Induction of endogenous tumor necrosis factor by OK-432 in ovarian cancer patients with ascites

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Abstract

To four ovarian cancer patients with malignant ascites, 10 KE of OK-432 was intraperitoneally administered four times at 2 day intervals for priming, and 40 KE of OK-432 was given on the 13th day after the first injection for triggering. The changes in blood monocyte and peritoneal macrophage levels and the production of tumor necrosis factor (TNF) by blood mononuclear cells (BMCs) and ascitic lymphoid cells (ALCs) were examined. In the two patients in whom TNF was induced in the ascites, TNF production by BMCs and ALCs was noted during priming. After triggering, increases in both the number of peritoneal macrophages and TNF production by ALCs were noted. In the other two patients, in whom TNF was not detected in the ascites, the ratio of peritoneal macrophages to ALCs did not change throughout the study period, and TNF production by the ALCs was not augmented. These findings suggest that OK-432 can exert a primary effect on both peritoneal macrophages and blood monocytes, and that OK-432 triggering can promote an increase in primed peritoneal macrophages and the release of TNF from these cells.

Abbreviations: ALCs, ascitic lymphoid cells; BMCs, blood mononuclear cells; FCS, fetal calf serum; IFN-γ, interferon-γ; i.p., intraperitoneal; KE, Klinische Einheit; LPS, lipopolysaccharide; PBS, phosphate buffered saline; Su-PS, Su-polysaccharide; TNF, tumor necrosis factor.

Introduction

Tumor necrosis factor (TNF) is an antitumor cytokine derived from macrophages [1], which has been shown to have a potent ability to damage tumor cells both in vitro [2] and in vivo [3]. Recombinant human TNFs have already been developed [4, 5], and are currently under clinical trial as an anticancer therapy [6, 7]. In addition, there have also been attempts to endogenously induce TNF in patients with cancer [8, 10]. OK-432, a streptococcal preparation, has been reported to exhibit endogenous TNF-inducing activity in tumor-bearing mice [9] and also in some cancer patients [8, 11], but the mechanism by which OK-432 acts remains to be clarified. In this study, in order to investigate the induction of endogenous TNF by OK-432, it was intraperitoneally administered to ovarian
cancer patients with malignant ascites. The changes in the numbers of blood monocytes and peritoneal macrophages, as well as TNF production by blood mononuclear cells (BMCs) and ascitic lymphoid cells (ALCs), were examined.

**Materials and methods**

**Subjects**

The subjects were four ovarian cancer patients with marked ascites (Table 1). Case 1 had not been previously treated. In the other three cases, recurrence had occurred after postoperative chemotherapy. More than 4 weeks had passed since the last course of chemotherapy in each case.

**Administration of OK-432**

OK-432 is a lyophilized preparation of the avirulent Su strain, group A *Streptococcus pyogenes* (Chugai Pharmaceutical Co. Ltd., Tokyo, Japan). The "Klinische Einheit (KE)" unit is used to express the strength of the preparation. One KE of OK-432 is equivalent to 0.1 mg of dried streptococci. It is known that two-step stimulation, priming and triggering, is necessary for the induction of endogenous TNF [8–10, 12]. Therefore, 10 KE of OK-432 was intraperitoneally injected four times at 2 day intervals for priming. Then on the 13th day after the first injection, a 40 KE intraperitoneal (i.p.) injection of OK-432 was given for triggering. The OK-432 was suspended in 100 ml of normal saline and infused into the abdominal cavity over 30 minutes.

**Su-polysaccharide (Su-PS) skin test**

The intradermal reaction to Su-PS, a polysaccharide fraction extracted from the cell wall of the Su strain of *Streptococcus pyogenes*, was tested both on the day before commencing i.p. OK-432 therapy, and on the day after triggering (day 14). One hundred μl of Su-PS (20 μg) was injected intradermally into the forearm, and the maximum and minimum diameters of the erythema induced were measured after 24 hours. The reaction was designated positive if the mean diameter was 10 mm or more.

**Table 1. Patients.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Stagea</th>
<th>Diagnosis</th>
<th>Therapy before i.p. OK-432</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
<td>III B</td>
<td>clear cell carcinomab</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>III B</td>
<td>serous cystadenocarcinoma</td>
<td>operation ↓ CAPc (2 courses)</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>III B</td>
<td>serous cystadenocarcinoma</td>
<td>operation ↓ CAPc (5 courses)</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>III B</td>
<td>serous cystadenocarcinoma</td>
<td>operation ↓ CAPc (5 courses)</td>
</tr>
</tbody>
</table>

*The clinical stage is in accordance with the FIGO ( Federation Internationale de Gynencologie et d'Obstetrique ) classification (1987).

b Pathological diagnosis was confirmed with tumor tissue obtained at diagnostic laparotomy after one course of i.p. OK-432 therapy.

c Combination therapy (cyclophosphamide, adriamycin and cisplatin).