are identified as CD4⁺CD25⁺ cells (4, 5). Several immune cell types have been proven to be potentially characterized by an immunosuppressive activity on the anticancer immunity, including T-reg cells themselves, macrophages, namely the M2 subtype (6) and myeloid-derived suppressor cells (7), consisting of myeloid precursors released from the bone marrow. However, according to recent discoveries, the end-result of the immunobiological interactions among the various immunosuppressive cells would consist of an abnormally enhanced generation of T-reg lymphocytes, which represent the most biologically active immune cells in suppressing the anticancer immune reaction through the release of immunosuppressive cytokines, namely interleukin (IL)-10 and transforming growth factor (TGF)-beta (4-7). Therefore, the inhibition of T-reg cell generation could constitute the fundamental key mechanism of the various immunotherapies of cancer in an attempt to reinduce an effective anticancer immune reaction capable of counteracting cancer progression. Several endogenous hormonal or immunological molecules capable of activating the anticancer immunity have been identified in recent years. In more detail, within these immunological substances, IL-2 (8) and IL-12 (9) would represent the most active anticancer cytokines in humans.

Within the endocrine system, the main endogenous anticancer hormones are the pineal indole hormones, the most investigated of which is melatonin (MLT), whose anticancer activity has already been well documented (10).
MLT may inhibit cancer cell proliferation through both antiproliferative and immunomodulating effects (11). In particular, within the immune effects of the pineal hormone, the most important anticancer immune activities of MLT would consist of the stimulation of IL-2 secretion through a direct effect on T-helper lymphocytes, by acting on specific MLT receptors (12) and the inhibition of macrophage-mediated immunosuppressive effects (13), whereas at present there are no data about the possible influence of MLT on T-reg lymphocytes. There are only some preliminary data, showing that MLT does not stimulate IL-10 secretion in response to IL-2 (11). The present preliminary study was performed in an attempt to evaluate the effect both in vitro and in vivo of MLT on the T-reg cell system.

Materials and Methods

In a preliminary study, we evaluated the in vivo effects of MLT as a palliative therapy of cancer in 20 patients with metastatic solid tumor (male/female: 12/8; median age 61, range 48-71 years), who failed to respond to conventional anticancer therapy and were not schedulable for other standard anticancer treatment. Tumor histotypes were as follows: non-small cell lung cancer: 8; colorectal cancer: 6; breast cancer: 4; prostate cancer: 1; pancreatic cancer: 1. According to previous clinical studies (14), MLT was given orally at a pharmacological dose of 20 mg/day every day in the evening, in accordance with its physiological light/dark circadian rhythm (10), without interruption. The treatment was continued for at least 3 consecutive months. The clinical response was evaluated according to WHO criteria. To evaluate T-reg cell number, venous blood samples were collected in the morning before and after 3 months of MLT therapy. No patient was under treatment with corticosteroids or with other immunosuppressive agents during the study. The clinical experimental protocol was explained to each patient and informed consent was obtained.

In a second study, we investigated the in vitro effects of MLT incubation on T reg cell number and percentage. Peripheral blood mononuclear cells were isolated from venous blood samples collected from healthy volunteers by centrifugation on Ficoll-Ipaque (Pharmacia, Uppsala, Sweden). After separation of monocytes by two-step Percoll density gradient centrifugation, cultures of pure lymphocytes were prepared in RPMI-1640 supplemented with 10% fetal calf serum. Cells were cultured in 24-well plates and each 1 million of cells was maintained in a final volume of 1 mL of medium. Cells were incubated in a humidified atmosphere of 5% carbon-dioxide in air at 37°C for 6 days with MLT at 10 pg/ml and at 100 pg/ml, corresponding to its physiological blood concentrations during the light and the dark period of the day, respectively (10). MLT was added daily until the end of the incubation or with medium alone, as control. In both studies, T-reg cell number was measured by a flow cytometric assay and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy), by identifying T-reg lymphocytes as CD4+CD25+ cells. Data are reported as mean±SE and statistically analyzed by chi-square test, Student’s t-test and analysis of variance, as appropriate. Normal values of T-reg lymphocytes determined in our laboratory (95% confidence limits) were below 240/mm², comprising less than 11% of the total lymphocytes.

Results

As far as the results of the first study are concerned, the clinical response to MLT consisted of a partial response (PR) in 1/20 patients and stable disease (SD) in 11/20 patients, whereas the remaining 8/20 patients had progressive disease (PD). Therefore, disease control (DC) (PR+SD) was achieved in 12/20 patients. Before MLT therapy, an abnormally high T-reg cell count was observed in 9/20 patients. MLT therapy induced a normalization of T-reg cell numbers in 4/9 patients who showed abnormally high values prior to therapy. Moreover, the percentage of MLT-induced normalization of T-reg cell count was significantly higher in patients with DC than in those who had a PD (4/6 vs. 0/38,
Discussion

The results of these preliminary studies, carried out to better clarify the neuroendocrine physiopathological regulation of the T-reg cell system, seem to show that the pineal hormone MLT, whose immunostimulatory effects on anticancer immunity have been well demonstrated (10-13), may actually counteract T-reg cell generation in vivo and this effect would simply be an epiphenomenon only, since it was associated with a control of the neoplastic disease. On the contrary, MLT does not seem to exert a direct action on T-reg cells themselves since no relevant effect was observed in vitro for cells under MLT incubation with respect to cells incubated in control medium alone, neither at 10 pg/ml, nor at 100 pg/ml. In the same way, the percentage of T-reg cells with respect to the total lymphocytes was also not significantly influenced by MLT incubation.

References


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