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CHROMOSOMAL ABERRATIONS IN HUMAN AMNIOTIC CELLS BEFORE THE COMPLETION OF A SINGLE GROWTH CYCLE OF MEASLES VACCINE VIRUS L-16

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Summary. — The L-16 vaccine strain of measles virus induces marked chromosomal alterations in continuous amniotic cell cultures. Different types of chromosomal lesions (single and multiple breakages, chromosome pulverization, "severe chromosomal abnormalities") were found at low multiplicities of infection (0.04 to 0.05 $TCID_{50}$ per cell) in 26 per cent of metaphase plates before the appearance of measles antigen and the onset of cytopathic effects. Karyometry has shown the interphase nuclei to be in a state of disintegrative swelling. The mechanisms and genetic implications of measles virus action on chromosomes are discussed.

Introduction

Although a number of data have been reported over the past few years about the effects of measles virus on chromosomes (Fjelde and Holterman, 1962; Nicols *et al.*, 1962, 1965, 1967; Grippenberg, 1965; Nicols, 1966; Abakova and Rappoport, 1967; and others), many aspects of these effects are insufficiently understood. There are also some contradictory reports concerning the action of measles vaccine and vaccine strains on chromosomes (Nicols, 1963; Nicols and Levan, 1965; Aula, 1965; Thun *et al.*, 1966).

Studies of the effects produced by vaccine strains on the cell chromosomal apparatus may be of practical interest (selection of the least "damaging" strains), but also of importance for the understanding of the mechanism of interaction between the various viral strains and the genetic apparatus of immunocompetent cells which are constantly one of the main sites of virus multiplication (Shroit, 1961; Sergiev *et al.*, 1966; Levenshtam *et al.*, 1966). Likewise unclear is the mechanism of measles virus action on chromosomes and the genetic implications of this phenomenon.

Materials and Methods

Cell culture. The AK-99 strain of amniotic cells was obtained from the Pasteur Institute of Leningrad. The cell line was a polyploid culture, with hyperploidy in the form of aneuploidy (54—60 chromosomes) predominating. Cells were grown on cover glasses in penicillin bottles, 100,000 cells being seeded into a bottle containing 90% medium 199 and 10% bovine serum with antibiotics.

Virus. In experiment 1, a dried vaccine strain of measles virus L-16 (Taros, 1963) adapted to a green monkey kidney tissue culture was used. In experiment 2, the same virus after it had undergone, in our laboratory, an additional passage on guinea pig kidney cells, was employed. The infectivity titre of both preparations on AK-99 cells was 10^4 $TCID_{50}$/ml. The haemagglutination titre was determined by the method of Norrby (1962) and was equal to 1 : 2. Infection was carried out on the second day of culture growth. Before infection, the growth medium was replaced by a medium of identical composition but with heated serum. The multiplicity of infection was 0.04 and 0.05 $TCID_{50}$ per cell in experiments 1 and 2, respectively.

Immunofluorescence studies. At 24 and 48 hours post infection (p.i.) the preparations of control and infected tissue cultures were fixed by absolute ethanol at 4° C for 18 hours. Staining was done by the direct method, using measles gamma-globulin labelled with fluorescein-isothiocyanate and rhodamine conjugated with bovine albumin, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology. The preparations were studied under the fluorescence microscope ML-2.

Cytopathic changes induced by measles virus were studied in preparations stained with haematoxylin and eosin. The mitotic index was expressed in per cent per 1000 counted nuclei.

Karyometric studies and statistical processing of their results were performed according to the procedure described by Khesin (1967).

Cytogenetic studies. At 20 hours p.i. colchicine was added for 4 hours to the bottle cultures. Then chromosome preparations were prepared by treatment with hypotonic solution and stained with acetic orcein. Chromosomes were studied and photographed by means of an MBI-6 microscope. A total of 460 metaphase plates were analyzed in the two experiments. The statistical significance of experimental and control results was determined using the "u" test (Urbakh, 1964).

Results

General characteristics of changes in cell cultures

At 24 hours p.i., no cytopathic or intranuclear inclusions or multinucleate symplasts were noted. Our attention was attracted by large nuclei and hypertrophied nucleoli present in most of the cells. Karyometric studies showed that the nuclei, which were distinguished in this cell line by relatively large sizes (because of marked polyploidy), increased by an average of 43.7% at 24 hours p.i. (Table 1). Along with morphologically intact cells, round cells that had lost their processes, cells with vacuolated protoplasm, and cells with pycnotic nuclei were observed. There were relatively few cells with changes suggestive of pronounced dystrophic processes. Necrotized cells occurred even less frequently.

Table 1. Changes in mean log of the area of amniotic cell nuclei 24 hours after infection with measles vaccine virus L-16 (per 100 nuclei)

Cells	M ± m	M in μ^2	Per cent increase	t
Control	2.1860 ± 0.01124	153.6		
Experimental	2.3430 ± 0.01606	220.3	43.7	8.0

The mitotic index of the infected tissue culture was 2.5%, compared with 5.5% in the control.

Twenty four hours p.i., no specific immunofluorescence characteristic of measles virus antigen was noted in the preparations. At that time, titration failed to detect any virus inside the cells or in the liquid phase of infected cultures.

A marked cytopathic effect and specific fluorescence of the measles virus antigen were observed only in those preparations incubated for 48 hours p.i.

Cytogenetic studies

As can be seen from the data in Table 2, which sums up the results of the two experiments, chromosomal abnormalities were detected in 2% of the dividing cells out of 250 metaphases studied in the uninfected (control) cell culture. In the infected cell culture 210 metaphases were examined, and

Table 2. Chromosomal aberrations in the amniotic cell culture infected with measles virus L-16

	Control	Experiments 1+2	"u"
Total No. of metaphases studied	250	210	
Metaphases with chromosomal abnormalities, including:	2%	26.15%	8.46
single chromatid breakages:			
(a) in 1—2 chromosomes	2%	8.5%	3.29
(b) in 3—5 chromosomes	0	2.9%	3.65
multiple chromosome breakages	0	0.95%	2.08
chromosome pulverization	0	4.3%	4.47
"severe chromosomal abnormalities"	0	9.5%	6.70

The difference between control and experimental results is significant with a probability of 99% at "u" >2.58 and with a probability of 95% at "u" >1.96.

abnormalities were detected in 26.15% of the dividing cells. This high percentage of pathology was observed at the multiplicity of infection of 0.04 to 0.05 $TCID_{50}$ per cell. The experimental material differed from the control one not only by the number of damaged cells, but also by the degree and pattern of detected changes. In the control, chromosomal abnormalities were limited to single breakages of chromatids (in 1—2 chromosomes of a plate) (Fig. 1). In contrast to this, breakages in 3—5 chromosomes as well as multiple chromosome breakages were noted in the infected cultures (Fig. 2). In 4.3% of the dividing cells the chromosomes were pulverized (Fig. 3), which was never observed in the control. In 9.5% of the infected cells changes occurred which we have provisionally called "severe chromosomal abnormalities" (Fig. 4). We found no description of such changes in the available literature devoted to the effects of measles virus on chromosomes. However, the fact that such changes were absent in the control, and also the fact that the affected plates were located in the immediate vicinity of unaltered plates (which rules out the possibility of their artifact origin) suggests that these changes resulted from the action of measles virus L-16.

Discussion

The absence of inclusions, marked cytopathic changes, or specific fluorescence suggests that the time period chosen by us for the study of chromosomal aberrations corresponded to the period of the first cycle of viral growth (until its completion), and this agrees with the data or Khesin *et al.* (1962), Mastyukova *et al.* (1963), and also of Rapp (1964). At that time there already were indications of lesions in the nuclear apparatus. The increase in nuclear size by an average of 43.7% is, as investigations of Khesin (1962, 1967) have shown, a reliable indicator of disintegrative swelling of cell nuclei developing under the action of viral infection.

The presence of chromosomal lesions in 26% of metaphase plates of infected cells (compared with 2% in the control cell culture) suggests a very active action of measles vaccine virus on chromosomes of the culture cells. The virus-induced chromosomal aberrations were demonstrable before the formation of virus antigen in the cells and before the onset of cytopathic changes.

It is noteworthy that the "aggressiveness" of the virus with respect to chromosomes is also manifested by vaccine strains. The only comparative study in this respect was undertaken by Aula (1965) on a pathogenic and the vaccine Edmonston strain; but this author compared the action on chromosomes of preparations with different infectivity titres.

Mauler and Hennesen (1965) think it uncertain whether chromosomal lesions are induced by direct viral action or by a toxin. In our experiments it is worthy of note that at a low multiplicity of infection (0.04—0.05 $TCID_{50}$ per cell), 26% of the metaphase plates had been affected until the single growth cycle of virus was completed. This seems to provide indirect evidence for the fact that chromosomal lesions were not induced by the infectious virus alone. Hence, it may be assumed that chromosomal changes arise not only in those cells where viral biosynthesis occurs, but also in those which have been just damaged by the virus, viral components or other substances.

As yet, it has not been ascertained in which particular period of nuclear development the virus-induced chromosomal aberrations occur in the cell. By analogy to what is known about the effect of ionizing radiation (Evans, 1962; and others) one can think that measles virus-induced isochromatid lesions can also arise during the interphase period.

There appears to be some association between the disintegrative swelling of interphase nuclei and the chromosomal lesions caused by measles virus and demonstrable in metaphase preparations. The features shared by both these processes is the absence of a distinct virus dose dependence (up to a certain limit) (data of Nicols *et al.*, 1965) and possibly also a common mechanism of occurrence. It appears that both these phenomena are due not so much to the reproduction of infectious virus as to the action of viral components or of other biologically active substances appearing in the virus-cell system and bringing about the disintegrative swelling of nuclei (Khesin and Ghendon, 1964; Khesin, 1967). It is believed (Ahnström and Natarajan, 1966) that the virus-induced breakage of chromosomal DNA

results from the reversal of the course of reaction due to a DNA polymerase. A different mechanism is responsible for chromosome pulverization. As Norrby *et al.* (1966) and Cantell *et al.* (1966) have demonstrated, it occurs very shortly after the cells have been exposed to a high-titre measles virus preparation or to a virus inactivated by ultraviolet radiation, and is associated with the haemolysin of measles virus which is identical with the "fusion factor" described by Cascado and Karzon (1965).

Of considerable interest is the question of genetic implications of chromosomal lesions induced by measles virus. Lesions that are different in character lead to different biological consequences. Chromosomal changes can be regarded as one of many and diverse manifestations of cytopathic viral action, as the result of which the cell loses its viability. Multiple chromosome breakage, chromosome pulverization, and "severe chromosomal abnormalities" should be referred to this group of lesions. The main biological implication of such lesions appears to be the death of the cell.

Of much greater importance can be those chromosomal alterations that do not lead to cell death. These primarily include single isolocus breakages and lesions which are not demonstrable morphologically. The chromosomes that have lost larger or smaller sections while retaining their centromeres, are capable of reduplicating in a changed form during cell division. The change in character induced by deletion is not inherited in this case. In most cases the lost section is reunited, but the genetic code may be disrupted. As a result, a virus-induced mutation can occur leading to the emergence of cells and cell clones with new properties. In measles, it seems that such changes can occur in somatic cells, including immunocompetent cells, where the virus multiplies. The possibility that this factor is involved in immunogenetic processes cannot be ruled out.

The above-mentioned aspects may be of practical importance in case virus strains are used to prepare live virus vaccines. It may well be that in the future it will be recognized desirable to evaluate vaccine strains not only in terms of their pathogenic and immunogenic effects, but also in terms of their mutagenic action.

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Explanation of Photomicrographs:

Different types of chromosome aberrations in AK-99 human amniotic cells infected with the L-16 vaccine strain of measles virus. Acetic orcein stain.
Fig. 1. Single breaks of chromatids in 1—2 chromosomes of metaphase plate; 24 hours p.i. × 3,600.
Fig. 2. Multiple breaks of chromosomes. × 2,600.
Fig. 3. Chromosome pulverization. × 2,600.
Fig. 4. "Severe chromosomal abnormalities". × 2,600.