KINETICS OF REPARATIVE DNA REPLICATION INDUCED IN CBA AND C57BL/6 MOUSE SPLEEN CELLS BY URETHANE AND INFLUENZA VIRUS

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The established fact of the cocarcinogenic action of influenza virus [5] has been confirmed by the work of several investigators [7]. However, the mechanism of this phenomenon is not yet clear and requires further study. It can be tentatively suggested that the repair mechanisms of cells takes part in the process of conversion of the normal cell into a malignant cell.

To shed light on this problem we investigated the reparative replication of DNA, induced by urethane and influenze virus, in C57BL/6 and CBA mice which, as was shown previously [6], have marked differences in tumor formation in response to the action of these factors. Cells of these lines of mice differ in their ability to repair injuries induced in DNA by several mutagens: 4-nitroquinoline-1-oxide, and salts of thallium, mercury, and lead. When the same dose of mutagen is used, far fewer induced breaks are formed in cells of CBA mice than in cells of C57BL/6 mice. On induction of breaks in DNA in cells of CBA mice, their resynthesis took place much more rapidly than in cells of C57BL/6 mice [3, 10].

EXPERIMENTAL METHOD

CBA and C57BL/6 mice each received two subcutaneous injections of 1 ml of 1% urethane and a single intranasal injection of influenza A virus (Hong Kong/68/H3N2) in a dose of 1.0 LD_{50}. The mice were decapitated 1, 3, and 6 days after these procedures, the spleen was removed, and lymphocytes obtained from it [4] and incubated for 2 h at 37°C in medium RPMI-1640 without serum, containing ^{3}H-thymidine in a concentration of 10 μCi/ml (specific activity 29.5 Ci/mmole). Reparative DNA replication, induced by urethane and influenza virus, was investigated by a liquid scintillation method based on measuring incorporation of ^{3}H-thymidine into the total mass of spleen cells, when replicative DNA synthesis was inhibited by hydroxyurea (10^{-2} M). The number of cells in each sample was 10^6. The intensity of reparative DNA replication was judged from the value of the stimulation index (SI), which is the ratio of the radioactivity in the spleen cells of the experimental animals to the corresponding parameters in cells of intact animals.

EXPERIMENTAL RESULTS

The level of reparative replication under the influence of influenza virus and urethane differed in mice of the two lines studied (Fig. 1). In CBA mice, for instance, a significant increase was observed in the parameters of reparative replication induced by influenza virus and urethane, and it could be recorded 24 h after exposure to these agents. This effect was manifested particularly clearly 3 days after the beginning of the experiment, when the parameters of reparative replication induced by influenza virus were 5.8 times higher than the control values and 4.1 times higher than those observed 24 h after infection. On the 6th day, higher activity of reparative replication induced by influenza virus still continued, but it was much lower than on the 3rd day.

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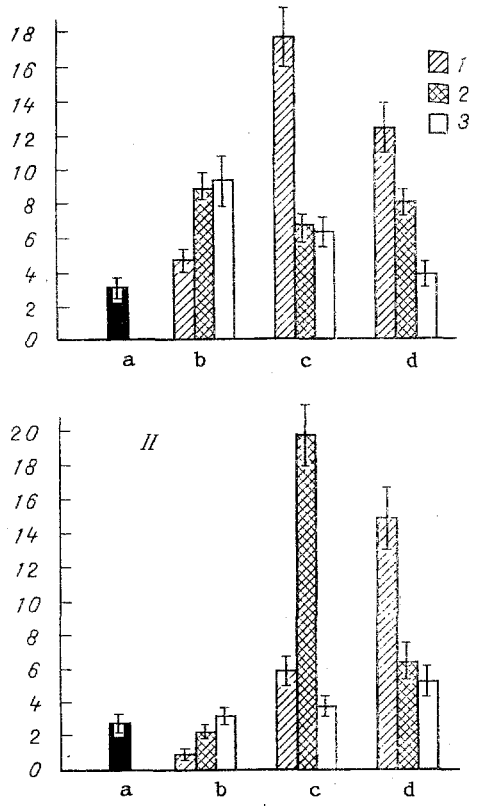

Fig. 1. Reparative replication of DNA in splenic lymphocytes of CBA (I) and C57BL/6 (II) mice. Ordinate, radioactivity (in cpm•10^{-3}). a) Control (intact mice); b, c, d) 1, 3, and 6 days, respectively, after exposure. 1) Infected with influenza virus, 2) treated with urethane, 3) combined treatment with influenza virus and urethane.

A characteristic feature of reparative replication stimulated by urethane was that its level was roughly the same at all times of investigation. The same differences were observed after combined exposure to urethane and influenza virus, but on the 3rd day reparative replication had a tendency to diminish.

These same parameters showed a different relationship when reparative DNA replication was analyzed in C57BL/6 mice. It was found that influenza virus, urethane, and a combination of both not only did not induce reparative activity, but actually inhibited it: when mice were infected with influenza virus its intensity was reduced by 3.5 times, when given urethane, it was reduced by 1.4 times, and after a combination of both, no effect was observed. On the 3rd day after its administration urethane caused marked induction of reparative replication (SI = 6.7). Incidentally, parameters of reparative DNA synthesis never reached this level in CBA mice.

The study of the kinetics of induction of reparative DNA replication in experiments with animals infected with influenza virus only revealed a low level of induction on the 3rd day (SI = 2.0) and a higher level by the 6th day (SI = 5.2), i.e., reparative replication in cells of C57BL/6 mice was delayed by comparison with that in CBA mice. Under the combined influence of influenza virus and urethane a tendency was observed for the level of reparative replication to rise on the 6th day above the control background.

Parallel with reparative replication of DNA in mouse spleen cells, reproduction of influenza virus in them also was studied. In C57BL/6 mice more rapid elimination of the virus was observed, and it could no longer be detected by the 3rd day, whereas in CBA mice it could be found on the 7th day after infection.

When the data are discussed it can be argued that the cocarcinogenic action of influenza virus when combined with urethane in C57BL/6 mice was accompanied by marked inhibition of reparative replication of DNA 24 h after their administration. Similar data were obtained in a system *in vitro*, when inhibition of the first stages of excision repair, induced by UV irrad-

iation, by 4-nitroquinoline-1-oxide, and by N-methyl-N-nitro-N-nitrosoguanidine, was observed in human diploid cells infected with mammalian leukemia virus [1, 2]. In a cell system infected by Rauscher leukemia virus [9] and herpes virus [8], inhibition of repair processes was found. Repression of repair processes is evidently an essential stage for conversion of the normal cell into a malignant cell. The system investigated *in vivo* confirms these data: In a highly sensitive system in which, during combined exposure to a carcinogen and influenza virus the yield of tumors is four times higher than that observed in response to the action of the chemical and biological agents separately [6], repression of one stage of repair also is observed.

Thus the hitherto unknown phenomenon of the stimulating and inhibitory effects of influenza virus on reparative DNA replication in cells depending on their genotype and sensitivity to virus was thus established. This correlates with the frequency of spontaneous tumors in mice of the lines studied. It can be postulated that the phenomenon now established is connected with the mechanisms of reproduction of influenza virus and their relations with cell DNA repair processes. Support for this hypothesis is given by data obtained during combined administration of influenza virus and urethane. Introduction of urethane into a system infected with influenza virus is known to sharply inhibit its reproduction [6]; under the experimental conditions which we used, this was accompanied by marked inhibition of the stimulating effect of the virus on the intensity of reparative DNA replication.

LITERATURE CITED

1. O. G. Andzhaparidze, G. D. Zasukhina, L. L. Matusevich, and L. G. Stepanova, Vopr. Virusol., No. 3, 300 (1972).
2. O. G. Andzhaparidze, G. D. Zasukhina, T. N. Shvetsova, et al., Vopr. Virusol., No. 6, 712 (1977).
3. G. N. Krasovskii, O. G. Chernov, G. N. L'vova, et al., Gig. Sanit., No. 3, 15 (1984).
4. T. Meo, in: Methods of Investigation in Immunology [in Russian], Moscow (1981), pp. 240-252.
5. A. F. Frolov, in: Problems in Experimental Oncology [in Russian], No. 2, Kiev (1966), pp. 52-59.
6. A. F. Frolov, Viruses and Carcinogenesis [in Russian], Kiev (1973).
7. R. Harris and G. Negroni, Br. Med. J., 1, 637 (1964).
8. A. K. Lorentz, K. Munk, and G. Daral, Virology, 82, 401 (1977).
9. R. Waters, N. Nishra, N. Bouck, et al., Proc. Natl. Acad. Sci. USA, 74, 238 (1977).
10. G. D. Zasukhina, I. M. Vasilyeva, N. I. Sdirkova, et al., Mutat. Res., 124, 163 (1983).