師大生物學報 第二十三期 中華民國七十七年十月

Protective effects of Heated-Killed *Clostridium butyricum* on experimental *Candida albicans* infections in mice

Hour-Young Chen

Department of Immunopathology, National Institute of preventive medicine, 161, Kun-yang Street, Nan-Kang, Taipei, Taiwan, 11513 Republic of China

Abstract

Bacterial vaccine, heat-killed *Clostridium butyricum* exhibits various immunomodulating activities including a strong protection activity against *Candida albicans* infection in mice.

The analyses of immunomodulating activities induced by the vaccine showed that the vaccine stimulated macrophage and natural killer cell activity. Stimulation of delayed type hypersensitivity, IgM antibody formation, induction of γ-type interferon and B-cell mitogenicity, were also observed. Among these immunomodulating activities, interferon was considered to be one of the important factors in the manifestation of *in vivo* anticandida activity. Further studies using recombinant α-A/D type interferon, confirmed an active role of interferon in the manifestation of the protection. The interferon production by the vaccine was also found to be dependent on the mouse strains used and most active in DDY mice.

Introduction

A butyric acid bacterium *Clostridium butyricum* MII 588 has been used clinically to prevent disturbances of microbial flora in intestine in Japan[1]. It has also been reported that oral administration of the bacterial spore improves clinical symptoms, such as diarrhea, constipation and abdominal distension [6]. During our studies on the mechanism of *C. butyricum's* anti-putrefactive action, we found that the bacterium showed strong an antimetastatic activity against B16-melanoma in mice, although it showed no direct cytotoxic effect on various tumor cell lines, such as L1210 and EL 4 leukemias (unpublished data by Chen *et al.*). These data indicate that the antitumor activity induced by the bacterium is host mediated and the bacterium may induce various immunomodulating activities.

In vivo protection activity against various microbial infections by BRMs (biological response modifiers), PS-K, MDP, LPS, OK-432 (Kaneda *et al.*, manuscript in preparation) has been reported. Since systemic candidiasis is a significant complication of immunodeficiency syndromes, surgical procedures, and immunosuppressive medical therapies[3]. We are interested in the immunomodulating activities of the vaccine.

In this paper, we report various immunomodulating activities including protection activity against *Candida albicans* in mice induced by the bacterium. We also discuss the possible effectors which play an important role in the manifestation of the anticandida activities.

Materials and Methods

Animals: Male Balb/c, BDF_1, C3H/He, DBA/2, DDY and ICR mice, 4 to 10 weeks old were purchased from Shizuoka Laboratory Animal Corporation Ltd., Hamamatsu, Japan and used throughout the study. For systemic infection, 4 weeks old DDY mice were used.

Preparation of CB vaccine: Clostridium butyricum: MII 588 was cultured for 24 hr at 37°C in PYG medium[4]. *C. butyricum*

was then harvested, washed several times with saline and suspended in saline. The cell suspension was heated for 1 hr at 100°C and lyophilized. CB, a lyophilized whole cell preparation of the *C. butyricum* MII 588 cells, was reconstituted with saline to give 20 mg/ml of the cells. MCB (methanol extract CB) fraction was prepared as follows: lyophilized whole cell preparation of CB was extracted with methanol and the solvent extract was evaporated *in vacuo* to dryness. The dried residue was dissolved in a small amount of methanol and chromatographed on Sephadex LH-20 column with methanol. The fast moving fraction with pale yellowish color was combined and evaporated to dryness. The dried residues were used as MCB fraction.

Media and cell lines: RPMI 1640 medium (Nissui Seiyaku Co., Ltd., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (Flow Laboratories, McLean, Virginia, USA), 40 µg/ml of gentamicin, and 3 µg/ml of glutamine. Eagle's minimum essential medium (MEM, Nissui Seiyaku) supplemented with 10% FCS, 60 µg/ml of kanamycin and 3 µg/ml of glutamine was also used. All cell cultures were carried out at 37°C in a humidified atmosphere with 5% CO_2. YAC-1 lymphoma cells of A/Sn origin were maintained in RPMI 1640 medium. EL-4 leukemic cells were maintained in the peritoneal cavities of C57BL/6 mice by weekly sequential transplantation. Mouse fibroblast cell line L929 was grown in MEM.

Systemic infection with C. albicans in mice: A highly virulent *C. albicans* IFM 40009 (ATCC 48130) strain was grown at 37 °C on Sabouraud dextrose agar (SDA, Difco Lab., Detroit, USA) for 18 hr. Mice were infected with intravenous injection of 5 x 10^5 blastospores of *C. albicans* IFM 40009.

Effect of CB, MCB or interferon (IFN) on C. albicans infection: In vivo anticandida activity was determined by the comparison of survival time with that of saline treated controls. Before *C. albicans* infection, mice were given an interaperitoneal injection of

CB on day -3, -2, and -1, *C. albicans* cells (5×10^5) in 0.2 ml of saline were inoculated into the tail vein of mice on day 0.

In case of IFN (A/D) (kindly given by Hoffman-La Roche, Inc., Japan), 1×10^4 U/day of IFN was administered intravenously on day -1.

Mitogenicity: Mitogenicity was determined by a slight modification of the method described by Kumazawa *et al.*[5] Briefly, spleen and thymus cell suspensions were prepared by gentle teasing, passed through a 200-gauge stainless steel sieve, and centrifuged. The packed cells were treated with ammonium chloride tris buffer to lyse red cells, and washed three times with Eagle's MEM. The cells were cultured at a density of 1×10^6 cells/ml in a total volume of 0.2 ml. After incubation at 37°C in a humidified atmosphere, ^{3}H-thymidine was added to each culture and further incubated for 4 hr. Cells were collected on glass fiber filters using a multiple cell harvester. Incorporation of ^{3}H-thymidine was determined by liquid scintillation counter. Lipopolysaccharide (LPS, Difco) and concanavalin A (Wako Pure Chemical Ind. Ltd., Japan) were used as controls.

Delayed-type footpad reaction: This reaction was determined by the method described by Yoshikai *et al.*[6] Ten-week old DDY mice were injected interperitoneally with 2×10^6 sheep red blood cells (SRBC) and at the same time with various doses of CB by the same route. Four days later 1×10^8 SRBC was given and 24 hr footpad swelling was measured.

PFC assay: Numbers of hemolytic plaque-forming cells (PFC) in the spleen were determined to study the effect of CB on antibody formation according to the method of Cunningham and Szenberg[7].

IFN assay and its induction by CB treatment: Mice were intraperitoneally given 1 mg of CB in 0.2 ml of saline. At intervals, groups of 5 mice were bled by cardiac puncture, the blood was pooled and sera were separated by centrifugation. The sera were frozen at

$-80°C$ until testing for IFN contents[8]. OK-432, a streptococcal whole cell preparation (Chugai Seiyaku Co. Ltd., Tokyo) was used as control IFN producers. The antiviral activity of the mouse serum (IFN titer) was determined using the phage plaque reduction method on mouse L-929 monolayer cell cultures by the method described previously[9]. IFN titer was expressed in units per milliliter as the reciprocal of the dilution that reduced cytopathic effect (CPE) by 50% as compared with the virus control. IFNα (A/D) was used as a standard.

Neutralization test: Anti-IFN (α / β) serum, a rabbit antiserum against mouse L-cell IFN was purchased from Lee Biomolecular Research, San Diego, CA, U.S.A. Neutralization tests[10] were performed by the method described by Yamamoto and Kawada[11].

Natural killer (NK) cell activity assay: The activity was determined by cytotoxic activity against YAC-1 target cells. Short term ^{51}Cr-release assay of NK cell activity was performed with fresh murine spleen cells used as the effector cells. Briefly YAC-1 target cells were labeled for 40 min at 37°C with 100 uCi of $Na_2{}^{51}CrO_4$[5]. In all assays, 2×10^4 target cells in a volume of 100 µl were seeded in round-bottom tissue culture plates (Costar). Appropriate concentrationsofspleen cells in a 100 µl volume were added. Radioactivity was counted in a gamma counter (LKB). The percent specific ^{51}Cr-release was calculated according to the formula:

Specific lysis(%) = (experimental ^{51}Cr release − spontaneous ^{51}Cr release) divided by (maximun ^{51}Cr release-spontaneous ^{51}Cr release)

The spontaneous ^{51}Cr-release from target cells incubated in medium alone was less than 10% of the total ^{51}Cr.

Macrophage activity: The activity was also determined by the cytostatic activity of macrophages against EL-4 leukemia. Macrophage fraction was prepared from peritoneal exudate cells (PEC) of CB treated mice by the plastic adhering method.

The *in vitro* cytostatic activity of macrophages was determied using the ^{3}H-thymidine incorporation inhibition method described by Kato *et al.* [12]. EL-4 leukemia cells were used as target cells. Results obtained as average counts per minute were expressed as percent incorporation calculated by the formula: [cpm (tumor + effector cell) - cpm(effector cell alone)] divided by cpm(tumor cell alone) x 100(%).

Phagocytic activity against opsonized SRBC: SRBC and anti-SRBC IgG were purchased from Nippon Bio-Test Laboratories, TOKYO and from Japan Immunoresearch Laboratories CO., Ltd., respectively. Assay method was the same with that of Kato *et al.* [12]. The number of SRBC phagocytized by peritoneal macrophages was counted microscopically and phagocytic activity was also determined as the number of SRBC phagocytized per 100 macrophages.

Results

Protection induced by CB against systemic C. albicans infection in mice:

Our previous antimetastatic studies of CB (Chen *et al.*, manuscript in preparation) indicated that CB is more active in prophylactic protection model. Therefore, CB was administered intraperitoneally to DDY mice as a premedication, *i.e.*, for three consecutive days before intravenous infection with *C. albicans.* The results were summarized in Fig. 1. In this experiment, all control mice died within 4 day. On the other hand, when CB doses ranging from 0.1 to 1.0 mg/mouse were administered, significant protection was observed at all doses tested. Optimun dose was around 0.5 to 1.0 mg/mouse and 40% of the group were survived after 30 day. These results indicate that CB could induce strong host mediated anticandida activity. MCB, methanol extract of CB, was also found to be active against the infection. Seventy percent of the mice were still survived after 30 day at the dose of 0.5 mg/mouse of MCB. These results indicate that active principles of CB were extractable by the solvent, although they are not

purified yet.

Mitogenicity of CB: Since CB was found to be one of the active BRMs against the infection, various immunomodulating activities of CB were studied. First, mitogenic responses to CB were determined by the enumeration of ^{3}H-thymidine incorporation into cultured spleen and thymic cells by pulse labeling. Both cell suspensions prepared from DDY mice were cultured with the indicated amount of CB in comparison with LPS and Con A. The results are shown in Table 1. Stimulation of mitogenic responses was observed to be dose-dependent. Maximum stimulation of thymidine uptake was seen with 5.0 μg of CB per 0.2 ml well. However, throughout the studies, the mitogenic response with CB was somewhat lower than those of LPS and Con A. On the other hand, no mitogenic activity with thymocytes was observed. Time course of mitogenic responses with the spleen cells induced by 5.0 μg of CB was studied and compared with that of LPS (Fig. 2). Mitogenic index increased with time, reached maximum at day 4 and then decreased. The incorporation profiles of thymidine were similar to those of LPS. These results indicate that CB has B-cell mitogenicity.

Effect of CB on antibody formation: Antibody formation after CB treatment in mice was determined using a PFC assay method with the spleen cells. DDY mice were immunized intraperitoneally with 1 x 10^{7} SRBC and concomitantly given intraperitoneally injections of 0.5, 1.0, 2.0 and 5.0 mg/mouse of CB. Four days later, the number of anti-SRBC IgM forming cells in the spleen was determined. As shown in Table 2, a marked enhancement of PFC numbers in the spleen was observed in groups of mice treated with 1.0 to 5.0 mg/mouse of CB: the maximum dose was 2.0 mg/mouse and the numbers of PFC declined with the dose of 5.0 mg/mouse.

Delayed-type footpad reaction: When delayed-type footpad swelling was measured with CB

doses ranging from 0.1 to 10.0 mg, a slight increase (statistically significant) in swelling was observed with CB doses from 1.0 to 5.0 (data not shown).

Time course of NK cell and macrophage activity: Activities of macrphages and NK cell after CB treatment were studied and the results were shown in Fig. 3. Maximum NK cell activity of spleen cells was observed on day 3 and then decreased rapidly. On the other hands, macrophage activity of PEC gradually increased and attained to maximum on day 9 to 10.

Effect on phagocytic activity by macrophage: To further characterize macrophage activation, phagocytic activity was measured as an index of macrophage activation. As shown in Table 3, when DDY mice were given an intraperitoneal injection of 1 mg/mouse of CB, phagocytic activity of macrophages was significantly enhanced and the increase was three times higher than that of control.

IFN production In the above experiments, we demonstrated highly stimulation of macrophage and NK cell functions. Since macrophage and NK cell have been reported to be stimulated by IFN, we attempted to determine the IFN inducing activity of CB. When DDY mice were given CB intraperitoneally at the doses of 0.1 to 10 mg/mouse, high titers of IFN were observed. Optimum dose for the IFN production was 1 mg/mouse as shown in Fig. 4. The maximum IFN activity was 2,560 units, while only 320 units of IFN titer with a positive control of OK-432. Time course of IFN production was shown in Fig. 5. IFN production occurred as early as 18 hr after administration of CB and reached a maximum at [illegible] hr. Thereafter IFN activity fell rapidly and return to normal level within 7 days.

Production and characterization of interferon in different mouse strains: In order to determine the response of different mouse strains to CB, 6 strains of mice i.e., C57

BL/6, C3H/He, Balb/c, BDF_1, ICR and DDY mice were used. These different mouse strains were treated with CB and IFN titers at 24 hr were determined. As shown in Table 4, there were significant differences in IFN production among these mouse strains tested. The highest response was observed with DDY and BDF_1, followed by C57BL/6 and ICR, and the lowest

Table 1. **Mitogenic responses of CB to lymphocytes from DDY mice**

Cell cultured	Sample (μg/well)	[^{3}H]Thymidine uptake (Mean cpm ± S.D.)**	Significance(p)***
Thymocyte*	None	585.3 ± 113.5	
	Con A(2.0)	44913.0 ± 1159.1	<0.001
	LPS (10.0)	1089.3 ± 128.2	<0.1
	CB (1.0)	306.3 ± 42.6	n.s.
	(5.0)	424.3 ± 7.0	n.s.
	(10.0)	619.0 ± 101.8	n.s.
Splenocyte*	None	5517.3 ± 923.5	
	ConA(2.0)	31823.3 ± 881.1	<0.005
	LPS (10.0)	39741.6 ± 1181.1	<0.001
	CB (1.0)	9244.0 ± 1396.4	n.s.
	(5.0)	17224.6 ± 1554.7	<0.01
	(10.0)	16701.3 ± 846.3	<0.01

*Thymocytes or splenocytes were cultured with the indicated doses of Con A, LPS and CB for 2 days in microtiter plates at cell density of 1×10^6 cells/ml in a total volume of 0.2 ml. After 2 days, incorporation of [^{3}H]-thymidine was determined.

**Mean cpm ± standard deviation.

***n.s.: not significant. Statistically different from control at the indicated p values.

Table 2. **Adjuvant effect of CB on antibody response to SRBC**

Stimulant	CB dose(mg/mouse)*	Anti-SRBC PFC/spleen (mean PFC ± S.D.)**	Significance(p)***
SRBC only	0	61213 ± 3882	
SRBC + CB	0.5	81082 ± 7226	n.s.
"	1.0	190492 ± 1445	<0.005
"	2.0	275569 ± 5336	<0.005
"	5.0	169655 ± 846	<0.005

*Five male DDY mice were immunized intraperitoneally with 1 x 10^7 SRBC and treated with the indicated doses of CB at the same time (on day 0). Four days later (on day 4). anti-SRBC antibody forming cells were determined as a hemolytic plaque.

**Mean PFC number in the spleen cells ± standard deviation.

***n.s.: not significant. Statistically different from control at the indicated P values.

Table 3. **Phagocytic acitivity of macrophages from CB treated DDY mice.**

Treatment regimen*	% Macrophage engulfing SRBC (Mean±S.D.)***	SRBC/macrophage** (Mean ± S.D.)***
Control	44.4 ± 5.3	151.0 ± 28.7
CB(1.0 mg/mouse x 3 days)	80.2 ± 6.7****	421.0 ± 80.7****

*CB was injected intraperitoneally for 3 consecutive days(days -3, -2 and -1), and after 2 days (on day 2), peritoneal macrophages (PEC) were harvested. Sheep erythrocytes (SRBC) coated with anti-SRBC antiserum were used.

**Number of SRBC found in 100 macrophages including both engulfing and non-engulfing cells.

***Mean number ± standard deviation.

****Statistically different from control at $p<0.05$.

Table 4. **Induction and characterization of IFN induced by CB treatment in various mouse strains CB**

	IFN titers(unit)				
	IFN produced*		Residual IFN titer (unit/ml) after tereatment with		
Mouse strain	Control	CB	pH 2	Heat	Anti-α/β**
DDY	<10	2560	<10	<10	2560
C57BL/6	20	1280	<10	<10	NT
C3H/He	40	320	<10	<10	NT
ICR	10	1280	<10	<10	NT
Balb/c	<10	640	<10	<10	NT
BDF1	<10	1280	<10	<10	NT

* Pooled sera were obtained from 6 to 8 DDY mice from each group 24 h after intraperitoneal injection of 1.0 mg/mouse of CB.
Each treated serum was assayed immediately for the antiviral activity.
**Result after neutralization by antiserum.

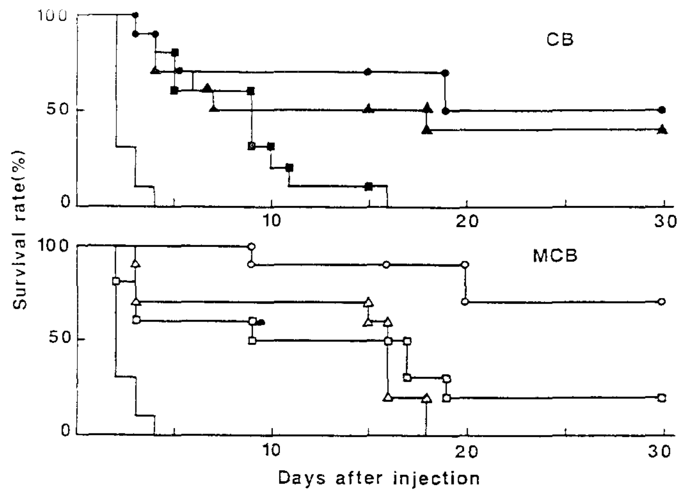

Fig. 1. Protection activity induced by CB and MCB against systemic *Candida albicans* infection in mice. Ten DDY mice were used in each group.
CB: (— control, ■—■ 0.1 mg/mouse, ●—● 0.5 mg/mouse ▲—▲ 1.0/mouse)
MCB: (— control, □—□ 0.1 mg/mouse, ○—○ 0.5/mouse △—△ 1.0 mg/mouse)

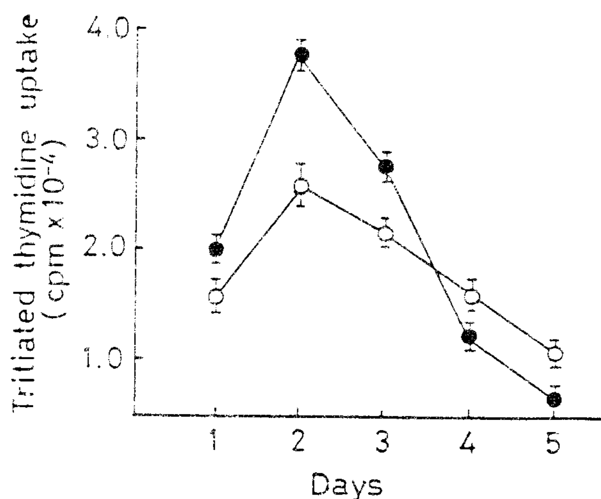

Fig. 2. Kinetics of mitogenic responses of splenocytes to CB. The spleen cells were cultured for 2 days in microtiter plate at a density of 1 x 10^6 cells/ml with 5 μg of CB (O) or 10 μg of LPS (●). [H] Thymidine was added to the culture and after 4 h incubation. Incorporation ratio of the activities were determined.

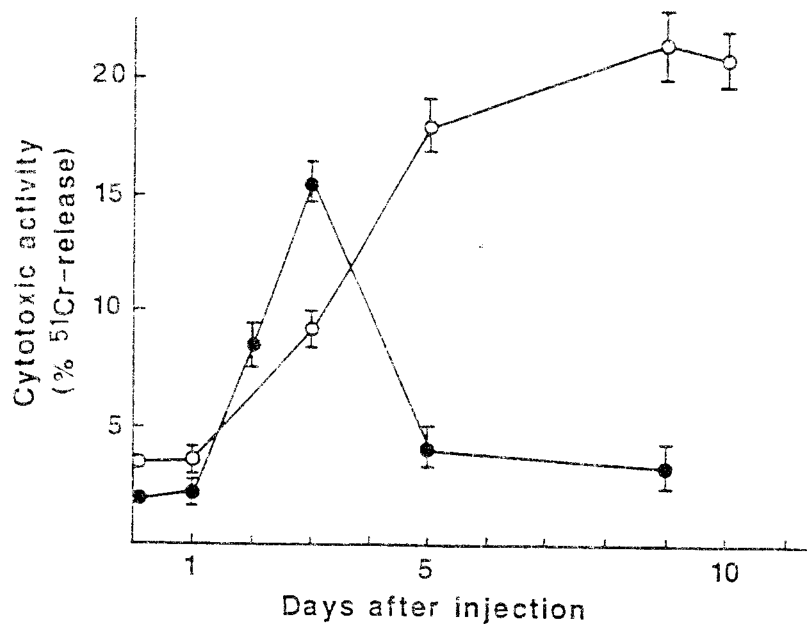

Fig. 3. Time course of NK-cell and macrophage activities after CB treatment. Macrophage (o——o) and NK cell (●——●) activities (mean standard derviation).

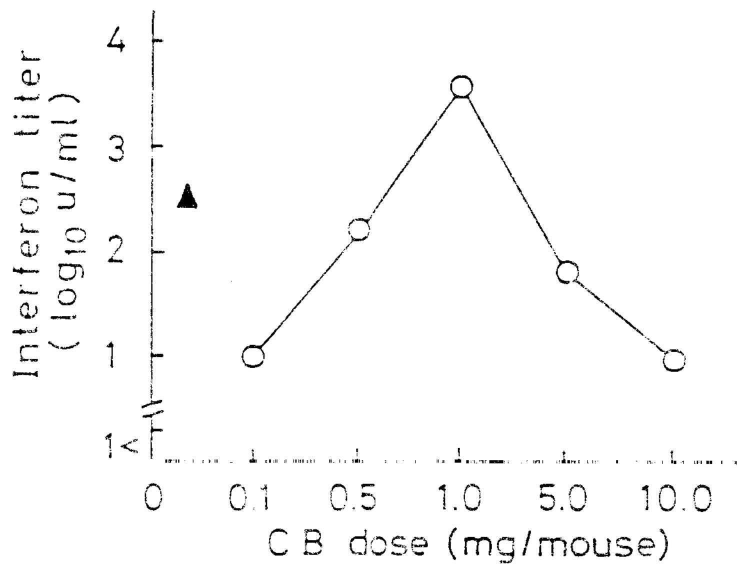

Fig. 4. Production of IFN in DDY mice by various doses of CB. pooled sera were obatined from DDY mice 24 h after intraperitioneal injection of various doses of CB (○) OK-432 (▲) was used as a reference. Five mice were used in each dose.

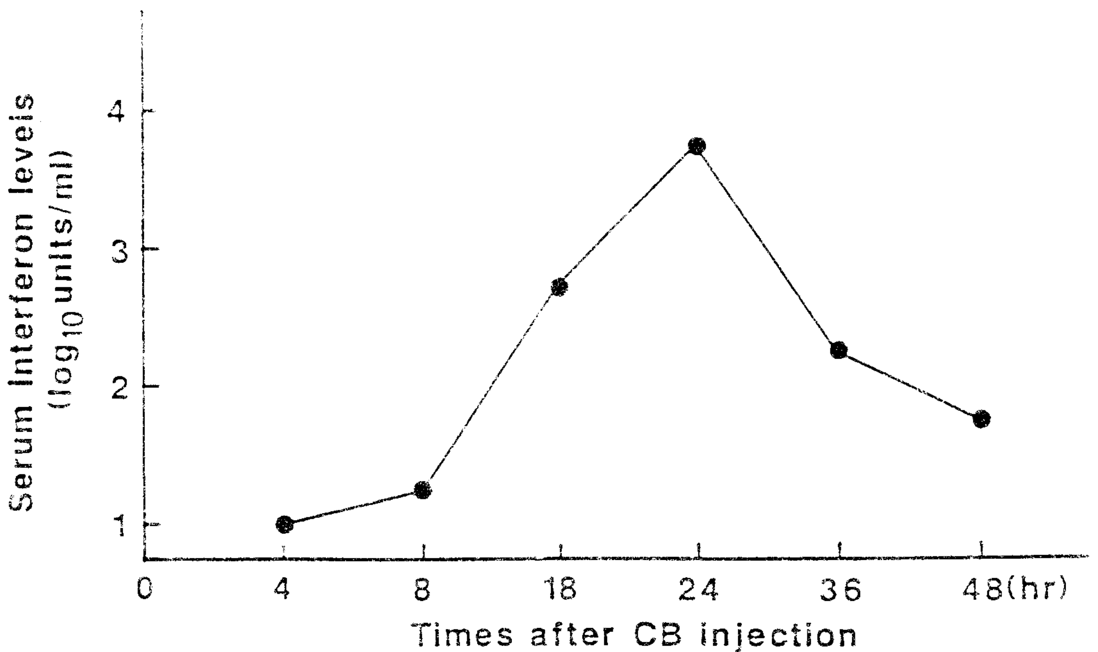

Fig. 5. Time couse of IFN production in DDY mice. Five mice were used in each experiment.

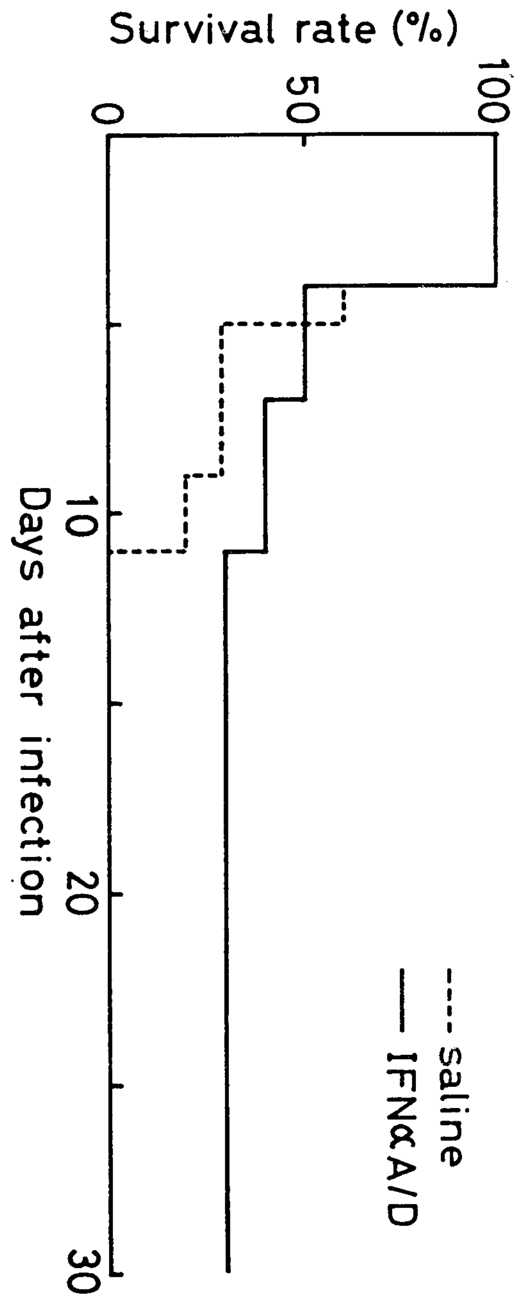

Fig. 6. Effect of recombinant IFN-α (A/D) on *Candida albicans* infection in mice. IFN (1 x 10^4 units of IFN-α (A/D) were adminstered intravenously into the tail vein of DDY mice.

response was observed with C3H/He and Balb/c mice. The difference was more than 4 times among them. In order to determine the type of IFN induced by CB, various physicochemical properties were compared. IFN activity was diminished by dialysis against 0.1 M glycine-HCl buffer (pH 2.0) for 24 hr at 4°C or incubation at 56°C for 60 min, while without decrease in IFN activity after neutralization with anti-IFN serum. The activity also disappeared with trypsin treatment, indicating that the present major IFN activity is similar to immune-type interferon, γ-IFN reported by Falcoff [13].

Effect of recombinant IFN-α (A/D) on C. albicans infection in mice:

Since it was found that high titer of IFN is produced after CB treatment in DDY mice, protection activity of recombinant - IFN against *Candida* infection was studied. As shown in Fig. 6. IFN showed a significant protection activity when administered intravenously. These data suggested an important role of IFN as an effector in the protection against Candida infection in mice.

Discussion

In the present studies, we first confirmed that CB has various immunomodulating activities which are alraeady reported in various bacterial BRMs such as bacillus Calmette-Guerin (BCG)[14], PSK[15], MDP[16], LPS[17] and OK-432[18].

However, it has been reported that there exist subtle differences in immunomodulating activity among these bacterial preparations[19]. Furthermore, living spores of *C. butyricum* have been used clinically over the past 40 years in Japan and their clinical seftyness has been confiremd[2]. Therefore, CB is considered worth of further evaluation as an active BRM for anticandida therapy, although detailed studies are nesessary to apply for clinically.

Adjuvant-like activity is also found with CB. This is reported with a number of different classes of BRMs which

can be classified into two groups: Poly ICLC and MVE-2 belong to one group and have ability to activate macrophage. Thymosin fraction 5 belongs to the other and these immunomodulators have an apparent direct effect on T-cells [19]. According to this definition, CB might belong to the former group, because it showed only a weak delayed-type hypersensitivity and mitogenic response with thymocytes.

Throughout the present studies, we used mainly heat-killed whole cell preparation of *C. butyricum* (CB). However, in the present studies, we could demonstrate that the active principles of CB are extractable with organic solvent. MCB fraction is partially purified by Sephadex LH-20 column chromatography. In our preliminary studies, MCB is found to be a potent NK-cell activator and has B-cell mitogenic activity. Now, further purification studies are in progress.

Since some bacterial preparation have been also reported to have IFN inducing activity both *in vitro* and *in vivo*[20], we attempted to identify the antiviral principle. The present experiments showed that IFN produced by CB treatment is mainly γ-type.

With reference to effectors, IFN was considered as a most possible candidate of anticandida activity in mice. Actually it was found that recombinant α-IFN showed a protection activity when administered intravenously. However, recently we found that lentinan[21] also exhibit strong protection against *C. albicans* infection (Kaneda *et al.*, manuscript in preparation). As already reported[21], and we also confirmend it, production of IFN by lentinan is very weak or negligible. Therefore, it is reasonable to consider that some other factors such as interleukins, in the present model might be olaying a role as effectors to protect mice against the infection.

We also found that IFN production by CB is strain dependent and DDY or BDF1 mice was a high responder in IFN

production by CB. Therefore, experimental model using DDY mice and CB vaccine are considered to be a suitable tool for analysis of protection mechanisms induced by CB treatment.

References

1. Miyairi, K. 1935, Effect of pH on the growth of intestinal microflora (in Japanese). J. Chiba Med. Soc., 13: 1102-1157.
2. Kobayashi, S., J. Tanami and T. Kitamura. 1968. Effect of oral administration of Miyarisan, a preparation composed of *Clostrodium butyricum* spores, on the intestinal bacterial flora of infants. J. Chiba Med. Soc., 44:186-192.
3. Thomas, E.R. and J.N. Galgiani. 1986. Activity of fluconazole (UK49, 853) and ketoconazole against *Candida albicans in vitro* and *in vivo*. Antimicrob. Agents and Chemother., 30: 418-422.
4. Maeda, A., K. Ishii, M. Tanaka, Y. Mikami and T. Arai. 1986. KM1, a bacteriophage of *Clostridium butyricum*. J. Gen. Microbiol., 132: 2271-2275.
5. Kumazawa, Y., K. Mizunoe and Y. Otsuka. 1982. Immunostimulating polysaccharide separated from hot water extract of *Angelica acutiloba* Kitagawa (Yamato Tohki). Immunology, 47: 75-83.
6. Yoshikai, Y., S. Miyake, T. Matsumoto, L. Nomoto and K. Takeya. 1979. Effect of stimulation and blockade of mononuclear phagocyte system on the delayed footpad reaction to SRBC in mice. Immunology, 38: 577-583.
7. Cunningham, A.J. and A. Szenberg. 1968. Further improvement in the plaque technique for detecting single antibody-forming cells. Immunology, 14: 599-601.
8. Kuwata, T., T. Oda, S. Sekiya and N. Morinaga. 1976. Characteristics of a human cell line successively transformed by Rous sarcoma virus and simian virus 40. J. Natl. Cancer Inst., 56: 919-926.
9. Armstrong, J.A. 1971. Semi-

micro, dye-binding assay for rabbit interferon. Appl. Microbiol., 21:723-726.

10. Kawada, Y. 1980. An analysis of neutralization reaction of interferon by antibody: A proposal on the expression of neutralization titer. J. Interferon Res., 1: 61-70.

11. Yamamoto, Y. and Y. Kawada 1980. Antigenicity of mouse interferon distinct antigenicity of the two L-cell interferon species. Virology, 103:80-88.

12. Kato, I., T. Yokokura and M. Mutai. 1983. Macrophage activation by *Lactobacillus casei* in mice. Microbiol. Immunol., *27:* 611-618.

13. Falcoff, R. 1972. Some properties of virus and immune-induced human lymphocyte interferons. J Gen. Virol., *16:* 251-253.

14. Sher, N.A., L.E. Chaparas Greenberg, and S. Bernard. 1975. Effect of BCG, *Corynebacterium parvum* and methanol-extraction residue in the reduction of mortality from Staphylococcus aureus and Candida albicans infection in immunosuppressed mice. Infect. Immunol., 12: 1325-1330.

15. Mayer, P. and J. Drews 1980. The effect of a protein-bound polysaccaraide from *Coriolus versicolor* on immunological parameters and experimental infections in mice. Infection, 8:13-21.

16. Chedled, L., M. Parant, F. Parant, P. Lofrancier, J. Choay and E. Lederer. 1977. Enhancement of non-sepcific immunity to *Klebsiella pneumoniae* infection by a synthetic immunoadjuvant (N-acetyl-muramyl-L-alanyl-D-isoglutamine) and several analogs. Proc. Natl. Acad. Sci., *74:* 2089-2093.

17. Kotani, S., H. Takada, M. Tsujimoto, T. Ogawa, I. Takahashi, T. Ikeda, K. Otsuka, H. Shimauchi, N. Kasai, J. Mashimo, S. Nagao, A. Tanaka, S., Tanaka, K. Harada, K. Nagaki, H. Kitamura, T. Shiba, S. Kusumoto, M. Imoto and H. Yoshimura. 1985. Synthetic lipid A with endotoxic and related biological activities comparable to those of a natural lipid A from an

Escherichia coli Re-mutant. Infect.Immun., *49:* 225-237.

18. Shiraishi, A., Y. Mikami and T. Arai. 1979. Protective effect of OK-432 (a streptococcal preparation) on experimental candidiasis. Microb. Immunol., 23: 549-554.

19. Talmadge, J. E. and R.B. Herberman. 1986. The preclinical screening laboratory: Evaluation of immunomodulatory and therapeutic properties of biological response modifiers. Cancer Treatment Rept., *70:* 171-182.

20. Yamaguchi, T., Y Kuroda, M Saito, T Ebina, F. Hoshino and N. Ishida. 1984. Immune interferon producing TH69, a lyophilized preparation of *Streptococcus faecalis* in murine spleen cell cultures. Microbiol. Immunol., *28:* 601-610.

21. Hamuro, J. and G. Chihara. 1982. Lentian, a T-cell-oriented immunopotentiator. In Immune Modulation Agents and Their Mechanisms.: Marcel Dekker Inc., New York., p.409-435.

酪酸菌對白色念珠菌感染症之防禦效果

陳 豪 勇
行政院衛生署預防醫學研究所

摘 要

經熱處理之酪酸菌（*Clostridium butyricum* MⅡ588 strain）菌體可增强老鼠之免疫機能，對真菌之一的白色念珠菌（*Candida albicans* 7N strain）之深在性感染症具高度的保護效果，經分析其免疫增强效果時發現，此菌體可促進細胞性遲延性過敏症反應（DTH）之發生，增加IgM抗體產生細胞數，促使巨噬細胞與NK細胞活性化，同時也可誘發產生免疫型干擾素（IFN-γ），而此干擾素之產生因老鼠品系而異，其中以DDY品系老鼠最佳。在in vivo之抗真菌過程中，此干擾素扮演著重要角色，因用基因工程生產之干擾素（IFNα-A/D）處理結果也證實干擾素確可增强保護真菌之感染，除此之外也發現酪酸菌是一種B細胞分裂原。