

Leukopenic and Lethal Effects of Slime from *Acinetobacter calcoaceticus*

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Abstract

Intravenous and intraperitoneal injections of slime from *Acinetobacter calcoaceticus* TMS266 induced leukopenia and death in mice. The leukopenia was characterized by a decrease in neutrophil count, followed by death of mice. This decrease of leukocytes was profound and supposed to contribute to the enhancement of virulence of other bacteria such as *Escherichia coli*. In mice treated with sublethal doses of the slime, the slime could not show its capacity to reduce circulating leukocytes.

Introduction

Acinetobacter calcoaceticus has been isolated from patients with urinary tract infections¹⁾, pneumonia²⁾, or septicemia³⁾. Although *Acinetobacter calcoaceticus* infections have been studied in recent years, relatively little is known about the pathogenesis concerned⁴⁾⁵⁾. Previous studies concerning the pathogenicity of *Acinetobacter calcoaceticus* have confirmed the role of slime of this bacterial species⁶⁾⁷⁾. It has been demonstrated that the slime produces antiphagocytic and lethal effects in mice.

In this study, the slime-induced leukopenic and lethal effects were further characterized.

Materials and Methods

Organism. *Acinetobacter calcoaceticus* TMS266, originally isolated from a human clinical specimen, was supplied by Prof. S. Goto (School of Medicine, Toho University, Tokyo).

Bacterial slime. Slime was obtained from the strain as indicated and was purified by methods described previously⁶⁾. Slime was extracted with saline from 48-hours bacterial cultures grown on sheets of cellophane overlying brain heart infusion agar (Difco). The crude slime was precipitated with cold ethanol. After treatment with chloroform-*i*-amylalcohol, 0.1 N sodium hydroxide, and cetylpyridinium chloride, in that order, the purified slime was obtained. Chemical components of the slime were determined by methods described previously⁶⁾. The slime had high contents of sugar, protein, and orcinol reaction-positive substances.

Animals. Four-week-old male mice of ddY strain weighing 17 to 18 g were used and were supplied with water and laboratory chow *ad libitum*.

Animal challenge. Groups of ten mice were injected intravenously or intraperitoneally with 0.2 ml of twofold dilutions of the slime. Animals were observed daily for up to five days and the LD₅₀ value was estimated by the Probit method.

Leukocyte counts. Leukocyte counts were made on peripheral blood samples collected retroorbitally. The results are presented in terms of average of five mice per group.

Results

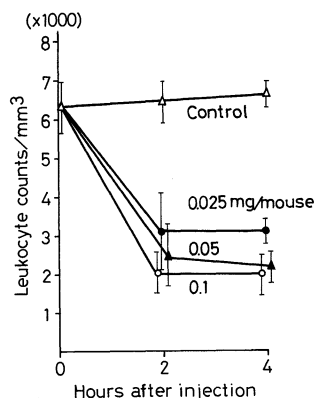
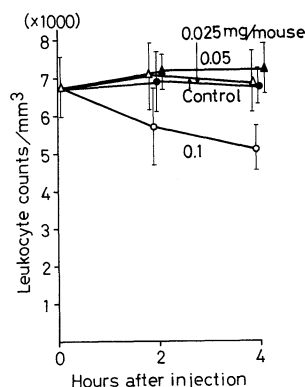
Toxicity of slime. The toxicity of slime in mice is shown in Table 1. The slime extracted from *Acinetobacter calcoaceticus* TMS266 showed high toxic effect when it was injected intravenously and intraperitoneally into mice. The LD₅₀ values for intravenous and intraperitoneal injections were 0.019

Table 1 Lethal activity of slime from *Acinetobacter calcoaceticus* TMS266

Route	Dose (mg/mouse)	Mortality (%)	Average interval between injection and death (min)	LD ₅₀ (mg/mouse)
i.v.	0.1	100	6.5 ± 7.8	0.019
	0.05	100	44 ± 23	
	0.025	80	—	
	0.0125	10	—	
i.p.	0.4	100	138 ± 13	0.062
	0.2	100	196 ± 132	
	0.1	90	—	
	0.05	20	—	
	0.025	0	—	
i.p. (heated) ^{a)}	0.4	100	N.D. ^{b)}	0.066
	0.2	100	N.D.	
	0.1	80	—	
	0.05	10	—	
	0.025	0	—	

a) 100°C, 15 min.

b) not done

Fig. 1 Leukopenia in the peripheral circulation of normal mice after intraperitoneal injection of slime from *Acinetobacter calcoaceticus* TMS266Fig. 2 Leukopenia in the peripheral circulation of slime-treated mice after intraperitoneal injection of slime from *Acinetobacter calcoaceticus* TMS266

mg/mouse and 0.062 mg/mouse, respectively. Heating to 100°C for 15 min did not alter the ability of the slime to induce lethality in mice.

Effect of slime on blood leukocyte counts. The leukocyte counts were determined on blood samples obtained at various time intervals after intraperitoneal injection of slime (Figure 1). Whereas saline-injected mice showed little, if any, change in total leukocyte count, mice receiving slime were found to undergo rapid, pronounced, and sustained leukopenia in the peripheral blood, followed by death. The leukopenia was due mainly to a decrease in the number of circulating polymorphonuclear leukocytes. Similar experiments were performed with mice treated with 0.04 mg/mouse of slime eight and four days before the experiment. The result indicates that the slime could not show its capacity to reduce circulating leukocytes in treated mice (Figure 2).

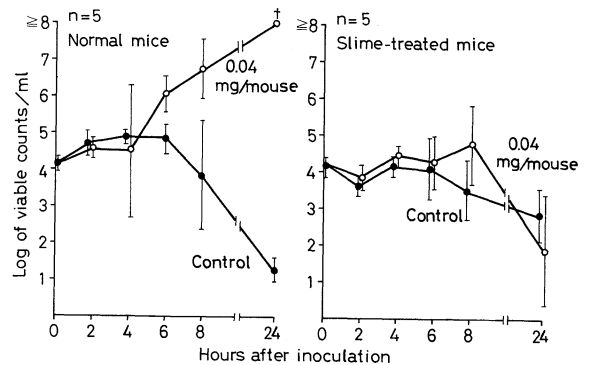
Virulence-enhancing activity of slime. The effect of slime on the virulence of *Escherichia coli* ST0198 in

Table 2 Effect of slime from *Acinetobacter calcoaceticus* TMS266 on the virulence of *Escherichia coli* ST0198 in normal and slime-treated mice

Slime concn. (mg/mouse)	LD ₅₀ (cells/mouse)*	
	Normal mice	Slime-treated mice
0	2.2×10^6	6.2×10^6
0.02	2.2×10^5	6.2×10^6
0.04	2.2×10	2.0×10^6

*Eight mice were used for each group in this experiment.

Fig. 3 Effect of slime from *Acinetobacter calcoaceticus* TMS266 after intraperitoneal injection into normal and slime-treated mice infected with *Escherichia coli* ST0198



mice is shown in Table 2. For normal mice, whereas the LD₅₀ value of *Escherichia coli* was 2.2×10^6 cells/mouse in control mice, the LD₅₀ values for mice receiving 0.02 mg and 0.04 mg of slime were 2.2×10^5 cells/mouse and 2.2×10 cells/mouse, respectively. Mice receiving slime was 10- to 10^5 -fold more susceptible than control mice. On the other hand, for mice treated with 0.04 mg/mouse of slime eight and four days before experiment, no significant differences in LD₅₀ could be detected between control mice and slime-receiving mice. The effect of 0.04 mg of slime on the bacterial counts in peripheral washings of mice infected intraperitoneally with *Escherichia coli* is shown in Figure 3. In normal mice, the bacterial counts of *Escherichia coli* in the control group showed a gradual decrease. However, in the slime receiving group, an increase in bacterial count and subsequent death of the mice were observed during the 24-hours period after injection. In mice treated with 0.04 mg/mouse of slime eight and four days before experiment, the bacterial counts in the control and slime-receiving groups showed a gradual decrease. The enhancement of the virulence of *Escherichia coli* was due mainly to a decrease in the number of circulating polymorphonuclear leukocytes, which play the most important role in acute infectious diseases.

Discussion

The present investigation studied the biological activities of slime from *Acinetobacter calcoaceticus*. Previous studies indicated that slime induces lethal and antiphagocytic effects in mice^{6,7}.

In this study, the slime from *Acinetobacter calcoaceticus* TMS266 was highly toxic. We observed that most of the mice given the slime died with haemorrhage in the thorax during the four-hour period after injection. Moreover, the lethal activity was retained after exposure to 100°C for 15 min. Therefore, the result suggests that the lethality might be due to heat-stable substances such as sugar components et al. However, we observed lack of correlation between the lethality of viable cells and the toxicity of the homologous slime⁶. No experimentally supported explanation exists as yet. Lynn et al.⁸ and Dimitracopoulos et al.⁹ reported potent lethal activity of the slime of *Pseudomonas aeruginosa* in relation to bacterial pathogenicity. In our study, we found that the slime of *Acinetobacter calcoaceticus* TMS266 was comparable to that of *Pseudomonas aeruginosa* in lethal activity for mice. We previously showed that *Acinetobacter calcoaceticus* slime interferes with polymorphonuclear leukocytes and macrophages^{6,7}. In this study, it was shown that the rapid and sustained leukopenia, induced by intraperitoneal injection of the slime, was characterized by a reduction in neutrophils. It seemed that the slime was able to induce the

leukopenia by quickly entering the blood from the peritoneal cavity. However, treatment with the slime protected mice from the leukopenia caused by injection of the homologous slime. The ability to maintain a large number of polymorphonuclear leukocytes in the circulating was an early indication that the mice would survive, whereas leukopenic mice usually died. No experimentally supported explanation is available as yet. Therefore, the enhancement of the virulence of other species was due mainly to a decrease in the number of circulating polymorphonuclear leukocytes. Lynn et al.⁸⁾ and Dimitracopoulos et al.⁹⁾ reported that the slime of *Pseudomonas aeruginosa* induces the leukopenia in mice. Furthermore, Schwarzmamm et al.¹⁰⁾, Laharrague et al.¹¹⁾, and Nadaud¹²⁾ reported that the slime inhibits phagocytosis of neutrophils and macrophages. Johnson et al.¹³⁾ also reported that the slime of *Staphylococcus epidermidis* interferes with granulocyte functions. The slime of *Acinetobacter calcoaceticus* was comparable to that of *Pseudomonas aeruginosa* and of *Staphylococcus epidermidis* in antiphagocytic activity. A substance like slime that induces leukopenia would constitute a virulence factor, whereas polymorphonuclear leukocytes and macrophages apparently play a key role in protection against several bacterial infections. Therefore, the results of this study support our previous conclusion⁶⁾ that the slime is an important pathogenic product of *Acinetobacter calcoaceticus* cells.

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Acinetobacter calcoaceticus の産生する slime の 白血球減少および致死作用について

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Acinetobacter calcoaceticus TMS266株から分離した slime は静脈あるいは腹腔内接種により、マウスに対して致死作用および白血球減少症を誘発することが認められた。白血球減少症は好中球数の減少によるものであり、続いてマウスは死亡

することが分かった。このような白血球減少は *Escherichia coli* などの他の菌種の菌力を亢進するものと思われた。また sublethal な濃度の slime をあらかじめ投与したマウスでは、slime による白血球減少作用は認められなかった。
