EXPERIMENTAL GENETICS

ABSENCE OF A RADIATION-INDUCED ADAPTIVE RESPONSE OF LYMPHOCYTES IN DOWN'S SYNDROME

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The adaptive response (AR) is a phenomenon which has been found both in prokaryotic cells and in eukaryotes. Its essence is that preliminary treatment with small, virtually nonmutagenic, doses of radiation or chemical compounds lowers the sensitivity of the cells to subsequent exposure to large, damaging doses of the same or other mutagens. The first report of the existence of AR in human peripheral blood lymphocytes was published in 1984 [6]. A decrease in the yield of chromosomal aberrations (CA) in response to irradiation in the G_2 and S phases was found in cells cultured with ^{3}H-thymidine. Later workers demonstrated a similar effect of treatment with other radioactive isotopes [8]. Preliminary x-ray irradiation in a dose of 0.01 J/kg body weight was shown to have a protective action [9], and the largest dose leading to the appearance of AR was 0.2 J/kg body weight. The appearance of AR was studied in relation to the stage of the cell cycle, the interval between stimulating and damaging doses, dose rate, and quality of radiation [9, 12]. In recent years reports have appeared on the hetogeneity of the human population with respect to induction of AR [4, 5, 8]. For instance, in groups of donors (clinically healthy individuals) studied some persons have been found in whose cells AR was absent. One possible explanation of this fact, in the opinion of the workers cited, is genetically determined differences between the cells of these individuals. It is accordingly interesting to study AR in patients with syndromes of chromosomal instability, linked with changes in radiosensitivity of the chromosomes, an immunodeficiency state, disturbances of the nervous and endocrine system, shortening of the life span, and increased risk of appearance of malignant tumors [1, 7].

The aim of this investigation was to study AR in the cells of patients with Down's syndrome (DS). Incidentally, AR in syndromes of chromosomal instability have not previously been described (except for a short communication relating to the study of this phenomenon in the cells of one patient with ataxia telangiectasia) [9].

EXPERIMENTAL METHOD

Experiments were carried out on lymphocytes from 6 patients with DS, cultured by the standard method for 54 h. The age of three patients (DS1, DS2, DS3) was 4-6 months, and of the other three (DS4, DS5, DS6) it was 14-16 years. Control donors were five clinically healthy persons aged 23-25 years. Previously the writers studied AR in cells of normal donors, using different experimental schemes [3]. From many different variants we selected the following irradiation schedule. Irradiation with an adapting dose (0.03 J/kg) of x-rays (190 kV, 6 mA) was given after 30 h of culture (at the beginning of the S-phase of the cell cycle). The damaging dose of γ-rays from a ^{60}Co source was 1.5 J/kg, and irradiation was given after 48 h in culture (in the G_2-phase). Analysis of CA included counting the frequency of chromosomal and chromatid exchanges and breaks (we considered it advisable to compare the total frequency of all types of aberrations). Gaps were disregarded. Statistical analysis was carried out by the t test at the 95% level of significance for the existence of AR.

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TABLE 1. Adaptive Response in Lymphocytes of Patients with Down's Syndrome

Donor	Control	Number of cells	Number of aberrations per cell		
			observed	expected	AR
Control	Control	400	0,03±0,01		
	3 rad (S)	295	0,03±0,01		
	150 rad (G_2)	730	0,22±0,03		
DS1	3 rad (S) + 150 rad (G_2)	462	0,12±0,02	0,22±0,02	+
	Control	50	0,01±0,01		
	3 rad (S)	150	0,05±0,02		
	150 rad (G_2)	45	0,09±0,04		
DS2	3 rad (S) + 150 rad (G_2)	123	0,13±0,03	0,13±0,05	—
	Control	40	0		
	3 rad (S)	51	0		
	150 rad (G_2)	88	0,18±0,05		
DS3	3 rad (S) + 150 rad (G_2)	151	0,15±0,03	0,18±0,05	—
	Control	50	0,01±0,01		
	3 rad (S)	98	0,07±0,02		
	150 rad (G_2)	56	0,18±0,06		
DS4	3 rad (S) + rad (G_2)	98	0,16±0,04	0,24±0,06	±
	Control	56	0,07±0,04		
	3 rad (S)	92	0,11±0,03		
	150 rad (G_2)	50	0,16±0,06		
DS5	3 rad (S) + 150 rad (G_2)	83	0,20±0,05	0,20±0,05	—
	Control	45	0		
	3 rad (S)	146	0,03±0,02		
	150 rad (G_2)	175	0,10±0,02		
DS6	3 rad (S) + 150 rad (G_2)	300	0,18±0,03	0,13±0,02	—
	Control	50	0		
	3 rad (S)	100	0,07±0,03		
	150 rad (G_2)	67	0,15±0,05		
	3 rad (S) + 150 rad (G_2)	53	0,32±0,08	0,22±0,02	—

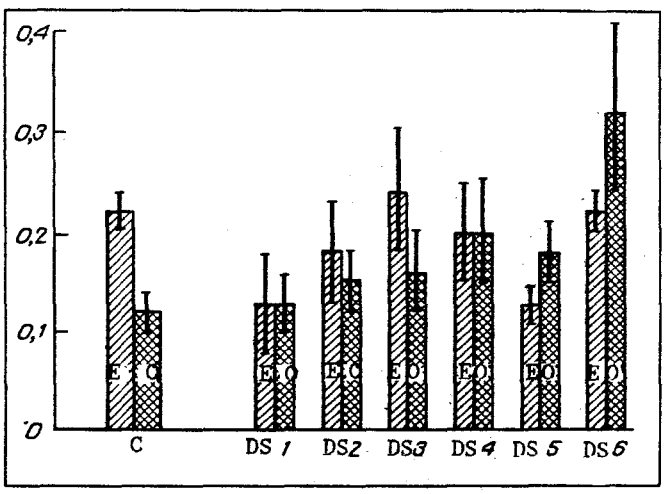

Fig. 1. Observed (O) frequency of CA, and frequency expected on the hypothesis that the effect is additive, in cells of control donors and patients with DS. Ordinate, number of aberrations per cell. C) control.

EXPERIMENTAL RESULTS

The results of irradiation of lymphocytes from the patients with DS are given in Table 1. Because no differences were found between the control donors, the results could be pooled, and from now on we shall speak of the control group

as a whole. Preliminary irradiation of cells of the control donors led to a reduction by almost half of the yield of CA after exposure to the damaging dose. As Table 1 and Fig. 1 show, in five cases the AR of the patients studied was absent. In one of them (DS3) the number of cells available for analysis did not allow any definite conclusion to be drawn regarding the presence or absence of AR ($0.90 < t < 0.95$).

We found no increase in the radiosensitivity of the cells of patients with DS compared with the control in the case of irradiation in the G_2-phase. It is interesting to note that in another study [5] variability of induction of AR likewise was not associated with any variability in the frequency of CA.

Despite active interest of research workers in the study of AR the mechanism of this phenomenon is still unknown. The hypothesis of selective death of the more radiosensitive cell subpopulation was not confirmed experimentally [11]. It can be concluded from data showing disappearance of AR after the addition of inhibitors of protein synthesis to the cell cultures that this process plays a role in adaptation [10]. Support for this view comes from data [13] on the appearance of new proteins in cells irradiated in a dose of 0.01 J/kg, which were absent in intact cells. Some workers [2] link AR with changes in the molecular structure of DNA. AR evidently arises at the level of regulation of cellular processes.

According to data in [4] the absence of AR in the normal control subjects was independent of the experimental conditions and it persisted throughout the period of investigation. It seems unlikely that this effect should depend on the physiological state of the donors; the investigators themselves attribute it to genetic differences between the donors.

Because of the genetic determination of the existence of AR, which the present investigation has confirmed, this test can be used to discover if a person belongs to a group with increased risk of developing the traits indicated above, which are linked with chromosomal instability.

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