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

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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 animals[11,27] and humans[13,21]. Its stimulates the host defense mechanism in terms of activation of macrophages[11], lymphocytes[13], granulopoiesis[9] and serum complement components[15,26], and production of interferon[17] and of serum antibody[33]. It was proved that *Mycobacterium bovis* (BCG)[4,36], *Propionibacterium acnes* (*Corynebacterium parvum*)[12,18] and OK-432[13,21] which are currently being used in cancer immunotherapy also enhance the resistance to experimental bacterial[1,19,28-31] and viral[6,10,14,16] 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. aeruginosa* infection in normal and dexamethasone-treated mice.

OK-432 was donated by Chugai Pharmaceutical Co., Tokyo. As the challenge inoculum, *P. aeruginosa* PAO 3047 and *Escherichia coli* 81 were used in some experiments. These organisms were incubated at 37°C for 24 h in heart infusion broth. Bacterial cells were harvested by centrifugation, washed three times, and diluted in saline. Female ddY mice 4 to 5 weeks old were purchased from the Shizuoka Union for Experimental Animals, Shizuoka, Japan. Each animal was given intraperitoneally (i. p.) 1 or 5 KE (Klinische Einheit) of OK-432 suspended in sterile physiological saline. Because 1 and 5 KE of OK-432 contain 2,690 and 13,450 units of penicillin G, respectively, equivalent amounts were added to each dose of saline inoculum administered to the control animals also. Ten mice each in the experimental and control groups were challenged i. p. with 0.1 ml of serial 5-fold dilutions of *P. aeurginosa* (2.8×10^8/ml) or *E. coli* 81 (1.2×10^7/ml) one day after injection with OK-432. The LD_{50} was determined by the method of Reed and Muench[23]. Mortality data were recorded 7 days after challenge. As shown in Fig. 1, LD_{50} values for *P. aeurginosa* and *E. coli* increased about 12- and 4-fold, respectively, as compared with those in control mice.

Twenty mice in the experimental and control groups were administered i. p. with or without 1 or 5 KE of OK-432. At various time intervals for up to 14 days, mice were infected i. p. with 0.1 ml of *P. aeruginosa* ($4.7-5.6\times10^7$/ml), and survival was recorded 7 days after infection. As shown in Fig. 2, the enhanced resistance in mice was gradually reduced and completely lost 10 days (1 KE) and 14 days (5 KE) after administration of OK-432. However, the resistance in both experimental groups to infection was significantly enhanced for 5 days thereafter ($P<0.05$).

Study was made to determine whether or not

*) 斎藤　肇，渡辺隆司，冨岡治明，佐藤勝昌，北川俊之：OK-432 投与マウスにおける緑膿菌感染抵抗性増強効果

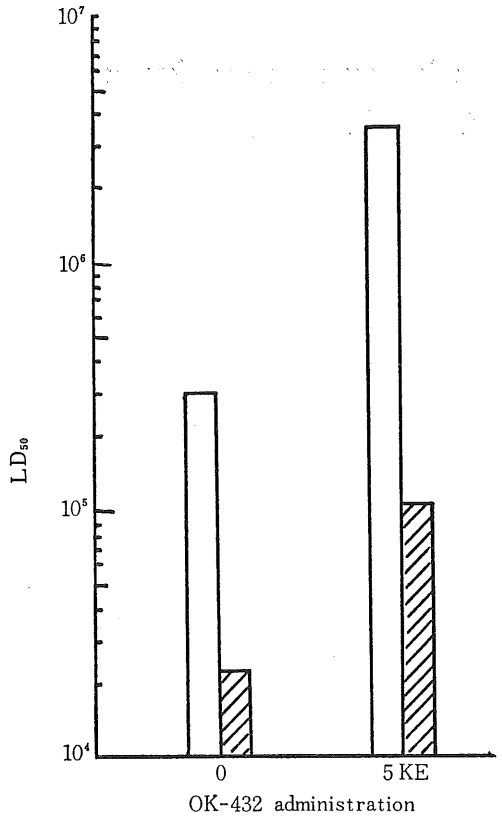

Fig. 1. The LD_{50} by intraperitoneal challenge with *P. aeruginosa* or with *E. coli* in mice pretreated with OK-432.
Mice (n=10) were injected i. p. with or without OK-432 (5 KE) one day before the intraperitoneal challenge with *P. aerugionsa* PAO 3047 (□) or *E. coli* 81 (▨). Survivals were recorded on day 7 afrer the challenge, and LD_{50} was determined[23].

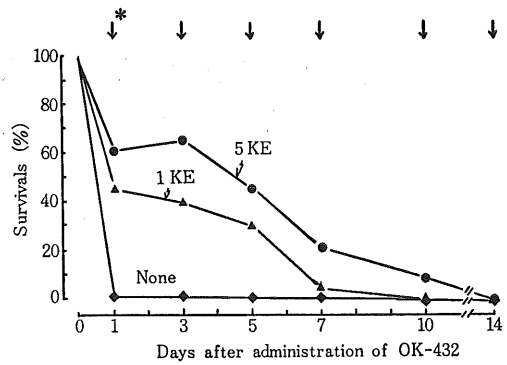

Fig. 2. Persistence of resistance against *P. aeruginosa* infection in mice pretreated with OK-432.
Mice (n=20) were injected i. p. with OK-432 (1 or 5 KE). At indicated intervals after the injection, mice were challenged i. p. with *P. aeruginosa* ($4.7-5.6\times10^6$), and survivals on day 7 were recorded.
* Arrows indicate infection with *P. aeruginosa*.

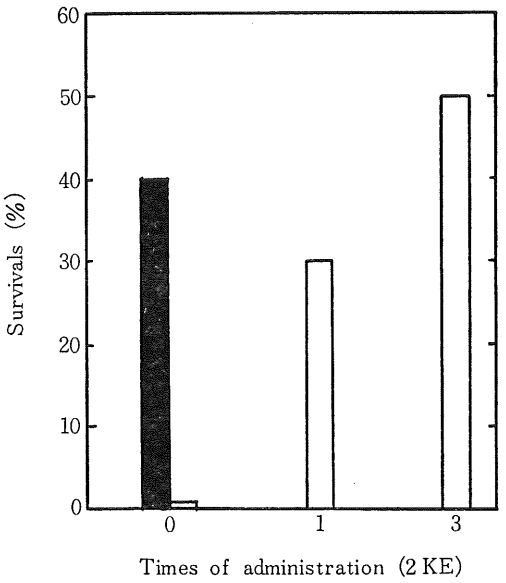

Fig. 3. Protection of OK-432 in dexamethasone-treated mice against *P. aeruginosa* infection.
Mice (n=10) pretreated with (□) or without (■) dexamethasone were injected once or three times with OK-432 (2 KE). One day after the injection with OK-432, mice were challenged i. p. with *P. aeruginosa* (1.1×10^6), and survivals on day 7 were recorded.

the reduced resistance against *P. aeruginosa* infection in immunosuppessed 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* (1.1×10^6) 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-

treated mice against *P. aeruginosa* was studied as described previously[28]. 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 Hanks' 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:

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

where Total CFU means CFU in supernatant fluid and cells. In addition, the number of cells in the peritonal 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 polymorphounuclear leukocytes (PMN) in peritoneal cells from dexamethasone-treated mice increased by the administration of OK-432 (Table 1).

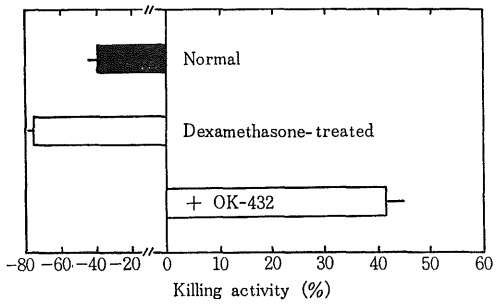

Fig. 4. *In vivo* killing of *P. aeruginosa* by peritoneal cells in normal and dexamethsone-treated mice administered with OK-432.
Mice (n=3) pretreated with or without dexamethasone were challenged i. p. with *P. aeruginosa* (1×10^5) 7 days after the administration of OK-432 (2 KE). After 3 h of challenge, the number of CFU in the peritoneal fluid was assayed, and the killing activity was determined. The details are described in the text.

Table 1. Nember and population of cells in the peritoneal fluids in normal and dexamethasone-treated mice administred with OK-432

Mice	Peritoneal or peritoneal exudate cells			
	Number of cells	Analysis (%)		
		Mφ	PMN	lymphocyte
Normal	5.1×10^6	58.1	3.4	38.5
Dexamethasone-treated	1.7×10^6	49.1	5.8	45.1
+ OK-432	2.0×10^7	59.1	10.9	30.0

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 colution.

Among the phagocytic cells, PMN plays the primary role for protection of the host against *P. aeruginosa* infection[8,32], while macrophages also assume an important role for the defense mechanism against pseudomonas infection[3,5,22]. 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[7], chemotaxis and microbicidal activity of monocytes[24,25] and migration, phagocytosis and microbicidal activity of macrophages[2,20,31,35]. In contrast, the migration and microbicidal activity of PMN are not affected by the agent[20,25,34]. 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

cancer treatment, but also for protection against the opportunistic infections in immunosuppressed patients.

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