Altered polyamine concentrations in cytomegalovirus-infected human cells

HELEN M. GARNETT

Summary

Polyamines have a regulatory effect on DNA and RNA synthesis and their levels are elevated in rapidly growing cells, including lymphoblasts. However, as shown in the current experiments, exposure to cytomegalovirus (CMV) reduces the polyamine levels in these cells, suggesting that the virus interferes with their metabolism. Studies have shown that the activity of ornithine decarboxylase is increased in CMV-infected fibroblasts and that there is an increased conversion of putrescine to spermidine and spermine. Thus it may be expected that the concentration of these molecules would increase in the infected cell. However, the results presented here demonstrate that only the concentrations of putrescine and spermidine are increased, the spermine concentration decreasing with infection.


It has been demonstrated that a continued presence of arginine is necessary in the medium of cytomegalovirus (CMV)-infected fibroblasts to maintain both the cytopathic effect and the production of virions. This observation led to the hypothesis that some of the arginine may be required for polyamine synthesis in the infected cells. It has been demonstrated that the activity of ornithine decarboxylase, which catalyses the conversion of ornithine to putrescine, rises rapidly between 9 hours and 24 hours after inoculation of human cells with CMV and this result supported the idea that polyamine synthesis may be elevated in the infected cells. Thus a study was undertaken to determine the polyamine concentrations in human embryonic fibroblasts 24 hours after infection. Because studies have indicated that this virus initiates replication in some human lymphoblasts and affects their functions, the effect of virus challenge on the polyamine levels in these cells was also assessed.

Material and methods

Cells

Human embryonic lung fibroblasts (HEF) were obtained from fetal lung by the method of Hayflick and Moorhead and used between passage 4 and 25. The cells were grown in Eagle's minimal essential medium (MEM) (Gibco, Grand Island, NY, USA) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml), 0.075% sodium bicarbonate and 10% heat-inactivated fetal calf serum. After 2 days the medium was replaced with MEM supplemented with antibiotics and 1% dialysed fetal calf serum or arginine-deficient MEM (arg-MEM) supplemented similarly. Twenty-four hours later the cells were infected with CMV.

Virus

Human CMV, strain AD 169, was grown in HEF and harvested when extensive cytopathic effect was observed. The cells were pushed from the glass and virus extracted by three cycles of freezing and thawing. The crude virus preparation was then clarified by low-speed centrifugation and the virus pelleted by spinning at 90,000 g at 4°C. The virus was resuspended in MEM or arg-MEM and used in a virus/cell ratio of 2:1, the titre being determined by the method of Wentworth and French.

Lymphocytes

Venous blood was collected from CMV antibody-negative donors, as determined by the complement fixation test. The blood was collected in a heparinised syringe and the mononuclear leukocytes collected by centrifugation on a Hypaque (9.8%)-Ficoll (6.4%) gradient. Monocytes were removed by incubating the total mononuclear fraction for 90 minutes at 37°C. The resultant non-adherent fraction consisted of > 98% lymphocytes as determined by differential staining (Diff-Quik, Industria, TVI). These cells were checked for viability by trypan-blue exclusion and adjusted to a concentration of 2 x 10⁶ cells/ml in RPMI 1640 (Gibco, Grand Island, NY), supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 20% autologous serum. Aliquots of 1 ml were dispensed in each of several culture tubes and 1 μg phytohaemagglutinin (PHA, Wellcome Laboratories, Beckenham, UK) added to some tubes. The cells were incubated at 37°C for 48 hours in a 5% carbon dioxide atmosphere and then the medium was removed, the cells washed twice in Hanks's balanced salt solution to remove all traces of medium and mitogen and virus added to the pelleted cells. After incubation for 2 hours at 37°C to allow adsorption, the cells were washed and resuspended in fresh medium with the identical composition to that in which the cells were previously incubated. Mock infected controls were treated similarly.

Polyamine extraction and analysis

The cells were washed twice with cold phosphate-buffered saline (pH 7.2). The fibroblasts were removed from the glass by exposing them to 0.25% trypsin for 5 minutes and then gently pushing them from the glass into 10 ml of buffer. For the studies on the lymphocytes the contents of 4 tubes were pooled to raise the cell concentration to 8 x 10⁶ cells/sample. Aliquots of all preparations were removed for total cell counts and the remainder of the cells pelleted. These were then resuspended in 0.5 ml ice-cold sulphosalicylic acid (5%) and subjected to ultrasonication. The protein was removed by centrifugation for 15 minutes at 10,000 g in a Beckman SW 50.1 rotor and the resultant supernatant collected. The polyamine analysis was performed on a Beckman Model 118B automatic amino-acid analyser using the procedure of Marton et al. Polyamine hydrochloride standards were obtained from Sigma and recrystallised three times from ethanol before use.

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Cell counts

These were determined using a Coulter Counter Model P equipped with a 70 µm probe.

Results

A linear response was obtained for the peak area resulting from increasing polyamine concentrations in the range 1-30 nmol, when analysed on the amino-acid analyser, the slope varying with the polyamine under consideration.

Polyamine concentrations of fibroblasts

The absolute polyamine concentrations of 4-day-old cultures of fibroblasts maintained on MEM supplemented with 1% dialysed fetal calf serum varied with the cell line. However, the concentration of putrescine was always less than that of spermidine, which in turn was less than the spermine concentration (Table I). When the levels of these molecules were assayed 24 hours after virus challenge, the levels of putrescine and spermidine were increased while the spermine concentration was decreased.

The arginine-depleted cells had lower levels of polyamines although virus infection did appear to alter the spermidine/spermine ratio slightly (Table I).

Polyamine concentrations in lymphocytes

Treatment of lymphocytes with PHA for 72 hours consistently increased the levels of polyamines in each of three experiments. When virus was added to these stimulated cells at 48 hours and the polyamine levels assessed 24 hours later, a distinct drop in the polyamine under consideration.

Discussion

It is interesting to note that polyamines have a regulatory effect on DNA and RNA synthesis and that they are elevated in differentiating tissues and actively growing cells, including lymphoblasts. Hence they may be expected to increase in virally infected cells, especially in cells infected with CMV, which has been shown to stimulate host cell DNA and RNA synthesis. A study by Tyms et al. demonstrated that there is an increased conversion of labelled putrescine to spermidine and spermine in CMV-infected fibroblasts 48 hours after infection. Thus one may expect the concentration of these molecules to rise in CMV-infected cells.

This idea is supported by the fact that an increase in the activity of ornithine decarboxylase has been reported for CMV-infected cells, the levels of this enzyme being almost maximal 24 hours after infection. However, the current study has demonstrated that although the putrescine and spermidine concentrations are elevated in the infected cells, the spermine concentration is decreased. That is, the spermidine/spermine ratio is increased (Table I).

Thus it is possible that there is an increased excretion of spermine and possibly also spermidine from the virally infected fibroblasts. In this context it is important to note that the synthesis of spermidine is elevated in experimental animal tumours, that tumour cell lines have a high spermidine/spermine ratio and that the levels of spermidine rise markedly in the serum of tumour patients, suggesting excretion from the tumour cells. If the maintenance of particular levels of polyamines is necessary for control of macromolecular synthesis in CMV-infected fibroblasts, then such cells may need to continuously synthesise these molecules. This may be one explanation for the constant need for arginine in the medium of infected cultures, removal of this amino acid having an effect on the polyamine synthesis. Indeed in the current study it was shown that cells maintained on arginine-depleted medium had very low levels of polyamines. However, the addition of virus did appear to increase the spermidine/spermine ratio (Table I). A similar effect has been observed in arginine-deprived herpes simplex-infected cells.

Therefore it appears that CMV infection of fibroblasts modifies the polyamine content of the cell. The question arises as to whether CMV challenge of other human cell types also modifies the levels of these substances.

Mitogen-induced lymphoblasts have been shown to have elevated levels of polyamines compared with control cells as

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<tr>
<th>TABLE I. POLYAMINE CONCENTRATION IN FIBROBLASTS (nmol/10^6 cells)</th>
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<tr>
<td>Putrescine</td>
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<td>Control</td>
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<tr>
<td>Infected</td>
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<tr>
<td>Arginine-control</td>
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<tr>
<td>Arginine-infected</td>
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*The results expressed here are for two different lines of fibroblasts. Although the absolute levels varied in each of five lines tested after 4 days in culture, the same trends were apparent with arginine depletion and/or exposure to CMV.

<table>
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<tr>
<th>TABLE II. POLYAMINE CONCENTRATION IN LYMPHOCYTES (nmol/10^6 cells)</th>
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<tbody>
<tr>
<td>Putrescine*</td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>PHA-stimulated</td>
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<td>PHA-stimulated + virus</td>
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*The results of three experiments with lymphocytes taken from the one donor. Although the absolute concentration of polyamines varied in lymphocytes taken from other donors, identical trends were apparent.
well as increased incorporation of thymidine into DNA. CMV has been shown to depress the thymidine uptake of virus-challenged lymphoblasts and in the current study a marked reduction in the polyamine content of these virus-challenged cells was noted, the spermidine/spermine ratio being decreased (Table II).

Morphological alterations occur in CMV-challenged lymphoblasts and immediate early antigens are expressed in some cells but few or no infectious virions seem to be produced by these cells. It is thus interesting to speculate that continued synthesis of polyamines must occur in productively infected cells, latent or abortive infections being established where continued synthesis of these molecules is not possible. This hypothesis is currently being investigated.

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REFERENCES


Kaaplandse plattelandse perinatale sterfes, Januarie — Desember 1985

G. B. THERON, R. C. PATTINSON, B. H. J. ENGBRECHT

Summary
In this study 18 rural hospitals in the Cape Province were prospectively surveyed in respect of the number of deliveries, perinatal deaths, low-birth-weight babies, and the hospital facilities available for routine antenatal, intrapartum and neonatal care. During the study period (1 January - 31 December 1985) there were a total of 19 274 deliveries (birth weight ≥ 1 000 g). The mean perinatal mortality rate was 27.3/1 000 deliveries (range 15.3 - 45.3). The prevalence of low-birth-weight babies ranged from 13.3% to 34.4% with a mean of 17.7%. By plotting the perinatal mortality rate against the prevalence of low-birth-weight babies a perinatal care index was established. A low perinatal mortality coupled with a high prevalence of low-birth-weight infants, for example, signifies proper care. The perinatal index was then used to compare the neonatal outcome of different hospitals. Outcome varied considerably, as did facilities and care. Serological tests for syphilis and blood groups were not known in the majority of patients at 4 hospitals involving 2 387 deliveries. No cervical cytology was done at 6 hospitals involving 5 462 deliveries. One hospital had no neonatal resuscitation equipment. There were no facilities for serum bilirubin measurements at 10 hospitals involving 8 867 deliveries. However, most problems identified are easily and cheaply rectifiable.

Die studie is ondernem om eerstens die perinatale sterfes word vandag wêreldwyd beset.2,3 Statistiek oor perinatale sterfes is tans houssaklik beperk tot opleidingshospitale en daar is 'n algemene gebrek aan inligting oor die situasie in buitestedelike gebiede.4 Die studie is onderneem om eerstens die perinatale sterfes in die Kaaplandse plattelandse hospitale gedurende 1985 te stel.

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