Lack of Oncogenic Potential of Measles Virus on Syrian Hamster Embryo Cell Cultures

GEORGE TH. DIAMANDOPOULOS

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

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It is known that oncogenic viruses (V. Defendi et al., J. Cellular Comp. Physiol. 66:351, 1965; G. Yerganian et al., Cytogenetics 1:314, 1962), chemical carcinogens (W. J. Burdette, Cancer Res. 15:201, 1955), and ionizing radiation (L. J. Cole, Science 150:1782, 1965) are capable of inducing chromosomal aberrations. If there were a direct relationship between chromosomal derangements on one hand and carcinogenesis on the other, it is possible that measles (W. W. Nichols, Am. J. Human Genet. 18:81, 1966) and other common viruses (H. F. Stich et al., Virology 22:439, 1964) which have recently been found to produce chromosomal lesions may, under certain circumstances, prove oncogenic. Although efforts to demonstrate, in the newborn Syrian hamster, a tumorigenic effect after inoculation of several ordinary viruses of human origin have yielded negative results (A. J. Girardi et al., Proc. Soc. Exptl. Biol. Med. 118:173, 1965), effects in vitro of prolonged interaction between any such agent and appropriate cell systems remain to be determined. Under these conditions, neoplastic cell transformation, which is absent or imperceptible in the animal because of inhibitory host factors, might occur. Results of experiments employing cells derived from embryonic Syrian hamsters and measles virus are presented.

The "Edmonston" strain (J. F. Enders et al., Proc. Soc. Exptl. Biol. Med. 86:277, 1954) of measles virus was employed. Infectivity titer of the stock virus was $10^{-24} \text{TCD}_{50}/0.1 \text{ml}$ as determined in human renal cells. Syrian hamster embryo cell cultures were prepared as previously described (G. Th. Diamandopoulos et al., Am. J. Pathol. 49:397, 1966). Serial cell passages from primary virus-inoculated and uninoculated control cultures (Table 1) were initiated between the 2nd and 3rd months after exposure to measles virus. The control lines thus established were utilized also in transformation experiments with simian virus 40 (SV40), earlier results of which have been published (G. Th. Diamandopoulos et al., Am. J. Pathol. 49:397, 1966). Cell suspensions ($10^4$ cells) of measles-infected and control lines were tested for oncogenic potential in cheek pouches of weaning Syrian hamsters.

Morphological evidence of the persistence of measles virus in primary Syrian hamster embryo cells was obtained consistently, and was confirmed by isolation of the agent in human amnion cell cultures. It was also found that the initial cellular cytopathic effect was followed by the occurrence of an invariable cell regrowth and the development of a virus-carrier state. In this state there was always a proportion of cells that would interact with the virus in a cytoidal manner. Sublines that were developed, although they showed persistence of the infection, exhibited gradual decrease in their cytopathic effect. There was also reduction in the amounts of infectious measles virus produced, as evidenced by the low infectivity titers of supernatant fluids from these cultures when tested in primary human amnion cells.

It appears (Table 1) that measles virus plays no decisive role in the in vitro malignant neoplastic transformation of the small proportion of measles-infected lines that eventually developed oncogenic properties. This conclusion is based on the fact that the acquisition of oncogenic potential did not take place in any one of the measles-exposed lines as late as 10 months after initiation of the cell cultures. One line was proven oncogenic in two out of six animals at 15 months and in six out of six animals 5 months later. Another line produced tumors in three out of six animals at 20 months. One of the control lines also became oncogenic in all six animals tested at 25 months. From experience based on the results with known oncogenic viruses, any in vitro neoplastic transformation that is virus-dependent usually takes place as early as 1 to 2 months (V. Defendi et al., J. Cellular Comp. Physiol. 66:351, 1965; H. M. Shein et al., Proc. Natl. Acad. Sci. U.S. 49:28, 1963). Moreover, neither the sections of any one of the tumors, all of which were sarcomas, nor the tumor cells grown in vitro showed morphological or serological evidence of the persistence of measles virus; neither did they carry any recognizable measles-related cellular antigens, as is usually the case with known oncogenic viruses (F. Rapp et al., Proc. Soc. Exptl. Biol. Med. 116:1131, 1964; K. K. Takemoto et al., Science 153:1122, 1966).
TABLE 1. History and oncogenic properties of measles-infected and control hamster embryo cell culture lines

<table>
<thead>
<tr>
<th>Source</th>
<th>Lines</th>
<th>Initiation of subcultivation (months)</th>
<th>Tests for oncogenicity in cheek pouch</th>
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<tbody>
<tr>
<td></td>
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<td>9-10 mo. 9 (15)</td>
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<td>Hamster embryo, measles-infected</td>
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<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Hamster embryo, control</td>
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<td>2 Ps</td>
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</table>

<sup>a</sup> Intervals in months after measles virus exposure or, in the case of control lines, intervals after addition of virus to exposed lines. Numbers inside parentheses indicate corresponding passage level.

<sup>b</sup> Injection of 10<sup>6</sup> cells into each cheek pouch of weanling Syrian hamsters; + = tumor growth, — = no tumor. Numbers inside parentheses indicate number of animals with tumors, over total number of animals inoculated.

It would seem, therefore, that the precursors of the oncogenic cells were among those few that were resistant to the infection by measles virus. In contrast, those cells in which the virus infection had persisted in a carrier state did not multiply or survive when transplanted into the homologous host. It is therefore unlikely that measles virus would be in any way etiologically related to the in vitro malignant neoplastic transformation of either one of the two measles-exposed cell lines. Therefore, the conclusion may be drawn that the delayed transformation of cells in the two measles-infected lines and in the one control line was of "spontaneous" character. Further inquiry is warranted to elucidate the true nature of such a process.

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