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Cytogenetic Adaptive Response Induced by Pre—exposure in Human Lymphocytes and Marrow Cells of Mice

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In 1977, Samson and Cairns found an inducible DNA repair process, the "adaptive repair" in Escherichia Coli[1]. Human lymphocytes exposed to low doses of χ—ray, γ—ray irradiation or endogenous irradiation in the form of incorporated thymidine (^{3}H—TdR), become less susceptible to chromatid damage induced by a subsequent high dose of χ—rays or γ—rays. This adaptive response to ionizing radiation is believed to be caused by the induction of a chromosomal break repair system[2,3,4]. Olivieri et. al. (1984)first reported that human blood lymphocytes pre—expossed to low levels of ^{3}H—TdR became less susceptible to chromatid breaks induced by a subsequent high dose of χ—rays[2], In present studies, we examined the question of whether adaptive response can be elicited by low—level irradiation from ^{3}H—TdR and by low does of χ—rays and γ—rays.

Materials and methods

1. Animals and irradiation

Swiss mice, b. w. 25 ~ 30g, males and females, were used in this test. The animals were firstly irradiated by χ—rays of 0.05 Gy or 0.1Gy(D_1). 3 h later the mice were irradiated by χ—rays of 1.50 Gy(D_2).

2. Lymphocytes and irradiation

Venous blood were collected from 9 healthly, non—smoking males. 0.3ml whole blood was added to 5ml RPMI1640 medium containing 20% new calf serum, 50 μl/ml PHA, penicillin 100IU/ml and streptomycin 100μg/ml. The cells were cultured in CO_2—incubator at 37℃. Conditions of irradiation: (1)^{3}H—Thymidine(^{3}H—TdR), 3.7 ×10^{2}Bq/ml; (2)χ—ray irradiation, dose rate: 0.14Gy/min; (3)^{60}Coγ—ray irradiation at dose rate: 0.80Gy/min. The cells received second irradiation of ^{3}H—TdR, χ—rays and γ—rays at their higher dosages, 3 h after first irradiation.

3. Slide preparation

For human blood lymphocytes, the cells were cultured for 54 h after second irradiation. 0.1ml of colcemid (final concentration: 2.7×10^{-5}M) was added to each culture for mitotic rest 4 ~ 6 h before harvest. The hypotonic treatment was performed with prewarmed 0.075M KCl for 15 min at 37℃. The air—dried slides were stained with Giemsa.

For animals, colcemid (4mg/g, b. w.) was injected into mouse abdominal cavity after second irradiation, the mice were killed 3 h later, the bone marrow cells were isolated, treated with 0.075M KCl, and air—dried slides were stained with Giemsa.

4. Chromosomal aberration analysis

200 cells from each treatment group were scored. Only cells with 46 chromosomes for human and 40 chromosomes for mice were included in the analysis.

5. Statistical analysis

The χ^2—test was used for frequencies of chromosomal aberrations(CA).

Results and discussion

Table 1 summarizes the results of CA analysis of the bone marrow cells from mice,it has shown that the marrow cells in vivo can become adapted to chronic low —level χ—irradiation as a consequence of which they become less sensitive to the chromosomal aberration effects of the challenging χ — ray dose delivered subsequently. The observed frequency of CA in the marrow cells pre—exposed to low—level χ—rays is very significantly lower than the expected. However,Jacobson —Kram and Williams(1988) reported that in the bone marrow cells of mice given an "adaptive" dose of 0. 125Gy and, 24 h later,a challenge dose of 1. 50Gy of γ— irradiation,the frequencies of chromatid breaks per cell were similar to those in the groups which received the challenge dose only[5]. The differences between our and their results may be due to irradiation and experimental conditions.

The results on human blood lymphocytes are indicated in tables 2～4. These data confirm that human peripheral blood lymphocytes can become adapted to low— level irradiation from ^{3}H—TdR or exposure to a low dose of χ—or γ—irradiation. Frequencies of CA in the lymphocytes pre—exposed to low-dose irradiation were significantly lower than those in the lymphocytes received challenging dose only.

It is also shown in tables 2～3,that the lymphocytes from one donor(donor 3) did not show adaptive response after prior treatment with ^{3}H — TdR or χ — ray irraiation. These findings suggest the existence of possible variations between individuals with respect to the induction of adaptive response in their lymphocytes. A similar conclusions has also been arrived at by Bosi et. al(1988)[6]. It is possible that some of this variability is related to differences between individuals in the degree of catabolism of the nucleosides(Bodycote and wolff,1986)[7].

In conclusion, the results of our study support the view that human blood lymphocytes in vitro and mouse marrow cells in vivo,pre—exposed to low—level irradiation, can become adapted so that they become less sensitive to the chromosomal damage effects of subsequent expoures. However, there are inter— individual differences with respect to the magnitude of the adaptive effect. The mechanism of the adaptive response to low—level irradtion remains unclear, and further study is needed.

Table 1

Effects of 0.005Gy, 0.01Gy, 0.05Gyor 0.10Gy of χ—rays Given Mice on The Frequencies of Chromosomal Aberrations Induced by a Subsequent χ—ray Dose of 0.75Gy Given after 3 h.

D_1(Gy)	D_2(Gy)	Number of cells scored	Chromosome Aberration N	%	Expected
0	0	435	6	1.37	
0	0.75	442	176	39.82	
0.005	0	400	7	1.75	
0.005	0.75	354	87	24.58 * *	40.20
0.01	0	476	14	2.94	
0.01	0.75	462	116	25.11 * *	41.39
0.05	0	400	14	3.50	
0.05	0.75	496	122	24.60 * *	41.95
0.10	0	478	28	5.86	
0.10	0.75	626	146	23.32 * *	44.31

* * The observed frequency is very significantly lower than expected

Table 2

Effects of Continuous Treatment With ^{3}H—TdR(0.01μci/ml)on the Frequencies of Chromosomal Aberrations Obtained in Human Lymphocytes Treated With 0.5Gy of χ—rays

Donor No.	Treatment group	Number of Cells Scored	Chromosome Aberration N	%	Expected
1	Control	400	1	0.25	
	^{3}H—TdR	300	17	5.67	
	0.50Gy of χ—rays	400	123	30.75	
	^{3}H—TdR+0.50Gy of χ—rays	344	101	29.36 *	36.17
2	Control	400	2	0.50	
	^{3}H—TdR	400	28	7.00	
	0.50Gy of χ—rays	400	138	34.50	
	^{3}H—TdR+0.50Gy of χ—rays	400	136	34.00 *	41.00
3	Control	450	2	0.44	
	^{3}H—TdR	400	19	4.75	
	0.50Gy of χ—rays	400	106	26.50	
	^{3}H—TdR+0.50Gy of χ—rays	400	141	35.25NS	30.81
4	Control	600	3	0.50	
	^{3}H—TdR	400	44	11.00	
	0.50Gy of χ—rays	600	258	43.00	
	^{3}H—TdR+0.50Gy of χ—rays	600	246	41.00 * *	53.50
5	Control	600	2	0.33	
	^{3}H—TdR	400	38	9.50	
	0.50Gy of χ—rays	400	128	32.00	
	^{3}H—TdR+0.50Gy of χ—rays	400	90	22.50 * *	41.17

* The observed frequency is significantly lower than expected.

* * The observed frequency is very significantly lower than expected.

NS The observed frequency is not significantly lower than the expected.

Table 3

Effects of 0.05 Gy of X—rays Given at 32 h after PHA Stimulation of Human *Lymphocytes on the Frequencies* of Chromosomal Aberrations Induced by a Subsequent X—ray Dose of 1.50 Gy at 48 h

Donor No.	Treatment group	Number of cells Scored	Chromosome Aberration		Expected
			N	%	
1	contor	400	1	0.25	
	0.005Gy	400	35	8.75	
	1.50Gy	400	350	87.50	
	0.005+1.50Gy	400	321	80.25 * *	96.00
2	cotrol.	400	2	0.50	
	0.0050Gy	400	14	3.50	
	1.50Gy	400	386	96.50	
	0.005+1.50Gy	400	376	94.00 * *	99.50
3	cotrol	450	2	0.44	
	0.005Gy	400	28	7.00	
	1.50Gy	400	338	84.50	
	0.005+1.50Gy	400	372	93.00NS	91.06
4	cotrol	600	3	0.50	
	0.005Gy	400	18	4.50	
	1.50Gy	600	472	78.67	
	0.005+1.50Gy	400	218	54.50 * *	82.67
5	cotrol	600	2	0.33	
	0.005Gy	500	62	12.14	
	1.50Gy	400	194	48.50	
	0.005+1.50Gy	400	140	35.00 * *	59.57

* The Observed frequency is significantly lower than expected

* * The observed frequency is very significantly lower than expected

NS The observed frequency is not significantly lower than expected.

Table 4

Effects of 0.05Gy or 0.10Gy γ—rays Given at 32 h after PHA Stimulation of Human Lymphocytes on The Frequencies of Chromosomal Aberrations Induced by a Subsequent γ—ray Dose of 1.50Gy Given at 48 h.

Donor No.	D_1(Gy)	D_2(Gy)	Number of Cells Scored	Chromosome Aberration		Expected
			N	%		
1	0	0	400	3	0.75	
	0	1.50	400	142	35.50	
	0.05	0	400	15	3.75	
	0.05	1.50	400	113	28.25 * *	38.50
	0.10	0	400	18	4.50	
	0.10	1.50	400	119	29.75 * *	39.25
2	0	0	450	3	0.67	
	0	1.50	400	139	34.75	
	0.05	0	400	13	3.25	
	0.05	1.50	400	108	27.00 * *	37.33
	0.10	0	400	16	4.00	
	0.10	1.50	400	110	27.50 * *	38.08
3	0	0	489	4	0.82	
	0	1.50	400	148	37.00	
	0.05	0	400	17	4.25	
	0.05	1.50	400	98	24.50 * *	40.43
	0.10	0	450	28	6.22	
	0.10	1.50	460	129	28.04 * *	42.40

* * Observed frequency is very significantly lower than expected

References

1. Samson,L. and J. Cairns (1977)Natwre,267,281,1977.
2. Olivieri,G. et. al. ,Science,223:594,1984.
3. Vijayalaxmi,et. al. , Mutat. Res. 221:1,1989.
4. Sankaranarayanan, K. et. al. ,Mutat. Res. ,211:2,1989.
5. Jacobson—Kram,D. ,et. al. ,Environ. Mol. Mutagen. ,11(suppl. 11),49—50,1988.
6. Bosi,R. ,et. al. ,Mutat. Res. 211:13,1988.
7. Bodycote,J. ,et. al. ,Proc. Natl. Acad. Sci. ,USA. ,83:4749,1986.