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Interleukin 10 (IL-10): an immunosuppressive factor and independent predictor in patients with metastatic renal cell carcinoma

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Summary Interleukin 10 (IL-10) is an immunosuppressive factor and has been detected in tumour cell cultures of renal cell carcinoma and of malignant melanoma. IL-10 has been described as a cytokine of the Th2 response; it is able to suppress antigen-presenting cells (APCs) and may lead to down-regulation of HLA class I and II molecules on dendritic cells and to anergy of T-lymphocytes. We evaluated pretreatment serum levels of soluble IL-10 and various clinical parameters to determine their prognostic value in 80 advanced renal cell carcinoma patients seen at our institution between May 1990 and April 1996. For statistical evaluation we used both univariate and multivariate Cox proportional hazards models. An elevated pretreatment serum level of IL-10 was a statistically independent predictor of unfavourable outcome ($P < 0.0028$), in addition to the well-known clinical and biochemical risk factors. These data support risk stratification for future therapeutic trials and identify a predictor which needs to be validated in prospective studies and may potentially influence decision making in palliative management of patients with metastatic renal cell carcinoma. These data also suggest a potential role of IL-10 in the development of advanced renal cell carcinoma and in the future design of therapeutic strategies.

Keywords: predictors, renal cell carcinoma, IL-10

Interleukin 10 (IL-10), originally defined as cytokine synthesis inhibitory factor produced by type 2 helper T-lymphocytes (Fiorentino et al, 1989), has subsequently been described as a potentially immunosuppressive cytokine. By interfering with the co-stimulatory function of antigen-presenting cells (APCs), e.g. regulation of class II MHC expression of monocytes (deWaal Malefyt et al, 1991), and co-stimulatory molecule expression of macrophages (Ding et al, 1993), IL-10 reduces antigen-specific T-cell proliferation. Moreover, a direct effect of IL-10 on IL-2 production by activated T-cells has been observed (deWaal Malefyt et al, 1993). It has been demonstrated that IL-10 blocks the development of predendritic cells into mature dendritic cells (DCs) in vitro (Buelens et al, 1997).

This IL-10-mediated immunosuppression represents an important escape mechanism of tumours and needs further clinical investigation.

Therefore, using a highly sensitive enzyme-linked immunosorbent assay (ELISA) recognizing both human and viral IL-10, we measured the serum concentrations of IL-10 in 80 consecutive patients with a fully documented clinicopathological history of metastatic renal cell carcinoma seen in our institution since May 1990. We assessed the potential correlation between serum IL-10 levels and the outcome of these patients.

METHODS

Patients and collections of samples

This study was approved by the institutional review board of the Medizinische Hochschule Hannover; written informed consent was obtained from all patients prior to entry into the study. At this time, we obtained samples of peripheral blood from 80 consecutive patients with metastatic renal cell carcinoma, seen at our institution at the Medizinische Hochschule Hannover, Germany since May 1990. Sera were frozen at –80° C until analysis.

Patient characteristics are summarized in Table 1; all patients had a Karnovsky performance status ≥70%, and presented with histologically confirmed metastatic renal cell carcinoma and with clinically progressive disease as demonstrated by standard radiographic procedures. Patients received systemic immunotherapy with subcutaneous IL-2 and interferon-alpha (Atzpodien et al, 1995); treatment was continued until disease progression occurred. Survival was measured from start of systemic therapy.

ELISA for IL-10

Pretreatment levels of soluble IL-10 were determined using an ELISA assay (IL-10, Medgenix, Ratingen, Germany). All analyses were performed in duplicate, strictly according to the procedures recommended by the manufacturers, and samples were analysed at a dilution resulting in measured concentrations within the range of the standard curves. Normal donor sera showed no measurable IL-10 serum levels in this assay. Sensitivity was at 0.208 pg ml^{-1} with a range of 0.78–50 pg ml^{-1}. Cross-reactivity was specified with less than 1%.

Statistical analysis

The statistical end point in our analysis was overall survival from time of entry into the study. We calculated univariate hazard ratios with 95% confidence intervals, using the Cox proportional hazards model. The simultaneous prognostic effect of various factors was determined in a multivariate analysis using a Cox

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Table 1 Patient characteristics

Variable	*n*	IL-10 ≤ 1 pg ml^{-1} *n*[a]	IL-10 > 1 pg ml^{-1} *n*
Patients	80	59	21
Sex			
Male	60	44	16
Female	20	15	5
Age (years)			
Mean	57	56	57
Range	32–74	37–72	32–74
Metastases			
Lung	59	43	16
Bone	23	17	6
Liver	20	13	7
Brain	11	8	3
Tumour sites			
1	28	24	4
> 1	52	35	17

[a]Serum IL-10 levels were assayed prior to systemic therapy.

proportional hazards model (forward and reverse selection of variables). The probability of overall survival was plotted over time according to the method of Kaplan and Meier (Kaplan et al, 1958). Differences in overall survival between groups were tested as dichotomized prognostic variables. For IL-10, erythrocyte sedimentation rate (ESR) and haemoglobin, Kaplan–Meier estimates were performed defining the best cut-off value for discrimination between poor and good overall survival. For lactate dehydrogenase (LDH), the institutional upper normal limits were chosen as the cut-off (240 U l^{-1}).

RESULTS

Univariate analysis of pretreatment variables and survival

We analysed the ability of various clinical factors and of serum levels of soluble IL-10 to predict clinical outcome. The mean period of follow-up for the surviving patients was 32+ months (range, 2–80+ months). The median survival of all 80 patients entering the study was 21+ months.

We calculated univariate *P*-values with the Kaplan–Meier model (Table 2). In this analysis, IL-10, LDH, haemoglobin and ESR had significant prognostic impact on overall survival. Elevated serum levels of IL-10 (> 1 pg ml^{-1}) were detected in 21 patients and were strongly associated with an unfavourable outcome. Median survival in these patients was 11+ months as opposed to patients with low IL-10 levels (≤ 1 pg ml^{-1}, $n = 59$) who had a median survival of 27+ months ($P < 0.0028$).

All other variables investigated (sex, age, time between diagnosis and progression, lung metastases, bone metastases, liver metastases, brain metastases and local relapse) were not statistically significant for the prediction of patient survival.

Multivariate analysis of risk factors

To identify the most powerful independent predictors, we established a multivariate Cox proportional hazards model containing those factors with significant prognostic value upon univariate analysis. All four univariate risk factors were found to be statistically independent: haemoglobin < 10 g dl^{-1} ($P = 0.0005$), ESR ≥ 50 mm h^{-1} ($P = 0.049$), serum LDH ≥ 240 U l^{-1} ($P = 0.0233$) and serum IL-10 > 1 pg ml^{-1} ($P = 0.0226$). The hazard ratios calculated with a model containing these prognostic factors are shown in Table 3.

Table 2 Impact of pretreatment serum IL-10 levels and clinical factors on patient survival in 80 consecutive patients with advanced renal cell carcinoma by univariate analysis

Variable	Categories compared[a]	*P*-value
Clinical factors		
Sex	Female vs. male	0.89
Age (years)	< 60 vs. ≥ 60	0.155
Time between diagnosis and progression (months)	> 12 vs. ≤ 12	0.16
Haemoglobin (g dl^{-1})	≥ 10 vs. < 10	0.0000
ESR (mm h^{-1})	< 50 vs. ≥ 50	0.0004
LDH (U l^{-1})	< 240 vs. ≥ 240	0.016
Lung metastases	Present vs. absent	0.86
Bone metastases	Absent vs. present	0.18
Liver metastases	Absent vs. present	0.92
Brain metastases	Absent vs. present	0.12
Local relapse	Absent vs. present	0.35
IL-10 (pg ml^{-1})	≤ 1 vs. > 1	0.0028

[a]For each variable, the prognostic significance of the first category listed was assessed by comparing that category with the reference category (the second category listed).

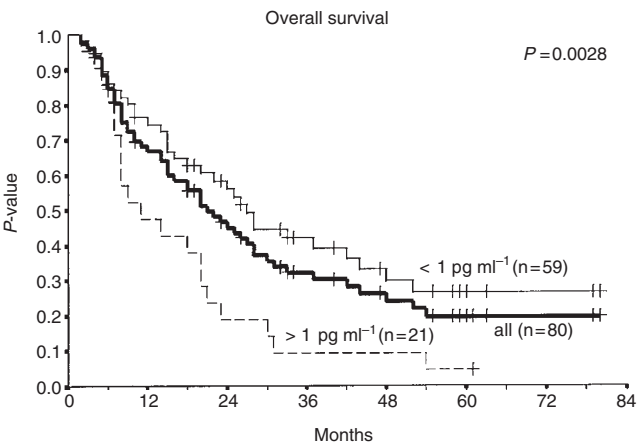

Figure 1 Serum IL-10 and survival in 80 patients with metastatic renal cell carcinoma. Survival curves (Kaplan–Meier estimate) of patients with either low (≤ 1 pg ml^{-1}) or elevated (> 1 pg ml^{-1}) serum levels of IL-10. *P*-value was determined by log rank test. Tick marks represent patients for whom data were censored.

DISCUSSION

Our study demonstrated that elevated pretreatment serum IL-10 is a potentially powerful and independent predictor of overall survival in patients with metastatic renal cell carcinoma. Both by univariate and multivariate analysis, serum IL-10 ($P = 0.0226$), serum LDH ($P = 0.0233$), haemoglobin ($P = 0.0005$) and ESR ($P = 0.049$) were predominant independent predictors of survival; by demonstrating statistic independence, IL-10 adds new information to previously identified risk factors.

Table 3 Hazard ratios associated with pretreatment predictors of overall survival in a multivariate analysis of 80 consecutive patients with metastatic renal cell carcinoma, using Cox proportional hazards models

Variable	Categories compared[a]	Hazard ratio (95% confidence interval)	*P*-value[b]
Clinical factors			
LDH (U l^{-1})	< 240 vs. ≥ 240	0.48 (0.26–0.89)	0.0233
ESR (mm h^{-1})	< 50 vs. ≥ 50	0.31 (0.15–0.65)	0.049
Haemoglobin (g dl^{-1})	≥ 10 vs. < 10	0.20 (0.09–0.45)	0.0005
Interleukin 10 (pg ml^{-1})	≤ 1 vs. > 1	0.50 (0.26–0.89)	0.0226

[a]For each variable, the prognostic significance of the first category listed was assessed by comparing that category with the reference category (the second category listed). [b]For the comparison of the hazard ratio shown with a hazard ratio of 1.0 (as postulated by the null hypothesis).

We have shown previously that elevated serum LDH, elevated ESR and decreased haemoglobin may correlate with tumour load and with poor outcome in patients with metastatic renal cell carcinoma (Lopez-Hänninen et al, 1995).

IL-10 has been detected in different human tumours, e.g. malignant melanoma and renal cell carcinoma (Wang et al, 1995). The location of the compartment of IL-10 secretion remains to be identified in humans with malignant disease. Tumour-infiltrating lymphocytes and tumour cells of renal cell carcinoma themselves have been detected as source for IL-10 (Dummer et al, 1996; Knoefel et al, 1997). The reason for the elevated IL-10 production still remains unclear. The elevated serum IL-10 levels that we found may be correlated with a high intratumoral IL-10 production. As Buer et al (1998) recently demonstrated, IL-10 can be produced by anergic T-lymphocytes and is able to contribute to T-cell anergy. Intratumorally produced IL-10 may therefore lead to anergy of tumour-infiltrating lymphocytes and thereby support intratumoral tolerance. This may be a reason for rapid progression of some patients with malignant disease.

Other malignant diseases have been demonstrated to show a similar mechanism. Thus, elevated serum IL-10 levels have been demonstrated as a negative prognostic factor in patients with active non-Hodgkin's lymphoma (Blay et al, 1993).

In conclusion, in the present study, elevated pretreatment serum levels of IL-10 correlate with poor outcome in renal cell carcinoma. These data may contribute to a better patient selection via risk stratification for future therapeutic trials. This factor may potentially influence the decision-making of available immunotherapeutic strategies of patients with metastatic renal cell carcinoma. In addition, an improved understanding of the pretreatment immunological status of patients with metastatic renal cell carcinoma may provide a lead for future therapeutic strategies focusing on the modulation of this cytokine and/or its receptor structure. Our results will have to be prospectively confirmed in future controlled studies.

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