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Activation of human B lymphocytes

VIII. DIFFERENTIAL RADIOSENSITIVITY OF SUBPOPULATIONS OF LYMPHOID CELLS INVOLVED IN THE POLYCLONALLY-INDUCED PFC RESPONSES OF PERIPHERAL BLOOD B LYMPHOCYTES

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Summary. The differential effect of various doses of irradiation on subpopulations of human peripheral blood lymphoid cells involved in the pokeweed mitogen (PWM) induced PFC response against sheep red blood cells (SRBC) was studied. The plaque forming B cells were quite sensitive to low doses of irradiation with complete suppression of responses at 300 to 500 rad. On the contrary, helper T-cell function was resistant to 2000 rad. Co-culture of irradiated T cells with autologous or allogeneic B cells resulted in marked enhancement of PFC responses consistent with the suppression of naturally occurring suppressor cells with a resulting pure helper effect. Irradiated T-cell-depleted suspensions failed to produce this effect as did heat killed T cells, whereas mitomycin C treated T cells gave effects similar to irradiated T cells. These findings are consistent with a lack of requirement of cell division for a T-cell helper effect and a requirement of mitosis or another irradiation sensitive, mitomycin C sensitive process for a T-suppressor cell effect. These studies have potential relevance in the evaluation of subpopulations of human lymphoid cells involved in antibody production in normal individuals and in disease states.

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INTRODUCTION

It is now well established that T lymphocytes exert critical regulatory influences on B-cell function in both animal and human systems (Gershon, 1974; Katz & Benacerraf, 1972; Waldmann & Broder, 1977). Distinct subpopulations of T cells manifesting helper or suppressor function have been identified in the mouse (Cantor & Boyse, 1975), and most recently Moretta, Webb, Grossi, Lydyard & Cooper (1977) have demonstrated functionally distinct subpopulations of human T cells which can be identified by the presence of an Fc receptor for either IgM (helper T cell) or IgG (suppressor T cell).

It is generally agreed that subpopulations of mononuclear cells differ in their sensitivity to ionizing radiation (Anderson & Warner, 1976). In that regard, using a system of pokeweed mitogen (PWM)-driven immunoglobulin production by human B cells, Moretta *et al.* (1977) have shown that their suppressor T cells were sensitive to radiation while helper T cells were radioresistant. In addition,

it has been demonstrated that co-culturing of irradiated purified human peripheral blood T cells with autologous or allogeneic B cells resulted in a significant enhancement of immunoglobulin production suggesting a selective inhibitory effect of radiation on suppressor as opposed to helper T-cell function (Siegal & Siegal, 1977).

Using a recently described haemolysis-in-gel plaque forming cell (PFC) assay to measure PWM-induced antibody production against sheep red blood cells (SRBC) by human peripheral blood B cells (Fauci & Pratt, 1976a), we have shown this system to be dependent on T cells (Fauci, Pratt & Whalen, 1976) and to operate by a balance between helper and suppressor phenomena (Haynes & Fauci, 1977; Fauci *et al.*, 1977).

In the present paper, we demonstrate the comparatively exquisite radiosensitivity of the plaque-forming B cell compared to the relative radioresistance of the helper function of unfractionated mononuclear or purified T-cell suspensions in this assay.

MATERIALS AND METHODS

Cell suspensions

Heparinized venous blood was obtained from normal adult donors and mononuclear cell suspensions were obtained by standard Hypaque-Ficoll density centrifugation. Mononuclear cell suspensions that were either enriched or depleted of T lymphocytes were obtained by sheep erythrocyte (E) rosetting of lymphocytes followed by separation of rosetted and non-rosetted cells by centrifugation over Hypaque-Ficoll gradients as previously described in detail (Fauci *et al.*, 1976). T-cell enriched suspensions generally contained between 95% and 100% T cells, while T-cell depleted suspensions contained less than 0·5% T-cells with approximately 45 to 53% B cells and an enrichment of monocytes to approximately 40 to 45%. Monocytes and subpopulations of lymphocytes were identified by previously described techniques (Fauci *et al.*, 1976).

Irradiation of cell suspensions

In various experiments, either unfractionated mononuclear cell suspensions, T-cell enriched or T-cell depleted suspensions were irradiated with a wide dose range of *X*-irradiation from 25 rad up to 10,000 rad. The source of radiation was a Philips 250 kVp dual head *X*-ray system.

Following radiation, cells were washed once in RPMI-1640 media (Grand Island Biological Co., Grand Island, NY) and resuspended in fresh media for culture as described below.

Mitomycin C treatment and heat killing

In certain experiments, cell suspensions were either pretreated with mitomycin C (Sigma Chemical Co., St Louis, MO), 40 μg/ml for 45 min at 37° or were heated at 56° for 45 min.

Cell cultures

Culture conditions for the generation of anti-SRBC PFC responses following polyclonal activation of human peripheral blood lymphocytes with PWM have been described in detail (Fauci & Pratt, 1976a). Briefly, cells were cultured in RPMI-1640 containing 1% trypticase soy broth, 2 mM L-glutamine, 100 u of penicillin per ml, 100 μg of streptomycin sulphate per ml, and supplemented with 10% pooled human AB or A serum absorbed twice with SRBC. Cultures were carried out in 12×75 mm plastic tubes (Falcon Plastics, Oxnard, CA) at a density of 2×10^6 cells in 1 ml. Cultures were incubated on a rocker platform (7 cycles/min) for 6 to 7 days at 37° in 5% CO_2 in air at 100% humidity. Cultures were stimulated either with PWM in a wide concentration range (1/20 through 1/10,000 final dilution) or media alone as control (background PFC). In experiments in which irradiated cells were co-cultured with unirradiated cells, 1×10^6 cells of each suspension were cultured to keep the cell density constant at 2×10^6 cells in 1 ml. The same was done in co-cultures with mitomycin C treated or heat killed cells.

Data are expressed as PFC per 10^6 cells. In certain of the co-culture experiments where indicated, data are expressed as expected PFC response per 10^6 cells compared to observed PFC response per 10^6 cells. Expected PFC response is based on the individual responses of each fraction of the co-culture when cultured alone.

Assay for PFC

At the end of the culture period (6–7 days), cells were harvested and assayed for direct PFC against SRBC by an ultrathin layer haemolysis-in-gel technique as previously described in detail (Fauci & Pratt, 1976a; Fauci & Pratt, 1976b).

Blastogenic responses

Blastogenic responses of unirradiated and irradiated lymphocytes to stimulation with the mitogens phytohaemagglutinin (PHA) and PWM were determined in microtitre plates by incorporation of tritiated thymidine as previously described (Fauci 1975).

Statistical analysis

Data were compared by the Student's *t* test or by the paired sample *t* test where indicated.

RESULTS

Effect of irradiation on PFC responses

The effect of directly irradiating the responding cell populations on the PWM-induced PFC responses is shown in Fig. 1. The PFC responses are quite

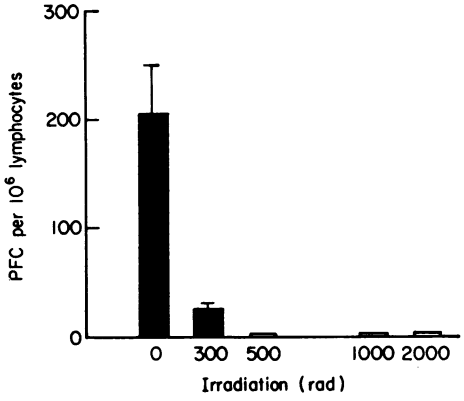

Figure 1. Effect of various doses of irradiation on PWM-induced PFC responses in human peripheral blood mononuclear cells. Unfractionated mononuclear cells were irradiated with from 300 to 2000 rad and subsequently cultured in the presence of PWM. After 6 days, cultures were harvested and assayed for PFC responses against SRBC. Data represent the mean (±SEM) of 10 separate experiments.

sensitive to low doses of irradiation with a marked diminution at 300 rad and a virtually complete suppression of PFC responses at 500 rad or greater. After exposure to 2000 rad, the cell viabilities remained between 75 and 95% after 6 days in culture with only a slight decrease in cell yield. Blastogenic responses to PHA and PWM were also markedly decreased by irradiation as shown in Fig. 2.

It is of interest that despite the fact that PFC responses were markedly decreased with 300 rad and completely suppressed with 500 rad in all individuals tested, quite variable results were seen at very low doses of irradiation. As shown in Table 1, some individuals had an enhancement of PFC responses at very low doses (25 to 100 rad) with the usual suppression at higher doses. However, when mean responses of several individuals at different lower doses of irradiation were examined, there was no dose at which significant enhancement was observed.

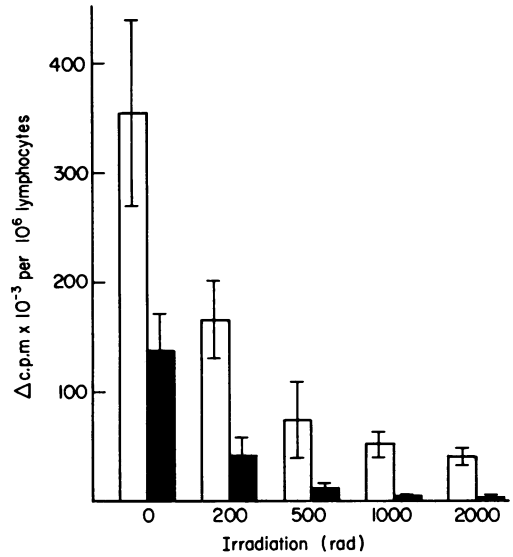

Figure 2. Effect of irradiation on the blastogenic response of human peripheral blood lymphocytes. Mononuclear cell suspensions were irradiated with various doses of irradiation and blastogenic responses were measured following stimulation with PHA (3 day cultures) and PWM (5 day). Blastogenic responses were measured by the incorporation of tritiated thymidine. Δc.p.m. equals the c.p.m. of stimulated cultures minus the c.p.m. of unstimulated cultures. Data represent the mean (±SEM) of 5 separate experiments. PHA, open columns; PWM, filled columns.

Effect of co-culturing unirradiated cells with various fractions of irradiated cells

The effect of co-culturing 1×10^6 unirradiated, unfractionated mononuclear cells with 1×10^6 irradiated autologous T cells is shown in Fig. 3. When unirradiated T cells were co-cultured with autologous unirradiated mononuclear cells there was no significant difference between expected and observed PFC responses. The co-culture of unirradiated cells with autologous T cells which had been

Table 1. Effect of low doses of *in vitro* irradiation on PFC responses in normal human peripheral blood

		PFC per 10^6 lymphocytes* (rad)					
Subject	No irradiation	25	50	100	200	300	500
1	140	725	120	225	90	18	0
2	6	45	195	600	33	0	0
3	156	195	219	99	57	36	0
4	100	54	2	0	0	0	0
5	93	176	30	19	0	48	0
6	49	79	141	114	12	15	2
7	85	27	18	16	1	20	0

* Cell suspensions were irradiated and then cultured in the presence of PWM for 6 days. Viabilities at the end of culture were 85 to 90%.

irradiated with 300 to 500 rad resulted in an obvious trend towards enhancement of responses which fell just short of statistical significance ($P > 0.05$) by a paired sample 't'-test. However, the co-culturing of unirradiated mononuclear cells with T cells pre-treated with 1000 to 2000 rad resulted in a marked enhancement of expected responses ($P < 0.01$). The same phenomenon was observed although less consistently when mononuclear cells were co-cultured with allogeneic irradiated T cells (Fig. 4).

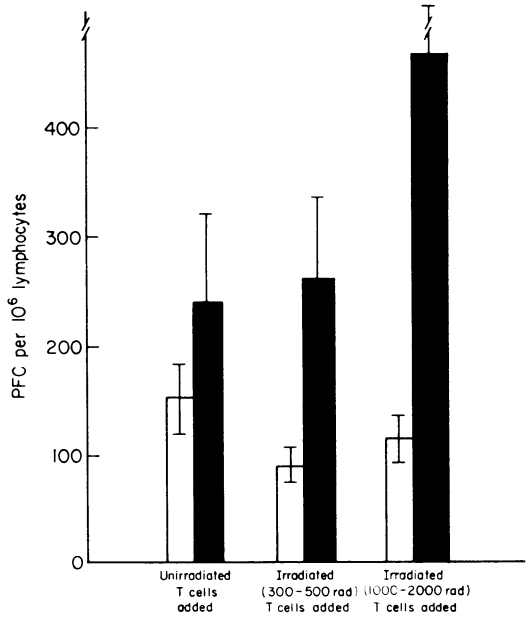

Figure 3. Effect of co-culture of irradiated T cells with autologous unfractionated mononuclear cells. Irradiated and unirradiated cells were co-cultured at a ratio of 1 : 1. Data are given as the comparison of the expected PWM-induced PFC response compared with the observed response. Data represent the mean (±SEM) of seven separate experiments. Expected PFC response, open columns; observed PFC response, filled columns.

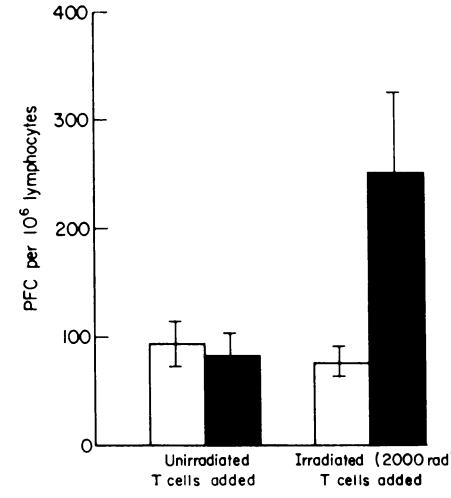

Figure 4. Effect of co-culture of irradiated T cells with allogeneic unfractionated mononuclear cells. Conditions were the same as in Fig. 3. Data represent the mean (±SEM) of nine separate experiments. Expected PFC response, open columns; observed PFC response, filled columns.

The addition of unirradiated allogeneic T cells to PWM stimulated mononuclear cells resulted in no significant differences in mean expected and observed PFC responses ($P > 0.2$). However, the co-culture of mononuclear cells with allogeneic T cells irradiated with 1000 to 2000 rad resulted in signifi-

cant enhancement over expected PFC responses ($P < 0.05$).

The same effect, although less consistently was observed when autologous, irradiated unfractionated mononuclear cells instead of T cells were used, i.e. significant enhancement was observed ($P < 0.01$). However, it should be pointed out, that as shown above, allogeneic irradiated T cells were essentially as effective as autologous irradiated T cells in enhancing PFC responses of B cells. This was not the case with allogeneic irradiated unfractionated mononuclear cells. They failed to consistently supply the same degree of enhancement as autologous irradiated unfractionated cells.

Irradiated T-cell depleted fractions, either autologous or allogeneic failed completely to enhance PFC responses, and in many cases actually suppressed the responses.

Mitomycin C treatment and heat killing

Similar enhancing effects were observed when T cells were treated with mitomycin C rather than irradiation prior to co-culture. As shown in Table 2, addition of mitomycin C treated T cells to culture resulted in enhanced PFC responses in two of three experiments.

Neither significant enhancement nor suppression of PFC responses was observed when heat killed T cells were co-cultured with PWM stimulated mononuclear cells.

Table 2. Effect of co-culture of unfractionated mononuclear cells with mitomycin C treated T cells on PFC responses

Subject	Cells added	PFC per 10^6 lymphocytes
1	T*	137
	T_{MC}†	260
2	T	167
	T_{MC}	480
3	T	125
	T_{MC}	129
4	T	75
	T_{MC}	375

* T, Fresh untreated autologous T cells preincubated with media alone.

† T_{MC}, Autologous T cells which had been preincubated with 40 μg/ml of mitomycin C for 45 min at 37°, washed four times and then added to culture.

DISCUSSION

The present study demonstrates that functional subpopulations of human peripheral blood lymphoid cells engaged in the PFC response following polyclonal activation are differentially sensitive to various levels of irradiation. The antibody forming cell itself is quite sensitive to relatively low doses of irradiation as 300 rad markedly suppressed PFC responses while 500 rad consistently abolished responses. We have previously shown this PWM-induced PFC system to be T-cell dependent (Fauci *et al.*, 1976) and to operate by a balance between 'helper' and 'suppressor' phenomena (Fauci *et al.*, 1977; Haynes & Fauci, 1977). In this regard, the present data clearly demonstrate that in co-culture experiments T-cell helper effects are radioresistant to as high as 2000 rad. These findings are in agreement with those reported for the radioresistance of helper T cells in PWM-induced intracytocytoplasmic immunoglobulin production (Siegal & Siegal, 1977) as well as for the radioresistance of functional helper T cells identified by the presence of an Fc receptor for IgM and the radiosensitivity of functional suppressor T cells identified by the presence of an Fc receptor for IgG (Moretta *et al.*, 1977).

We have further shown that irradiated T cells can also enhance the PFC responses of allogeneic B cells. This allows a convenient and reproducible method for separately evaluating B-cell function and helper T-cell function in various patient groups by allogeneic co-culture experiments.

Since unfractionated mononuclear cells contain predominantly T cells, one might expect that co-culture of irradiated unfractionated mononuclear cells with unirradiated unfractionated mononuclear cells would also result in enhancement of responses by a selective helper effect. Indeed, we found this to be the case. However, significant and consistent enhancement was only seen with autologous co-cultures of unfractionated mononuclear cells and not with allogeneic co-cultures as was seen with allogeneic irradiated T cells. The reason for this is unclear; however, it is possible that the strong mixed leucocyte reaction which one would expect against the non-T cells in the allogeneic irradiated unfractionated mononuclear cells and not against the

pure T cells in the allogeneic irradiated T cell suspensions masked the helper effect.

It is of interest that in some individuals very low doses of irradiation delivered directly to the responding cell population (Table 1) resulted in enhancement of PFC responses with complete suppression of responses at higher doses of irradiation. Although it seems, in general, that human B cells are more sensitive to irradiation than are suppressor T cells, it is possible that a subpopulation of suppressor cells in some individuals are even more sensitive to irradiation than are B cells, resulting in the occasional enhancement of responses seen at doses of irradiation lower than those which directly suppress B-cell function.

The similarity of this helper effect with mitomycin C treated T cells and not with heat killed T cells strongly suggests that live T cells whose division or mitosis has been blocked are necessary for shifting the balance towards pure helper effect. However, it must be emphasized that irradiation and mitomycin C treatment besides interfering with mitosis can have multiple effects on cells, many of which are not understood. Thus, it is possible but not absolutely certain that the mechanism of supplying 'pure help' with irradiated T cells is by interfering with the mitosis which is necessary for suppressor T cells to fully express their function. The complexity of this issue of mitosis and suppressor cells has recently been emphasized in the mouse model (Tse & Dutton, 1977).

It should also be pointed out that other non-specific factors may simulate helper effects. The occasional enhancement seen when heat-killed cells or cells irradiated with 10,000 rad resulting in markedly decreased viability (data not shown) were added in co-culture may well be due to the non-specific adjuvant effects of nucleic acid degradation products released by dead cells (Braun, 1965). However, it is clear that a consistent and reproducible helper effect is seen in co-cultures with normal irradiated (2000 rad) autologous or allogeneic T cells whose viabilities are not significantly decreased.

Thus, this system has potential clinical relevance in the evaluation of functional capabilities of subpopulations of human lymphoid cells involved in antibody production.

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