Enhanced Resistance to Pseudomonas aeruginosa Infection in Mice Pretreated with OK-432*3

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ABSTRACT

The resistance to Pseudomonas aeruginosa infection in normal mice was markedly enhanced by intraperitoneal administration of streptococcal preparation, OK-432. OK-432 reduced the mortality and enhanced the in vivo killing activity of peritoneal cells against P. aeruginosa infection in immunosuppressed mice treated with high doses of dexamethasone.

A streptococcal preparation, OK-432, developed by Okamoto et al.,21) has been known to have potent antitumor activities in experimental animals11,27) and humans13,21). It stimulates the host defense mechanism in terms of activation of macrophages11, lymphocytes13, granulopoiesis9) and serum complement components15, 26), and production of interferon17) and of serum antibody33). It was proved that Mycobacterium bovis (BCG)4,36), Propionibacterium acnes (Corynebacterium parvum)12,18> and OK-43218, 21) which are currently being used in cancer immunotherapy also enhance the resistance to experimental bacterial1,19,28-31) and viral9,10,14,16 infections in animals. Among these, little is known of its enhanced resistance to bacterial infections in animals. In the present study, we examined the effect of this agent on P. aerugino­

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Fig. 1. The LD₅₀ by intraperitoneal challenge with *P. aeruginosa* or with *E. coli* in mice pretreated with OK-432. Mice (n=10) were injected i. p. with or without OK-432 (5 KE) one day before the intraperitoneal challenge with *P. aeruginosa* PAO 3047 (□) or *E. coli* 81 (△). Survivals were recorded on day 7 after the challenge, and LD₅₀ was determined.

The reduced resistance against *P. aeruginosa* infection in immunosuppressed animals is enhanced by OK-432. Mice were daily treated i. p. with 0.2 ml of dexamethasone (0.2 mg/ml, Decadron®, Japan Merck Co., Tokyo) for 3 days. One day after the last administration of dexamethasone, mice were given OK-432 i. p. once or three times with or without 2 KE, followed by intraperitoneal challenge with *P. aeruginosa* (1.1 × 10⁶) after 24 h. As shown in Fig. 3, the survival rate of dexamethasone in treated (infected) mice was 40%, while none survived in the dexamethasone-treated (infected) mice. This reduced resistance to *P. aeruginosa* infection in dexamethasone-treated mice was significantly enhanced by OK-432, and the degree of enhancement was higher in the three dosage groups than in the one dosage group.

The effect of OK-432 on *in vivo* killing activity of peritoneal cells in dexamethasone-

Fig. 2. Persistence of resistance against *P. aeruginosa* infection in mice pretreated with OK-432. Mice (n=20) were injected i. p. with OK-432 (1 or 5 KE). At indicated intervals after the injection, mice were challenged i. p. with *P. aeruginosa* (4.7-5.6 × 10⁶), and survivals on day 7 were recorded.

* Arrows indicate infection with *P. aeruginosa*.

Fig. 3. Protection of OK-432 in dexamethasone-treated mice against *P. aeruginosa* infection. Mice (n=10) pretreated with (□) or without (△) dexamethasone were injected once or three times with OK-432 (2 KE). One day after the injection with OK-432, mice were challenged i. p. with *P. aeruginosa* (1.1 × 10⁶), and survivals on day 7 were recorded.
treated mice against *P. aeruginosa* was studied as described previously. Briefly, dexamethasone-treated mice were challenged i.p. with *P. aeruginosa* (10^5) 7 days after i.p. administration of OK-432 (2 KE). Three hours after challenge, 2.5 ml of Hank’s balanced salt solution containing 4 units heparin per ml were injected into the peritoneal cavity of mice after gently massaging their abdomen, and the peritoneal fluid was collected. The fluid was separated into supernatant fluid and cell pellets by centrifugation, and the cells were disrupted with 5 ml of distilled water to release the *P. aeruginosa*. The number of colony-forming units (CFU) in the supernatant fluid and the cells was determined on nalidixic acid-cetrimide (NAC) agar plates. The killing activity was calculated by means of the following formula:

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\text{Killing activity (\%) = } \frac{\text{Total CFU at 0 time} - \text{Total CFU after 3h}}{\text{Total CFU at 0 time}} \times 100
\]

where Total CFU means CFU in supernatant fluid and cells. In addition, the number of cells in the peritoneal fluid before challenge with *P. aeruginosa* was counted with a hemocytometer and the peritoneal fluid was smeared, stained with Giemsa’s solution and analyzed. As shown in Fig. 4, the killing activity of peritoneal cells from dexamethasone-treated mice against *P. aeruginosa* was lower than that of cells from normal mice. The reduced killing activity of peritoneal cells from dexamethasone-treated mice was markedly enhanced by the intraperitoneal administration of OK-432. The number and the percentage of macrophages and polymorphonuclear leukocytes (PMN) in peritoneal cells from dexamethasone-treated mice increased by the administration of OK-432 (Table 1).

Among the phagocytic cells, PMN plays the primary role for protection of the host against *P. aeruginosa* infection, while macrophages also assume an important role for the defense mechanism against pseudomonas infection. It is well known that patients and experimental animals treated with corticosteroid become susceptible to opportunistic pathogens. The agent mainly suppressed the host cell-mediated immunity such as mitosis and lysis of lymphocytes, chemotaxis and microbicidal activity of monocytes and migration, phagocytosis and microbicidal activity of macrophages. In contrast, the migration and microbicidal activity of PMN are not affected by the agent. The resistance to *P. aeruginosa* infection in normal mice was markedly enhanced by intraperitoneal injection of OK-432, which also reduced the mortality due to *P. aeruginosa* infection in immunosuppressed mice treated with a high dose of dexamethasone. The reduced *in vivo* killing activity of peritoneal cells from the dexamethasone-treated mice was enhanced by the administration of OK-432. These actions of OK-432 can be explained by its enhancing ability of PMN and macrophage accumulation in the infection site. Our findings suggest that OK-432 may serve not only for

**Table 1. Number and population of cells in the peritoneal fluids in normal and dexamethasone-treated mice administered with OK-432**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Peritoneal or peritoneal exudate cells</th>
<th>Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cells</td>
<td>MΦ</td>
</tr>
<tr>
<td>Normal</td>
<td>5.1x10^6</td>
<td>58.1</td>
</tr>
<tr>
<td>Dexamethasone- treated</td>
<td>1.7x10^6</td>
<td>49.1</td>
</tr>
<tr>
<td>+ OK-432</td>
<td>2.0x10^7</td>
<td>59.1</td>
</tr>
</tbody>
</table>

Mice pretreated i.p. with dexamethasone (40 µg/day, for 3 days) were injected i.p. with OK-432 (2 KE). Seven days after injection with OK-432, the cells in intraperitoneal fluid of normal and experimental mice were counted, and analyzed after staining with Giemsa’s solution.

**Fig. 4.** In vivo killing of *P. aeruginosa* by peritoneal cells in normal and dexamethasone-treated mice administered with OK-432.

Mice (n=3) pretreated with or without dexamethasone were challenged i.p. with *P. aeruginosa* (1x10^7) 7 days after the administration of OK-432 (2 KE). After 3 h of challenge, the number of CFU in the peritoneal fluid was assayed, and the killing activity was determined. The details are described in the text.
cancer treatment, but also for protection against the opportunistic infections in immunosuppressed patients.

REFERENCES


