Serum Level of Interleukin 6 as a Prognosis Factor in Metastatic Renal Cell Carcinoma

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ABSTRACT

Interleukin (IL) 6 was measured in the serum of 138 patients with metastatic renal carcinoma before the initiation of IL-2 treatment. IL-6 was detectable in 66 patients with renal cancer (48%) and in only 8 of 70 normal adults (11%). Serum C reactive protein (CRP) and IL-6 levels are correlated, suggesting that IL-6 is involved in CRP increase in these patients. The interval between diagnosis of the primary tumor and metastasis was shorter in patients with a detectable serum IL-6 and/or serum CRP level >50 mg/liter. Serum IL-6 and CRP levels were higher in subgroups of patients previously defined as having a poor life expectancy according to the Eastern Cooperative Oncology Group criteria.

Pretreatment concentrations of IL-6 and CRP were higher in patients who experienced progressive disease after IL-2 treatment. Patients with detectable IL-6 had a shorter survival from the beginning of IL-2 treatment than patients without circulating IL-6 (median, 8 versus 16 months). Similarly, the median survival from the beginning of IL-2 therapy of patients with CRP levels >50 mg/liter was 6 months, compared to 16 months in those with CRP levels below this threshold. None of the 21 patients with serum IL-6 concentrations >300 pg/ml achieved response to any of the three IL-2 regimens. This subgroup has a median survival of 5 months after IL-2 treatment and consisted of 15% of the patients in our series. These results indicate that serum IL-6 and CRP levels are adverse prognosis factors in patients with metastatic renal cell carcinoma. Serum IL-6 level could help in the selection or stratification of the patients in future IL-2 trials.

INTRODUCTION

Clear cell renal carcinoma represents 3% of adult malignancies (1). Nephrectomy is curative in about 80% of the cases of small (Robson A or B) limited tumors (1, 2). However, about 50% of the patients with renal carcinoma develop metastasis and are then usually considered incurable (1, 2). None of the cytotoxic drugs tested yet has demonstrated any significant and reproducible efficacy (1). The median survival of patients with metastatic renal carcinoma is 8 months in most series (3–5). Prognosis factors include performance status, time between the diagnosis of the primary tumor and metastasis, the number of metastatic sites, weight loss, and previous chemotherapy (4, 5). A combination of these criteria was used by the ECOG to define five risk groups with different survival prognoses (5).

Treatment with IL-2 yields partial or complete responses in 10 to 35% of the patients with some long-lasting remissions (6–13). However, IL-2 is responsible for multiple side effects, including fever, capillary leak syndrome, skin, hepatic, and cardiac toxicities (6, 7, 14, 15). Pretreatment criteria predictive of the response to IL-2 would thus be useful to exclude patients who are unlikely to benefit from such treatment.

IL-6 is a multipotent cytokine exerting numerous biological activities (16–22). It regulates the proliferation and differentiation of immunocompetent cells including T- and B-lymphocytes, NK cells, and normal hematopoietic progenitors, epitelial and neural cells (17, 18, 23–28). IL-6 stimulates the differentiation of major histocompatibility-restricted and non-major histocompatibility-restricted cytotoxic cells (25, 28). IL-6 induces the expression of the p55 chain of the IL-2 receptor on T-lymphocytes and synergizes with IL-2 to enhance the cytotoxic activity of T-lymphocytes (25, 29). IL-6 is also a potent proinflammatory cytokine. It acts as an endogenous pyrogen and induces the expression of acute phase protein genes including the CRP gene (30, 31). In addition to its physiological properties, IL-6 is involved in the pathophysiology of various neoplasias such as multiple myeloma, non-Hodgkin's lymphoma, and Kaposi's sarcoma (17, 18, 32–36). Fresh renal carcinoma cells and cell lines have been reported to express IL-6 mRNA and to produce IL-6 in the supernatants (37, 38). Furthermore, IL-6 receptor and its specific mRNA have been detected in renal carcinoma cells (37, 38). In vitro, anti-IL-6 antibodies block the proliferation of renal carcinoma cell lines, indicating that IL-6 is an autocrine growth factor for these cell lines (37). Serum IL-6 increase has been previously reported for patients with severe burns and patients with multiple myeloma (32, 39). To date, however, serum IL-6 levels in patients with renal cell carcinoma have not been investigated.

In this study, IL-6 was detectable in 48% of the 138 patients with metastatic renal cell carcinoma. Our results indicate that serum CRP levels are highly correlated with IL-6 levels and that patients with detectable IL-6 and/or CRP levels >50 mg/liter have a poor response rate to IL-2 and a shorter survival.

PATIENTS AND METHODS

Patients

A total of 138 patients with metastatic renal cell carcinoma were included in a protocol of immunotherapy with IL-2 after written informed consent. All patients belonged to risk groups 1, 2, and 3 defined by the ECOG (5). Patients from groups 4 and 5 were not eligible for these IL-2 protocols. Characteristics of the patients are shown in Table 1. A total of 127 patients were evaluable for response to IL-2. Twenty-three patients achieved partial or complete responses, 41 had stable diseases, and 63 had progressive diseases. Eleven patients were not evaluable for response because of death due to toxicity during IL-2 treatment (2 patients), interruption of the treatment before completion because of patient refusal (2 patients), major toxicity (3 patients), no evaluable lesion (2 patients), and rapid progression (2 patients). IL-2 schedules are presented in Table 2. Of the 138 patients, 66 received continuous i.v. IL-2 infusion with (n = 22) or without (n = 44) LAK cell reinjection, as previously described (9, 12). Thirty-two patients

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The abbreviations used are: ECOG, Eastern Cooperative Oncology Group; CRP, C reactive protein; IL, interleukin; LAK, lymphokine-activated killer cell; PR/CR, partial/complete response; SD, stable disease; PD, progressive disease; TNF, tumor necrosis factor; NK, natural killer.

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received a protocol of i.v. bolus high doses of IL-2 and α-interferon with LAK cells. Forty patients received a combination of low doses of IL-2 and α-interferon given as s.c. injections as previously reported (13). Response rates of these 3 regimens were not significantly different.

Methods

Serum Sampling and Storage. Serum samples were obtained for all patients on the day prior to the beginning of IL-2 and stored at −70°C until the assay. Serum samples were also obtained from a control population of 70 normal healthy donors with comparable sex and age distribution characteristics.

Determination of IL-6 Serum Concentration. Serum IL-6 concentration was measured in the samples from 138 patients using an enzyme-linked immunosorbent assay (Intertest 6; Genzyme, Boston, MA) according to the kit procedure. The limit of detection of the test was 76 pg/ml, and lower levels were considered as undetectable.

Serum IL-6 levels obtained with the enzyme-linked immunosorbent assay method were controlled in 50 patients with the B9 bioassay as previously described (32). Results obtained with the two methods are highly correlated (data not shown).

Determination of Serum CRP Concentration. CRP concentration was measured using a fluorescence polarization immunoenzyme assay (Abbott Laboratories, Chicago, IL). The limit of detection of the CRP assay is 5 mg/liter. The determination was performed on an aliquot of the same serum sample from 129 patients including 124 of the 127 patients evaluable for response to IL-2. CRP concentration could not be determined from the serum of 9 patients because quantities of serum available were not sufficient. IL-6 and CRP were measured by two different investigators in a double-blind manner.

Statistical Analysis. Statistical analyses were performed using Yates corrected χ², Fisher's exact test (two tailed), the nonparametric Fisher-Yates test, and the Pearson's test for the coefficient of correlation. Survivals were calculated according to the method of Kaplan and Meier. Survival curves were compared using the log rank test.

RESULTS

Serum IL-6 Concentrations in Patients with Renal Carcinoma: Correlation with Serum CRP Levels. Of 138 patients, 66 (48%) with metastatic renal carcinoma had detectable serum IL-6 concentrations before the beginning of IL-2 treatment (Fig. 1). In contrast, IL-6 was detectable in the serum of only 8 of 70 (11%) normal donors (χ² = 26.8, P < 10⁻⁵). Since CRP production is stimulated by IL-6 in vitro (31), we studied the possible correlation between serum IL-6 and CRP concentrations. A highly significant correlation between CRP and IL-6 levels was observed (r = 0.63, P < 10⁻⁵). Patients with undetectable levels of IL-6 in the serum had a mean CRP level of 27 mg/liter, whereas it was 43 mg/liter for patients with detectable IL-6 levels <300 pg/ml and 117 mg/liter for patients with IL-6 levels >300 pg/ml. As shown in Fig. 2, CRP concentrations in each subgroup were significantly different.

Correlation between Prognosis Factors and Serum Levels of IL-6 and CRP. The time between the diagnosis of primary tumor and the appearance of metastasis was compared in patients with or without circulating IL-6. This interval was significantly longer for the 72 patients with undetectable IL-6 than for the 66 patients with detectable IL-6 (mean, 22 versus 7 months; Z = 2.67, P < 0.01). Similarly, the 90 patients with a serum CRP level <50 mg/liter had a longer interval than the 39 patients with CRP >50 mg/liter (mean, 20 versus 7 months; Z = 2.75, P = 0.005). No correlation was found between serum IL-6 level and the performance status prior to IL-2 treatment or with the number of metastatic sites (data not shown).

We compared the serum concentrations of IL-6 and CRP in the different risk groups previously defined by the ECOG (5). None of the patients in our series belonged to risk group 4 or 5 since these patients were not eligible for inclusion in the IL-2 therapy protocols. IL-6 was detectable in the serum of 28, 50, and 67% of the patients of risk groups 1, 2, and 3, respectively. Serum IL-6 levels were more frequently detectable in the sera of patients with poor life expectancy (Fig. 3). Mean CRP levels were 50 and 79 mg/liter in patients from risk groups 2 and 3, respectively, compared to 25 mg/liter in patients from risk group 1 (P < 0.01).

Correlation of Pretreatment Serum IL-6 and CRP Concentrations with the Response to IL-2. The pretreatment serum concentrations of IL-6 were compared in patients who experienced PR/CR, SD, and PD after IL-2. As shown in Fig. 4, patients with PD after IL-2 had significantly higher pretreatment IL-6 than patients with PR/CR or SD. Pretreatment IL-6 levels were also significantly higher for patients with SD than for patients with PR/CR after IL-2. Pretreatment IL-6 and CRP levels had a prognosis value for both response to IL-2 and survival in all of the three IL-2 regimens used in this study (data not shown).

Of 72 patients with undetectable IL-6 in serum, 19 (26%)

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* Patients received a priming dose of IL-2 (9 × 10⁶ IU/m² by bolus i.v. infusion) during 2 days the week before the beginning of IL-2/α-interferon.
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Fig. 1. Serum concentrations of IL-6 of 138 patients with metastatic renal carcinoma and 70 normal individuals. IL-6 is detectable in 8 (11%) of the normal subjects and 66 (48%) of patients with renal carcinoma (Yates corrected $\chi^2 = 25.9, P < 10^{-4}$).

achieved PR/CR compared to 4 of 66 (6%) patients with detectable IL-6 ($\chi^2 = 8.83, P = 0.003$). Of 66 patients with circulating IL-6, 40 (62%) experienced disease progression after IL-2, compared to 23 of 72 (31%) of the patients with undetectable IL-6 ($\chi^2 = 10.27, P = 0.001$). The response rate to IL-2 was 0% for 21 patients with pretreatment serum IL-6 levels $>300$ pg/ml and 20% for the 117 remaining patients ($P < 0.05$).

We also compared pretreatment CRP levels according to patient status after IL-2 treatment. Mean pretreatment CRP levels were significantly higher in patients with PD after IL-2 than in patients with PR/CR or SD after IL-2 ($56 \text{ versus } 36 \text{ mg/liter}, P < 0.001$).

Of 90 patients with serum CRP concentrations $<50$ mg/liter, 22 (24%) achieved PR/CR compared to one of 39 (3%) patients with higher CRP levels ($\chi^2 = 7.5; P = 0.006$). Furthermore, 34% (31/90) of the patients with serum CRP $<50$ mg/liter experienced disease progression after IL-2 compared to 74% (29/39) of the patients with higher CRP levels ($\chi^2 = 15.8; P < 10^{-4}$).

Serum IL-6 and CRP Levels as Prognosis Factors for Survival. The survival from the beginning of IL-2 treatment was analyzed in the subgroups of patients with or without detectable serum IL-6. The results presented in Fig. 5 show that patients without circulating IL-6 had a significantly longer survival than patients with detectable IL-6 in serum (median survival, 16 versus 8 months; log rank = 4.85, $P < 0.03$). Of note, patients with a serum concentration of IL-6 $>300$ pg/ml had a very poor survival compared to that of patients with lower IL-6 levels (median survival, 5 versus 13 months; log rank = 7.6, $P < 0.01$).

As shown in Fig. 6, patients with a serum CRP level $>50$ mg/liter also had a significantly shorter survival than patients with a lower CRP (median survival, 6 versus 16 months; log rank = 23.3, $P < 10^{-5}$). These results indicate that IL-6 and CRP are prognostic factors for survival in patients with metastatic renal carcinoma treated with IL-2.

**DISCUSSION**

Fresh renal carcinoma cells and cell lines have been reported to express IL-6 mRNA and to produce IL-6 in the supernatant (37, 38, 40). Our results are consistent with these observations and demonstrate that IL-6 levels are increased in the serum of patients with metastatic renal carcinoma. Preliminary observations obtained in our laboratory indicate that IL-6 levels are 10-fold higher in the vein of a renal carcinoma compared to...
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Fig. 5. Survival from the first day of IL-2 treatment in patients with (----) and without (-----) detectable serum IL-6 before IL-2 treatment. Log rank = 4.85, $P < 0.03$.

Fig. 6. Survival from the first day of IL-2 treatment in patients with pretreatment serum CRP concentration >50 mg/liter (-----) and <50 mg/liter (----). Log rank = 23.28, $P < 10^{-5}$.

Peripheral blood. This suggests that IL-6 is indeed produced by tumor cells and released in the circulation in patients with renal cancer. IL-6 is an endogenous pyrogen and induces the production of acute phase proteins, including CRP, by liver cells (17, 30, 31, 39). It has recently been shown that serum CRP levels are increased in patients with renal carcinoma whose tumor expresses high levels of IL-6 mRNA (37). Our data are consistent with these observations and further indicate that serum CRP is correlated with serum IL-6 in patients with renal carcinoma. IL-1 and TNF also regulate the production of acute phase proteins by liver cells in vitro (41, 42).

These cytokines have been found at normal levels in patients with metastatic renal cell carcinoma before the beginning of IL-2 treatment (43, 44). Taken together, these data strongly suggest that IL-6 is involved in the pathogenesis of the inflammatory syndrome associated with renal cell carcinoma.

We observed that patients with high serum IL-6 levels had a shorter time between the diagnosis of their primary tumor and the development of metastasis, as well as a shorter survival from the beginning of IL-2 treatment. No correlation was found between serum IL-6 level and the performance status prior to IL-2 treatment or with the number of metastatic sites. This is consistent with a recent report showing that the level of IL-6 mRNA expression in the tumor is not correlated with the presence of distant metastasis (38). Interestingly, patients belonging to the high-risk groups previously defined by the ECOG (5) were found to have higher IL-6 levels. These results indicate that IL-6 is a marker of the aggressiveness of renal cell carcinoma and suggest that it is involved in tumor progression in vivo.

Two hypotheses could account for these observations. It was previously reported that IL-6 is an autocrine growth factor in renal carcinoma cells (37). Fresh renal carcinoma cells and cell lines express IL-6 mRNA and IL-6 receptor mRNA and produce detectable concentrations of IL-6 in the supernatant (37, 38). The addition of anti-IL-6 antibody in the culture medium has shown to inhibit the proliferation of two renal carcinoma cell lines (37). However, this has not consistently been found for all renal carcinoma cell lines (40). Therefore, addi-
tional data are required to demonstrate an autocrine role of IL-6 in this model. A phase II study of anti-IL-6 antibody in metastatic renal carcinoma was recently initiated in our group. This study should provide interesting information concerning this potential role for IL-6 in vivo.

Alternatively, IL-6 could exert an adverse effect through a modulation of the antitumor response. In vitro, IL-6 is a potent costimulator of the proliferation of T-lymphocytes and enhances the cytotoxic function of cytotoxic T-lymphocytes and natural killer cells (24–28). IL-6 synergizes with IL-2 to enhance the cytotoxic activity of peripheral blood T-lymphocytes and induces the expression of the p55 chain of the IL-2 receptor on T-lymphocytes (25, 29). The inverse correlation between serum IL-6 levels and response to IL-2 is thus paradoxical in view of the stimulatory effect of IL-6 on the differentiation of cytotoxic effectors in vitro.

However, in vivo, IL-6 has been shown to enhance tumor progression in a murine model (45). Tumor cell lines transfected with an IL-6 retroviral vector, and producing high amounts of IL-6 in mouse serum, exhibit a higher tumorigenicity in this model (45). The tumorigenicity of these transfected cell lines is correlated with the level of IL-6 production in the culture supernatant in this model and is possibly due to the inhibitory effect of the very high concentrations of IL-6 (>10^4 units/ml) on the immune response directed against the tumor (45). IL-6 could conceivably exert a similar role in patients with renal cell carcinoma. We have previously shown the lack of correlation between the NK and LAK activities of peripheral blood lymphocytes and the response to IL-2 (46). Furthermore, we observed no correlation between the pretreatment level of IL-6 and the NK and LAK activities of peripheral blood lymphocytes collected before, during, and after IL-2 treatment (data not shown). Thus, IL-6 does not appear to enhance or block the NK or LAK activity of peripheral blood lymphocytes in patients treated with IL-2. However, it would be interesting to investigate the cytotoxic function of tumor-infiltrating lymphocytes which could be blocked by very high local concentrations of IL-6 at tumor sites (45). It has recently been shown that serum IL-6 levels increase transiently after IL-2 administration in 40–50% of patients (44, 47). However, the increase of IL-6 consistently lasts until the end of IL-2 infusion and reaches 300 pg/ml in <5% of the cases. No correlation with the clinical response to IL-2 was observed (44).

Ten to 35% of the patients achieve a response to IL-2. However, most of the patients experience severe side effects during the administration of high doses of IL-2 (6–15). It was thus important to determine a pretherapeutic criterion in order to identify patients in whom IL-2 treatment is likely to be inefficient. We previously reported that responders to IL-2 have higher serum TNF levels 48 h after the end of the first course of continuous i.v. IL-2 infusion (43). These results permitted us to define a threshold level of TNF under which no response was observed. However, this criterion can only be determined after the beginning of IL-2 treatment. In the present study, we show that patients with pretreatment serum concentrations of IL-6 >300 pg/ml have a very poor prognosis and a 0% response rate to the IL-2 regimens used in this study. Although additional studies are required to confirm these observations, the benefit of IL-2 treatment for these patients remains to be demonstrated.

Pretreatment IL-6 levels had a prognosis value in all of the three regimens used in this study, suggesting that the mode of administration of IL-2 does not influence the value of IL-6 as a prognosis factor. Furthermore, given the diversity of IL-2 regimens used in this study, it seems unlikely that any other method of IL-2 administration could affect the prognosis value of IL-6 and CRP. The correlation between the response rate to IL-2 and pretreatment serum IL-6 level suggests that it could be interesting to use this criterion to stratify patients in future clinical trials of IL-2. This procedure could help reduce the discrepancies between the rates of response to IL-2 previously reported in the literature.

In conclusion, we have shown that serum IL-6 levels are increased in patients with renal cell carcinoma and correlate with CRP levels. Serum IL-6 concentration is a prognosis factor for survival and correlates with the response to IL-2. None of the patients with serum IL-6 levels >300 pg/ml achieved a response to the IL-2 regimens used in this study. These results suggest that IL-6 plays an important role in renal carcinoma progression in vivo. The role of IL-6 in the immune antitumor response and in the proliferation of renal carcinoma in vivo are currently under investigation.

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