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# EFFECT OF NEURAMINIDASE ON THE DURATION OF CONTACT WITH CON A IN LYMPHOCYTE STIMULATION*)

By

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## ABSTRACT

Neuraminidase-treatment greatly increased $^{3}$H-thymidine incorporation by human lymphocytes at lower concentrations of Con A. An irreversible sequence after 24 hr of incubation in the presence of the optimal concentration of Con A was found. However, at a suboptimal concantraiton of Con A, neuraminidase-treated lymphocytes exposed to Con A for 72 hr exhibited a greater magnitude of $^{3}$H-thymidine incorporation in contrast to the neuraminidase-treated lymphocytes exposed to Con A for 24 hr, indicating that the enancement effect of neuraminidase was not due to the increased binding of Con A to cell surfaces.

## INTRODUCTION

Neuraminidase, which splits the glycosidic linkage between sialic acid and mucopolysaccharide in membrane glycoproteins, is capable of initiating proliferation of chick fibroblasts[1] and human lymphocytes[2]. This enzyme enhances the lymphocyte response to specific antigens and to some mitogens[3-6].

There has been considerable disagreement about the duration of contact with mitogen required to produce irreversible stimulation[7-10]. This paper reports a study of the duration of contact with Con A in the stimulation of neuraminidase-treated human lymphocytes.

## MATERIALS AND METHODS

*Cell preparation.* Purified peripheral blood lymphocytes were obtained from heparinized venous blood of normal adults by Ficoll-sodium diatrizoate centrifugation for 30 min at 400 g. After washing three times, the cells were suspended in RPMI 1640 (GIBCO) containing 20% heat-inactivated fetal calf serum (GIBCO) supplemented with 100 u/m*l* penicillin and 50 $\mu$g/m*l* streptomycin.

*Neuraminidase treatment.* Cells at a concentration of $1\times10^{7}$/m*l* in phosphate-buffered saline (PBS) were incubated at 37°C for 20 min with 0.1 u/m*l* of neuraminidase (from Cl. perfringens, Type V, Sigma). The control cells were similarly treated with an equal volume of neuraminidase-free PBS. The cells were washed three times and suspended in culture medium. Studies of the viability of cells after neuraminidase-treatment showed no evidence of cytotoxicity by the trypan blue exclusion tast.

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*Culture conditions.* Lymphocytes, $1\times10^6$ in 1.0 m*l* of culture medium were cultured in round-bottomed culture tubes in the presence of various concentrations of Con A (Sigma, Type IV). Culture tubes were incubated at 37°C in a humidified atmospere of 95% air and 5% $CO_2$ for 72 hr. α-Methyl-D-mannoside (αMM, Nakarai) in RPMI 1640 was added at appropriate times in aliquots of 1.0 m*l* to give a final concentration of 0.1 M. After incubation for 10 min at 37°C, the cells were centrifuged. The supernant was removed and 1.0 m*l* of complete medium without Con A was added. Six hours before harvesting, 1.0 μCi of tritiated thymidine was added to each well. At the end of the labelling period, the cells were collected on glass fiber filter paper (Whatman GF/C, Whatman), and the radioactivity associated with washed and dried filters was determined with a liquid scintillation counter (Beckman LS 230).

## RESULTS

The responses of neuraminidase-treated and control lymphocytes to Con A are shown in Fig. 1. $^3$H-thymidine incorporation of lymphocytes was markedly enhanced by neuraminidase-treatment at 1 μg/m*l* and 5 μg/m*l* of Con A ($p<0.01$).

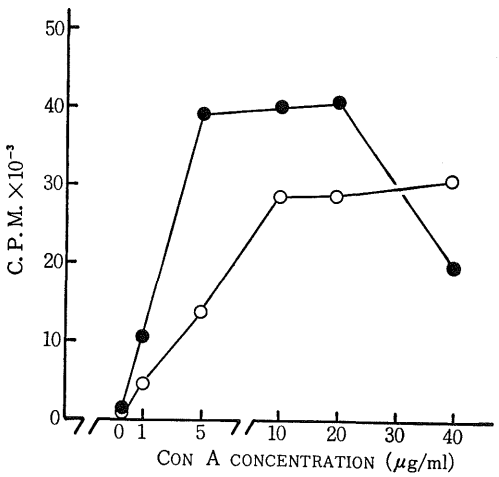


**Fig. 1.** Effect of neuraminidase on the $^3$H-thymidine incorporation by lymphocytes. Neuraminidase-treated lymphocytes (●) or control lymphocytes (○) were cultured in the medium containing various concentrations of Con A.

At concentrations of 10 μg/m*l* and 20 μg/m*l* of Con A, $^3$H-thymidine incorporation was also enhanced, but the differences were not significant ($p>0.1$). However, $^3$H-thymidine incorporation was less than the control level at the higher concentration of Con A (40 μg/m*l*). In relation to the lympocyte subpopulations, the T lymphocytes obtained by E rosette formation increased $^3$H-thymidine incorporation by neuraminidase treatment whereas non-T lymphocytes which had been treated by neuraminidase or PBS failed to respond to Con A (data not shown).

The effect of the removal of Con A by αMM was studied (Fig. 2). If Con A was removed 12 hr after the addition of Con A, the incorporation of $^3$H-thymidine measured at 72 hr was at least partially inhibited. No inhibition was observed when Con A was removed 24 hr after the beginnging of culture with the optimal concentration of Con A (20 μg/m*l*). At a

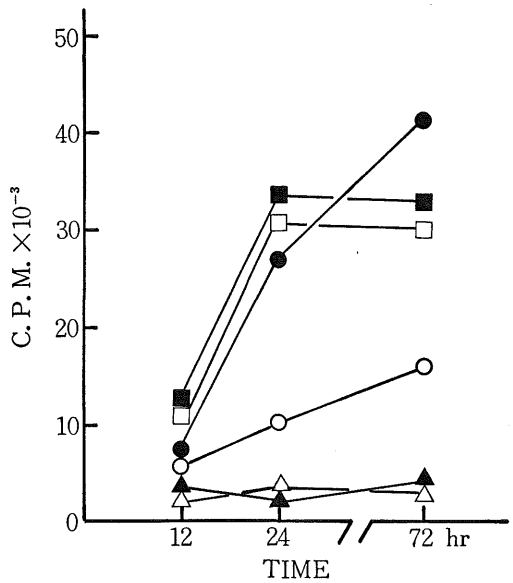


**Fig. 2.** Effect of length of exposure to Con A on the kinetics of DNA synthesis in human lymphocyte cultures. Neuraminidase-treated lymphocytes were cultured in the presence of Con A 0 μg/m*l* (▲), 5 μg/m*l* (●) or 20 μg/m*l* (■). PBS-treated lymphocytes were cultured in the presence of Con A 0 μg/m*l* (△), 5 μg/m*l* (○) or 20 μg/m*l* (□). The cultures were inhibited with 0.1 M αMM at various times, pulsed $^3$H-thymidine between 66 hr and 72 hr and harvested for scintillation counting as described in the text.

concentration of Con A 5 $\mu$g/m*l*, the level of response of cultures in which Con A was removed at 24 hr was much smaller than that of cells cultured in the presence of Con A for 72 hr.

## DISCUSSION

Considerable interest in the surface receptors of lymphocytes and their relationship to the cellular state of differentiation has been manifest. The importance of oligosaccharide units as constituents or modifiers of these surface groups has been noted in several observations. It is well known that sialic acid-containing membrane molecules are critical for growth control. Neuraminidase-treated lymphocytes showed a remarkable increase in $^3$H-thymidine incorporation when stimulated by suboptimal concentrations of Con A.

The mechanism of enhancement of $^3$H-thymidine incorporation by lymphocytes remains unknwon. One may assume that neuraminidase-treatment enhances the binding of Con A to lymphocyte cell surface receptors and results in an increase of $^3$H-thymidine incorporation. Lymphocytes became irreversibly stimulated after 24 hr of exposure to the optimal concentration of Con A. Neuraminidase-treated lymphocytes exposed to Con A 5 $\mu$g/m*l* for 24 hr exhibited the same magnitude of $^3$H-thymidine incorporation compared to that of untreated lymphocytes exposed to the optimal concentration of Con A. However, at a suboptimal concentration of Con A, neuraminidase-treated lymphocytes which were exposed to Con A for 72 hr exhibited a greater magnitude of $^3$H-thymidine incorporation than did neuraminidase-treated lymphocytes exposed to Con A for 72 hr, indicating that the enhancement effect of neuraminidase was not due to the increased binding of Con A to cell surfaces. Some alterations of lymphcoyte metabolism caused by neuraminidase-treatment may modify the kinetics of lymphocyte proliferation.

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