Pretreatment with Interleukin-2 Modulates Perioperative Immunodysfunction in Patients with Renal Cell Carcinoma

(immunomodulation / kidney cancer / IL-2 / complementary medicine)

M. BÖHM[1], A. ITTENSON[2], T. KLATTE[1], K. F. SCHIERBAUM[1], F.-W. RÖHL[3], S. ANSORGE[2], E. P. ALLHOFF[1]

[1]Department of Urology, [2]Institute of Experimental Internal Medicine and [3]Institute of Biometrics and Medical Informatics, Otto-von-Guericke-University, Magdeburg, Germany

Abstract. Complex perioperative immunodysfunction occurs in patients with renal cell carcinoma undergoing nephrectomy. Here, the effect of pretreatment with IL-2 is addressed. Of 63 patients who underwent tumour nephrectomy, 26 patients received four doses of 10 Mio IE/m^2 IL-2 b.d. s.c. (i.e. a total of 40 Mio IE/m^2) a week before operation, 37 did not. Parameters of cellular and humoral immunity (differential blood count, T-cell markers CD2, CD3, CD4, and CD8, B-cell markers CD19 and CD20, monocyte markers CD13 and CD14, NK-cell marker CD16, activation markers CD25, CD26, CD69 and HLA-DR, and cytokines IL-1-receptor antagonist (IL-1RA), IL-2, soluble IL-2-receptor (sIL-2R), IL-6, IL-10, and TGFß) were measured in peripheral venous blood. Blood was drawn before IL-2, one day before and immediately after the operation, and on the 1st, 3rd, 5th, and 10th postoperative day.

All patients showed postoperatively elevated leukocyte and granulocyte counts, and elevated serum levels of cytokines IL-6 and IL-10. T-cell and activation markers were decreased. However, all these alterations were less accentuated in patients who had been pretreated with IL-2. Monocyte counts and IL-2 and TGFß levels were decreased, but IL-1RA and sIL-2R levels were elevated in pretreated patients. IL-2-related toxicity was WHO grade I-II in all patients, grade III in one patient. The anaesthetic regimen had no measurable effect. IL-6 concentrations were higher in renal venous than in venous pool blood, indicating IL-6 production in the tumour *in vivo*. Tumour-specific survival was better in pretreated patients with tumours extending beyond the kidney.

Pretreatment with IL-2 modulates perioperative immunodysfunction in patients undergoing tumour nephrectomy. This affects in particular T-cell-mediated immunity and levels of cytokines IL-10 and IL-6. The IL-2 application scheme used here was followed by distinct counter regulation including monocytes, IL-2, sIL-2R, IL-1RA and TGFß. Taken together, pretreatment with IL-2 may complement surgery in the treatment of patients with renal cell carcinoma, and may help close the therapeutic gap between neo-adjuvant and adjuvant immunotherapy.

Received January 20, 2003. Accepted February 24, 2003.

Clinical Science Award 2002

Corresponding author: Malte Böhm, Department of Urology, Otto-von-Guericke-University, Leipziger Straße 44, 39120 Magdeburg, Germany. Tel.: +49-391-67-15036; fax: +49-391-67-15094; e-mail: malte.boehm@medizin.uni-magdeburg.de.

Abbreviations: CD – cluster of differentiation, ECOG – Eastern Cooperative Oncology Group, EDTA – ethylenediaminetetraacetate, IL – interleukin, LAK – lymphokine-activated killer, NK cell – natural killer cell, TGFß – transforming growth factor beta, UICC – Union Internationale Contre le Cancer, WHO – World Health Organization.

Surgery is the mainstay of therapy of localized renal cell carcinoma. This approach can be curative if the disease is diagnosed and treated while being confined to the kidney. Surgery (Parry-Billings et al., 1992; Allendorf et al., 1997; Hensler et al., 1997; Kuntz et al., 1998; Gitzelmann et al., 2000; Leaver et al., 2000; Ogawa et al., 2000;), including renal surgery (Böhm et al., 2001), and related measures, however, appear to be associated with distinct perioperative immunodysfunction. This may be a reason why more patients with organ-confined disease appear to die from relapse after complete resection of the tumour than are expected to have micrometastases at the time of operation (Giuliani et al., 1990; Herrlinger et al., 1991; Sánchez de la Muela et al., 1991), in particular those with initially small tumours less than 6.5 cm in diameter (Hultén et al., 1969). Dissemination of tumour cells into the blood pool is not a rare event after all in renal cell carcinoma (McKiernan et al., 1999; Ashida et al., 2000; Bilkenroth et al., 2001), but only the minority of disseminated tumour cells appear to be able to attach to and survive in body tissue and form metastases. The immune system being impaired in the early postoperative period may facilitate the implantation of disseminated tumour cells and their early metastatic growth.

Interleukin-2 (IL-2) is an established modulator of the immune system. The mature 15.5 kDa glycoprotein protects T cells from glucocorticoid-induced apoptosis and enhances immunoglobulin production. *In vivo* T cells – in particular activated T-helper (CD4$^+$) type 1 cells – are the major source of IL-2. IL-2 activates a variety of cells in the immune system, including

T-helper cells, cytotoxic T cells, B cells, macrophages, natural killer (NK) cells and lymphokine-activated killer (LAK) precursors. IL-2 can also act as an autocrine growth factor for the rapid clonal expansion of antigen-activated cells. Thus, it boosts both cellular and humoral immunity. Recombinant IL-2 is therefore being used as a key agent in the immunotherapy and combined immuno/chemotherapy of a variety of tumour entities, including renal cell carcinomas. Newer schemes using dendritic cells emerge which use IL-2 as a co-stimulant. IL-2 has been used to attenuate postoperative immunosuppression in colorectal cancer (Nichols et al., 1992; Nichols et al., 1993; Bovo et al., 1995; Deehan et al., 1995; Brivio et al., 1996; Brivio et al., 1997), and non-small cell lung cancer (Masotti et al., 1998), and a prognostic benefit has been attributed to its use (Brivio et al., 1996; Brivio et al., 1997; Masotti et al., 1998). It has to date, however, not yet been used as a perioperative agent in renal cell carcinomas. Here, the effects of preoperative administration of subcutaneous IL-2 in patients with renal cell carcinoma are addressed.

Material and Methods

Patients' selection

Patients who underwent tumour nephrectomy were offered preoperative immunomodulation with IL-2. Of 32 patients, 26 gave their informed consent. These patients were given a total of four doses of 10 Mio IE per m^2 IL-2 (ProleukinR, Chiron) b.d. s.c. a week before operation together with an antipyretic co-medication of 1 g paracetamol tds. A high IL-2 dosage was chosen in order to initiate a measurable immunomodulation. The administration was started a week before surgery in order to allow the patient's immune system enough time to respond (see discussion). The administration was discontinued several days before the operation so that IL-2-related toxicity did not interfere with the operation. Performance was assessed using the ECOG score, and tumours were staged using the UICC-TNM classification. IL-2-related toxicity was assessed according to WHO criteria (World Health Organization, 1979). Thirty-seven patients who received no IL-2 pretreatment and had given informed consent to blood withdrawal served as a control. Peripheral venous blood was collected before administration of IL-2, one day before and immediately after the operation, and on the 1^{st}, 3^{rd}, 5^{th}, and 10^{th} postoperative day. Open surgery was performed under general anaesthesia, and no preoperative haemodilution was used. Only patients between 35 and 80 years of age were included in order to minimize an age bias. Also, septic patients, patients who did not consent, and emergencies were excluded. The study has been approved by the local ethics committee.

In order to assess the effect of anaesthesia, additional blood was drawn from a subset of patients (N=18) before and after the induction of anaesthesia, but before a skin incision was made.

In an attempt to discriminate whether the changes originate within the tumour-bearing kidney or are rather a phenomenon of redistribution between various body compartments, in a subset of patients (N=19) renal venous blood and a sample from the general blood pool (peripheral or central) were collected at the same time during operation.

Immunological analyses

Parameters of cellular and humoral immunity (differential blood count, T-cell markers CD2, CD3, CD4, and CD8, B-cell markers CD19 and CD20, monocyte markers CD13 and CD14, NK-cell marker CD16, activation markers CD25, CD26, CD69 and HLA-DR, as well as the cytokines IL-1-receptor antagonist, IL-2, soluble IL-2-receptor, IL-6, and IL-10) were measured using a standard technique or commercially available kits. Cellular parameters: whole blood (100 µl, EDTA) was stained with monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin chlorophyll protein (PerCP). All antibodies were purchased from Becton Dickinson (Becton Dickinson, Heidelberg, Germany). Erythrocytes were lysed by incubation with FACS lysing solution (Becton Dickinson) for 15 min, and leukocyte subsets were determined by flow cytometry using a FACS Calibur (Becton Dickinson). Cytokines and soluble cytokine receptors: serum was harvested from whole blood (0.105 M sodium citrate) after centrifugation at 800 *g* and stored at –70°C until analysis. Enzyme immunoassays (R&D Systems GmbH, Wiesbaden, Germany) were used for the analyses according to the recommendations of the manufacturer. The TGFß assay was performed as described (Danielpour, 1993) with minor alterations. For this assay relative values were calculated because of a base line shift due to a change of batches.

Statistical analyses

Group differences were calculated using a t-test for each time point. A t-test was also performed for the analysis of renal venous data and for the analysis of data before and after induction of anaesthesia. A P value of 0.05 and less was considered significant. Survival was calculated using the log rank module. The statistical software SAS, version 6.12, PROC GLM (SAS Institute, Cary, NC, USA, SAS Institute Inc., 1994) was used.

Results

The two groups appeared comparable: ECOG performance score on admission was 1 or 2 for all patients and did not differ between the groups. Tumour stages of

the patients who did not receive IL-2 were: organ-confined (T1-2N0M0) 23/37, beyond Gerota's fascia (T3N0M0) 10/37, and metastasized (N+M+) 4/37. Tumour stages of the patients who received IL-2 were: organ-confined (T1-2N0M0) 15/26, beyond Gerota's fascia (T3) 8/26, and metastasized (N+M+) 3/26. All patients who received IL-2 suffered from IL-2-related toxicity WHO grade I-II, one patient suffered from toxicity WHO grade III. Toxicity subsided usually the day after the last IL-2 injection. Operation had to be postponed once because of elevated liver enzymes.

Pretreated patients showed a marked reduction of their postoperative monocyte and CD13/CD14 counts. In constrast, the postoperative depletion of intravasal lymphocytes was attenuated. This effect is caused by both a preoperative increase (day -7 vs. day -1) and a less accentuated T-cell depression (CD2, CD3, CD4, and CD8). It is noteworthy that this effect is more clearly seen with total T cells (CD3) and T-helper cells (CD4) than with cytotoxic T cells (CD8). NK cells (CD16) and B cells (CD19, CD20) showed a trend to increase after pretreatment with IL-2 (day -7 vs. day -1), but were not significantly different from non-pretreated patients. Activation markers increased significantly after the administration of IL-2 (day -7 vs. day -1) and remained above the levels of non-pretreated patients.

The postoperative increase of cytokines IL-6 and IL-10 on days 0 and 1 was less accentuated in IL-2-pretreated patients. As expected, IL-2 levels were increased after the administration of IL-2, but downregulated during the early postoperative period. Following the IL-2 challenge, levels of the soluble IL-2 receptor were elevated during the postoperative period with a tendency to approach pretreatment levels on day 10. This was mirrored by the CD25, i.e. membrane-bound IL-2 receptor changes. However, both soluble IL-2 receptor and membrane-bound CD25 were elevated in IL-2-pretreated patients.

IL-1 receptor antagonist levels were elevated after IL-2 pretreatment. TGFß levels were initially lower in pretreated patients: 2.3 ng/mL on day -7 in pretreated patients vs. 16.4 ng/mL on day -1 in non-pretreated patients. Pretreatment with IL-2 increased TGFß levels perioperatively.

The general anaesthetic regimen used in this study seemed to have little effect on the parameters of immunity measured. None of the parameters measured changed significantly after the induction of anaesthesia.

Renal vein measurements corroborate our previous finding (Böhm et al., 2001) that IL-6 is present in the tumour-bearing kidney in higher concentrations than in the general venous blood pool (P = 0.028).

Tumour-specific survival after a mean follow-up of 27 months appeared to be better in pretreated patients (mean survival of 0.87 vs. 0.76, P = 0.127, Fig. 1). If T1 tumours or organ-confined (T1/2) tumours were excluded, tumour-specific survival of pretreated patients appeared to be better: P = 0.065.

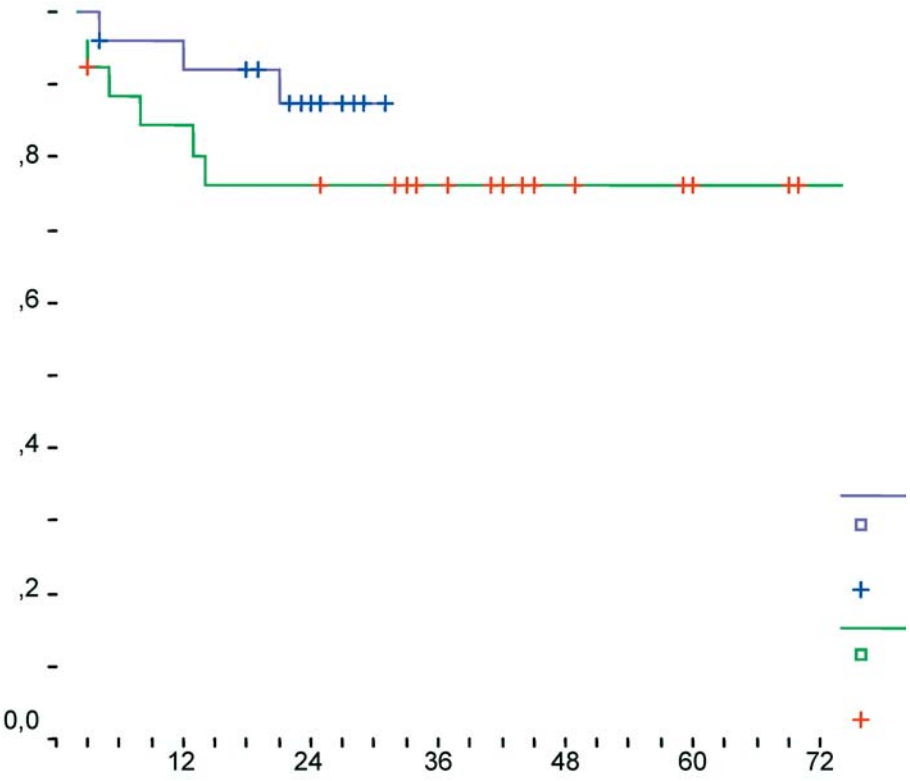

Fig. 1. Tumour-specific survival of patients undergoing tumour nephrectomy. Patients receiving (blue) or not receiving (red) pre-operative immunomodulation with IL-2.

Discussion

The main finding of this study is that pretreatment with the IL-2 can modulate the postoperative immunodysfunction in patients undergoing tumour nephrectomy. In particular is the depression of T-cellular immunity counteracted by pretreatment with IL-2, and the postoperative increase of cytokines IL-6 and IL-10, which are known to have also negative immunomodulatory properties, is attenuated. This appears to benefit predominantly patients with tumours extending beyond the kidney. However, the IL-2 application scheme used here is moderately well tolerated. Frequency and severity of side effects were within the reported range for the dosage used (Brivio et al., 1992; Brivio et al., 1993; Brivio et al., 1996). Considering that the IL-2 appli-

cation scheme used was also followed by a distinct counter-regulation of IL-2, a less toxic scheme but extending into the postoperative period might benefit the patients. In fact, a dose of 1.8 Mio IE/m^2 b.d. s.c., i.e. about one fifth of the dosage used in this study, seemed to be well tolerated when administered until the early postoperative period (Nichols et al., 1992).

The elevated renal venous IL-6 levels suggest that the IL-6 changes are associated with the tumour-bearing kidney in a subset of patients. This is consistent with the finding that IL-6 expression is elevated in primary renal cell carcinomas (Takenawa et al., 1991). IL-6 is a prominent mediator of an acute phase reaction with immunosuppressive properties (Biffl et al., 1996). It has an ability to enhance growth of human renal cell carcinomas (Miki et al., 1989; Koo et al., 1992; Takenawa et al., 1995), and elevated IL-6 levels have been associated with an adverse prognosis in renal cell carcinoma patients (for review see Ulchaker and Klein, 1996). However, the authors did not measure renal venous cytokine levels from the contralateral kidney for ethical reasons. Therefore, a possibility remains that the IL-6 difference was also caused by IL-6 production in the contralateral healthy kidneys in these patients.

The authors realize that measurements and data pertain only to the blood compartment, but that immunological responses and processes also occur within the tissue, such as the proposed production of IL-6 in the operative field (Hisano et al., 1997). This gives room to speculate that the perioperative changes shown may simply reflect a redistribution of cell populations between blood and tissue. A simultaneous investigation of blood and tissue compartment could answer this question. However, when considering the possibility of haematogenous dissemination of tumour cells, which is a major path of metastasis in renal cell carcinoma, the perioperative status of the immune system within the blood stream would be essential.

The authors are equally aware that some of their data are phenotypic. Functional assays such as proliferative responses and cytokine production in allogenic mixed leukocyte reactions (MLR) or polyclonal stimulations of mononuclear cells using phytohaemagglutinin (PHA) or *Staphylococcus aureus* superantigen could provide more information on the functional capacity of the immune system (Lodge et al., 2000). However, if the leukocytes are from peripheral blood, these assays would also monitor intravasal immune status only. For this reason, the authors chose a spectrum of immune parameters that are suitable for monitoring the functional status of the immune cells. Humoral markers, such as some of the cytokine levels measured in this study (IL-2, sIL-2R, IL-6, and IL-10) do indicate on the functional status of the immune system.

Although a variety of T-cell subsets with very diverse functions are being identified, the parameters CD25, CD26, CD69, and HLA-DR viewed together are suitable for monitoring the effect of immunomodulators. Recently, a small subset of CD4+CD25+ T-cells was identified that contribute to negative immunoregulation (Roncarolo et al., 2001) and can suppress the induction of autoimmune disease. This subset comprises an estimated 5 to 10% of peripheral T cells (Roncarolo and Levings, 2000), a fraction that does not compromise the usefulness of parameter CD25, in particular if it is viewed together with other cellular activation markers, such as CD26, CD69, and HLA-DR.

Anaesthesia can alter the immune system (for review see Sheeran and Hall, 1997; Hunter, 1999). However, different general anaesthetic regimens did not influence perioperative IL-6 and IL-10 plasma levels in patients undergoing abdominal hysterectomy (Gilliland et al., 1997) or upper abdominal surgery (Kato et al., 1998). The dosage of opioid had also no significant effect during cardiac surgery (Brix-Christensen et al., 1998). In fact, none of the parameters measured differed significantly after the induction of anaesthesia. This suggests that the effect of the anaesthetic regimen used here was small and did not obscure the effects of IL-2 pretreatment. It appears that anaesthesia does also have only little effect on lymphocyte function as measured as lymphoproliferative response which was not related to the anaesthetic regimen in breast cancer patients (Stanojevic-Bakic et al., 1999).

Surgical stress impairs cellular immunity. Recent studies suggest that the impairment of the immune system parallels the degree of the surgical trauma and tissue injury (Parry-Billings et al., 1992; Allendorf et al., 1997; Hensler et al., 1997; Kuntz et al., 1998; Gitzelmann et al., 2000; Leaver et al., 2000; Ogawa et al., 2000). In a mouse model, postoperative immune function varied inversely with the surgical trauma (Allendorf et al., 1997; Gitzelmann et al., 2000), and postoperative tumour growth was associated with the extent of the operative trauma (Southall et al., 1998). A laparoscopic approach appears to reduce the systemic acute phase reaction associated with cholecystectomy (Gitzelmann et al., 2000) or nephrectomy (Fornara et al., 2000). Blood transfusions (Heiss et al., 1997; Ishijima and Suzuki, 1998) and sepsis (Song et al., 1999; Steinhauser et al., 1999) do also have a capacity to modulate the immune response. Here, the degree of surgical trauma and blood loss was comparable between the two groups, and septic patients were excluded from the study. Therefore, the authors conclude that the differences between IL-2-pretreated and non-pretreated patients are not related to the degree of surgical trauma, blood loss, or sepsis.

Taken together, pretreatment with IL-2 might be a feasible, yet investigational approach to complement surgical therapy and modulate some alterations of the immune system that occur perioperatively in patients undergoing tumour nephrectomy (Böhm et al., 2002). It appears to benefit predominantly patients with tumours

no longer confined to the kidney. Functional data are needed to corroborate the phenotypic data presented here, before a definite recommendation can be given for these patients. In particular is the concept of seeding and circulation of tumour cells during an operation with subsequent formation of micrometastases, however compelling, speculative (Böhm et al., 2001; Böhm et al., 2002) and needs corroboration with more clinical survival data. IL-2 treatment should be initiated about one week before operation in order to allow the patients' immune system enough time to respond to the challenge. However, optimal IL-2 dosage, duration of administration and possible beneficial co-stimulatory agents remain to be determined.

Complementary and alternative medical approaches are receiving increased attention in oncology, and the American Cancer Society has compiled a guide to complementary and alternative medicine (American Cancer Society, 2000). Complementary measures seem to have beneficial effects on the immune system of patients suffering from prostate cancer (for review see Fair, 1999). Such measures might also benefit patients with renal cell carcinoma, because sophisticated surgery, including nephron-sparing and laparoscopic techniques, alone or in combination with radio- or chemotherapy cannot cure metastasized disease.

References

Allendorf, J. D., Bessler, M., Whelan, R. L., Trokel, M., Laird, D. A., Terry, M. B., Treat, M. R. (1997) Postoperative immune function varies inversely with the degree of surgical trauma in a murine model. *Surg. Endosc.* **11,** 427-430.

American Cancer Society (2000) *American Cancer Society's Guide to Complementary and Alternative Cancer Methods*. American Cancer Society, Atlanta.

Ashida, S., Okuda, H., Chikazawa, M., Tanimura, M., Sugita, O., Yamamoto, Y., Nakamura, S., Moriyama, M., Shuin, T. (2000) Detection of circulating cancer cells with von Hippel-Lindau gene mutation in peripheral blood of patients with renal cell carcinoma. *Clin. Cancer. Res.* **6,** 3817-3822.

Biffl, W. L., Moore, E. E., Moore, F. A., Peterson, V. M. (1996) Interleukin-6 in the injured patient – marker of injury or mediator of inflammation? *Ann. Surg.* **224,** 647-664.

Bilkenroth, U., Taubert, H., Riemann, D., Rebmann, U., Heynemann, H., Meye, A. (2001) Detection and enrichment of disseminated renal carcinoma cells from peripheral blood by immunomagnetic cell separation. *Int. J. Cancer* **92,** 577-582.

Bovo, G., Brivio, F., Brenna, A., Fumagalli, L., Perego, P., Brivio, O., Uggeri, F., Lavorato, F., Bratina, G. (1995) Preoperative interleukin-2 immunotherapy induces eosinophilic infiltration in colorectal neoplastic stroma. *Pathologica* **87,** 135-138.

Böhm, M., Ittenson, A., Philipp, C., Röhl, F.-W., Ansorge, S., Allhoff, E. P. (2001) Complex perioperative immunodysfunction in patients with renal cell carcinoma. *J. Urol.* **166,** 831-836.

Böhm, M., Ittenson, A., Schierbaum, K. F., Röhl, F.-W., Ansorge, S., Allhoff, E. P. (2002) Pretreatment with interleukin-2 (IL-2) attenuates perioperative immunodysfunction in patients with renal cell carcinoma. *Eur. Urol.* **41,** 458-468.

Brivio, F., Brivio, O., Lissoni, P., Emanuelli, G., Girlando, M., Uggeri, F. (1997) Presurgical neuroimmunotherapy with melatonin and interleukin-2 in cancer patients. In: *Therapeutic Potential of Melatonin. Front. Horm. Res.*, eds. Maestroni, G. J. M., Conti, A., Reiter, R. J., pp. 137-148, Karger, Basel.

Brivio, F., Lissoni, P., Alderi, G., Barni, S., Lavorato, F., Fumagalli, L. (1996) Preoperative interleukin-2 subcutaneous immunotherapy may prolong the survival time in advanced colorectal cancer patients. *Oncology* **53,** 263-268.

Brivio, F., Lissoni, P., Barni, S., Tancini, G., Ardizzoia, A., Erba, L., Alderi, G., Nociti, V. (1993) Effects of a preoperative course of interleukin-2 on surgical and immunobiological variables in patients with colorectal cancer: a phase 2 study. *Eur. J. Surg.* **159,** 43-47.

Brivio, F., Lissoni, P., Tisi, E., Erba, L., Barni, S., Tancini, G., Rescaldani, R., Alderi, G., Nociti, V. (1992) Effects of a preoperative therapy with interleukin-2 on surgery-induced lymphocytopenia in cancer patients. *Oncology* **49,** 215-218.

Brix-Christensen, V., Tønnesen, E., Sørensen, I. J., Bilfinger, T. V., Sanchez, R. G., Stefano, G. B. (1998) Effects of anaesthesia based on high versus low doses of opioids on the cytokine and acute-phase protein responses in patients undergoing cardiac surgery. *Acta Anaesthesiol. Scand.* **42,** 63-70.

Danielpour, D. (1993) Improved sandwich enzyme-linked immunosorbent assays for transforming growth factor $ß_1$. *J. Immunol. Methods* **158,** 17-25 .

Deehan, D. J., Heys, S. D., Simpson, W., Broom, J., McMillan, D. N., Eremin, O. (1995) Modulation of the cytokine and acute-phase response to major surgery by recombinant interleukin-2. *Br. J. Surg.* **82,** 86-90.

Fair, W. R. (1999) Back to the future – the role of complementary medicine in urology. *J. Urol.* **162,** 411-420.

Fornara, P., Doehn, C., Seyfarth, M., Jocham, D. (2000) Why is urological laparoscopy minimally invasive? *Eur. Urol.* **37,** 241-250.

Gilliland, H. E., Armstrong, M. A., Carabine, U., McMurray, T. J. (1997) The choice of anesthetic maintenance technique influences the antiinflammatory cytokine response to abdominal surgery. *Anesth. Analg.* **85,** 1394-1398.

Gitzelmann, C. A., Mendoza-Sagaon, M., Talamini, M. A., Ahmad, S. A., Pegoli, W. J., Paidas, C. N. (2000) Cell-mediated immune response is better preserved by laparoscopy than laparotomy. *Surgery* **127,** 65-71.

Giuliani, L., Giberti, C., Martorana, G., Rovida, S. (1990) Radical extensive surgery for renal cell carcinoma: long-term results and prognostic factors. *J. Urol.* **143,** 468-474.

Heiss, M. M., Fraunberger, P., Delanoff, C., Stets, R., Allgayer, H., Strohlein, M. A., Tarabichi, A., Faist, E., Jauch, K. W., Schildberg, F. W. (1997) Modulation of immune response by blood transfusion: evidence for a differential effect of allogeneic and autologous blood in colorectal cancer surgery. *Shock* **8,** 402-408.

Hensler, T., Hecker, H., Heeg, K., Heidecke, C.-D., Bartels, H., Barthlen, W., Wagner, H., Siewert, J. R., Holzmann, B. (1997) Distinct mechanisms of immunosuppression as

a consequence of major surgery. *Infect. Immun.* **65,** 2283-2291.

Herrlinger, A., Schrott, K. M., Schott, G., Sigel, A. (1991) What are the benefits of extended dissection of the regional lymph nodes in the therapy of renal cell carcinoma? *J. Urol.* **146,** 1224-1227.

Hisano, S., Sakamoto, K., Ishiko, T., Kamohara, H., Ogawa, M. (1997) IL-6 and soluble IL-6 receptor levels change differently after surgery both in the blood and in the operative field. *Cytokine* **9,** 447-447.

Hultén, L., Rosencrantz, M., Seeman, T., Wahlqvist, L., Åhrén, C. (1969) Occurrence and localization of lymph node metastases in renal carcinoma. *Scand. J. Urol. Nephrol.* **3,** 129-133.

Hunter, J. D. (1999) Effects of anaesthesia on the human immune system. *Hosp. Med.* **60,** 658-663.

Ishijima, N., Suzuki, H. (1998) Blood transfusion and postoperative serum interleukin-6 levels in colorectal cancer patients. *Hepatogastroenterology* **45,** 1011-1013.

Kato, M., Honda, I., Suzuki, H., Murakami, M., Matsukawa, S., Hashimoto, Y. (1998) Interleukin-10 production during and after upper abdominal surgery. *J. Clin. Anesth.* **10,** 184-188.

Koo, A. S., Armstrong, C., Bochner, B., Shimabukuro, T., Tso, C.-L., DeKernion, J. B., Belldegrun, A. (1992) Interleukin-6 and renal cell cancer: production, regulation, and growth effects. *Cancer Immunol. Immunother.* **35,** 97-105.

Kuntz, C., Wunsch, A., Bay, F., Windeler, J., Glaser, F., Herfarth, C. (1998) Prospective randomized study of stress and immune response after laparoscopic vs conventional colonic resection. *Surg. Endosc.* **12,** 963-967.

Leaver, H. A., Craig, S. R., Yap, P. L., Walker, W. S. (2000) Lymphocyte responses following open and minimally invasive thoracic surgery. *Eur. J. Clin. Invest.* **30,** 230-238.

Lodge, P., Jones, L., Bader, R., Murphy, G., Salgaller, M. (2000) Dendritic cell-based immunotherapy of prostate cancer: immune monitoring of a phase II clinical trial. *Cancer Res.* **60**, 829-833.

Masotti, A., Morandini, G., Ortolani, R., Fumagalli, L. (1998) Phase-II randomized study of pre-operative IL-2 administration in operable NSCLC. *Lung Cancer* **20,** 191-202.

McKiernan, J. M., Buttyan, R., Bander, N. H., de la Taille, A., Stifelman, M. D., Emanuel, E. R., Bagiella, E., Rubin, M. A., Katz, A. E., Olsson, C. A., Sawczuk, I. S. (1999) The detection of renal carcinoma cells in the peripheral blood with an enhanced reverse transcriptase-polymerase chain reaction assay for MN/CA9. *Cancer* **86,** 492-497.

Miki, S., Iwano, M., Miki, Y., Yamamoto, M., Tang, B., Yokokawa, K., Sonoda, T. , Hirano, T., Kishimoto, T. (1989) Interleukin-6 (IL-6) functions as an autocrine growth factor in renal cell carcinomas. *FEBS Lett.* **250,** 607-610.

Nichols, P. H., Ramsden, C. W., Ward, U., Sedman, P. C., Primrose, J. N. (1992) Perioperative immunotherapy with recombinant interleukin 2 in patients undergoing surgery for colorectal cancer. *Cancer Res.* **52,** 5765-5769.

Nichols, P. H., Ramsden, C. W., Ward, U., Trejdosiewicz, L. K., Ambrose, N. S., Primrose, J. N. (1993) Peri-operative modulation of cellular immunity in patients with colorectal cancer. *Clin. Exp. Immunol.* **94,** 4-10.

Ogawa, K., Hirai, M., Katsube, T., Murayama, M., Hamaguchi, K., Shimakawa, T., Naritake, Y., Hosokawa, T., Kajiwara, T. (2000) Suppression of cellular immunity by surgical stress. *Surgery* **127,** 329-336.

Parry-Billings, M., Baigrie, R. J., Lamont, P. M., Morris, P. J., Newsholme, E. A. (1992) Effects of major and minor surgery on plasma glutamine and cytokine levels. *Arch. Surg.* **127,** 1237-1240.

Roncarolo, M.-G., Levings, M. K. (2000) The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr. Opin. Immunol.* **12**, 676-683.

Roncarolo, M.-G., Levings, M. K., Traversari, C. (2001) Differentiation of T regulatory cells by immature dendritic cells. *J. Exp. Med.* **193**, F5-F9.

SAS Institute Inc. (1994) *SAS/STAT User's Guide, Version 6*; SAS Institute, Cary, North Carolina.

Sánchez de la Muela, P., Zudaire, J. J., Robles, J. E., Rosell, D., Aguera, L., De Castro, F., Isa, W., Berián, J. M. (1991) Renal cell carcinoma: vena caval invasion and prognostic factors. *Eur. Urol.* **19,** 284-290.

Sheeran, P., Hall, G. M. (1997) Cytokines in anaesthesia. *Br. J. Anaesth.* **78,** 201-219.

Song, G. Y., Chung, C.-S., Chaudry, I. H., Ayala, A. (1999) What is the role of interleukin 10 in polymicrobial sepsis: anti-inflammatory agent or immunosuppressant? *Surgery* **126,** 378-383.

Southall, J. C., Lee, S. W., Bessler, M., Allendorf, J. D., Whelan, R. L. (1998) The effect of peritoneal air exposure on postoperative tumour growth. *Surg. Endosc.* **12,** 348-350.

Stanojevic-Bakic, N., Vuckovic-Dekic, L., Radomirovic, S., Juranic, Z., Jovanovic, N. (1999) The influence of surgery and anesthesia on lymphocyte functions in breast cancer patients: in vitro effects of indomethacin. *Neoplasma* **46,** 54-60.

Steinhauser, M. L., Hogaboam, C. M., Kunkel, S. L., Lukacs, N. W., Strieter, R. M., Standiford, T. J. (1999) IL-10 is a major mediator of sepsis-induced impairment in lung antibacterial host defense. *J. Immunol.* **162,** 392-399.

Takenawa, J., Kaneko, Y., Fukumoto, M., Fukatsu, A., Hirano, T., Fukuyama, H., Nakayama, H., Fujita, J., Yoshida, O. (1991) Enhanced expression of interleukin-6 in primary human renal cell carcinomas. *J. Natl. Cancer Inst.* **83,** 1668-1672.

Takenawa, J., Kaneko, Y., Okumura, K., Yoshida, O., Nakayama, H., Fujita, J. (1995) Inhibitory effect of dexamethasone and progesterone *in vitro* on proliferation of human renal cell carcinomas and effects on expression of interleukin-6 or interleukin-6 receptor. *J. Urol.* **153,** 858-862.

Ulchaker, J. C., Klein, E. A. (1996) Biology of metastasis and its clinical implications: renal-cell cancer. *World J. Urol.* **14,** 175-181.

World Health Organization (1979) *WHO Handbook for Reporting Results of Cancer Treatment*, World Health Organization, Geneva.