EFFECT OF CYTOCHALASIN B ON LYMPHOCYTE STIMULATION INDUCED BY CONCANAVALIN A OR PERIODATE

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SUMMARY

The effects of cytochalasin B on lymphocyte stimulation induced by concanavalin A (Con A) and by periodate were investigated. At low concentrations (0.1 - 1 μg/ml) cytochalosin B greatly potentiated the responses to these two mitogens. Cytochalasin B was most effective when added with the mitogens at the beginning of incubation. The action of cytochalasin B at low concentration was suggested to be on an early process of DNA synthesis induced by these mitogens.

INTRODUCTION

Little is known about the mechanism of lymphocyte stimulation induced by various mitogens (1-3). From recent experiments on binding inhibition by specific sugar (2) and experiments using mitogen covalently attached to Sepharose (4, 5) it was suggested that the mitogen first interacted with the cell membrane. It was also suggested that movement of the cell membrane could take part in various functions of the cell (6). To elucidate the reaction occurring after interaction of mitogen with the cell membrane, the influence of a substance which affected the early stage of lymphocyte stimulation by mitogens was studied. In the present work two mitogens with different properties were used: concanavalin A (Con A), known as a phytomitogen (2, 5) and periodate (3, 7 known as an oxidizing agent.

Cytochalasin B was found to potentiate the responses of lymphocytes to these two mitogens. Cytochalasin B is a fungal product (8) and has effects on cellular function (8), morphology (9), and transport of sugar (10) and nucleotide (11). The effect of cytochalasin B is thought to result from its action upon microfilaments which partici-

pate in movement of the membrane. Cytochalasin B has been used in studies on the function of the cell related to the membrane (9, 12).

MATERIALS AND METHODS

Chemicals. Tritiated thymidine (thymidine 6-T, specific activity 5 Ci/m mole) was obtained from Daiichi Pure Chemicals Co., Tokyo, Japan. Cytochalasin B (Imperial Chemical Industries Ltd., England) was dissolved at 2 mg/ml in 50 % dimethyl sulfoxide (DMSO) in calcium and magnesium-free phosphate buffered saline (PBS) and diluted with PBS. Eagle's minimal essential medium (MEM) was obtained from Chiba Serum Institute, Chiba, Japan. Heat inactivated fetal calf serum was purchased from Gland Island Biological Co., New York, U. S. A. Sodium periodate ($NaIO_4$) was obtained from Merck, Darmstadt, Germany. Con A was prepared from jack bean meal (Sigma Chemical Co., St. Louis, U. S. A.) by the method of Agrawal and Goldstein (13).

Cells. Mesenteric lymph node cells were obtained from rabbits from Hoshino Animal Corporation, Tokyo. A male rabbit, weighing 2-3 Kg, was exsanguinated from a carotid artery and the mesenteric lymph node was quickly removed. The node was teased apart with forceps in medium (MEM, 10 % heat inactivated fetal calf serum, 10 mM HEPES, 100 μg/ml streptomycin). The cells were suspended by pipette and passed through a stainless steel mesh (pore size, 50 μm). The suspension was centrifuged and the precipitated cells were washed once and suspended in the above medium. Duplicate suspensions, usually containing 2.0×10^6 cells/1.0 ml of medium, were placed in small test tubes with rubber stoppers and incubated at 37°C.

Treatment with $NaIO_4$. Lymph node cells were suspended at a concentration of 1×10^7 cells/ml in PBS containing $NaIO_4$ at the concentrations specified in the figures, and incubated at 23° C for 10 min (3, 7). The cell suspensions were then diluted with two volumes of medium, centrifuged to remove excess $NaIO_4$, and resuspended in medium at a concentration of 2.0×10^6 cells/1.0 ml.

^{3}H-Thymidine incorporation. ^{3}H-Thymidine incorporation was measured as described previously (5). Three hours before the end of incubation, 1.0 μCi of ^{3}H-thymidine (5 Ci/m mole) was added. After incubation the mixture was washed with PBS and treated with cold 5 % trichloroacetic acid (TCA). The precipitate was dissolved in 0.1 N NaOH and treated with cold 6.7 % TCA. Then the precipitate was applied to a glass filter (Whatman GF/C) and the filter was washed with ethanol and ether, dried and placed in toluene scintillator fluid. The radioactivity was measured in a liquid scintillation counter (Beckman LS-150).

RESULTS

Effect of cytochalasin B on lymphocyte stimulation induced by Con A. Con A stimulated incorporation of ^{3}H-thymidine into lymphocytes. Incorporation reached a maximum with 10 μg/ml of Con A and decreased with higher concentrations of Con A. The effect of cytochalasin B on stimulation of lymphocytes induced by two concentrations

of Con A was investigated. This system contained less than 0.5 % of DMSO which was added to dissolve the cytochalasin B. But this did not affect the stimulation of incorporation of thymidine into lymphocytes by either Con A or periodate. Fig. 1 shows that

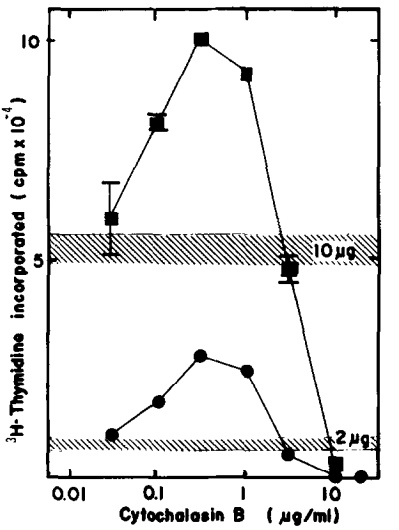

Fig. 1. Effect of cytochalasin B on rabbit mesenteric lymph node cells stimulated with 2 μg/ml (●) and 10 μg/ml (■) of Con A. Stumulations by Con A in the absence of cytochalasin B are shown by shaded zones. Con A and cytochalasin B were added initially and cultures were incubated for 48 hr at 37°C and pulse labeled with ^{3}H-thymidine during the last 3 hr.

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the patterns of the effect of cytochalasin B were similar at the two concentrations of Con A. In both cases cytochalasin B at low concentrations (0.1 - 1 μg/ml) potentiated the response to Con A, while at high concentrations (3 μg/ml) it inhibited the response. Maximum potentiation of incorporation of ^{3}H-thymidine was observed at 0.3 μg/ml of cytochalasin B, while 10 μg/ml caused almost complete inhibition. With 40 μg/ml of Con A the pattern of the effect of cytochalasin B was similar to those with 2 and 10 μg/ml of Con A.

Effect of cytochalasin B on lymphocyte stimulation induced by periodate. The incorporation of ^{3}H-thymidine increased with increase in the concentration of $NaIO_4$

to 5 x 10^{-4} M and decreased at higher concentrations of $NaIO_4$. Fig. 2 shows that the patterns of the effect by cytochalasin B with three concentrations of periodate were

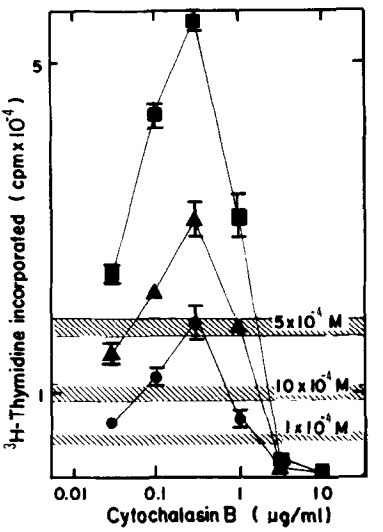

Fig. 2. Effect of cytochalasin B on rabbit mesenteric lymph node cells stimulated with 1 x 10^{-4} M (●), 5 x 10^{-4} M (■) and 10 x 10^{-4} M (▲) of $NaIO_4$. Stimulations by $NaIO_4$ in the absence of cytochalasin B are shown by shaded zones. Cytochalasin B was added initially and cultures were incubated for 48 hr at 37° and pulse labeled with ^{3}H-thymidine during the last 3 hr.

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similar to those with Con A. At low concentrations (0.1- 1 µg/ml) cytochalasin B caused potentiation while at concentrations above 3 µg/ml it was inhibitory. Maximum potentiation was observed with 0.3 µg/ml of cytochalasin B and incorporation of ^{3}H-thymidine was almost completely inhibited with 10 µg/ml of cytochalasin B. Thus the effects of cytochalasin B were similar with the two mitogens, although these mitogens had different properties.

Effective time of addition of cytochalasin B. Stimulation of thymidine incorporation was only observed on incubation for at least 24 hours after addition of Con A or treatment with periodate and maximum stimulation was observed between 48 and 96 hours

after treatment with the mitogens.

Cytochalasin B at the concentration causing maximum potentiation (0.3 µg/ml) was added at various times after treatment with Con A or periodate. Fig. 3 shows the

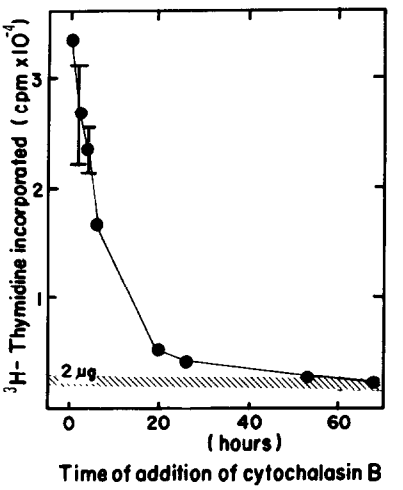

Fig. 3. Effect of cytochalasin B on rabbit mesenteric lymph node cells stimulated with 2 µg/ml of Con A. Stimulation by Con A in the absence of cytochalasin B is shown by a shaded zone. Con A was added initially and cytochalasin B (0.3 µg/ml) various times during incubation. After the last addition of cytochalasin B (at 68 hr) cultures were pulse labeled with ^{3}H-thymidine for 3 hr.

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effect of cytochalasin B on stimulation induced by 2 µg/ml of Con A. The potentiating effect was greatest when cytochalasin B was added at the same time as Con A. When added 6 hours after Con A, it was half as effective, when added 20 hours later it was approximately 10 % as effective and when added 20 hours after Con A it was ineffective. Similar results were obtained with 10 µg/ml of Con A.

On the contrary, the inhibitory effect was the same when 10 µg/ml of cytochalasin B was added initially as when it was added after 24 hours.

Fig. 4 shows the effect of cytochalasin B on stimulation induced by periodate. The potentiating effect was greatest when cytochalasin B was added immediately after treat-

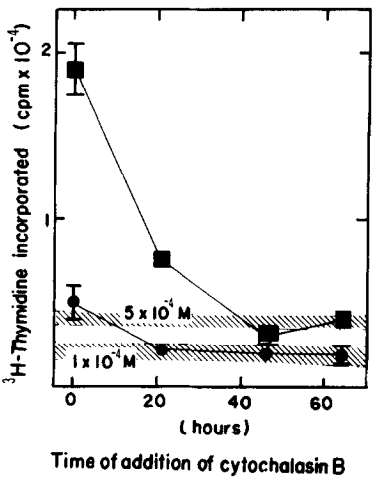

Fig. 4. Effect of cytochalasin B on rabbit mesenteric lymph node cells stimulated with 1×10^{-4} M (●) and 5×10^{-4} M (■) of $NaIO_4$. Stimulations by $NaIO_4$ in the absence of cytochalasin B are shown by shaded zones. Cytochalasin B (0.3 µg/ml) was added at various times after $NaIO_4$ treatment. Cultures were pulse labeled with ^{3}H-thymidine for 3 hr after the last addition of cytochalasin B (after 64 hr).

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ment with either 1×10^{-4} or 5×10^{-4} M periodate. When added 21 hours after treatment with 1×10^{-4} M or 5×10^{-4} M periodate it was 7 or 26 %, respectively as effective.

Time course of the effect of cytochalasin B. Fig. 5 shows the time courses of ^{3}H-thymidine incorporation stimulated by Con A and Con A plus cytochalasin B. Potentiation by cytochalasin B was observed at all times tested.

DISCUSSION

It has been suggested that lymphocyte stimulation by Con A is primarily due to binding of Con A to the cell surface (2, 4, 5). It has also been suggested that oxidation of sugar, and especially of the sialic acid moiety at the cell membrane may be involved in the mechanism of stimulation by periodate (7, 14).

In the present experiments, 0.3 µg/ml of cytochalasin B greatly potentiated the response to these two mitogens while at concentrations above 3 µg/ml cytochalasin B was inhibitory. These two mitogens have different properties but the potentiating effects of

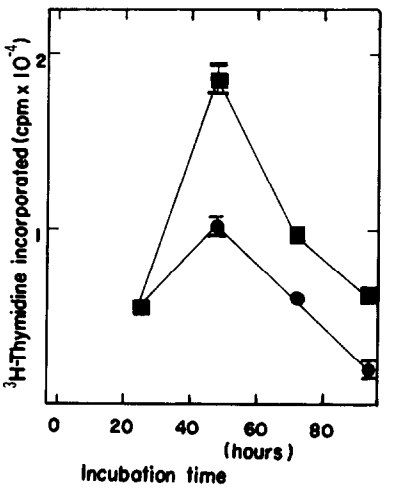

Fig. 5. Time course of the effect of cytochalasin B on rabbit mesenteric lymph node cells stimulated with Con A. Con A (2 μg/ml) (●) and Con A (2 μg/ml) plus cytochalasin B (0.3 μg/ml) (■) were added initially and cultures were pulse labeled with ^{3}H-thymidine for 3 hr.

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cytochalasin with them were similar. Thus the effect of cytochalasin B is probably not related to the primary actions of these mitogens but to some process occurring after the cell surface has been modified by the primary actions of these mitogens.

Using rat lymph node cells Yoshinaga et al. (15) investigated the effect of cytochalasin B on stimulation by several mitogens (i.e. PHA-P, Con A, lipopolysaccharide endotoxin, and antigen). Cytochalasin B was most effective at a concentration of 0.1 to 1 μg/ml with PHA-P or Con A, but at a concentration of 2 μg/ml with lipopolysaccharide. It did not potentiate the effect of antigen.

Stimulation of thymidine incorporation by mitogens was only observed after at least 24 hours incubation. Figs. 3 and 4 show that cytochalasin B was most effective when added at the same time as the mitogens. So the potentiation by cytochalasin B is related to an early process in lymphocyte stimulation induced by the two mitogens.

One of the actions of cytochalasin B on cells is thought to be on microfilaments (8, 9, 12). From these findings it seems possible that cytochalasin B may modulate the

reactions of microfilaments after the primary interaction of the mitogens with the cells. However cytochalasin B caused potentiation in a relatively narrow concentration range (0.1 - 1 μg/ml) and this range differs from that at which most reactions of microfilaments occur. On the other hand, inhibition of transport (10, 11) and stimulation of chemotaxis (16) are known to occur at concentrations of 0.1 to 1 μg/ml of cytochalasin B. Thus it is possible that potentiation of the actions of mitogens by cytochalasin B is not due to its action on microfilaments, but on some other system.

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