Adaptive Response in the Reparation-Defective Gout Cells

G. D. Zasukhina, T. A. Sinel'shchikova, and G. D. L'vova

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Adaptive response of fibroblasts from skin biopsy specimens from patients with gout has been studied; these cells are characterized as reparation-defective during exposure to mutagens N-methyl-N'-nitro-N-nitrosoguanidine and 4-nitroquinolin-N-oxide. Adaptive response formed during exposure to nitrosoguanidine. The data indicate that the formation of adaptive response does not depend on DNA reparation.

Key Words: *adaptive response; DNA reparation; gout*

Adaptive response (AR) is a mechanism of cell protection from endo- and exogenous stress factors, such as radiation, H_2O_2, radioactive thymidine, heat shock, and some alkylating compounds. Three-five hours after exposure to a low-dose stress factor, the cells become more resistant to radiation and chemical mutagens. The mechanism of AR is not clear. Some scientists believe that reparation processes are involved in AR [14], others observed AR in both reparation-competent and reparation-defective cells [16]. To elucidate whether DNA reparation is involved in the formation of AR, a wide spectrum of reparation-defective cells should be examined.

The number of diseases associated with DNA reparation is constantly increasing. Besides the well-known reparation defects in xeroderma pigmentosum, Fanconi's anemia, ataxia-telangiectasia, progeria, etc., disorders of DNA reparation were detected in lymphocytes of patients with diabetes mellitus, ankylosing spondilitis [10], systemic lupus erythematosus, and rheumatoid polyarthritis [1,11]. Studies of AR in atactic cells showed contradictory results. In cells from patients with hyperradiosensitivity AR was observed after preliminary low-dose X-ray exposure followed by high-dose irradiation [13], while other scientists detect no AR in patients with ataxia and Down's syndrome [5,12]. We studied AR in reparation-defective gout cells characterized by defective repair of DNA breaks induced by 4-nitroquinolin-N-oxide (4NQO) and N-methyl-N'-nitro-N-nitrosoguanidine (NG) [4]. AR was evaluated by the decrease in induced DNA breaks, increased reactivation rate, inhibition of induced mutagenesis of vaccinia virus, and the appearance of mutagen resistant DNA synthesis in cells preirradiated in a low dose and subsequently exposed to detrimental mutagen doses.

Laboratory of Mutagenesis and Reparation, N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow

MATERIALS AND METHODS

Normal human embryo fibroblasts (strain 1214) from the collection of Medical Genetic Research Center of the Russian Academy of Medical Sciences and fibroblasts from skin biopsy specimens of gout patients were used. Cells were cultured in Eagle's medium with 10% cattle serum and 5% human umbilical serum.

DNA breaks after NG exposure were evaluated as previously described [8]. The number of induced DNA breaks was determined by a modified method of alkaline elution of cell lysates from membrane filters [9]. Cells were labeled with ^{3}H-thymidin (7.4×10^4 Bq/ml) for 24 h before low-dose (0.1 Gy) γ-irradiation generated by ^{137}Cs (6.22 Gy/min). Treatment with mutagens was performed 3 h after irradiation. The cells were collected on membrane filters (1.5 μ, 24 mm), lyzed, and eluted with EDTA (pH was adjusted to 12.45 with 20% tetrapropylammonium hydroxide) for 2 h at an elution rate of 0.15 ml/min. Four 30-min fractions were collected into tubes, trichloroacetic acid

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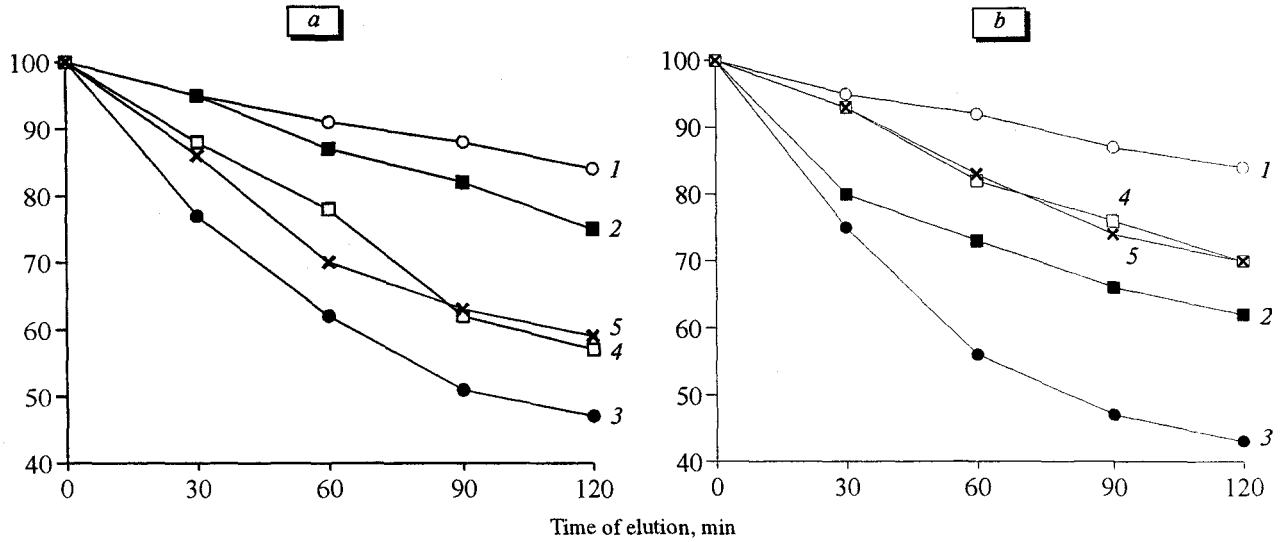

Fig. 1. Induction of DNA breaks in normal (*a*) and gout (*b*) fibroblasts. *1*) alkaline elution of control cells; *2* and *3*) cells treated with N-methyl-N-nitro-N-nitrosoguanidine (NG) in a dose of 5 μg/ml for 30 and 60 min, respectively; *4* and *5*) cells preirradiated with γ-rays in a dose of 10 cGy and after 3 h treated with NG for 30 and 60 min, respectively. Ordinates: radioactivity of filters, %.

was added to a concentration of 5%, and the cells were sedimented on filters.

Suppression of replicative DNA synthesis reflecting the genotoxic effects of mutagens was assessed by incorporation of labeled thymidine for 2 h in phosphate buffer immediately after mutagen treatment (24 h after cell subculturing). Radioactivity was counted in a toluene scintillator after sedimentation of radioactive material on nitrocellulose filters (per 10^6 cells).

Since viruses utilize host cell reparation systems for repair of their genome, we investigated the survival and mutations of vaccinia virus, reflecting the potentially lethal DNA damage.

For studies of the virus survival, intracellular virus was treated with 4NQO (10^{-5} M) and NG (100 μg/ml) for 1 h or exposed to γ-radiation 3 h after preirradiation of cells in a dose of 100 Gy. Virus was titered by the plaque-forming cell method in chick embryo fibroblast culture. Virus survival was determined for each specimen in lg of titers of plaque-forming units/ml. Mutation rate was evaluated by the size of plaque; plaques less than 2 mm were considered small.

Results were statistically processed using Student's *t* test.

RESULTS

Gout cells (lymphocytes and fibroblasts) were characterized as reparation-defective after exposure to NG and 4NQO by the formation of induced DNA breaks, reparative DNA synthesis, and reaction of vaccinia virus [4].

In normal and gout fibroblast cultures, preirradiated cells treated with NG were eluted slower than non-irradiated cells (Fig. 1), which correlates with a lesser number of induced DNA breaks. Control specimens were characterized by the lowest elution rate: 85% radioactive material remains on filters during 2 h. These data indicate the presence of AR to mutagens in both cell strains.

The better survival of the virus in preirradiated cells may reflect the induction of proteins accelerating the repair of virus genome.

In experiments with preirradiated normal fibroblasts (γ-radiation+4NQO) we observed a high reac-

TABLE 1. Suppression of Replicative DNA Synthesis in Preirradiated Normal Fibroblasts and Gout Cells Treated with 4NQO and NG, % (*M*±*m*)

Cells	4NQO, 30 min		NG, 5 μg/ml	
	5×10^{-7} M	5×10^{-6} M	30 min	60 min
Normal fibroblasts	65±6	38±7	66±6	50±5
Preirradiated normal fibroblasts	80±6	68±6	74±4	60±5
Gout cells	53±4	49±6	61±4	46±4
Preirradiated gout cells	82±4	89±5	96±7	65±6

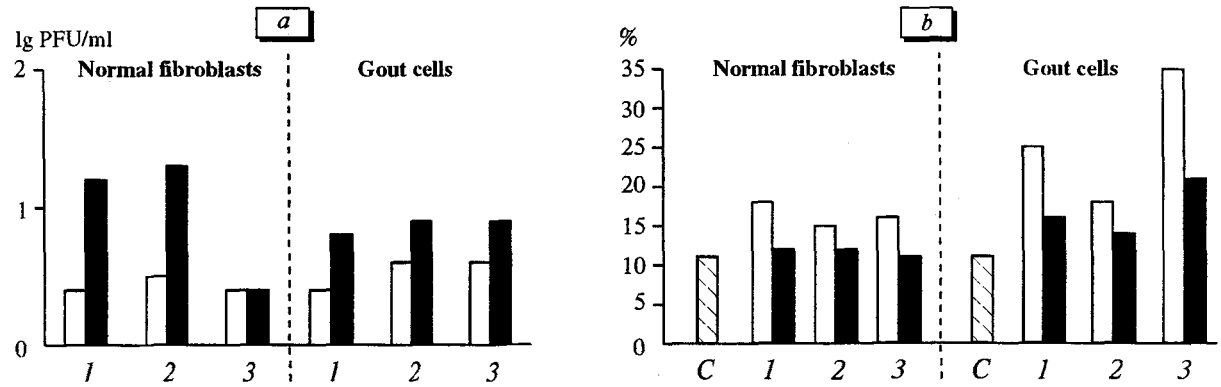

Fig. 2. Survival (*a*) and mutations (*b*) or vaccinia virus in normal fibroblasts and gout cells after preirradiation (dark bars) and without it (light bars). Cells exposed to γ-radiation (*1*), treated with 4-nitroquinolin-N-oxide (*2*), and N-methyl-N-nitro-N-nitrosoguanidine (*3*). C: control cells; PFU: plaque-forming units.

tivation rate of potentially lethal damage in vaccinia virus, while in experiments with NG the rate of reactivation was virtually the same as in intact cells (Fig. 2, *a*). In gout cells, the virus reactivation was increased after exposure to all mutagens.

The level of induced mutagenesis of the virus replicating in normal fibroblasts decreased in preirradiated cells treated with both mutagens (Fig. 2, *b*). Hence, both potentially lethal damages induced by γ-rays, 4NQO, and NG and premutation injuries formed during exposure were repaired in these cells. The same regularity was observed in gout cells, maximum inhibition of mutagenesis was observed after exposure to NG (from 35 to 21%).

Therefore, previously detected disorders in reparative activity of gout cells induced by NG did not correlate with reactivation of potentially lethal injury to the virus treated with the same mutagen. Increased reactivation rate of the virus in preirradiated cells can be considered as an indirect indicator of cell capacity to form AR. These data point out that DNA repair and mechanisms involved in virus reactivation after exposure to NG are unrelated.

Study of replicative DNA synthesis in preirradiated cells treated with 4NQO and NG showed the appearance of mutagen-resistant synthesis (Table 1). The effect persisted during co-culturing 3 h after preirradiation. In the reparation-defective cells of xeroderma pigmentosum, preirradiation produced no such effect [2].

In experiments with NG, AR developed in normal fibroblasts and gout cells, which indicates that DNA repair and AR are unrelated. The same regularity was observed in the homocystinuria cells defective by repair of γ- and 4NQO-induced DNA mutation [3]. AR was not observed in these cells after γ-irradiation and did not differ from the control after exposure to 4NQO.

Therefore, studies of a spectrum of reparation-defective cells differing by DNA repair defects are needed for elucidating the mechanisms of AR. The use of 1-2 types of reparation-defective cells can lead to erroneous conclusions on the necessity of DNA repair functioning for the formation of AR. AR was not observed in atactic [12], Down's syndrome [5], or xeroderma pigmentosum [2] cells. Thus, we found a cell system for identification of NG-induced DNA damage. These injuries are not corrected by the DNA repair systems but through the mechanism of AR formation. This proves that the formation of AR does not depend on the DNA repair.

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