Group G Chromosomes and the Susceptibility of Cells of Human Origin to Coxsackie B Viruses

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SUMMARY

Comparative karyological studies have been made on the J-96 line of human leukaemic cells, which are susceptible to enteroviruses, and on cell strains derived from this line which are persistently infected with Coxsackie B5 virus or free from infective virus but possessing high specific resistance to Coxsackie B3 or B5 viruses. It was shown that the karyotypes of these cell strains were characterized by reduced numbers of small acrocentric chromosomes of group G. It is suggested that group G chromosomes in cells of human origin incorporate genes which control alkaline phosphatase activity and the production of specific substances essential to adsorption and intracellular development of Coxsackie B viruses.

INTRODUCTION

The infection of cell cultures with cytopathogenic viruses is known to result sometimes in cell strains with inheritable and reduced susceptibility, or complete resistance, to repeated infection by homologous viruses. The resistance to virus thus acquired is accompanied infrequently by changes in the karyotype. Thus, changes in the modal number of chromosomes in cell strains resistant to various viruses were reported by Vogt (1959); Chessin & Hirschhorn (1961); Ebina et al. (1969), and Kusano et al. (1970). More detailed investigations showed that changes in karyotype affected certain groups of chromosomes (Ebina et al. 1969; Gulevitch, Bahutashvily & Grinberg, 1970; Wang et al. 1970; Khesin & Amchenkova, 1972). Such findings elucidate the mechanisms by which cells acquire specific antivirus resistance.

It was of interest to relate the development of specific resistance to viruses to the changes in karyotype of the cell cultures. With this purpose, we compared the karyotype of J-96 cells during several years of cultivation with the karyotypes of cell strains persistently infected with Coxsackie B5 virus and consequently resistant to superinfection, and with the karyotypes of two cell strains derived by other methods which were free from infective virus but were specifically resistant to the cytopathogenic effects of Coxsackie B3 or B5 viruses.

METHODS

Cells. The J-96 stable line of human leukaemic cells was that obtained by Osgood & Brooke (1955) from a patient with monocytic leukaemia. This cell line is highly sensitive to enteroviruses. Karyological investigations of J-96 cells were started at the Moscow Antiviral
Preparations Institute in 1964 (Varshaver & Gulevitch, 1964; Gulevitch et al. 1970) and in parallel at our laboratory from 1967.

The J-41 cell strain was obtained by Gulevitch et al. (1970) by treating J-96 cells with large doses of Coxsackie B3 virus. The strain is free of infective virus but resists infection by the homologous virus, although still fully susceptible to other viruses which are cytopathogenic to the original cell line.

The J-36 cell strain was derived by Sovjetova et al. (1970) after combined treatment of J-96 cells with small doses of Coxsackie B5 virus and an immune serum prepared against J-96 cells infected with the same virus. The cells strain contains no infective virus and, like J-41 cells, is completely resistant to infection with the homologous virus.

The J-52 cell strain was obtained simultaneously with the J-36 strain (Sovjetova et al. 1971) and was studied at the stage of persistent infection; the cells survived infection with 0.5 to 1.0 TCD50/cell of the homologous virus.

Karyological analysis of cell cultures. This was according to the method of Moorhead et al. (1960), with modifications for particular cultures. Cells were grown in 250 ml flasks in Parker's 199 medium supplemented with 10% heated bovine serum. Colchicine was added for 1 to 3 h at a final concentration 0.2 μg/ml; Hanks's salt solution diluted in distilled water (1:4) was used as hypotonic medium; smears were flame dried and stained with azur-eozine. The distribution of chromosome numbers and the modal numbers were based on calculations from 100 metaphase plates without overlappings. The Denver nomenclature with letter indices was followed in constructing chromosome sets (Patau, 1960; Robinson, 1960). It should be noted that the use of the Denver system was optional, because the karyotypes of the J-96 cells differ from that of the typical human chromosome in number and morphology.

RESULTS

It is evident from Fig. 1 that cells of the original J-96 line, as well as cells of strains derived from it, are heteroploid with wide individual variations of chromosome numbers.

In J-96 cells, the number varied from 36 to 66 chromosomes and only 2% of cells possessed 46. The modal number was 60 and 51% of the cells contained chromosome sets with 58 to 63 chromosomes.

Over the last ten years, our own and other investigations of the J-96 karyotype have shown the limits of variation of chromosome number to move towards smaller numbers; the modal number fell from 64 to 58-60, and the percentage of cells within the mode declined.

Fig. 2 and Table 1 show the distributions of chromosomes between groups. In J-96 cells of modal chromosome number we chose 7 chromosomes for group A, 6 of which could be arranged in pairs with the non-paired chromosome similar to that of the first pair although considerably different in size; group B included 2 pairs of large submetacentric chromosomes; group C contained 21 or 22 chromosomes, metacentric and submetacentric, and of uncertain individual characterization; group D contained 6 or 7 large acrocentric chromosomes; groups E and F contained 6 and 6 or 7 chromosomes; group G contains 8 small chromosomes of acrocentric type.

We examined several metaphase plates of J-96 cells with chromosome numbers within the mode. The distributions for cells of 58, 60 or 61 chromosomes are shown in Table 1. Generally the J-96 virus sensitive cell line was characterized by constant chromosome numbers in groups A, B, E and G and by the presence of a large metacentric chromosome in group A; the numbers of chromosomes varied by 1 or 2 in groups C, D and F.
Coxsackie B viruses and human G chromosomes

Fig. 1. Distributions of chromosomes in cells of strain J-96, susceptible to Coxsackie B viruses: strain J-41, specifically resistant to Coxsackie B3 virus; strain J-36, specifically resistant to Coxsackie B5 virus; and strain J-52, chronically infected with virus Coxsackie B5. Modal and polyploid classes are shown by dark bars.

If we consider (Fig. 1) results for the karyotypes of cell strains exhibiting specific resistance to Coxsackie B viruses, the following differences arise: cells of the resistant lines J-41, J-36 and J-52 are heteroploid to much the same degree as cells of the J-96 line, and are characterized by modal numbers which are 6 to 8 chromosomes less than that for cells of the original sensitive line. The greatest shift in the mode towards smaller numbers was in cells of the J-52 strain in which specific resistance was associated with the stage of persistent virus infection. Table 1 and Fig. 2 show that the reduction of modal number for all the resistant strains occurred primarily at the expense of 5 or 6 small acrocentric chromosomes of group G. These changes in the karyotype, associated with the acquisition of specific antivirus resistance, were already evident at the stage of persistent virus infection.

The establishment of resistance to Coxsackie B viruses was accompanied in our studies by loss of the large metacentric chromosome in group A; the chromosome was maintained, however, in J-52 cells which contained infective virus. For groups B and E the chromosome numbers and their morphological characteristics were identical in cells of strains J-41, J-36 and J-52, while chromosome numbers in groups C, D, F and G differed by 1 or 2 chromosomes.

Table 1 includes for comparison the distribution of chromosomes in the L-34-2 cell line, which originated from J-96 cells and is resistant to Coxsackie B3 virus (Gulevitch et al. 1970) and showed changes in karyotype very similar to those reported in this paper.
Fig. 2. Chromosome sets for cells of strains indicated in Fig. 1.
Table 1. Distribution of chromosomes between groups in J-96 cells susceptible to Coxsackie B viruses and in virus-resistant cell strains derived from it

<table>
<thead>
<tr>
<th>Group of chromosomes</th>
<th>Chromosome distribution in three metaphase plates of susceptible cell line J-96</th>
<th>Chromosome distribution in cell strains resistant to: Coxsackie B3 virus L-34-2*</th>
<th>Coxsackie B5 virus J-41</th>
<th>J-36</th>
<th>J-52†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 7 7†</td>
<td>7† J-41</td>
<td>6 6 6 7†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4 4 4</td>
<td>4 4 4</td>
<td>4 4 4 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21 22 22</td>
<td>22 23 23</td>
<td>23 22 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6 6 7</td>
<td>6 6 7§</td>
<td>7 6 7 6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>6 6 6</td>
<td>8 6</td>
<td>6 6 6 6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>F</td>
<td>6 7 7</td>
<td>6 5</td>
<td>6 7 6 4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>8 8 8</td>
<td>3 3</td>
<td>3 3 2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total chromosome number</td>
<td>58 60 61</td>
<td>55 54</td>
<td>54 52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A resistant cell line described by Gulevitch et al. (1970).
† Resistant cell strain persistently infected with virus.
‡ Large metacentric chromosome.
§ Large acrocentric chromosome.

DISCUSSION

Comparative karyological examinations of a cell line sensitive to Coxsackie B viruses, and of cell strains derived from it which are specifically resistant to Coxsackie B3 and B5 viruses, have shown clearly a correlation between cell karyotype and susceptibility to virus infection.

The acquisition by cells of specific antivirus resistance was accompanied in our studies by a reduction of the modal chromosome number, due mainly to loss of small acrocentric chromosome of group G.

We have shown (Khesin & Amchenkova, 1972) that cells sensitive to Coxsackie B viruses have high activity of alkaline phosphatase. The cell strains in our experiments, which were specifically resistant to Coxsackie B3 and B5 viruses, were characterized by a sharp decline in this enzymic activity (Amchenkova & Gulevitch, 1968; Amchenkova & Sovjetova, 1968; Soloviev & Khesin, 1970).

According to Alter et al. (1963); De Carli, Maio & Nuzzo (1963); Königsberg & Nitowsky (1964), and other authors, cultures of human cells which possess lower activity of alkaline phosphatase than the parent cells are distinguished by fewer small acrocentric chromosomes of group G (21st and 22nd pairs). This, with our results, supports the suggestion by these authors that small acrocentric chromosomes carry genes which control alkaline phosphatase activity in human cells. Our observations of a decline in alkaline phosphatase activity were correlated invariably with the disappearance of some group G chromosomes.

The question arises whether the changes in karyotype and enzymic activity are the result of selection of cells resistant to viruses, or are in some degree induced by virus infection. During the development of resistance to Coxsackie B3 or B5 viruses our cell strains first became persistently infected. Later, some cells became free of infective virus but retained specific antivirus resistance.

Walker (1964) suggested that a major process was the selection by virus of cells genetically unsusceptible to it. However, in our studies, the state of persistent infection developed within 1 or 2 passages if cells were treated with an antiserum to virus-infected cells and later
infected with small doses of virus: there was no gross death of cells (Sovjetova et al. 1970) and, as shown in the present study for J-52 cells, their karyotype was characterized by the same type of changes as those for strains containing no infective virus but specifically resistant to infection with Coxsackie B viruses. We consider that the synchronous development of these karyotypic changes shows that virus infection not only leads to the selection of genetically resistant cells, but also induces changes in the karyotype leading to modified cellular metabolism.

REFERENCES


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