Title	Long-term outcome from a study on the prevention of postoperative recurrence in patients with renal cell carcinoma: could PBL be a predictor of postoperative recurrence in patients postoperatively treated with IFN-gamma?
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LONG-TERM OUTCOME FROM A STUDY ON THE PREVENTION OF POSTOPERATIVE RECURRENCE IN PATIENTS WITH RENAL CELL CARCINOMA : COULD PBL BE A PREDICTOR OF POSTOPERATIVE RECURRENCE IN PATIENTS POSTOPERATIVELY TREATED WITH IFN-γ ?

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This study examined the outcome of postoperative recurrence therapy on renal cell carcinoma (RCC) prevention involving treatment with single doses of interferon-γ (IFN-γ). From 1990–2000, 37 patients with no distant metastasis at the time they underwent a nephrectomy were enrolled in this investigation. Subcutaneous IFN-γ was administered once a week. Total and differential white blood cells were counted before the pre-administration of IFN-γ and then monthly thereafter for all patients. Blood lymphocyte subsets were analyzed phenotypically by direct immunofluorescence.

Disease-free survival rates (DFSR) at 5 and 10 years were 81.7% and 75.9%, respectively. To clarify the effects of preoperative peripheral blood lymphocyte (PBL) and NK activity on DFSR, we categorized the patients into two groups according to the median number of PBL before the administration of IFN-γ. Except for CD11b, PBL level had no effect on DFSR. Multiple logistic regression analysis showed that CD11b levels greater than 16.5% were associated with 25.35 odds ratio increase in the risk of postoperative recurrence.

A multivariate analysis found that CD11b may be an independent factor for postoperative recurrence. In terms of preventing postoperative recurrence, our results showed that an elevated CD11b level may indicate patients who can benefit from further combination therapy.

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Key words : Renal cell carcinoma, Postoperative recurrence, PBL, Interferon-γ

INTRODUCTION

Our understanding of the mechanism by which interferon (IFN)-γ mediates the antineoplastic action of immunomodulation is incomplete. Although the mechanism producing an antineoplastic effect remains unclear, IFNs are known to process a variety of immunoregulatory characteristics[1], including the activation of macrophages and monocytes[2], increased natural killer (NK) cell activity[3], the induction of specific antigen expression on cell surfaces[4] and an enhancement of cytotoxic lymphocyte activity[1].

IFN-γ is produced by T-lymphocytes after their activation by a variety of antigens and cytokines. It appears to act on cell surface receptors other than those shared by IFN-α and IFN-β[2]. Peripheral blood sample studies performed over the last 10 years have been unable to clarify whether the lymphocyte subset plays a role during immunomodurative therapy in patients with renal cell carcinoma (RCC). Aulitzky et al. recently demonstrated a 15% overall response rate

and a 13-month median survival in patients with advanced RCC. They concluded that low-dose IFN-γ is effective in a small proportion of patients with disease limited to a single site[3].

Regarding peripheral blood lymphocytes (PBL), Bukowski reported that PBL has no correlation with a favorable prognosis[4]. It is well known that approximately 25% of patients with localized RCC experience postoperative recurrence after undergoing nephrectomy[5]. Although IFN-α, IFN-γ, antitumor agents and Interleukin 2 are often administered to prevent postoperative recurrence, the usefulness of these treatments remains unclear for localized RCC.

We investigated the anti-RCC host immune environment before the administration of IFN-γ and changes to PBL resulting from the postoperative administration of IFN-γ. To study the tumor-host interactions using flow cytometry (FCM), we examined the immunophenotype of the PBL in patients with RCC. Our study was based on the hypothesis that a host's immunological reaction to a tumor is reflected in both

the clinical status of the patient and changes in the lymphocyte subpopulations in the PBL. The objective of this study was to evaluate the clinical and immunological effectiveness of long-term follow-up using IFN-γ treatment in preventing the postoperative recurrence of RCC.

METHOD

Patients

From January 1990 to December 2000, 37 patients with no distant metastasis at the time they underwent a nephrectomy were enrolled in this investigation. Before entry, all of the patients were evaluated by physical examination, computed tomographic (CT) scan of the chest and abdomen, and radionuclide bone scan (BS). No other malignancy was apparent. Other inclusion criteria were patient age less than 80 years and no renal hepatic dysfunction. Exclusion criteria consisted of evidence of any serious active infection. All patients had a performance status of either grade 0 or 1 (Karnofsky index).

The clinicopathologic factors were examined based on the criteria of the Japanese Urological Association[6].

Treatment

In an out-patient setting, subcutaneous IFN-γ was administered once a week. IFN-γ (Immuno max; Shionogi Tokyo, Japan) was administered at a dose of 5 million Japan Reference Units (JRU)/body over at least a one-year period. All patients were treated on an out-patient basis[7-9]. None of the patients received any other concomitant chemotherapy.

Immunological effects

The IFN-γ treatment regimen was started approximately 10 days after nephrectomy. Total and differential white blood cells were counted before the pre-administration of IFN-γ and then monthly thereafter for all patients. Blood lymphocyte subsets were analyzed phenotypically by means of direct immunofluorescence. The cells were analyzed by FCM using a FACScan

apparatus (Cytoron Absolute). The following monoclonal antibodies were used : CD3 (Dako), CD4 (Dako), CD8 (Nichirei), CD11b (Becton Dickson), HLA-DR (Becton Dickson), CD16 (Becton Dickson), and CD 57 (Becton Dickson). NK activity was determined by a standard 4-hour ^{51}Cr release assay. Effector cells (peripheral blood mononuclear cells) were added to yield an effector cell to target cell ratio of 20 : 1.

Toxicity

The patients were carefully monitored each month. Serum total aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltransferase levels were assessed to test for hepatic toxicity. Renal toxicity was evaluated based on changes in serum creatinine levels. Toxicity was graded according to World Health Organization (WHO) criteria[10].

Method of follow-up

Throughout the study our postoperative follow-up protocol included chest radiography and CT scans every 3 months and BS once a year for 3 years.

Statistical analysis

Statistical analysis was performed using the χ^2 analysis. Disease-free survival rate (DFSR), defined as the interval from entry to the study until identification of metastasis, was calculated using the Kaplan-Meier method, and statistical significance was analyzed by log rank test. Significant factors were analyzed by computer, using multiple logistic regression analysis and Firth's method[11] to determine the prognostic value. All analyses were performed using STATVIEW (Abacus Concepts, CA, USA) and JMP 3.2 (SAS Institute Inc. NC, USA). Differences between the groups were considered to be statistically significant when p was less than 0.05.

RESULT

Although all 37 patients demonstrated grade I–II toxicity in the form of fever and fatigue (grade 1–2), most toxic effects were self-limited and rapidly reversed

Table 1. Clinicopathologic characteristics of the 37 patients

Median age (yr)	59 (33–76)						
Gender (n)	Male 24 (67) Female 13 (33)						
Tumor size (cm)	Median size 7 (2–20)						

Tumor stage	Vessel invasion		Capsular invasion		Nuclear grade		
	Negative	Positive	Negative	Positive	1	2	3
T1a	1/2 (50)	0/2 (50)	1/4(25)	0/0(0)	0/2	1/2	0
T1b	0/1 (0)	0/3 (0)	0/4(0)	0/0 (0)	0/2	0/1	0/1
T2	0/5(0)	2/5 (40)	2/8(25)	0/2 (0)	1/6	1/3	0/1
T3	3/15 (20)	1/4 (25)	0/4 (0)	4/15 (27)	1/7	2/11	1/1
p value	P=0.081		p=0.012		p=0.75		

Median follow-up (months)	60.0 (5–141)						

The numerator indicates the number of recurrent cases, while the denominator indicates the total number of cases in each category. Statistical analysis was performed using χ^2 test. Numbers in parentheses indicate percentage, except for median age, median tumor size and median follow-up, for which numbers in parentheses indicate the range.

regardless of the treatment regimen.

The median follow-up period was 60 months. The characteristics of the patients are shown in Table 1. Seven patients experienced postoperative recurrence, with a median recurrence of 19 months (5–74 months). DFSR at 5 and 10 years were 81.7% and 75.9%, respectively.

With respect to clinicopathologic factors, DFSR at 5 and 10 years for patients with high nuclear grade RCC (nuclear grade $\geqq 3$) were 75% and 75%, respectively, and for patients with low nuclear grade RCC (nuclear grade $\leqq 2$) were 83.3% and 76.9%, respectively (p= 0.30). In addition, DFSR at 5 and 10 years for patients with capsular invasion RCC were 73.0% and 73.0%, and for patients with RCC without capsular invasion were 89.7% and 78.5%, respectively (p=0.55).

To clarify the effect of these factors on DFSR, the patients were divided into two groups according to whether each patient's PBL levels were above or below the median for the group as a whole before the administration of IFN-γ. As shown in Table 2, excluding CD11b, no PBL (CD3, CD4, CD8, CD16, CD57 and NK activity) had any effect on DFSR.

To assess which categories might affect postoperative disease-free status using IFN-γ for the prevention of postoperative recurrence of RCC, we compared the PBL findings with clinicopathologic factors including nuclear grade and capsular invasion. An evaluation of HLA-DR was excluded from this analysis because it influenced other PBL. As shown in Table 3, multivariate analysis
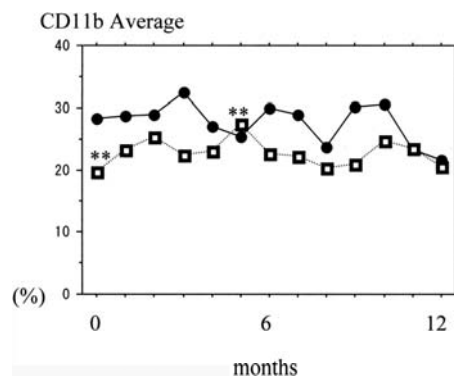
Table 3. Multivariate analysis of PBL and clinicopathological factors for 37 patients with RCC

| Category	Risk ratio	Confidence interval	P value
CD3<66.2 (20)			
$\geqq$66.2 (17)	9.23	−0.35–6.64	0.094
CD4<46.5 (19)			
$\geqq$46.5 (18)	0.21	−6.26–0.90	0.23
CD8<23.3 (19)			
$\geqq$23.3 (18)	2.24	−1.41–3.98	0.46
CD11b<16.5 (19)			
$\geqq$16.5 (18)	25.35	0.56–8.69	0.011
CD16<16.8 (18)			
$\geqq$16.8 (19)	0.38	−3.84–1.81	0.48
CD57<20.7 (17)			
$\geqq$20.7 (20)	1.02	−2.97–2.56	0.98
NK activity<13 (19)			
$\geqq$13 (18)	0.25	−4.70–0.61	0.19
Grade 1.2 (34)			
3.4 (3)	1.47	−2.88–3.39	0.75
Capsular invasion − (20)			
+ (17)	2.20	−1.52–3.68	0.51

Numbers in parentheses indicate the number of patients in each category.

suggests that CD11b and NK activity have predictive potential for postoperative recurrence. Multiple logistic regression analysis showed that CD11b greater than 16.5% was associated with a 25.35 odds ratio increase in the risk of postoperative recurrence. Conversely, NK activity greater than 13% was associated with a 0.25 odds ratio decrease in the risk of postoperative local recurrence.

Regarding PBL, we studied the change in the level of PBL before the administration of IFN-γ each month. At 4 and 5 months postoperatively, CD11b levels in disease-free patients increased significantly (p=0.0031, Student's t test), while patients with postoperative recurrence did not show any significant changes in CD11b

Table 2. Comparison of 5- and 10-year disease-free survival rates (DFSR)

	Recurrence		DFSR		
	(+) n	(−) n	5-year %	10-year %	p value
CD3<66.2	2	18	88.8	88.8	0.25
$\geqq$66.2	5	12	74.7	67.2	
CD4<46.5	4	15	74.2	74.2	0.62
$\geqq$46.5	3	15	88.9	88.9	
CD8<23.3	2	17	87.2	87.2	0.21
$\geqq$23.3	5	13	75.4	62.9	
CD4/8<2.09	5	12	72.8	54.6	0.11
$\geqq$2.09	2	18	88.4	88.4	
CD11b<16.5	1	18	93.8	93.8	0.029
$\geqq$16.5	6	12	69.2	57.7	
HLA-DR<27.2	2	15	94.1	83.7	0.26
$\geqq$27.2	5	15	71.5	71.5	
CD16<16.8	4	14	82.6	74.4	0.85
$\geqq$16.8	3	16	79.6	79.6	
CD57<20.7	2	15	86.5	86.5	0.28
$\geqq$20.7	5	15	77.3	64.5	
NK<13	4	15	77.5	77.5	0.76
$\geqq$13	3	15	85.9	73.6	
Total	7	30	81.7	75.9	

Fig. 1. Changes in CD11b levels during the 12-month follow-up. ** p<0.01. ●: Recurrence (+) n=7. □: Recurrence (−) n=30.

NK activity　Average
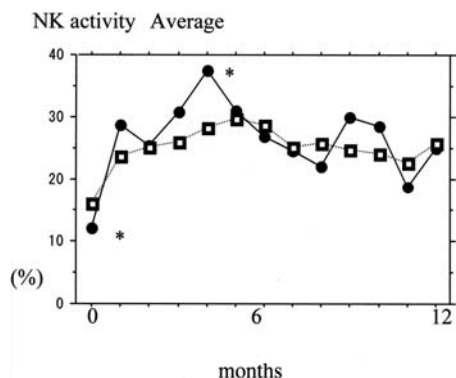
Fig. 2. Changes in NK activity during the 12-month follow-up, * p<0.05. ●: Recurrence (+) n=7. □: Recurrence (−) n = 30. NK activity in patients experiencing recurrence increased significantly between the start of treatment and 4 months of treatment. Postoperative recurrence free cases showed a significant increase in NK activity each month following the administration of IFN-γ (p <0.05).

levels (Fig. 1). Conversely, NK activity in patients experiencing recurrence increased significantly between the start of treatment and 4 months of treatment. In addition, the level of NK activity in all of the disease-free patients during the 12-month follow-up was greater than that observed before administration (Fig. 2).

DISCUSSION

Following radical nephrectomy, the 5-year survival rate for patients with stage I RCC ranges from 60% to 82%, compared with a 5-year survival rate for patients with stage II RCC that ranges from 47% to 80%[12-14].

Minerivini et al. showed that the identification of patients with a high nuclear grade is prognostically important for determining the metastatic potential of pT2 cases, because this group of patients may benefit from adjuvant immunotherapy[15].

Immunotherapy offers the ability to activate the natural immune effector mechanism in a way that allows for the destruction of tumor cells in the body. In addition, immunotherapy efficacy increases in patients with good prognostic factors, that is, a small tumor burden and limited metastatic invasion indicate better median survival rates than a large tumor burden and multiple metastases[16]. Several investigators have attempted to improve the DFSR of postoperative RCC patients through the administration of IFNs. Takahashi et al. demonstrated the efficacy of adjuvant treatment by administering IFN-α for non-metastatic RCC. In that study, 17 of 20 patients remained free of metastasis during follow-up periods ranging from 37 to 57 months[17].

Migliari et al.[18] described the efficacy of postoperative adjuvant immunochemotherapy in which 30 patients (pT2-pT3) received a combination of IFN-α and vinblastine following surgery. Metastases were found in 5 of the 30 patients (16.6%) with a median interval to progression of 24 months, while 15 of 32 patients who had not received combination therapy developed distant metastasis and 2 of 32 patients experienced local recurrence. These findings suggest the theoretical advantages of adjuvant immunochemotherapy for patients with radically resected stage II, III RCC.

We demonstrated the therapeutic effects of IFN-γ and the outcome of a curative resection by analyzing changes in the PBL subsets to identify prognostic indices. Questions remain, however, as to why IFN-γ appears to be effective as a postoperative adjuvant therapy for patients with localized RCC. Answering these questions requires an investigation of the relationship between PBL and postoperative recurrence.

Our results were compared with those of other investigators researching IFNs as an adjunctive treatment for localized RCC. Our study found that 30 of the 37 patients (81%) showed no postoperative recurrence at examinations ranging from 5 to 141 months after surgery. The number of recurrent cases was, however, similar to those reported by other investigators[17,18].

The percentage of CD8 cells in postoperative blood samples has been thought to be a useful indicator of a poor prognosis[19]. Because white blood cells were transiently removed from blood circulation following the subcutaneous administration of IFN-γ, the CD8 positive cells counts declined to approximately 70% of their pretreatment values 24 hours after administration of IFN-γ therapy[8]. In addition, Characiejus et al. reported that the percentage of CD8 high CD57+ lymphocytes in the CD8 subset may have a predictive value in selecting patients with a better chance of favorable prognosis as a result of treatment with IFN[20].

In the immune system, IFN-γ augments the NK cell cytolytic function. Our results in terms of NK activity are consistent with those of other investigators, in which the significance of the NK function in IFN-γ treated RCC patients was demonstrated[7,16,20]. Previously, we found a significant correlation between CD11b and Factor VIII by means of a histopathological analysis and hypothesized that CD 11b may positively affect the angiogenesis of RCC[22]. Mcfarland reported that CD11b promotes cytotoxic T cell activation and may also be a marker of such activation[23]. Regarding relationships between IFN-γ and CD11b, Momose failed to detect whether or not IFN-γ had a significant effect on CD11b expression[24].

As discussed above, CD11b may therefore play a multifunctional role. Thus, further randomized studies, including patients with low- and high-stage disease, are needed to examine the effects of postoperative adjuvant therapy.

CONCLUSION

We analyzed the results of a postoperative adjuvant

treatment regimen for patients with localized RCC using single doses of IFN-γ. Our results also showed that CD11b is potentially an independent factor of DFS in patients with RCC. In terms of preventing postoperative recurrence, our results showed that an elevated CD11b level (greater than 16.5%) may indicate patients who can benefit from further combination therapy.

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和文抄録

インターフェロン γ の腎細胞癌術後再発予防の長期成績
—末梢血リンパ球は術後再発の指標となりえるか—

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[目的] 腎細胞癌術後再発に対するインターフェロンγの単剤投与の有用性について検討した.

[対象と方法] 1990〜2000年までの間に原発巣摘除時に転移病巣のない腎細胞癌37例を対象とした．インターフェロン γ を毎週連続で皮下投与して末梢血のリンパ球の分画について，投与前，投与後各 1 カ月ごとに flow cytometry を用いて検討した．[結果] 37例中 7 例に再発が認められ，非再発率は 5 年が81.7％，10年が75.9％であった．術後再発に関わる影響を調べるため末梢血リンパ球と NK 活性について投与前の中央値でおのおの 2 群に分けた．CD11b と NK 活性を除いて術後再発に関連は認められなかった．多変量解析の結果，投与前 CD11b が16.5％以上だと16.5％未満の症例に比較して25.35倍術後再発が高く，逆に投与前 NK 活性が13％以上の症例は0.25倍術後再発が減少する．[結語] 多変量解析の結果術後に補助療法としてインターフェロン γ 投与した症例では，CD11b と NK 活性が術後再発に関わる独立した因子であることが判明した．また腎細胞癌の術後再発予防という観点から CD11b が高く，NK 活性が低い症例には，さらなる combination therapy が必要と考えられる．

（泌尿紀要 **52**：603-608，2006）