IMMUNE CYTOLYSIS OF MALIGNANT CELLS—EFFECT OF NEURAMINIDASE ON CERVICAL CANCER CELLS

By

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A hypothesis has been put forward that pericellular sialic acid of cancer cells acts as an electrochemical barrier to immunologically competent cells in many, if not all, forms of malignancy (Currie and Bagshawe, 1967). The possibility exists that sialic acid coating of cancer cells can mask cell-surface antigens and prevent their detection by the immune system.

The present study was undertaken to determine the effect of neuraminidase on human malignant cells. If the above hypothesis is correct, immune lymphocytes ought to be able to attack and destroy cancer cells more easily when they are treated with neuraminidase.

Radioactive chromium is taken up by cancer cells on incubation and this is released to the surrounding medium when the cells are lysed. The radioactivity in cell-free supernatant is thus expected to be proportional to the destruction of cancer cells by immune lymphocytes.

The experimental methodology in our work was as follows :

Cells from human uterine cervix cancer biopsy were dispersed and suspended in Eagle's minimum essential medium. 250 units of neuraminidase were added to 1 million cancer cells and incubated for 30 minutes at 37°C. After incubation the cells were washed 4 times in Eagle's medium. 30 μc of labelled sodium chromate was added to 1 million enzyme-treated cells and incubated at 37°C for 1 hour. The pellet was washed after centrifugation 5 times with the medium containing cold chromium.

Cancer cell suspensions similarly labelled with radioactive chromium, in which neuraminidase treatment was omitted, served as controls.

Immune lymphocytes were isolated from blood of patients suffering from cancer cervix. Cells were resuspended in Eagle's medium.

Normal lymphocytes obtained from patients free from any form of cancer were also resuspended as above.

0·5 ml of labelled cancer cell suspension was incubated with 0·5 ml of lymphocyte preparation at 37°C for 12 hours, keeping the ratio of cancer cells to lymphocytes as 1 : 100. At the end of incubation, the tubes were centrifuged and supernatant measured for radioactivity in a scintillation counter. The percentage of chromium release was calculated as :

$$\frac{\text{CPM of Cr released by cytolysis}}{\text{CPM of total Cr incorporated}} \times 100$$

Uptake of chromium by cancer cells during the labelling was noted against time ; and it was found that incubation with radioactive chromium for 1 hour sufficed.

Activity of lymphocytes on cancer cells was studied in 10 cases. It was found that the cytolytic effect of lymphocytes on cancer cells increased approximately two fold after treatment of cancer cells with neuraminidase. The cytolytic efficiency was higher with lymphocytes of patients with cancer cervix than with normal lymphocytes.

As a control, effect of normal lymphocytes on normal cells from uterine cervix was also studied. In these cases, treatment with neuraminidase produced a negligible effect.

In Summary

1. Cancer cells were incubated with radioactive chromium. They were further treated with neuraminidase and then subjected to attack by lymphocytes. The radioacivity in cell free supernatant indicated the degree of cytolysis.

2. Cytolytic effect was increased two times after treatment with neuraminidase.

3. The cytolysis was more marked when lymphocytes from cases of cancer were used.

REFERENCE

Currie, G. A. and Bagshawe, K.D. (1967) Lancet, **i**, 780.

DISCUSSION

G. Bazaz-Malik : Was the tumour studied histologically before taking the tumour cells for study ?

D.M. Vasudevan : Yes. Only frankly malignant cases were included in this study.

G. Bazaz-Malik : How was the cell suspension prepared ?

D.M. Vasudevan : By dispersing with Trypsin EDTA solution.

G. Bazaz-Malik : Cervical carcinomas are of different grades of malignancy. Many cells in less differentiated tumours are almost normal. How were these excluded ?

D.M. Vasudevan : The cases in the present study were of anaplastic varieties, mainly Grade IV.

G. Bazaz-Malik : Carcinoma of cervix being superficial has severe secondary inflammatory reaction. How were these inflammatory cells, mostly lymphocytes and plasma cells excluded ?

D.M. Vasudevan : These cells were not excluded in this study.

S.N. Choudhury : Do you hypothesise the cancer cells are immunologically different from normal cells ?

D.M. Vasudevan : Yes.

S.N. Choudhury : Do you think that there are specific histo-antigenic changes in carcinogenetic process *e.g.*, blood group antigenic changes ?

D.M. Vasudevan : Our hypothesis is that tumour specific antigens are masked by sialic acid. It needs further investigation to prove this hypothesis.

K. Saha : I appreciate the question of Dr. S.N. Choudhury, "Can cancer change T antigens ?" There is documentary evidence that blood group antigens change in Hodgkin's dermal cancer, leukemias, etc.

Mihir Bagchi : Sialic acid is one of the normal constituents of the cell membrane. Can you tell how pericellular sialic acid of cancer cell differs from normal sialic acid, say in its molecular configuration ?

D.M. Vasudevan : This is a preliminary communication and we have not analysed sialic acid content in our studies. In the literature there is plenty of evidence to suggest that pericellular sialic acid content of cancer cells is increased markedly as compared with their normal counterparts. But all these studies are with reference to quantitative changes only. Whether there are any actual qualitative changes or any configurational changes of the attached protein molecules have not yet been studied.