Variable Response of Human Cells to Polyinosinic-Polycytidylic Acid

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Human cells derived from various organs of a single embryo responded variably to polyinosinic-polycytidylic acid with respect to induction of antiviral resistance and interferon.

Complexes of polyinosinic and polycytidylic acids (poly rI-rC) stimulate the synthesis of interferon in cell cultures from many species of animals (3, 4). Also, a state of antiviral resistance is established in cell cultures incubated with this complex (2, 3, 5, 8). Several workers have noted that the concentration of poly (rI-rC) required to induce a given level of antiviral resistance or interferon in cell cultures varied depending upon the type of cells used (3, 4). The present report deals with experiments which indicate that variability in the response to poly (rI-rC) exists also in cell cultures originating from different organs of a single human embryo.

Samples of 12-week-old human embryos were supplied by Flow Laboratories, Rockville, Md. Cells from various organs of the embryo were prepared and grown in tissue culture. Cultures were prepared by trypsinization of various tissues from a single embryo. The cells were grown to confluency in tubes (16 by 125 mm) by incubation at 37 °C in a humidified incubator provided with 4% CO₂. The growth medium was Eagle minimal essential medium (MEM) with 10% fetal calf serum. The monolayers reached confluency after 96 h of incubation at 37 °C and then were used for experiments. Over 90% of the cells appeared to have fibroblastic morphology. On an average, each culture contained approximately 1.3 x 10^6 cells at the start of the experiment. Induction of antiviral resistance and production of interferon were carried out as follows. Cell cultures were washed twice with phosphate-buffered saline (PBS) and incubated with 1 ml each of MEM (with no serum) containing a mixture of various concentrations of poly (rI-rC) and 100 μg of diethylaminoethyl (DEAE)-dextran/ml (molecular weight, 2 x 10^4; Pharmacia, Uppsala, Sweden). A single batch of poly (rI-rC) preparation (obtained from the Antiviral Substance Program, National Institutes of Health, Bethesda, Md.) was used in all experiments. DEAE-dextran was used because it is known to enhance the production of interferon by poly (rI-rC) in cell cultures (7). The poly (rI-rC)-DEAE-dextran mixtures were incubated for 30 min at 37 °C before they were added to cultures. Three cultures were used for each concentration of the compound tested. The cultures were incubated for 1 h at 37 °C, at the end of which time they were washed twice with PBS and then incubated with MEM (with 1% fetal calf serum) for 12 h at 37 °C. Then the supernatant fluid from each culture was harvested and assayed for interferon. The cells from each culture were used for antiviral resistance assay.

Antiviral resistance in cell cultures was determined by incubating the cultures with Sindbis virus (input multiplicity of 10 plaque-forming units [PFU] per cell) for 30 min, at the end of which time the cultures were washed twice with PBS and then incubated with 1 ml each of MEM containing 2% fetal calf serum for 24 h at 37 °C. Several cultures receiving no poly (rI-rC) served as virus controls. The virus present in the cultures at the end of 24 h after infection was assayed by the hemagglutination method (1). The concentration of poly (rI-rC) required for a 50% reduction in the yield of Sindbis virus was calculated on the basis of the virus yield in cell cultures receiving no poly (rI-rC). Interferon present in supernatant fluids from cultures was assayed as follows. Samples were incubated with 50 μg of pancreatic ribonuclease/ml for 1 h at 37 °C to remove any residual poly (rI-rC). Twofold dilutions of the samples were made in MEM containing 2% fetal calf serum. Samples of 1 ml of the various dilutions were added to confluent monolayers of MA-308 human cells (Microbiological Associates, Inc., Bethesda,
Md.) and incubated at 37°C for 24 h. Then the cultures were washed three times with PBS and inoculated with 1,000 PFU of Indiana-type vesicular stomatitis virus (VSV) in 100 μlitsers of serum-free medium. Appropriate virus and cell controls were included. After incubation for 30 min at 37°C, MEM with 2% fetal calf serum was added to each culture. The cultures were then scored microscopically for cytopathic effect at 24 h after virus inoculation, a time at which the cells in virus controls were completely destroyed by the virus. The highest dilution of the sample causing approximately 50% protection of cells was considered the end point. An internal laboratory standard of human interferon was included in each titration. The internal interferon standard used was obtained from human cells (MA-308) induced with poly (rI·rC) and contained 200 units of interferon/ml when calibrated against a sample of the British human interferon standard. The titers of interferon produced in the cell cultures were calculated on the basis of the above internal standard. The data presented in Table 1 indicate the minimal concentration of poly (rI·rC) required for the production of 10 units of interferon per culture. The choice of the challenge virus for assaying interferon was based on the observation that cytopathic effect in infected cultures was complete by 24 h with VSV, whereas similar effects were maximal only by 48 h with Sindbis virus.

The results presented in Table 1 show the minimal amount of poly (rI·rC) required for the induction of antiviral resistance and interferon in the human cells from various tissues. Production of 10 or more units of interferon per culture was arbitrarily chosen as an index for comparing the response of cells from different organs. It can be seen that there was considerable variation in the amount of the compound required for eliciting a given level of response in cells obtained from various organs. The cells derived from the lung responded poorly to poly (rI·rC), whereas those from the foreskin elicited the best response. The amount of poly (rI·rC) required for the detection of extracellular interferon in the culture medium always exceeded that necessary for the induction of antiviral resistance intracellularly, a finding similar to that reported by others (3, 5, 8). It appears that a good correlation exists between a cell culture's capacity to become resistant to virus and its ability to produce interferon. Thus, the amount of poly (rI·rC) required for eliciting comparable levels of interferon or antiviral resistance in lung cultures was approximately 100- or 1,000-fold higher than that required for similar re-

<table>
<thead>
<tr>
<th>Origin of cell culture</th>
<th>Minimal amount of poly (rI·rC) required to induce</th>
<th>Antiviral resistance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>10 or more units of interferon</th>
</tr>
</thead>
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<tr>
<td>Lung</td>
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<td>25</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
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<td>2.5</td>
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<tr>
<td>Tonsil</td>
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<td>2.0</td>
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<tr>
<td>Spleen</td>
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<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
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<td></td>
</tr>
<tr>
<td>Foreskin</td>
<td>0.001</td>
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<tr>
<td>Whole embryo</td>
<td>0.05</td>
<td>2.0</td>
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</tbody>
</table>

<sup>a</sup> The cell cultures were incubated with poly (rI·rC) at different concentrations, and after incubation for 24 h supernatant fluids were collected and assayed for interferon. The antiviral resistance induced in poly (rI·rC)-treated cells was assayed by challenging them with Sindbis virus. The results presented are average values from three independent determinations made with three different embryos. The variation from experiment to experiment did not exceed more than 25% of each value.

TABLE 1. Response of various human cells to poly (rI·rC)

The observed variation in the cells' response was not due to their differing susceptibility to the challenge virus (Sindbis or VSV) since little variation existed in the capacity of cells to support viral multiplication. The yield of Sindbis virus or VSV in the various cultures receiving no poly (rI·rC) was approximately 500 PFU/cell. These results suggest that cells derived from various organs of a given host respond variably to poly (rI·rC). Other results (not presented here) indicate that similar variability in response to poly (rI·rC) exists in human cell cultures from various organs even after six continuous passages in tissue culture.

Variability similar to that reported here on induction of interferon by poly (rI·rC) has been shown to exist in cells derived from other animals such as mice and rabbits (6, 9). However, as far as I am aware, this report is the first instance demonstrating the differing responses of various human cells originating from a single embryo to poly (rI·rC). It is possible that the observed variability may be organ-specific since primary cell cultures were used. However, direct evidence on the true response of various organs to poly (rI·rC) may be obtained through the use of organ-transplant cultures. The present results or those reported by others do not explain the basis of the variability in the response of cell cultures to poly(rI·rC).
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LITERATURE CITED