

SHORT COMMUNICATION

The Effect of Irradiation on the Release of Lymphocyte-Activating Factor (LAF)

B. GEIGER, RUTH GALLILY, AND I. GERY

Departments of Immunology and Medical Ecology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

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Peritoneal cells from irradiated mice released two to three times more lymphocyte-activating factor (LAF) than did the cells from untreated controls. Similar results were obtained with unfractionated or enriched adherent cells. On the other hand, irradiation of adherent cells *in vitro* did not affect the level of factor released.

INTRODUCTION

Several functions of macrophages damaged by total body X-irradiation include the capacity to digest chicken red cells (1), and to retain or kill bacteria (2). In some experimental systems total body irradiation impaired the immunogenic function of macrophages (3-5). On the other hand, phagocytosis and clearance of colloidal particles by phagocytes of the reticuloendothelial system were unaffected by irradiation (6, 7).

Adherent cells, consisting mainly of macrophages, are capable of releasing soluble factors that potentiate the response of lymphocytes to mitogenic stimulants. The activity of the potentiation factors was demonstrated in the mixed lymphocyte reaction (8) and in the stimulation of thymocytes by various mitogens (9-11). The later factor was termed "lymphocyte-activating factor" (LAF). This paper presents data concerning the effect of irradiation on the release of LAF by peritoneal adherent cells.

MATERIALS AND METHODS

Irradiation in vivo. Female BALB/c mice, 3-4 months old, were exposed to total body X-irradiation (550 or 800 R) in a Picker-Vanguard X-ray machine (Physical conditions: 250 kV, 15 mA with 1 mm Cu filter, FSD 50 cm, dose rate: 100 R/min).

Collection of peritoneal cells (PC). Normal or irradiated mice (48 hr postirradiation) were killed by cervical dislocation and injected intraperitoneally with 3-4 ml of Hanks' balanced salt solution (HBSS). The animals were vigorously

shaken and the peritoneal fluid collected into ice cold tubes. The total yield of PC was $4.0\text{--}5.0 \times 10^6$ per normal mouse with about 30% macrophages, or $1.5\text{--}2.0 \times 10^6$ per irradiated animal, with about 85% macrophages, characterized by their morphology.

Enrichment of adherent cells. The collected PC were centrifuged (200g, 5 min), resuspended in HBSS containing 10% newborn calf serum (Microbiological Associates, Bethesda, MD), and cultured in petri dishes (30 × 10 mm, NUNC, Denmark) in aliquots of 2 ml containing $5\text{--}10 \times 10^5$ macrophages. After incubation for 40 min at 37° C in 5% CO₂ and 95% air, the nonadherent cells were removed by rinsing with HBSS. The remaining cells, which adhered firmly, consisted of 90–95% macrophages and about 5% polymorphonuclear cells with some lymphocyte-like cells. The macrophages were identified by their ability to engulf *Staph. albus*.

Irradiation in vitro. Monolayers of adherent PC were irradiated (550, 800, or 5000 R) by using Rich, Seifert & Co. Dermovolt X-ray machine (Physical conditions: 56 kV, 20 mA with a 1 mm Al filter, FSD: 10 cm, dose rate: 750 R/min).

Culture supernatant fluids (SUP). Cultures of peritoneal cells, unfractionated or enriched, were set in petri dishes in HBSS with 10% newborn calf serum to yield $5\text{--}10 \times 10^5$ adherent cells per dish. The number of adherent cells was calcu-

TABLE 1
THE EFFECT OF IRRADIATION *in vivo* ON LAF RELEASE BY PERITONEAL CELLS

Expt no.	Irradiation ^a of macrophage donors (R)	Peritoneal cells		Thymocytes	
		Cell type	Number of adherent cells/culture	³ HTdr uptake (cpm) ^b SUP alone	SUP + PHA
1	—	None	—	144	1,443
	—	Unfractionated ^c	8×10^5	187	6,287
	550	Unfractionated	8×10^5	452	11,818
	800	Unfractionated	8×10^5	481	9,430
2	—	None	—	61	613
	—	Unfractionated	8×10^5	160	5,615
	550	Unfractionated	8×10^5	163	10,807
	800	Unfractionated	8×10^5	195	10,193
3	—	None	—	183	1,944
	—	Adherent ^d	9×10^5	275	7,552
	550	Adherent	9×10^5	517	11,300
	800	Adherent	9×10^5	407	10,417
4	—	None	—	173	2,083
	—	Adherent	8×10^5	234	6,798
	550	Adherent	8×10^5	512	15,028
	800	Adherent	8×10^5	1,028	18,715

^a Irradiation of macrophage donors was carried 48 hr before PC withdrawal.

^b Incorporation of ³H-thymidine by $3\text{--}4 \times 10^6$ thymocytes during the last 24 hr in the presence of 0.2 ml SUP (with or without PHA). Each value represents the mean of duplicate cultures.

^c Total population of cells withdrawn from the peritoneal cavity of mice.

^d Cells remaining attached to culture dishes after 40-min incubation of unfractionated cell population; nonadherent cells were removed by rinsing.

TABLE 2
THE EFFECT OF X-IRRADIATION *in vitro* ON LAF RELEASE
BY PERITONEAL ADHERENT CELLS

Expt	Irradiation (R)	Peritoneal cells		Thymocytes	
		Number of adherent cells	Incubation time ^a post irradiation (hr)	³ HTdr Uptake (cpm)	
				SUP alone	SUP + PHA
1	—	None	—	61	613
	—	5×10^5	—	126	1,957
	550	5×10^5	0-24	82	2,098
	800	5×10^5	0-24	83	1,389
	5,000	5×10^5	0-24	226	2,026
2	—	None	—	173	2,083
	—	7×10^5	—	236	7,284
	550	7×10^5	0-24	285	7,460
	800	7×10^5	0-24	330	6,016
	5,000	7×10^5	0-24	234	5,730
3	—	None	—	61	613
	—	5×10^5	—	105	1,568
	550	5×10^5	24-48	234	1,939
	800	5×10^5	24-48	173	2,655
	5,000	5×10^5	24-48	172	2,045
4	—	None	—	173	2,083
	—	7×10^5	—	211	6,682
	800	7×10^5	24-48	167	4,410
	5,000	7×10^5	24-48	152	4,412

^a The release of LAF during 24 hr of incubation either immediately after irradiation (0-24) or 24 hr later (24-48 hr).

lated by counting representative fields of known surface area. After incubation for 24 hr, the supernatant fluids (SUP) were removed, centrifuged (500*g* for 10 min), filtered through 0.22- μ m Millipore filters, and stored at -20° C.

Assay for LAF activity. The ability of the SUP preparations to stimulate mouse thymocytes and to potentiate their response to phytohemagglutinin (PHA) was measured as described elsewhere (9). Thymus cells ($3-4 \times 10^6$) from 6-week-old female BALB/c mice were cultured for 72 hr in 0.8 ml minimal essential medium (MEM-S, Microbiological Associates Israel, Jerusalem), supplemented with 6% normal human serum. The response of the thymocytes, with or without PHA, was determined by the incorporation of ³H-thymidine (³HTdr) and expressed as the mean counts per minute values of duplicate cultures. The differences between the duplicate values usually did not exceed 10%.

RESULTS AND DISCUSSION

The levels of LAF activity in supernatant fluids from PC cultures of irradiated mice are summarized in Table 1. The supernatant fluids obtained from cultures of unfractionated PC or enriched adherent cells were similar active and markedly

potentiated the mitotic response of thymocytes to PHA. In all experiments, culture supernatant fluids of PC from irradiated mice were two to three times more active than those derived from the corresponding unirradiated controls. Moreover, in some experiments, supernatant fluids from cultures of irradiated mice increased significantly the incorporation of $^3\text{HTdr}$ by thymocytes in the absence of PHA (Table 1, Expts. 1, 3, 4).

In contrast, no consistent changes in LAF release was detected after irradiation of the adherent cells *in vitro* (Table 2). Irradiation doses of 500, 800, and 5000 R caused inconsistent small changes in the levels of LAF. No difference was detected between levels of LAF released from the cells immediately after irradiation and 24 hr later.

This study supports the previous findings, that LAF is made by adherent cells. Unfractionated PC and the corresponding enriched macrophages were similarly active in releasing the substance.

The reason for the increase in release of LAF by irradiation *in vivo* is not understood. The fact that irradiation *in vitro* does not change the LAF release suggests that the effect is indirect. There are two main possibilities: Irradiation may enhance the ability of peritoneal cells to produce or release LAF. This suggestion is in agreement with the findings of an increase in activity of several functions of macrophages which follows irradiation (12, 13). Alternatively, irradiation may select a subpopulation of PC which possesses an increased capacity to produce LAF, as Allison and his collaborators have found at least two subpopulations among the adherent cells of the peritoneal cavity (personal communication). In addition, irradiation has a transient influence on the kinetics of macrophage differentiation (14).

The increase in release of LAF may be related to an attempt of the organism to overcome the damaging effect of irradiation. LAF causes the proliferation of lymphocytes needed for regenerating the depleted lymphoid system. This hypothesis is now under investigation.

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