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Melphalan Treatment of Human Peripheral T Cells Promotes Ig Production by B Cells *in Vitro**

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Abstract—*Human blood B lymphocytes were cocultured with autologous T lymphocytes which were pretested with melphalan or adriamycin in the presence of pokeweed mitogen (PWM). Melphalan treatment of the cells produced an increased secretion of IgG and IgM, whereas adriamycin did not.*

INTRODUCTION

THE MAJORITY of cytostatic agents used in the chemotherapy of malignant diseases have immunosuppressive properties [1-6]. Thus cytostatics may cause depletion of the number of circulating blood lymphocytes with regard to both T and B lymphocytes and impairment of different immune responses [1-6].

Previous studies have demonstrated that B cells are more vulnerable to the action of melphalan and adriamycin than T cells, as measured by the cells' ability to express receptors for C'3 and sheep erythrocytes [7]. Since the elicitation of an immune response frequently represents an end product of an interaction between different T cell subsets and B cells, it was considered of interest to investigate the effect of melphalan and adriamycin treatment of human peripheral T cells on their capacity to interact with B cells in PWM-induced Ig synthesis.

MATERIALS AND METHODS

Blood sampling

Heparinized venous blood was obtained from 17 healthy staff members.

Separation of lymphoid cells

Lymphoid cells were separated by centrifugation of blood on Ficoll-Isopaque [8]. The cells were washed four times by centrifugation in a balanced salt solution (BSS) and twice in RPMI 1640 medium supplemented with L-glutamine, antibiotics and 20% heat-inactivated fetal calf serum (FCS). Cell preparations were depleted of monocytes by adherence in a plastic bottle for 30 min at 37°C.

Lymphocyte subpopulations

T- and B-enriched cell fractions were separated from such purified lymphocyte preparations by a rosette sedimentation technique using neuraminidase-treated sheep red blood cells (SRBC) [9]. The SRBC-lymphocyte mixture was incubated in 50% FCS at 4°C overnight. After gradient centrifugation T cells were recovered from the pellet and SRBC were lysed with NH_4Cl. This T cell-enriched suspension contained 96-100% SRBC rosette-forming cells (E-binding cells). The B cell-enriched fraction was recovered from the interphase and contained less than 3% of E-binding cells.

Cytotoxic drugs

Melphalan was purchased from Wellcome (The Wellcome Foundation Ltd, London, U.K.). The concentrations used were 0.04, 0.4 and 4 μg/ml. Adriamycin (doxorubicin hydrochloride) was a gift from Farmitalia Carlo Erba (Farmitalia Carlo Erba AB, Täby, Sweden). The concentrations employed were 0.01, 0.05 and 0.1 μg/ml. Adriamycin concentrations higher than 0.5 μg/ml resulted in a pronounced cell death (>50%) within 24 hr after treatment. These concentrations of both melphalan and adriamycin are in the same range as used in the *in vitro* drug sensitivity assay [10].

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PWM

Vials of pokeweed mitogen (PWM) were purchased from Grand Island Biological Co., Grand Island, NY. The same batch was used for all tests. The contents of each vial were dissolved in 5 ml of distilled water. Lymphocytes were stimulated with PWM at a final dilution of 0.2 mg/ml.

Experimental design

The T cell-enriched cell preparations were incubated in RPMI 1640 medium at a concentration of 10^6 cells/ml with or without melphalan or adriamycin. The time of exposure to the drug was 2 hr in a 5% CO_2-air atmosphere at 37°C. The cells were then washed three times and resuspended in RPMI 1640 medium. The frequency of viable cells was higher than 95%, as determined by the Trypan blue exclusion test. Treated T cells ($2.5 = 10^5$) were co-cultured with $2.5 = 10^5$ untreated autologous non-T lymphocytes in 1 ml RPMI 1640 with 10% FCS. The Ig production was stimulated with PWM. Control cultures did not receive PWM. The cells were incubated for 7 days in a 5% CO_2-air atmosphere at 37°C, and after centrifugation at 200 **g** the supernatants were collected. All cultures were set up in duplicate.

Determination of immunoglobulin concentrations

The amounts of IgM and IgG in the culture supernatants were determined by an enzyme-linked immunoadsorbent assay (ELISA). The technique, which is a modification of the method of Voller *et al.* [11], has been previously described. The concentrations were expressed in μg/100 ml. The variability within duplicates usually did not exceed 25%.

Data processing and statistical evaluation

The mean values of released Ig of untreated cell cultures were put as 100% and those of cultures treated with different concentrations of melphalan and adriamycin were related to this value. The mean values and standard deviations (S.D.) of released Ig were calculated on an arithmetic basis. Statistical evaluations were performed using Student's *t* test.

RESULTS

Table 1 shows that melphalan pretreatment of peripheral T cells increased their capacity to promote a PWM-induced IgG and IgM secretion of B cells. This increase was directly related to the concentration of melphalan, i.e. the largest increase was registered at the highest concentration of melphalan used in these experiments.

Table 2 shows that the PWM-induced IgM secretion was significantly impaired by pretreatment of the cocultured T cells with adriamycin at the two lower concentrations whereas the IgG secretion was not significantly changed.

Spontaneous IgG and IgM release in the absence of PWM did not exhibit any consistent changes following the exposure to cytostatics *in vitro*.

DISCUSSION

Differential effects of radiation and various cytotoxic drugs on the human peripheral lymphocyte population have been demonstrated previously. B cells were shown to be relatively more sensitive to the cytocidal action of both agents than T cells [5, 7, 12, 13]. This has been found following therapeutic use of ionizing

Table 1. IgG and IgM release of B lymphocytes co-cultured with autologous T cells pretreated with melphalan

Melphalan concentration (μg/ml)	IgG %	No. of observations	IgM(%)	No. of observations
0.04	128 ± 31 $P < 0.005$	17	116 ± 21 $P < 0.01$	17
0.4	156 ± 23 $P < 0.001$	17	147 ±34 $P < 0.001$	17
4.0	219 ± 23 $P < 0.001$	17	226 ± 48 $P < 0.001$	17

The values are expressed as a percentage of the corresponding PWM-stimulated control cultures containing B cells and autologous T cells which were not pretreated with melphalan. Mean values ± S.D. are presented.* P-values indicate the degree of statistical significance in Ig production between untreated and melphalan treated cultures.

*PWM-induced IgG production in the untreated cell cultures was 2575 ± 736 μg/100 ml and IgM release was 5917 ± 1263 μg/ml. These corresponding values obtained in cultures not stimulated with PWM was 265 ± 39 μg/100 ml and 292 ± 41 μg/ml respectively.

Table 2. IgG and IgM release of B lymphocytes co-cultured with autologous T cells pretreated with adriamycin

Adriamycin concentration (μg/ml)	IgG(%)	No. of observation	IgM(%)	No. of observations
0.01	119 ± 24 N.S.*	8	81 ± 15 $P < 0.01$	8
0.05	118 ± 23 N.S.	8	88 ± 12 $P < 0.005$	15
0.1	112 ± 27 N.S.	15	105 ± 21 N.S.	15

*N.S., no significance.

radiation and cytostatics [5, 12], as well as after exposure of lymphoid cells to these agents *in vitro* [7, 13].

It is currently well recognised that T lymphocytes are phenotypically and functionally heterogeneous, containing at least two subsets, namely one with receptors for IgM (Tμ) which exerts helper activity for the proliferation and differentiation of PWM-activated B cells and another, smaller subset displaying receptors for IgG (Tγ) which mediates suppressor effects [14]. Gupta and Good have demonstrated that corticosteroids and irradiation are relatively more cytotoxic to Tγ cells then to Tμ cells [15]. In the present study it was found that exposure of human peripheral T cells to melphalan *in vitro* significantly increased their capacity to promote a PWM-induced secretion of IgM and IgG of autologous B cells. Such an effect could not be observed when the T cells were pretreated with adriamycin. Such a treatment slightly reduced the release of IgM. Since B cells were not exposed to the drugs, the effects observed have to be ascribed to the drugs' influence on the T cell subsets. A limited number of tests showed that there was no preferential killing effect on any of the subpopulations of T cells as determined by the monoclonal antibodies Leu-2 and Leu-3 24 hr after drug exposure (data not presented). Hence it is possible that the increase of the cells' capacity to contribute to an increased IgG and IgM production by B cells reflects an action on the cell function rather than a shift in the proportions of the respective subsets. Theoretically, this could be due to a stimulation of the helper function or conversely to an inhibition of suppressor function of T cells. A stimulation of the cellular function (helper activity), which *per se* requires an active metabolism, by a cytotoxic agent seems rather unlikely, whereas a selective impairment of the suppressor function is more compatible with the inhibitory action of such drugs. A similar effect on PWM-induced Ig secretion was observed in a previous study in which peripheral T cells were exposed to ionizing radiation *in vitro* [16].

As an increased incidence of leukemias and non-Hodgkin's lymphomas was demonstrated in patients who had been treated with alkylating agents for ovarian cancer [17, 18], gastrointestinal tumors [19] and Hodgkin's disease [20], it is tempting to inquire if this could be related to possible changes in the functional properties of T cells. Interestingly enough, no increased frequency of secondary malignancies has so far been observed in Hodgkin's disease patients treated with adriamycin [21]. A reduction of T cell-mediated suppressor function in the presence of an unchanged activity of helper T cells might theoretically enable an uncountered proliferation of a transformed malignant cell clone.

An investigation of PWM-induced IgG and IgM synthesis of lymphocytes from patients with ovarian carcinoma treated either with melphalan or adriamycin is being planned. Such a study might shed some light on the possible clinical relevance of the above-reported findings.

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