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损伤淋巴细胞的作用

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IL-2 ON IRRADIATED HUMAN PERIPHERAL
BLOOD LYMPHOCYTES

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摘　要

用 1～40 Gy 不同剂量 γ 线照射人外周血淋巴细胞，加 PHA 刺激剂培养淋巴细胞，制备含 IL-2 的上清标本，以观察内源性 IL-2 的动力学；加纯化 IL-2 制剂观察外源性 IL-2 对损伤细胞的效应。以 CTLL 细胞测定 IL-2 的活性单性，用 ^{3}H-TdR 掺入法测定细胞增殖和单克隆抗体检测 T 细胞亚群。实验结果表明：受照射的淋巴细胞 DNA 合成降低，但在 1～10 Gy 范围内可被加入纯化 IL-2 而部分逆转。2.5 Gy 照射的淋巴细胞，各种 T 细胞亚群的数目均减少，CD_4^+/CD_8^+ 细胞比值升高，当加 IL-2 后，T 细胞的数目和亚群比值均有所恢复。IL-2 动力学显示，1～10 Gy 照射淋巴细胞，IL-2 产量随照射剂量的加大而增加，大于 10 Gy 时 IL-2 产量逐步下降，其峰值在 10 Gy。

EFFECTS OF EXOGENOUS AND ENDOGENOUS IL-2 ON IRRADIATED HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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ABSTRACT

Human peripheral blood lymphocytes were irradiated with 1 to 40 Gy of γ-ray, and then cultured with PHA to prepare supernatant containing IL-2 for observation of kinetics of endogenous IL-2 production and reversion of lymphocyte proliferation after adding a highly purified IL-2. IL-2 activity was determined by the ability to sustain IL-2 dependent cell line (CTLL), lymphocyte proliferation was defermined by ^{3}H-TdR incorporation and T lymphocyte subsets by monoclonal antibodies. The experimental **results** showed that lymphocytes exposed to ^{60}Co synthesized less DNA than nonirradiated lymphocytes. The inhibitory effect can partially reversed by purified IL-2 at the γ-ray dose range of 1 to 10 Gy, while irradiation with 2.5 Gy resulted in a reduction of T cells and T subsets, and increase in CD_4^+/CD_8^+ ratio. The ratio of subsets recovered after adding IL-2. The kinetics of IL-2 production showed that the endogenous IL-2 production rose markedly with increasing dose of irradiation at the range of 1 to 10 Gy, and the peak of IL-2 production was at the γ-ray dose of 10 Gy.

In vivo and in vitro studies have demonstrated the high radiosensitivity of the small lymphocytes. After γ-ray irradiation[1-2], any immune functions are severely damaged and the ability to combat infection and tumor is much lowered. Therefore, whether the damaged immune functions can be restored completely or partially is one of the important research subjects.

IL-2 has multiple immunoenhancing properties[3-4]. Recent studies in rodents and human demonstrated that decreased immune responsiveness in vitro can be reversed by IL-2[5-6]. The resumption of in vitro reactivity of cells from patients with immune defects such as Nezoloff's syndrome, patients with leprosy, cancer, and of irradiated cells from rodents has been demonstrated by adding IL-2.

1 METHODS

1.1 Cell culture medium

The culture medium was RPMI 1640 (GIBCO) supplemented with 25 mM HEPES, 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 u/ml penicillin, 100 μg/ml streptomycin, 10% new born calf serum, Which was known as the complete RPMI 1640 medium.

1.2 Lymphocyte preparation and irradiation[7-8]

Human peripheral blood lymphocytes (HPBL) from heparinized blood of healthy blood donors were enriched by Ficoll Hypaque gradient of lymphocyte separation media. The HPBL were washed three times with Ca^{2+} and Mg^{2+} free Hanks solution, then adjusted to a concentration of 4×10^{6} cells/ml in complete RPMI 1640 medium.

Cell suspensions in culture flasks were irradiated with ^{60}Co γ-ray at doses of 1, 2, 5, 5, 10, 20 and 40 Gy respectively, and 0 Gy was used as control sample.

1.3 Preparation of IL-2-containing supernatant sample

Equal volume of complete RPMI 1640 medium containing 200 μg/ml of mitogen (PHA) was added, to lymphocyte suspensions irradiated with various doses. This PHA concentration was selected previously to yield maximum lymphocyte stimulation index. Cell suspensions at 2×10^{6} cells/ml were placed in an incubator with humidified atmosphere containing 5% Co_2 at 37 C for 24, 48, 72 and 96 h. The supernatant of mitogen stimulated HPBL culture was obtained by centrifugation at 2000 r. p. m. for 20 min, then passed through 0.22 μm Millipore filter membrane, and samples were stored at 20 C for IL-2 assay.

1.4 IL-2 assay

The biological activity of IL-2 was determined by IL-2 concentration-dependent stimulation of proliferation of a cloned murine cytotoxic T lymphocyte line (CTLL)[9-10].

The cell proliferation ability was measured by ^{3}H-TdR incorporation. The dilution of a sample and a standard preparation yielding 50% of the maximum CTLL ^{3}H-TdR incorporation were determined. The IL-2 activity (units per ml) was calculated by the following formula:

$$\text{IL-2 activity of the sample (u/ml)} = \frac{\text{Sample 50\% max. dilution}}{\text{Standard 50\% max. dilution}} \times \text{Standard IL-2 activity (u/ml)}$$

1.5 Use of IL-2 for counter acting the effect of irradiation on lymphocytes

Irradiated and nonirradiated lymphocytes were resuspended in complete RPMI 1640 medium containing PHA 200 μg/ml to form suspensions at 1×10^6 cells/ml. The cell suspensions were added in triplicate to 96-well microtitration plates (Linbro USA) and 100 μl (50 u) of highly purified human IL-2 was added to each well. The plates were then incubated at 37°C for 72 h. The 37 kBq ^{3}H-TdR was added for the last 16 h of incubation to each well. Sample radioactivity was measured by liquid scintillation counting[11,12].

1.6 Determination of T cell subsets by immunofluorescent method

The lymphocytes were enriched on a Ficoll Hypaque gradient to prepare 5×10^6 cells/ml suspension, which was damaged by 2.5 Gy ^{60}Co irradiation, and 0 Gy was used as control. The culture cell suspensions were collected on time, washed twice in PBS containing 0.1% NaN_3 and 1% goat serum and transferred into plastic tubes at concentration 1×10^6 cells/tube. Monoclonal antibodies CD_3, CD_4 and CD_8 (Ortho USA) 10 μl each were added on ice for 1 h. 1×10^6 cells in 100 μl were incubated with monoclonal antibodies CD_3, CD_4 and CD_8 10 μl on ice for 1 h. After washing, the cells were incubated with FITC-coupled goat anti-mouse Ig antibody for another 1 h on ice, then washed twice and the immunofluorescent stained cells were examined with a Zeiss fluorescent microscope by counting percentage of positive cells in 200 lymphocytes.

2 RESULTS

2.1 Influence of γ- irradiation on IL-2 production

When human peripheral blood lymphocytes were exposed to γ-ray at the dose range of 0 to 10 Gy, IL-2 production was markedly increased with increasing doses of irradiation (Fig. 1). IL-2 production was maximal at 10 Gy irradiation. When human peripheral blood lymphocytes were exposed to 20 to 40 Gy, IL-2 production was decreased with increasing doses of irradiation (Statistical significance of differences: 10 Gy vs 0, 1, 2, 5 and 40 Gy, $P < 0.01$; 10 Gy vs 5 Gy, $P < 0.05$; 10 Gy vs 20 Gy, $P < 0.05$).

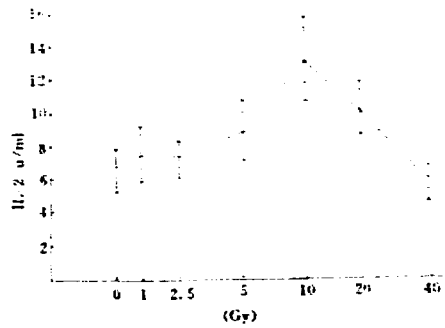

Fig. 1 Effect of different doses of irradiation on the production of IL-2 by human peripheral blood lymphocytes

The kinetics of IL-2 production by the human peripheral blood lymphocytes were altered, the peak of IL-2 production changed from 24 h of culture for non-irradiated cells to 48 h of culture for irradiated cells (Fig. 2).

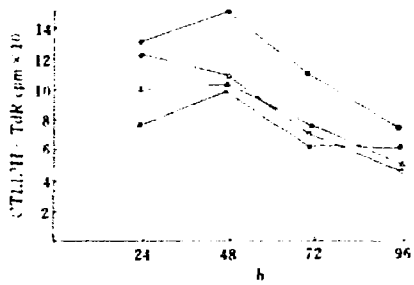

Fig. 2 Effect of irradiation on kinetics of IL-2 production by human peripheral blood lymphocytes

0 Gy; △ 2.5 Gy; ● 10 Gy; ▲ 40 Gy.

2.2 The restoration of inhibitory effect of proliferation on irradiated lymphocytes by IL-2

The human peripheral blood lymphocytes exposed to 1 to 40 Gy of γ-ray incorporated less ^{3}H-TdR than non-irradiated peripheral blood lymphocytes when subsequently cultured with PHA. The inhibitory effect of irradiation on proliferation of human peripheral blood lymphocytes can partially be counteracted by highly purified IL-2 at the dose range of 0 to 10 Gy. However, when human peripheral blood lymphocytes were exposed to radiation at doses higher than 10 Gy, this inhibition could not be reversed

by adding IL-2 (Table 1) (Statistical significance of differences: 1 and 2.5 Gy, $P <$ 0.01; 5 and 10 Gy, $P < 0.05$; 20 and 40 Gy, $P > 0.05$).

Table 1 Effect of IL-2 on proliferation of irradiated human peripheral blood lymphocytes

Dose (Gy)	PHA (100ug/ml)		PHA plus IL-2		P-value
	cpm	%	cpm	%	
0	15021±3325	100	17111±2473	100	
1	12618±2781	82.2± 3.6	15622±2647	91.7± 6.7	<0.01
2.5	11307±2788	74.9± 7.1	14431±2117	86.8± 5.9	<0.01
5	8438±1986	56.9±12.1	11206±1829	64.5± 7.5	<0.05
10	5729±1127	38.5± 7.6	7965±1934	47.0±11.0	<0.05
20	4331±1692	28.6± 8.6	5432±1187	31.3± 5.7	>0.05
40	4113±1892	27.7± 8.9	4991±1196	29.4± 5.7	>0.05

Irradiated and non-irradiated PBL were cultured for 4 days with PHA (100ug/ml) in the absence or presence of IL-2 (20u/ml). Results were average values of six donors.

P values were determined by Student's t-test.

2.3 Influence of γ-ray irradiation and IL-2 on T lymphocyte subsets

The change of CD_3, CD_4, and CD_8 subsets in human peripheral blood T lymphocytes damaged by 2.5 Gy ^{60}Co gamma rays was studied with indirect immunofluorescent method in the presence and absence of IL-2. The specific binding ability of CD_3, CD_4 and CD_8 monoclone antibodies with membrane antigen of the lymphocyte subsets was determined. The purpose of this experiment was to observe the effect of IL-2 on T lymphocyte subsets damaged by irradiation. T cell subsets of human peripheral blood in irradiated culture were compared with non-irradiated control culture. The results showed that 2.5 Gy irradiation resulted in a reduction of CD_3, CD_4 and CD_8 cells (Statistical significance of differences: CD_3 cells, $P < 0.001$; CD_4 cells, $P < 0.002$, CD_8 cells, $P < 0.001$). T cell CD_4/CD_8 ratio (helper/suppressor ratio) rose, indicating that the percentage of CD_8 cells declined more dramatically (Fig. 3) (Non-irradiated T cell CD_4/CD_8 ratio = 1.65±0.25; Irradiated T cell CD_4/CD_8 ratio = 1.83±0.53). When T cells exposed to 2.5 Gy were cultured for 96 h in the presence of IL-2, the percentage of CD_3, CD_4 and CD_8 cells rose markedly, being higher than that in the absence of IL-2 ($P < 0.01$) as shown in Fig 1, 5 and 6.

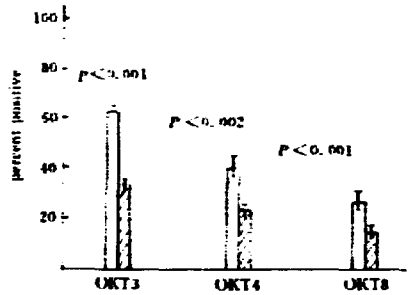

Fig. 3 Effect of irradiation on T cell subsets in human peripheral blood lymphocytes

□ 0 Gy OKT4 R=1.65±0.25 ■ 2.5Gy OKT4 R=1.83±0.53

Irradiated and non-irradiated T cells cultured for 4 h were identified by indirect immunofluorescence.

The results were average values of five donors.

Significant differences between mean values were determined by Studen's t-test.

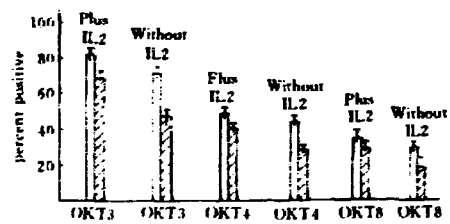

Fig. 4 Effect of IL-2 containing supernatant on irradiated subsets in human PBL.

□ 0 Gy ■ 2.5 Gy

Irradiated and non-irradiated T cells cultured for 96 h in the absence or presence of IL-2 were identified by indirect immunofluorescence.

Results were average values of five donors.

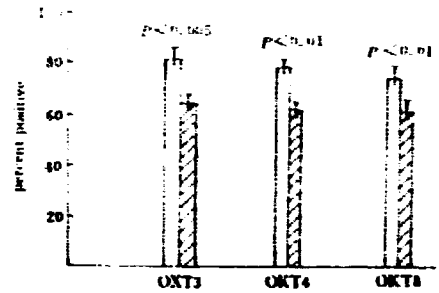

Fig. 5 Effect of IL-2 containing supernatant on relative percentage of irradiated T cell subsets

□ — plus IL-2 ■ — without IL-2

Relative percentage was calculated by the formula:

$$\frac{\text{The percentage of irradiated T cell subsets (with or without IL-2)}}{\text{The percentage of non-irradiated T cell subsets (with or without IL-2)}} \times 100\%$$

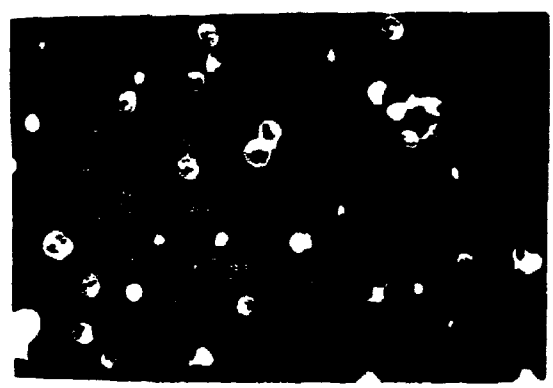

Fig. 6 Positive T cell subsets by indirect immunofluorescent method

3 DISCUSSIONS

Human peripheral blood lymphocytes were irradiated with γ-ray in order to study the effect of IL-2 on irradiated lymphocytes. IL-2-containing supernatants were prepared by PHA-stimulated peripheral blood lymphocytes and a purified IL-2 preparation for parallel control sample was used. IL-2 activity in preparation was determind by the ability to sustain IL-2 dependent cell line (CTLL). Cell proliferation was measured by tritiated thymidine uptake and IL-2 activity was observed. It should be noted that irradiation at the experimental doses led to decrease in the proliferation response to PHA as compared with the non irradiated peripheral blood lymphocytes. It was also found that IL-2 could reverse

the decreased proliferation. The reversion rate reached 20% ~ 30%. The results agree with Manori's opinion[13,14]. The expression of surface membrane antigens on irradiated peripheral blood lymphocytes was visualized by indirect immunofluorescence using McAb of anti-CD_3, CD_4 and CD_8. From the results mentioned above it is reasonable to conclude that IL-2 has apparent effect on the recovery of peripheral blood lymphocytes damaged by γ-irradiation.

It is evident that each T cell subset has radiosensitive T lymphocytes and relatively less radiosensitive cells. Both T cell subsets are able to proliferate in the presence of IL-2. The above finding suggest that the use of IL-2 appears to hold some promise for the treatment of radiation injury.

4 CONCLUSION

(1) Human peripheral blood lymphocytes exposed to ^{60}Co γ-irradiation showed less incorporation of ^{3}H-TdR than non-irradiated lymphocytes when cultured with PHA subsequently.

(2) The inhibitory effect of radiation on proliferation of lymphocytes can be partially reversed by highly purifed IL-2 at the dose range of 0.1 to 10 Gy. When lymphocytes exposed to higher than 10 Gy irradiation, the inhibition could not be reversed by adding IL-2.

(3) 2.5 Gy irradiation resulted in reduction of CD_3, CD_4 and CD_8 cells. When T cells exposed to 2.5 Gy were cultured for 96 h in the presence of IL-2, the percentage of above T subsets rose markedly as compared with that in the absence of IL-2.

(4) When the lymphocyte exposed to ^{60}Co at 0.1 to 10 Gy, endogenous IL-2 production rose markedly with increasing dose of irradiation. IL-2 production decreased when the radiation dose was higher than 10 Gy.

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