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Short Communication

Interleukin-12 in Early or Advanced Cancer Patients

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Interleukin-2 (IL-2) and interleukin-12 (IL-12) may represent the most important antitumour cytokines in human neoplasms. IL-2 blood levels decrease in advanced solid malignancies, but currently there are no data on IL-12 secretion in cancer patients. This study was performed to obtain preliminary data about IL-12 secretion in patients with solid malignant tumours, either in relation to the extension of disease, or to other cytokines, including IL-2, IL-6 and IL-10. The study included 40 solid cancer patients, 24 of whom showed distant organ metastases. Cytokine serum levels were measured by an enzyme immunoassay of blood samples collected during the morning. No patient had abnormally low levels of IL-12, but the levels were high in 14/40 (35%) patients. Mean levels of IL-12 were significantly higher in metastatic patients compared with non-metastatic patients ($P < 0.05$). Moreover, metastatic patients with high blood concentrations of IL-12 showed significantly lower levels of IL-10 than metastatic patients with normal IL-12 values, while no difference was seen in IL-2 mean concentrations. IL-6 mean levels were lower in metastatic patients with increased IL-12 levels, but this was non-significant. This preliminary study shows that advanced solid cancers are not characterised by a diminished secretion of IL-12, but rather IL-12 levels tend to be abnormally high in metastatic cancer patients. © **1997 Elsevier Science Ltd.**

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INTRODUCTION

THE RECENT availability of methods to measure blood levels of the most important cytokines have allowed the characterisation of their secretory behaviour either in physiological conditions or in human diseases, including cancer. Several alterations in cytokine secretions and in biological response markers have been observed in human malignant neoplasms. To define more precisely the role of cytokines in the pathogenesis of cancer-related immune disorders, it is essential to differentiate those cytokines responsible for the immune alterations themselves from the alterations involving other cytokines or biological response markers, which are simply correlated with the main immunopathological events.

The most important cancer-related suppressive events documented to date would be represented by the evidence of abnormally high blood levels of IL-6 and IL-10, which reflect macrophage-mediated and T helper-type 2 (TH2)-mediated immunosuppression, respectively [1–3]. IL-2 [4] and IL-12 [5] seem to represent the two most important antitumour cytokines in humans. Therefore, the simultaneous measurement of IL-2, IL-12, IL-6 and IL-10 blood concentrations could allow the concomitant analysis of the status of both anticancer immunoactivation and immunosuppression in cancer patients. Abnormally low blood levels of IL-2 have been described in advanced cancer patients [6], and is associated with a poor prognosis and with a lower survival. The role of IL-12 secretion in human malignant neoplasms has still to be established. Preliminary results would suggest that cancer progression may be associated with endogenous IL-12 deficiency [7].

Currently, it is still unknown whether IL-2 and IL-12 deficiencies reflect different pathological conditions, or whether they simply reflect a common immunopathological disorder. As far as cancer-related markers of immunosuppression are concerned, it has been demonstrated that

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Table 1. Characteristics of solid tumour patients

Characteristics	*n*
n	40
M/F	23/17
Median age (years)	61 (39–77)
Tumour histotypes	
Lung cancer	7
Epidermoid cancer	3
Adenocarcinoma	3
Small cell cancer	1
Colorectal Cancer	6
Breast cancer	6
Head and neck cancer	4
Uterine cervix cancer	4
Gastric cancer	4
Biliary tract cancer	2
Renal cell cancer	2
Hepatocarcinoma	2
Ovarian cancer	1
Prostate cancer	1
Soft tissue sarcoma	1
Sites of disease	
Locally limited disease	16
Metastatic disease	24
Soft tissues	2
Bone	4
Lung only	8
Liver only	4
Lung + liver	2
Serous	4

abnormally high blood levels of IL-6 and IL-10, which reflect macrophage- and TH2-mediated immunodeficiency, respectively, may occur independently in advanced cancers [8]. Other biological modifier markers, in particular, elevated concentrations of neopterin, tumour necrosis factor-alpha (TNF) and soluble IL-2 receptors (SIL-2R) could simply reflect macrophage hyperactivation, as shown by the evidence of concomitant abnormally high values of IL-6 [9]. Lymphocytopenia is another common biological marker of cancer progression [10], but it still needs to be investigated in relation to alterations of cytokine production.

This study was performed to investigate IL-12 blood levels in human solid malignancies, in relation either to disease extension, or to the secretion of the most important suppressive cytokines, IL-10 and IL-6, as well as that of the other main antitumour cytokine, IL-2.

PATIENTS AND METHODS

The study included 40 consecutive patients suffering from locally limited ($n = 16$) or metastatic ($n = 24$) malignant solid neoplasms. The clinical characteristics of patients are reported in Table 1. Patients with locally limited or metastatic cancer were analysed before surgery or before the onset of chemotherapy, respectively. Moreover, no patient was under therapy with drugs influencing cytokine secretion, such as steroids or interferon, for at least 1 month prior to the study.

To evaluate serum levels of cytokines, venous blood samples were collected in the morning after an overnight fast. Serum levels of IL-12, IL-10, IL-6 and IL-2 were measured by an enzyme immunoassay and commercially available kits (Medgenix Diagnostics, Brussels, Belgium). All samples were measured in duplicate, and intra-assay and inter-assay coefficients of variations were less than 4% and 5%, respectively. Normal values obtained in our laboratory (95% confidence limits) were 9–89 pg/ml for IL-12, 0.1–6 pg/ml for IL-10, 2–31 pg/ml for IL-6 and 0.1–0.4 IU/ml for IL-2.

Results were reported as mean ± SEM, and statistically analysed by Student's *t*-test and the coefficient of correlation, as appropriate.

RESULTS

Cytokine mean serum levels observed in non-metastatic cancer patients and in those with metastatic disease are reported in Table 2. No patient had abnormally low concentrations of IL-12, but normal levels were observed in 26/40 (65%) patients, and abnormally elevated levels in 14/40 (35%) patients. Moreover, no patient with locally limited disease showed high levels of IL-12, whereas 14/24 (58%) metastatic cancer patients had abnormally increased levels. IL-12 mean concentrations were significantly higher in metastatic patients than in those with locally limited disease ($P < 0.05$). Moreover, metastatic patients showed significantly higher concentrations of IL-6 ($P < 0.025$) and IL-10 ($P < 0.01$), and significantly lower levels of IL-2 ($P < 0.025$) with respect to those found in nonmetastatic patients. Table 3 shows cytokine mean levels observed in metastatic patients with or without high levels of IL-12. Metastatic patients with elevated concentrations of IL-12 showed significantly lower levels of IL-10 compared with those with normal IL-12 levels ($P < 0.05$), even though no significant correlation was seen between IL-12 and IL-10 blood concentrations ($r = -0.4$). Metastatic patients with increased IL-12 values had lower levels of IL-6 than those with normal IL-12 values, although this was not significant. In contrast, no significant difference was observed in IL-2 mean values between metastatic patients with or without high IL-12 concentrations. Finally, the mean lymphocyte count was higher in metastatic patients with increased IL-12 levels than in those with normal IL-12 secretion (1641 ± 123 versus 1228 ± 114/mm^3), although the difference was not significant.

DISCUSSION

In contrast to our expectation and to results in other studies [4, 6, 10], this study shows that there was no deficiency in IL-12, either in patients with locally limited disease or in those with metastatic solid neoplasms. On the contrary, IL-

Table 2. Cytokine serum concentrations (mean ± SEM) observed in nonmetastatic and metastatic solid tumour patients

Patients	*n*	IL-12 pg/ml	IL-10 pg/ml	IL-6 pg/ml	IL-2 IU/ml
Non-metastatic patients	16	59 ± 7	0.4 ± 0.1	37 ± 7	0.22 ± 0.01†
Metastatic patients	24	108 ± 16*	3.4 ± 0.9‡	130 ± 31†	0.14 ± 0.02

*$P < 0.05$, †$P < 0.025$, ‡$P < 0.01$.

Table 3. IL-12 serum levels in metastatic patients in relation to other cytokines (mean ± SEM)

Patients	*n*	IL-10 pg/ml	IL-6 pg/ml	IL-2 IU/ml
Patients with high IL-12	14	1.3 ± 0.6*	83 ± 21	0.16 ± 0.03
Patients with normal IL-12	10	5.3 ± 1.3	173 ± 32	0.14 ± 0.02

*$P < 0.05$ versus patients with normal IL-12

12 concentrations tended to increase with disease progression, with higher levels in metastatic patients than in those with limited disease. Moreover, this study would suggest that there is no apparent relationship between IL-12 and IL-2 endogenous production, since no difference in IL-2 levels was observed between patients with normal or increased IL-12 levels. Although the low number of patients and the different histotypes do not allow us to draw definite conclusions, this study seems to suggest that two different subpopulations of metastatic solid cancer patients may be recognised concerning IL-12 secretion, characterised by normal or increased IL-12 endogenous production. These two subgroups of patients would also seem to be differentiated in terms of IL-10 secretion, with high levels of IL-12 associated with lower concentrations of IL-10, and normal IL-12 values associated with abnormally elevated concentrations of IL-10.

The apparent association between high levels of IL-12 and normal or low concentrations of IL-10 in metastatic disease could reflect important immunopathological mechanisms. In fact, since IL-12 has been proven to inhibit TH2-lymphocyte functions, including IL-10 secretion [7], the lack of high levels of IL-10 in patients with high values of IL-12 could depend on the inhibitory effect of IL-12 on IL-10 release. Therefore, because of the immunosuppressive action of IL-10 on host anticancer immune defences [3], the increased IL-12 secretion could be considered as a compensatory feedback mechanism to protect against an exaggerated release of the immunosuppressive cytokine IL-10. Alternatively, on the basis of the fact that IL-10 may inhibit IL-12 secretion [3, 7], the lack of increased IL-12 production as a protective immune reaction in metastatic disease could be simply due to the enhanced production of IL-10 itself. Therefore, it still has to be established what is the cause and what is the effect in the anomalous secretions of IL-12 and IL-10 in metastatic solid neoplasms. Moreover, long-term studies, where endogenous IL-12 production is monitored during the clinical course of metastatic solid tumour patients, will be required to establish whether increased IL-12 secretion is associated with a more favourable prognosis compared with those metastatic patients in whom IL-12 production is not enhanced. This prognostic question is justified by the well-documented antitumour activity of IL-12 [5, 7].

Moreover, the evidence of an association between high levels of IL-12 and normal values of IL-10 in metastatic cancer patients could have important prognostic significance during IL-2 cancer immunotherapy to predict the efficacy of IL-2 itself. In fact, in the presence of enhanced pretreatment levels of IL-12, the exogenous injection of IL-2 could reproduce the synergistic antitumour action between IL-12 and IL-2 demonstrated in experimental conditions [5]. Normal pretreatment values of IL-12, which are generally associated with abnormally enhanced production of IL-10, could correlate with resistance to IL-2, which has been shown to be reduced in advanced cancer patients [6, 10].

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