Note

Actions of Teleocidins on *in Vitro* CFU-GM and CFU-E Colony Formation

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Granulocytes, monocyte-macrophages, and erythrocytes are derived from the proliferation and differentiation of a common hematopoietic stem cell. The CFU-GM (colony forming units granulocyte-macrophage), the committed granulo-monopoietic stem cells, form CFU-GM derived colonies in the presence of CSF (colony stimulating factors) in semi-solid culture of bone marrow cells.¹⁾ On the other hand, the CFU-E (colony forming units erythroid) form CFU-E derived colonies in the presence of erythropoietin.

In the screening of substances which stimulate CFU-GM colony formation of mouse bone marrow cells, we found that the extracts from the mycelium of strain F-186 stimulate colony formation of CFU-GM without added

CSF. After purification, we identified the active substances as teleocidin A-1 (F186-A) and teleocidin B-4 (F186-B).²⁾ In this paper we describe the actions of teleocidins on *in vitro* CFU-GM and CFU-E colony formation compared to those of TPA (12-*O*-tetradecanoylphorbol-13-acetate).

The CFU-GM colony formation activities of teleocidin A-1 and teleocidin B-4 are shown in Fig. 1. These compounds stimulated CFU-GM colony formation in the absence of added CSF. At the final concentration of 5×10^{-7} M, teleocidin A-1 and teleocidin B-4 formed 38 ± 4 and 36 ± 4 CFU-GM colonies/ 1×10^5 bone marrow cells, respectively, while 20 units/ml of CSF-CHUGAI formed 27 ± 3 colonies/ 1×10^5 bone marrow cells. The ability of teleocidin A-1 to form colonies was similar to that of teleocidin B-4. Moreover, the activities of these compounds were almost the same as that of the tumor-promoting phorbol ester, TPA.

Recently, it was reported that bryostatins stimulated normal human hematopoietic cells to form CFU-GM colonies *in vitro*.³⁾ As to the effects of TPA on CFU-GM colony formation, some mechanisms have been reported^{4~11)}; a) TPA enhances the responsiveness of CFU-GM to CSF, b) TPA induces secretion of CSF, and c) others. Teleocidins and bryostatins may stimulate bone marrow cells by similar mechanisms to those of TPA. On the other hand, Mass *et al.* reported that a TPA-type tumor promoter enhanced colony formation of rat tracheal epithelial cells. Although the mechanism is considered to be based on the proliferative activation of attached cells,¹²⁾ the effects of TPA were 100-fold less than teleocidin in the culture system.

Further, the relative activities of teleocidin A-1 and teleocidin B-4 on CFU-E colony formation in the presence of erythropoietin were measured. Figure 2 shows that the addition of teleocidin A-1 and teleocidin B-4 to the cultures inhibited CFU-E colony formation stimulated by erythropoietin. At teleocidin A-1 and teleocidin B-4 concentrations of 5×10^{-7} M, the number of CFU-E colonies

Fig. 1. Effects of Teleocidin A-1 (F186-A) and Teleocidin B-4 (F186-B) on CFU-GM Colony Formation. Mouse bone marrow cells were cultured at 1×10^5 cells/dish in the presence of CSF (■) at 20 U/ml, teleocidin A-1 (○), teleocidin B-4 (●), or TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) (△) at various concentrations. Each circle represents the mean of triplicate data with S.E.

Fig. 2. Effects of Teleocidin A-1 and Teleocidin B-4 on CFU-E Colony Formation.

Mouse bone marrow cells (1×10^5 cells/dish) were cultured with 0.1 U/ml erythropoietin in the absence (■) or presence of teleocidin A-1 (○), teleocidin B-4 (●), or TPA (△) at various concentrations. Each circle represents the mean of triplicate data with S.E.

formed by addition of 0.1 U/ml erythropoietin decreased to 40% and 54%, respectively. The magnitudes of the inhibitory effects on CFU-E colony formation among teleocidin A-1, teleocidin B-4 and TPA were similar.

Just before this paper was written, Broudy *et al.* reported that TPA caused a decrease in the number of erythropoietin receptors without altering the affinity of the receptor for erythropoietin.¹³⁾ It is interesting that concentrations of teleocidins which inhibit CFU-E colony formation coincide with those which induce CFU-GM colony formation in our experiments.

Experimental

Assay of CFU-GM colony formation. Methylcellulose cultures were used for the CFU-GM assay. Bone marrow cells were obtained from 6- to 10-week-old male ICR mice. The cells (1×10^5) were suspended in alpha medium (α -MEM) containing 0.8% methylcellulose, 30% horse serum, 1% bovine serum albumin, 1×10^{-4} M 2-mercaptoethanol, and a test sample in a final volume of 1 ml, in 35×10 mm plastic dishes. The plates were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 7 days. At the end of the incubation, colonies of more than 50 cells were counted under an inverted microscope. The mean number of colonies in three plates was calculated.

Assay of CFU-E colony formation. The CFU-E colonies were cultured in methylcellulose. The culture medium was similar to that of the CFU-GM culture system, except for 30% fetal calf serum instead of horse serum, and the addition of 0.1 U/ml erythropoietin. The plates were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 2 days. After benzidine staining, benzidine-positive colonies containing more than 8 cells were scored.

Isolation of teleocidin A-1 and teleocidin B-4. The mycelium obtained from 20 l of a culture of strain F186 was extracted with acetone, and then AcOEt. The AcOEt extract was chromatographed on silica gel using toluene-acetone, and then purified by C-8 reversed-phase column chromatography by elution with acetonitrile-H₂O (1:1). The active components, F186-A (28 mg) and F186-B (33 mg), were eluted successively. The F186-A analytical data were: mp 77~82°C. $[\alpha]_D^{25} -113.0^\circ$ ($c=0.5$, EtOH). FAB-MS $[m/z: 438 (M+H)^+]$. HR-EIMS C₂₇H₃₉N₃O₂ (Found $m/z: 437.3042$, Calcd. $m/z: 437.3042$). The UV, IR, ¹H-NMR, and ¹³C-NMR were similar to those of lyngbyatoxin A.^{14,15)} F186-B analytical data were: mp 123~129°C. $[\alpha]_D^{25} -164.5^\circ$ ($c=1.0$, EtOH). FAB-MS $[m/z: 452 (M+H)^+]$. HR-EIMS C₂₈H₄₁N₃O₂ (Found $m/z:$

451.3181, Calcd. $m/z: 451.3199$). The UV, IR, ¹H-NMR, and ¹³C-NMR agreed with those of teleocidin B-4.^{16,17)}

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