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Relationship between Psychoncology and Psychoneuroendocrinoimmunology (PNEI): Enhanced T-Regulatory Lymphocyte Activity in Cancer Patients with Self-punishement, Evaluated by Rorschach Test

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Abstract. *Background: Psychological studies have documented the presence of a self-punishment profile in cancer patients. Recent immuno-oncological studies have shown that within the group of $CD4^+$ cells, which play a fundamental role in the generation of anticancer immunity, there is a subtype of cells that in contrast mediates the suppression of the anticancer immunity, the so-called T-regulatory cells (T-reg), which may be identified as $CD4^+CD25^+$ cells. Patients and Methods: On this basis, we performed a psychoncological study to evaluate $CD4^+CD25^+$ cell numbers in relation to the response to Rorschach's test in a group of 30 cancer patients suffering from the most frequent tumor histotypes. Results: Normal values obtained in our laboratory (95% confidence limits) of T-reg lymphocytes and $CD4^+/CD4^+CD25^+$ were $<240/mm^3$ and $>4mm^3$, respectively. The psychological profile of self-punishment was found in 18/30 patients (60%). The percentage of patients with abnormally high $CD4^+CD25^+$ values observed in the group with self-punishment was significantly higher than that found in patients without self punishment (11/18 vs. 3/12 (25%), $p<0.05$). In the same way, the percentage of patients with abnormally low $CD4^+/CD4^+CD25^+$ ratios was significantly higher in the group with self-punishment (16/18 vs. 4/12, $p<0.01$). The mean numbers of T-reg lymphocytes observed in the group with self-punishment was significantly higher than that found in patients who had no self-punishment (314 ± 39 vs. 173 ± 27, $p<0.05$). In addition, the mean $CD4^+/ CD4^+CD25^+$ ratio was significantly lower in patients with self-punishment than in the other group (2.6 ± 0.2 vs. 5.2 ± 0.8, $p<0.025$). On the contrary, no significant difference was seen in the mean number of $CD4^+$ lymphocytes. Conclusion: The study suggests that self-punishment may inhibit the generation of an effective anticancer immune response by stimulating the activation and proliferation of T-reg lymphocytes, which in turn stimulate tumor dissemination by suppressing anticancer immunity. The abnormally high number of T-reg lymphocytes in patients with self-punishment would suggest a specific immune alteration, as suggested by the evidence of a normal profile for other immune parameters, such as total $CD4^+$ lymphocytes.*

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The existence of a psychological influence on cancer growth was been some time ago (1, 2). However, only recently with the development of the field of psychoneuroendocrino-immunology (PNEI) has it been possible to establish that the psychological influence on cancer growth is mediated by the immune system and to better define the immune and psychoneuroendocrine mechanisms responsible for such an influence on the patient's prognosis (3). Even though it is known that a patient's psychological status may influence the prognosis of the neoplastic disease, the investigation of the psychic profile is not generally included in the clinical management of cancer patients, or it is limited to the evaluation of patient compliance and quality of life. According to the data available, the psychological profile may be expected to affect the clinical history of cancer, mainly by influencing the immune status of patients. In particular, it has been demonstrated that the secretion and the biological activity of interleukin-2 (IL-2), which is the main

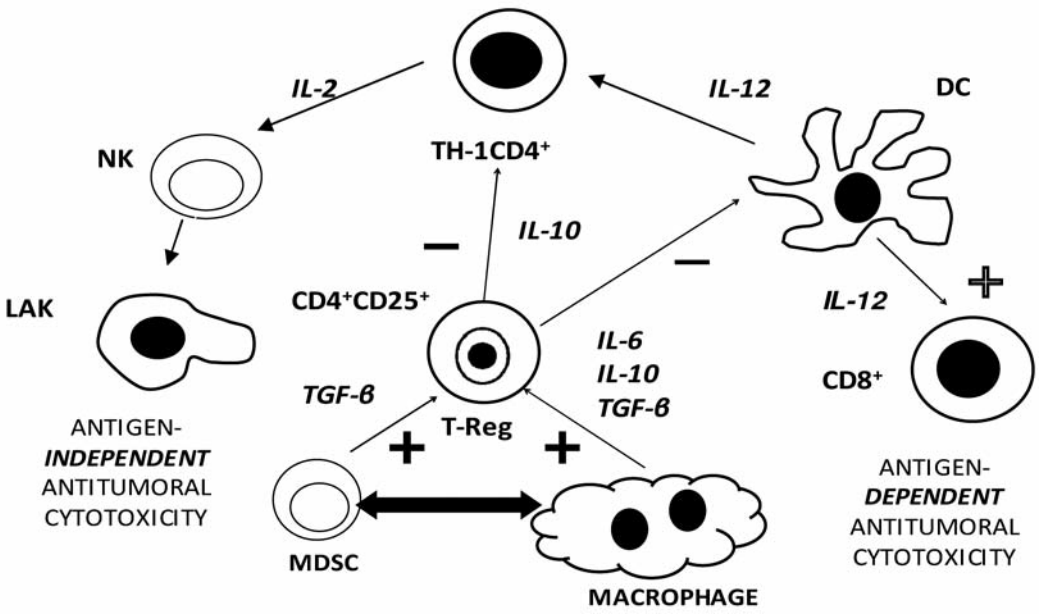

Figure 1. *Anticancer immunity and the fundamental role of T-reg lymphocytes. NK, Natural killer cells; LAK, lymphokine-activated killer cells; MDSC, myeloyd-derived suppressor cells; DC, dendritic cells; IL, interleukin; TGF, transforming growth factor; T-reg, T-regulatory lymphocyte.*

antitumour cytokine in humans (4), is inhibited by stress-related substances, such as catecholamines and opioid peptides, whereas these processes are stimulated by pleasure-related substances, namely pineal indoles, gamma-aminobutyric acid and endocannabinoids (5, 6). In fact, it is know that the antitumor efficacy of IL-2 does not depend on its direct action on cancer cell proliferation, but it is due to a biological antitumour response induced by IL-2 itself (4). Moreover, according to recent knowledge acquired from psychoneuroimmunology, it is also known that the immune responses, including anticancer immunity, are physiologically under psychoneuroendocrine control, which represents the chemical mediation of the emotions and of the states of consciousness. Finally, preliminary clinical studies showed that anxiety negatively influences the clinical efficacy of IL-2 cancer immunotherapy (7). As far as the psychological profile of cancer patients is concerned, our previous clinical studies showed that suppression of sexual interest and sexual identity, as evaluated using Rorschach's test, would constitute one of the most evident cancer-related psychological characteristics (8). In more detail, several psychoncological studies have demonstrated that cancer is associated with the progressive decline in the perception of pleasure and with a self-punishment profile (1, 2, 9). In addition, our previous studies had shown that the repression of pleasure is associated with a more evident immune suppressive status in cancer patients, as shown by the evidence of lymphocytopenia and a decrease in the number of T-helper lymphocytes ($CD4^+$) (10). Finally, recent immuno-oncological studies have shown that within the group of $CD4^+$ cells, which play a fundamental role in the generation of anticancer immunity, there is a subtype of $CD4^+$ cells that in contrast mediates the suppression of the anticancer immunity, the so-called T-regulatory (T-reg) cells, which may be identified as $CD4^+$ $CD25^+$ cells (11, 12). The regulation of the T-reg cell system is illustrated in Figure 1. On this basis, a study was planned to evaluate the relation between T-reg cell function and the evidence of self-punishment, as assessed by Rorschach's test.

Patients and Methods

The study included 30 consecutive cancer patients affected by the more frequent tumor histotypes, 21 of whom had metastatic disease. The clinical characteristics of patients are reported in Table I. Rorschach's test was administered using 10 cards, each containing a complicated inkblot pattern, five in black and grey, two in black and red, and three in various pastel colours. Participants looked at the cards one at a time and described what each inkblot resembled. They were instructed to look at the shape, shading and colour of the inkblots. After viewing all 10 cards, the examiner usually goes back over the responses for additional information. The participant may be asked to clarify some responses or to describe which features of each inkblot prompted the response. There is no one correct response to any inkblot card, although there certain common responses to some cards. Despite criticism, Rorschach's test is considered to be able to contribute to the psychological diagnosis and to the study of the subconscious profile of the personality (13).

As far as the immunological evaluation is concerned, venous blood samples were collected in the morning before the onset of the

Table I. *Clinical characteristics of patients.*

Overall patients		Self-punishment	No self-punishment
N	30	18	12
Male/female	15/15	8/10	7/5
Median age	58 (21-72)	61 (21-72)	59 (44-71)
Disease extension			
Metastatic	21	13/18	8/12
Non-metastatic	9	5	4
Tumor histotype			
Lung cancer	9	4	5
Breast cancer	11	9	2
Ovarian cancer	1	1	0
Renal cell cancer	3	2	1
Prostate cancer	2	0	2
Colon cancer	2	1	1
Pancreatic cancer	1	0	1
Soft tissue sarcoma	1	1	0

different oncological treatments. For each blood sample, the absolute number of total lymphocytes, T-helper ($CD4^+$), T-cytotoxic ($CD8^+$) cells, natural killer (NK) cells ($CD16^+CD56^+$), T lymphocytes ($CD3^+$) and T-reg cells ($CD4^+CD25^+$) were assessed. Lymphocyte subsets were measured by a flow cytometric assay and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy). The results were statistically evaluated using the Chi- square test and Student's *t*-test, as appropriate.

Results

Normal values obtained in our laboratory (95% confidence limits) of T-reg lymphocytes and $CD4^+/CD4^+CD25^+$ were $<240/mm^3$ and $>4mm^3$, respectively. Psychological profile of self-punishment occurred in 18/30 patients (60%). The percentage of patients with abnormally high $CD4^+CD25^+$ values observed in the group with self-punishment were significantly higher than that found in patients without self-punishment (11/18 *vs*. 3/12, $p<0.05$). In the same way, the percentage of patients with abnormally low values of $CD4^+/CD4^+CD25^+$ was significantly higher in the group with self-punishment (16/18 *vs*. 4/12, $p<0.01$). Moreover, as illustrated in Figure 2, the mean numbers of T-reg lymphocytes observed in the group with self-punishment was significantly higher than that found in patients who had no self-punishment ($314\pm39/mm^3$ *vs*. $173\pm27/mm^3$, $p<0.05$), whereas no significant difference was seen in the mean number of $CD4^+$ cells. In addition, as shown in Figure 3, the mean $CD4^+/CD4^+CD25^+$ ratio was significantly lower in patients with self-punishment than in the other group (2.6 ± 0.2 *vs*. 5.2 ± 0.8, $p<0.025$). Finally, as reported in Table II, no significant difference in the mean number of total lymphocytes, $CD3^+$, $CD8^+$ and NK cells was seen between patients with or without self-punishment.

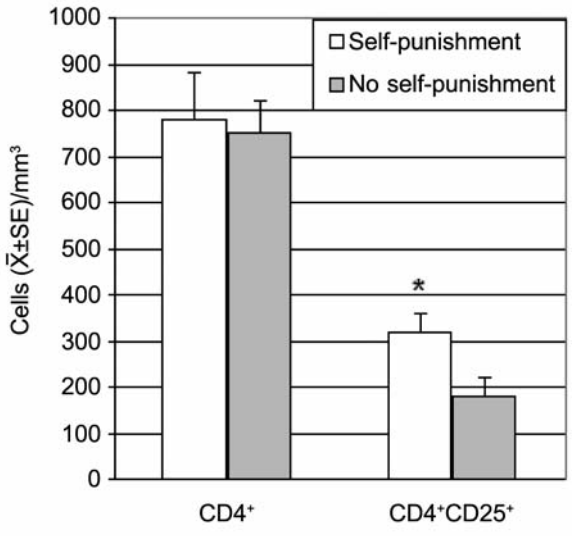

Figure 2. *Mean numbers of T-regulatory lymphocytes ($CD4^+$ $CD25^+$) and T-helper lymphocytes ($CD4^+$) in 30 patients with non-metastatic and metastatic solid tumor, with or without presence of self-punishment (*$p<0.05$ vs. no self-punishment).*

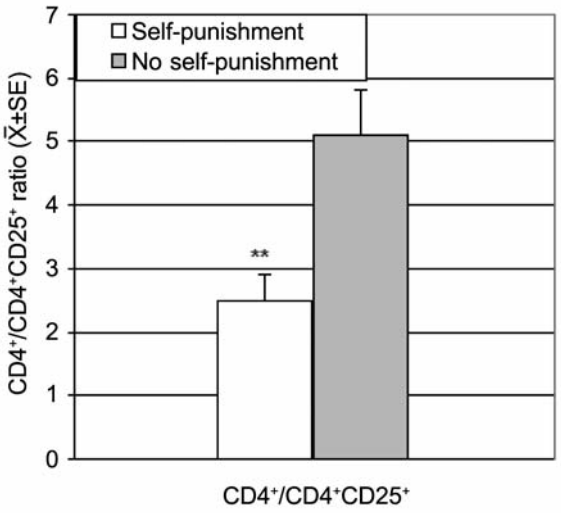

Figure 3. *Mean $CD4^+/CD4^+CD25^+$ ratio in 30 patients with non-metastatic and metastatic solid tumor, with or without presence of self punishment (**$p<0.025$ vs. no self-punishment).*

Discussion

In addition to previous study, showing various immune alterations in cancer patients with psychological disturbances (14), this preliminary study would suggest that the evidence of self-punishment is mainly associated with an abnormally enhanced T-reg cell population. Because of the evidence of no difference between patients with or without self-punishment regarding the number of the other main

Table II. *Mean numbers of total lymphocytes, T-cytotoxic lymphocytes, NK cells and CD3+ cells in cancer patients with or without self-punishment.*

Lymphocytes (n/mm^3) X±SE	No self-punishment	Self-punishment
Total lymphocytes	1678±161	1693±91
NK	253±50	269±39
CD3	1230±124	1302±115
CD8	414±49	449±43

lymphocytes subsets, enhanced T-reg cell activity would represent a specific immune alteration of cancer patients with self-punishment, as the major psychological profile. Moreover, because of the suppressive activity of anticancer immunity exerted by T-reg cells, these results would suggest that a self-punishment psychological profile may negatively influence the clinical course of the neoplastic disease by stimulating the generation of T-reg cells, which are able to suppress anticancer immune action. If further studies confirm this evidence, a psychological approach to remove the self-punishment condition could influence the prognosis of the neoplastic disease by reducing the hyperactivity of T-reg cell system. Therefore a psychological approach could serve not only a supportive function but also have a therapeutic impact. In this case, the monitoring of T-reg cell numbers during psychological therapy could constitute a useful parameter to measure the efficacy of the psychological treatment itself.

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