Stage-dependent Reduction in T Colony Formation in Hodgkin's Disease

COINCIDENCE WITH MONOCYTE SYNTHESIS OF PROSTAGLANDINS

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ABSTRACT Prostaglandin synthesis and T lymphocyte colony formation have been examined in previously untreated patients with Hodgkin's disease. Mononuclear cells have been isolated from peripheral blood and spleens of these patients. Significant augmentation in prostaglandin E levels were noted in the mononuclear cell cultures from Hodgkin's disease patients compared with controls (1.64±0.29 vs. 0.39±0.09 ng/10^6 cells, $P < 0.005$). Measured prostaglandin E levels increased with advancing stage of disease. Virtually all of the prostaglandins were synthesized by the adherent monocyte cell population. Prostaglandin E was the major product. Clonal expansion of a T lymphocyte precursor cell, which gives rise to colonies >50 cells, was determined by a layered soft agar method. T colony formation was significantly reduced in patients with stage II, III, and IV disease. There were progressively reduced colony numbers seen with advancing stage of disease (609±209, 416±158, 207±58 compared with normals 2,274±360 colonies/10^6 cells plated; $P < 0.005$). The addition of inhibitors of endogenous prostaglandin synthesis resulted in significant augmentation of T colony number. However, a consistent relative decrease in T colony number was seen even when endogenous prostaglandin E synthesis was blocked. It would appear that both the prostaglandin-dependent and independent T colony precursor cells are lost with progressive stage of disease. A causative role of augmented prostaglandin synthesis in this stage-dependent reduction of T colony formation could not be established.

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INTRODUCTION

Impaired cell-mediated immunity is a well-reported occurrence in patients with Hodgkin's disease (1–6). Several of the in vitro assays used to assess mature T lymphocyte function in Hodgkin's disease have been shown to be inhibited by prostaglandins of the E series (7, 8). Marked elevation of prostaglandin E (PGE)[1] levels have been measured in immune cell cultures of patients with Hodgkin's disease (9). The addition of a prostaglandin synthetase inhibitor to lectin-activated lymphocyte cultures augmented thymidine incorporation to control levels (9). Monocytes have been shown to be the prime immune cell producers of prostaglandins (10, 11) and have also been identified as suppressors of T cell function in Hodgkins' disease (6, 9, 12). It seems plausible to assume that monocytes may mediate immune cell suppression in part through their synthesis of prostaglandins; however, the mechanism(s) by which monocytes affect the profound and progressive immunosuppression seen in Hodgkin's disease has yet to be elucidated.

Recently, we have demonstrated that the clonal expansion of a circulating precursor T lymphocyte could be inhibited by synthetic PGE (13). Further, it could be shown that monocyte derived PGE was an endogenous regulator of the proliferation and differentiation of this circulating precursor T cell (13). Because this latter cell gave rise to mature T lymphocytes whose functional activities could be measured, it seemed appropriate to determine to what extent endogenous monocyte-derived PGE was inhibiting the proximal immune cell compartment in patients with Hodgkin's disease. Thus, we have studied T lymphocyte colony formation in untreated patients with Hodgkin's dis-

[1] *Abbreviations used in this paper*: PGE, prostaglandin E; PBS, phosphate-buffered saline; TXB_2, thromboxane B_2.

ease. T colony formation by peripheral- and spleen-derived cells was found to be significantly reduced and the reduction correlated with the stage of disease. Prostaglandin synthetase inhibition significantly augmented colony number but did not restore colony formation to control levels. Thus, although our data confirmed that augmented monocyte PGE_2 synthesis occurs in such patients, we could not establish a causal relationship.

METHODS

Subjects. The patient population consisted of 50 unselected consecutively studied patients with Hodgkin's disease seen at the Memorial Sloan-Kettering Cancer Center between 1977 and 1979. None of the patients had received any prior radiation or chemotherapy. All subjects were studied during the initial phase of their diagnostic evaluation for extent of disease. Staging of disease followed the recommendations of the Ann Arbor Conference (14); histological classification was in accord with the recommendations made at the Rye Conference (15). None of the patients had received medication that might interfere with any of the studies performed for at least 48 h before evaluation.

Mononuclear cell preparation. Mononuclear cells from peripheral blood drawn into sterile heparinized tubes were isolated by Ficoll-Hypaque density centrifugation (16). The cells were washed three to five times with autologous plasma diluted 1:3 with saline. Splenocytes were obtained from patients who underwent splenectomy during a staging laparotomy. Spleen slices grossly free of tumor involvement were minced in Dulbecco's phosphate-buffered saline (PBS), calcium, and magnesium-free (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.), and then sequentially drawn by syringe through 15, 18, and 22-g needles to obtain single cell suspensions. Cells were finally suspended in McCoy's 5A modified media (Gibco Laboratories) supplemented with essential and nonessential amino acids, glutamine, asparagine, sodium pyruvate, and fetal calf serum (Microbiological Associates, Walkersville, Md.) or autologous serum, 15% by volume. Human lymph nodes were obtained from patients undergoing mastectomy for biopsy-proven breast cancer. Lymph node cells were prepared by the same procedure used to obtain splenocytes.

Consent for all studies and the use of patient materials were obtained in accordance with the Clinical Investigation Committee guidelines of Memorial Hospital.

Monocytes. Monocytes were isolated from the mononuclear cell populations on the basis of their adherence to tissue culture-treated plastic dishes as previously described (10). Between 10 and 20×10^6 mononuclear cells in McCoy's media with 10% fetal calf serum were allowed to adhere to 35-mm plastic dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in a 5% CO_2 atmosphere at 37°C for 1.5 h. The nonadherent cells were decanted and cultured in McCoy's media with 15% fetal calf serum; the monocytes on the dishes were washed with cold PBS at least five times to remove any nonadherent cells. Fresh McCoy's media with no serum or 15% fetal calf serum was placed over the adherent cells and the cultures kept in a humidified 5% CO_2 atmosphere at 37°C for 24 h. The cell-free media from adherent and nonadherent cultures were harvested for PGE determination by a double antibody radioimmunoassay as described previously (10). There is a 7% cross-reactivity with PGA_2 and virtually no cross-reactivity with $PGF_{2\alpha}$ in this assay. All samples were fractionated on silicic acid columns to remove PGA and $PGF_{2\alpha}$, thereby minimizing cross-reacting species. The assay does not discriminate between PGE_1 and PGE_2. The plates were washed with PBS to remove excess protein. The adherent cell protein was solubilized by adding a 0.1% Triton-X-100 saline solution to the culture dishes and subjecting the plate to several freeze/thaw cycles. Protein was determined by the method of Lowry et al. (17).

Histology. Histological review of peripheral and spleen mononuclear cell suspensions and adherent cell cultures was carried out using tetrachrome and nonspecific esterase stains (10). Cell viability was determined by trypan blue dye exclusion. Mononuclear cell phagocytosis was kindly performed by Dr. R. Schulof, (Memorial Hospital) using uniform sized (0.8 μ) latex particles (Dow Chemical Co., Midland, Mich.).

T colony assay. T lymphocyte colony assays were performed as previously described (13). Briefly, the Ficoll-Hypaque-derived blood mononuclear cells, splenocytes, or lymph node cells were suspended at 10^6 cells/ml in McCoy's media supplemented with 15% autologous or fetal calf serum. Phytohemagglutinin (The Wellcome Research Laboratories, Kent, England) at 0.5 $\mu g/10^6$ cells, previously determined to yield optimum colony formation (13), was added to the cell cultures. The cells were placed in a humidified 5% CO_2 atmosphere at 37° for 18 h. At the end of liquid culture, cell counts were performed and a cell-free aliquot was removed for radioimmunoassay of PGE levels. A small aliquot of liquid agar (Difco Laboratories, Detroit, Mich.) was added to the cell cultures to give a final cell and agar concentration of 10^6 cells and 0.3% (wt/vol) agar, respectively. The agar-cell suspension was overlayed onto previously gelled 0.5% (wt/vol) cell-free agar and underlayers containing McCoy's media supplemented with 15% fetal calf serum and 1.6 μg phytohemagglutinin/plate of 35-mm gridded plastic dishes (Lux Scientific Corp., Newbury Park, Calif.). The overlayers were allowed to gel at room temperature to yield a suspension of isolated cells. The cultures were placed at 37°C in a controlled atmosphere of 10% CO_2 for 7 d. At the end of the incubation period, the number of colonies that contained >50 cells were scored using an inverted microscope. At least three plates were used for each experimental point and the data expressed as the mean±SEM. To give a final concentration of 1–2 μM, indomethacin was added to parallel cultures 30 min before the addition of phytohemagglutinin at the beginning of the liquid phase of cell culture.

Tritiated prostaglandin release. The pattern of prostaglandins synthesized by the adherent cell population derived from four subjects was determined. Approximately 1 μCi of tritiated arachidonic acid ([5,6,8,9,11,12,14,15-^{3}H] arachidonic acid, Amersham Corp., Arlington Heights, Ill.) was added to ~10^6 adherent monocytes in 35-mm plastic dishes. After 2–3 h, ~30–50% of the radiolabel can be shown to be incorporated into membrane phospholipids, mainly phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine (18).[2] After label incorporation, the cells were washed four to five times with fresh McCoy's media and the cell cultures placed at 37°C in a 5% humidified CO_2 atmosphere for 4 h. At the end of incubation, cell-free supernates were acidified with citric acid and the labeled prostaglandins extracted with chloroform-methanol (3:1); the organic phase was backwashed with methanol-water (1:1); then the organic phase was taken to dryness under a stream of nitrogen gas. The extracted prostaglandins were resolubilized with ethylacetate-methanol (3:1) and mixed with authentic

[2] Bockman, R. S. Prostaglandin synthesis by human monocytes. Manuscript submitted for publication.

prostaglandin standards ($PGF_{2\alpha}$, 6-$KPGF_{1\alpha}$, thromboxane B_2 [TXB_2], PGE_2, PGD_2, and PGA_2), which were the generous gift of Dr. J. Pike (Upjohn Co., Kalamazoo, Mich.). Prostaglandin recovery by this technique was found to be 52±3% (mean±SEM). A single sample was spotted onto silica gel plates (Analtech, Inc., Newark, Del.). The plate was developed with solvent No. 1: chloroform-methanol—acetic acid-water (90:9:1:0.6); rotated 90°, then run with solvent No. 2: ethylacetate-acetone-acetic acid (90:10:1). The prostaglandins were visualized in an iodine chamber. The spots corresponding to the separated authentic standards were scraped and the radioactivity that cochromatographed with the known compounds was measured in a liquid scintillation counter. The relative migration in solvent 1:solvent 2 for 6 $KPGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2, TXB_2, PGD_2, PGA_2, and arachidonic acid was determined from 27 separate plates as 0.21:0.06, 0.18:0.19, 0.31:0.27, 0.28:0.41:0.37:0.46, 0.52:0.55, and 0.74:0.66, respectively; the SEM for all measurements was ±0.01. Excellent correlation of radioimmunoassay for PGE with quantitative measurements of tritiated arachidonic acid conversion has been demonstrated for the two systems (18). Indomethacin, arachidonic acid, aspirin, and zymosan were purchased from Sigma Chemical Co., St. Louis, Mo.; flurbiprofen was a gift from Dr. G. J. Frank, Boots Pure Drug Co. Ltd., Nottingham, England.

RESULTS

Patient population. The patients studied (22 women, 28 men) ranged in age from 16 to 64 yr with a median of 27 yr. As summarized in Table I, the majority of patients were stage II or III, and nodular sclerosis was the predominant histological classification. Over half of the spleen samples studied were involved with disease. All patients had biopsy-proven disease and all were studied within 1 mo of initial diagnosis. All staging was determined after complete diagnostic evaluation.

Cell preparations. The compositions of the peripheral blood mononuclear cell preparations from normal subjects and the Hodgkin's disease patients with stage II–III disease were not significantly different. Cytological examination in Hodgkin's disease patients revealed 74±3% (mean±SD) lymphoid cells, 23±8% monocytes, and <5% granulocytes. The determinations of monocyte number using cytologic or phagocytic methods were in close agreement. A relative or absolute difference in monocyte number in the Ficoll-Hypaque preparations of the stage II and III patients with Hodgkin's disease compared with normal subjects was not seen (19). Therefore, it is unlikely that the differences in the functional activities to be described are caused by discrepancies in the proportion of monocytes in the test cell preparation. The spleen cell and lymph node preparations were similar and both predominantly comprised of lymphoid cells, with <10% of the cells identifiable as monocytes. The adherent cell preparations were performed as previously described (10). More than 90% of the cells were nonspecific esterase positive on staining. Approximately 90% of the cells phagocytized three or more latex particles per cell as determined by scanning 200 cells (10). Platelet contamination of day 1 monocyte preparations was not sufficient to account for any significant amount of PGE measured. By day 3 of culture, virtually no platelets were seen. Although the increment in PGE synthesis measured by radioimmunoassay is the same for day 1 and 2 of culture, the starting levels of PGE measured at the inception of culture on day 1 were greater than the near zero levels measured for freshly washed day 2 cultures.[2] It is possible that the

TABLE I
Patient Population

Stage	No. of patients	Sex		Histological classification			Splenic involvement
		Male	Female	NS*	MC*	LP§	
I							
A	7	4	3	4	2	1	0/2
B	0	—	—	—	—	—	—
II							
A	11	3	8	10	1	—	0/5
B	7	4	3	7	—	—	0/3
III							
A	10	8	2	6	4	—	3/4
B	8	7	1	7	1	—	4/4
IV							
A	2	1	1	2	—	—	—
B	5	1	4	5	—	—	1/1

* NS, nodular sclerosis.
‡ MC, mixed cellularity.
§ LP, lymphocyte predominant.

PGE levels measured at the initiation of monocyte cell culture were derived from release after burst synthesis by contaminating platelets or during monocyte adherence. To correct for PGE initially present when comparing basal synthetic rates of cultures, "de novo synthesis" was determined by measuring the difference between paired cultures with and without 1–2 μM indomethacin. In virtually every case measured there was >90% inhibition of prostaglandin synthesis when indomethacin or flurbiprofen were added to the cultures.

T colony formation. T colony formation was evaluated in 36 patients with Hodgkin's disease. Marked reduction in colony number was evident in all stages of disease in peripheral blood as well as in the spleen preparations when compared with blood mononuclear cells from normals or lymph node cells derived from mastectomy patients (Table II). The addition of indomethacin (2 μM) caused a significant (as determined by paired t test) increase in measured colony formation in the Hodgkin's disease specimens, but caused no significant augmentation in the comparable normal peripheral blood cells or mastectomy-derived lymph node cells. The data examined for individual patients showed that the addition of indomethacin resulted in a significant increase in colony number in 23 of 36 patients. As was previously reported (13), the addition of indomethacin caused significant augmentation of colony number in only 3 of the 13 normal subjects, but overall, colony formation for the 13 normal subjects was not significantly increased by the addition of indomethacin (13). With the lymph node cells derived from patients undergoing mastectomy, 4 of 28 showed a significant increase in T colony number when indomethacin was added. Three of four of the latter patients had metastatic involvement of lymph nodes; 12 of the 28 patients had tumor metastatic to axillary lymph nodes. No significant augmentation of colony formation was seen when the data from the 28 mastectomy subjects were compared by the paired t test or the t test of means. Therefore, the effect of a prostaglandin synthetase inhibitor in peripheral blood and spleen-derived mononuclear cells of Hodgkin's disease patients differs from the peripheral blood cells of normal subjects and the lymph node cells of patients with breast cancer. The numbers of T colonies formed in patients with Hodgkin's disease appeared to be related to the stage of disease. With advancing stages of disease, fewer colonies were measured Fig. 1. A significant negative correlation was noted in the absence and presence of indomethacin when T colony formation was plotted vs. stage of disease, Fig. 2, and underscores the progressive loss of the precursor T cells that undergo clonal expansion to give colonies. It should also be noted that even in the presence of a prostaglandin synthetase inhibitor, progressive reduction of T colony number was observed. Furthermore, there was little change in the relative increase in colony formation seen for the various disease stages. This might suggest that there was concomitant loss of the prostaglandin "sensitive" as well as the prostaglandin "resistant" subpopulations of T precursor cells with advancing stages of disease.

The quantitative reduction in colony number did not appear to be correlated with a decrease in colony size or an increase in cell clusters containing <50 cells. Colony size for any given individual tended to be relatively uniform. The addition of indomethacin or flurbi-

TABLE II
*T Colony Counts**

	Stage					
	I	II	III	IV	Normals	Lymph nodes
Peripheral blood	1,173±566 ($P < 0.05$)‖	609±209‡ ($P < 0.005$)	416±158‡ ($P < 0.005$)	207±58§	2,274±360	—
With indomethacin	1,811±511	1,358±355¶	659±165‡	384±194**	2,666±378	—
Number of patients	4	14	14	4	13	
Spleen	1,675±234 ($P < 0.05$)§	631±151‡ ($P < 0.001$)	525±153‡ ($P < 0.02$)	427±301¶		2,303±200
With indomethacin	2,319±260	1,083±179§	973±271§	651±412¶		2,324±188
Number of patients	4	8	8	2		28

* Given as mean No. colonies±SEM/10^6 cells plated in autologous serum.
‡ Differs from normal by t test of means $P < 0.001$.
§ Differs from normal by t test of means $P < 0.005$.
‖ Differs from normal by paired t test±indomethacin.
¶ Differs from normal by t test of means $P < 0.02$.
** Differs from normal by t test of means $P < 0.01$.

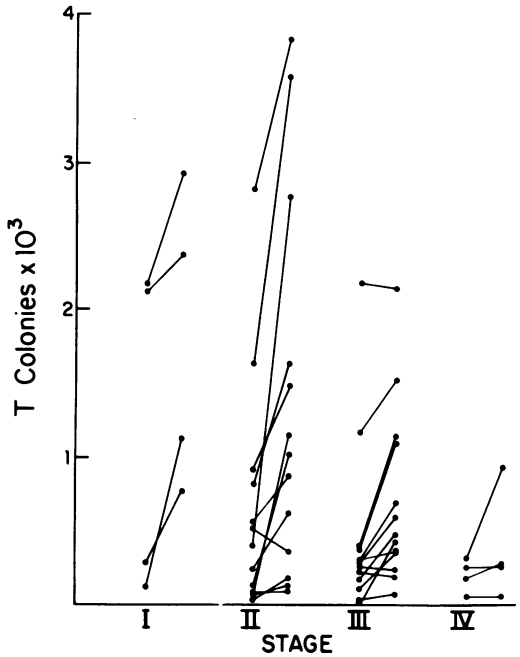

FIGURE 1 T colony number is plotted as a function of stage of disease. T colony formation per 10^6 peripheral blood mononuclear cells plated in media containing 15% autologous serum is measured in the absence and presence of indomethacin (2 μM). Straight lines reflect the change in colony number for an individual patient±indomethacin, left-hand dot represents patient minus indomethacin, right-hand dot represents patient plus indomethacin.

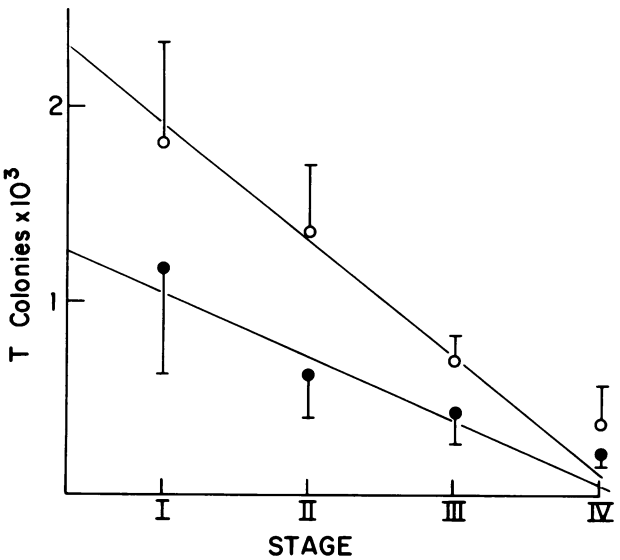

FIGURE 2 T colony number (mean±SEM) is plotted as a function of stage of disease. ●, average colony number per stage in autologous serum; ○, average colony number per stage in autologous media with indomethacin (2 μM, final concentration). The correlation coefficient and significance for the straight line curves fitted by linear regression are $r = -0.35, P < 0.05$ for the data from parallel experiments performed in the absence of indomethacin and $r = -0.45, P < 0.01$ in the presence of indomethacin. All data points $n = 35$ were used for the straight line curves.

profen did not significantly increase individual colony size. Functional activity of colony-derived cells was not tested. Other inhibitors of prostaglandin synthetase that are structurally unrelated to indomethacin caused similar augmentation of colony number in parallel Hodgkin's disease cultures, Table III. The latter finding supports the concept that indomethacin acts by inhibiting prostaglandin synthesis.

When mean colony numbers from patients with the nodular sclerosis classification were compared with colony formation by patients with the mixed cellularity classification, no significant difference was noted.

PGE levels. PGE levels were measured by radioimmunoassay in the cell-free supernate media from the various cell preparations. De novo (PGE level in culture—PGE in parallel culture with indomethacin) prostaglandin synthesis increased in a progressive fashion with advancing stage of disease for cells cul-

TABLE III
Effect of Various Prostaglandin Synthetase Inhibitors

Patient	Control	Indomethacin*	Flurbiprofen*	Aspirin*
	T colony number±SEM/10⁶ cells plated			
A	460±54	508±33	778±45‡	—
B	411±183	736±70	830±76‡	562±72
C	1,080±49	1,472±60‡	922±46§	1,081±94

* Indomethacin, flurbiprofen, and aspirin were added 20 min before phytohemagglutinin at the initiation of liquid culture. The agents were added to give a final concentration of 1 μM, 1 μM, and 10 μM, respectively.

‡ Difference compared with control significant, $P < 0.02$ as determined by the t test of means.

§ Difference compared with control significant, $P < 0.05$ as determined by the t test of means.

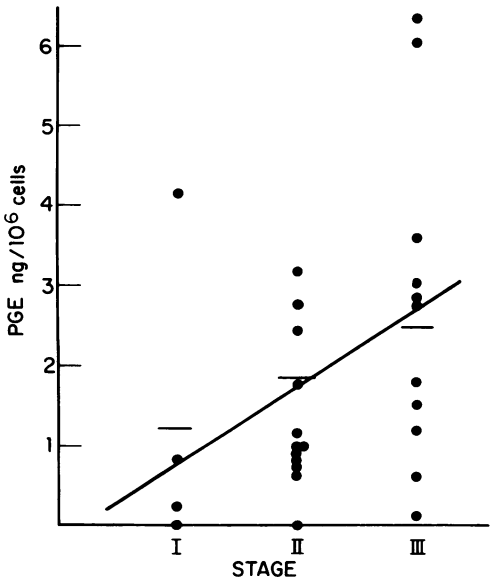

FIGURE 3 PGE levels measured by radioimmunoassay are plotted as a function of stage of disease. ●, PGE levels from the mononuclear cell cultures of 27 individual patients whose cells were cultured in autologous serum. The straight line curve fitted by linear regression analysis has a correlation coefficient of $r = 0.42, P < 0.05$. The horizontal bar represents the mean at each stage.

tured in a standard fetal calf serum or cells cultured in autologous serum, Fig. 3 and Table IV. The correlation of measured PGE level with stage of disease was positive and significant for cells cultured with autologous serum, $n = 27, r = 0.42, P < 0.05$, Fig. 3, but not significant for cells cultured in fetal calf serum, $n = 31$, $r = 0.28$. The difference in the mean levels of PGE measured in mononuclear cell-conditioned media from Hodgkin's patients compared with control subjects was significant whether fetal calf or autologous serum were used. When paired data for patient cells cultured in fetal calf serum or autologous serum were compared using the paired t test, significant differences were noted between stage II and III as well as the summarized data.

Fractionation of the mononuclear cells derived from the blood mononuclear cells of patients with Hodgkin's disease revealed that the adherent cells accounted for the increased prostaglandin synthesis, Table V. Paired data from 15 patients was compared, revealing that nonadherent cell synthesis of PGE did not differ significantly from controls. Adherent cell synthesis did differ significantly from the nonadherent cells. Furthermore, the cells cultured in the presence of serum (only fetal calf serum was tested) had higher measured levels of PGE than cells cultured in serum-free media. As with the T colony data, no significant difference was noted in PGE levels measured when the mononuclear cell cultures derived from patients with the nodular sclerosis histological classification were compared with cell cultures from patients with the mixed cellularity classification.

A comparison of 40 paired samples from cultures supplemented with autologous serum showed that T colony number and PGE levels were significantly correlated ($r = 0.43$, $P < 0.01$). Similar analysis of the cell cultures in which fetal calf serum-supplemented media was used failed to demonstrate a significant correlation coefficient ($r = 0.20$) between T colony number and PGE level. The latter data is summarized graphically in Fig. 4 where, for the purposes

TABLE IV

PGE Levels Determined by Radioimmunoassay Lymphocyte-conditioned Media‡*

	Stage						
	I	II	III	IV	Average	Paired t test	t-test of means
Hodgkin's disease							
Fetal calf serum	0.49±0.26 (7)§	0.85±0.32 (13)	1.20±0.45 (11)	1.35±0.59 (5)	0.78±0.17	$P < 0.001$ (21)	$P < 0.01$ (43)
Autologous serum	1.32±0.96 (4)	1.86±0.59 (12)	2.48±0.50 (12)	—	1.64±0.29		
Controls							
Fetal calf serum (17)					0.29±0.11	—	$P < 0.05$‖
Autologous serum (6)					0.39±0.09	—	$P < 0.005$‖

* Data expressed as ng PGE/10^6 cells cultured, mean±SEM.

‡ Cell-free media from 24 h cultures of mononuclear cells; PGE levels represent the difference between parallel cultures±indomethacin (2 μM).

§ Number of subjects studied in parentheses.

‖ Difference between control group and Hodgkin's disease patients cultured under similar conditions.

TABLE V
*Adherent vs. Nonadherent Cell Synthesis of PGE**

Conditioned media	Mean±SEM	Difference by paired *t* test
	mg/10⁶ cells	
Nonadherent cells (15% fetal calf serum)	0.31±0.13	
Adherent cells		$P < 0.001$
0% serum	1.31±0.30	
15% serum	3.63±0.73	$P < 0.005$

* Paired data from 15 patients with Hodgkin's disease.

of simplifying the presentation, mean T colony counts are plotted as a function of the mean PGE level for each stage of disease. Both sets of data appear to fall along a "best fit" straight line; however, this fit is significant only for the data from cultures supplemented with autologous serum. These graphs imply that greater colony numbers were measured in the presence of higher endogenous levels of PGE when autologous serum was present (see Discussion for comment).

Tritiated prostaglandin release. The classes of prostaglandins released from the adherent cells of peripheral blood and spleen mononuclear cells were studied by two-dimension, thin layer chromatography. Using authentic standards, the efficacy of separation is demonstrated in Fig. 5. The amount of tritiated PGE_2, PGA_2, and TXB_2 released, which cochromatographed with authentic standards, is given in Table VI. The pattern of tritiated prostaglandins released were similar for the two control subjects and the two Hodgkin's

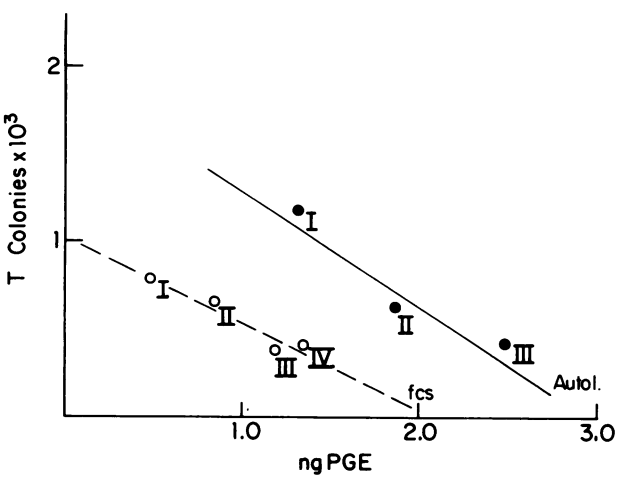

FIGURE 4 Averaged T colony counts are plotted as a function of averaged PGE levels measured by radioimmunoassay for Hodgkin's disease patients grouped by stage of disease. The data from cell cultures supplemented with fetal calf serum (fcs) are compared with cell cultures containing autologous serum (autol.), see text.

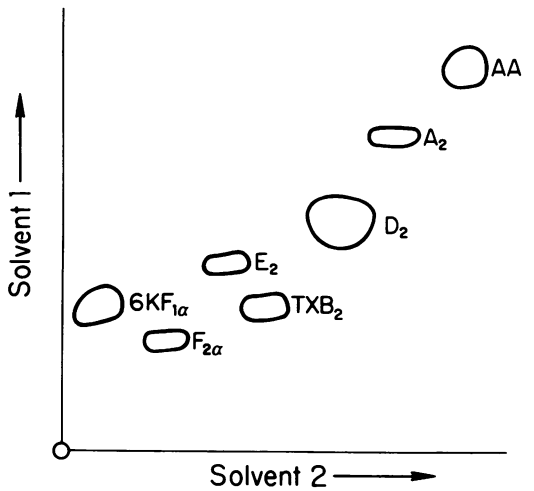

FIGURE 5 Two-dimensional thin layer chromatography showing separation of major prostaglandin classes.

disease patients studied. Greater conversion of tritiated arachidonic acid into prostaglandins was consistently observed for the Hodgkin's samples. PGE_2 was consistently the major prostaglandin product, followed ty TXB_2. All tritiated PGA_2 released could be accounted for by the nonenzymatic conversion of PGE_2 to PGA_2.[2]

DISCUSSION

Earlier studies have demonstrated that colonies of mature T lymphocytes can arise by clonal expansion from a circulating precursor cell (13, 20, 21). The lymphocytes isolated from pooled colonies could be shown to have different functional activities (13). Synthetic as well as monocyte-derived PGE inhibits this clonal proliferation (13). Reports of elevated levels of PGE and suppressed mitogen response in mononuclear cells cultured from six patients with Hodgkin's disease suggested that prostaglandins may have contributed to the impaired immunity seen in such patients (9). It is relevant to note that the disturbances in immune function that have been described in patients with Hodgkin's disease (1–6) involve several functional classes of T lymphocytes. Further, with progression of disease, immune cell depletion becomes more evident (6). These latter features suggest that the pathophysiological mechanism leading to depressed immunocompetence in Hodgkin's disease may operate at the level of a precursor T lymphocyte.

When T colony formation was examined in our patients, significant depression of colony number was noted in the blood and spleen cell cultures as compared with control samples. Progressive reduction in colony number was recorded with advancing stages of disease. For all stages, significant augmentation in

TABLE VI
Monocyte Synthesis of Tritiated Prostaglandins

	Subject	Cell source	PGE_2	PGA_2	TXB_2	Total cpm released
			cpm released/mg of cell protein			
Control	1	PBL*	2,796	322	710	78,231
	2	PBL	1,638	446	793	45,458
		PBL + zymosan	2,353	1,097	1,289	45,185
		PBL + zymosan + indomethacin	537	332	251	69,386
Hodgkin's disease	1	PBL	21,913	2,887	7,708	61,377
	1	Spleen	5,615	1,635	9,215	53,931
	2	PBL	12,679	1,780	7,831	50,066
	2	Spleen‡	28,117	4,625	17,790	108,799

* PBL, peripheral blood lymphocytes.
‡ Spleen involved with Hodgkin's disease.

colony counts was seen when a potent inhibitor of prostaglandin synthetase was added to the cell culture. Monocyte-derived PGE levels were significantly elevated in Hodgkin's disease cell cultures compared with normals. The pattern of tritiated prostaglandins released by monocytes from Hodgkin's disease patients showed increased amounts of PGE_2 when compared with monocytes from control subjects. The tritiated PGA_2 we measured does not appear to be a primary cyclooxygenase product of monocytes (11, 22) and probably arises from the conversion of preformed PGE_2 (23). Conversion of PGE to PGA, which in our system ranged from 11–23%, may account for the variability in PGE levels measured by radioimmunoassay and in part account for the inability to significantly correlate levels with stage of disease. The balance between synthesis and conversion of PGE reveals the complexity of endogenous prostaglandin modulation of immune cell function.

Greater levels of PGE and higher colony counts were mesured in cultures supplemented with autologous serum. These findings would appear to be in conflict. Previously we have described that the proliferation and differentiation of the committed stem cell that gives rise to monocytes/macrophages can be regulated by agents with opposing actions (24). The positive or promoting agent for the latter system is species specific and can overcome inhibition by PGE (24). A parallel mechanism may exist for T cell precursors; in fact, a species-specific soluble agent(s) promoting T lymphocyte proliferation has been described (25–27). The presence of such an agent in the autologous serum could account for the relative refractoriness of precursor T cells to endogenous PGE levels.

Whereas there appeared to be a strong correlation between augmented monocyte synthesis of PGE and depressed T colony counts, this does not mean that depressed T colony formation was caused by the increased prostaglandin production. It was evident that blocking prostaglandin synthesis did not restore T colony numbers of the normal range in patients with stage II, III, or IV disease, Table II. In fact, what was observed was a consistent relative increase at each stage of disease when indomethacin was added. If increased prostaglandin synthesis was the direct cause of depressed T colony formation, then the addition of cyclooxygenase inhibitors should have resulted in greater augmentation of T colony formation with advancing stages of disease. The latter condition was not supported by the data obtained. Excluding those patients in whom the addition of indomethacin restored T colony formation to normal levels, it must be assumed that some other mechanism was involved in the depression of T colony counts seen in the patients with advanced stages of disease. Yet it is still possible to consider that persistent exposure to a suppressor substance such as prostaglandin may result in the continuous diminution of the precursor T cell pool.

In sum, the data showed a progressive loss of a precursor T cell with advancing stage of disease but did not identify the mechanism or mediator that caused this to occur. Whatever the pathophysiological mechanism, suppression and depletion at the level of the precursor cell probably plays an important role in the depressed immunocompetence seen in patients with Hodgkin's disease.

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