Immunologic Effects of Recombinant Interferon-gamma in Patients with Renal Cell Carcinoma

Ken Marumo, Masaru Mura, Nobuhiro Deguchi, Kouichi Ikeuchi and Hiroshi Tazaki

Department of Urology, School of Medicine, Keio University, Tokyo, Japan
Department of Urology, National Defence Medical College, Tokorozawa, Japan

(Received for publication on January 23, 1990)

Abstract. Immunologic and antitumor effects of human recombinant interferon-gamma were studied in patients with renal cell carcinoma. A daily dose of 6 to $10^6$ units/m$^2$ of interferon-gamma was given by intravenous drip infusion or intramuscular injection to nine patients over a period varying from two to 16 weeks. Antibody-dependent cell-mediated cytotoxicity and OKIa1-positive monocytes count increased significantly after the therapy was started. Interferon-gamma transiently increased OKT3- and OKT4-positive lymphocyte count. Tumor regression was not observed when clinical response was evaluated in seven patients. Two others, who had no measurable metastases, were not evaluated, because interferon-gamma were given to them as post-operative adjuvant therapy. Our results indicate that interferon-gamma stimulated monocytes and enhanced cell-mediated cytotoxicity; they also suggest the necessity of combining monoclonal antibodies and other biological response modifiers that effect tumor-associated antigens. (Keio J Med 39 (2): 97–101, June 1990)

Key words: NK activity, ADCC activity, lymphocyte, monocyte, surface antigen

Introduction

Interferon (IFN), discovered by Isaacs and Lindenmann in 1957 and originally categorized as an antiviral substance, was later used to treat many kinds of cancer, after its anticancer effects were discovered. Renal cell carcinoma (RCC), a target for clinical trial with IFN since 1982, is among the tumors least sensitive to radio-therapy and anticancer chemotherapy. IFN therapy clearly seems to induce a response in a limited group of patients with metastatic RCC and more effective than hormonal therapy or chemotherapy against this disease. Results of experiments in vitro indicate that the anticancer effects of IFN against RCC include inhibition of the growth of the tumor cells and stimulation of the immune system, though the mechanism of these effects in patients with malignant disease remain unclear. The immunologic and antitumor effects of human recombinant IFN-γ in nine patients with RCC are reported here.

Patients and Methods

Patient selection

IFN-γ was given to nine patients with advanced RCC (Table 1), seven of whom had distant metastases before the therapy was begun. IFN-γ was administered as an adjuvant therapy to two patients who had no measurable metastases; case 2, that of a patient who received bilateral partial nephrectomy for RCC in both kidneys and lung lobectomy for pulmonary metastases of RCC; and case 5, in which metastasis was observed in a specimen obtained at para-aortic lymphnode dissection.

Preparation of IFN-γ

Specific activity of IFN-γ was more than $10^7$ units/mg protein. The titer of IFN-γ was expressed in accordance with the titer of human IFN-γ standard received from National Institute of Health (USA). IFN-γ, produced by Toray Industries, Inc., Japan, was used in case 1 through 6, and IFN-γ, produced by Biogen, Inc., Switzerland, was used in case 7, 8, and 9. One-hour intravenous drip infusions containing 6 to $10^6$ units/m$^2$ of IFN-γ were given daily while the patients were hospitalized, and IFN-γ was given intramuscularly six days a week on an outpatient basis after at least 14 days of inpatient treatment.
Table 1 Characteristics of patients treated with rIFN-γ

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Metastases</th>
<th>PS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Weeks of therapy</th>
<th>Response&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>53 M</td>
<td>Lung</td>
<td>1</td>
<td>6</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>54 M</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>6</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>67 M</td>
<td>Bone</td>
<td>2</td>
<td>6</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>66 M</td>
<td>Duodenum</td>
<td>1</td>
<td>6</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>42 F</td>
<td>ND</td>
<td>0</td>
<td>3</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>56 M</td>
<td>Lung</td>
<td>0</td>
<td>12</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>57 F</td>
<td>Lung</td>
<td>2</td>
<td>4</td>
<td>PD</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>55 M</td>
<td>Bone</td>
<td>1</td>
<td>16</td>
<td>NC</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> ECOG performance status.
<sup>b</sup> CR indicates complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluated.
<sup>c</sup> ND indicates not detectable.

Mononuclear cell separation

Peripheral blood mononuclear cells were isolated, following the method of Böyum, from heparinized blood obtained from the patients.

Cytotoxicity assay

To measure natural killer (NK) activity, a four-hour ⁵¹Cr-release assay was used to determine cell-mediated cytotoxicity against K562 cells, as previously described. Antibody-dependent cell-mediated cytotoxicity (ADCC) was measured similarly, using a six-hour ⁵¹Cr-release assay. Chicken red blood cells (CRBC) labeled with ⁵¹Cr were used as target cells. To assay for ADCC, rabbit anti-CRBC serum at a final dilution of 1:10,000 was added to each well. Values are presented as the mean of triplicated samples.

Analysis of lymphocyte and monocyte surface antigen

Cell surface antigens, as revealed by monoclonal antibodies (MoAb), were enumerated by direct or indirect immunofluorescence assay. For direct immunofluorescence, the fluorescein isothiocyanate (FITC)-conjugated MoAb against OKT3 (mature T cells), OKT4 (helper/inducer T cells), OKT8 (suppressor/cytotoxic T cells), OKT11 (pan T cells), Leu 7 (NK cells), Leu 11 (NK cells), IL-2R (human interleukin-2 receptor) were used to stain the mononuclear cells. The expression of the antigens on cell surfaces were determined using FITC-labeled MoAb and flow cytometry. For indirect immunofluorescence, cells were similarly treated with MoAb against OKIa1 (Ia Frame) or OKM1 (NK cells, mature monocytes), incubated with fluoresceinated goat anti-mouse IgG for 30 min at 4°C, washed twice, and brought up in 1.0 ml for flow cytometry. The OKT reagents were prepared at Ortho Pharmaceutical Corp., Raritan, NJ, USA. The Leu reagents and MoAb against IL-2R were prepared at Becton Dickinson Monoclonal Center Inc., Mountain Views, CA, USA.

Response criteria

Clinical response was evaluated using response criteria adopted by Japan Society for Cancer Therapy. Complete response was defined as disappearance of all known disease. Partial response was 50 percent or greater decrease in the product of diameters (width × length) in measurable lesions. No lesion could have progressed nor any new lesion appear. No change was defined as less than 50 percent decrease or less than 25 percent increase for at least four weeks in measurable lesion. Progressive disease was defined as an increase of at least 25 percent in measurable lesion or appearance of new lesions.

Statistical analysis

The results were compared using the paired t test. A p value of less than 0.05 was considered statistically significant.

Results

IFN-γ had limited objective effects on NK activity, which had decreased transiently three days after IFN therapy was started, recovered in two weeks and increased slightly by the fourth week after the start of therapy. These changes were not significant, however. On the other hand, ADCC activity significantly increased in two weeks and four weeks after start of the therapy (Table 2).

OKT3-positive lymphocytes and OKT4-positive lymphocytes increased transiently in three days after the start of therapy, but returned to its initial level in two weeks and four weeks after the start of therapy (Table 3). Mean proportion of OKIa1-positive lymphocytes appeared to increase in two weeks and four weeks after start of the therapy, though these changes were not significant. OKIa1-positive monocytes significantly increased in three days, two weeks, and four weeks after start of the therapy (Table 4).

IFN-γ did not influence the phenotype in the OKT8-OKT11-, Leu7-, Leu11-, IL-2R-, OKIa1- or OKM1-positive lymphocytes, or in the OKM1-positive monocytes.
Clinical response was evaluated in seven patients who had measurable metastases. Tumor regression was not achieved; five patients showed no change; and two others had progressive disease (Table 1). Two patients were not evaluated, because they had no measurable lesion and IFN-γ was administered as post-operative adjuvant therapy.

Discussion

IFN is known to produce tumor regression in patients with RCC, however, a response rate, which was noted in 12 trials using IFN-α, was 16%8. These results are by no means satisfactory. Prospects for therapy include trials of IFN with other biological response modifiers (BRMs) or chemotherapeutic agents.

The antitumor activity of IFN is thought to depend mainly on direct growth-inhibiting effects on tumor cells and immunomodulatory effects. On the assumption that IFN would directly suppress tumor-cell proliferation in tumor-bearing patients, several studies have been performed evaluating combinations of IFN and chemo-
Table 4  OKIa1-positive lymphocytes and OKIa1-positive monocytes in peripheral blood mononuclear cells, before and after start of rIFN-γ therapy

<table>
<thead>
<tr>
<th>Case No.</th>
<th>OKIa1-positive lymphocytes (%)</th>
<th>OKIa1-positive monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td>28.5</td>
<td>24.1</td>
</tr>
<tr>
<td>2</td>
<td>22.8</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>7.8</td>
<td>13.9</td>
</tr>
<tr>
<td>4</td>
<td>18.4</td>
<td>32.7</td>
</tr>
<tr>
<td>5</td>
<td>25.5</td>
<td>32.7</td>
</tr>
<tr>
<td>6</td>
<td>16.0</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Mean: 19.9 22.3 46.1 37.3 83.3 97.5* 95.5* 95.0*

*p < 0.01, when compared with value before rIFN-γ therapy.

ND indicates not determined.

therapeutic agents including vinblastine,⁹,¹⁰ doxorubicin,¹¹ and cyclophosphamide¹² for the treatment of renal cell carcinoma. These studies have failed to prove enhanced antitumor activity over treatment with IFN alone. On the other hand, they have generally demonstrated significantly greater toxicity due to intolerable flu-like side effects, myelosuppression, and liver dysfunction. These results prompted clinical trial with IFN combined with BRMs¹³,¹⁴ rather than with cytotoxic chemotherapeutic agents, which are generally thought to suppress the immune system, which should be properly stimulated by administration of IFN. To determine the optimum combination of these agents, the biological effects of IFN must be clarified in patients, as well as in vitro and in animal experiments.

This study showed that ADCC activity was enhanced during the early period of administration of IFN-γ, and a high level of activity was maintained throughout the therapeutic period. Since cytotoxicity was measured using peripheral blood mononuclear cells in this study, macrophages and K cells, which are thought to be in similar population of NK cells, are presumed to be the effector cells for ADCC activity. Because the changes in ADCC activity paralleled changes in the ratio of OKIa1-positive monocytes, and because IFN-γ produced no significant change in NK activity or in ratios of Leu7- or Leu11-positive lymphocytes, which are thought to be specific for NK cells, we surmise that the ADCC activity activated by IFN-γ can be attributed largely to the cytotoxicity of macrophages.

IFN-γ was not observed to reduce tumor size, perhaps because the number of cases studied here was so small. IFN-γ is thought to have less antitumor efficacy than IFN-α,¹⁵,¹⁶ in cases of RCC. Consequently, IFN-γ alone may provide only minimal benefit to patients with RCC. IFN-γ has been shown to have stronger monocyte-activating effects than IFN-α or IFN-β,¹⁷ however. Furthermore, our results suggest, when monoclonal antibodies against tumor-associated antigens become clinically available, increase in the antitumor activity of monoclonal antibodies associated with concomitant use of IFN-γ can be expected by augmentation of ADCC activity.

We conclude that studies of the effect of combination with other therapeutic modalities that would take advantage of the biological characteristics of IFN-γ will provide the basis for better ways to treat RCC.

References

10. Fosd SD, De Garis ST, Heier MS, Flokkmann A, Lien HH, Salveson A, Moe B: Recombinant interferon alpha-2a with or