Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma

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As regulators of malignant cell behaviour and communication with stroma, cytokines have proved useful in understanding cancer biology and developing novel therapies. In renal cell carcinoma, patients with inflammatory reactions are known to have poor prognosis. In order to elucidate the relation between renal cell carcinoma and the host, serum levels of inflammatory cytokines, interleukin-6, tumour necrosis factor α, interleukin-1β, were measured. One hundred and twenty-two patients with renal cell carcinoma and 21 healthy control subjects were studied, and serum cytokine levels were measured using a highly sensitive ELISA kit. As a result, in the control group, interleukin-6, tumour necrosis factor α and interleukin-1β levels were 1.79 ± 2.03, 2.74 ± 0.94 and 0.16 ± 0.17 pg ml⁻¹, respectively. In the renal cell carcinoma patients, they were 8.91 ± 13.12, 8.44 ± 4.15 and 0.53 ± 0.57 pg ml⁻¹, respectively, and significantly higher. In the comparison of stage, interleukin-6 level was significantly higher in the stage IV group compared to the other stage groups including the control group, while tumour necrosis factor α level was significantly higher in each stage group compared to the control group. As for grade, interleukin-6 level was significantly higher in the grade 3 group compared to the control, grade 1 and grade 2 groups, while tumour necrosis factor α level was significantly higher in each grade group compared to the control group. All cytokines had a positive correlation with tumour size. In regard to the correlation with CRP, all cytokines had a positive correlation with CRP, while interleukin-6 had a particularly strong correlation. In conclusion, interleukin-6 may be one of the factors for the poor prognosis of patients with renal cell carcinoma. In addition, tumour necrosis factor α may be useful in the early diagnosis of renal cell carcinoma and post-operative follow-up.

Renal cell carcinoma (RCC) is the most common malignant tumour of the kidney. As regulators of malignant cell behaviour and interaction with the host, cytokines have proved useful in understanding cancer biology and developing novel therapies. Among these cytokines, interleukin-6 (IL-6) has been frequently studied in renal cell carcinoma (RCC). Miki et al. (1989) reported that freshly isolated RCC tissues expressed mRNA of IL-6 and secreted active IL-6, and that IL-6 antiserum specifically inhibited in vitro tumour growth. Takenawa et al. (1991) reported that primary RCC tissues and RCC cell lines expressed mRNA of IL-6 and IL-6 receptors, and that patients with a high level expression of IL-6 had significantly greater incidences of lymph node metastasis and a larger increase in serum c-reactive protein (CRP) than those without it. In addition, fresh RCC cells and cell lines have been reported to produce IL-6 (Blay et al., 1994; Chang et al., 1997). These results suggest that IL-6 acts as an autocrine growth factor for RCC cells. Blay et al. (1992) reported that serum IL-6 was detectable in 66 of 138 patients (48%), and that serum CRP correlated with IL-6 levels. The interval between diagnosis of the primary tumour and metastasis was shorter in patients with a detectable serum IL-6 and/or serum CRP level >50 mg l⁻¹, and they concluded that serum IL-6 and CRP levels were adverse prognosis factors in patients with metastatic renal cell carcinoma. They also reported that the presence of high pretreatment serum IL-6 levels inversely correlated to the response to IL-2 and to survival after immunotherapy with IL-2 in patients with RCC (Blay et al., 1990). Tsukamoto et al. (1992) reported that serum IL-6 was detected in 18 out of 71 patients (25%). They indicated that although IL-6 level did not directly correlate with tumour volume and differentiation grade of the carcinoma, the positive rate increased with progression of the stage, and that serum IL-6 level affected the 5-year survival of patients without distant metastasis. Furthermore, Stadler et al. (1992) and Dosquet et al. (1994) reported that serum IL-6 was a good marker for metastases in RCC patients. However, as these studies did not use a high sensitivity method, the obtained values were lower than the minimum detectable levels in many of the patients. In this study, we used a highly sensitive ELISA kit in order to study in more detail the significance of serum IL-6 in RCC patients.

Tumour necrosis factor α (TNFα) and interleukin-1β (IL-1β) are pleiotropic mediators of biologic responses related to infection, immunity, and inflammation (Le and Vileček, 1987). IL-1, TNFα and IL-6 are cytokines with overlapping biological properties which form a complex network of interactive signals. They are considered major mediators of fever and the production of acute phase proteins (Baumann and Gauldie, 1990). There have been few reports on serum TNF and serum IL-1 in RCC. In multivariate analysis for proportional hazard regression, Dosquet et al. (1997)
reported that TNFα was an independent prognostic indicator, with a normal plasma TNFα being highly predictive for a good prognosis in patients with untreated renal cell carcinoma. Determining the levels of these cytokines in the serum may not only help in understanding the biology of the tumour, but they may also be useful as a diagnosis. In this study, we measured the serum levels of the cytokines in RCC patients using a highly sensitive ELISA kit and also investigated these cytokines in the supernatant of short-term and long-term established RCC cells.

**MATERIALS AND METHODS**

**Patients**

One hundred and twenty-two patients with RCC (mean age: 62.4 years) were studied. Histological examination of their tumours indicated RCC in all cases. Tumour stages were classified according to Robson’s classification. The patients were also divided into three groups according to grade. They did not receive any treatment before the admission to the study. Twenty-one healthy control subjects at several cutoffs.

**Assay of IL-6, TNFα and IL-1β in serum**

Serum from the peripheral venous blood of the patients and healthy control subjects was frozen at −80°C until use. Serum cytokine levels were measured using a highly sensitive ELISA kit (Genzyme Techne Analyza Immunoassay System human IL-6, TNFα, IL-1β). The minimum detectable concentrations were estimated to be 0.094 pg ml⁻¹ for IL-6, 0.17 pg ml⁻¹ for TNFα and 0.1 pg ml⁻¹ for IL-1β.

**Short-term and long-term established RCC lines**

Tumour cells were isolated by enzymatic digestion of surgically removed tumour tissues. Short-term cultured RCC were established by cultivating the tumour cell suspension in a 24-well plastic tissue culture cluster (Corning Inc., NY, USA) in RPMI 1640 (Nikken Bio Medical Laboratory, Japan) supplemented with 10% foetal calf serum (FCS) (Equitech-BIO, Inc., Ingram, TX, USA). Cells were grown for 2 weeks and then split by the use of tripsin. Tumour cells that had been through 4 to 5 passages were characterised by immunostaining with antibodies to RCC (Monosen, San Francisco, CA, USA) and Uro2 (Signet, Dedham, MA, USA). In some of the patients, long-term cell culture of 20 passages or more were possible.

**Table I**

| Serum cytokine levels in relation to tumour stage, grade and metastasis in 124 patients with RCC |
|----------------------------------|------------------|----------------|------------------|
| **IL-6 (pg ml⁻¹)**               | **TNFα (pg ml⁻¹)** | **IL-1β (pg ml⁻¹)** |
| Mean ± s.d. | Median (range) | P value | Mean ± s.d. | Median (range) | P value | Mean ± s.d. | Median (range) | P value |
| Control subjects | 1.79 ± 2.03 | 1.0 (0.2 – 7.8) | | 2.74 ± 0.94 | 2.7 (1.0 – 4.7) | | 0.16 ± 0.17 | 0.08 (0 – 0.61) | |
| RCC patients | 8.91 ± 13.12 | 2.9 (0.2 – 62.4) | | 8.44 ± 4.15 | 7.1 (1.0 – 23.4) | | 0.53 ± 0.57 | 0.2 (0 – 4.5) | |
| Stage | | | | | | | | |
| I | G1 | 4.81 ± 6.44 | 2.8 (0.4 – 34.8) | NS | 7.50 ± 3.22 | 7.2 (1.5 – 23.4) | | 0.75 ± 0.25 | 5.8 (1.4 – 10.7) | NS |
| II | G2 | 3.36 ± 3.24 | 1.9 (12.6) | NS | 7.75 ± 2.55 | 8.2 (1.4 – 16.5) | | 0.53 ± 0.75 | 0.15 (0 – 2.4) | NS |
| III | G3 | 9.61 ± 13.2 | 3.6 (10.4 – 46.5) | NS | 7.99 ± 3.75 | 7.5 (2.4 – 16.5) | | 0.53 ± 0.63 | 0.2 (0 – 1.8) | NS |
| IV | | 21.8 ± 20.9 | 9.4 (10.6 – 62.4) | NS | 9.40 ± 4.92 | 8.7 (1.6 – 19.2) | | 0.59 ± 0.51 | 0.55 (0 – 1.5) | NS |
| Grade | | | | | | | | |
| I | G1 | 3.94 ± 4.64 | 2.6 (0.8 – 20.1) | NS | 6.46 ± 6.15 | 6.3 (1.4 – 11.5) | | 0.31 ± 0.77 | 0.14 (0 – 3.3) | NS |
| II | G2 | 6.32 ± 9.76 | 2.4 (0.4 – 50) | NS | 7.85 ± 7.05 | 8.2 (1.5 – 13.9) | | 0.60 ± 0.89 | 0.25 (0 – 4.5) | NS |
| III | G3 | 13.7 ± 17.1 | 5.3 (1.5 – 47.3) | NS | 8.38 ± 14.5 | 8.4 (3.0 – 16.5) | | 0.28 ± 0.39 | 0.11 (0 – 1.1) | NS |
| Metastasis | | | | | | | | |
| Non-metastasis | 6.83 ± 10.2 | 1.8 (0.2 – 34.8) | NS | 7.94 ± 0.09 | 7.5 (1.4 – 21.5) | | 0.57 ± 0.89 | 0.2 (0 – 4.5) | NS |
| Metastasis | 22.7 ± 20.9 | 3.7 (1.0 – 62.4) | NS | 9.85 ± 4.71 | 9.7 (1.6 – 23.4) | | 0.69 ± 0.54 | 0.65 (0 – 1.6) | NS |

Values are expressed as mean ± s.d., median and range. *P<0.05, **P<0.01, ***P<0.001.

**Statistical analysis**

The data are presented as mean ± standard deviation. The data were analysed by multiple comparison (Fisher’s PLSD) after one-factor ANOVA. Regression lines were fitted by the method of least squares. Receiver operating characteristic (ROC) curves were constructed for IL-6, TNFα and IL-1β by calculating their sensitivity and specificity in differentiating RCC patients from healthy control subjects at several cutoffs.

**RESULTS**

Serum levels of IL-6, TNFα and IL-1β in the RCC patients and healthy control subjects are summarised in Table 1. In the control subjects, IL-6 level was 1.79 ± 2.03 pg ml⁻¹, TNFα level was 2.74 ± 0.94 pg ml⁻¹ and IL-1β level was 0.16 ± 0.17 pg ml⁻¹, while in the RCC patients, IL-6 level was 8.91 ± 13.12 pg ml⁻¹, TNFα level was 8.44 ± 4.15 pg ml⁻¹ and IL-1β level was 0.53 ± 0.57 pg ml⁻¹ and significantly higher (IL-6: P<0.05, TNFα: P<0.0001, IL-1β: P<0.05). RCC patients were also divided according to stage (I, II, III, IV), grade (G1, G2, G3) and with or without lymph node or distal metastasis (metastasis, non-metastasis). When compared according to stage, IL-6 level was significantly higher in the stage IV group compared to the other stage groups including the control group, while TNFα level was significantly higher in each stage group compared to the control group. There was no significant difference in IL-1β level in among the groups. When compared according to grade, IL-6 level was significantly higher in the G3 group compared to the control, G1 and G2 groups, while TNFα level was significantly higher in each grade group compared to the control group. IL-1β level was significantly higher in the G2 group compared to the control group. In the comparison of with or without metastasis, IL-6, TNFα and IL-1β levels were significantly higher in the metastasis group.
compared to the control and non-metastasis groups. TNF\(\alpha\) level was also significantly higher in the non-metastasis group compared to the control group (Table 1).

There was a positive correlation between tumour size and IL-6, TNF\(\alpha\) and IL-1\(\beta\) levels (IL-6: \(r=0.42, P<0.0001\); TNF\(\alpha\): \(r=0.31, P<0.001\); IL-1\(\beta\): \(r=0.41, P<0.005\)) (Figure 1). There was also a positive correlation between CRP and IL-6 and TNF\(\alpha\) levels (IL-6: \(r=0.83, P<0.0001\); TNF\(\alpha\): \(r=0.43, P<0.0001\)) (Figure 2).

The ROC curve analysis was used for the comparison of cytokine levels in the evaluation of RCC diagnosis. In this analysis, area under the curve (AUC) of serum TNF\(\alpha\) was best compared to the other cytokines (Figure 3).

Cytokine levels of the culture supernatants of short-term and long-term established RCC lines are shown in Tables 2 and 3. IL-6 level was high in all patients, and while TNF\(\alpha\) and IL-1\(\beta\) levels could be detected in some of the cells, these levels were low in all cases and under minimum detectable concentrations in most cases. IL-6 tended to be lower in the long-term established RCC lines compared to the short-term established RCC lines.

**DISCUSSION**

IL-6 is a pleiotropic cytokine secreted by a wide variety of cell types including lymphocytes, monocytes and tumour cells (Le and Vilcek, 1989; Kishimoto et al, 1992). There have been several reports that RCC can produce IL-6 and act as an autocrine growth factor (Blay et al, 1994; Chang et al, 1997). In our study of RCC using short-term and long-term established cell culture, IL-6 was also produced at high levels. These observations support the hypothesis that IL-6 may be a marker of tumour aggressiveness. Blay et al (1992) reported that serum IL-6 was positive in 48% of patients with RCC. Tsukamoto et al (1992) reported that it was positive in 25% and Hamao et al (1994) reported that it was positive in 53%. In our study, serum IL-6 could be detected in all patients in the control group and RCC group by using a highly sensitive ELISA kit in our present study. Tsukamoto et al
Furthermore, IL-1 is recognised as a potentially powerful immune mediator and inflammatory cytokine, which can activate the production of acute-phase proteins including CRP. This process is mediated by the complex network of interactive signals (Le and Vilcek, 1987) and forms a complex network of interactions with overlapping biological properties (Le and Vilcek, 1987) and are produced mainly by cells of the monocytes–macrophage series (Nathan, 1987). It has been reported that TNFα is produced from RCC cells (Gogusev et al., 1993), but Waase et al. (1992) reported that mRNA of TNF was expressed in infiltrating monocytes–macrophages in RCC but never by the tumour cells, and that the degree of TNF-α production in RCC depends on tumour spread to the draining lymph nodes. In our study, when the levels in the culture supernatant of short-term and long-term established cells were measured, TNFα and IL-1β levels were lower than the minimum detectable concentrations in many cases, suggesting that they are mainly produced by monocytes–macrophages. There are very few studies on serum TNFα and IL-1β in RCC patients. Bichler et al. (1990) reported that when serum TNFα level was measured in 29 RCC patients, patients with metastatic disease could exhibit increased levels of serum TNFα and that the decrease in TNFα may be associated with a better prognosis.

Dosquet et al. (1997) reported that plasma TNFα level was higher in RCC patients compared to healthy controls, and that it was higher in the disseminated group compared to the undissemi­nated group. They also reported that in multivariate analysis for proportional hazard regression, only TNFα was an independent prognostic indicator, with a normal plasma TNFα being highly predictive for a good prognosis in patients with RCC. (Dosquet et al., 1997). Hamao et al. (1994) reported that serum TNFα was elevated in 6 out of 32 patients (18.8%) and serum IL-1α was elevated in 1 out of 32 patients (3.1%). They also reported that the number of serum TNFα-positive patients increased with stage. In this study, we were able to measure serum TNFα and serum IL-1β levels in all the control subjects and RCC patients by using a highly sensitive ELISA kit. Serum TNFα level was significantly higher in the stage I, II, III and IV groups compared to the control group, and unlike serum IL-6, it was already increased in the low stage. There was no significant difference in serum IL-1β level among the groups. As for grade, it was higher in the grade 1, 2 and 3 groups compared to the control group, and unlike serum IL-6, it was already increased in grade 1. In the ROC curve analysis, AUC of serum TNFα was best compared to other cytokines. These results indicate that the production of IL-6 in RCC patients is related to parameters of tumour progression such as tumour size and the existence of metastasis. As for TNFα, it was already higher in the stage I group and grade 1 group compared to the control group, so that it may be useful in early diagnosis and post-operative follow-up. TNFα has been directly shown to serve as anticancer bioactive molecules. In addition, TNFα indirectly exerts antitumour effects by activation of T cells, NK cells and macrophages. The increased TNFα secretion in RCC patients may be considered as a favourable compensatory mechanism to counteract tumour growth. However, TNFα mediates the catabolism of the body substrate. The increased TNFα secretion may induce a hypermetabolic status that may be a cause of malnutrition and cancer cachexia. Further studies on the role of these cytokines in tumour progression should provide some insight into RCC treatment.

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Figure 3 ROC curve of cytokines in healthy control subjects and patients with RCC.

| Table 3 Cytokine levels of long-term established RCC lines culture supernatant |
|Pt. no. | Age (years) | Sex | Stage | Grade | IL-6 (pg ml⁻¹) | TNFα (pg ml⁻¹) | IL-1β (pg ml⁻¹) |
|-------|-------------|-----|-------|-------|---------------|----------------|----------------|
| 1     | 58          | M   | II    | G2    | 8060          | 39             | 0              |
| 2     | 71          | F   | I     | G1    | 2290          | 0              | 0              |
| 3     | 62          | M   | I     | G1    | 1230          | 0              | 0              |
| 4     | 70          | M   | I     | G2    | 2650          | 0              | 0              |

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