Vol. 100, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1981 Pages 888-893

ACTIVATION OF HUMAN LYMPHOCYTES BY TUMOR PROMOTER TELEOCIDIN

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Received April 20, 1981

SUMMARY: Teleocidin from Streptomyces caused rapid aggregation of human lymphocytes. Lymphocyte fractionation experiment demonstrated that the cell aggregation occurred preferentially in T-enriched lymphocytes. Teleocidin had a mitogenic effect on both T- and B-enriched lymphocytes, but had no stimulatory effect on the immunoglobulin synthesis. The effects of teleocidin on lymphocyte subpopulations resembled those of 12-O-tetradecanoylphorbol-13-acetate(TPA), while differences existed between teleocidin and lectins in stimulating functions of human lymphocytes. These observations suggest that experiments comparing the effects of tumor promoters and lectins may help to understand the mechanism of action of tumor-promoting teleocidin.

INTRODUCTION

Teleocidin, an indole alkaloid, is a toxic substance isolated from the mycellia of Streptomyces(1, 2). Recently, Fujiki et al. and Nakayasu et al. reported that teleocidin and dihydroteleocidin B stimulated ornithin decarboxylase activity of skin and inhibited terminal differentiation of Friend erythroleukemia cells(3, 4). We also reported that teleocidin inhibited the binding of epidermal growth factor(EGF) to cellular receptors probably by the same mechanism as TPA(5). These and other available data indicate that teleocidin is a new type of promoter of carcinogenesis(6).

On the other hand, a possible modulation of lymphocyte functions by tumor promoting agents is of great interest considering a role of immune response in carcinogenesis(7). TPA has been reported to stimulate DNA synthesis of lymphocytes(8, 9), but very little is known about the effect of teleocidin on the immune system(10). In the present study, effects of teleocidin on cultured human lympnocytes were examined.

MATERIALS AND METHODS

Chemicals. Teleocidin isolated from mycellia of Streptomyces was a gift from Fujisawa Pharmaceutical Industries, Ltd., Osaka, Japan(1, 2).

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TPA was purchased from Sigma(St. Louis). Teleocidin and TPA was dissolved in 50% ethanol and dimethyl sulfoxide respectively(5). These stock solutions were diluted with the culture medium just before the use. The final concentration of solvents in the culture medium was less than 0.01%. Phytohemagglutinin P(PHA-P) and pockweed mitogen(PWM), obtained as vials of sterile powder from Difco Laboratories(Detroit, Mich.), were diluted to 5 ml with sterile water.

Lymphocyte preparation. Heparinized peripheral blood from healthy adults was diluted 1/1 with RPMI 1640 medium(Nissui Seiyaku, Tokyo), layered on Ficoll-Paque(Pharmacia, Uppsala, Sweden), and spun at 400 x G(11). Cells harvested from the interface(unfractionated lymphocytes) were washed and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated autologous serum and 1% penicillin-streptomycin solution(GIBCO, N.Y.). Unfractionated lymphocytes were depleted of macrophages by 2 hours of incubation at 37°C in plastic culture dish(Falcon #3002). Non-adherent cells($1x10^7$) were incubated for 1 hour with neuraminidase-treated sheep red blood cells (sRBC, $2x10^8$, Japan Immunoresearch Lab. LTD), layered on Ficoll-Paque gradient and spun at 400xG for 30 minutes. Pelleted rosetting lymphocytes were treated with Tris-buffered ammonium chloride solution(0.83%) to lyse sRBC, washed three times in RPMI 1640 medium and used as the final T-enriched lymphocytes(12). Non-rosetting cells were harvested from the interface of the Ficoll-Paque gradient, washed three times in the medium, and used as the final B-enriched lymphocyte population(12).

Lymphocyte culture. Culture medium was RPMI 1640 containing 10% autologous serum and 1% penicillin-streptomycin solution. Lymphocytes($1x10^5$ in 0.2 ml of medium) were seeded into the well of Falcon Micro Test II tissue culture plate. Teleocidin, TPA and lectins were added at the start of culture. Cultures were carried out at 37°C in a humidified atmosphere containing 5% CO_2.

The degree of cell aggregation was estimated as described by Hoshino et al.(10).

Mitotic response of lymphocytes was quantified by measuring the incorporation of [^{3}H]thymidine. After 3 days of culture with various mitogens, 0.2 μCi of [^{3}H]thymidine(New England Nuclear, 20 Ci/mmol) was added to each well (1 μCi/ml) and the incubation was continued for further 24 hours at 37°C. [^{3}H]thymidine incorporation into acid-precipitable materials was measured as described elsewhere(13).

Immunoglobulin synthesis was estimated by measuring the amount of [^{3}H]leucine incorporated into materials reactive with protein A(14). Lymphocytes were cultured with or without promoters or mitogens. After 7 days of culture, [^{3}H]leucine(51 Ci/mmole, New England Nuclear) was added into the medium(10 μCi/ml) and the incubation was continued for further 24 hours. The cells and medium were separated by centrifugation. Medium was applied to a protein A-Sepharose column(0.9x3 cm, preequilibrated with 0.1 M phosphate buffer, pH 7.5, Pharmacia), and the radioactive immunoglobulin adsorbed to the column was eluted with 0.1 M sodium acetate(pH 3.5) as described(14). The radioactivity of the protein A-bound immunoglobulin was determined as described previously(13).

RESULTS

Cell aggregation. Morphological appearances of human lymphocytes cultured with teleocidin are demonstrated in Fig. 1. T-enriched lymphocytes show remarkable aggregation after 12 hours of culture with 10 ng/ml of

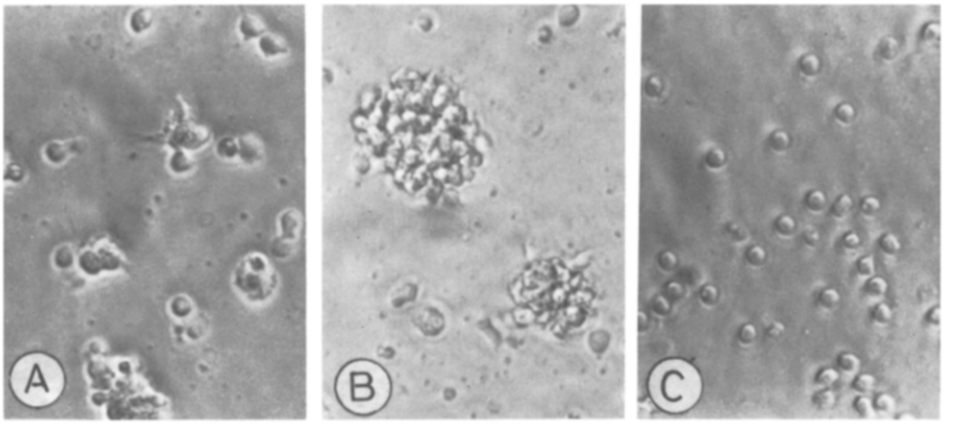

Fig. 1. Aggregation of human lymphocytes induced by teleocidin. Lymphocytes(1 x 10^5/well) were cultured for 12 hours with 10 ng/ml of teleocidin. A, unfractionated; B, T-enriched; C, B-enriched(magnification, x 100).

teleocidin(Fig. 1B). The same treatment brought moderate aggregation of unfractionated lymphocytes(Fig. 1A) but had no detectable effect on B-enriched lymphocytes(Fig. 1C).

Fig. 2A shows that the aggregation of T-enriched lymphocytes became apparent within 2 to 3 hours of incubation with teleocidin and reached maximum within 24 hours. At this point, most of B-enriched lymphocytes remained to be free-floating individual cells. The B-enriched lymphocytes gradually increased the number of aggregated cells, but the ratio of aggregated cells to the total cells was at most 0.3 after 4 days of culture(Fig. 2A). In addition, most of the aggregated and free cells of B-enriched lymphocytes adhered relatively firmly to the surface of culture plates, showing a contrast with the aggregates of T-enriched lymphocytes which did not adhere to the substratum.

Dose response curve of Fig. 2B shows that, at lower concentrations, teleocidin was more effective in inducing cell aggregation than TPA. Teleocidin and TPA aggregated at most 80% of T-enriched lymphocytes, while PHA-P caused cell aggregation in more than 90%(Fig. 2C).

Mitogenic effect of teleocidin. Teleocidin acted as a mitogen of human peripheral blood lymphocytes(Table 1). Approximately 3-fold increase in

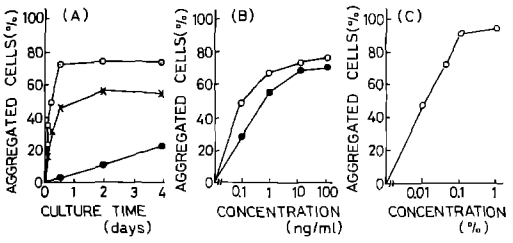

Fig. 2. Lymphocyte aggregation by teleocidin. (A) Time course: Lymphocytes (1 x 10^5/well) were cultured with 10 ng/ml of teleocidin for indicated time. o—o, T-enriched; •—•, B-enriched; x—x, unfractionated. (B) Dose response: T-enriched lymphocytes(1 x 10^5/well) were cultured for 4 days with different concentrations of teleocidin(o—o) or TPA(•—•). (C) Dose response: T-enriched lymphocytes(1 x 10^5/well) were cultured for 4 days with different concentrations(percent, V/V) of PHA-P(o—o).

[^{3}H]thymidine incorporation was observed when T- and B-enriched lymphocytes were cultured for 4 days with 10 ng/ml of teleocidin(Table 1). The stimulation of [^{3}H]thymidine incorporation was recognized at the teleocidin concentration of 0.5 ng/ml and reached maximum at 10 ng/ml. The mitogenic action of teleocidin was comparable to that of TPA. Table 1 also demonstrates that the potency of teleocidin as a lymphocyte mitogen appears to be greater than that of PWM but is weeker than that of PHA-P.

Effect of teleocidin on immunoglobulin synthesis. Table 2 demonstrates that the amount of radioactive immunoglobulin bound to protein A-Sepharose was not significantly different between control lymphocytes and those treated

Table 1. Effect of teleocidin on [^{3}H]thymidine incorporation by human peripheral lymphocytes.

culture condition	[^{3}H]thymidine incorporation(cpm/well)		
	unfractionated	T-enriched	B-enriched
none	2,258 ± 159	1,782 ± 289	1,648 ± 169
teleocidin(10 ng/ml)	6,627 ± 991	6,426 ± 144	6,937 ± 315
TPA(10 ng/ml)	6,857 ± 1,019	6,739 ± 1,214	6,203 ± 313
PWM(1 %)	5,443 ± 501	5,302 ± 540	5,018 ± 1,065
PHA-P(1 %)	11,363 ± 1,047	8,398 ± 593	5,301 ± 936

Lymphocytes(1 x 10^5/well) were cultured with teleocidin or other growth stimulaters for 4 days. The lymphocytes were incubated for the last 24 hours with 1 μCi/ml of [^{3}H]thymidine.

Table 2. Effect of teleocidin on immunoglobulin synthesis by human lymphocytes.

culture condition	[^{3}H]leucine incorporation into immunoglobulin*	
	unfractionated	B-enriched
none	370 ± 25.5	425 ± 113
teleocidin(10 ng/ml)	411 ± 34.0	423 ± 54.2
TPA(10 ng/ml)	377 ± 23.0	482 ± 29.0
PWM(1 %)	744 ± 177	570 ± 70
PHA-P(1 %)	825 ± 97.8	453 ± 23.9

*, cpm/well

Lymphocytes(1 x 10^5/well) were cultured with teleocidin or with other mitogens for 7 days. The lymphocytes were incubated with 10 μCi/ml of [^{3}H]leucine for further 24 hours. The amounts of tritium-labelled immunoglobulin were determined as described in Materials and Methods.

with teleocidin or TPA. On the contrary, PWM and PHA-P brought 2- to 3-fold increase in the immunoglobulin synthesis by unfractionated lymphocytes(Table 2).

DISCUSSION

Our present data show that cell aggregation in response to teleocidin or TPA was more remarkable in T-enriched lymphocytes than in B-enriched lymphocytes. The aggregates of B-enriched lymphocytes adhered more firmly to the substratum than those of T-enriched lymphocytes. Therefore, both qualitative and quantitative differences of cell aggregation existed between T- and B-enriched lymphocytes. On the other hand, teleocidin brought aggregation of approximately 70 to 80% of T-enriched lymphocytes, while PHA-P caused aggregation of more than 90% of T-enriched lymphocytes(Fig. 2). This may suggest that the subsets of T-lymphocytes responsive to teleocidin may not be completely the same as those responsive to PHA-P(8).

Our results also demonstrated that teleocidin was a mitogen for both T- and B-enriched lymphocytes. Our results resembled those of Abb et al., showing that TPA enhanced DNA synthesis of T- and B-enriched lymphocytes(9). However, Touraine et al. reported that TPA acted as a mitogen selective for T lymphocytes(8), and the reason for this discrepancy remains to be studied.

As shown in the present study, teleocidin and TPA had no stimulatory effect on the immunoglobulin synthesis. This appears not to be due to a technical

failure, since stimulation of immunoglobulin synthesis was observed in unfractionated lymphocytes cultured with PWM or PHA-P. Therefore, a nice shade appeared to exist between tumor promotes and lectins in stimulating human lymphocytes.

Present report shows that teleocidin modulates functions of human lymphpcytes in tissue culture. Further studies comparing the effects of teleocidin with those of TPA and lectins on human lymphocytes may provide valuable informations about the molecular mechanism of tumor promotion by teleocidin.

ACKNOWLEDGMENT

We thank Dr. T. Sugimura, National Cancer Center, for his valuable suggestions.

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