AMPHOTERICIN B-INDUCED RESISTANCE TO *Pseudomonas aeruginosa* INFECTION IN MICE

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We evaluated the effects of amphotericin B (AmB) against *Pseudomonas aeruginosa* (*P. aeruginosa*) infection in mice. Pretreatment with 2 mg/kg of AmB 24 hours before infection significantly increased the survival rates of mice intraperitoneally infected with either *P. aeruginosa* or *Escherichia coli*. To evaluate the mechanism of this AmB-induced resistance to infection, we conducted a number of experiments. Peritoneal macrophages exposed *in vitro* to AmB showed superior bactericidal activity compared to that of control macrophages. Interleukin-1 production by peritoneal macrophages from mice pretreated with 2 mg/kg of AmB was significantly higher than that in control mice. Serum tumor necrosis factor level after intravenous injection of *P. aeruginosa* was also higher in mice pretreated with 2 mg/kg of AmB than in control mice. These data indicate that AmB induces resistance to *P. aeruginosa* in mice. Furthermore AmB-induced activation of peritoneal macrophages and their production of interleukin-1 and tumor necrosis factor appeared to play important roles in this phenomenon.

Amphotericin B (AmB) is a polyene antifungal antibiotic. This drug is frequently used in patients with deep mycotic infections, and also in immunocompromised hosts as a prophylactic.

Some reports have already described an immunopotentiating activity of AmB, including AmB-induced resistance to *Schistosoma mansoni*[1,2], *Listeria monocytogenes*[3,4], and *Candida albicans*[5]. With respect to immunocompromised hosts, the incidence of *Schistosoma* or *Listeria* infection appears to be rare[6]. In contrast, *Pseudomonas aeruginosa* (*P. aeruginosa*) infection is a major problem in immunocompromised hosts, because *P. aeruginosa* is resistant to many commonly used antibiotics[7] and frequently causes severe infections such as sepsis or pneumonia[6~9]. It is not known whether AmB can induce resistance to *P. aeruginosa*. Therefore, the present studies were performed to determine whether AmB influences host resistance to *P. aeruginosa*, and to examine the mechanism of this resistance.

Materials and Methods

Reagents

A commercial preparation of AmB (Fungizone; Bristol-Myers Squibb Co., Tokyo) containing 50 mg of AmB and 41 mg of sodium deoxycholate (DOC) per vial was used. The drug was dissolved with sterile

pyrogen-free water into the desired concentration. Recombinant human tumor necrosis factor (TNF) was kindly supplied by Dainippon Pharmaceutical Co., Ltd., Osaka.

Bacterial Strain

A *P. aeruginosa* D4 strain isolated from the cardiac blood of a mouse with bacteremia was used[10]. *Escherichia coli* (*E. coli*) ATCC 25922 strain which is included in Bactrol Disk Set A (Difco Laboratories, Detroit, Mich., U.S.A.) was used. These strains were grown at 37°C on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md., U.S.A.). After 24-hour culture, the microorganisms were suspended in sterile saline, and adjusted to a suitable concentration using optical density. The number of bacteria was subsequently quantitated by colony counts.

Mice

Male, specific pathogen-free ICR mice, 5 weeks old, were obtained from Charles River Japan, Inc., Kanagawa. All mice were housed in sterilized cages, and were given food and water *ad libitum*.

Influence of AmB on the Survival of Mice after Infection with either *P. aeruginosa* or *E. coli*

Mice were given a single intraperitoneal injection of 2 mg/kg of AmB 24 hours before infection. Control mice were simultaneously administered DOC by equivalent volume to which included administered AmB. Subsequently, AmB-pretreated and control mice were given a single intraperitoneal inoculation with either 6.0×10^7 cfu of *P. aeruginosa* or 2.0×10^8 cfu of *E. coli* on day 0. The effects of AmB treatment were monitored by survival rates. Survival of the mice was observed for 2 weeks after infection.

Animal experiments were conducted under "The Guideline of Animal Care and Use of Committee of Nagasaki University".

Influence of AmB on Inflammatory Cell Recruitment after Intraperitoneal Inoculation with Heat-killed *P. aeruginosa*

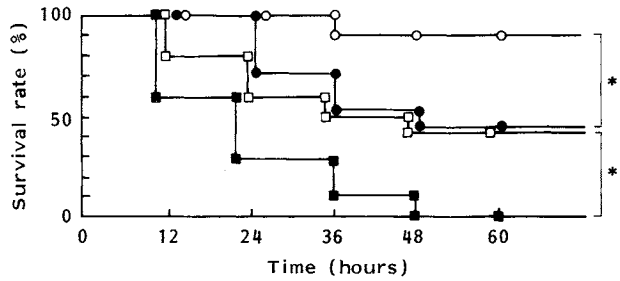
Mice were intraperitoneally injected with 2 mg/kg of AmB 24 hours before inoculation with 1.2×10^7 cfu of heat-killed bacteria in sterile saline. Control mice were intraperitoneally administered DOC by equivalent volume to which included 2 mg/kg of AmB. Peritoneal lavage fluids were obtained in the above-mentioned manner at various times after inoculation. The number of peritoneal exudate cells was measured after staining with Turk solution. Cell differentiation was investigated by examining 100 cells after Wrights and Giemsa staining.

Preparation and Cell Culture of Peritoneal Macrophages and Peritoneal Exudate Cells

Each mouse was sacrificed with ether. Three ml of HANKS' solution (Nissui Pharmaceutical Co., Ltd., Tokyo) was injected into the peritoneal cavity and peritoneal lavage fluids were obtained after gentle massage. The obtained cells were called peritoneal exudate cells. After 1 hour of culturing in plastic petridishes (Falcon 3001; Becton Dickinson and Co., Rutherford, N.J., U.S.A.), nonadherent cells were

Fig. 1. Influence of AmB on survival rates of mice after infection with either *P. aeruginosa* or *E. coli*.

○ AmB-treated and infected with *P. aeruginosa*, ● DOC-treated and infected with *P. aeruginosa*, □ AmB-treated and infected with *E. coli*, ■ DOC-treated and infected with *E. coli*.



* $P<0.01$, AmB-treated *versus* DOC-treated.

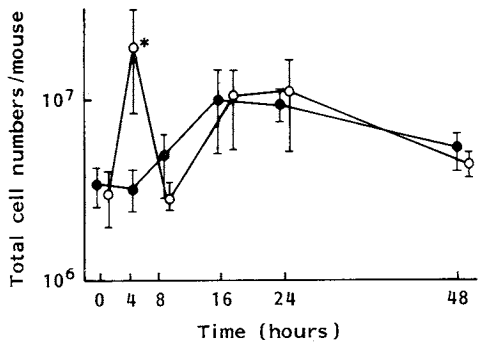
removed by washing. Adherent cells were recovered by scraping the dish with a rubber policeman and washing and suspending them (viability, 80 to 90%) in RPMI 1640 medium (Nissui). These cells were called peritoneal macrophages and cultured in a humidified atmosphere of 5% CO_2 at 37°C.

Bactericidal Activity of Peritoneal Macrophages

The bactericidal studies with peritoneal macrophages were performed according to the modified method of BISTONI *et al.*[5]. Peritoneal macrophages obtained from 5 to 10 normal noninduced mice in the above-mentioned manner were placed on 24-well tissue culture plates (Falcon 3047; Becton Dickinson and Co.). These cells were cultured with several concentrations of AmB or DOC in RPMI medium with 10% fetal calf serum for 24 hours. *P. aeruginosa* were opsonized by incubating in sterilized saline with 10% decomplemented normal human serum. Then the macrophages, washed and incubated in RPMI without serum, were infected with *P. aeruginosa* by using an infection (bacteria/macrophages) ratio of 1:10. Infected macrophages were incubated statically for 2 hours. Then, each well was washed three times and incubated for 1 hour with RPMI containing 100 μg/ml of streptomycin to kill the remaining extracellular bacteria. After washing each well with sterile saline for three times, sterile distilled water was added and dilution plate counts were made on Trypticase soy agar plates for detection of viable bacteria. The number of cfu was determined after 18 hours of incubation at 37°C.

Fig. 2. Changes in the number of peritoneal exudate cells after intraperitoneal inoculation with *P. aeruginosa*.

○ AmB-treated, ● control.



The results are expressed as the means ± standard deviations of five experiments.

* $P<0.05$, AmB-treated *versus* controls.

Interleukin-1 (IL-1) Production by Peritoneal Macrophages

Twenty-four hours after the intraperitoneal

Table 1. Bactericidal activity of peritoneal macrophages after *in vitro* exposure to AmB.

Pretreatment dose of AmB (μg/ml)	% survival[a]
20	39.5 ± 24.2[c]
5	59.2 ± 20.0
1	86.8 ± 19.2
0.2	90.7 ± 19.0
Control[b]	100.0 ± 16.3

[a] Percent survival of bacteria incubated with AmB-treated macrophages compared with the level of DOC-treated macrophages (100%). The titer assigned to each sample is the mean ± standard error (n = 6).

[b] DOC 9 μg/ml (equivalent volume to which included 20 μg/ml of AmB) treated.

[c] $P<0.01$, AmB-treated *versus* controls.

Table 2. IL-1 production by peritoneal macrophages.

Pretreatment dose of AmB (μg/kg)	^{3}H-Thymidine incorporation
2,000	9,509 ± 1,308[b]
200	4,481 ± 398
20	4,837 ± 192
Control[a]	2,548 ± 363

^{3}H-Thymidine incorporation is expressed as counts per minute. The titer assigned to each sample is the mean ± standard error (n = 4).

[a] DOC 900 μg/kg (equivalent volume to which included 2 mg/kg of AmB) treated.

[b] $P<0.001$, AmB-treated *versus* controls.

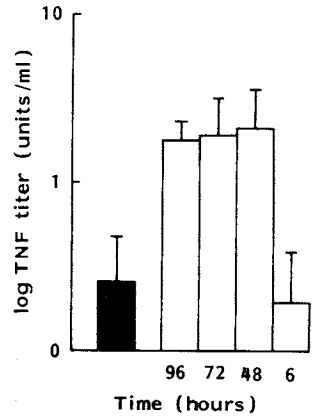
injection of various doses of AmB, peritoneal macrophages were obtained in the above-mentioned manner. Then, 3.6×10^6 cells/dish of adherent cells were incubated with 3.0×10^7 cfu/dish of heat-killed *P. aeruginosa* for 24 hours at 37°C in 5% CO_2. The IL-1 activities of culture supernatants were investigated by thymocyte co-stimulating assay[11]. The counts per minute were determined to measure ^{3}H-thymidine incorporation with a liquid scintillation system (LSC-900, Aloka, Tokyo).

TNF Production after Intravenous Injection of Heat-killed Bacteria

Mice were intraperitoneally administered 2 mg/kg of AmB 6, 48, 72, and 96 hours before intravenous inoculation with heat-killed *P. aeruginosa* (1×10^8 cfu/mouse). Six hours after inoculation, mice were sacrificed with ether and the cardiac blood was obtained. The serum was separated by centrifugation at $1,500 \times g$ and pooled at −80°C. Serum TNF levels were assayed for TNF-induced cytotoxicity using L929 murine fibroblasts and methylene blue staining, according to the procedure described by RUFF and GIFFORD[12]. TNF levels were determined as equal units of recombinant TNF.

Fig. 3. TNF production after intravenous injection of heat-killed bacteria.

■ Control, □ AmB-pretreated.



Data are presented as the mean titer + standard deviations of five experiments.

Statistical Analysis

Differences in survival rates were analyzed by chi-square test. Differences in the number of recruited inflammatory cells were determined by the student *t* test. Differences in cfu in the bactericidal assay and differences in ^{3}H-thymidine incorporation in the thymocyte co-stimulating assay were determined by one-way analysis of variance with pairwise comparison by Bonferroni method. A level of 5% was considered to be significant. Each experiment was repeated at least three times.

Results

Influence of AmB on the Survival of Mice After Infection with either *P. aeruginosa* or *E. coli*

Fig. 1 shows the survival kinetics of AmB treated and control mice after infection with either *P. aeruginosa* or *E. coli*. Among the groups infected with *P. aeruginosa*, the survival rate of AmB-treated mice was significantly higher than that of DOC-treated mice ($p < 0.01$). Furthermore, among the groups infected with *E. coli*, the survival rate of AmB-treated mice was also significantly higher than that of DOC-treated mice ($p < 0.01$). Therefore, AmB significantly enhanced the survival rates of the AmB-treated groups compared with that of the control groups not only for *P. aeruginosa* infection but also for *E. coli* infection.

Influence of AmB Pretreatment on Inflammatory Cell Recruitment after Intraperitoneal Inoculation with Heat-killed *P. aeruginosa*

Fig. 2 shows the changes in the number of peritoneal exudate cells up to 48 hours after inoculation with *P. aeruginosa*. In the control group the number of total cells gradually increased and peaked 16 hours after inoculation. In contrast, there was significant rise in the number of inflammatory cells of AmB-treated mice after 4 hours in comparison with controls ($p < 0.05$). We also found that this abrupt rise was mainly caused by an increase in neutrophils (data not shown).

Bactericidal Activities of Peritoneal Macrophages

Table 1 shows the results of the bactericidal assay with AmB pretreated macrophages and control macrophages from normal noninduced mice. It appears that the bactericidal activity of macrophages pretreated with 20 μg/ml of AmB was significantly higher than that of control macrophages ($p<0.01$).

IL-1 Production by Peritoneal Macrophages

The results are shown in Table 2. ^{3}H-Thymidine incorporation by macrophages from groups pretreated with 20 and 200 μg/kg of AmB and from the control group did not differ significantly from each other. However, ^{3}H-thymidine incorporation by peritoneal macrophages pretreated with 2 mg/kg of AmB was significantly higher than that of control macrophages.

TNF Production after Intravenous Injection of Heat-killed Bacteria

Fig. 3 shows the serum TNF levels of each group. The serum TNF levels of groups treated with AmB at -48, -72 and -92 hours was higher than that of a control group. However, there was no significant difference between the group treated with AmB at -6 hours and the control group.

Discussion

Since the studied *P. aeruginosa* D4 strain and *E. coli* ATCC 25922 strain were able to grow in broth containing over 1,000 μg/ml of AmB, the direct bactericidal activity of AmB against these organisms is excluded (data not shown).

Some reports have already described immunopotentiating effects[13~20] and antitumor activity[21] of AmB. The immunopotentiating effects of AmB appear to be induced by IL-1 and TNF produced by macrophages or mononuclear cells after stimulus with AmB[22~24]. It was also demonstrated that optimal doses of IL-1 and TNF enhance resistance to bacterial infection in murine experimental models[25~27].

Our study revealed that IL-1 production by peritoneal macrophages pretreated with AmB was superior to that of control macrophages. TNF levels in serum after intravenous injection of *P. aeruginosa* were also high in AmB-pretreated mice, as compared with controls. We found that the peritoneal macrophages from the AmB-pretreated mice demonstrated remarkable morphological changes such as enlargement and spreading (data not shown). In addition, the number of bacteria associated with macrophages were higher in AmB-pretreated group compared with that in controls (data not shown). The present study shows that *in vitro* application of AmB induce superior bactericidal activity of peritoneal macrophages and AmB also enhance inflammatory cell recruitment after peritoneal inoculation with *P. aeruginosa*. We think that these events may be related to the increase in resistance to infection with *P. aeruginosa*.

However, some reports indicate that the immunopotentiating effects of AmB are induced by oxidation-dependent events[3,13,15]. We therefore speculate that at least one of the mechanisms of immunopotentiation by AmB could be explained by our results. We think it is necessary to determine the main mechanism of this immunopotentiation.

Since we could confirm the effects of AmB against not only with *P. aeruginosa* infection but also with *E. coli* infection, we think this immunopotentiating activity is not limited to infection of *P. aeruginosa*.

Thymocyte co-stimulating assay is a sensitive bioassay for measurement of IL-1 and can detect IL-1 in concentrations as low as 10 to 100 pg/ml. However IL-2, TNF, and IL-6 can mimic IL-1 in this assay[28]. Therefore we could not neglect the influence of cytokines other than IL-1 on the result of this bioassay.

Some reports describe immunosuppressive properties of AmB[29~32]. Why do such conflicting results exist? We speculate that it is because certain experimental factors differed. At least four differences must be listed: 1) dosage of AmB, 2) cell types used *in vitro* studies, 3) the type of mice strain used, 4) methods for pretreating with AmB to investigate neutrophil function, and 5) drug administration schedules.

With respect to dosage, the kinetics of serum AmB concentration after intravenous administration in humans has already been clarified[33,34], and it was reported that using high-dose AmB may be toxic for

all eukaryotic cells[15]. The optimal dosage required to induce the immunopotentiating effects of AmB has not been determined in humans. It is difficult to clarify the optimal dosage for inducing antibacterial resistance in all strains of mice, because many strains differ in their sensitivity to AmB[15,35]. Therefore, one of the mechanism of the immunosuppressive propeties of AmB may be due to high-dose administration of this drug.

When considering cell types for use in *in vitro* studies, it is important to think about which cell types mainly contribute to the analyzed phenomenon. FERRANTE *et al.* examined the effects of AmB on mitogen-induced lymphocyte proliferation and the results showed that AmB inhibited the lymphocyte response to mitogens[30]. However, we mainly used peritoneal macrophages for our *in vitro* evaluation of the mechanism of this antibacterial resistance because macrophages and neutrophils play important roles in the early protection against bacterial infections.

Some mice strains show low response to AmB[14,15]. All strains bearing the C57BL background gene, such as C57BL/6 or C57BL/10, failed to show adjuvant effects to any dose of AmB. Therefore, using high responder mice is recommended when evaluating the immunoadjuvant effects of AmB.

Some investigators have reported that AmB inhibits neutrophil function[29~32]. They studied neutrophil chemotaxis *in vitro* by preincubating neutrophils with AmB, concluding that AmB inhibited neutrophil chemotaxis. In contrast, we examined the number of peritoneal exudate cells and cell differentiation after intraperitoneal inoculation with *P. aeruginosa*. After peritoneal inoculation with heat-killed *P. aeruginosa*, the number of peritoneal exudate cells from AmB-pretreated mice increased rapidly (4 hours after inoculation). We also found that most of the observed cells were neutrophils (data not shown). Therefore, it was inferable that neutrophil function is inhibited by direct exposure to AmB *in vitro*, but that neutrophil recruitment is enhanced *in vivo*. We speculate that this response was caused by hormonal factors such as interleukin-8 production by AmB-activated macrophages. It is necessary to study the mechanism of this diverse reaction.

With respect to administration schedules of AmB, there were major differences between the reports supporting immunopotentiating effects of AmB and those supporting immunosuppressive effects. Single-dose injections of AmB tended to have immunopotentiating effects[18,20,36], while multiple-dosage schedules showed immunosuppressive effects[20, 30]. We confirmed that administration of AmB after, not before, inoculation with *P. aeruginosa* led to increased mortality in mice (data not shown). It is therefore inferable that the observed immunopotentiating effect of AmB reflects a priming effect. Our results showed that this priming effect was induced in mice 24 hours after the administration of AmB. The administration schedule of AmB is an important factor when debating the immunological effects of this drug. Since AmB can induce positive or negative immunologic reactions under the same conditions, it is necessary to determine an administration schedule for AmB that will have immunopotentiating effects in the host without inducing immunosuppressive effects.

Our results suggest that we can use AmB to induce resistance to *P. aeruginosa* infection. However, AmB may also cause immunosuppressive changes in the host even using the recommended mode of administration. We therefore feel that it is very important to think about the immunological effects of AmB when treating immunocompromised hosts with the drug.

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References

1) GORDON, B. L. & P. A. S. JOHN: Amphotericin B in the chemotherapy of experimental *Schistosomiasis mansoni* in Swiss mice. Nature 200: 790~791, 1963
2) OLDS, G. R.; S. J. STEWART & J. J. ELLNER: Amphotericin B-induced resistance to Schistosoma mansoni. J.

Immunol. 126: 1667~1670, 1981
3) BRAJTBURG, J.; S. ELBERG, G. S. KOBAYASHI & G. MEDOFF: Toxicity and induction of resistance to Listeria monocytogenes infection by amphotericin B in inbred strains of mice. Infect. Immun. 54: 303~307, 1986
4) THOMAS, M. Z.; G. MEDOFF & G. S. KOBAYASHI: Changes in murine resistance to *Listeria monocytogenes* infection induced by amphotericin B. J. Infect. Dis. 127: 373~377, 1973
5) BISTONI, F.; A. VECCHIARELLI, R. MAZZOLLA, P. PUCCETTI, P. MARCONI & E. GARACI: Immunoadjuvant activity of amphotericin B as displayed in mice infected with *Candida albicans*. Antimicrob. Agents Chemother. 27: 625~631, 1985
6) HUGHES, W. T.; D. ARMSTRONG, G. P. BODEY, R. Feld, G. L. MANDELL, J. D. MEYERS, P. A. PIZZO, S. C. SCHIMPFF, J. L. SHENEP, J. C. WADE, L. S. YOUNG & M. D. YOW: Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained faver. J. Infect. Dis. 161: 381~396, 1990
7) FLICK, M. R. & L. E. CLUFF: Pseudomonas bacteremia: review of 108 cases. Am. J. Med. 60: 501~508, 1976
8) BRYAN, C. S.; K. L. REYNOLDS & E. R. BRENNER: Analysis of 1,186 episodes of gram-negative bacteremia in non-university hospitals: The effects of antimicrobial therapy. Rev. Infect. Dis. 5: 629~638, 1983
9) KREGER, B. E.; D. E. CRAVEN, P. C. CARLING & W. R. MCCABE: Gram-negative bacteremia: III. Reassessment of etiology, epidemiology and ecology in 612 patients. Am. J. Med. 68: 332~343, 1980
10) HIRAKATA, Y.; K. TOMONO, K. TATEDA, T. MATSUMOTO, N. FURUYA, K. SHIMOGUCHI, M. KAKU & K. YAMAGUCHI: Role of bacterial association with kupffer cells in occurrence of endogenous systemic bacteremia. Infect. Immun. 59: 289~294, 1991
11) GERY, I.; R. K. GERSHON & B. H. WAKSMAN: Potentiation of the T lymphocyte response to mitogens. I. The responding cell. J. Exp. Med. 136: 128~142, 1972
12) RUFF, M. R. & G. E. GIFFORD: Purification and physico-chemical characterization of rabbit tumor necrosis factor. J. Immunol. 125: 1671~1677, 1980
13) BRAJTBURG, J.; W. G. POWDERLY, G. S. KOBAYASHI & G. MEDOFF: Amphotericin B: current understanding of mechanisms of action. Antimicrob. Agents Chemother. 34: 183~188, 1990
14) LITTLE, J. R.; E. J. PLUT, J. KOTLER-BRAJTBURG, G. MEDOFF & G. S. KOBAYASHI: Relationship between the antibiotic and immunoadjuvant effects of amphotericin B methyl ester. Immunochemistry 15: 219~223, 1978
15) LITTLE, J. R. & J. E. WOLF: Immunologic effects of amphotericin B. Clin. Immunol. News. 8: 183~185, 1987
16) MEHTA, R. T.; K. MEHTA, G. LOPEZ-BERESTEIN & R. L. JULIANO: Effects of liposomal amphotericin B on murine macrophages and lymphocytes. Infect. Immun. 47: 429~433, 1985
17) SARTHOU, P.; D. PRIMI & P. A. CAZENAVE: B cell triggering properties of a nontoxic derivative of amphotericin B. J. Immunol. 137: 2156~2161, 1986
18) SHIRLEY, S. F. & J. R. LITTLE: Immunopotentiating effects of amphotericin B. I. Enhanced contact sensitivity in mice. J. Immunol. 123: 2878~2881, 1979
19) SHIRLEY, S. F. & J. R. LITTLE: Immunopotentiating effects of amphotericin B. II. Enhanced in vitro proliferative responses of murine lymphocytes. J. Immunol. 123: 2883~2889, 1979
20) VECCHIARELLI, A.; G. VERDUCCI, S. PERITO, P. PUCCETTI, P. MARCONI & F. BISTONI: Involvement of host macrophages in the immunoadjuvant activity of amphotericin B in a mouse fungal infection model. J. Antibiotics 36: 846~855, 1985
21) CHAPMAN, H. A. Jr. & J. B. HIBBS Jr: Modulation of macrophage tumoricidal capability by polyene antibiotics: support for membrane lipid as a regulatory determinant of macrophage function. Proc. Natl. Acad. Sci. U.S.A. 75: 4349~4353, 1978
22) CHIA, J. K. S. & M. POLLACK: Amphotericin B induces tumor necrosis factor production by murine macrophages. J. Infect. Dis. 159: 113~116, 1989
23) CHIA, J. K. S. & E. J. MCMANUS: In vitro tumor necrosis factor induction assay for analysis of febrile toxicity associated with amphotericin B preparations. Antimicrob. Agents Chemother. 34: 906~908, 1990
24) GELFAND, J. A.; K. KIMBALL, J. F. BURKE & C. A. DINARELLO: Amphotericin B treatment of human mononuclear cells in vitro results in secretion of tumor necrosis factor and interleukin-1. Clin. Res. 36: 456A, 1988
25) CROSS, A. S.; J. C. SADOFF, N. KELLY, E. BERNTON & P. GEMSKI: Pretreatment with recombinant murine tumor necrosis factor-α/cachectin and murine interleukin 1 protects mice from lethal bacterial infection. J. Exp. Med. 169: 2021~2027, 1989
26) CRUPRYNSKI, C. J. & J. F. BROWN: Recombinant murine interleukin-1 enhancement of nonspecific antibacterial resistance. Infect. Immun. 55: 2061~2065, 1987
27) HAVELL, E. A.: Evidence that tumor necrosis factor has an important role in antibacterial resistance. J. Immunol. 143: 2894~2899, 1989
28) MUEGGE, K. & S. K. DURUM: UNIT 6.2. Measurement of soluble and membrane-bound interleukin 1. *In* Current Protocols in Immunology. *Ed.*, J. E. COLIGAN *et al.*, Greene Publishing Assoc. and John Wiley & Sons, Inc., New York, 1991

29) BJORKSTEN, B.; C. RAY & P. G. QUIE: Inhibition of human neutrophil chemotaxis and chemiluminescence by amphotericin B. Infect. Immun. 14: 315~317, 1976
30) FERRANTE, A.; B. ROWAN-KELLY & Y. H. THONG: Suppression of immunological responses in mice by treatment with amphotericin B. Clin. Exp. Immunol. 38: 70~76, 1979
31) MARMER, D. J.; B. T. FIELDS, Jr, G. L. FRANCE & R. W. STEELE: Ketoconazole, amphotericin B, and amphotericin B methyl ester: Comparative in vitro and in vivo toxicological effects on neutrophil function. Antimicrob. Agents Chemother. 20: 660~665, 1981
32) THONG, Y. H. & D. NESS: Antifungal drugs and neutrophil chemotaxis. Lancet II: 568, 1977
33) ATKINSON, A. J., Jr. & J. E. BENNET: Amphotericin B pharmacokinetics in humans. Antimicrob. Agents Chemother. 13: 271~276, 1978
34) FIELDS, B. T., Jr.; J. H. BATES & R. S. ABERNATHY: Amphotericin B serum concentrations during therapy. Applied Microbiology. 19: 955~959, 1970
35) LITTLE, J. R.; A. ABEGG & E. PLUT: The relationship between adjuvant and mitogenic effects of amphotericin methyl ester. Cell. Immunol. 78: 224~235, 1983
36) STEWART, S. J.; P. J. SPAGNUOLO & J. J. ELLNER: Generation of suppressor T lymphocytes and monocytes by amphotericin B. J. Immunol. 127: 135~139, 1981