

# Effect of Neuraminidase on Lymphocyte Function in Chronic Lymphatic Leukaemia (CLL)

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## Abstract

Neuraminidase treated T-lymphocytes from normals and CLL gave an enhanced response to PWM when compared to untreated T-lymphocytes. However the overall response of CLL T-lymphocytes was much lower as compared to normals. CLL T-lymphocytes showed an imbalance of T-subsets, where the  $T_H$  (Helper) were not only reduced  $20.4 \pm 2.96$  but also diluted by increased  $T_S$  (Suppressor)  $45.2 \pm 5.35$ , when compared to the normal T-subsets  $T_H$   $53.6 \pm 2.64$  and  $T_S$   $21.0 \pm 1.16$ . In co-cultures of normal autologous  $T_H$  and B lymphocytes, mitomycin blocked B-lymphocytes increased the response of  $T_H$ -lymphocytes to PWM and this response was further enhanced by neuraminidase treated mitomycin blocked B-lymphocytes. However mitomycin blocked, CLL B-lymphocytes in co-cultures with autologous or normal T-lymphocytes failed to enhance their response to PWM even after neuraminidase treatment. This study shows that CLL T-lymphocytes have the same surface membrane properties as normal T-lymphocytes when responding to mitogen and suggests that the low response they manifest is due to the imbalance of T-subsets. It also shows that CLL B-lymphocytes unlike normal B-lymphocytes do not collaborate with either autologous or normal T-lymphocytes in their response to mitogen and suggests that this failure to collaborate is due to surface membrane defects (JPM 32:88, 1982).

## Introduction

Lymphocyte Cell Surface plays an important role in ligand-receptor and cell-cell interactions. Treatment of normal lymphocytes by neuraminidase results in a number of structural alterations of the cell membrane. These changes result in the potentiation of functional responses of lymphocytes, as shown by the enhancement of lymphocyte response to mitogens (Sakano, 1980; Novogrodsky, 1974), potentiation of human mixed Lymphocyte reaction (Han, 1972) and the facilitation of cell-cell interactions (Sakano, 1980). In chronic Lymphatic leukaemia (CLL) Lymphocytes, a number of cell membrane abnormalities have been reported (Godal et al., 1978; Wiley et al., 1979; Boumsell et al., 1978; Zucker-Franklin et al., 1979). Furthermore functional abnormalities have also been reported both in T and B Lymphocytes in CLL (F'oa et al., 1980; Chiorazzi et al., 1979; Weisbart et al., 1980; Han and Barbara, 1979). This study is aimed at elucidating the functional properties of CLL lymphocytes after altering their cell membrane with neuraminidase. We have therefore investigated firstly, the response of CLL  $T_H$ -lymphocytes to PWM (pokeweed mitogen), and secondly, determined the effect of mitomycin blocked CLL B lymphocytes, on the response of autologous or normal T-lymphocytes to PWM.

## Material and Methods

### Cell separation

Peripheral blood mononuclear cells from 6 normals and 8 CLL patients were separated on Lymphoprep (Nyergaard). T-lymphocytes were purified by rosette formation followed by lymphoprep separation (Wybran et al., 1973). In CLL lymphoprep separation of rosettes was repeated 2 times and after the last

separation sheep RBC were lysed with distilled water. To remove adherent cells from non-rosetting fraction, cells were suspended in medium TC 199 (Wellcome) with 20% fetal calf serum (FCS) at a concentration of  $5 \times 10^6$ /ml and incubated at 37°C for 2 hr in 30 x 15 mm petri dishes. Non-adherent cells from this fraction were removed, washed and suspended in medium. To determine the purity of sheep rosette (ER) positive T-lymphocyte and ER negative B-lymphocytes fractions, they were tested for their ability to form (a) ER (b) mouse RBC rosette (MR) by the method of Slathopoulos and Elliott (1974) and (c) the number of monocytes, by morphology and non-specific Esterase. T-subsets in purified Tlymphocytes were determined by the method of Lauria et al. (1980).

#### **Neuraminidase Treatment**

$10 \times 10^6$  cells/ml were suspended in phosphate buffered saline (PBS) and incubated at 37°C for 20 min. with 20 units/ml neuraminidase type five from *Cl. perfringens* (Sigma). Control cells were treated in the same way with PBS only. After treatment cells were washed three times and suspended in medium TC 199.

#### **Mitomycin Treatment**

$10 \times 10^6$  cells/ml were suspended in PBS and treated with mitomycin 40 ug/ml (Kyowa Hakko Kogyo Ltd) at 37°C for 30 min. Cells were washed three times suspended in medium.

#### **T-lymphocyte Cultures**

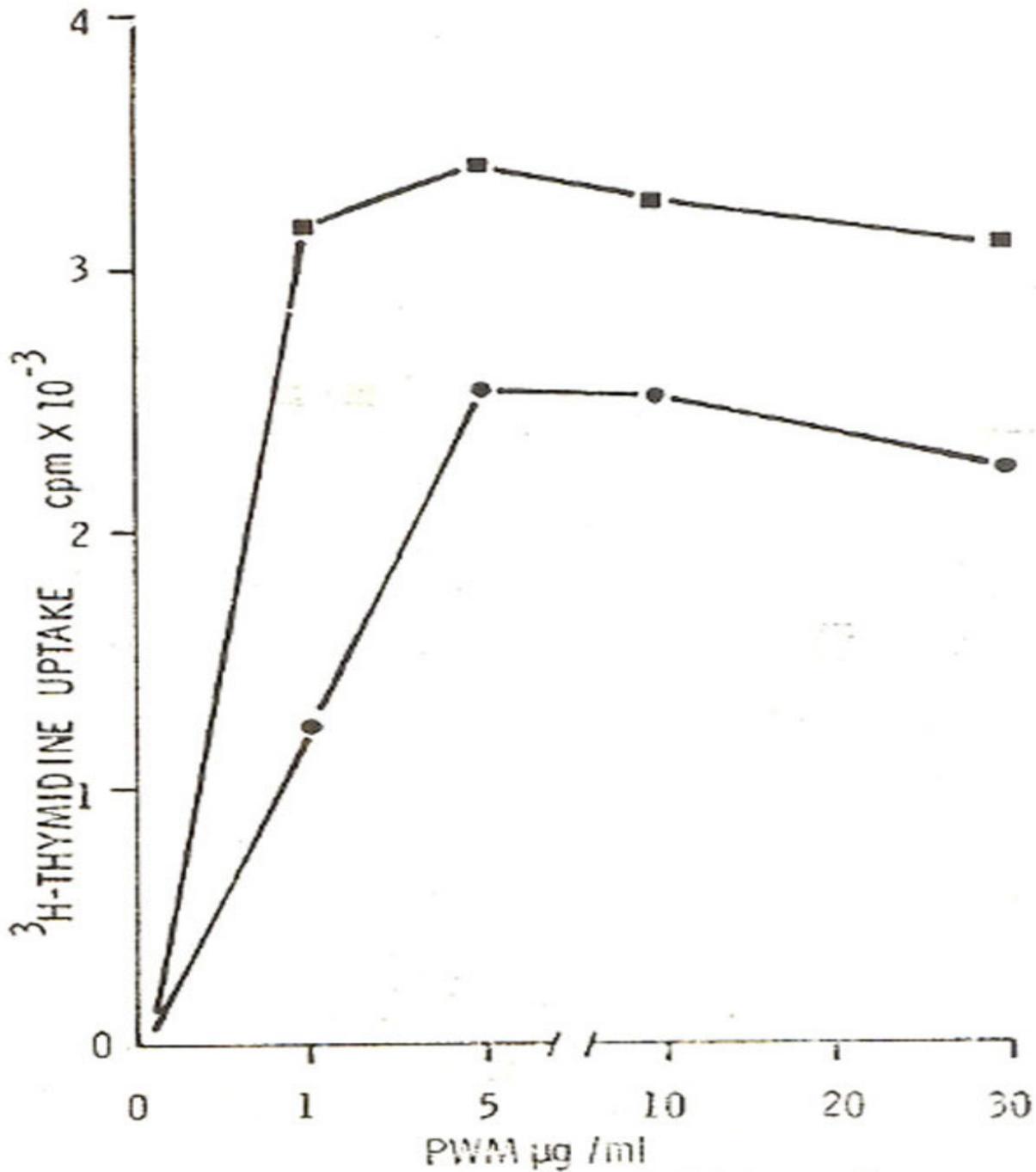
$10^6$  cells in 1 ml of medium TC 199 with 20% FCS (Gibco) were cultured in the presence of pokeweed mitogen (PWM) (Gibco) at 0, 1, 5, 10, 20 and 30 ug/ml for 72 hr at 37°C in a humidified atmosphere. 1 uCi <sup>3</sup>H-thymidine (Amersham) was added to each culture six hours before harvesting. After incubation cells were washed once in PBS, twice in 5% trichloroacetic acid and once in methanol. Dissolved in 0.1 ml 1 M NaOH and counted in an inter-technique counter using a toluene based scintillant.

#### **Co-cultures with T and B lymphocytes**

$10^5$  T-lymphocytes were cultured in the presence of  $10^5$  B-lymphocytes blocked mitomycin treatment. B-lymphocytes were either treated or untreated with neuraminidase. Cells were suspended in 1 ml medium with 20% FCS and cultured with PWM at 0 and 10 ug/ml for 72 hr at 37°C in a humidified atmosphere. 1 uCi <sup>3</sup>H-thymidine was added as in Tlymphocyte cultures and samples counted as before.

## **Results**

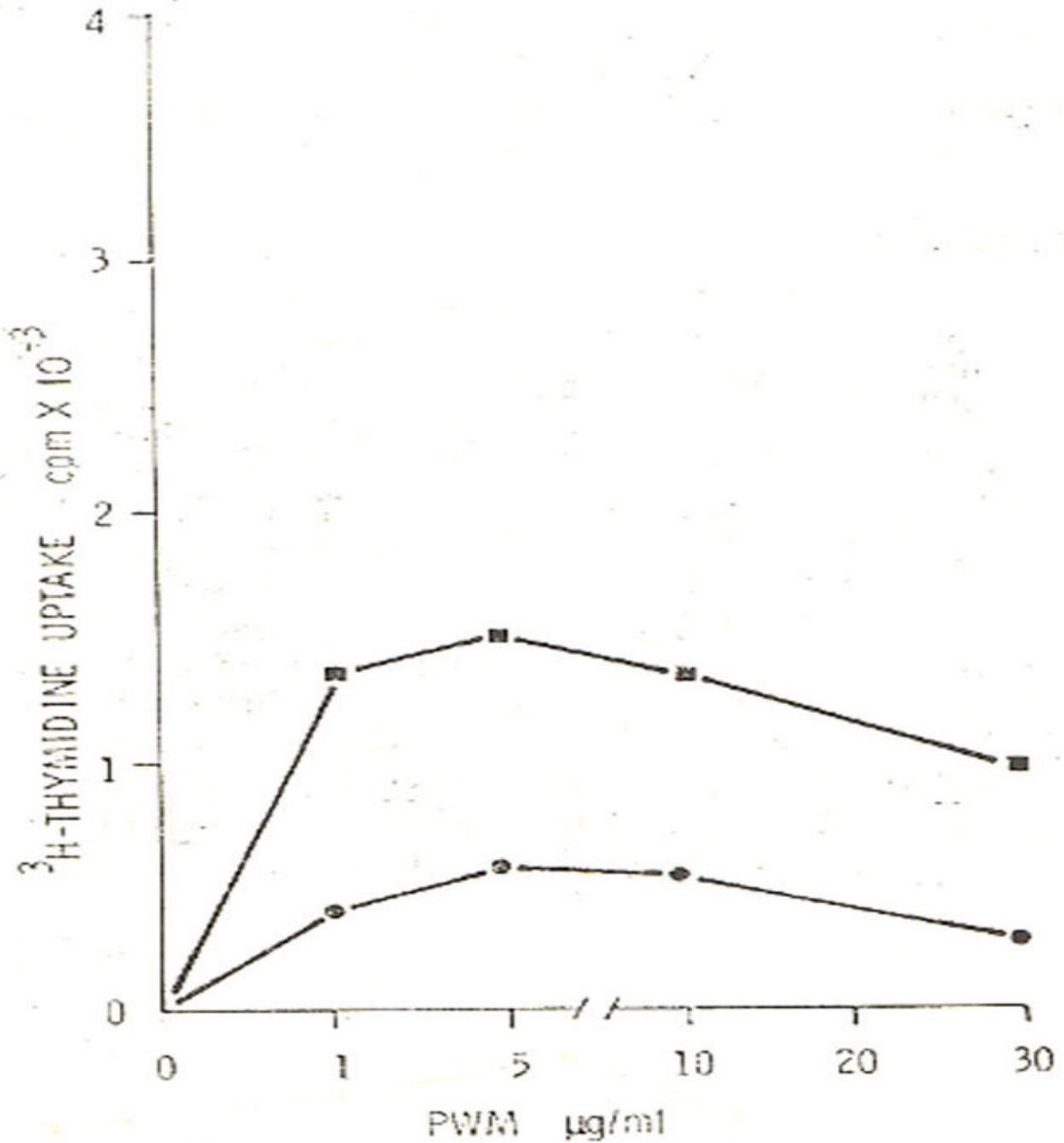
T-lymphocyte populations from normals contained low numbers of monocytes  $0.4 \pm 0.53\%$  and MR  $0.2 \pm 0.34\%$  while majority of the cells were ER positive  $88.0 \pm 3.2\%$ . The % of T-subsets in these cells were Tu (Helper)  $53.5 \pm 2.64$  and 'F?' (Suppressor)  $21.0 \pm 3.16$ . Normal T-lymphocytes gave a good response to PWM with a maximal response at 5-10 ug/ml, however neuraminidase treatment greatly enhanced this response to PWM (Fig. 1).



*Fig. 1:* Response of normal T-lymphocytes to PWM (cpm) (6 subjects) T-lymphocytes from normals, either treated  $\blacksquare$  or untreated  $\bullet$  with neuraminidase, were cultured in the presence of PWM.

T-lymphocyte population from CLL also contained low numbers of monocytes  $0.6f \pm 0.81\%$  and MR  $8.0 \pm 4.5\%$  while most of the cells were ER positive  $75.3 \pm 11.6\%$ . The % of 'F-subsets in CLL were reversed as compared to normals, red uccd Tu (Helper)  $20.4 \pm 2.96$  and increased T? (Suppressor)  $45 \pm 5.35$ . T-lymphocytes in CLL 'gave a low response to PWM as compared to normals, however,

neuraminidase treatment doubled their response to PWM (Fig. 2).



*Fig. 2:* Response of CLL T-lymphocytes to PWM (cpm) (8 patients) T lymphocytes from CLL patients either treated  $\blacksquare$  or untreated  $\bullet$  with neuraminidase, were cultured in the presence of PWM.

B-lymphocytes (ER negative) population from normals contained  $1.8 \pm 1.16\%$  monocytes,  $2.0 \pm 1.1\%$  ER and mostly MR  $55.1 \pm 5.6\%$ . In -cues of normal T and B-lymphocytes, mitomycin blocked B-

lymphocytes increased the response of T-lymphocytes to PWM as compared to T-lymphocytes alone ( $p < 0.05$ ), however mitomycin blocked, neuraminidase treated B-lymphocytes markedly increased the response to PWM ( $p < 0.05$ ) (Table 1).

## Table 1

Effect of Mitomycin Blocked B-lymphocytes on T-lymphocyte Response to PWM in Normal Co-cultures (6 Normals)

Culture Conditions	PWM $\mu\text{g/ml}$	
	0 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$
T-lymphocytes	350.5 $\pm$ 142.3	1035.3 $\pm$ 118.4
T-lymphocytes +		
PBS treated B-lymphocytes	409.3 $\pm$ 114.2	2278.0 $\pm$ 247.6
T-lymphocytes +		
neuraminidase treated B-lymphocytes	328.3 $\pm$ 167.1	5942.0 $\pm$ 332.3
PBS treated B-lymphocytes	316.0 $\pm$ 151.4	342.6 $\pm$ 60.3
neuraminidase treated B-lymphocytes	295.4 $\pm$ 132.6	350.6 $\pm$ 78.6

Normal T-lymphocytes were Co-cultured with mitomycin blocked B-lymphocytes treated or untreated with neuraminidase in the presence of PWM.

B lymphocytes from CLL contained 0.8  $\pm$  0.5%, monocytes, 0.4  $\pm$  0.45% ER while majority of the cells were MR positive 85.1  $\pm$  6%. In CLL autologous Co-cultures, B-lymphocytes failed to enhance the response of T lymphocytes to PWM, either before ( $p > 0.4$ ) or after neuraminidase treatment ( $p > 0.4$ ) as

compared Vu T-lymphocytes alOaè (Table II).

## Table II

Effect of Mitomycin Blocked B-lymphocytes on T-lymphocytes Response to PWM in CLL Co-cultures (8 Patients)

<i>Culture Conditions</i>	<i>PWM ug/ml</i>	
	<i>0 ug/ml</i>	<i>10 ug/ml</i>
T-lymphocytes	300.0 ± 160.3	775.7 ± 385.9
T-lymphocytes + PBS treated B-lymphocytes	290.5 ± 154.1	894.0 ± 315.3
T-lymphocytes + neuraminidase treated B-lymphocytes	374.7 ± 173.0	1005.0 ± 319.0
PBS treated B-lymphocytes	275.5 ± 123.0	351.0 ± 176.0
neuraminidase treated B-lymphocytes	247.5 ± 143.2	376.5 ± 169.6

CLL B lymphocytes also failed to enhance the response of normal T-lymphocytes to PWM, either before or after neuraminidase treatment (Table III).

# Table III

Effect of Mitomycin Blocked CLL B-lymphocytes on Normal T-lymphocytes response to PWM (4 Normals and 6 Patients)

Culture Conditions	PWM ug/ml	
	0 ug/ml	10 ug/ml
T-lymphocytes	250.7 ± 9.91	1009.8 ± 119.6
T-lymphocytes + PBS treated B-lymphocytes	213.3 ± 61.3	964.7 ± 301.5
T-lymphocytes + neuraminidase treated B-lymphocytes	197.1 ± 84.9	979.0 ± 347.4
PBS treated B-lymphocytes	195.2 ± 53.9	221.6 ± 78.9
neuraminidase treated B-lymphocytes	210.5 ± 44.3	196.9 ± 50.3

CLL T-lymphocytes were Co-cultured with B-lymphocytes which were blocked with mitomycin and treated or untreated with neuraminidase in the presence of PWM

## Discussion

Neuraminidase exerts its effect on the lymphocyte cell membrane by removing sialic acid residues from glycoproteins. This results in the reduction of surface charges, and exposure of receptor sites leading to facilitation of ligand-receptor and cell-cell interactions. Since neuraminidase treatment enhanced the response of T-lymphocytes to PWM both in normals and CLL, it suggests that the latter

cells have normal cell membrane properties. Therefore the low responses observed with CLL T-lymphocytes are not due to membrane defects which manifest as functional abnormalities, but possibly due to the imbalance of T-subsets. Infact this is confirmed by the observations that only Tu (Helper) have the capacity to respond to mitogens and form T-colonies (McCann et al., 1980; Victorino and Hodgson, 1980; Foa et al., 1980). These observations indicate that T-lymphocytes in CLL are probably functionally normal and the low responses they manifest here and in other reports are due to the low numbers of reactive Tu (helper) lymphocytes.

Normal B-lymphocytes have been shown to collaborate with T-lymphocytes in their response to mitogen and to participate in mixed lymphocyte reactions (Weisbart et al., 1980). Since neuraminidase treated normal B-lymphocytes enhanced the response of T-lymphocytes to PWM, it confirms that the enzyme by altering the surface membrane, exposes receptor sites and facilitates cell-cell interactions. The failure of CLL B-lymphocytes to enhance the response of both normal and autologous T-lymphocytes to PWM even after neuraminidase treatment, confirms that their surface membrane is abnormal, and that the enzyme does not increase the number of receptor sites. CLL B-lymphocytes have been shown to have reduced  $964.7 \pm 301.5$  numbers of receptor sites on their surface membrane (Wiley et al., 1979; Sheppard et al., 1977).

Therefore the failure of CLL B-lymphocytes to collaborate with T-lymphocytes is probably due to a reduced number of receptor sites which are crucial for cell-cell interactions. This reduction of receptor sites may also explain the reported inability of CLL B-lymphocytes to participate in mixed lymphocyte reactions (Weisbart et al., 1980) and to respond to mitogen (Zafar et al., 1981). Our study shows that CLL T-lymphocytes have similar surface membrane properties as normal T-lymphocytes and confirm suggestions that CLL T-lymphocytes respond poorly to mitogens due to an imbalance of T-subsets. It also shows that CLL B-lymphocytes fail to collaborate with T-lymphocytes even when their membrane was altered with neuraminidase, thus confirming that CLL B-lymphocytes have cell membrane abnormalities.

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