T-Helper / T-Regulator Lymphocyte Ratio as a New Immunobiological Index to Quantify the Anticancer Immune Status in Cancer Patients

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Abstract. *Background: The evaluation of the immune status of cancer patients is not routinely included in clinical oncological practice mainly because of the great number of candidate immune parameters that could potentially be the best index of the status of anticancer immunity. Until recently, the T-helper/T-suppressor lymphocyte ratio (CD4/CD8) was considered to be an index of immunosuppression in cancer patients. Successive studies documented the existence of several subtypes of CD4$^+$ lymphocytes, as well as showing that CD8$^+$ cells were not in fact suppressive, but cytotoxic lymphocytes. More recently, the existence of a subtype of T-helper lymphocytes has been demonstrated provided by an evident suppressive activity on anticancer immunity. These are the so-called T-regulator (T-reg) lymphocytes, which may be detected as CD4$^+$CD25$^+$ cells. Materials and Methods: A study was carried out to evaluate CD4$^+$/CD4$^+$CD25$^+$ ratio, corresponding to the T-helper/T-reg cell ratio (TH/TR), in a group of 50 cancer patients in relation to their disease extension and in 20 healthy controls. Results: The mean TH/TR ratio observed in patients with metasytases was significantly lower with respect to that found in both patients without metastases and controls. On the contrary, the absolute mean number of T-reg cells was higher in patients with metastases than in those without, but the difference was not statistically significant. Conclusion: The evaluation of T-reg cells in terms of their proportion with respect to T-helper cell total number seems to be more appropriate than the simple measurement of their absolute count, in order to quantify cancer-related immunosuppression. Thus, the TH/TR ratio could represent a useful biological marker to explore the immune status of cancer patients.*

The existence of an antitumor immune response, potentially capable of counteracting cancer growth, has been confirmed by several experimental studies (1-5). However, until about twenty years ago, the clinical evaluation of anticancer immune status in individual cancer patients was still an empirical investigation, since it generally consisted of skin reaction tests. The first laboratory immune parameter which was found to be able to predict the prognosis of cancer patients by correlating with the extension of disease was represented by the simple total lymphocyte count in the peripheral blood (6). In fact, lymphocytopenia was associated with a poor prognosis in cancer patients (6-8). Obviously, at those times, it was not possible to explain in detail the mechanisms responsible for the prognostic significance of the lymphocyte count.

Successive studies demonstrated the existence of several subpopulations of lymphocytes, providing different immune functions and characterized by a different expression of cell surface markers, the so-called cluster of differentiation antigens (CD) (9). In particular, the CD4$^+$ and CD8$^+$ lymphocyte subpopulations were identified as providing helper or suppressive effects, respectively, on the activation of an effective anticancer immune response. This response was then expressed by the T-helper/T-suppressor cell ratio (CD4/CD8 or T4/T8) (10). Moreover, a progressive decline in T4/T8 ratio was observed in relation to tumor metastatic dissemination (11). The T4/T8 ratio was thus proposed as an immunobiological marker capable of evaluating the status of the immune system in individual cancer patients (10-12). The prognostic value of the T4/T8 ratio was confirmed by the evidence that both AIDS patients (13) and metastatic cancer patients (11) were characterized by a progressive decline in the T4/T8 ratio. Further studies demonstrated that CD8$^+$ cells were essentially cytotoxic lymphocytes, whereas CD4$^+$ lymphocytes were shown to be characterized by at least two

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distinct subpopulations, the so-called T-helper 1 (TH1) and T-helper 2 (TH2) lymphocytes, responsible for the activation of the cellular or the humoral immune responses, respectively.

In the same way, the discovery of the interleukins (ILs) (13) as the main factors responsible for immune cell communication and modulation of the anticancer immune response itself, with both inhibitory and stimulatory effects (14-16), completely modified the clinical investigation of the immune status in cancer patients. In particular, the evidence of low blood levels of IL-2 (17) or that of abnormally high concentrations of IL-6 and IL-10 (18, 19) appear to be associated with a poor prognosis in cancer patients, as expected considering that IL-2 is the main antitumor cytokine in humans (14), whereas both IL-6 and IL-10 have been shown to suppress anticancer immunity (15, 16). However, no single interleukin blood concentration has been proposed for use as a index to quantify the immune status in neoplastic disease.

Recent experimental and clinical investigations have demonstrated the existence of a new lymphocyte subpopulation within the $CD4^+$ cell group consisting of $CD4^+CD25^+$ cells, providing an inhibitory effect on both T-helper and T-cytotoxic functions (18-20) with a subsequent suppressive activity on anticancer immunity, which have been defined as T-regulator lymphocytes (T-reg). The CD25 antigen corresponds to the alpha chain of the whole IL-2 cell surface receptor (14), which may also be released into the blood as soluble IL-2 receptor (sIL-2R) (14). Hence, $CD4^+CD25^+$ cells could theoretically simply represent the activated T-helper lymphocytes, since T-lymphocyte activation is consistently associated with IL-2 receptor expression (14).

From an experimental point of view, T-reg lymphocytes need to be better defined by the detection of other immune parameters, namely the intracellular concentration of Fox-p3 (21) and/or CD152 cell surface expression (22), since the inhibition of Fox p3 expression and the block of CD152 antigen by monoclonal antibodies appeared to abolish the suppressive activity (21, 22). However, from a clinical point of view, the evidence of CD4-CD25 positivity may be considered sufficient to identify the T-reg lymphocyte subpopulations (23, 24). In fact, preliminary clinical studies would suggest an increase in $CD4^+CD25^+$ cells in metastatic cancer patients with respect to that found in patients with locally limited disease (23, 24). However, there are controversial data in the literature regarding T-reg cell numbers at the different clinical stages of neoplastic disease (23-25), since the simple $CD4^+CD25^+$ lymphocyte number may be influenced by the total lymphocyte count, which is low in metastatic disease (6-8).

On this basis, a study was performed to evaluate the proportion of $CD4^+CD25^+$ cells using the T-helper/T-regulator ratio in patients with locally limited or metastatic disease, in an attempt to define its possible clinical relevance in the anticancer immune status in patients with neoplasms.

Table I. *Clinical characteristics of cancer patients.*

Characteristic	N
Male/Female	29/21
Median age (years)	61 (43-76)
Tumor histotype	
Non-small cell lung cancer	19
Epidermoid carcinoma	8
Adenocarcinoma	11
Colorectal carcinoma	18
Breast cancer	14
Disease extension	
Locally limited disease	22
Metastatic disease	28

Materials and Methods

The study included 50 consecutive cancer patients with locally limited or metastatic disease, suffering from the most frequent tumor histotypes, namely non-small cell lung cancer (NSCLC), breast cancer and colorectal carcinoma. Eligibility criteria were as follows: histologically proven locally limited or metastatic NSCLC, breast cancer or colorectal cancer; measurable lesions; no double tumor; no brain metastases; no important medical illnesses other than cancer; no previous chemotherapy or radiotherapy and no chronic concomitant treatment with drugs influencing the immune system, namely corticosteroids and opioids. The clinical characteristics of patients are shown in Table I. The control group consisted of 20 age- and sex-matched healthy individuals.

For immune detection, venous blood samples were collected in the morning after an overnight fast. In each blood sample, evaluation was made of the absolute numbers of total lymphocytes, T-helper lymphocytes ($CD4^+$), T-reg lymphocytes ($CD4^+CD25^+$), and the T-helper/T-reg ratio. Lymphocyte subpopulations were measured with a cytofluorimetric assay and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy), respectively directed against CD4 antigen and CD25 antigen, which correspond to the alpha chain of the whole IL2-receptor (16-19). Data were expressed as mean±SE and statistically analyzed by the Student's *t*-test, the analysis of variance and the chi-square test, as appropriate.

Results

The mean numbers of total lymphocytes, $CD4^+$ cells and $CD4^+CD25^+$ cells found in patients are illustrated in Figure 1. As shown, the mean lymphocyte number in patients with metastases was significantly lower than that found in patients with locally limited disease ($p<0.01$). In the same way, patients with metastases showed a significantly lower number of CD4+ lymphocytes than those without ($p<0.05$). On the other hand, the mean number of $CD4^+CD25^+$ lymphocytes was higher in patients with metastases than in those with locally limitated disease, but this difference was not statistically significant. Expressing the values of
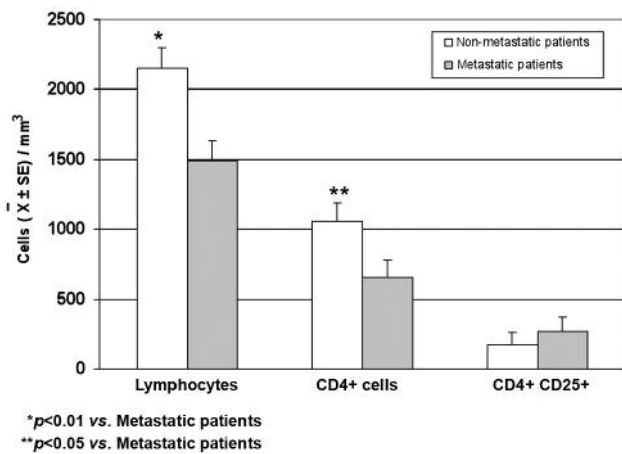
Figure 1. *Mean number of total lymphocytes, T-helper (CD4$^+$) lymphocytes and T-reg (CD4$^+$CD25$^+$) lymphocytes in cancer patients with and without metastases.*
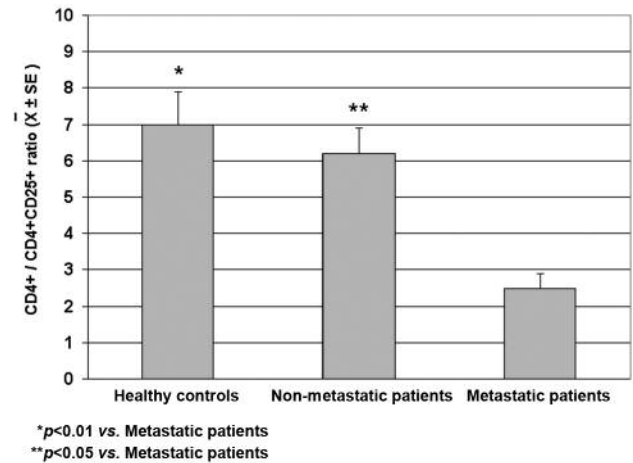

Figure 2. *CD4$^+$/CD4$^+$CD25$^+$ ratio observed in patients with metastases and those without metastases.*

CD4$^+$CD25$^+$ cells in terms of the total number of CD4$^+$ lymphocytes, as illustrated in Figure 2, the ratio of CD4$^+$ cells to CD4$^+$CD25$^+$ cells in patients with metastases was statistically significantly lower than that found in patients without ($p<0.01$) and in healthy controls ($p<0.005$). Moreover, the CD4$^+$/CD4$^+$CD25$^+$ ratio was higher in healthy controls than in patients without metastases, however without statistically significant differences. The proportion of patients with a CD4$^+$/CD4$^+$CD25$^+$ ratio less than 4.1 was significantly higher in patients with metastases than in those without (21/28 *vs.* 6/22, $p<0.005$).

Discussion

In accordance with previous data reported in the literature (6-9), this study confirms the occurrence of lymphocytopenia in metastatic neoplastic disease. The problem, however, of cancer-related lymphocytopenia is to establish if it is characterized by a general decline in the overall lymphocyte subsets or if it may depend on a deficiency of a particular cell subset. The results of this study, by showing an increase in the proportion of T-reg lymphocytes in metastatic patients, identified as CD4$^+$CD25$^+$ cells, would suggest that the decline in lymphocyte number related to cancer progression is due to a selective deficiency of immune cells responsible for the generation of an effective antitumor immunoreaction, such as T-helper lymphocytes. Moreover, with respect to the results of others showing an association between cancer progression and increase in T-reg generation (23-27), the study demonstrates that an increase in the absolute number of T-reg lymphocytes may only be observed in metastatic patients, with normal lymphocyte count, whereas lymphocytopenic cancer patients may present a normal or a paradoxically low number of T-reg cells. In contrast, by considering the percentage of T-reg cells in relation to that of total lymphocyte and T-helper cells, the study confirm that metastatic disease is constantly associated with an abnormally increased generation of T-reg cells, which are responsible for the suppression of an effective anticancer immune reaction. Therefore, the evaluation of T-reg lymphocyte numbers in terms of their percentage with respect to the total number of CD4$^+$ lymphocytes would be a more appropriate clinical immunological parameter than the measurement of their absolute numbers. The CD4$^+$/CD4$^+$CD25$^+$ ratio could constitute a new simple clinical immunological index capable of assessing the anticancer immune function in cancer patients.

Since no patient had been treated by chemotherapy or radiotherapy, the observed lower CD4$^+$/CD4$^+$CD25$^+$ ratio in patients with metastases would not simply represent a consequence of various antitumor treatments, but it would reflect the actual immune status of patients, which directly depends on the physiopathology of the neoplastic disease. On the contrary, it has to be remarked that the previous studies carried out by most others (23-27) did not consider the possible influence of the anticancer therapies on the immune status of patients, hence it is not possible to establish whether advanced cancer-related increased T-reg cells may depend on the neoplastic disease itself or on the effect of the anticancer therapies, namely chemotherapy and radiotherapy.

In any case, further longitudinal studies monitoring changes in the percentage of T-reg cells in patients under treatment with chemotherapy or radiotherapy be will be needed to establish whether the efficacy of the anticancer therapies may be mediated, at least in part, by changes in the immune status, particularly in T-reg proportion itself, being responsible for the suppression of the anticancer immunity.

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