Neuraminidase Treatment Enhances the Lysolecithin Induced Intercellular Adhesion of *Amoeba proteus*

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Egg lysolecithin induces intercellular adhesion of *Amoeba proteus*. Pre-treatment of cells with *Vibrio cholerae* neuraminidase (VCN) increases the adhesive property of cells as was evidenced in their formation of larger cell aggregates than controls. A possible role of VCN exposed receptor sites in cellular adhesion is suggested.

Until very recently cell fusion, to obtain homo- and hetero-karyocytes, was usually performed using sendai virus as an inducing agent. Lucy [2] formulated a hypothesis on cell fusion based on membrane fusion induced by lysolecithin and vitamin A. Poole and colleagues [3] investigated the effect of egg-lysolecithin (ELL) on avian erythrocytes and could actually get multinucleated cells. Lysolecithin induced cell fusion was also observed in various mammalian cell types [4]. Spies *et al.* [5] demonstrated ELL-induced cellular aggregation in an intestinal parasite, *Entamoeba invadens* and in a free-living soil amoeba, *Acanthamoeba castellanii*. We report here that ELL can induce intercellular adhesion in a large, free-living protozoa, *Amoeba proteus* and that *Vibrio cholerae* neuraminidase (VCN) pretreatment increase this cellular adhesion.

Amoeba proteus were cultured [6] in media containing 0.036 mM $CaHPO_4$, 0.08 mM KCl and 0.033 mM $MgSO_4$, pH 6.8. VCN obtained from Behringwerke AG, Marburg, Lahn, West Germany, contains 500 units/ml and certified as having no detectable proteinase, aldolase or lecithinase C activity. One unit of enzyme activity is defined as being equivalent to the release of 1 μg of N-acetyl neuraminic acid from a glycoprotein substrate at 37 °C in 15 min at pH 5.5. That VCN can liberate sialic acid from cell surface [7] and its effect is inhibited in presence of the negative feed back inhibitor N-acetyl neuraminic acid [8] were demonstrated earlier.

A measured aliquot of cell suspension from a stock culture was dispensed in a syracuse watch glass. The cells were allowed to settle down. Excess media was sucked off and 25 units of VCN/0.1 ml of media was added. The sham control cells were kept under identical conditions, but without VCN. Cells were incubated at 37 °C for 20 min and then washed thoroughly several times with excess of media. During incubation with VCN, cells come off the bottom of the syracuse watch glass and assume a spherical shape. So after incubation they were suspended in media until most of the cells adhere to the watch glass and show normal streaming pseudopodial movement. At 37 °C, some cells from the sham control group also come off the bottom surface. Both treated and sham treated cells were, therefore, allowed to settle down before they were subjected to lysolecithin treatment. It should be mentioned that the concentration of VCN used for the study is non-cytolytic and does not induce any aggregation of cells as such.

Egg Lysolecithin (ELL) obtained from V. P. Chest Institute, Delhi, India, was dissolved in chloroform and then the solvent was evaporated. Finally media was added to it and mixed thoroughly over a vortex mixer to have a colloidal solution. Lysolecithin is not cytotoxic up to a concentration of 2 mg/ml. Cells kept up to 24 hours in presence of lysolecithin (2 mg/ml) were found to have almost normal morphological shape, pseudopodial movement etc. Though ELL induces cellular adhesion at a concentration of 500 μg/ml, the optimal amount of adhesion was obtained at 2 mg/ml concentration.

Measured number of VCN treated and sham-treated cells were transferred in small test tubes and centrifuged to have a compact cell pellet. Lysolecithin (0.2 ml) was added to it and then kept as such for 15 – 20 min at room temperature (22 °C). The cell bottom was transferred on slides taking sufficient care that it is not vigorously disturbed. The liquid was evenly spread on the slides with the help of a wide mouth pipette and numbers of both single and clumped cells were counted in light microscope having a squared grid in the ocular and at a magnification of ca. X 60. The number of free cells, 2 cells-, 3 cells-, and 4 cells-clumps were recorded. Clumps containing more than 4 cells were included in the group of 4 cell clumps.

Results are presented in Table I. It is clear that ELL induces cellular adhesion in *A. proteus*. It is extremely significant that in cells pretreated with VCN cellular adhesion is facilitated in that the number of larger cell clumps ($\geqq 4$) is more and single non-aggregated cells is very few compared to sham treated controls. The results are highly reproducible.

The exact mechanism of lysolecithin induced cell-adhesion is not clearly understood. It is believed that lysolecithin helps forming globular micellar

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Table I. Effect of VCN on the ELL induced adhesion in *Amoeba proteus*.

Experiment number	Cell treatment **	Number of cellular aggregates *			
		Single	Two	Three	Four or more
I	None	30	14	8	13
	VCN	15	9	22	37
II	None	39	25	14	19
	VCN	20	18	27	43
III	None	46	28	11	15
	VCN	21	10	10	33
IV	None	51	15	3	1
	VCN	27	21	14	20

* The number of free cells and the cell aggregates were counted with the help of an eye piece graticule. The total area counted ranged from ca. $8.7-9.2 \times \mu m^2$.

** In all the experiments cellular inocula used for VCN and sham treatment contained identical numbers of cells taken out from stock culture and varied between 350—500 cells/ml.

structure in the membrane lipids [2, 3]. Aggregation of various types of cells by VCN treatment was also shown by a number of laboratories [9–11], and the phenomenon was described to be dependent on the sialic acid content of the cell surface. At present the exact mechanism of increased lysolecithin induced cellular adhesion of VCN-treated cells remains to be established. It is not known, if sialic acid has any precise role in the phenomenon studied.

Earlier we have shown that the removal of surface sialic acid by VCN treatment unmasks newer receptor sites on cell surfaces [12, 13] and the possibility of participation of these newly-exposed sites in the cell aggregation has recently been suggested by Llyod and Cook [9]. Thus it may not be unwise to suggest a similar mechanism which might be responsible for the observed effect in neuraminidase treated *A. proteus*.

[1] H. Harris and J. F. Watkins, Nature **205**, 640 [1965].
[2] J. A. Lucy, Nature **227**, 814 [1970].
[3] A. R. Poole, J. I. Howell, and J. A. Lucy, Nature **27**, 810 [1970].
[4] C. M. Croce, W. Sawicki, D. Kritchevsky, and H. Koprowski, Exp. Cell Res. **67**, 427 [1971].
[5] F. Spies, P. F. Elbers, and W. A. M. Linnemans, Cytobiologie **6(3)**, 327 [1972].
[6] S. Chatterjee and M. V. N. Rao, Exp. Cell Res. **84**, 235 [1974].
[7] P. K. Ray and R. L. Simmons, Cancer Res. **33**, 936 [1973].
[8] P. K. Ray, H. Gewurz, and R. L. Simmons, Clin. Exp. Immunol. **11**, 441 [1972].
[9] C. W. Llyod and G. M. Cook, J. Cell Sci. **15**, 575 [1974].
[10] M. G. Vicker and J. G. Edwards, J. Cell Sci. **10**, 759 [1972].
[11] J. J. Deman, E. A. Bruyneel, and M. M. Marcel, J. Cell Biol. **60**, 641 [1974].
[12] P. K. Ray and R. L. Simmons, Proc. Soc. Exp. Biol. Med. **142**, 217 [1973].
[13] P. K. Ray and K. Sundaram, Clin. Exp. Immunol. **19**, 529 [1975].