Mammalian Cell-Virus Relationship. III. Poliovirus Production by Non-primate Cells Exposed to Poliovirus Ribonucleic Acid.* (24798)

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Ability of primate cells in culture to adsorb poliovirus quantitatively and suffer infection, and the corresponding incapacity of non-primate cells, is determined by specific surface receptor material possessed only by susceptible cells(1,2). This finding suggested that limitation of poliovirus infectivity to primate cells was governed primarily by specific interaction between viral coating protein and cell surface receptors. This communication shows that insusceptible non-primate cells *in vitro* or *in vivo* can produce infectious poliovirus when exposed to viral ribonucleic acid (RNA) as a means of bypassing specific surface virus-cell interaction.

Materials and methods. Cell culture stocks, media and cultivation methods have been described(1). Maitland-type suspensions of liver tissue in 10% calf serum in YEM (yeast extract medium) were prepared from liver gently minced into 3-5 mm fragments and washed thoroughly before exposure to RNA. *Poliovirus,* Type 1 (Mahoney, Connaught Medical Labs, Toronto) was prepared by disrupting infected HeLa cells, sedimenting cell debris at 20,000 x g for 10 minutes, and concentrating virus at 105,000 x g. (Experiments with purified and concentrated Type 1 poliovirus kindly supplied by Dr. Ralph B. Houlihan of Cutter Labs will be reported elsewhere.) Concentrated virus, suspended in 0.2 M NaCl and stored at –20°C, contained between 0.6 and 1.0 x 10^{10} plaque-forming units (PFU)/ml in different pools. *Virus assays* by plaque count with HeLa cells adapted to growth in calf serum have been described (1). *Extraction of ribonucleic acid* from poliovirus was accomplished by Gierer-Schramm (3) phenol extraction method, as applied by Colter *et al.*(4) to animal viruses, and improved by Alexander *et al.*(5) for infection of cultivated cells by suspension of RNA in hypertonic saline. Versene (5 x 10^{-4} M) was added to virus before extraction to prevent metal-ion inactivation(6). Small lots of virus were triply extracted with equal volumes of water-saturated phenol (Mallinckrodt analytical reagent, liquefied); extractions involved vigorous shaking for 5 minutes at room temperature, chilling of mixtures in ice bath, and centrifugation to separate supernatant aqueous layer for 2 further extractions. The final aqueous phase was extracted 5 times with equal volumes of ether (Merck, fresh and washed with distilled water), and dissolved ether evaporated by bubbling nitrogen gas at 0°C. Animals were inoculated directly with RNA freshly so prepared; for infection of cell cultures, pH was adjusted to 7.2 with sodium bicarbonate solution and phenol red indicator, and sterile 5 M NaCl was added to make the RNA solution about 1 M(5). *Infection of animals and cells with RNA.* Mice were injected with 0.03 ml and rabbits, guinea pigs, chicks and hamsters with 0.05 ml intracerebrally. Cell monolayers (about 2 x 10^6 cells) triply washed with 0.5 M NaCl and thoroughly drained were exposed to 0.1 ml amounts of RNA for 15 minutes at room temperature. The inoculum was redistributed frequently by rocking of the bottles. For plaque-count assay of produced poliovirus, exposed bottles were overlaid with 5 ml of semi-solid agar medium; for assays of released virus, bottles were overlaid with 5 ml of 10% calf serum in YEM. Bottles were incubated at 37°C. Liquid-medium cultures were treated at intervals by separation of cells and medium, disruption of cells by 3 cycles of freezing and thawing, and storage of medium and disrupted cells at –20°C. *Anti-poliovirus serum* was obtained from monkeys repeatedly inoculated with live virus. *Ribonuclease* (crystalline, Nutritional Biochemicals Corp.) was dissolved in 0.14 M NaCl (1 mg/ml) to

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give a 10x stock solution which was sterilized by filtration and stored at 4°C.

Results. For control purposes, response of primate cell monolayers to poliovirus RNA (5) was determined by counting plaques appearing in agar-overlaid cultures of HeLa, human amnion and monkey kidney. Table 1 shows that primate cell monolayers could be infected by poliovirus RNA; infectivity was destroyed completely by pretreatment of RNA with ribonuclease. Results illustrate variation in infectivity of 2 lots of RNA prepared at different times with the same poliovirus pool, and diversity in susceptibility of different cells.

TABLE I. Production of Type 1 Poliovirus by Primate Cell Cultures Exposed to Poliovirus RNA.

Cell type*	Plaque counts/monolayer inoc. with 0.1 ml poliovirus RNA†	
	RNA lot 1	RNA lot 2
HeLa	171, 108, 154	26, 41, 18, 20
Human amnion	3, 0, 7	0, 0, 0, 0
Monkey kidney	>100‡	19, 15, 25, "

* Different batches of cultures were used for each experiment.

† Prior treatment of viral RNA with ribonuclease for 1 min. at room temperature destroyed infectivity (0 PFU from 21 seedings on 7 monolayer cultures of each cell type). RNA lots were prepared at different times from same virus pool.

‡ Confluent cytopathic effect on all 3 monolayers.

Response of non-primate cells to poliovirus RNA. Freshly prepared RNA was added to monolayer cultures or suspensions of non-primate cells ordinarily insusceptible to poliovirus infection. At intervals after exposure, total number of PFU per culture vessel was assayed by HeLa cell plaque counts. Table II shows results typical of many experiments; infectious poliovirus was produced by non-primate cells in amount comparable to that produced under the same conditions by primate cells. Non-primate cells exposed to ribonuclease-treated RNA produced no infectious virus, and capacity of RNA-exposed cells to produce infectious virus was lost when cells were heated for 2 min at 60°C before exposure. Absence of infectious virus 3 hours after exposure of cells to RNA suggested that a latent period preceded production of new virus, as it does when susceptible cells are infected with intact virus. In different experiments, non-primate cells also varied considerably in response to RNA. Some cells (untransformed embryonic rabbit kidney (ERK-2) kindly supplied by Dr. J. C. N. Westwood, domestic rabbit fibroblast, rabbit skin cells in primary culture) consistently produced good yields of poliovirus following RNA infection, while other cells (cottontail rabbit papilloma

TABLE II. Production of Type 1 Poliovirus by Naturally Susceptible and Insusceptible Mammalian Cells to Poliovirus RNA.

Test system	Poliovirus PFU/culture vessel at indicated times after RNA infection*		
	3 hr	18 hr	24 hr
Primate			
HeLa	0	7×10^4	
Primary human amnion	0	5×10^2	
" monkey kidney	0	9×10^4	
Non-primate†			
Domestic rabbit fibroblast	0	7×10^3	9×10^3
Cottontail " epithelium	0	5×10^2	5×10^2
" " papilloma	0	9×10^4	8×10^4
ERK-2	0	1×10^5	9×10^4
Heat-killed ERK-2	0	0	0
L strain mouse fibroblasts	0	2×10^4	1×10^4
Heat-killed L strain	0	0	0
Primary rabbit skin	0	3×10^4	
Chick liver Maitland culture	0	4×10^3	8×10^2
Guinea pig liver Maitland culture	0	8×10^3	5×10^4
Hamster liver Maitland culture	0	1×10^3	

* Monolayer cultures of about 2×10^6 cells were treated with 0.1 ml RNA; 0.5 ml was added to washed tissue fragments in Maitland-type suspension. When RNA was treated with ribonuclease prior to inoculation, each cell system yielded 0 PFU.

† Ref. (1).

TABLE III. Distribution of Type 1 Poliovirus in Non-primate Cell Cultures Exposed to Poliovirus RNA.

Test system	Distribution of new virus at indicated times after RNA inoculation*		
	10 hr	18 hr	24 hr
	%		
Domestic rabbit fibroblast:			
New virus in cells	>95	>95	47
" " " medium	< 5	< 5	53
ERK-2:			
New virus in cells	>95	61	34
" " " medium	< 5	39	66

* At indicated intervals after exposure to 0.1 ml poliovirus RNA, monolayer-culture cells were washed and disrupted by freeze-thaw cycles; disrupted cells and harvested medium were assayed separately for poliovirus PFU.

epithelial cells, human amnion cells in primary culture) invariaby yielded no or small numbers of PFU. As with susceptible cells infected with intact virus, non-primate cells exposed to RNA accumulated infectious virus intracellularly before releasing it into the medium (Table III). Non-primate cells exposed to poliovirus RNA were not visibly cytopathically affected.

Response of non-primate animals to poliovirus RNA. Animals of species ordinarily insusceptible to poliovirus infection yielded infectious virus 18 hours after intracerebral inoculation of viral RNA (Table IV). Virus production was prevented by prior treatment of RNA with ribonuclease, and was preceded by a latent period.

TABLE IV. Production of Type 1 Poliovirus by Brain Cells of Non-primate Animals Inoculated Intracerebrally with Poliovirus RNA.

Animal	Poliovirus PFU/brain at indicated times after RNA inoculation*		
	3 hr	18 hr	36 hr
Mouse	0	9×10^3	5×10^3
Rabbit	0	2×10^1	
Guinea pig	0	7×10^1	
Chick	0	3×10^3	7×10^3
Hamster	0	8×10^2	4×10^2
Any of above inoc. with ribonuclease-treated RNA	0	0	0

* Mice were inoculated with 0.03 ml and other animals with 0.05 ml RNA. At indicated times after inoculation, animals were killed, brains were removed, disrupted in a Potter-Elvehjem homogenizer and assayed for virus PFU. Ribonuclease treatment for 1 min. at room temperature prior to inoculation destroyed RNA infectivity.

Discussion. Naturally insusceptible non-primate cells, after a latent period following exposure to poliovirus RNA, *in vitro* or *in vivo* produced infectious poliovirus. RNA activity was destroyed by ribonuclease treatment. RNA-exposed non-primate cells in culture accumulated infectious virus intracellularly before release. By use of viral RNA, it was possible to bypass specific receptor mechanisms of the cells to infect both primate and non-primate cells to about the same extent. Findings are similar to those with T2 coliphage(7) indicating that bacterial-virus host range also may be dependent primarily upon specific complementariness of cell surface and virus coat protein (or phage-tail tip). Extracellular release of infectious poliovirus by non-primate cells lacking specific receptors suggests that such receptors are not essential for poliovirus release. Present experience indicates that infectious poliovirus produced by RNA-exposed insusceptible cells and animals is indistinguishable from the virus donating the RNA; progeny virus has been found readily neutralized by homotypic antiviral serum, unaffected by ribonuclease, and unable to infect such non-primate cells as produced it. Reasons for variation in receptivity of various cell types to the same or different lots of viral RNA are not known, but do not appear connected with susceptibility of cells to intact virus. Human amnion cells in primary culture, which are very sensitive to infection by intact poliovirus, generally are relatively unreceptive to RNA although occasionally membranes have yielded cells as able to produce virus from RNA as are HeLa and monkey kidney cells.

Summary. Exposure of non-primate cells *in vitro* and *in vivo* to poliovirus RNA resulted in production of infectious poliovirus, without induction of apparent cytopathogenic effect *in vitro* or disease *in vivo*.

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