Mucus Secretion–Stimulating Activity in Human Lymphoblastoid Cells

Abstract. *Two fractions isolated from cultured lymphoblastoid cells stimulated mucus secretion from the urn cell complex of the marine invertebrate* Sipunculus nudus. *The activity detected in the nuclear fraction was trypsin-sensitive, and it increased in response to specific nucleotides.*

Substances that stimulate mucus secretion have been identified in a number of invertebrate, vertebrate, and human body fluids, including tears and serum, through an assay developed by Cantacuzène (*1*) and Bang and Bang (*2*, *3*). The assay involves utilization of the urn cell of the marine invertebrate *Sipunculus nudus*. The urn cell is a detached epithelial cell complex that has been shown to produce mucus in vitro in response to defined mucus-stimulating substances (MSS's).

Using this assay, we have found mucus-stimulating activity in sonicated human lymphoblastoid cells. The diploid lymphoblastoid cell lines MGL-8 (*4*) and GM 0130 (*5*), established from the peripheral blood of apparently healthy individuals, were used as sources of MSS's. The cells were cultured in RPMI 1640 medium with 10 to 20 percent fetal calf serum and passaged every 2 or 3 days to maintain their density between 2×10^5 and 1×10^6 cells per milliliter. Cells (7.6×10^7 per milliliter) were washed with isotonic phosphate buffer, resuspended in phosphate-buffered saline, and disrupted by sonic oscillation (Polytron).

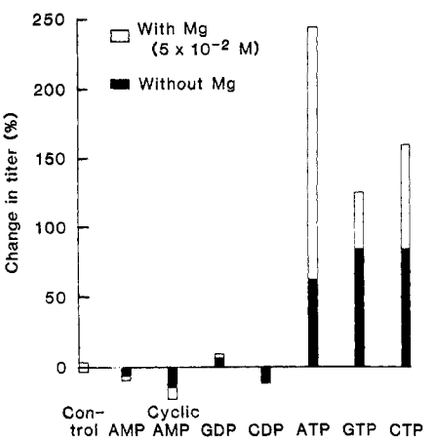

Fig. 1. Effect of nucleotides on mucus-stimulating activity in sonicated lymphoblastoid cells. The final concentration of each nucleotide in the incubated samples was $10^{-2}M$. All titers were calculated in relation to the incubated control with and without Mg. Mean increases in MSS titers after incubation of the cells with the nucleoside triphosphates and Mg^{2+} were: ATP, 224 ± 26 percent; GTP, 125 ± 25 percent; and CTP, 159 ± 32 percent. With lower concentrations of these nucleotides, a significant increase in activity was not detected.

No intact cells or nuclei were seen after sonication. To determine the amount of MSS present in a sample, we tested a 10-μl portion on urn cells (*2*). For each experimental point, at least three replicate samples were analyzed. The length of the mucus tail produced was an indication of the amount (titer) of MSS. Activity was calculated in urn units: one urn unit is the amount of MSS which induces the urn cell to produce a tail equal in length to the vertical diameter of the cell.

The temperature sensitivity of the mucus-stimulating activity was then determined. Freezing followed by thawing decreased mucus-stimulating activity in the sonicated cells 50 percent. Incubation at 37°C for 30 minutes did not significantly change the titer (mean increase, 18 percent). Heating to 85°C for 4 minutes increased the titer by an average of 196 percent. The activity found by Bang and Bang (*2*) in human serum was also enhanced by heating to 85°C for 4 minutes. All changes in titer are expressed as percentages because different sets of urn cells may respond differently to the same stimulus. Differences between the titers of control and treated samples were always based on assays with the same preparation of urn cells.

Addition of crystalline deoxyribonuclease I (up to 2 mg/ml) (*6*) increased the MSS titer 28 to 200 percent, depending on the amount added (incubation was at 37°C for 30 minutes). The increase might only have been due to a reduction in the viscosity of the sample, allowing serial dilution of the sample. For example, deoxyribonuclease I has no effect on MSS in human serum. Nevertheless, the 5′ nucleotides, some of which are products of deoxyribonuclease action on DNA, were tested for their effect on mucus-stimulating activity in the sonicated cells. The titer of mucus-stimulating activity increased in response to the addition of three 5′-nucleoside triphosphates: adenosine triphosphate (ATP), guanosine triphosphate (GTP), and cytidine triphosphate (CTP) (Fig. 1). This effect was enhanced by the addition of Mg^{2+}. Neither the 5′-monophosphates, adenosine monophosphate and adenosine 3′,5′-monophosphate, nor the 5′-diphosphates, guanosine diphosphate and cytidine diphosphate, increased the titer. The nucleotides were tested in final concentrations of 10^{-2}, 10^{-3}, and $10^{-4}M$. The Mg^{2+} added was 1.5 times the amount of the nucleotides. The sonicated cells (100 μl) and the appropriate dilution of nucleotides (10 μl) were mixed and incubated at 37°C for 30 minutes. The deoxyribonuclease I and the nucleotides were neither toxic to the urn cells nor inducers of mucus production.

The nucleus and cytoplasm of the cells were separated before testing in order to determine the location of the MSS's. Homogenization of 5.3×10^7 cells suspended in 2 ml of hypotonic buffer was performed with a Dounce homogenizer until about 96 percent of the cells were destroyed, but the cell nuclei were left intact. After centrifugation at 1000*g*, no nuclei were seen in the supernatant. The nuclear fraction was sonicated and the supernatant was brought to isotonicity with NaCl before testing. Approximately 65 percent of the activity recovered was associated with the nucleus and 35 percent was associated with the cytoplasm. Total recovery was more than 90 percent.

Incubation with trypsin (600 U) at 37°C for 30 minutes decreased the titer of mucus-stimulating activity 52 percent. The activity in the nuclear fraction was sensitive to trypsin (Fig. 2), while that in the cytoplasmic fraction was not. In addition, mucus-stimulating activity in the nuclear fraction was increased by the addition of deoxyribonuclease I; this enzyme had no effect on the cytoplasmic fraction.

Our demonstration of mucus-stimulating activity in lymphoblastoid cells may

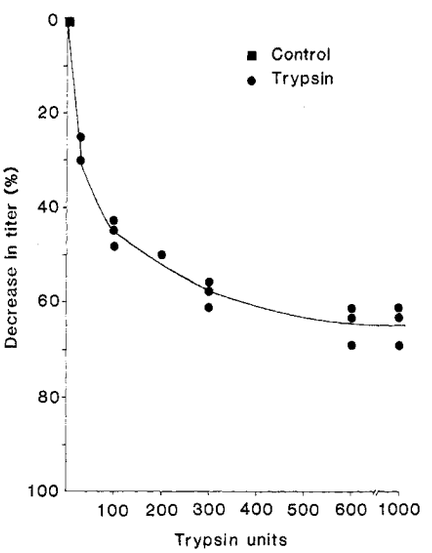

Fig. 2. Effect of trypsin on mucus-stimulating activity in the nuclear fraction of cells. Deoxyribonuclease I (2 mg/ml) was added to reduce the viscosity of the samples.

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give a clue to understanding the stimulation of mucus release in the body. Although we did not find mucus-stimulating activity in the culture fluid of the cells—even after heating to 85°C for 4 minutes—MSS might be released from lymphoblastoid cells into the serum in vivo. With a different assay involving the use of isolated rabbit endocervical cells, Nicosia *et al.* (*7*) found an MSS in rabbit serum. Fetal calf serum in the culture medium of the lymphoblastoid cells was not found to contain MSS's, even after heating.

The finding that trypsin and deoxyribonuclease I affects the activity in the nuclear fraction but not in the cytoplasmic fraction suggests the presence of at least two different MSS's in the cell. The effects of the nucleotides seem to indicate the involvement of energy-dependent processes in the activation of MSS's. Although the concentration at which the maximal response was obtained, $10^{-2}M$, is fairly high, it seems to be specific for the nucleoside triphosphates and for the Mg-nucleotide complex. The increase in titer after addition of deoxyribonuclease I may also be due to the endogenous formation of nucleoside triphosphates, since it occurs only with the nuclear fraction. The relation between the two MSS fractions can be determined only after further characterization and purification.

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References and Notes

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2. B. G. Bang and F. B. Bang, *Johns Hopkins Med. J.* **145**, 209 (1979).
3. ———, *Biol. Bull. (Woods Hole, Mass.)* **159**, 571 (1980).
4. Line MGL-8 was obtained from J. W. Littlefield, Department of Pediatrics, Johns Hopkins University School of Medicine.
5. Line GM 0130 was obtained from the Human Genetic Mutant Cell Repository, Camden, N.J.
6. The deoxyribonuclease I (Sigma) used was derived from bovine pancreas. It contained about 2000 Kunitz units per milligram of protein and was substantially free of ribonuclease.
7. S. V. Nicosia, E. J. Streibel, L. Mogul, *In Vitro* **17**, 205 (1981).
8. We thank B. G. Bang for advice on the urn cell assay and for providing urn cells, P. C. Huang for advice on cell homogenization, and J. W. Littlefield for providing MGL-8 cells. Supported by NIH grant 5P50 HL-19157 and NCI grant CA-00640.

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Transformation of Human Leukocytes by Cocultivation with an Adult T Cell Leukemia Virus Producer Cell Line

Abstract. *The transmission of adult T cell leukemia virus, a human retrovirus, into fresh leukocytes from normal humans was examined. One of three virus-carrying cell lines, tested after being subjected to lethal x-irradiation, consistently transformed leukocytes from adult peripheral blood and umbilical cord blood. All the transformed cell lines expressed adult T cell leukemia virus–associated antigen, but transformed lines originating from adult and umbilical cord blood exhibited T cell and non-T, non-B cell surface natures, respectively. Efforts to transform human leukocytes with cell-free virus were unsuccessful.*

In 1977 Takatsuki and his colleagues (*1*) reported the clinical and hematological characters of a new type of leukemia, adult T cell leukemia (ATL). Since then, many investigators have confirmed the existence of this malignancy and have proposed that the disease also be called adult T cell leukemia/lymphoma because of its leukemic, lymphomatous nature (*2*). The disease is principally found in areas of southwestern Japan.

Recently, Hinuma *et al.* (*3*) reported that an antigen associated with a long-term culture of ATL cell line MT-1 reacted with serum from all ATL patients tested and also with serum from about 25 percent of the healthy adults tested in areas where ATL is endemic. The antigen (ATLA) reacted with very few serum samples from subjects living in areas not affected by ATL. This antigen is also found in two other cell lines, MT-2 (*4*) and MT-4 (*5*), each of which was obtained by coculturing peripheral leukemic cells from ATL patients with normal umbilical cord leukocytes. Both MT-2 and MT-4 were shown to be of cord cell origin by sex chromosome analysis. Furthermore, ATLA was detectable in short-term cultures of peripheral leukemic cells from ATL patients (*6*). Thus ATLA was consistently detected in long- or short-term cultures of ATL cells but not in 14 other human lymphoblastoid cell lines, including T cell, B cell, and non-T, non-B cell lines (*3*).

Virus particles with C-type morphology were found in all the ATLA-positive cell cultures by electron microscopy (*3, 4, 6, 7*). We have demonstrated and characterized type C retrovirus by biochemical techniques (*8*) and have named the virus adult T cell leukemia virus (ATLV). However, the biological activities of this retrovirus are largely unknown. We now report the transmission of ATLV genomes into normal human adult peripheral and umbilical cord blood leukocytes by cocultivation with x-irradiated MT-2 cells (*9*). Transmission was revealed by the transformation of and expression of ATLA in the recipient cells.

Cells of line MT-1, MT-2, or MT-3 were cultured in RPMI-1640 medium (supplemented with 20 percent fetal calf serum and antibiotics) at 37°C in an incubator with CO_2. About half the medium was changed twice a week. The cells were lethally x-irradiated (9000 R), centrifuged, and suspended in fresh medium to reduce the concentration of free radicals generated in the medium during irradiation. Blood was obtained from healthy adults and from umbilical cords at full term. Mononuclear leukocytes (lymphocytes and macrophages) were prepared by Ficoll-Conray gradient centrifugation (*10*). Samples of freshly prepared leukocyte suspension (1×10^6 cells per milliliter) or cells that had been cultured for 1 day were mixed with an equal volume of the suspension of irradiated MT-2 cells (1×10^6 cells per milliliter). Samples (1.0 ml) of the mixtures were cultured in plastic wells (Falcon model 3008). Control cultures consisted of leukocytes or irradiated MT-2 cells alone. The cultures were examined at least once a week for cell transformation, which was defined as the appearance of scattered cell aggregations and a subsequent increase in their size and number. Transformation was confirmed by continuous growth of the cells during serial subcultivation.

In cocultures with irradiated MT-2 cells, transformation and continuous growth of "recipient" cells from adults or newborns were observed after 2 to 4 weeks of incubation. In control cultures the irradiated MT-2 cells did not show any evidence of proliferation, and cultures containing only leukocytes showed only transient proliferation of macrophages or lymphoid cells. All 20 of the transformed cell lines tested were positive for ATL virus.

Table 1 shows properties of 12 transformed cell lines derived from six preparations of adult peripheral blood leukocytes (PBL) and six of umbilical cord blood leukocytes (CBL). The lines show variable proportions of ATL antigen–positive cells (from 17 to over 90 percent). All the lines tested were negative

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