Enhancement of Host Resistance to Bacterial Infections in Normal and Immunosuppressed Mice with Actinobacillus suis

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Abstract

A single intraperitoneal (ip) inoculation of heat-killed Actinobacillus suis ATCC 15557 (AS 15557) into normal and immunosuppressed (dexamethasone-treated) mice led to remarkable nonspecific resistance to ip challenge with lethal doses of opportunistic pathogens such as Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus and Candida albicans. The duration of this enhanced protective action and the minimal effective dose, in normal mice, induced by AS 15557 were superior to those induced by other bacterial immunostimulants such as heat-killed Lactobacillus casei YIT 9018 (LC 9018) and penicillin-treated Streptococcus pyogenes, Su (OK-432). In immunosuppressed mice, the reduced in vivo killing activity of peritoneal exudate cells (PECs) against P. aeruginosa infection was markedly augmented by ip injection of AS 15557. The degree of PEC augmentation induced by AS 15557 was higher than that induced by LC 9018 or by OK-432. The toxicity and histopathological changes associated with AS 15557 were very low, as compared with those by produced by LC 9018 and OK-432. The results suggest that AS 15557, which showed a strong resistance-enhancing capacity against opportunistic bacterial infections, may be a useful bacterial immunostimulant.

Introduction

Bacterial immunostimulants such as Mycobacterium bovis (BCG), Propionibacterium acnes (Corynebacterium parvum) and an attenuated Streptococcus pyogenes are well known to have strong antitumor and antimicrobial actions in experimental animals and patients1-9. However, it is essential to pay careful attention to side reactions in clinical applications for cancer therapy and/or infectious diseases since these bacteria are naturally pathogenic. As an example, a side reaction such as penicillin-shock due to the S. pyogenes cell preparation (OK-432) can occur easily because the attenuation of OK-432 is achieved by treatment with a high dose of penicillin G10. The author and colleagues11-13 and Mutai et al.14,15 have reported that a heat-killed Lactobacillus casei cell preparation (LC 9018) has strong antimicrobial and antitumor activities in experimental animals. However, L. casei cells having long and polymorphous rod-shapes may cause a secondary side reaction such as enlargement and necrosis of host organs (for instance: the spleen and liver) via bacterial clumping16.

Actinobacillus suis, one member of the genus Actinobacillus (A. ligniersii, A. suis, A. equuli, A.
capsulatus and A. actinomyctemcomitans), is a gram-negative capnophilic coccobacillus belonging to the Pasteurellaceae family\(^{17}\). A. suis is found as a commensal agent in alimentary, respiratory and genital tracts of several normal animals such as swine, cattle and horses\(^{17-20}\).

There have been no reports, to date, indicating that the genus Actinobacillus cells show immunopotentiating actions against microbial infections. The objective of the present study was to determine whether or not a heat-killed A. suis cell preparation shows resistance-enhancing effects on bacterial infections in normal and immunosuppressed animals.

Materials and Methods

Animals

Five-week-old female ddY mice (weighing 24 to 25 g) were purchased from the Shizuoka Agricultural Cooperative for Experimental Animals, Shizuoka, Japan. Immunosuppressed mice were created by intraperitoneal (ip) administration of 0.2 ml of dexamethasone (decadron\(^{®}\), Nippon-Merk Co., Tokyo) in 1 mM sodium phosphate buffer (pH 7.2) once daily for 3 days (1 mg/ml; total inoculum: 0.6 mg/mouse).

Bacterial immunostimulants

Cells of A. suis ATCC 15557 (AS 15557) and A. actinomyctemcomitans ATCC 29522 (AA 29522) grown in brain-heart infusion broth (Eiken Chemicals Co., Tokyo) were washed 3 times with saline by centrifugation, suspended in saline, treated in a water-bath at 80°C for 30 min, lyophilized and stored at 4°C until use. LC 9018 (a lyophilized preparation of heart-killized L. casei cells YIT 9018) and OK-432 (penicillin G-treated S. pyogenes Su) were donated by Yakult Central Institute for Microbiological Research (Tokyo) and Chugai Pharmaceutical Co. (Tokyo), respectively.

Challenge pathogens

Wild types of P. aeruginosa, methicillin-resistant S. aureus (MRSA) and C. albicans were isolated from patients in Mie University Hospital, Mie, Japan. These pathogens grown in brain-heart infusion broth at 37°C for 18 hr were washed 3 times with saline and suspended in saline. The colony-forming units (CFUs) of P. aeruginosa, MRSA and C. albicans were estimated on nalidixic acid-cetrimide (NAC) agar plates, No. 110 agar plates and Sabourud-Glucose agar plates, respectively.

Evaluation of protective actions of immunostimulants in normal mice

Normal mice, in groups of 10 each, were injected ip with either 0.2 ml (0.5 mg/ml; 0.1 mg/mouse; 4 mg/kg) or 0.2 ml (2.5 mg/ml; 0.5 mg/mouse; 20 mg/kg) of one of four kinds of immunostimulants 3 days before ip challenge with 0.2 ml of P. aeruginosa (1.8 \times 10^7 CFUs/mouse), MRSA (4.4 \times 10^7 CFUs/mouse) or C. albicans (1.3 \times 10^8 CFUs/mouse). The survival rates were recorded on the 10th day after infection. As controls, mice (n=10) were injected ip with 0.2 ml saline instead of each immunostimulant. For statistical comparison of final survival rates between experimental and control groups, P values were calculated by the chi-square Student’s \(t\)-test. A value of \(P <0.05\) was considered to be statistically significant.

Duration of protective actions of immunostimulants against P. aeruginosa infection in normal mice

Mice (n=10) were challenged ip with 1.8 \times 10^7 CFUs of P. aeruginosa 3, 7, 10, 14 or 21 days after ip inoculation with 0.5 mg of AS 15557, AA 29522 or LC 9018. The survivors were recorded on day 10 after the challenge.

Protective actions against P. aeruginosa infection in normal mice based on administration routes of immunostimulants

Mice (n=10) were challenged ip, intravenously (iv) or subcutaneously (sc) with P. aeruginosa (1.6 - 5.2 \times 10^7 CFUs) 3 days after ip, iv or sc injection of either AS 15557 (0.5 mg) or LC 9018 (0.5 mg). The survivors were recorded on day 10 after the challenge.
Minimal effective immunostimulant dose against *P. aeruginosa* infection in normal mice

Three days before the ip challenge with *P. aeruginosa* (1.8 × 10⁷ CFUs), mice (n=10) were inoculated ip with various doses (0.01, 0.05, 0.1, 0.2, 0.5 and 1.0 mg) of the immunostimulants tested. The survivors were recorded on the 10th day after the challenge. The minimal effective dose of each immunostimulant was determined by the chi-square Student’s *t*-test.

Comparison of protective actions of immunostimulants against *P. aeruginosa* infection in immunosuppressed mice

One day after the last ip treatment with dexamethasone (DM), DM-treated mice (n=10) were injected ip with or without two doses (0.1 and 0.5 mg) of As 15557, AA 29522, LC 9018 or OK-432. Three days after the injection of each immunostimulant, mice were challenged ip with *P. aeruginosa* (6.2 × 10⁶ CFUs), and the survivors were recorded on day 10 after the challenge.

Assay for *in vivo* killing activity of PECs from immunosuppressed mice against *P. aeruginosa* infection

This assay system has been described previously. In brief, mice immunosuppressed by DM treatment (n=3) were challenged ip with *P. aeruginosa* (1.0 × 10⁶ CFUs) 3 days after the ip inoculation with 0.1 mg of AS 15557, LC 9018 or OK-432. Three hours later, 2.5 ml of Hanks balanced salt solution (HBSS) containing 4 units heparin/ml was inoculated into the peritoneal cavities of DM-treated mice, and peritoneal fluid was then harvested after gently massaging the abdomen. This procedure was repeated once. As controls (at zero time), the same number of *P. aeruginosa* was inoculated into the peritoneal cavity of normal (DM-untreated) control mice. The peritoneal fluids were harvested immediately and centrifuged at 170 × g for 10 min. The pooled cell pellets were disrupted with 5 ml of 0.85% NH₄Cl solution at room temperature for 20 min to release the phagocyted bacteria from PECs. Each sample was serially diluted 10-fold with HBSS, and the number of CFUs was determined by plating on NAC agar plates. The PECs were counted with a hemocytometer under a microscope, and the cell populations of PECs were calculated by means of the following formula: Killing activity (%) = Total CFUs (at zero time after 3 hr)/Total CFUs at zero time × 100. In this formula, total CFUs indicates CFUs in the supernatant fluid plus PEC CFUs. The lethal dose (LD₅₀) values of immunostimulants

Normal mice in groups of 5 were each given ip, iv or sc injections, or an oral dose, of either serially 2-fold diluted AS 15557, LC 9018 or OK-432. Mortality was recorded on day 50 after the inoculation. The LD₅₀ value was determined by the method of Reed and Muench.

Histopathological observations

Mice (n=3) were injected ip with 0.5 mg (20 mg/kg) of AS 15557, LC 9018 or OK-432. The liver and spleen were removed on the 10th day after inoculation and fixed in 10% formalin. As controls, mice (n=3) were injected ip with saline (0.2 ml). Tissue sections of these organs were made from all fixed preparations and stained with hematoxylin and eosin solution.

Results

Comparison of protective actions of immunostimulants against infections with bacterial pathogens in normal mice

As shown in Table 1, normal mice manifested significantly enhanced resistance to *P. aeruginosa*, MRSA and *C. albicans* infections with all four kinds of immunostimulants (AS 15557, AA 29522, LC 9018 and OK-432) tested. Among them, the protective actions of the genus *Actinobacillus* (AS and AA) were comparatively stronger than those of LC 9018 and OK-432. In particular, AS 15557 exhibited the highest host resistance-enhancing capacity.

Duration of protective action of immunostimulants against *P. aeruginosa* infection in normal mice
Table 1 Comparison of protective actions of bacterial immunostimulants against infection with opportunistic pathogens in normal mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/mouse)</th>
<th>Survivors (%)</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P. aeruginosa</td>
<td>MRSA</td>
</tr>
<tr>
<td>AS 15557*</td>
<td>4 (0.1)</td>
<td>90**</td>
<td>60*</td>
</tr>
<tr>
<td>administered</td>
<td>20 (0.5)</td>
<td>100**</td>
<td>90**</td>
</tr>
<tr>
<td>AA 29522*</td>
<td>4 (0.1)</td>
<td>60*</td>
<td>60*</td>
</tr>
<tr>
<td>administered</td>
<td>20 (0.5)</td>
<td>80**</td>
<td>80**</td>
</tr>
<tr>
<td>LC 9018*</td>
<td>4 (0.1)</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>administered</td>
<td>20 (0.5)</td>
<td>70*</td>
<td>70*</td>
</tr>
<tr>
<td>OK-432*</td>
<td>4 (0.1)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>administered</td>
<td>20 (0.5)</td>
<td>70*</td>
<td>40</td>
</tr>
<tr>
<td>Control (none)</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mice (n=10) were injected ip with one of the test immunostimulants 3 days before ip challenge with *P. aeruginosa*(1.8×10⁷ CFUs), *MRSA*(4.4×10⁷ CFUs) or *C. albicans*(1.3×10⁷ CFUs). Survival rates were recorded on the 10th day after the challenge. a) AS 15557 (*A. suis* ATCC 15557), b) AA 29522 (*A. actinomycetemcomitans* ATCC 29522), c) LC 9018 (*L. casei* YIT 9018), d) OK-432 (penicillin G-treated *S. pyogenes*, Su).

*p<0.05, **p<0.01.

Table 2 Protective actions against *P. aeruginosa* infection in normal mice based on immunostimulant administration routes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ip</td>
</tr>
<tr>
<td>AS 15557-administered</td>
<td>90**</td>
</tr>
<tr>
<td>iv</td>
<td>30</td>
</tr>
<tr>
<td>sc</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LC 9018-administered</th>
<th>70*</th>
<th>20</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>iv</td>
<td>20</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>sc</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

Mice (n=10) preinoculated ip, iv or sc with AS 15557 (0.5mg) or LC 9018 (0.5mg) were challenged (1.8×10⁷ CFUs), iv (1.6×10⁷ CFUs) or sc (5.2×10⁷ CFUs) with *P. aeruginosa* 3 days after inoculation of one of the two immunostimulants. Survival rates were recorded on day 10 after the challenge.

*p<0.05, p<0.01.

![Fig. 1 Durations of protective actions of immunostimulants against *P. aeruginosa* infection in normal mice](image)

**Mice (n=10) were challenged ip with *P. aeruginosa* (1.8 × 10⁷ CFUs) 3, 7, 10, 14 or 21 days after ip inoculation with or without 0.5 mg of AS 15557 (●), AA 29522 (○) or LC 9018 (▲). The survival rates were recorded on day 10 after the challenge. *P<0.05, **P<0.01.**
Fig. 1 shows the changes in survival rates of mice (%) infected ip with *P. aeruginosa* at indicated intervals after ip administration of AS 15557, AA 29522 or LC 9018 (0.05 mg/mouse: 2 mg/kg). All control group mice died within 24 hr after the infection, while 60% of mice survived if they were challenged on the 10th day after the ip administration of AS 15557 (*P* < 0.05). On the other hand, significant numbers of survivor mice were noted on the 7th day with AA 29522 and on the 3rd day with LC 9018.

Effective actions against *P. aeruginosa* infection in normal mice based on routes of immunostimulant administration

Mice (*n* = 10) were infected ip, iv or sc with *P. aeruginosa* on the 3rd day after the ip, iv or sc administration of AS 15557 or LC 9018 (Table 2). In the case of the same routes of inoculation and infection (ip via ip, iv via iv and sc via sc), host resistance-enhancing capacities were seen in the AS 15557-administered and the LC 9018-administered groups. However, with differing routes such as ip via iv, ip via sc and iv via sc, there was no significant protective effect against *P. aeruginosa* infection.

### Table 3 Minimal effective dose of immunostimulants against *P. aeruginosa* infection in normal mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Survivors (%)</th>
<th>Dose of inoculum; mg/mouse (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>AS 15557-administered</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>LC 9018-administered</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Mice (*n* = 10) were inoculated ip with or without the various doses of AS 15557 and LC 9018 indicated, and then infected ip with *P. aeruginosa* (*1.8* × 10⁸ CFUs) 3 days after the inoculation. The survival rates were recorded on day 10 after the challenge. *p < 0.05, **p < 0.01.

### Table 4 Comparison of protective actions of immunostimulants against *P. aeruginosa* infection in immunosuppressed mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Survivors (%)</th>
<th>mg/mouse (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Untreated normal</td>
<td>20</td>
<td>(0)</td>
</tr>
<tr>
<td>DM-treated alone</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>+ AS 15557</td>
<td>60*</td>
<td>(0)</td>
</tr>
<tr>
<td>+ AA 29522</td>
<td>30</td>
<td>(0)</td>
</tr>
<tr>
<td>+ LC 9018</td>
<td>20</td>
<td>(0)</td>
</tr>
<tr>
<td>+ OK-432</td>
<td>10</td>
<td>(0)</td>
</tr>
</tbody>
</table>

One day after the last ip treatment with dexamethasone (DM; total inoculum: 0.6 mg/mouse), mice (*n* = 10) were injected ip with one of two concentrations (0.1 and 0.5 mg) of AS 15557, AA 29522, LC 9018 or OK-432. Three days after injection of these immunostimulants, mice were challenged ip with *P. aeruginosa* (*6.2* × 10⁸ CFUs) and the survival rates were recorded on day 10 after the challenge. *p < 0.05, **p < 0.01.
Minimal effective immunostimulant dose against *P. aeruginosa* infection in normal mice

Mice (*n*=10) were inoculated ip with or without various doses of AS 15557 or LC 9018, and then infected ip with *P. aeruginosa* 3 days after the inoculation. As shown in Table 3, the minimal effective doses of AS 15557 and LC 9018 were 0.05 mg/mouse (2 mg/kg) and 0.2 mg/mouse (8 mg/kg), respectively, that is, the minimal effective dose of AS 15557 was a quarter that of LC 9018.

Comparison of protective actions of immunostimulants against *P. aeruginosa* infection in mice immunosuppressed with dexamethasone (DM)

As shown in Table 4, 20% of normal control mice survived when they were challenged ip with *P. aeruginosa* (6.2 × 10^6 CFUs), while there were no survivors in the DM-treated group. In contrast, improved survival rates of DM-treated mice which had been inoculated ip with one of the four kinds of immunostimulants (0.5 mg) were observed in all experimental groups, and the host resistance-enhancing capacities of these immunostimulants were dose-dependent. When DM-treated mice were inoculated with 0.1 mg of these immunostimulants, a significant survival rate (60%) was seen only in the AS 15557-administered group.

In vivo killing activity against *P. aeruginosa* infection of PECs from immunosuppressed mice injected ip with immunostimulants.

As shown in Fig. 2, the in vivo killing activity against *P. aeruginosa* (1.0 × 10^6 CFUs/mouse) of PECs (−28 ± 9%) from DM-treated mice was lower than that of PECs (−9 ± 3%) from untreated normal mice. The reduced killing activity of PECs from DM-treated mice was markedly restored when these mice were injected ip with either AS 15557, LC 9018 or OK-432 3 days before the ip challenge. However, the PEC-enhancing capacity of AS 15557 was significantly greater than that of LC 9018 or OK-432 (*P* < 0.05), that is, the mean killing activities of PECs induced by AS 15557, LC 9018 and OK-432 were 82, 65 and 51%, respectively. In addition, there were no remarkable differences in the number of PECs or the PEC cell population elicited by AS 15557, LC 9018 and OK-432 (2.8 × 10^7 cells/mouse, macrophage= Mφs; 66.2%, polymorphonuclear cells= PMNs: 9.6%), LC 9018 (3.2 × 10^7 cells/mouse, macrophage= Mφs; 66.2%, polymorphonuclear cells= PMNs: 9.6%), LC 9018 (3.2 × 10^7 cells/mouse,

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**Fig. 2 In vivo killing activity against *P. aeruginosa* infection of PECs from immunosuppressed mice injected ip with immunostimulants**

Mice (*n*=3) immunosuppressed with dexamethasone (DM) were challenged ip with *P. aeruginosa* (1.0 × 10^6 CFUs) 3 days after ip inoculation with (□) or without (■) 0.1 mg of each immunostimulant. Untreated normal mice (*n*=3) were also challenged with *P. aeruginosa* (■). Killing activities of PECs from these mice 3 hr after infection were determined as described in “Materials and Methods”.

平成8年6月20日
Table 5 Histopathological liver and spleen findings of mice given ip immunostimulants

<table>
<thead>
<tr>
<th>Organs</th>
<th>Item</th>
<th>None (Control)</th>
<th>Immunostimulant administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AS 15557</td>
<td>LC 9018</td>
</tr>
<tr>
<td>Liver</td>
<td>bacterial clumping in histiocytes</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>monocytes, PMN infiltration of sinusoids</td>
<td>(−)</td>
<td>(±)</td>
</tr>
<tr>
<td></td>
<td>necrosis of hepatic cells</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>reconstruction of liver acinus</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Spleen</td>
<td>red pulp (giant cells)</td>
<td>(±)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>white pulp</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

(−): negative, (±): false positive, (+): positive, (++): strongly positive. Mice (n=3) were injected ip with either 0.5mg (20mg/kg) of AS 15557, LC 9018 or OK-432. Livers and spleens were removed on the 10th day after inoculation, and fixed in 10% formalin. As controls, mice (n=3) were injected ip with saline (0.2ml). Tissue sections of each organ were made from all fixed preparations and stained with hematoxylin and eosin solution.

Mφs: 67.0%, PMNs: 8.9%) or OK-432 (2.5 × 10⁶ cells/mouse, Mφs: 64.8%, PMNs: 7.8%).

Histopathological features of liver and spleen from mice injected ip with immunostimulants

Histopathological findings of the liver and spleen from mice (n=3) 10 days after ip administration of AS 15557, LC 9018 or OK-432 (0.5 mg/mouse) are summarized in Table 5. In the livers of mice inoculated with LC 9081, a large number of small and intermediate nodular infiltrations consisting of inflammatory cells, mainly monocytes together with neutrophils, were prominent in sinusoids with an irregular distribution to lobules. The major lesions were composed of necrosis of hepatic cells, that is, severe necrotizing inflammatory lesions of the septic type. Histopathological changes in the livers of mice inoculated ip with AS 15557 or OK-432 were relatively mild as compared to those observed with LC 9018. On the other hand, the appearance of giant cells in the red pulp of the spleen was evoked by the administration of all of the immunostimulants tested, though the degree was more severe with LC 9018 and OK-432 than with AS 15557. In contrast, none of the experimental cases showed histopathological changes in the white pulp.

LD₅₀ values of immunostimulants in normal mice

In the case of ip administration, LD₅₀ values of AS 15557, LC 9018 and OK-432 in normal female mice were 812, 561 and 141 mg/kg, respectively. LD₅₀ values of AS 15557, LC 9018 and OK-432 utilizing iv administration were 200, 177 and 31 mg/kg, respectively. The LD₅₀ value of AS 15557 with oral administration was 3000 mg/kg or more. Thus, the toxicity of AS 15557 was markedly lower than that of LC 9018 or OK-432 (data not shown).

Discussion

This study was concluded to determine whether or not a single ip inoculation of heat-killed AS 15557, which is known to be present as a commensal agent in several animals¹⁷-²⁰, would lead to enhanced host defense mechanisms against opportunistic infections involving P. aeruginosa, MRSA and C. albicans.
Effect of *Actinobacillus suis* on bacterial infections

Despite there being no differences from normal mice in the numbers of PECs and peritoneal Mφs ip with AS 15557, LC 9018 or OK-432, the duration of enhanced resistance to *P. aeruginosa* infection in AS 15557-administered normal mice was statistically superior to that in LC 9018-administered or OK-432-administered normal mice. The minimal effective dose of AS 15557 was also lower than that of LC 9018.

The in vivo killing activity of Mφs-rich PECs against *P. aeruginosa* 3 days after ip administration of AS 15557 was stronger than that of PECs from mice pretreated ip with LC 9018 or OK-432. Thus, the host resistance-enhancing capacity of AS 15557 was comparatively higher than those of the other bacterial immunostimulants tested. The strong protective effect of AS 15557 cannot be convincingly explained by the results obtained. A possible explanation may be the presence of heat-stable specific constituents in the cell wall and/or in the cell membrane of AS 15557, and if so, these constituents would play an important role in the efficient enhancement of resistance to bacterial infections. In light of this speculation, studies on extraction, purification and the biological actions of various cellular fractions of AS 15557 cells will be undertaken.

Previous investigators have reported that cellular immunity is not important for protection against *P. aeruginosa* infection, because the main effector cells are PMNs, and their functions are enhanced in the presence of specific immunoglobulins\(^{22,23}\). On the contrary, some authors attribute the protective mechanism against *P. aeruginosa* infection to cellular immunity, proposing in particular that Mφs play a critical role in the control of this infection\(^{24-26}\). In our preliminary studies, it was found that a high percentage of PMNs was observed in the mouse peritoneal cavity at 3 to 24 hr after ip adminnistration of AS 15557 (52 ± 2%) and drastically decreased thereafter, whereas the mean percentage of Mφs began to increase 2 days after the injection of AS 15557 (69%), peaked on the 5th to 7th day (86%) and was maintained at a high level even on the 14th day (75%). These observations may suggest that the early stage of protection against *P. aeruginosa* infection in AS 15557-administered mice is due to inflammatory PMNs rapidly accumulating in the infected sites, while the enhanced resistance in the late stage may be attributable to Mφs mediated by AS 15557. Our speculation appears to be supported by the findings that host resistance to *P. aeruginosa* infection in granulocytopenic animals is enhanced by the administration of BCG in complete Freund's adjuvant\(^{25}\). The acquisition of enhanced resistance to *P. aeruginosa* infection was also explained by the difference in the accumulation and/or activation of PECs (mainly Mφs) at the site of infection.

Corticosteroids strongly suppress cell-mediated immunity, and consequently the susceptibility of drug-treated patients or animals to opportunistic infections may be relatively higher than that of normal subjects\(^{27-29}\). In the present study, survival rates of DM-treated mice infected ip with *P. aeruginosa* were lower than those of untreated normal mice, though the survival rates of DM-treated mice were elevated by the administration of AS 15557. The suppressed in vivo killing activity against *P. aeruginosa* of PECs from DM-treated mice was markedly enhanced by the administration of AS 15557, because corticosteroids dramatically suppress various biological properties of Mφs but do not affect PMN functions\(^{11,27}\). These speculations are strongly supported by the report of Stinnett et al.\(^{30}\), which showed that the protective effect of *C. parvum* vaccine on mice receiving high doses of cortisone acetate was attributable to the activation of Mφs induced by this immunostimulant.

The histopathological changes observed in the livers and spleens of AS 15557-administered mice were very mild as compared with those in LC 9018-administered mice. The differences in histopathological changes between AS 15557 and LC 9018 may be due to the morphological differences between these bacteria\(^{16,17}\).

Based on the results presented herein, it is reasonable to propose that the AS 15557 preparation be added to the armamentarium of bacterial immunostimulants.

平成 8 年 6 月20日
References


Effect of *Actinobacillus suis* on bacterial infections


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*Actinobacillus suis* の正常および免疫不全マウスにおける
細菌感染抵抗性増強効果

鈴鹿医療科学技術大学，保健衛生学部，医療栄養学科

渡 邊 隆 司

要 目

加熱処理 *A. suis* ATCC 15557（AS 15557）の0.5mg（4 mg/kg）を正常およびデキサメゾン処置マウス腹腔内(ip)に投与したのち，患者由来線粒菌，メチシリン耐性黄色ブドウ球菌（MRSA）あるいは *Candida albicans* をマウス ip 内に感染させたところ，非投与対照群におけるよりも高い生存率を示すと共に，免疫増強能を有することが知られている加熱処理 *Lactobacillus casei* YIT 9018（LC 9018）およびベニシリン処理 *Streptococcus pyogenes* Su（OK-432）よりも高い値を示した。AS 1557の緑膿菌感染抵抗性持続効果並びに最小有効量は LC 9018およびOK-432のそれらよりも優れていた。デキサメゾン処理によって低下した腹腔浸出細胞（特にマクロファージ）の生体内線粒菌殺菌能は AS 15557を投与することによ り著しく回復した。AS 1557による毒性および病理組織学的変化は LC 9018およびOK-432におけるよりも極めて軽度であった。以上の結果より，AS 1557は日和見菌感染に対して優れた宿主抵抗増強作用を有する細菌性免疫増強剤であることが示唆された。

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