ASSOCIATION BETWEEN GROUP G CHROMOSOMES, ESPECIALLY CHROMOSOME G21, AND SUSCEPTIBILITY OF HUMAN CELLS TO COXSACKIE B VIRUSES

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The R method of differential staining of chromosomes by length was used for a comparative study of karyotypes of human cells of the J-96 strain and the J-41 cell line obtained from it, which is specifically resistant to Coxsackie B viruses. It was shown with a high degree of statistical significance ($P < 0.0001$) that the G21 chromosome was eliminated from the chromosome sets of the resistant cells. The number of other chromosomes in individual cells of the cultures varied, but they were constantly present in most cells of both lines tested. It is concluded that the susceptibility of human cells to Coxsackie B viruses is mainly linked with the G21 chromosome.

KEY WORDS: *Coxsackie B virus; antiviral immunity; chromosomes.*

The study of the association between susceptibility of cells to viruses and template activity of particular chromosomes began in the West in 1970. The use of the method of interspecific hybridization of cells *in vitro* with the aid of Sendai virus and the use of differential staining of chromosomes by length showed which chromosomes were in fact responsible for the susceptibility of human cells to a particular virus. As yet only isolated papers on this problem have been published. Miller et al. [14], for instance, after studying the susceptibility of clones of hybrid human–mouse cells to poliovirus compared this property with the karotype and concluded that the genes coding cell receptors for poliovirus are located in the F19 chromosome. A group of French workers has studied a similar problem [9]. After a detailed virological, biochemical, and karyological study of hybrid clones these workers concluded that besides chromosome F19, chromosome G21 also plays a definite role in the susceptibility of cells to poliomyelitis virus. In this connection it should be noted that as long ago as in 1970, Kusano et al. [13], using old methods to stain chromosomes, linked the localization of the gene responsible for susceptibility of human cells to poliomyelitis virus with a group of small acrocentric chromosomes, which is known to include chromosome G21.

Carrit and Goldfarb [8] showed that susceptibility to herpes virus in hybrid human–mouse clones is linked with the presence of human A3 chromosome in the karyotype. Finally, in a review of human gene mapping published in the Proceedings of the 1975 Baltimore Conference [11] it is stated that susceptibility of human cells to ECHO-11 virus is linked with the presence of the F19 chromosome.

However, in the USSR investigations of this type began much earlier. Since 1963 under Academician of the Academy of Medical Sciences of the USSR V. D. Solov'ev's direction an extensive virological, morphological, cytochemical, and karyological study has been undertaken of cell cultures acquiring specific resistance, as a result of experimental procedures, to the cytopathogenic action of certain viruses [3, 4]. In the course of these investigations it was found that acquisition of resistance to Coxsackie B viruses by cells is accompanied by characteristic changes in cell metabolism, coupled with a decrease in the modal number of chromosomes; a decrease in the number of small acrocentric chromosomes of the G group, i.e., the 21st and 22nd pairs, was most constantly observed [6, 7, 12].

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TABLE 1. Comparison of Number of Chromosomes of Group G in Culture of J-96 Cells Susceptible to Coxsackie B Viruses and in Cell Line J-41 Resistant to These Viruses Obtained from It

Number of chromosomes	J-96	J-41
0	0	12
1	0	13
2	8	7
3	13	3
4	9	0
5	5	0
$M \pm m$	3,31±0,16	1,03±0,16

Legend. Number of metaphase plates containing corresponding number of group G chromosomes in 35 plates in each culture is shown

TABLE 2. Comparison of Number of Chromosomes of 21st and 22nd Pairs in Cultures of J-96 Cells Susceptible to Coxsackie B Viruses and J-41 Cells Resistant to These Viruses

Number of chromosomes	J-96		J-41	
	21	22	21	22
0	0	0	28	12
1	16	15	5	13
2	17	15	2	7
3	2	5	0	3
$M \pm m$	1,57±0,10	1,71±0,12	0,26±0,09	1,03±0,16

Legend. Number of metaphase plates containing corresponding number of G21 and G22 chromosomes in 35 plates in each culture is shown.

The object of the present investigation was to undertake a detailed study of chromosomes of the G Group in a cell line which had acquired resistance to Coxsackie B viruses, using modern methods of differential linear staining.

EXPERIMENTAL METHOD

Strain J-96 of human reticular cells, highly susceptible to Coxsackie B viruses (a heteroploid culture with modal class of 58-62 chromosomes) and cell line J-41 obtained from this culture, specifically resistant to the same viruses (a heteroploid culture with modal class of 52-57 chromosomes), were used for the investigation. The last cell line was obtained by Solov'ev and Gulevich [5] by the action of Coxsackie B3 virus on J-96 cells.

Preparations for karyological analysis were obtained by the usual method with modifications due to the special features of the object. After comparing the results of the R and G methods of differential linear staining of chromosomes [10, 15] the first was chosen, for it enabled the 21st and 22nd pairs of chromosomes to be more clearly distinguished in the cells.

The specimens were treated during the first 3 days after fixation of the chromosomes in accordance with the following scheme: 1) rinsing in distilled water and immersion in Earle's solution (10 times concentrated Earle's solution, diluted 1:10 with distilled water, and adjusted to pH 6.5 with phosphate buffer) at 87.5°C for 40 min; 2) washing in tap water and then in distilled water; 3) staining by Giemsa's method for 12 min (4 ml of the standard stain + 4 ml phosphate buffer, pH 6.5, + 92 ml distilled water). In each culture 35 metaphase plates with a chromosome number lying within the modal class was chosen.

EXPERIMENTAL RESULTS

In the first part of the investigation the number of chromosomes of the 21st and 22nd pairs was counted in susceptible and resistant cultures. It will be clear from the data in Table 1 that cells of the initial susceptible J-96 culture contained from 2 to 5 chromosomes of the G group. The resistant J-41 culture contained a large number of metaphase plates without chromosomes of the G group or contained only one chromosome each of this group. Statistical analysis showed that the mean number of chromosomes of the G group in the resistant culture J-41 was significantly smaller ($P < 0.0001$) than in the susceptible J-96 culture. It must be pointed out here that Buzhievskaya [2], who used the G method of differential staining of chromosomes, failed to find any chromosomes of the G group in the metaphase plates of resistant J-41 cells which she studied.

By the use of the new methods of linear staining of chromosomes the writers' previous view [6, 7, 12] regarding the link between susceptibility of human cells to Coxsackie B viruses and chromosomes of the G group, was thus confirmed and given added precision. To this it can be added that the number of chromosomes of the G group evidently is independent of the total number of chromosomes in the cell. For example, the maximal number of chromosomes of the G group in these observations, namely five was found in J-96 cells containing both 58 and 62 chromosomes.

In the next part of the investigation the object was to determine with which chromosome of the G group, the 21st or 22nd, susceptibility of the cells to Coxsackie B viruses is connected. The data in Table 2 show that cells of the susceptible J-96 strain contain about equal numbers of chromosomes of the 21st and 22nd pairs. In cells of the resistant J-41 strain, however, the number of chromosomes of the 21st pair was statistically significantly smaller ($P < 0.0001$).

The number of chromosomes of the remaining groups in the cultures studied varied from one cell to another, as is generally the case with individual cells of stable cell lines. However, for none of these chromosomes could it be said of the G21 chromosome that it was present in most of the susceptible and absent in most of the resistant cells. Consequently, the data given above show with a high degree of statistical significance that the susceptibility of human cells to Coxsackie B viruses is mainly linked with the G21 chromosome.

As regards about 20% of the resistant cells in whose chromosome sets chromosome G21 was still present, there is reason to suppose that these chromosomes or, at least, their loci connected with coding of the cell receptors for Coxsackie B viruses, are in a state of deep repression. This assumption follows logically both from the data given in this paper and also from data published elsewhere [9, 14]. This of course does not mean that the susceptibility or resistance of human cells is linked exclusively with one G21 chromosome taken in isolation. On the contrary, the whole experience of modern genetics is evidence that the phenotypic manifestation of any feature, more especially such a compelx feature as susceptibility to a given virus, "depends on all the genes of the genotype as a whole, even if dependence on some genes was imperceptible" [1]. Elucidation of the role of other chromosomes in the manifestation of features of cell metabolism coded by genes of the G21 chromosome, which ultimately determine the susceptibility or resistance of human cells to Coxsackie B viruses, will engage the writers' attention in the near future.

In conclusion a few words must be said about methods used to study the role of definite chromosomes in the formation of susceptibility or resistance of cells to viruses. It was stated above that the few investigations so far conducted into this problem have used the method of interspecific cell hybridization. The method used in the present writers' experiments is similar in essence to that just mentioned: It also is based on comparison of the chromosomal sets of cells which possess or have lost their susceptibility to a given virus. However, during interspecific hybridization of cells chromosomes of the other species of animal interact with the human chromosomes, whereas in the objects used in the present investigation the specific interchromosomal interactions are preserved. In the writers' view, preservation of physiological interchromosomal interactions plays an important role in the objective sutdy of the nature and mechanisms of specific resistance of cells to viruses.

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GEOMETRIC COMPLEMENTATION OF THE PRIMARY MOLECULAR STRUCTURES OF HISTONES H2A, H2B, H3, AND H4 AND SOME POSSIBLE CONSQUENCES OF THIS PHENOMENON

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Conformity between the geometries of arrangement of extended sequences of basic and nonpositively-charged amino acids along the polypeptide chains of histone H2A, H2B, H3, and H4 molecules, forming the protein skeleton of the nucleosomes, discovered for the first time, are described in this paper. The number of histone packing variants in the tetramer satisfying the conditions of complementation discovered is 10^4, and in a histone octamer 10^8 respectively. It is postulated that the structural heterogeneity of the nucleosomes may have functional significance and that the choice of packing variant of their protein skeleton can take place by mechanisms of allosteric regulation or of the primary structure of that part of the DNA molecule which is a component of the nucleosome.
KEY WORDS: *nucleosome; histones; code; regulation; complementation.*

In recent years substantial progress has been made in the elucidation of the structure of the elementary deoxyribonucleoprotein (DNP) fibril which lies at the basis of organization of the eukaryote chromosome. This fibril has been shown to be a chain of repeating DNP subunits (nucleosomes) with a definite number of DNA molecules between them. Yet information at present available provides no grounds for the determination of the specific functions of the nucleosomes. They are ascribed mainly a structural role (DNA packing). This suggestion is based on at least two theses: a) the constancy of composition of the proteins in nucleosomes and b) the structure of nucleosomes is independent of the primary structure of their DNA components.

However, these theses do not take into account a further possibility, to be described below, which is a consequence of relationships discovered by the writer between the geometries of arrangement of extended sequences of nonpositively-charged amino-acid residues along the polypeptide chains of four types of histones participating in the formation of the protein skeleton of nucleosomes.

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