Impaired Induction of Type II Interferon in Tumor-bearing Mice

Shuzo Matsubara, Midori Suzuki, and Nakao Ishida

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ABSTRACT

The response of mice to type II interferon inducers such as purified protein derivative of tuberculin or OK-432 was significantly impaired after either the implantation of tumor cells or the injection of cell-free tumor ascitic fluids. Complete inhibition of type II interferon induction occurred in 3 to 5 days. This inhibitory effect persisted until the death of mice implanted with tumors or for 7 days after i.p. injection of 0.2 ml of tumor ascitic fluid. The inhibitory activity was seen even with 0.2 ml of the 10,000-fold dilution of the fluid and with its filtrate passed through a Diaflo UM-10 membrane (>10,000-dalton exclusion). However, such tumor ascitic fluids did not affect the induction of type I interferon in mice by polyriboinosinic-polyribocytidylic acid or tilorone-HCl. Moreover, adjuvant-induced nontumorous ascitic fluid had no inhibitory activity. These results indicate that the in vivo induction of type II interferon is specifically impaired by tumor-mediated factor(s) with molecular weights of less than 10,000.

INTRODUCTION

A high-titered type II (immune type) interferon was induced in mice (10) that were treated with OK-432, a Streptococcus preparation with proven anticancer effectiveness (9, 13). For the induction of type II interferon by OK-432, the involvement of both T-cells (3) and macrophages was required (10). However, we have recently noticed that the type II interferon-inducing activity of OK-432 is significantly reduced in mice with progressively growing tumors. Since the immunological functions of T-cells and macrophages are generally suppressed in tumor bearers (8), this impairment of type II interferon induction by OK-432 may correlate with the suppressed functions of such immunocompetent cells.

In the present study, the responses of tumor-bearing mice to type II and type I interferon inducers were compared. Only the induction of type II interferon was impaired by tumor-mediated factor(s).

MATERIALS AND METHODS

Mice. Six- to 8-week-old inbred DDI mice, maintained free of both Sendai virus and Mycoplasma infections, were obtained from the Experimental Animal Center of Tohoku University. Two weeks before use for the induction of type II interferon by PPD, one group of these mice was immunized by i.v. injection of 10^7 viable BCG organisms (19).

Tumor Ascitic Fluids. Ehrlich and Sarcoma 180 ascites

RESULTS

Impaired Interferon Induction in Tumor-bearing Mice. The interferon responses of mice to OK-432 were examined at various intervals after i.p. or s.c. implantation of 10^6 tumor cells. These mice and normal control mice received a single injection of OK-432 (5 mg/kg i.p.), and 24 hr later sera were obtained for the interferon assay. In normal mice, OK-432 induced a serum interferon titer of 5120 units/ml (Table 1). In contrast, mice implanted with either Ehrlich or Sarcoma 180 tumors showed a gradual reduction with time in their interferon response to OK-432 despite the different tumors and inoculation sites. No detectable interferon was induced 5 to 8 days after tumor implantation. This hyporeactivity lasted until the death of the mice.


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The abbreviations used are: T-cells, thymus-derived lymphocytes; PPD, purified protein derivative of tuberculin; BCG, Bacillus Calmette-Guerin; poly(l-C), polyriboinosinic-polyribocytidylic acid.

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Inhibitory Effect of Cell-free Tumor Ascitic Fluids. To test the inhibitory effect of cell-free tumor ascitic fluids, 0.2 ml of the fluid was injected i.p. into normal recipient mice 3 days before inducer challenge. Both tumor ascitic fluids completely inhibited the induction of type II interferon by PPD or OK-432 (Table 2). However, the fluids did not affect the induction of type I interferon by poly(I-C) or tilorone-HCl. No inhibitory activity was found in the adjuvant-induced ascitic fluid. In the next experiment, tumor ascitic fluids were subjected to pressure ultrafiltration through a UM-10 membrane (>10,000-dalton exclusion). The in vivo analysis for inhibitory activity of type II interferon induction demonstrated that the activity was recovered in the filtrates without appreciable loss (data not shown). This result indicates that the specific inhibition of type II interferon induction is caused by ascites constituent(s) with molecular weights of less than 10,000.

The impaired response of type II interferon in mice treated with tumor ascitic fluids was further confirmed by pursuing the interferon titer not only at 24 hr but also for a period of 4 to 48 hr. No interferon activity was found in recipient sera during this period, in repeated experiments. In addition, to test whether the antiviral activity of either PPD- or OK-432-induced serum interferon is directly reduced by ascitic fluids or sera obtained from tumor-bearing mice on Day 11, 1.6 ml of each interferon preparation were mixed with 0.4 ml of such tumor-derived fluids. One half of the mixture was tested for its interferon activity immediately, and another half was tested after 3 hr incubation at 37°. However, the antiviral activity of both interferon preparations was never neutralized in our interferon assay system. This circumstantial evidence suggests that the hyporeactivity may actually be induced by tumor ascitic fluids in mice by affecting the type II interferon-producing target cells.

Kinetics of Inhibition of Type II Interferon Induction. To detect the time lag required before the appearance of such inhibitory activity, mice received a single i.p. injection of 0.2 ml of cell-free Ehrlich ascitic fluid at various intervals before inducer challenge (Table 3). A slight but definite inhibitory effect was observed even when both the fluid and inducers were injected simultaneously. Complete inhibition of the induction was found in mice treated with the fluid 3 days before inducer challenge. Thus, complete inhibition occurred 3 days after treatment with ascitic fluid. The inhibitory effect persisted for as long as 7 days, but not for 10 days, after the injection of ascitic fluid. These results may suggest the presence of a barely reversible mechanism of the impairment caused by tumor ascitic fluid.

Dose Response of Ehrlich Ascitic Fluid. To test the potency of inhibitor(s) in cell-free Ehrlich ascitic fluid, 0.2 ml of each of the serial 10-fold dilutions was injected i.p. into normal recipient mice 3 days before OK-432 challenge. Even with a dilution as high as 1:10,000, significant inhibition of interferon induction resulted (Table 4). The inhibitory activity was not apparent when the ascitic fluid was diluted by 1:100,000. Almost the same potency was found with Sarcoma 180 ascitic fluid (data not shown).

DISCUSSION

The present study demonstrates that the induction of type II interferon by PPD or OK-432 was completely inhibited in mice 5 days after implantation of tumor cells or 3 days after injection of the cell-free tumor ascitic fluid. The inhibition persisted until the death of tumor bearers or for 7 days after treatment with 0.2 ml of tumor ascitic fluid. In addition, the hyporeactivity to type II interferon inducers was induced by tumor ascites constituent(s) with molecular weights of less than 10,000 but not by a nontumorous ascitic fluid obtained from adjuvant-stimulated mice. However, in vivo induction of type I interferon by poly(I-C) or tilorone-HCl was not affected by the pretreatment with tumor ascitic fluid. These results indicate that the tumor-mediated low-molecular-weight factor(s) may specifically in-

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Table 1

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Method of inoculation</th>
<th>Interferon titer (units/ml) at following days after tumor implantation</th>
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</thead>
<tbody>
<tr>
<td>Ehrlich</td>
<td>i.p.</td>
<td>1280 160 &lt;20 &lt;20 &lt;20 &lt;20</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>i.p.</td>
<td>640 80 &lt;20 &lt;20 &lt;20 &lt;20</td>
</tr>
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<td>Phosphate-buffered saline (control)</td>
<td>i.p.</td>
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Table 2

<table>
<thead>
<tr>
<th>Fluid</th>
<th>PPD</th>
<th>OK-432</th>
<th>Poly(I-C)</th>
<th>Tilorone-HCl</th>
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<tr>
<td>Ehrlich ascites</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>2,560</td>
<td>5,120</td>
</tr>
<tr>
<td>Sarcoma 180 ascites</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>2,560</td>
<td>5,120</td>
</tr>
<tr>
<td>Adjuvant-induced ascites</td>
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<td>5,120</td>
<td>5,120</td>
<td>5,120</td>
</tr>
<tr>
<td>Phosphate-buffered saline (control)</td>
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<td>5,120</td>
<td>5,120</td>
<td>5,120</td>
</tr>
</tbody>
</table>

Table 3

<p>| Interferon titers (units/ml) at following days of treatment with the fluid |
|-------------------------------|------------------|------------------|-----------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>OK-432</th>
<th>Poly(I-C)</th>
<th>Tilorone-HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>5120</td>
<td>5120</td>
<td>2560</td>
</tr>
<tr>
<td>Phosphate-buffered saline</td>
<td>2560</td>
<td>2560</td>
<td>1280</td>
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hibit the in vivo induction of type II interferon. This inhibition appears to be caused by impairment of the interferon production system in mice because of the following circumstantial evidence. When a possible delay of interferon production was examined in mice receiving tumor ascitic fluid, interferon activity did not appear during the sampling period extended up to 48 hr. When the presence of neutralizing antagonist or degrading substance of interferon activity was examined by mixing or incubating type II interferon preparations with both ascitic fluid and serum obtained from tumor-bearing mice on Day 11, neither antagonistic nor degrading activity was found. Thus, the hyporeactivity to type II interferon inducers was assumed to be caused by the impairment of the production system, although further detailed studies are required.

In general, the persistent infections of viruses and microorganisms and the repeated administration of potent interferon inducers are known to bring about reduced responses in interferon-producing cells (7, 16). However, establishment of hyporeactivity is not restricted to such situations. Mice with leukemia produce markedly less type I interferon despite stimulation with powerful interferon inducers such as poly(I-C) and Newcastle disease virus (3, 17). In human cancer patients, Armstrong et al. (1) also noted that induction of type I interferon by herpes zoster infection was significantly impaired compared to noncancer patients. In addition, although plant mitogens have been known to induce type II interferon by stimulating the lymphocytes in vitro (5, 18), less responsiveness was found in the lymphocytes from patients with chronic lymphocytic leukemia (6). This evidence may indicate that the induction of both type I and type II interferons is generally impaired in tumor bearers. In these studies, hyporeactivity has been shown to be associated with the suppressed functions of the interferon-producing cells. Therefore, the tumor-induced hyporeactivity directed only to type II interferon inducers may also be explained by the lowered functions of interferon-producing cells. To identify the target cells of the interferon inducers used in this study, PPD was shown to elicit type II interferon during blastogenesis of T-cells obtained from BCG-sensitized mice (18). It also appeared that OK-432 required the cooperation of T-cells and macrophages for interferon induction, since it was completely inhibited when mice were pretreated with either whole-body X-irradiation or immunosuppressive agents such as hydrocortisone acetate, carrageenan, or trypan blue and since it was not found in athymic nude mice (10). On the other hand, both poly(I-C) and tilorone-HCl have been shown to induce type I interferon through stimulation of the reticuloendothelial system, probably of macrophages (3, 14). Consequently, impaired induction of only type II interferon may reflect the suppressed functions of the T-cells rather than of the macrophages, a very frequently observed occurrence in tumor bearers (8).

Crate et al. (2) recently reported noteworthy data that the antitumor activity of type II interferon was about 100 times more powerful than that of type I interferon. Furthermore, Sonnenfeld et al. (15) suggested the possibility that the primary biological activity of type II interferon may be exhibited in the modulation of the host immunosurveillance system rather than in the virus inhibition. Therefore, our observation that the induction of only type II interferon is gradually impaired in mice with progressively growing tumors may help to elucidate the mechanism of progressive immunosuppression in tumor bearers. The isolation and chemical characterization of the inhibitor(s) of type II interferon induction contained in tumor ascitic fluids could be of great diagnostic and therapeutic importance.

Acknowledgments

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REFERENCES


Table 4

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Interferon titer (units/ml)</th>
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<tr>
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<td></td>
</tr>
<tr>
<td>Original</td>
<td>&lt;20</td>
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<tr>
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<tr>
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<td>160</td>
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<tr>
<td>1:100,000</td>
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<tr>
<td>Adjuvant-induced asctes</td>
<td></td>
</tr>
<tr>
<td>Original</td>
<td>5120</td>
</tr>
<tr>
<td>Phosphate-buffered saline (control)</td>
<td>5120</td>
</tr>
</tbody>
</table>

* Diluted with phosphate-buffered saline.
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