

Human-Mouse Hybrid Cell Lines and Susceptibility to Poliovirus¹

I. Conversion from Polio Sensitivity to Polio Resistance Accompanying Loss of Human Gene-Dependent Polio Receptors

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Received for publication 5 January 1970

A number of human-mouse somatic hybrid cell lines have been prepared, containing from 3 to 12 human biarmed chromosomes. These lines were susceptible to poliovirus type 1, producing viral yields comparable to those of the human parental cells. A small proportion of the cells of these lines survived the polio infection, and their progeny were solidly resistant to reinfection with the virus. Both sensitive and resistant hybrids produced virus following infection with viral ribonucleic acid, indicating that the cytoplasm of the resistant hybrids was able to support viral multiplication. Viral adsorption studies carried out at 4 C showed that the resistant sublines had negligible ability to adsorb the virus. It was concluded that the hybrid cells became resistant to polio through loss of the human chromosome bearing the gene for the receptor substance.

Human fibroblasts are susceptible to infection by poliovirus, but mouse cells are not (6). For this reason, the first human-mouse hybrid line was tested for susceptibility (23) and found to be unaffected by the virus. Since that time, a number of new human-mouse hybrids have been made which do support infection by poliovirus (R. Pollack et al., *in preparation*), and in which the permissive state is therefore dominant. Non-permissive sublines were derived from these, and the nature of the human gene product by which they differ from sensitive lines was examined.

MATERIALS AND METHODS

Lines and culture conditions. Cell cultures were maintained in Dulbecco's modification of Eagle's medium, supplemented with 10% calf serum and equilibrated with 10% carbon dioxide.

The human parental cells were WI38, an embryonic diploid strain (4); KL, a diploid strain bearing the X-linked mutation hypoxanthine-guanine phosphoribosyl transferase minus (HGPRT⁻) (17), kindly provided by Rody Cox; and D98/AH₂ (21), an established line, HGPRT⁻, presumably originated from HeLa (3). The mouse parental line was 3T3-4E (15).

The human-mouse hybrids were obtained by the double selective system of Littlefield (10), or the

half-selective system of Davidson and Ephrussi (2). The hybrids of D98/AH₂ were HLE-C [reported previously (15)] and two of its sublines, HLE-C (I) and HLE-C (D). The hybrid of KL was designated as KLE-H, and that of WI38, as WE-2. A number of subclones of these hybrids were isolated and tested for poliovirus sensitivity. The most sensitive subclones were employed in the studies reported here.

Since all biarmed (metacentric and submetacentric) chromosomes in these hybrids originated from the human parent, their number gives the minimal human contribution (Table 1). The number of acrocentric and telocentric chromosomes gives essentially the mouse contribution, but probably includes as well a small number of human acrocentrics.

Poliovirus infection. Most experiments were carried out with type 1 poliovirus (vaccine strain). The virus was plaque-purified and grown on cells of the BSC-1 monkey line. This strain of poliovirus grew to a somewhat higher titer in the monkey line than in any human line tested. Viral stocks for experiments were prepared from clarified infected cell supernatant fluids by sedimenting the virus at 40,000 rev/min for 2 hr and then resuspending it. Petri dish cultures containing 10⁵ cells were washed with serum-free medium and infected for 1 hr at 37 C with 0.2 ml of virus [5 × 10⁶ plaque-forming units (PFU)/ml], for an input multiplicity of 10. Controls were mock-infected when the fraction of cells surviving infection was to be determined (see below). After infection, the cultures were washed twice and 4 ml of medium was added. To harvest the virus, plates were frozen and thawed, and the detached cells and medium were sonically treated. Residual virus was measured in each

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experiment from a plate frozen immediately after infection and washing (time zero).

Plaque assay. Titrations were done on BSC-1 monolayers. Medium was removed from the monolayers, and 0.2 ml of the sonic extract, or a dilution in serum-free media, was added for 1 hr at 37 C. Each plate then received 6 ml of medium at 45 C, containing horse serum (5%), agar (0.9%), neutral red (10^{-4} , w/v), and nystatin (50 units/ml). The plates were permitted to cool for 15 min at room temperature, and then were incubated at 37 C in the dark for 3 to 4 days. No new plaques developed after this time.

Infection with poliovirus ribonucleic acid (RNA). RNA was extracted from concentrated poliovirus (20). Infection of cells with the RNA was carried out in the presence of diethylaminoethyl (DEAE)-dextran (16). By this method, the infectivity of poliovirus RNA was 10^{-3} to 10^{-4} that of whole virus. In the absence of the dextran, the infectivity of the RNA fell to 10^{-8} that of whole virus. A 0.2-ml amount of a phosphate-buffered saline solution of RNA (0.1 PFU/cell) and DEAE-dextran (1.7 mg/ml) was added to cultures for 15 min at room temperature. The virus yield (PFU/cell) after infection with RNA was assayed in the absence of DEAE-dextran; under these conditions, the infectivity of any residual of RNA was too low to give plaques.

Estimation of surviving fraction of poliovirus-infected cells. The surviving fraction of HLE-C hybrid sublines was determined directly by colony counts 10 to 15 days after viral infection. The plating efficiency of the human diploid-3T3 hybrids was too low to permit the number of polio-resistant survivors to be estimated in this way. The problem was dealt with by inoculating a known number of hybrid cells in a mixture with 10^4 BSC cells acting as feeders. After 4 days, when microcolonies of hybrid cells had formed, the cultures were infected with the virus. The BSC cells were destroyed, together with colonies of any susceptible hybrid cells, and the surviving polio-resistant colonies were scored 8 days later, when they had grown to visible size.

Adsorption of ^{32}P -labeled virus. Highly purified poliovirus labeled with ^{32}P was obtained through the kindness of Benjamin Mandel (14). Cells to be tested for ability to adsorb the virus were grown on cover slips (18×18 mm) to almost confluent monolayers containing on the order of 10^6 cells. The dishes containing the cover slips were incubated at 4 C. The cover slips were washed with medium containing 2% fetal calf serum, the bottom side of them was blotted dry, and they were placed on a dry surface. The culture was infected with 0.025 ml of ^{32}P -labeled virus suspension containing 2.5×10^7 PFU and 2.5×10^4 to 5.7×10^4 counts/min in medium, and then was placed in a 10% CO_2 incubator. Every 10 min, the virus suspension on the cover slips was mixed by tilting. At the end of the adsorption period (60 to 120 min), the cover slips were washed four times by dipping into fresh medium, inserted into a vial containing a scintillation mixture, and counted. A time zero value was obtained for each experiment by washing the cover slips immediately after the addition

of virus. These values were subtracted from those obtained after incubation. The counts obtained in experiments on different days were corrected for ^{32}P decay, and the results were expressed as PFU adsorbed per square centimeter of cover slip surface.

RESULTS

Table 1 shows the results obtained by infecting parental and hybrid cell populations with the virus, at an input multiplicity of 10 PFU per cell. All parental human cell types were sensitive to the virus. D98 and WI38 gave only slightly lower yields than BSC-1, but KL, another diploid fibroblast strain, produced comparatively little virus, though the cells were killed. The mouse parental line, 3T3-4E, was totally unaffected by the virus, which declined continuously in amount after infection.

All of the hybrid lines, HLE-C (I), KLE-H, and WE-2, were sensitive to the infection and produced as much virus as their human parental cells. Viral growth curves obtained after infection of a susceptible hybrid line WE-2 with 10 PFU/cell were not appreciably different from those of human diploid strains or the monkey line BSC-1 (Fig. 1). Some virus was liberated by 8 hr after infection, and the maximal yield was obtained by 24 hr.

Viral infection of the susceptible human parental and monkey lines resulted in killing of all cells in the populations; no virus-resistant survivors were obtained. However, in the case of the hybrid lines just described, numbers of cells survived the infection and grew into clones. Resistant survivors varied between 0.1 and 1% of infected HLE-C (I) hybrids. In the case of hybrids of human diploid cells, the surviving fraction was always greater. For example, about 40% of the population of KLE-H cells gave rise to colonies after viral infection.

All clones originating from surviving cells appeared to be fully resistant to reinfection with poliovirus, as shown in Table 1 for HLE-C (I)/Po, KLE-H/Po, and WE-2/Po. There was no detectable viral yield or cell killing even after infection with 400 PFU per cell.

Resistance to polio was also found to develop in nearly all cells of a previously sensitive hybrid in the course of long-term serial propagation, during which some human chromosomes were eliminated from the line. This is shown in the case of HLE-C (D), a subpopulation of HLE-C cultivated serially over a period of several months, during which the number of human chromosomes declined from 35 to 3 (Table 1). HLE-C (D) was found to be completely resistant to the virus (Table 1). Resistant populations can therefore be

TABLE 1. Poliovirus yield of human-mouse hybrids

Cell line	No. of metacentric and submetacentric chromosomes	No. of acrocentrics and telocentrics	Yield (PFU/cell)		Fraction of resistant cells in population
			0 hr	48 hr	
Human parental					
D98 (presumptive HeLa)	51	12	0.15	150	0
K1	36	10	0.18	22	0
WI38	36	10	0.40	190	0
Mouse parental					
3T3-4E	0	69	0.10	0.008	1.0
Monkey					
BSC-1			0.16	560	0
Hybrid					
D98 × 3T3-4E					
HLE-C (I)	12.5	129	1.3	180	0.01
HLE-C (I)/Po	12.9	121	0.9	0.05	1.0
HLE-C (D)	3.1	118	0.10	0.08	1.0
K1 × 3T3-4E					
KLE-H	6.4	141	0.5	34	0.4
KLE-H/Po	4.0	137	0.006	0.001	1.0
WI38 × 3T3-4E					
WE-2	3.8	132	0.07	280	0.4
WE-2/Po	3.5	126	0.024	0.003	1.0

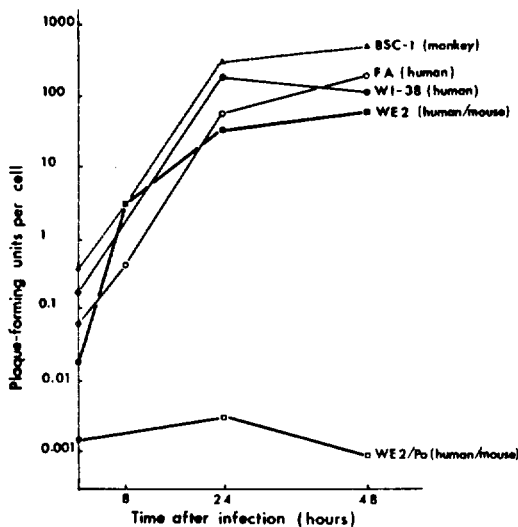


FIG. 1. Yields of poliovirus by monkey and human cells, by a virus-sensitive mouse-human hybrid (WE-2), and by its resistant subline WE-2/Po.

obtained spontaneously (nonselectively) as human chromosomes are lost, or selectively at any stage, by viral elimination of the susceptible cells.

Susceptibility of hybrids to poliovirus RNA. Parental and hybrid lines were tested for their ability to release poliovirus after infection with viral RNA. Cultures were infected with 0.1 PFU of RNA per cell, together with DEAE-

dextran. At various times after infection, the cultures were harvested and sonically treated, and their content of whole poliovirus was assayed on BSC monolayers in the absence of dextran. Table 2 shows that, whereas no cultures of either parental or hybrid types gave plaque-forming extracts immediately after infection (time zero), within 8 hr all cultures, whether mouse, human, or hybrid, produced significant numbers of infectious particles. Although the amounts produced were quite variable, there were no consistent differences in this respect between the different cell types or between hybrids sensitive or resistant to whole virus. In lines sensitive to whole virus, subsequent multiplication cycles led to synthesis of much larger quantities of virus by 24 hr after infection.

Ability of virus susceptible and nonsusceptible hybrids to adsorb poliovirus. Poliovirus receptor activity of different cell lines was assayed by measuring the amount of purified ³²P-labeled virus adsorbed to cell monolayers during a 60- or 120-min period at 4 C. At that temperature, adsorption occurs but no penetration or viral breakdown follows (8, 13).

The results obtained for different lines, expressed as PFU taken up per square centimeter of exposed cell surface, are shown in Table 3. The human parental lines D98/AH₂, WI38, and KL all adsorbed virus, whereas the mouse parental line adsorbed less than 3% as much as the least active human strain (KL). D98/AH₂

TABLE 2. Poliovirus multiplication in cells infected with viral RNA

Cell line	Virus yield (PFU per 10 ⁶ cells)	
	8 hr ^a	24 hr ^a
Monkey		
BSC.....	1,100	24,000
Human		
D98.....	0	168
	0	40
	5	50
WI38.....	0	310
	3	170
KI.....	7	100
	30	240
Mouse		
3T3-4E.....	2	9
	40	6
Hybrids		
HLE-C (I).....	134	3,540
	300	1,500
	120	
	8	24
HLE-C (I)/Po.....	16	250
	2	100
	200	25
WE-2.....	0	26
	70	320
WE-2/Po.....	7	26
	15	30
KLE-H.....	34	1,200
		9,500
KLE-H/Po.....	0	89
	7	150

^a Time after infection.

adsorbed considerably more virus than the human diploid strains. All polio-sensitive hybrid lines adsorbed virus, and though, as might be expected, they were less active than their respective human parental lines, they adsorbed up to 16 times more virus than the polio-resistant sublines. The small amount taken up by the resistant lines was probably not specifically adsorbed, especially since the amount did not increase with adsorption times longer than 60 min (HLE-C/Po).

DISCUSSION

Poliovirus is able to infect all serially cultivated primate cells, but not those of nonprimates, such as the mouse (6). This is due to the fact that primate cells possess polio receptors on the cell membrane and therefore adsorb the virus (5, 8), whereas murine cells do not possess receptors and do not adsorb virus. The viral receptors

TABLE 3. Adsorption of ³²P-labeled poliovirus by sensitive and resistant cells

Cell lines		Period of adsorption (min)	PFU taken up per cm ² of surface
Parental	Hybrid		
D98/AH2		60	21.7 × 10 ⁻⁵
3T3-4E		60	0.09 × 10 ⁻⁵
3T3-4E		60	0.05 × 10 ⁻⁵
	HLE-C	60	3.26 × 10 ⁻⁵
	HLE-C/Po	60	0.23 × 10 ⁻⁵
	HLE-C	60	1.58 × 10 ⁻⁵
	HLE-C/Po	60	0.39 × 10 ⁻⁵
	HLE-C	60	2.05 × 10 ⁻⁵
		120	4.13 × 10 ⁻⁵
	HLE-C/Po	60	0.51 × 10 ⁻⁵
		120	0.25 × 10 ⁻⁵
WI38		60	4.29 × 10 ⁻⁵
KL		60	3.69 × 10 ⁻⁵
		110	4.83 × 10 ⁻⁵
	WE-2	60	2.94 × 10 ⁻⁵
	WE-2/Po	60	0.79 × 10 ⁻⁵
	KLE-H2	60	1.68 × 10 ⁻⁵
	KLE-H2/Po	60	0.14 × 10 ⁻⁵
	KLE-H9	60	1.33 × 10 ⁻⁵
	KLE-H9/Po	60	0.19 × 10 ⁻⁵

appear to be protein or lipoprotein (24, 12). Infection with viral RNA was shown to lead in both primate and mouse cells to viral synthesis (7, 9).

Human cells have not been shown to undergo mutation to complete polio resistance. Vogt and Dulbecco (22) found that serial selection of HeLa cell populations by repeated infection with low multiplicities of type 3 poliovirus permitted the survival of cells with a reduced probability of initiating virus multiplication. An approximately 10-fold higher concentration of infecting virus was necessary to kill an equal proportion of the resistant subline. This line continued to adsorb virus normally, and presumably had undergone no change in its virus receptors. A similar line isolated from HeLa without selective methods also had greater resistance to infection, presumably due to a defect in uncoating (1). No fully resistant sublines were reported in either study.

In our experiments, in which infection was carried out at relatively high multiplicity, no human cell populations gave rise to survivors. From this and the other studies just mentioned, we may conclude that mutational loss of ability to synthesize polio receptor must be very rare in human cells. [A polio-resistant monkey cell line has been evolved over a very long period of selection with virus (18), and more recently shown not to adsorb the virus (19).] On the

other hand, spontaneous events in a small proportion of all hybrid populations led to complete and permanent resistance to infection by whole virus. Since this resistance did not extend to infection with viral RNA, it cannot be attributed to difficulty in the multiplication process itself.

A direct measurement of receptor activity was made at low temperature, at which adsorption takes place but penetration does not follow (8, 13). Under these conditions, cells susceptible to infection adsorb large amounts of virus whereas insusceptible (nonprimate) cells can adsorb only a small amount of virus nonspecifically (9). All polio-resistant hybrids studied in our experiments retained only very small amounts of virus, probably held nonspecifically in pockets between the large hybrid cells. All sensitive hybrids adsorbed much greater amounts, though not as much as that adsorbed by parental human cells, predominantly for the following reasons: (i) sensitive hybrid populations contain a small proportion of resistant cells, which would not contribute to virus uptake; (ii) probably only one homologue of the human chromosome bearing the polio gene is present per sensitive hybrid cell; (iii) there is a large (preponderant) contribution of mouse chromosomes, whose gene products likely dilute those of human origin in the cell membrane, as elsewhere.

Loss of the receptor gene from hybrid cells is spontaneous. Some resistant cells are present in all the hybrid cell populations, but they are more numerous in hybrids (WE-2 and KLE-H) which possess fewer human chromosomes than the HLE-C hybrids, and may become preponderant in any line after sufficient elimination of human chromosomes has occurred, a process probably affecting both the acrocentrics and the biarmed. A close analogy may be drawn between the single-step viral selection for receptor (-) hybrids and the bromodeoxyuridine selection for thymidine kinase (-) hybrids (23). It seems most likely that, as in the bromodeoxyuridine selection and the human thymidine kinase gene, the virus-resistant hybrids are those in which there has been a loss of the human chromosome bearing the gene for the receptor substance.

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