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Monocyte PGE_2 secretion in Hodgkin's disease and its relation to decreased cellular immunity

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SUMMARY

The secretion of PGE_2 from monocytes of newly diagnosed patients with Hodgkin's disease (HD) was compared to that of patients in remission, who were not receiving either chemotherapy or radiotherapy, and normal controls. We found that monocyte monolayers of some patients, both newly diagnosed and those in remission, secreted markedly elevated levels of PGE_2. The lymphocyte proliferative response to PHA was increased to a similar extent in both newly diagnosed patients and those in remission when cultured in the presence of indomethacin. PGE_2 concentrations in the medium of mononuclear cultures correlated with the lymphocyte proliferative response to PHA ($P < 0{\cdot}05$). However, no correlation of monocyte PGE_2 production with decreased E rosette forming lymphocytes, anergy or clinical stage could be demonstrated. We suggest that PGE_2 secretion by monocytes is indicative of an 'activated' state of these cells. It is, however, unlikely that PGE_2 is the only molecular species responsible for the decreased cellular immune function in HD. 'Activated' monocytes may be part of the immune response in this disease and may be responsible for the decreased cellular immunity.

INTRODUCTION

Patients with Hodgkin's disease (HD) have decreased cellular immune functions. The severity of these impaired cellular responses correlate with the stage of the disease but is only poorly correlated with the prognosis (Kaplan, 1980). Long term survivors of HD who have been treated by either chemotherapy or radiotherapy have persistent decreased immunological functions for many years after cessation of therapy (Fuks *et al.*, 1976; Fisher *et al.*, 1980). It has, therefore, been suggested that these decreased cellular immune functions may be an inherent characteristic of the susceptible individual who develops HD (Fisher *et al.*, 1980).

It is now, well established in both human and animal systems that monocytes or macrophages potentiate the lymphocyte proliferative response to both antigens and mitogens (Unanue, 1978). In addition large numbers of mononuclear phagocytes or activation of these cells results in suppression of the blastogenic response (Keller, 1975; Baird & Kaplan, 1977). Activated monocytes or macrophages secrete large amounts of prostaglandins and the production of prostaglandin E, in particular, has been correlated with decreased lymphocyte proliferative responses (Kurland & Bockman, 1978; Passwell, Dayer & Merler, 1979; Passwell, Rosen & Merler, 1980; Goodwin, Bankhurst & Messner, 1977).

Recent studies in HD patients have shown that depletion of adherent cells or incubation of

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mononuclear cells in the presence of indomethacin restored the lymphocyte function to normal (Goodwin *et al.*, 1977; Schechter & Soehnlen, 1978). These findings were only present in some of the patients studied and in others a suppressor T cell population has been implicated in the decreased lymphocyte responses (Twomey *et al.*, 1980).

In this study we have compared the immune function of newly diagnosed untreated patients with HD to a group of clinically disease free patients who had completed therapy at least 1 year prior to study. The role of monocytes and PGE_2 production and their possible effect on the decreased cellular immune function in these two groups was studied.

MATERIALS AND METHODS

Patient population. Seventeen males and 16 females with HD were studied. Their ages ranged from 8 to 63. Fourteen patients were newly diagnosed, and had not received treatment. Nineteen had completed their treatment regimes at least 1 year prior to study and were considered to be in remission. Staging and histological criteria were performed according to the Ann Arbor clinical staging classification. Remission was determined by clinical staging which included physical examination, chest X-ray and ultrasound abdominal scanning. Complete blood cell count, serum immunoglobulin levels and skin tests to tuberculin (Lederle Labs, Pearl River, New York, USA), candida (Hollister Stier Labs, Berkley, California, USA) and mumps antigens (Lilly Labs, Indianapolis, Indiana, USA) were performed by standard laboratory procedure. Skin tests were measured at 48 hr and read as positive if the diameter of the area of induration exceeded 10 mm. The clinical and immunological data of these patients are shown in Tables 1 & 2. Twenty healthy laboratory personnel aged 20–45 years volunteered to serve as normal controls.

Cell cultures. Blood was drawn in preservative free heparin and the mononuclear cells were separated by Ficoll-Hypaque density centrifugation at 400*g* for 20 min. The percentage of monocytes was determined by Giemsa and non-specific esterase stains on cytocentrifuge preparations of the cell suspensions. The mononuclear cell layer was washed three times in Hank's balanced salt solution. Cell cultures were done using RPMI 1640 medium (GIBCO) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), L-glutamine (100 mM) (GIBCO) and 2% pooled AB heat-inactivated millipore filtered serum. T cells were measured by the unmodified E rosette technique. Only cells that attached at least three sheep red cells were considered E positive (Ramot *et al.*, 1981)

Table 1. Clinical characteristics of patients studied

	Group 1 (untreated)	Group 2 (remission)
Total number	14	19
Age (median)	37·9	35·6
Sex ratio M:F	9:5	8:11
Histology NS;* MC†	8;6	12;7
Pathological stage I;II;III;IV	0;6;4;4	4;12;2;1
Remission (median)	—	6·4
Total peripheral WBC ($\times 10^9$/ml)	10·6 ± 1·3	6·4 ± 0·5
Monocyte count (mm^3)	751 ± 146	454 ± 76
Skin tests (% positive)‡	30	47

* Nodular sclerosis.

† Mixed cellularity.

‡ Indicates the total percentage of positive skin tests, i.e. more than 10 mm induration 48 hr after subcutaneous injection of three antigens.

Table 2. Immunological tests in patient groups

	Normal	Group 1 (untreated)	Group 2 (remission)
Serum immunoglobulins (mg/dl)			
IgG	1,154±20	1,583±126(14)	1,357±113(19)
IgA	266±7	211±24(14)	266±34(19)
IgM	74±2	128±27(14)	88±12(19)
E rosettes (%)	62·4±2·8(20)	56·2±4·7(11)	44·9±2·4(19)
% Monocytes in mononuclear preparation	14·5±1·2(15)	21·5±2·4(13)†§	15·1±2·4(11)
Lymphocyte proliferative response PHA (c.p.m. $\times 10^{-3}$)			
0·5 μg/ml	43·35±6·29(16)	30·80±7·27(11)	37·36±8·55(12)
1 μg/ml	93·89±11·02(16)	46·41±8·10(11)†	66·10±14·89(11)*
% Increase with indomethacin (1 μg/ml)	13·9±2·4(11)	51·7±9·8(11)†	82·5±19·5(7)‡

Numbers in parentheses indicate the number of individuals tested.

Significant difference from control *$P<0·05$; †$P<0·005$; ‡$P<0·001$; §Also significantly increased compared to group 2 $P<0·05$.

Lymphocyte proliferative responses. Phytohaemagglutinin (PHA) (Pharmacia Fine Chemicals, Uppsala, Sweden) dose–response curves were done in microtitre plates (Falcon Chemical Co., Oxnard, California, USA). One hundred thousand cells in 200 μl of medium. Lymphocyte proliferation was assayed by incorporation of ^{3}H-thymidine (1 μCi/culture; Nuclear Research Centre, Negev, Israel) (specific activity 3,000 mCi/mM) added 16–24 hr prior to termination of the 72 hr incubation period. Cells were harvested and the radioactivity incorporated by the cells was counted in a scintillation counter. Results are expressed as mean counts per minute (c.p.m.) of at least quadruplicate cultures ± standard error of the mean (s.e.m.) corrected by subtracting the appropriate control counts.

The effect of indomethacin on the lymphocyte proliferative response was determined by adding the drug to the mononuclear cell suspensions. Indomethacin (1 μg/ml; Merck, Sharpe & Dohme, West Point, Pennsylvania, USA) was added 1 hr prior to the PHA and kept in culture for the duration of the experiment.

Monocyte monolayers. The mononuclear cell concentration was adjusted to 5×10^6 cells/ml and aliquots of 0·4 ml were pipetted into wells of tissue culture trays (16 mm diameter, Costar, Cambridge, Massachusetts, USA). Adherence of monocytes was facilitated by gentle rocking at 37°C for 45 min, after which the non-adherent cells were removed by washing vigorously three times (Passwell *et al.*, 1979). The resultant monocyte monolayers were cultured in complete medium supplemented with 2% AB serum in the presence or absence of concanavalin A (Con A, 50 μg/ml; Calbiochem, Santiago, California). Con A binds to a specific plasma membrane receptor and results in marked endocytosis of the monocyte plasma membrane (Edelson & Cohn, 1974). Supernatants were harvested after 48 hr and stored at −20°C until assayed. The monocyte monolayers were lysed with 1% sodium dodecyl sulphate (Eastman Kodak Co., Rochester, New York, USA) in water and assayed for DNA content as described previously (Einstein, Schneeberger & Colten, 1976).

Prostaglandin assay. The concentration of PGE_2 in the mononuclear cells and monocyte monolayers supernatants was determined by radioimmunoassay (Levine *et al.*, 1971). Specific antiserum which shows no cross-reactivity with F prostaglandins was used (Miles Yeda, Kiryat Weizmann, Rehovot, Israel), The supernatant of the mononuclear cells was withdrawn from the culture at the time of the ^{3}H-thymidine pulse and used for the assay. Supernatants from the monocyte monolayers were collected for assay after 48 hr in culture. Lysozyme was measured as described previously (Gordon, Todd & Cohn, 1974).

Statistical analysis. Results in the Tables are expressed as mean ± s.e.m. The numerical data between the study groups were analysed using Student's *t*-test. Spearman rank correlation coefficients were used to study the relationship between the PGE_2 concentration in the medium, percentage monocyte content and the lymphocyte proliferative response to PHA.

RESULTS

The newly diagnosed patients (group 1) and those in long term remission (group 2) were comparable as regards clinical staging, histology, age and sex. There were more positive skin test responses in group 2. Serum immunoglobulins and total monocyte counts were not different from normal controls; however the newly diagnosed patients (group 1) had significantly increased numbers of monocytes in their mononuclear cell preparations after Ficoll-Hypaque separation compared to controls ($t = 2{\cdot}72$; $P < 0{\cdot}005$) and group 2 ($t = 1{\cdot}87$; $P < 0{\cdot}05$). E rosette formation was decreased in patients of both groups, but in this relatively small group of patients were not significantly different from the control group.

Lymphocyte proliferative responses

The lymphocyte proliferative responses of both groups of patients were decreased from the controls at both PHA doses tested. This reached significance at PHA dose 1 μg/ml (group 1, $t = 3{\cdot}17$; $P < 0{\cdot}005$; group 2, $t = 1{\cdot}53$; $P < 0{\cdot}05$). However, no differences in PHA responses between group 1 and group 2 patients were apparent. Incubation of the mononuclear cells in the presence of indomethacin resulted in a similar significant increase of the lymphocyte proliferative response to PHA in both groups of patients (group 1, $t = 3{\cdot}75$; $P < 0{\cdot}005$; group 2, $t = 4{\cdot}48$; $P < 0{\cdot}005$) (Table 2).

Monocyte monolayers

Spontaneous monocyte production of PGE_2 was markedly increased in some patients of both groups compared to the controls (Fig. 1). The mean levels of PGE_2 production were significantly greater in both group 1 ($t = 2{\cdot}81$; $P < 0{\cdot}005$) and group 2 ($t = 3{\cdot}41$; $P < 0{\cdot}005$), however several patients had PGE_2 levels within the normal range (Fig. 1). Monocyte monolayers from both groups of patients with Hodgkin's disease and the controls responded to a similar degree by an increase of PGE_2 production following stimulation with Con A (Table 3). Lysozyme production and DNA content of the monocyte monolayers did not vary within the three study groups.

PGE_2 concentration in the extracellular medium of the mononuclear cell suspension stimulated with PHA (1 μg) was significantly increased in the patients ($4{\cdot}23 \pm 0{\cdot}89$ ng/ml/culture) as compared to normal controls ($1{\cdot}05 \pm 0{\cdot}14$ ng/ml/culture) ($t = 4{\cdot}59$; $P < 0{\cdot}001$). The PGE_2 concentration in the extracellular medium of these mononuclear cells correlated with the amount of suppression of the lymphocyte proliferative response to phytohaemagglutinin (Fig. 2). However, no correlation of the

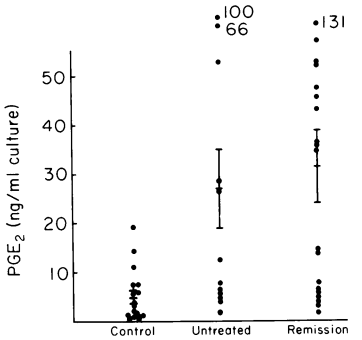

Fig. 1. Comparison of the basal secretion of PGE_2 from monocyte monolayers of Hodgkin's disease patients and controls.

Table 3. PGE_2 and lysozyme secretion from monocyte monolayers in Hodgkin's disease patients

	Normal	Group 1 (untreated)	Group 2 (remission)
PGE_2 (ng/ml/culture)	5·0±1·3	26·6±8·9*	31·5±8·8*
+Concanavalin A (50 μg/ml) (fold increase)	4·6±1·3	4·2±0·9	6·5±2·2
Lysozyme (μg/ml/culture)	4·8±0·5	5·9±1·3	5·2±2·3
DNA/culture (μg/ml)	4·1±0·5	3·9±0·9	4·2±0·8

Significant difference from control *$P < 0.005$.

monocyte monolayer PGE_2 production with either the lymphocyte proliferative response or the PGE_2 content in the mononuclear cell supernatant could be demonstrated. The number of monocytes in the mononuclear preparation of the patients with Hodgkin's disease did correlate with the degree of suppression of the lymphocyte proliferative response to PHA (Fig. 3).

No correlation was found between the increase in lymphocyte proliferative response to PHA following addition of indomethacin and the amount of PGE_2 in the cultures. Neither did the number of E rosette forming cells correlate with the lymphocyte proliferative response nor with the amount of PGE_2 produced by either monocyte monolayers or by the mononuclear cell cultures. There was insufficient data to establish a clear correlation with a decreased response to skin recall antigens.

DISCUSSION

These studies have shown that both newly diagnosed patients with HD (group 1) and those in remission (group 2) secrete increased amounts of PGE_2 from monocyte monolayers cultures. However, in several individuals from both study groups PGE_2 production was not greater than the control group. The observed decrease of lymphocyte proliferative response to PHA in both untreated patients and those in long term remission are in accord with previous studies (Kaplan,

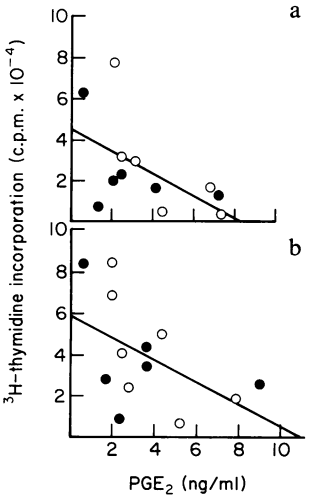

Fig. 2. Correlation of PGE_2 content of the extracellular medium of mononuclear cell suspension and their proliferative response to phytohaemagglutinin. (top) = 0·5 μg PHA ($r = 0.53$; $P < 0.05$); (bottom) = 1 μg PHA ($r = 0.54$; $P < 0.05$). ● = untreated patients; ○ = patients in remission.

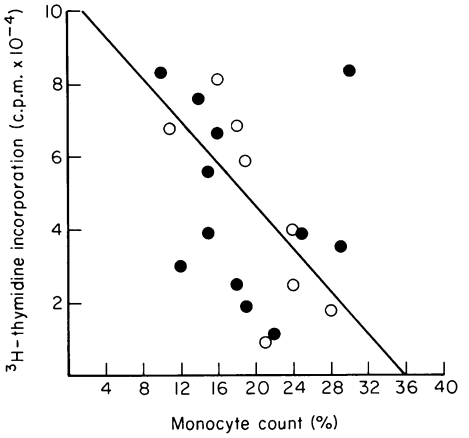

Fig. 3. Correlation of lymphocyte proliferative response to PHA (1 μg/ml) in patients with Hodgkin's disease and the percentage of monocytes in the mononuclear cell preparation. ($r=0{\cdot}55$; $P<0{\cdot}01$) ● = untreated patients; ○ = patients in remission.

1980; Fuks *et al.*, 1976; Fisher *et al.*, 1980). We have also corroborated the findings of other investigators that the addition of indomethacin to mononuclear cells results in an increased lymphocyte proliferative response in newly diagnosed patients (Goodwin *et al.*, 1977; Schechter & Soehnlen, 1978). In addition we found that HD patients in remission also increased their lymphocyte proliferative response to a similar extent in the presence of indomethacin.

An increased percentage of monocytes was found in the mononuclear cells of untreated patients with HD. Twomey *et al.* (1980) also recorded this finding. The number of monocytes in the mononuclear cell population correlated with a decreased lymphocyte proliferative response. In addition, PGE_2 concentration in the extracellular medium of the mononuclear cell suspension correlated with the decreased lymphocyte responses.

The interpretation of these findings and relation of excess PGE_2 production to the lymphocyte proliferative response is complex. Monocytes are the major source of PGE_2, however they may be stimulated to increase production by several stimuli including lymphokines from activated T cells (Kurland & Bockman, 1978; Passwell *et al.*, 1979). Circulating immune complexes are commonly present in HD and they, too, may induce monocyte prostaglandin production via activation of the Fc receptor (Brandeis *et al.*, 1980; Bonney *et al.*, 1979). The production of PGE_2 has variable effects on T cell populations and preferential induction of suppressor T cells has been reported (Stobo, Kennedy & Goldyne, 1979; Fischer, Durandy & Griscelli, 1981). In addition, lymphocyte proliferative response has been shown to be more sensitive to PGE_2 after stress and in elderly individuals (Goodwin *et al.*, 1981; Goodwin & Messner, 1979). Thus, the excess of PGE_2 in the extracellular medium is probably due to the amount of activation of the monocytes and their subsequent effect on the lymphocyte proliferative response is dependent on the sensitivity of the peripheral blood lymphocytes to PGE_2 and the relative effect of PGE_2 on the T subpopulations.

We were unable to correlate PGE_2 concentration in the mononuclear supernatants with the percentage increase of lymphocyte proliferative response in the presence of indomethacin. This suggested that factors other than PGE_2 contributed to the decreased blastogenic responses in HD. In addition the amount of PGE_2 found in the extracellular medium of the mononuclear cultures, while in excess of those seen in normal cultures was still 100-fold less than the amount of exogenous PGE_2 required to induce similar amounts of suppression (Passwell *et al.*, 1980). However, other prostaglandin molecules which are also synthesized and secreted by macrophages (Goldstein *et al.*, 1978) may have a more potent suppressive action (Kelly, Johnson & Parker, 1979). Recently the oxygen metabolites derived from activated monocytes have been shown to exert a suppressor effect on the lymphocyte proliferative response in HD (Deshazo *et al.*, 1981). We have shown that in normal individuals increasing the numbers of activation of monocytes *in vitro* resulted in

suppression of the lymphocyte proliferative response to PHA and the amount of PGE_2 present in the extracellular medium correlated with the amount of suppression. However, when equal numbers of macrophages (derived from monocytes grown in culture) were used no suppression of the lymphocyte proliferative response occurred despite higher levels of PGE_2 in the extracellular medium (Passwell *et al.*, 1982). Therefore, while PGE_2 production from monocytes is a convenient marker of stimulated monocytes, whether this is the molecular species associated with decreased cellular immunity in this disease has not been established.

Our data were not sufficient to demonstrate a clear correlation of cutaneous anergy with the monocyte PGE_2 production. However, since it is well known that PGE_2 enhances cyclic AMP production and reagents that increase cyclic AMP production result in decreased monocyte chemotaxis (Gallin *et al.*, 1978); it is possible that the decreased chemotaxis that has been demonstrated in HD monocytes could be the consequence of excess PGE_2 production and contribute to the skin anergy (Leb & Merrit, 1978).

Our findings do support the notion that at least in some patients activated monocytes are present and probably account for their decreased lymphocyte proliferative responses. It is of particular interest that the same abnormality was present in long term survivors of this disease. This may suggest that the host's response is in fact a normal response to the disease and may be beneficial. Therefore, treatment directed at altering these functions such as indomethacin may have undesirable effects.

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