The Effect of Mumps Virus on the Resistance of Burkitt Lymphoma Cell Lines to Various Viruses

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SUMMARY

Infections by certain paramyxoviruses were shown by others to increase the susceptibility of cell cultures to various unrelated viruses. Burkitt tumor cell lines were exposed therefore to mumps virus in an attempt to enhance replication of the Epstein-Barr herpes group virus (EBV) present in a small proportion of the cells. Persistent mumps virus infections were readily established in every line tested with nearly every cell (>95%) synthesizing viral antigen. Average cellular growth rates were reduced by as little as 10% and no more than 50% as compared to uninfected control populations maintained in parallel. After an initial peak, mumps virus titers persisted at levels of 10^4 to 10^5.5, 50% tissue culture infective doses/ml of culture for at least 3 months. The mumps viral carrier state increased the susceptibility of Burkitt tumor cell cultures to vesicular stomatitis virus. It did not reduce autogenous or recombinant virus particles, the majority of which lack part or all of the nucleoid (7). Many of the cell lines, whether they contain EBV or not, yield an interferon-like inhibitor (4, 12, 13, 24, 27) which, since it may be synthesized without apparent stimulation, has been termed autogenous interferon (Zajac et al., in preparation). All attempts have failed to transmit EBV to host systems other than cultures of cells from the hematopoietic system in which again mainly low-grade persistent infections are established (16). Efforts were made therefore to improve EB viral replication in existing carrier lines. Several reports have indicated that paramyxoviruses (mumps, parainfluenza, Newcastle disease viruses) may lower the resistance of cell population to various unrelated viruses (10, 17, 21-23, 31) and that this effect may be due to reduced interferon synthesis (23) or prevention of interferon action (2, 11) or both (17, 18). Consequently, selected cell lines of Burkitt tumor or leukemic origin were exposed to mumps virus in order to determine its effect upon the resistance of the cultures to various viruses and on the EBV viral carrier state.

MATERIALS AND METHODS

Cells. The Burkitt cell lines selected for these studies were the (a) the EB-1 line (6), which contained EBV and produced autogenous interferon; (b) the EB-3 line (7), which carried the virus but did not produce detectable amounts of autogenous interferon; (c) the Raji line (26), which neither contained virus particles nor produced autogenous interferon (5); and (d) the Ogun line (Osunkoya and Pulvertaft, personal communication), which contained rare cells with EBV particles, yet produced autogenous interferon. In addition, 2 EBV-free cell lines derived from peripheral leukocytes of leukemic patients were used for some of the experiments. These were the SK-L1 line (3), which produced autogenous interferon, and the RPMI 6410 line (20), which did not. The Burkitt cell lines were kindly supplied by M. A. Epstein, Bland Sutton Institute, Middlesex Hospital, London, England. The SK-L1 line was furnished by M. B. Clarkson, Sloan-Kettering Institute, New York, New York, and the RPMI 6410 line was furnished by J.

Abbreviations used: EBV, Epstein-Barr virus; HEK, human embryonic kidney; VSV, vesicular stomatitis virus; TCD50, 50% tissue culture infective doses; ID50, 50% infectious doses; L(MCN), Earle's strain L cell cultures; NDV, Victoria strain of Newcastle disease virus; HBSS-2, Hanks' balanced salt solution supplemented with 2% fetal calf serum.

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All lines were maintained on Medium #1629 (Baltimore Biological Laboratories, Baltimore, Maryland), supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units of penicillin, and 100 μg streptomycin/ml. The lines were subcultured weekly by seeding 1 × 10^5 viable (trypan blue exclusion) cells/ml of fresh medium.

Human diploid cells (WI-38) were obtained from the Wistar Institute, Philadelphia, Pennsylvania, and HEK cultures from Flow Laboratories, Rockville, Maryland. Primary rabbit kidney cultures were kindly supplied by T. Tokumaru, of this hospital, fat head minnow cells by R. Malsberger, Lehigh University, Allentown, Pa., and turtle heart cell cultures by D. T. Karzon, Children’s Hospital, Buffalo, New York. The above cell cultures were maintained on Eagle’s basal medium in Hanks’ balanced salt solution containing 2—10% fetal calf serum, glutamine, penicillin, and streptomycin. The pH of the media was adjusted to 7.4 with sodium bicarbonate. L(MCN) were maintained as described (29).

Typical case of parotitis by amniotic inoculation of 8-day-old chick embryos. Amniotic fluids of the 7—9th consecutive passages were used. The stock virus preparations contained approximately 10^8.5 ID_{50} for chick embryos and 10^3 hemagglutinating units/ml.

Stocks of the Indiana strain of VSV were derived from infected chick embryo fibroblast cultures. These preparations contained between 10^8 to 10^9 TCD_{50} /ml when assayed on HEK or L(MCN) cultures respectively.

NDV had been propagated for an undetermined number of allantoic passages in embryonated chicken eggs. These preparations contained 10^9.0 to 10^9.5 ID_{50} /ml for chick embryos.

Reovirus Type 3 was obtained from S. Dales, Public Health Research Institute of the City of New York, and passaged in HEK cultures. The stock preparation contained 10^8.2 TCD_{50} /ml when assayed in HEK cultures.

Infection of Cell Cultures and Virus Assays. For infection, 5 × 10^6 cells were sedimented and resuspended in 1 to 2 ml of virus, appropriately diluted to yield the desired input multiplicity. After incubation with intermittent shaking for 1 to 2 hr at 37°C, the cells were sedimented again and resuspended in growth medium to a concentration of 2 × 10^5 cells/ml. Uninfected control cultures were handled and maintained in parallel. Samples were removed at intervals for cell counts, infectivity titrations, and preparation of cell smears for immunofluorescence tests.

Serial two-fold dilutions of interferon samples were made in HBSS-2, and 0.5-ml aliquots were added to duplicate cultures of HEK cells. After 24 hr incubation at 37°C, interferon-treated and control cultures were challenged with 0.2 ml of VSV suspension containing 1000 TCD_{50}. In assays of virus-induced interferons, the cultures were refed prior to challenge with VSV. After 40 hr at 37°C, the controls showed total destruction of the cells. The endpoints of interferon activity were taken as the highest initial dilution of sample which gave 50% suppression of viral cytopathic effects. Reovirus-induced interferons were diluted in HBSS-2 which contained a 1:800 dilution of monkey anti-reovirus Type 3 serum obtained from the Virus Research Resources Branch of the NIH, Bethesda, Maryland. A dilution of 1:10,000 of this serum neutralized 10^3 TCD_{50} of reovirus when assayed in HEK cultures.

RESULTS

Course of Mumps Virus Infection in Burkitt Tumor Cell Lines. Cells of the EB-1, EB-3, and Raji lines were exposed to mumps virus at an input multiplicity of 5, and the resulting cultures as well as uninfected controls were observed thereafter for cellular growth, percentages of cells containing mumps antigen, and yields of infectious mumps virus. As seen in Chart 1, the reduction in cellular growth rate was most marked in the EB-3 mumps culture, ranging from 18 to 63% at various intervals with an average of 48%. The EB-1 mumps and Raji mumps cultures showed an average reduction in cell replication of 20 and <10% respectively. The titers of mumps virus in the initial period rose at different rates to varying peaks depending upon the cell line, but after establishment of an equilibrium the titers persisted at levels between 10^4 and 10^5.5 TCID_{50} /ml as determined in HEK cultures. The actual quantities of mumps virus were probably 100-fold higher, since comparative titrations of the virus in HEK cultures and
Chart 1. Course of mumps virus infection in EB-1, EB-3, and Raji cells. VP, virus particles; FA, fluorescent antibody.

in the chick embryo amnion generally revealed differences of 2 log_{10} units in favor of the latter assay system. Even with corresponding corrections, the titers obtained were low in view of the fact that within 5 to 10 days after exposure, and continuing thereafter, practically all cells contained mumps antigen, as evident from mumps-specific immunofluorescence. It is obvious that infected cells retained their capacity to divide since the cultures continued to grow at only slightly reduced rates. Only a few of the cells contained sufficient mumps antigen to render the whole cell intensely fluorescent. Such cells were seen mainly during the early incubation period. The great majority of cells contained well-delineated aggregates of antigen of varying sizes (Fig. 1), often amounting to no more than minute granules. Infection of other cell lines, i.e., the Ogun line of Burkitt tumor, and the SK-Li and RPMI-6410 lines of leukemic origins, yielded similar results.

The above observations bear a striking resemblance to the persistent mumps viral infection of Chang’s human conjunctiva cells as reported by Walker and Hinze (34). Later experiments (35, 36) indicated that a reduction in serum concentration in the culture medium increased the virus yields and the number of cells showing hemadsorption. Similar experiments with the EB-1 line failed to show differences in the various parameters studied, whether the cultures were fed medium with 10 or 2% fetal calf serum immediately after exposure to mumps virus or after establishment of the carrier state.

**Increased Susceptibility of Mumps Carrier Cultures to VSV.** In preliminary experiments it was found that EB-1 mumps cultures were more susceptible to VSV compared to uninfected controls and that the susceptibility to VSV appeared to increase with prolongation of the mumps viral carrier state. The enhanced susceptibility to VSV was evident from a marked reduction in the cellular growth rates, a 5- to 20-fold increase in the number of cells showing VSV-specific immunofluorescence, and a similar increase in the VSV titers obtained within 3 days after challenge. In order to establish these observations firmly, EB-1 cells were exposed to mumps virus as described. At various intervals thereafter, cells from this culture, as well as from uninfected control populations, were sedimented and challenged with VSV at an input multiplicity of 2. The resulting sublines of the EB-1 and EB-1 mumps cultures were monitored by cell counts, VSV and mumps virus specific immunofluorescence, and infectivity titers. The results (Chart 2) showed a gradually increasing susceptibility of the EB-1 mumps culture to superinfection with VSV. The enhancement, as measured by reduction in cellular growth, was not dramatic before the 52nd day following exposure to mumps virus. At that time and thereafter, the effect of VSV on cellular growth was rapid and pronounced. VSV-specific immunofluorescence tests revealed low degrees of enhancement already in the first week after infection of the culture with mumps virus, and the differences between the EB-1 and EB-1 mumps cultures became more striking as time progressed. At each time of challenge, the enhancement of the VSV-superinfection was most pronounced within the first 3 ensuing days. Thereafter, the percentages of cells with VSV antigen declined to lower levels. All the experimental cultures in this experiment survived as triple carrier populations, harboring EBV, mumps virus, and VSV. Another EB-1 mumps carrier culture, which had been established and maintained in the same manner, succumbed to VSV when challenged on the 66th day after exposure to mumps virus (Chart 3).

Similar, but less extensive experiments, were carried out with EB-3 and Raji cells. The results obtained with the EB-3 mumps cultures were comparable to those described for EB-1 mumps populations. Raji mumps cultures failed to reveal significant differences in response to VSV, as compared to the controls, when challenged either on the 21st or 90th day after exposure to mumps virus.

**Effect of Mumps Virus Infection upon Interferon Synthesis.** Cell lines of Burkitt tumor or leukemic origin which either did or did not produce autogenous interferon were exposed to mumps virus and, after establishment of the carrier state, examined for its effect upon interferon synthesis. The results (Table 1) revealed that the mumps infection failed to induce per se detectable interferon production and to reduce autogenous interferon yields.

The effect of mumps virus infections on production of virus-induced interferon was examined by exposure of mumps carrier and control cultures to either NDV or reovirus Type 3. The EB-1 and Ogun lines could not be stimulated by NDV to produce interferon in excess of autogenous inhibitor synthesis. The experiments with NDV (Table 2) were restricted therefore to cell lines devoid of the capacity to yield autogenous interferon (Raji and RPMI 6410) and to the SK-L1 line capable of producing interferon in excess of autogenous interferon. The mumps viral carrier state had different effects on NDV-induced interferon synthesis depending upon the line under test. It prevented interferon production in the Raji culture, reduced synthesis 8-fold in SK-L1, but only insignificantly in RPMI...
6410 cells. NDV-specific immunofluorescence tests on cell smears prepared at the time of harvest of the interferons clearly showed that the mumps carrier state did not enhance infection by another paramyxovirus, but rather reduced it 2.5- to 10-fold. The percentages of cells with NDV antigen in all 3 mumps carrier cultures, however, were similar; yet the yields of interferon varied from <4 to 128 units. When the same cell lines were exposed to reovirus Type 3 at different input multiplicities, the yields of interferon were similar in the control and mumps virus-infected cultures (Table 3). Test for reovirus-specific immunofluorescence again failed to show enhancement of the superinfection by the mumps viral carrier state, but, in contrast to the results with NDV, there was no reduction in the number of reovirus-infected cells.

**Effect of Mumps Virus Infection on the Protective Action of Extraneous Interferon.** EB-1 mumps cultures, infected 66 days previously, and controls were exposed to interferon produced by SK-Li cells on stimulation by ultraviolet-inactivated NDV (Zajac et al., in preparation). Cells were sedimented (5 × 10⁶), resuspended in 2.5 ml of the interferon preparation (170 units/ml), incubated for 24 hours at 37°C, and then challenged with VSV at an input multiplicity of 2. After an adsorption period of 2 hours, the volume of the culture was adjusted to yield 2 × 10⁵ cells per ml. Control cultures, carried in parallel, were treated with (a) VSV only, (b) interferon only, and (c) medium only. The experiment was monitored by cell counts, VSV-specific immunofluorescence, and infectious VSV titers. The results (Chart 4) showed that in the EB-1 mumps cultures, whether treated with interferon or not, the cellular growth rates, VSV-specific immunofluorescence, and VSV-infectivity titers were closely similar. In contrast, EB-1 cultures free of mumps virus and treated with interferon showed good protection on the basis of all 3 parameters when compared to the controls which were not exposed to interferon.

**Effect of Mumps Virus on the Indigenous EB Virus Infection.** EB-1 and EB-3 cultures were tested 2, 5, 7, 14, and 50 days after infection with mumps virus for EBV-specific immunofluorescence, and samples taken at these intervals were inoculated into various types of tissue cultures and newborn and weanling animals in an effort to detect enhancement of the indigenous agent. For the first approach, cell smears from mumps-infected and control cultures were stained for mumps antigens as well as EBV, using a fluorescein-labeled human mumps hyperimmune globulin before and after exhaustive adsorption with mumps virus. The adsorbed conjugate failed to detect the characteristic mumps antigen aggregates but retained its capacity to stain those EB-1 and EB-3 cells which harbored EBV (1—2% and 3—5% respectively). The mumps virus-infected Burkitt cell lines failed to show significant increases in the percentages of EBV-positive cells at any time in the course of the experiments.

The various samples of cell suspension, which had been preserved at −70°C until assay, were exposed to sonication for 3 minutes (Disintegrator-Forty, Ultrasonic Industries, Inc., Plainview, New York) and then mixed with equal volumes of a 1:5 dilution of equine antiserum to mumps virus, kindly supplied.
harvested and processed for EBV-specific immunofluorescence. None of the inoculated cell cultures showed cytopathic effects referable to EBV. In a few instances cytopathic effect was noted, however, which was found to be due to unneutralized mumps virus. Cultures without cytopathic effect were maintained by feeding and reseeding for as long as 40 days. These showed no evidence of interference at any time when challenged with VSV. The coverslip preparations failed to reveal any immunofluorescence specific for EBV.

Mixtures of cell suspension and equine anti-mumps serum were inoculated also into litters of 1- and 6-day-old hamsters or mice by the intraperitoneal or intracerebral routes. The animals were checked daily for illness or death in the early period and then at longer intervals for development of tumors. None of the mice inoculated at birth or later developed signs of illness. About 12% of the hamsters infected at the newborn stage became ill or were found dead after incubation periods of 10 to 17 days. The preparations involved were shown to contain unneutralized mumps virus. Passages of brain or spleen homogenates from these animals into newborn animals and cell cultures failed to produce illness or to show cytopathic effects. Surviving animals of the primary and passage series were kept for 18 months. No tumor formation became evident.

**DISCUSSION**

The persistant mumps virus infection of cell lines derived from Burkitt's lymphoma or peripheral leukocytes of leukemic patients resembles the mumps carrier state in Chang's human conjunctiva cell line as described by Walker and Hinze (34). Even at a high multiplicity of infection there was relatively little evidence of cell loss, and the cultures showed merely a reduction in growth rates which were most marked in the EB-3 line but barely perceptible in Raji cultures. Practically all cells produced mumps antigen, but mostly in segregated, well-delineated masses of variable sizes. The cells evidently were not greatly disturbed and continued to divide yielding infected descendants. This type of carrier state has been termed "regu- lated infection" (33) or "endosymbiosis" (9) and has been observed, in addition to mumps virus, with parainfluenza Type 1 (19), measles (29), rubella (28), and rabies viruses (9). The exact mechanism by which this type of carrier state is maintained is presently unknown.

Infection of cell cultures with paramyxoviruses, i.e., mumps, parainfluenza, and Newcastle disease viruses, were shown to be accompanied by enhanced susceptibility to other, unrelated viruses (cf. 1). These observations were confirmed in that EB-1 mumps, and EB-3 mumps cultures were less resistant to VSV and also herpes simplex virus (Henle and Henle, unpublished) than the corresponding controls free of mumps virus. Raji mumps cultures, however, failed to show enhanced susceptibility to VSV, nor did they or SK-Li mumps and RPMI 6410 mumps cultures reveal increased susceptibility to reovirus Type 3. Challenged with NDV, these cultures even showed a reduction in susceptibility which in part may be based on destruction by mumps virus of cell receptors needed for adsorption of NDV. Obviously, enhanced susceptibility appears to be limited to certain cells as well as to certain viruses.
The reduction in cellular resistance has been ascribed to suppression of interferon production (23) and/or prevention of interferon action (2, 11, 17, 18). The data presented here have shown that autogenous interferon synthesis is not suppressed by a persistent mumps infection. The effects of a mumps viral carrier state on virus-induced interferon production may be influenced by the type of virus used as well as by the cultures under test. With NDV as inducer, the results varied from complete inhibition to no inhibition of interferon synthesis. This variability was apparently unrelated to differences in adsorption of the virus onto the cells, as already discussed, since similar numbers of cells were infected by NDV in every instance. When reovirus Type 3 was used as inducer, interferon synthesis was similar in mumps viral carrier cultures and their controls. Inhibition of interferon synthesis thus may be an occasional, but not a general, result of mumps virus infections. The experiments with EB-1 mumps cultures clearly indicated that the action of extraneous human interferon was prevented by the persistent mumps infection.

Despite the evidence for increased susceptibility of EB-1 mumps and EB-3 mumps cultures to VSV and HSV and for prevention of interferon action, no evidence was obtained that the indigenous EBV infection of these cell lines was enhanced in any detectable way. The percentage of cells producing EBV antigen remained essentially stationary, except for minor fluctuations also seen in controls, and efforts to transmit the virus from mumps carrier cultures to other host systems failed, as did earlier attempts with materials from stock cultures (8).

### Table 2

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control Interferon (units/ml)</th>
<th>NDV-specific immunofluorescence (% + cells)</th>
<th>Mumps-infected Interferon (units/ml)</th>
<th>NDV-specific immunofluorescence (% + cells)</th>
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</thead>
<tbody>
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<td>Raji</td>
<td>0</td>
<td>&lt;4</td>
<td>0.0</td>
<td>&lt;4</td>
</tr>
<tr>
<td>SK-L1</td>
<td>0</td>
<td>8</td>
<td>30.0</td>
<td>64</td>
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<tr>
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<td>0</td>
<td>&lt;4</td>
<td>53.0</td>
<td>&lt;4</td>
</tr>
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</table>

Effect of mumps virus infection on the production of NDV-induced interferon. NDV, Victoria strain of Newcastle disease virus.

### Table 3

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control Interferon (units/ml)</th>
<th>Reovirus-specific immunofluorescence (% + cells)</th>
<th>Mumps-infected Interferon (units/ml)</th>
<th>Reovirus-specific immunofluorescence (% + cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji</td>
<td>0</td>
<td>&lt;4</td>
<td>0.0</td>
<td>&lt;4</td>
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<tr>
<td>SK-L1</td>
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<td>8</td>
<td>24.3</td>
<td>240</td>
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<td>&lt;4</td>
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<td>&lt;4</td>
</tr>
</tbody>
</table>

Effect of mumps virus infection on the production of reovirus-induced interferon. nt, not tested.
ACKNOWLEDGMENTS

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REFERENCES


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Fig. 1. Mumps-specific immunofluorescence in EB-1 cells infected 15 days previously. × 1000.
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