ROLE OF CATHEPSIN D IN INDUCTION OF MICRONUCLEI IN LUNG CELLS OF MICE IMMUNIZED WITH LIVE INFLUENZA VIRUS

T. K. Kliment'eva, O. N. Samoilova, and N. N. Il'inskikh

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Live viral vaccines can induce considerable cytogenetic lesions in the cells of man and experimental animals [2, 5]. However, the mechanisms of the onset of chromosomal aberrations under these circumstances are far from clear. In 1965 Allison and Malucci postulated a role of lysosomal DNases in the genesis of chromosomal aberrations. Experimental investigations have either supported [12] or contradicted [14] this hypothesis. There is no doubt that not only DNases, but also other enzymes such as proteinases, may play a definite role in the induction of cytogenetic disturbances. Attention from this point of view has been drawn to catheps in D which, according to our own observations, can affect the level of cells with micronuclei [15].

The aim of this investigation was to study the level of cytogenetic disturbances and cathepsin D activity in the lung cells of mice immunized with live influenza vaccine.

EXPERIMENTAL METHOD

Noninbred male mice were immunized intranasally (0.01 ml/10 g body weight) with influenza vaccine (from Stavropol Research Institute of Vaccines and Sera). The efficacy of immunization was monitored by determining the antibody titer in the animals' blood serum by the hemagglutination inhibition test (HIT). Activity of cathepsin D in mouse lung homogenate was determined 3, 6, 12, and 18 h and 1, 3, 5, 7, 9, and 12 days after immunization. The frequency of cells with micronuclei was determined in films made form this tissue [7].

In one series of experiments immunization was carried out after administration of lysosomal inhibitors: hydrocortisone 80 mg/kg, ammonium chloride 54 mg/kg, contrykal 50,000 U/kg, chloroquine 30 mg/kg, and chloromercuribenzoate 3 mg/kg body weight. Hydrocortisone and contrykal were injected intraperitoneally, the remaining substances intravenously [10].

Free activity of the enzyme was determined without delay after homogenization of the tissue, total activity after preincubation with Triton X-100. Hemoglobin (Reanal, Hungary) was used as the substrate for cathepsin D. Protein was determined by the microbiuret method. The results were subjected to statistical correlation analysis by Spearman's method.

EXPERIMENTAL RESULTS

The use of the vaccine significantly increased free cathepsin D activity in the lungs of the mice on the 3rd, 5th, and 7th days after immunization; total activity of this enzyme remained high until the 9th day of the experiments. The maximal increase in total activity was observed 3 days after immunization of the animals and amounted to 187% of the control level (Fig. 1).

Free activity of lysosomal enzymes is determined by hydrolases, which are released from lysosomes or present on their membrane, i.e., they are prepared for contact with the substrate. The increase in free activity is usually connected with functional activation of the lysosomal apparatus [3]. There is evidence that during reproduction of influenza virus functional activity of the lysosomes is enhanced [4, 8]. The increase in total cathepsin D activity in mice immunized with influenza vaccine may be

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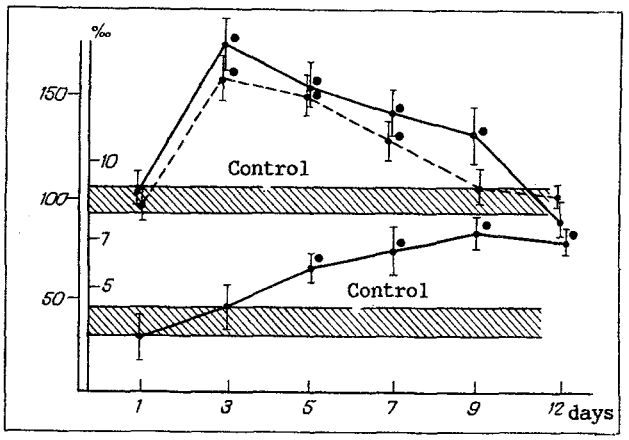

Fig. 1. Changes in cathepsin D activity and frequency of cells with micronuclei in lungs of mice immunized with influenza vaccine. Abscissa, enzyme activity (in % of control); ordinate, days after immunization. Here and in Figs. 2 and 3: filled circles $p < 0.05$; 1) free enzyme activity; 2) total activity; 3) level of micronuclei.

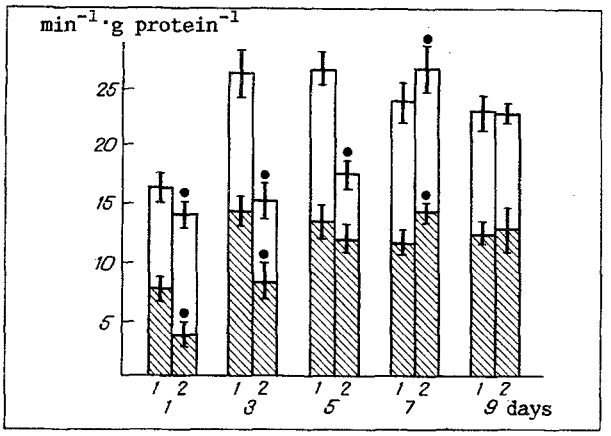

Fig. 2. Changes in cathepsin D activity after immunization of mice with influenza vaccine, preceded by injection of hydrocortisone. 1) Immunization with influenza vaccine; 2) immunization preceded by injection of hydrocortisone (in μM).

evidence that this vaccine can induce biosynthesis of the enzyme or that the number of lysosomes in the cell is increased during immunization. Meanwhile, the possibility cannot be ruled out that the sharp rise of total cathepsin B activity in the lungs may be due to migration of macrophages into the organ damaged by viruses.

Immunization with influenza vaccine caused a considerable increase in the number of cells with micronuclei in the mouse lungs. A high level of these cells was observed 5 days after injection of the vaccine and continued until the end of the experiment. Correlation analysis demonstrated positive correlation between cathepsin D activity and the frequency of cells with micronuclei. Moreover, as Fig. 1 shows, cathepsin D activation preceded the appearance of a large number of cells with cytogenetic disturbances. Cathepsin D is a very sensitive and quickly reacting protease, and it is this enzyme which can bring about contact between lysosomes and the nuclear membrane and chromatin [13]. It is by no means impossible that activation of cathepsin D leads to proteolysis of the proteins of the achromatin apparatus of dividing cells [9, 11], giving rise to deletion of individual chromosomes or their fragments and the formation of micronuclei in interphase.

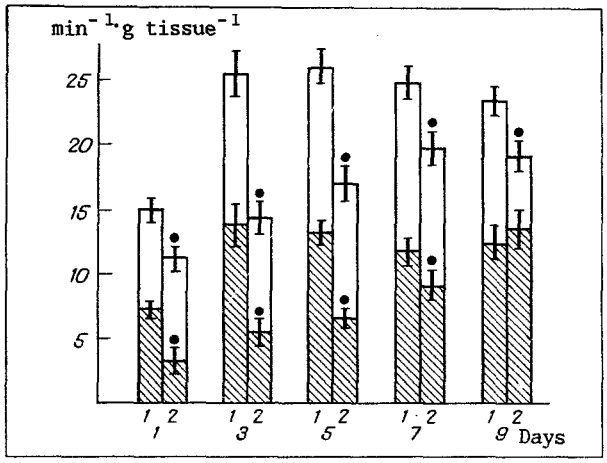

Fig. 3. Changes in cathepsin D activity after immunization of mice with influenza vaccine preceded by injection of ammonium chloride. 1) Immunization with influenza vaccine; 2) immunization preceded by injection of ammonium chloride (in μM).

To study the contribution of activation of cathepsin D to the appearance of chromosomal aberrations, in the next series of experiments immunization was carried out after previous injection of inhibitors of activity of the lysosomal apparatus, differing in their mechanism of action, contrykal, chloroquine, chloromercuribenzoate, ammonium chloride, and hydrocortisone. Of the inhibitors mentioned, hydrocortisone and ammonium chloride had the strongest effect on activity of the lysosomal enzymes, reducing both free and total activity of cathepsin D in homogenate of the lungs of intact mice. Hydrocortisone, by preventing contact between substrate and enzyme, caused a decrease in both free and total cathepsin D activity: free activity on the 1st-3rd days, total activity on the 1st-5th days after immunization of the mice. The membrane effect of hydrocortisone was brief and reversible, probably due to recycling of the membranes [3]. In fact, in the later stages of the experiment the effect of hydrocortisone was reversed: on the 7th day a considerable increase in both free and total cathepsin D activity was observed, followed by normalization of its activity (Fig. 2). Accordingly, the marked decrease in antibody production which we observed after injection of hydrocortisone may be due to intensification of protein catabolism and the immunodepressive action of this hormone [1].

Ammonium chloride, a lysosomotropic substance, is known to accumulate in the matrix of the organelles, where it nonspecifically inhibits acid hydrolases through reduction of the pH in the matrix of the lysosomes [1]. In the present experiments inhibition of cathepsin D activity by ammonium chloride lasted longer and was more stable than the action of hydrocortisone, as regards both free and total enzyme activity (Fig. 3). Under these circumstances ammonium chloride caused a significant decrease in the number of cells with micronuclei in the lungs of mice immunized with influenza vaccine.

The results are evidence that immunization of animals with influenza vaccine leads to activation of cathepsin D and to an increase in the number of cells with micronuclei in the mouse lung. The positive correlation between these parameters indicates that activation of lysosomal proteinase (cathepsin D) may be the cause of disturbance of the division spindle and of the appearance of micronuclei.

Suppression of lysosomal function of immunized animals reduced the number of cells with cytogenetic disturbances.

LITERATURE CITED

1. T. I. Buzhievskaya, Virus-Induced Mutagenesis in Mammalian Cells [in Russian], Kiev (1984).
2. A. G. Bulychev, O. A. Assinovskaya, and E. G. Semenova, Vopr. Med. Khim., No. 5, 20 (1987).
3. Yu. Z. Gendon, Vopr. Virusol., No. 4, 429 (1977).
4. D. B. Golubev, Enzymes in Systems of Virus-Infected Cells [in Russian], Leningrad (1979).
5. N. N. Il'inskikh, E. F. Bocharov, and I. N. Il'inskikh, Infectious Mutagenesis [in Russian], Novosibirsk (1984).
6. N. N. Il'inskikh, I. N. Il'inskikh, and V. N. Nekrasov, Tsitol. Genet., 22, No. 1, 67 (1988).

7. T. P. Korolenko, Biochemical Aspects of Lysosomotropism [in Russian], Novosibirsk (1983).
8. L. E. Panin and N. N. Mayanskaya, Lysosomes: Role in Adaptation and Restoration [in Russian], Novosibirsk (1987).
9. R. V. Petrov and V. M. Man'ko, Immunodepressors [in Russian], Moscow (1971).
10. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
11. O. V. Sablina, Genetika, No. 7, 131 (1974).
12. O. V. Sablina and N. Yu. Ramul', Genetika, No. 9, 150 (1974).
13. V. A. Filov, V. Ya. Shats, and D. B. Golubev, Tsitologiya, **12**, No. 5, 561 (1970).
14. N. N. Il'inskikh (N. N. Ilyinskikh) and D. R. Urmancheev, EEMS XVIII Annual Meeting: Abstracts (1988), pp. 110-111.
15. Nicols, cited in [5].

SURFACE EXCLUSION SYSTEMS OF F-LIKE PLASMIDS OF *E. coli* AND THEIR GENETIC CONTROL

V. P. Shchipkov, S. L. Sokolova, O. B. Gigani, K. S. Krivskaya, R. G. Khamidullina, and A. P. Pekhov

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Surface exclusion of plasmid F and of F-like plasmids is controlled by traS and traT genes [5, 7, 9, 11]. The problem of the distribution of the different types of surface exclusion systems (Sfx systems) determined by these genes and of their location in the genome of different plasmids remains unsolved.

The aim of the investigation was to identify Sfx systems determined by genes of F-like plasmids in cells of natural strains of *E. coli,* to study their specificity, and to determine the possible localization of these genes in genomes of individual plasmids.

EXPERIMENTAL METHOD

We studied 17 F-like drd-plasmids, including their transposon-containing variants [1-4]. As standard strains we used plasmids Flac, R124, R1, and R100, determining known types of Sfx systems [12]. We also used plasmids pAP53::Tn5 and pAP53::Tn9, shown to belong to the SfxII group (Tables 1 and 2). As host cells for the plasmids we used cells of strains of *E. coli* AP115 (Lac, Met, Nal) and C600 (Lac, Thr, Leu, Rif). Competent cells of *E. coli* HB101 were used in the transformation experiments.

Surface exclusion was studied in standard (direct and reverse) conjugation crosses of bacteria containing plasmids. The surface exclusion index (SEI) was determined as the ratio of the number of plasmid transconjugants found by the use of a plasmid-free recipient strain to their number obtained for an isogenic strain containing the plasmid. Plasmid DNA was isolated by the method in [10], the purified cell lysates being centrifuged in a CsCl density gradient. Restriction of plasmid DNA by endonucleases EcoRI and SalI, elution of the restriction fragments from the gel, and subsequent molecular cloning of these fragments in the composition of vector plasmid pBR325 were carried out by the usual methods [8]. The restriction fragments were fractionated in 0.65% agarose gel by horizontal slab electrophoresis. The dimensions of the restriction fragments of DNA were determined by comparing their mobility in agarose gel with that of DNA fragments from phage λ [6].

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