# WS1279, A NOVEL LIPOPEPTIDE ISOLATED FROM *Streptomyces willmorei*

## BIOLOGICAL ACTIVITIES

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WS1279, a new lipopeptide isolated from the fermentation broth of *Streptomyces willmorei* No. 1279, stimulated the proliferation of mouse bone marrow cells *in vitro* and accelerated the recovery of granulocyte counts in bone marrow from leukopenia induced by mitomycin C (MMC) in mice. The glycerylcysteine moiety of WS1279 is necessary and the lipid peptide structure is required for manifestation of full stimulating activity *in vitro*. WS1279 was the most effective on the proliferation of bone marrow cells among the tested immunostimulants *in vitro*. However, the effect of WS1279 on restoration of reduced granulocyte counts in MMC-induced leukopenia in mice was less than that of FK-565, lipopolysaccharide, picibanil or forphenicinol. WS1279 augmented host resistance to infection with *Staphylococcus aureus* 47 in normal and immunosuppressed mice.

Bone marrow suppression is the most important side effect limiting the dose of cancer chemotherapy or radiotherapy[1]. A major impediment to dose escalation is infection during the associated myelosuppression[2,3]. Therefore, drugs that prevent the infection or accelerate recovery from iatrogenic myelosuppression are clinically useful. The hematopoietic growth factors, colony-stimulating factors (CSFs), are proving effective *in vivo* stimulation of granulocytopoiesis in clinical situations associated with myelosuppression[4]. In the course of our screening program for myelopoietic substances, WS1279 (**1**) was isolated from the culture broth of *Streptomyces willmorei* No. 1279[5]. The structure of WS1279 was established on the basis of chemical and physical evidence as *S*-[2,3-bis(palmitoyloxy)propyl]-$N^{\alpha}$-palmitoyl-Cys-Asn-Ser-Gly-Gly-Ser-OH and was confirmed by synthesis[6,7]. In the present study we investigated the myelopoietic activity of WS1279 *in vitro* and the ability to restore the number of granulocytes from leukopenia and to recover the host resistance to microbial infection in immunosuppressed mice by WS1279 *in vivo*.

## Materials and Methods

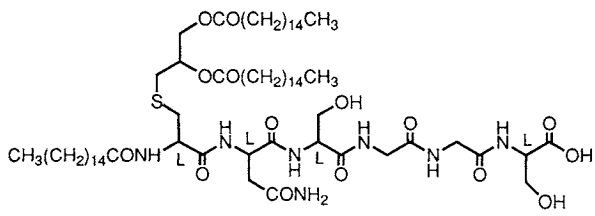
### Animals

Specific pathogen-free female $BDF_1$ mice were purchased from Charles River Japan.

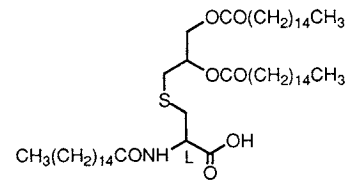
### WS1279 and Synthetic Compounds

Natural WS1279 was isolated from the culture broth of *Streptomyces willmorei* No. 1279[5]. *S*-[(2*RS*)-

Fig. 1. Structures of WS1279 and synthetic intermediates.



WS1279 (**1**)



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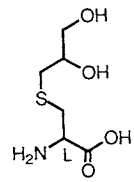

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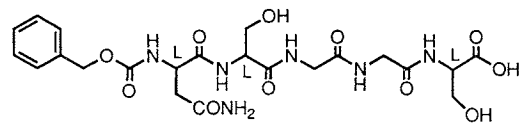

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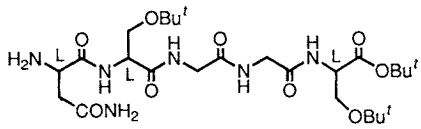

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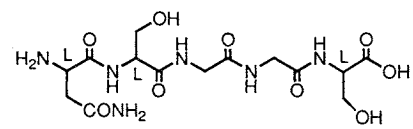


**6**

OBu$^t$: *tert*-butyl ester

2,3-bis(palmitoyloxy)propyl]-*N*-palmitoyl-Cys-OH (**2**) was purchased from Boehringer Mannheim GmbH. Synthetic WS1279 and its synthetic intermediates, *S*-[(2*RS*)-2,3-dihydroxypropyl]-Cys-OH (**3**), benzyloxycarbonyl-Asn-Ser-Gly-Gly-Ser-OH (**4**), H-Asn-Ser(Bu$^t$)-Gly-Gly-Ser(Bu$^t$)-OBu$^t$ (**5**) and H-Asn-Ser-Gly-Gly-Ser-OH (**6**), were obtained as described previously[7].

Drugs

Mitomycin C (MMC) was purchased from Kyowa Hakko Kogyo Co., Ltd. FK-565 and forphenicinol were prepared in our laboratories. Lipopolysaccharide (LPS, *Escherichia coli* 055: B5), picibanil, muramyl dipeptide (MDP) and bestatin were obtained from Difco Laboratories, Chugai Pharmaceutical Co., Ltd., Sigma Chemical Co. and Nippon Kayaku Co., Ltd., respectively.

Preparation of Mouse Bone Marrow Cells

Bone marrow cells of $BDF_1$ mice, 6~9 weeks of age, were prepared from their femurs. The bone marrow cells ($3 \times 10^6$ cells/ml) were suspended in DULBECCO's modified EAGLE's medium (DMEM) containing 10% heat inactivated fetal bovine serum (FBS), 100 U/ml benzylpenicillin, 100 $\mu$g/ml streptomycin, $5 \times 10^{-5}$ M 2-mercaptoethanol and 2.5% L929 cell-conditioned medium (LCM).

Proliferation of Bone Marrow Cells

Bone marrow cell suspension (25 $\mu$l) and sample solution (50 $\mu$l) in DMEM were mixed per well of 96-well flat-bottomed microtiter plates (Sumitomo Bakelite) and incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in air for 4 days. The proliferation of bone marrow cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method[8]. The reduced MTT was dissolved in 0.04 N HCl with isopropanol, and then, absorbance was measured at 550 nm with reference at 660 nm using a microplate reader (MTP100, Corona Electric).

Mouse Leukopenia Model

Female $BDF_1$ mice, 7 weeks of age, were used. MMC was given in a single intraperitoneal dose of 4.6 mg/kg.

Flow Cytometry

Bone marrow cells from femur were suspended in phosphate-buffered saline and separated using a FACS440 flow cytometer (Becton Dickinson) on the basis of their light scattering properties[9]. The forward light scatter was collected with the obscuration bar set as flat as possible at an angle between approximately $0.5° \sim 13°$ and the perpendicular light scatter was simultaneously collected within a cone of approximately $70° \sim 110°$ angle to the laser direction. The argon ion laser was operated at 488 nm using 300 mW of power. A minimum of 30,000 events was collected in list mode on a Consort 30 Data Management System. The absolute number of granulocytes in bone marrow was calculated from the total number of bone marrow cells and the differential count of granulocytes measured by flow cytometry. Total number of bone marrow cells was counted with a Micro-Cell Counter CC-130 (Sysmex).

Systemic Infections in Mice

*Staphylococcus aureus* 47, maintained in our laboratory, was grown on Nutrient Broth Agar (Difco) overnight at 30°C.

Normal female $BDF_1$ mice, 6 weeks old, were intraperitoneally infected with $1.8 \times 10^8$ cells of *S. aureus* 47 suspended in 5% mucin (1/2MLD) on day 0. Synthetic WS1279 was suspended in 0.5% methylcellulose and intraperitoneally administrated daily for the three consecutive days ($-3$ to $-1$ day) before infection.

Immunosuppressed $BDF_1$ mice produced by a single intraperitoneal treatment with 4.6 mg/kg of MMC three days prior to infection were infected intraperitoneally with $1.2 \times 10^7$ cells of *S. aureus* 47 in 5% mucin suspension (1/2MLD). Synthetic WS1279 suspended in 0.5% methylcellulose was intraperitoneally administrated 1 hour after MMC injection and daily for the two consecutive days.

The mortality and the body weight of the survived mice were recorded on day 3 after infection.

Induction of CSF

Synthetic WS1279 suspended in 0.5% methylcellulose was administrated subcutaneously into female $BDF_1$ mice (7 weeks old). Five hours after the administration of WS1279, the level of CSF in the serum was determined by measuring the proliferation of bone marrow cells as described above[8]. The serum was added at 10% concentration in the DMEM without 2.5% LCM.

Statistical Analysis

Statistical analysis was carried out by STUDENT's *t*-test.

## Results

### Myelopoietic Activity of WS1279 *In Vitro*

Natural and synthetic WS1279 were investigated for their ability to stimulate the proliferation of mouse bone marrow cells according to the method described in Materials and Methods. The mitogenic effects of natural and synthetic WS1279 on bone marrow cells of $BDF_1$ mice can be seen from the dose response plots in Fig. 2. The $ED_{50}$ values (50% increase over control) of natural and synthetic WS1279 on the proliferation of bone marrow cells of $BDF_1$ mice were 0.33 $\mu$g/ml and 1.3 $\mu$g/ml, respectively.

In order to determine more precisely the minimum structural requirements of WS1279 (**1**) for myelopoietic activity *in vitro*, various synthetic fragments of WS1279 were examined. As shown in Fig. 3, *S*-[(2*RS*)-2,3-bis(palmitoyloxy)propyl]-Cys-OH (**2**), glycerylcysteine moiety of WS1279, stimulated the proliferation of bone marrow cells dose-dependently. The $ED_{50}$ value of **2** was 12.5 $\mu$g/ml and less than that of WS1279. On the othre hand, *S*-[(2*RS*)-2,3-dihydroxypropyl]-Cys-OH (**3**), benzyloxycarbonyl-Asn-Ser-Gly-Gly-Ser-OH (**4**), H-Asn-Ser($Bu^t$)-Gly-Gly-Ser($Bu^t$)-$OBu^t$ (**5**) and H-Asn-Ser-Gly-Gly-Ser-OH

Fig. 2. Dose response of natural and synthetic WS1279 on the proliferation of bone marrow cells of $BDF_1$ mice.

□ Natural WS1279, ○ synthetic WS1279. Values are means±SE (n=3).

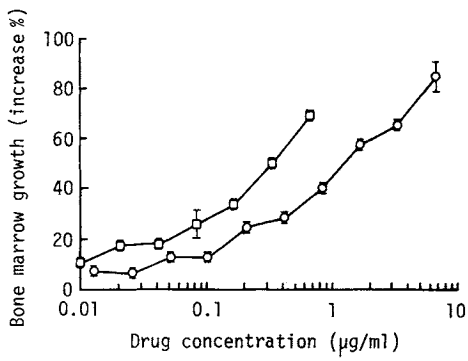
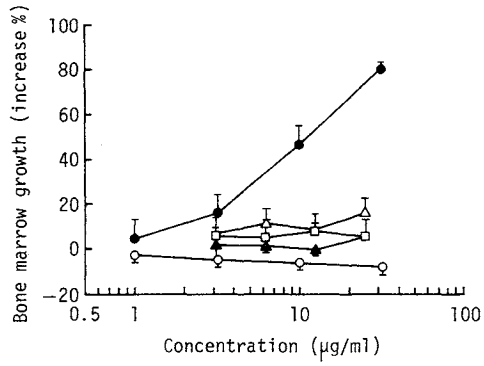



Fig. 3. Effects of synthetic intermediates of WS1279 on the growth of bone marrow cells of $BDF_1$ mice.

● **2**, ○ **3**, ▲ **4**, △ **5**, □ **6**. Values are means±SE (n=3).




(**6**) did not exhibit any myelopoietic activity.

## Effect of WS1279 on Restoration of Granulocytes in Bone Marrow

We established the MMC-induced myelosuppressive model in mice to estimate the usefulness *in vivo* of myelopoietic substances isolated in the screening program. It is known that MMC is one of the drugs useful for cancer chemotherapy but causes severe myelosuppression as a side effect[1]. When $BDF_1$ mice were given with MMC intraperitoneally, the number of granulocytes in bone marrow decreased and reached a nadir on day 3 after injection, and then began to increase on day 4. On day 5, the granulocyte counts increased over that of non-treated control mice (data not shown). We examined the ability of WS1279 to enhance the recovery of granulocyte counts in bone marrow in the MMC-induced mouse leukopenia model.

Effects of natural and synthetic WS1279 on the granulocyte counts in the MMC-induced mouse leukopenia model are shown in Fig. 4. Natural and synthetic WS1279 were suspended in 0.5% methylcellulose. $BDF_1$ mice were administrated WS1279 intraperitoneally once a day for 3 days after MMC treatment, and bone marrow cells from their femurs were harvested on day 3. The absolute number of granulocytes in bone marrow was measured by flow cytometry as described in materials and methods. Natural and synthetic WS1279 increased the number of granulocytes dose-dependently. The $ED_{50}$ values (50% restoration from MMC-treated control) of natural and synthetic WS1279 were 0.13 mg/kg and 0.8 mg/kg, respectively. The restoration of granulocyte counts after administration of synthetic WS1279 (10 mg/kg) in MMC-treated mice is shown in Fig. 5. Synthetic WS1279 augmented the decreased number of granulocytes to the normal level on day 3 after MMC treatment. While in MMC-treated mice without WS1279, granulocyte counts reached a nadir on day 2 and did not change on day 3. Granulocytes were stimulated to proliferate, when WS1279 was administrated alone.

## Prophylactic Effect of Synthetic WS1279 on a Systemic Mouse Infection Model

The prophylactic effect of synthetic WS1279 on *S. aureus* 47 infection in normal mice and MMC-induced immunosuppressed mice is shown in Table 1. WS1279 enhanced the protective effect to *S. aureus* 47 infection at doses of 1.1 mg/kg to 10 mg/kg in normal mice. Five out of 15 mice died within 3 days

Fig. 4. Effects of natural (A) and synthetic (B) WS1279 on restoration of the number of granulocytes in mitomycin C (MMC)-treated mice.

Values are means±SE (n=5). Significant differences from MMC-treated control value; * $P<0.05$, ** $P<0.01$.

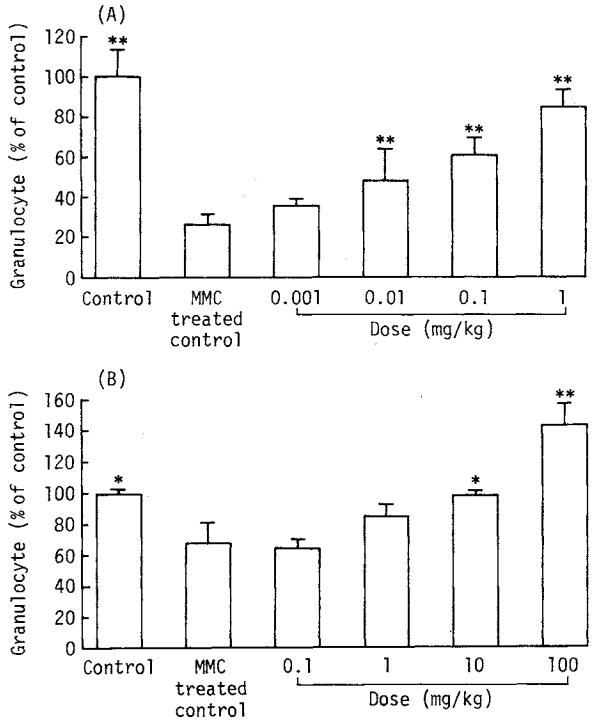
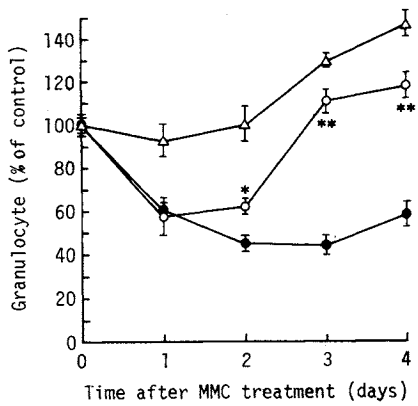



Fig. 5. Time course of restoration of granulocyte counts after mitomycin C (MMC) treatment with synthetic WS1279 administration.

● MMC-treated control, ○ MMC+WS1279, △ WS1279 alone. Values are means±SE (n=5). Significant differences between MMC-treated control and WS1279-injected mice; * $P<0.05$, ** $P<0.01$.




following infection with $1.8\times10^8$ cells of *S. aureus* 47 in normal mice. WS1279-treated mice were all alive on day 3 post-infection and their body weight were significantly different from vehicle-treated control.

Furthermore, in MMC-induced immunosuppressed mice, WS1279 augmented the host defense to *S. aureus* 47 infection at doses of 1.1 mg/kg to 10 mg/kg. Nine out of 15 mice died within 3 days after infection with $1.2\times10^7$ cells of *S. aureus* 47 in MMC-induced immunosuppressed mice. On the other hand, WS1279-administrated mice were all alive on day 3 after infection and their body weight were significantly different from vehicle-treated immunosuppressed mice.

## Induction of CSF

As it had been known that CSFs stimulated the

Table 1. Prophylactic effect of synthetic WS1279 on *Staphylococcus aureus* 47 infection in mice.

| Synthetic WS1279 (mg/kg) | Normal mice | | Immunosuppressed mice | |
|---|---|---|---|---|
| | Mortality[a] | Body weight[b] (g) | Mortality[a] | Body weight[b] (g) |
| Control | 5/15 | 16.4±0.3 | 9/15 | 15.9±0.2 |
| 1.1 | 0/8 | 18.5±0.3[c] | 0/8 | 18.5±0.2[d] |
| 3.3 | 0/8 | 18.8±0.3[d] | 0/8 | 18.9±0.3[d] |
| 10.0 | 0/8 | 18.8±0.4[d] | 0/8 | 18.1±0.4[d] |

All mice were weighed and then divided to the groups with a mean body weight of 17.9±0.2 g on day −3 before infection.

[a] Number of dead mice/number of treated mice.

[b] mean±SE.

[c] $P<0.01$, by STUDENT's *t*-test.

[d] $P<0.001$, by STUDENT's *t*-test.

Fig. 6. Time course of colony-stimulating factor (CSF) induction in the serum of $BDF_1$ mice after subcutaneous injection with synthetic WS1279.

Values are means±SE (n=5). Significant differences from control (0 hour) value, ** $P<0.01$.

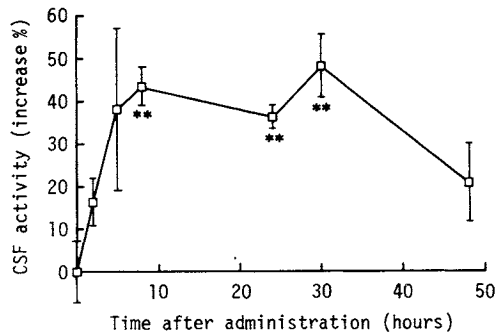
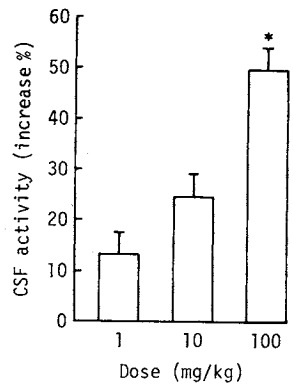



Fig. 7. Dose response of synthetic WS1279 on the induction of colony-stimulating factor (CSF) in the serum of $BDF_1$ mice.

Values are means±SE (n=5). Significant difference from vehicle treated (control) value, * $P<0.05$.




proliferation of bone marrow cells[10], we investigated the CSF-inducing activity of WS1279 in the serum in $BDF_1$ mice by subcutaneous administration. As shown in Fig. 6, the CSF level increased in 2 hours after administration of WS1279 (10 mg/kg), reached a maximum in 5 hours and kept plateau for 30 hours. WS1279 induced CSF in mouse serum in a dose-dependent manner (1 to 100 mg/kg, Fig. 7).

## Effects of Various Immunostimulants on Bone Marrow Proliferation

Immunostimulants such as FK-565[11], LPS[12], picibanil[13], MDP[14], bestatin[15] and forphenicinol[16] have been reported to have CSF-inducing activity. Additionally, bestatin[17,18] and muroctasin (MDP-Lys)[19] have shown to have the hematopoietic effect in immunosuppressed animals. We examined the effects of WS1279 and various immunostimulants on the proliferation of bone marrow cells *in vitro* and *in vivo*. The results are shown in Table 2. Synthetic WS1279 was the most effective on the proliferation of bone marrow cells. No proliferating activity of FK-565, picibanil and bestatin was observed *in vitro*. The $ED_{50}$ values of MDP, LPS and forphenicinol could not be determined *in vitro*, because their efficacies were under 50%. On the other hand, FK-565, LPS, picibanil and forphenicinol were more effective than

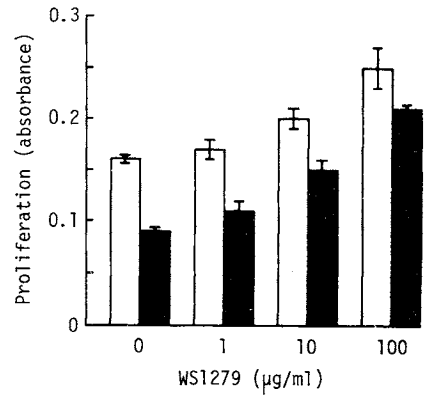
Table 2. Effects of several drugs on the proliferation of bone marrow cells *in vitro* and the restoration of granulocyte counts in mitomycin C-treated mice *in vivo*.

| Drug | $ED_{50}$ | |
|---|---|---|
| | *In vitro* (μg/ml) | *In vivo* (mg/kg) |
| WS1279 | 1.3 | 0.8 |
| FK-565 | ND[c] | 0.0007 |
| MDP[a] | ND[d] (5.0) | >10 |
| LPS[b] | ND[d] (0.01) | 0.015 |
| Picibanil | ND[c] | 0.038 |
| Forphenicinol | ND[d] (2.5) | 0.018 |
| Bestatin | ND[c] | >10 |

[a] MDP: muramyl dipeptide.
[b] LPS: lipopolysaccharide.
[c] None of the stimulative activity was observed.
[d] Efficacy was under 50%, $ED_{50}$ could not be determined.
The number in parentheses represents maximum response at the indicated dose.

Fig. 8. Proliferation of the bone marrow cells of $BDF_1$ mice in DULBECCO's modified EAGLE's medium with or without L929 cell-conditioned medium (LCM).

Open bars: LCM 2.5%, closed bars: LCM free. Values are means ± SE (n = 3).




synthetic WS1279 on restoration of granulocyte counts in bone marrow in MMC-induced leukopenic mice. The $ED_{50}$ values of MDP and bestatin were above 10 mg/kg *in vivo*.

## Discussion

WS1279 is a novel lipopeptide of *S*-[2,3-bis(palmitoyloxy)propyl]-$N^{\alpha}$-palmitoyl-Cys-Asn-Ser-Gly-Gly-Ser-OH structure. Natural and synthetic WS1279 stimulated the proliferation of mouse bone marrow cells *in vitro*. When WS1279 was administrated intraperitoneally in MMC-treated mice, the reduced number of granulocytes in bone marrow was restored. Though the synthetic WS1279 was identical with the natural product on thin layer chromatography and HPLC[7], their activities *in vitro* as well as *in vivo* in the proliferation of bone marrow cells were rather discrepant. These differences in activity between the synthetic sample and the natural product may be due to the fact that the synthetic sample was a diastereomeric mixture. KURIMURA *et al.*[20] reported that (2*R*)-propyl type of WS1279 had a higher B-lymphocyte mitogenic activity than (2*S*)-propyl type of WS1279.

*S*-[(2*RS*)-2,3-dihydroxypropyl]-Cys-OH (**3**), a synthetic intermediate of WS1279, did not stimulate the growth of bone marrow cells *in vitro*. *S*-[(2*RS*)-2,3-bis(palmitoyloxy)propyl]-*N*-palmitoyl-Cys-OH (**2**) exhibited growth stimulating activity, but its activity was less than that of WS1279. Furthermore, benzyloxycarbonyl-Asn-Ser-Gly-Gly-Ser-OH (**4**), H-Asn-Ser(Bu$^t$)-Gly-Gly-Ser(Bu$^t$)-OBu$^t$ (**5**) and H-Asn-Ser-Gly-Gly-Ser-OH (**6**) did not exhibit any stimulating activity (Fig. 3). These results suggest that a lipid peptide structure with a certain chain length is required for manifestation of full stimulating activity.

We tried to determined whether the addition of 2.5% LCM would increase the proliferating activity of WS1279. WS1279 stimulated the growth of bone marrow cells dose-dependently in DMEM without LCM. The data show that 2.5% of LCM has an incremental effect on the proliferation of bone marrow cells *in vitro* (Fig. 8).

WS1279 increased the number of granulocytes in MMC-induced leukopenic mice. This effect may be due to the following mechanisms of WS1279; 1) an effect mediated through CSF induction, or 2) a direct effect on granulocytes. Recently we reported that FK-565 accelerated recovery of the number of granulocytes in MMC-treated mice and this result may be mediated through CSF induction[11,21]. WS1279 induced CSF in mouse serum (Figs. 6 and 7), but the CSF level was very low as compared with that induced by FK-565 (data not shown). Their CSF-inducing ability may be related to their granulopoietic activity *in vivo*. It remains a problem for the future to determine the mechanism of granulopoiesis stimulated by WS1279.

Bestatin was reported to restore leukocyte counts in myelosuppressed mice[17,18], however, bestatin

did not stimulate granulopoiesis in MMC-induced leukopenic mice (Table 2). This discrepancy may be due to the difference in experimental animal models.

In addition, WS1279 enhanced host resistance to infection with *Staphylococcus aureus* 47 in normal and immunosuppressed mice (Table 1). These results suggest that prophylactic effect of WS1279 on the infection may be induced by an increase in granulocyte numbers.

## References


1) GALE, R. P.: Antineoplastic chemotherapy myelosuppression: mechanisms and new approaches. Exp. Hematol. 13: 3~7, 1985
2) PIZZO, P. A.: Granulocytopenia and cancer therapy; past problems, current solutions, future challenges. Cancer 54: 2649~2661, 1984
3) GERSON, S. L.; G. H. TALBOT, S. HURWITZ, B. L. STROM, E. J. LUSK & P. A. CASSILETH: Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. Ann. Intern. Med. 100: 345~351, 1984
4) MOORE, M. A. S.: The clinical use of colony stimulating factors. Annu. Rev. Immunol. 9: 159~191, 1991
5) TANAKA, M.; Y. HORI, H. UEDA, M. EZAKI, T. GOTO, M. HASHIMOTO & M. OKUHARA: WS1279, a novel lipopeptide isolated from *Streptomyces willmorei*. Fermentation, isolation and physico-chemical properties. J. Antibiotics 46: 858~860, 1993
6) TANAKA, M.; N. SHIGEMATSU, Y. HORI, T. GOTO, M. HASHIMOTO, Y. TSUDA & Y. OKADA: Structure and synthesis of an immunoactive lipopeptide, WS1279, of microbial origin. Chem. Pharm. Bull. 38: 1793~1795, 1990
7) TSUDA, Y.; Y. OKADA, M. TANAKA, N. SHIGEMATSU, Y. HORI, T. GOTO & M. HASHIMOTO: Structure and synthesis of an immunoactive lipopeptide, WS1279, of microbial origin. Chem. Pharm. Bull. 39: 607~611, 1991
8) MONNER, D. A.: An assay for growth of mouse bone marrow cells in microtiter liquid culture using the tetrazolium salt MTT, and its application to studies of myelopoiesis. Immunol. Lett. 19: 261~268, 1988
9) WATT, S. M.; A. W. BURGESS, D. METCALF & F. L. BATTYE: Isolation of mouse bone marrow neutrophils by light scatter and autofluorescence. J. Histochem. Cytochem. 28: 934~946, 1980
10) CLARK, S. C. & R. KAMEN: The human hematopoietic colony-stimulating factors. Science, 236: 1229~1237, 1987
11) NAKAMURA, K.; K. NAKAHARA & H. AOKI: Induction of colony-stimulating factor (CSF) by FK-156 and its synthetic derivative, FK-565. Agric. Biol. Chem. 48: 2579~2580, 1984
12) CHERVENICK, P. A.: Effect of endotoxin and postendotoxin plasma on *in vitro* granulopoiesis. J. Lab. Clin. Med. 79: 1014~1020, 1972
13) HIRAOKA, A.; M. YAMAGISHI, T. OHKUBO, Y. YOSHIDA & H. UCHINO: Effect of a streptococcal preparation, OK-432, on murine hematopoietic stem cells. Acta. Hematol. Jpn. 44: 860~863, 1981
14) BROUDY, V. C.; K. KAUSHANSKY, S. G. SHOEMAKER, B. B. AGGARWAL & J. W. ADAMSON: Muramyl dipeptide induces production of hemopoietic growth factors *in vivo* by a mechanism independent of tumor necrosis factor. J. Immunol. 144: 3789~3794, 1990
15) OKAMURA, S.; F. OMORI, K. HAGA, H. BABA, K. SUGIMACHI & Y. NIHO: Influence of bestatin on production of granulocyte-macrophage colony-stimulating factor from human peripheral blood mononuclear cells *in vitro*. Acta Oncol. 29: 795~797, 1990
16) OHHARA, N.; S. OKAMURA, T. OTSUKA, M. HARADA & Y. HINO: Stimulatory effect of forphenicinol on normal human bone marrow granulocyte-macrophage progenitor cells mediated by T-lymphocytes. Biomed. Pharmacotherapy 41: 425~428, 1987
17) UCHIDA, K.; F. KOHI, S. YORIMITSU, I. TAKAHASHI, K. KITAJIMA & I. KIMURA: Acceleration of recovery from cyclophosphamide (CYC)-induced myelosuppression by bestatin. Jpn. J. Cancer Chemother. 9: 220~224, 1982 (in Japanese)
18) HORIUCHI, K. & T. MIYAMOTO: Radioreductive effect of bestatin (Ubenimex) in BALB/c mice. Int. J. Radiat. Biol. 62: 73~80, 1992
19) TAKADA, M.; M. FUKUOKA, N. TAKIFUJI, N. SAKAI, S. RYU, N. MASUDA, K. MATSUI, S. Negoro, Y. KUSUNOKI & E. TSUBURA: Restorative effect of muroctasin, MDP-Lys (L18), on leukopenia caused by anticancer chemotherapy in lung cancer. Comparative study by envelope method. Jpn. J. Cancer Chemother. 15: 3095~3101, 1988 (in Japanese)
20) KURIMURA, M.; A. OCHIAI & K. ACHIWA: Stereospecific synthesis and mitogenic activity of lipopeptide WS1279 and its derivatives. *In* Peptide Chemistry 1991. *Ed.*, A. SUZUKI, pp. 361~366, Protein Research Foundation, Osaka, 1992
21) TANAKA, M.; F. SAKAI, T. GOTO & M. OKUHARA: Acceleration of recovery from mitomycin C-induced leukopenia by FK-565. Biosci. Biotech. Biochem. 57: 1602~1603, 1993