Urological Research 2, 69-71 (1974)

Immunosuppressive Effect of Estrogen on Thymic Dependent Lymphocytic Blastogenesis

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Received: May 11, 1974

Summary. Suppression of the in vitro blastogenic stimulation of thymic dependent lymphocytes (T-cells) from healthy adult males in the presence of diethylstilbestrol diphosphate suggests that further investigation of estrogenic hormones as potential immunosuppressants in selected instances is warranted. Furthermore, preliminary evidence of the suppression of blastogenesis of T-cells in the presence of autologous serum from patients with prostatic cancer receiving hormonal therapy suggests that the palliative effects of such endocrine manipulation may be countered by impairment of these patient's cell-mediated immunologic responsiveness to their malignancy.

Key words: Blastogenesis, estrogen, immunosuppression, thymic dependent lymphocytes.

Sex hormones, in particular estrogens, may modulate, depending upon the nature of the neoplastic tissue, the growth or regression of tumour cells (1) and have striking effects on lymphoid tissue (2) and non-specific (3-5) as well as specific parameters of cellular and humoral mediated immunologic responsiveness (6-16). In view of recent reports (15, 16) of the effects of alterations of the endocrine environment on allograft rejection, it was thought communication of some of our preliminary findings of the suppression of lymphocytic blastogenesis to phytohaemagglutinin (PHA) by diethylstilbestrol diphosphate (DEP-S) would be of interest.

Materials and Methods

PBL from seven healthy adult males, from 18 to 33 years of age, were obtained by centrifugation of heparinized blood on a Ficoll-Isopaque gradient. Two ml cultures containing 5.0 x 10^5 PBL/ml of 80% RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York) supplemented with 20% foetal calf serum (Grand Island Biological Company, Grand Island, New York) containing 100 U/ml penicillin G and 100 μg/ml streptomycin with and without purified PHA (Burroughs Wellcome, Beckenham, England) reconstituted in phosphate buffered saline, pH 7.2, at a concentration of 5 ug protein/ml culture were prepared in triplicate and incubated for 68 hrs at 37°C in a 5% CO_2 in air mixture. Viability was assessed by trypan-blue dye exclusion. Four μCi of ^{3}H-thymidine (^{3}HTdR, specific activity 66 Ci/mM, ICN Pharmaceuticals, Inc., Irvine, California) was added 4 hrs before harvesting. Blastogenesis was defined as incorporation of ^{3}HTdR in the trichloroacetic acid insoluble fraction measured by liquid scintillation counting (Mark IV, Searle, Chicago, Illinois) and expressed as counts per min (cpm) per 10^6 PBL.

The effect of estrogen on blastogenesis was evaluated by the addition of 500 μg/ml culture of DEP-S, (Dome Laboratories, West Haven, Connecticut), determined as the optimal inhibitory

Table 1. Incorporation of ^{3}H-thymidine of phytohaemagglutinin (PHA) stimulated human peripheral blood Lymphocytes (PBL) in the presence and absence of diethylstilbestrol diphosphate (DEP-S)[a]

PBL + PHA + DEP-S	PBL + PHA	Significance (P)
$3.3 \pm 2.3 \times 10^4$	$7.5 \pm 4.6 \times 10^4$	0.008

a Data expressed as mean $\pm$ 1 S.D. counts per min per 10^6 PBL of triplicate determinations on seven adult males

doseage from a dose-response curve for PHA-stimulated PBL cultured in varying concentrations of DEP-S (17), to PBL cultured in the presence and absence of PHA.

Results and Comment

The mean $\pm$ 1 S.D. of triplicate determinations of the incorporation of ^{3}HTdR of PHA-stimulated human PBL from seven healthy adult males in the presence and absence of DEP-S is shown in Table 1. Analysis of this data by the "sign test" indicates that the thymidine incorporation of PHA-stimulated PBL was significantly suppressed (P 0.008) when PBL were cultured in supplemented medium containing DEP-S. That this observed suppression of lymphocytic blastogenesis to PHA in the presence of DEP-S was not due to a lymphocytotoxic effect of DEP-S, was shown by the observation that the viability (as determined by trypan-blue exclusion) on PBL incubated for 72 hrs in the supplemented culture medium alone and the supplemented culture containing 500 μg/ml of DEP-S were identical.

At present these observations suggest that as the response of lymphocytes to PHA is closely associated with the thymic dependent axis of immunologic responsiveness, i.e., thymus dependent lymphocytes (T lymphocytes, T-cells), that suppression of lymphocytic blastogenesis by estrogen and the absence of its depressive effects on the bone marrow (15), should receive, despite the tendency towards the development of secondary female sexual characteristics following long term therapy, further investigation as a potential immunosuppressant in selected instances. In support of this suggested use of estrogen as an immunosuppressant and of perhaps equal importance, particularly in view of preliminary studies demonstrating depressed blastogenesis of PBL to PHA in the presence of autologous serum from patients with prostatic cancer following estrogenic therapy (18), is the further indication from the present observations as recently discussed (19), that the heretofore palliative effects of endocrine manipulations in the clinical treatment of patients with hormonally-dependent tomours, e.g., of the breast and prostate (20-22), may be countered by the antiandrogenic impairment of the host's immunologic responsiveness to malignancy.

While, the results of the present study are particularly interesting, it is of importance that we are cognizant that there exist a variety of substances, including steroid (23) and protein hormones (24), and as yet ill defined factors present in the plasma and serum, e.g., alpha globulin (25, 26), prolactin (27), which may inhibit lymphocytic activity. As such, we must prior to drawing any definitive conclusions in reference to the present study, delineate the specificity of the observed suppression.

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