

Human-Mouse Hybrid Cell Lines and Susceptibility to Poliovirus¹

II. Polio Sensitivity and the Chromosome Constitution of the Hybrids

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A number of human-mouse hybrid cell lines with partial human chromosome complements were sensitive to poliovirus because the cells contained the viral receptor substance of human origin. Infection of the lines with one type of poliovirus regularly led to the survival of a few cells, whose progeny were found to be resistant to all types of poliovirus. Comparison of the chromosomes of sensitive hybrids and their resistant sublines showed no consistent difference in the number of banded human chromosomes of any group. The number of acrocentrics was always lower in the resistant hybrids than in the corresponding sensitive lines. It is suggested that the human chromosome bearing the polio receptor gene is an acrocentric.

Study of a number of human-mouse hybrid lines possessing human chromosome complements and sensitive to poliovirus, and of the resistant sublines derived from them after virus infection, has shown that the polio receptor substance, which is of human origin, is present in the sensitive hybrids but not in the resistant sublines (6). Since human-mouse hybrids eliminate human chromosomes spontaneously, the most likely basis for loss of the human polio receptor would be in the loss of the chromosome bearing the receptor gene. A study of the chromosomes was therefore undertaken in an attempt to identify the human chromosome bearing this gene.

MATERIALS AND METHODS

The cell lines employed, their origin, and their behavior with respect to the virus have been described (6). The poliovirus used for most experiments, including those in which chromosomes were studied, was the type 1 Sabin strain. For the cross-sensitivity experiments, the other strains used were type 2 (MEF-1) and type 3 (Saukett) kindly provided by B. Mandel. Stocks of all of the polioviruses were grown on BSC cells.

The chromosomes were prepared according to Rothfels and Siminovitch (5), with the use of colchicine to accumulate metaphases. The spreads were photographed, and the chromosomes were cut from

prints and classified as acrocentrics plus telocentrics and banded (metacentrics plus submetacentrics). The banded were further divided according to the Denver classification. Approximately 15 metaphases were counted for each value in Table 2, where the mean number of each type of chromosome is given.

RESULTS

Cross-resistance of hybrids to different polio types. Since hybrid cell populations predominantly sensitive to poliovirus type 1 were shown to give rise after infection to survivors resistant to that virus (6), it was of interest to determine whether the survivors were also resistant to poliovirus of other types. It was found that infection of the type 1 survivors with poliovirus type 2 or type 3 did not produce any cytotoxic effect. This was true for the type 1-resistant hybrids originating both from human diploid cells and from D98. A more precise study of this cross-resistance was carried out with HLE-C. Cultures were inoculated with poliovirus type 1, 2, or 3, and resistant populations were grown from the small number of surviving cells. Petri dishes were inoculated with 200 or 400 cells of each virus resistant type, and 5 days later the cultures were infected with 0.5 ml of an undiluted stock of poliovirus of the other types. Control cells were mock-infected. Plates were fixed and stained for colony count 8 days after infection. Table 1 shows that when HLE-C was infected with any of the poliovirus types very

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TABLE 1. *Nonsusceptibility of virus-resistant HLE-C to all poliovirus types*

Cell line	No. of cells inoculated	[No. of surviving colonies]			
		Control	Infected with		
			Polio type 1	Polio type 2	Polio type 3
HLE-C.....	200	35.5	2.5	0.5	0
	400	63.5	4.5	1.5	0.5
HLE-C/Po-1...	200	53		51	51
	400	108		95.5	95.5
HLE-C/Po-2...	200	48	53		45
	400	92	89		92
HLE-C/Po-3...	200	49.5	46	53.5	
	400	102	91.5	100	

few colonies survived. Sublines of HLE-C selected for resistance to any one type of poliovirus produced as many colonies after infection with the other poliovirus types as did uninfected cells. This finding indicates that either all poliovirus types utilize the same receptor substance or that the same chromosome contains genes determining receptors for all the types.

Analysis of the chromosomes of sensitive and resistant hybrids. Hybrid populations most likely to be sensitive to the virus are those with large numbers of human chromosomes. However, for the purpose of making chromosome assignments for the receptor gene, it was thought advisable to use virus-sensitive populations in which the total number of human chromosomes was as low as possible. Since these hybrid populations are not homogeneous with respect to human chromosome content, randomly isolated hybrid clones with low human chromosome number are also especially likely to contain a fraction of cells resistant to the virus. The sensitive clones described in Table 2 were therefore selected for susceptibility to the virus from a larger number of clones isolated. In the case of HLE-C and its subclones, populations were obtained in which virtually all of the cells were sensitive; in some hybrid populations deriving from human diploid cells, nearly 90% of the cells were sensitive (KLE-H2, KLE-H9), but in others (KLE-H6 and WE2) the proportion of sensitive cells was as low as 60%. This heterogeneity must be kept in mind when evaluating the chromosome results.

Table 2 shows a summary of the chromosome data obtained. The biarmed chromosomes in all of the hybrids are assumed to be of human origin. The mouse parental cell 3T3-4E contains only acrocentric and telocentric chromosomes, and

most chromosomes of this type in the hybrids are of mouse origin. There are nearly twice as many such chromosomes in the hybrids as in 3T3-4E, a doubling of the 3T3-4E complement being very often observed in human-mouse hybrids (2, 7). It is important to note that no attempt has been made to distinguish human acrocentric chromosomes from those of the mouse, so that any human acrocentrics present in the hybrids are included in the total number of acrocentrics and telocentrics.

Comparing the total mean number of biarmed chromosomes of virus-sensitive and virus-resistant clones, we found that three of the eight pairs showed a decline of one chromosome or more in the resistant clone, and five did not. As this result was rather inconclusive, the chromosomes assignable to each of the human groups of biarmed chromosomes were examined separately. Two criteria were used to eliminate any group as responsible for the polio receptor.

(i) Absence of any member of the group in a virus-sensitive line: by this criterion, A-group chromosomes were eliminated by WE-2 and HLE-C6; B-group chromosomes, by virtually every hybrid; the no. 16 chromosome, by KLE-H6 and WE2; and F-group chromosomes, by WE-2.

(ii) Absence of an adequate difference between sensitive and resistant lines in the mean number of chromosomes in any group: if a given group included a chromosome determining sensitivity, the mean number of chromosomes in the group should be lower in the resistant line by approximately 1.0, if nearly all infected cells were sensitive, or by a slightly smaller number where not all of the infected cells were sensitive. By this criterion, all of the remaining groups were eliminated. For chromosomes of group A, seven of eight pairs of hybrids showed no adequate difference in number; for groups E and F, in six of the eight pairs of hybrids there was not an adequate difference; for group C, five of the eight pairs showed no adequate difference, and in one of these the mean number of C-group chromosomes in the sensitive hybrid was so low (1.0) that comparison with the resistant hybrid should have most clearly revealed any difference in number of chromosomes of this group.

The only value for which there was a consistent difference between the sensitive and resistant hybrids was the total number of acrocentric and telocentric chromosomes. The resistant hybrids had a mean number lower by at least four and in some cases by much more. As indicated earlier, it was not possible to distinguish reliably the human acrocentrics from those of the mouse.

TABLE 2. Chromosomes of sensitive and resistant hybrids

Hybrid line	Fraction of cells sensitive	Total biarmed	A	B	C	E	16	17-18	F	Acrocentric plus telocentric
KLE-H.....	0.60	6.4	0.62	0.05	3.4	2.0	0.48	1.5	0.38	141.3
KLE-H/Po.....	0	4.0	0.50	0.10	1.9	1.4	0.15	1.2	0.05	137.2
KLE-H6.....	0.62	8.4	1.5	0.38	4.4	1.5	0.18	1.4	0.73	151.7
KLE-H6/Po.....	0	4.7	0.6	0	2.9	1.0	0	1.0	0.20	127.0
KLE-H2.....	0.84	10.2	3.3	0	4.0	1.5	0.32	1.1	1.5	158.2
KLE-H2/Po.....	0	10.3	3.4	0	4.1	1.9	0.45	1.5	0.91	151.5
KLE-H9.....	0.88	11.2	3.3	0	5.0	1.7	0.33	1.3	1.3	158.7
KLE-H9/Po.....	0	10.1	2.8	0	4.6	1.8	0.27	1.5	0.91	153.5
WE-2.....	0.60	3.8	0.15	0	1.0	2.6	0.08	2.5	0.08	131.7
WE-2/Po.....	0	3.5	0.12	0.12	1.0	2.1	0.12	2.0	0.12	125.7
HLE-C(I).....	0.99	12.5	0.78	0.22	6.67	3.89	2.3	1.56	1.22	128.6
HLE-C(I)/Po.....	0	12.9	1.20	0.15	7.10	2.05	0.9	1.05	0.80	121.3
HLE-C4.....	Nearly all	7.1	0.30	0.05	3.10	3.30	0.9	2.25	1.10	126.7
HLE-C4/Po.....	0	6.8	0.06	0.06	2.75	3.31	1.3	2.00	0.31	121.0
HLE-C6.....	Nearly all	8.4	0.05	0	5.2	2.5	1.2	1.3	0.70	117.6
HLE-C6/Po.....	0	9.3	0.30	0	3.9	2.6	1.1	1.5	0.70	112.6

DISCUSSION

The following hypotheses may be considered to explain the lack of association of the receptor gene with any biarmed human chromosome.

(i) Loss of sensitivity to the virus comes about through mutation, and therefore is not related to chromosome loss. Since no such mutants have been found in human lines (*see* 6), this explanation seems unlikely unless such a mutation were lethal for the human cells and nonlethal for hybrid cells containing complete mouse genomes. The fact that the development of resistance in hybrid clones is so common, and becomes preponderant in clones which have spontaneously lost many human chromosomes (6) makes mutation very unlikely as the basis for resistance.

(ii) Chromosome rearrangement is so extensive that the biarmed chromosome bearing the relevant gene becomes unrecognizable. While chromosome arrangement does occur in somatic cell hybrids, and may mask the identity of a human chromosome (4), it does not occur with a high frequency and has not yet been an important obstacle to making gene assignments (3, 4). A rearrangement affecting a single biarmed chromosome in all eight clones originating from three independent hybrids seems most unlikely. A rearranged chromosome was occasionally seen in mouse human hybrids of the type described here, but none was noted in the clones described in Table 2.

(iii) Genes for the polio receptor are duplicated at several sites located on different biarmed chromosomes, so that a unique locus does not exist. Under this hypothesis, absence of a given

chromosome from a sensitive hybrid does not eliminate it as one of the bearers of a receptor gene, since another chromosome bearing the same gene might be present. However, since all such chromosomes would have to be eliminated in order to achieve resistance, the greater the number of chromosomes bearing such genes, the greater should be the difference in human chromosome number between sensitive and resistant hybrids, particularly where the total human chromosome number in the sensitive hybrid is large. But hybrid HLE-C showed no difference in total number of biarmed chromosomes between sensitive and resistant lines, even when 12 biarmed chromosomes were present in the sensitive hybrid. This hypothesis can probably be excluded.

(iv) The gene for the polio receptor is located on one of the five acrocentric chromosomes (groups D and G), which could not be distinguished with confidence from those of the mouse. The decline in number of acrocentrics in the resistant hybrids is consistent with this hypothesis. If the polio receptor gene has a unique locus, then the loss of one or at the most two acrocentrics should be sufficient to produce resistance. The decline is much larger than this, suggesting that the human acrocentric bearing the polio gene is most likely to be lost in a single event in which a number of other acrocentrics are lost as well. Such an effect has already been noted in other hybrid cells (1). The possibility exists that two or more complementary genes are required for the synthesis of the receptor substance (or site), and that the

absence of any one gene would result in absence of the receptor. If these genes were located on different chromosomes, at least one of them should be an acrocentric.

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