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**Research Article**

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# Prostaglandin E Inhibition of T-Lymphocyte Colony Formation

## A POSSIBLE MECHANISM OF MONOCYTE MODULATION OF CLONAL EXPANSION

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ABSTRACT Prostaglandin and monocyte modulation of a T-lymphocyte cell capable of undergoing clonal expansion was studied. Circulating human mononuclear cells were isolated by density centrifugation. After 24 h in culture with phytohemagglutinin present, the cells were mixed with 0.3% agar and overlayed onto a 0.5% agar layer that contained media and phytohemagglutinin. At day 6, colonies that contained >50 cells were counted. These colonies represented clonal proliferation of a phytohemagglutinin-responsive T-lymphocyte precursor. This responder cell accounted for <0.3% of the starting cell population. Colonies were comprised of cells which, when isolated, formed E rosettes. These colony cells could be shown to have helper or suppressor function as measured by their ability to promote or inhibit immunoglobulin synthesis. By these latter criteria the colony cells were considered to be mature T lymphocytes. The addition of prostaglandin E to the cultures demonstrated a linear, $r = 0.82$, dose-dependent inhibition of colony formation with a 50% point of inhibition ($I_{50}$) = 0.18 $\mu$M. Low numbers of normal monocytes when added to the cultures mimicked the effect of synthetic prostaglandin $E_2$. A highly significant correlation could be shown for endogenous prostaglandin E levels and colony counts. It appears that monocytes through their synthesis of prostaglandin $E_2$ can restrict the clonal expansion of a circulating T-lymphocyte precursor.

### INTRODUCTION

Recent in vitro studies have demonstrated that physiological concentrations of prostaglandin (PG)[1] $E_2$ ($PGE_2$) can inhibit the clonal expansion of immune precursor cells. When measured by the technique of colony formation in semisolid agar culture, the committed granulocyte/macrophage precursor cell (colony-forming unit-culture [CFU-c]) (1) and the B-lymphocyte precursor cell (2) were inhibited in a dose-dependent fashion by synthetic $PGE_2$. For both the CFU-c (3) and the B-lymphocyte precursor cell (2), monocytes and macrophages inhibited colony formation presumably through the synthesis and release of prostaglandin (4–6) because this effect correlated with $PGE_2$ levels (3). Similar immunomodulator controls are presumed to exist for the T lymphocyte as suggested by the results of Goodwin et al. (7). These authors have presented evidence that adherent cell-derived $PGE_2$ can inhibit phytohemagglutinin (PHA)-induced, tritiated thymidine ($^3$H-tdr) incorporation into T lymphocytes (7).

In the CFU-c system, where the most information is available, PGE has been postulated to be part of a coregulatory mechanism which controls myelopoiesis (3). A number of agents cause colony-stimulating factor release which results in myeloid cell proliferation and differentiation (8). As part of a probable physiological control to prevent runaway myelopoiesis, monocyte/macrophage $PGE_2$ synthesis is initiated (3, 9). When highly purified colony-stimulating factor was tested it could be shown to directly cause $PGE_2$ synthesis (10). Thus myelopoiesis appears to be regulated by two diffusable, monocyte/macrophage-derived agents with the specificity residing in the positive limb of the dual feedback loop. Macrophage-derived agents which induce T-lymphocyte activation and proliferation have been described (11–17). When T-lymphocyte differentiation and proliferation was studied in clonal

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[1] *Abbreviations used in this paper*: CFU-c, colony-forming unit-culture; F/H, Ficoll-Hypaque; $^3$H-tdr, tritiated thymidine; $I_{50}$, 50% point of inhibition; IM, indomethacin; PG, prostaglandin(s); PHA, phytohemagglutinin(s); RBC, erythrocyte(s).

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assays, inhibitory and facilitative factors from monocytes/macrophages were reported to be released (18, 19).

In this study we describe a soft agar, colony system for studying clonal expansion of T lymphocytes derived from blood and lymph nodes of human subjects. Evidence is presented which shows that synthetic $PGE_2$ but not $PGD_2$ or $PGF_{2\alpha}$ inhibits the initiation of clonal expansion, and further, that autologous monocytes can inhibit T-colony formation through their synthesis of prostaglandins. These data suggest a regulatory function for monocyte/macrophage control of clonal T-lymphocyte expansion through the elaboration of inhibitory prostaglandins.

## METHODS

Human mononuclear cells were obtained from the peripheral blood of 13 healthy volunteers, 8 men and 5 women aged 21–57 yr with a median age of 31 yr. None of these normal subjects had taken any drug which might affect cyclooxygenase activity for at least 4 d before testing. The blood of these consenting human donors was drawn into sterile, heparinized test tubes. Mononuclear cells were isolated by Ficoll (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) -Hypaque (Winthrop Laboratories, New York) (F/H) density centrifugation (20), washed 3–5 times with diluted autologous plasma, and then suspended in McCoys 5A modified media supplemented with 10–15% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.) or autologous serum. Histological review of F/H cell preparations was performed on Giemsa-, tetrachrome-, and nonspecific esterase-stained smears. Viability was determined by trypan blue exclusion. T- and non-T-lymphocyte subpopulations were kindly provided by Dr. F. and Ms. M. Siegal of the Sloan-Kettering Institute, New York using a previously described sheep erythrocyte (RBC)-rosette method (21). To determine thymidine incorporation, $1-2 \times 10^5$ F/H cells were aliquoted into microtiter wells (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) with the predetermined optimum concentration of PHA (Wellcome Reagents Ltd. Beckenham, England). After 72 h at 37°C in a humidified, 5% $CO_2$ atmosphere, the cells were pulsed for 18 h with $\cong 0.1$ $\mu Ci$ of $^3H$-tdr, (6.0 Ci/mM sp act; New England Nuclear, Boston, Mass.) The $^3H$-tdr nucleic acids were harvested on glass fiber filters with a MASH II cell harvester (Microbiological Associates, Walkersville, Md.). Human lymph nodes were excised from patients with biopsy-proven breast cancer who were undergoing mastectomy and from one patient for diagnostic purposes who was subsequently shown to have no disease. None of these subjects had taken cyclooxygenase inhibitors at least 4 d before study. Consent for the use of tissue samples was obtained. The lymph nodes were rapidly sliced into 1- to 2-mm slices under sterile conditions, teased apart, and then the released cells were expelled through a 22-g needle and suspended in McCoys 5A modified media with 15% fetal calf serum.

Human monocytes were isolated from the mononuclear cell populations on the basis of their adherence to tissue culture-treated plastic dishes as previously described (6). Briefly, 10- to 20 million mononuclear cells in McCoys 5A modified media supplemented with 10–15% fetal calf serum were allowed to adhere to 60-mm plastic dishes (Falcon Labware) for 1.5–2 h in 5% $CO_2$ atmosphere at 37°C. The dishes were then washed with cold Dulbecco's phosphate buffered saline (Grand Island Biological Co.) at least five times to remove nonadherent cells. The adherent cells were then released from the dish with the aid of a rubber policeman. The cells were washed and then resuspended in fresh media. Cell counts, viability determinations, phagocytic activity, and nonspecific esterase staining were carried out as previously described (6).

Monocyte-conditioned media was prepared by harvesting the cell-free supernatant media from the adherent cells which had been cultured for 24 h at 37°C in a 5% $CO_2$, humidified atmosphere. Adherent cell underlayers were prepared by allowing various numbers of F/H cells to adhere to plastic dishes, washing away the nonadherents, and then overlaying the cells with 1 ml of 0.5% agar in supplemented McCoys 5A modified media. Such plates were then used in the T-colony assay to be described. In this way an intervening, noncell-containing agar layer existed between the adherent cells and the overlayed F/H cells.

T-Colony assays were carried out with a double agar-layering technique. Mononuclear cells including subfractions and mixtures of adherent cells or monocyte-conditioned media were placed in McCoys 5A modified media which was supplemented with 15% fetal calf serum as well as essential and nonessential amino acids, glutamine, asparagine, and sodium pyruvate (Microbiological Associates) as well as 0.001–0.1 mg of PHA/1 million cells; the cells were counted, then 3% agar (Bacto-Agar, Difco Laboratories, Detroit, Mich.) in liquid phase was added to give a final agar concentration of 0.3%. The cells were then overlayed onto previously prepared and gelled 0.5% agar underlayers which were supplemented with McCoys 5A modified media plus 15% fetal calf serum and PHA (1.6 $\mu g$/plate) in 35-mm gridded Petri dishes (Lux Scientific Corp., Newbury Park, Calif.). The overlayers gel rapidly at room temperature to yield a suspension of isolated cells. The plates containing the two agar layers 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 which contained >50 cells were scored with an inverted microscope. At least three plates were used for each experimental point, and the data is expressed as the mean±SEM.

PGE levels of cell-free supernates were determined by a double antibody radioimmunoassay as previously described (6). Synthetic $PGE_1$, $PGE_2$, $PGF_{2\alpha}$, and $PGD_2$ which were used in these studies were the generous gift of Dr. John Pike of The Upjohn Co., Kalamazoo, Mich. Indomethacin was obtained from Sigma Chemical Co., St. Louis, Mo. and was prepared for each experiment as a 10-mM solution in 100% ethanol and then diluted to final concentration with McCoys 5A modified media. Indomethacin was added at the initiation of cell culture 30–60 min before the addition of the PHA. Autologous monocytes separated by their adherence to plastic were added to the mononuclear cell cultures at the beginning or end of liquid culture.

## RESULTS

### *Cell preparations*

The composition of the F/H cell preparations from the 13 normal subjects was as previously described (20). Cytological examination of the F/H cell preparations after 24 h in liquid culture in the presence of PHA revealed $\cong 84 \pm 6\%$ (mean±SD) lymphoid cells, with $\cong 60\%$ of these cells showing blast-like changes, $14 \pm 5\%$ monocytes, and $\cong 2\%$ polymorphonuclear cells. The T-cell subfraction isolated by sheep RBC rosetting (21) showed $86 \pm 6\%$ lymphoid cells ($\cong 70\%$ blast cells) with $14 \pm 6\%$ monocytes. The non-T cells consisted of

69±4% lymphoid cells (≅20% blasts) with 26±7% monocytes. The lymphocytes derived from ipsilateral axillary nodes (negative for metastases of breast cancer) were shown by marker studies to consist of 85±2% (mean±SEM) T lymphocytes, 12±5% B lymphocytes, and <5% of the cells phagocytized latex particles, (measurements kindly performed by Dr. S. Gupta, Sloan-Kettering Institute, New York). Of the cells which adhered to plastic, >95% were capable of phagocytizing latex and zymosan particles as determined by counting 200 cells with an inverted microscope with phase contrast. More than 95% of the cells stained strongly with α-napthylesterase. Constitutive $PGE_2$ synthesis could be demonstrated in the adherent cell cultures; by contrast, no detectable levels of PGE could be found in the isolated nonadherent mononuclear fractions. These latter properties of the adherent cells have previously been reported (6) and are characteristic of monocytes.

## *T-Colony assay*

*Peripheral blood mononuclear cells.* Colony growth, expressed as the mean number of colonies per $10^6$ mononuclear cells (F/H) plated for the individuals studied, is summarized in Table I. The ideal conditions for maximal colony growth were achieved when the time in liquid culture exceeded 7 h, the plated cell number was between 0.5 and 1.0 × $10^6$ cells, and the PHA in the upper and lower gel phases were 0.5 μg/$10^6$ cells and 0.8 μg/ml, respectively. No colony formation occurred when <0.25 × $10^6$ cells were plated. When indomethacin (IM) (1 μM final concentration) was added at the inception of liquid culture, there was an overall 23±9% (mean±SEM) increase in colony counts seen. Nine subjects showed an increase in colony counts (range 12–96%) and this rise was statistically significant in three of the subjects. The average increase in colony counts was similar to

TABLE I
*T-Colony Counts*

| Peripheral blood | Number of times studied | Age | Sex | Colony counts | Colony counts cultured with IM |
|---|---|---|---|---|---|
| | | | | *colonies/$10^6$ mononuclear cells plated ±SEM* | |
| 1 | 17 | 30 | M | 1,395±201 | 2,116±367* |
| 2 | 8 | 25 | M | 1,710±520 | 1,750±227 |
| 3 | 8 | 36 | M | 1,381±390 | 1,707±318 |
| 4 | 5 | 40 | M | 3,552±1,182 | 4,685±1,692‡ |
| 5 | 6 | 25 | F | 2,736±476 | 2,404±424 |
| 6 | 3 | 35 | F | 5,493±501 | 4,994±331 |
| 7 | 3 | 21 | M | 2,484±422 | 3,067±540 |
| 8 | 3 | 40 | F | 1,870±252 | 1,688±483 |
| 9 | 3 | 57 | M | 3,038±280 | 2,719±478 |
| 10 | 3 | 48 | M | 2,733±236 | 4,329±597 |
| 11 | 3 | 31 | M | 684±37 | 921±69‡ |
| 12 | 3 | 27 | F | 1,733±226 | 3,404±599 |
| 13 | 3 | 24 | F | 747±80 | 875±133 |
| Totals | 68 | | | 1,989±156 | 2,450±203§ |
| Lymph node | | | | | |
| A (Normal) | 1 | 20 | F | 4,043±373 | 3,556±111 |
| B (Breast cancer) | 1 | 58 | F | 3,388±638 | 2,492±89 |
| C (Breast cancer) | 1 | 66 | F | 1,492±334 | 1,478±318 |
| D (Breast cancer) | 1 | 47 | F | 2,757±54 | 2,796±360 |
| E (Breast cancer) | 1 | 48 | F | 2,647±31 | 2,908±225 |
| F (Breast cancer) | 1 | 56 | F | 2,346±423 | 2,615±42 |
| Totals | 6 | — | — | 2,779±357 | 2,636±277§ |

* Difference±IM significant $P < 0.02$ in 2-tailed paired $t$ statistic.
‡ Difference±IM significant $P < 0.05$ in 2-tailed paired $t$ statistic.
§ Difference±IM not significant by $t$ test of means, paired $t$ statistic or the Wilcoxon matched pairs signed ranks test.

the increase in $^3$H-tdr uptake caused by the addition of 1 $\mu$M IM, 32±11% in the six subjects (Table I; numbers 1–4, 6, and 7) tested. The difference in augmentation measured by T-colony formation or $^3$H-tdr uptake was not significant by the $t$ test of means or paired $t$ test. In the cumulative analysis of normal subjects, no significant IM effect was noted on T-colony formation as tested by the $t$ test on the means, paired $t$ test, or by the nonparametric, Wilcoxon matched pairs signed ranks test (22). Colony size was generally uniform for a given individual whose cells were cultured at the same time under the same conditions. In almost all cases colonies contained >100 cells, with various numbers of separate cell clusters containing <8 cells also being seen. Such cell clusters did not form colonies in prolonged cultures. There did not appear to be a continuum in the size of colonies containing 50 cells to colonies having >200 cells in any of the cases tested.

*Lymph node cells.* Colony formation could be demonstrated to occur in the noncancer-involved lymph node cells from five patients undergoing surgery for breast cancer or the lymph node removed from the normal subject (Table I). No augmentation of colony number was noted when IM was added to these cultures. As noted previously, the lymph node cells contained <5% monocytes as compared with the F/H preparations which averaged 14%.

*T Lymphocytes.* E-rosette-isolated T lymphocytes gave rise to colonies, whereas the nonrosetting cells, which included B lymphocytes, monocytes, and null cells, failed to give rise to measurable colonies (Fig. 1). No colonies were seen when $<2 \times 10^5$ T cells were plated. As noted previously, ≅20% (equivalent to $2 \times 10^5$ cells) of the non-T-cell subpopulation appeared to contain PHA-responsive cells. Sham-rosetted cells, i.e., mononuclear cells exposed to sheep RBC but not separated into the subfractions, appeared to yield greater numbers of colonies compared with the starting mononuclear cells, but this difference was not significant. Reconstituting the T- and non-T-cell populations to near their starting ratio gave colony numbers comparable to the starting cell population and the T-cell subpopulation.

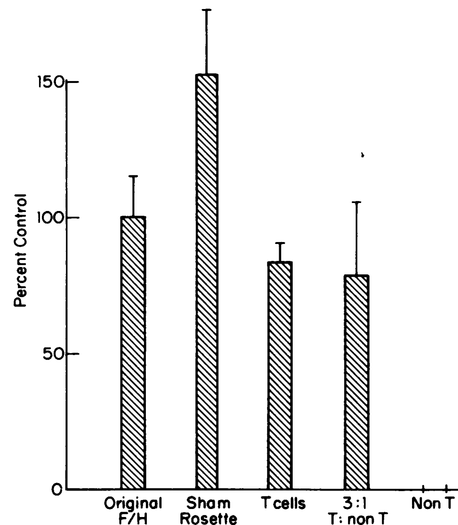


FIGURE 1 Colony counts expressed as a percentage of colonies formed by $10^6$ control F/H cells. F/H cells incubated in the cold overnight with sheep RBC (original F/H), then lysed without separating the subpopulations of F/H cells (sham rosette); mononuclear cells isolated after rosetting with Sheep RBC (T cells) (21); reconstituted subpopulations comprising T and non-T cells at a final ratio of 3:1; and finally those cells which did not form rosettes with sheep RBC (non-T cells) are compared. These data are based on three separate experiments in which the untreated F/H cells gave rise to an average±SEM of 1,322±447 colonies.

## *Colony cells*

Cell clusters representing clones of proliferating cells destined to form colonies were apparent at day 3 of culture at the time when most of the single, nonproliferating cells were disintegrating. Most of the colonies could be found at the surface of the 0.3% agar (Fig. 2), which facilitated counting and colony abstraction. Most of the colony cells were easily removed by washing the 7-d-old plates with cold saline. Each plate yielded $\cong 10^4$–$5 \times 10^4$ cells in which <30% stained with trypan blue. Cytocentrifuge-prepared slides revealed the recovered cells to be mononuclear with nongranular cytoplasm; many blast cells were seen. The nuclei of the colony cells were Giemsa positive, their cytoplasm peroxidase and nonspecific esterase were negative. Greater than 95% of the recovered viable

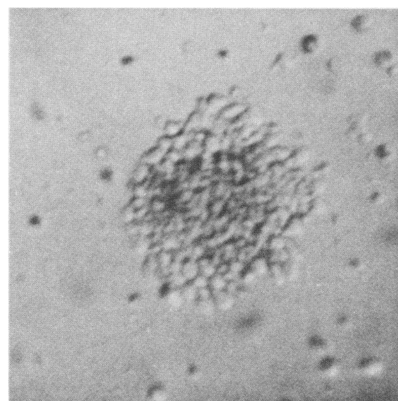

FIGURE 2 Photomicrograph of a T colony on the surface of the 0.3% agar after 6 d in culture. Approximately ×45.

cells formed sheep RBC rosettes both at 4° and 37°C. These findings are similar to previous reports (19, 23–25). Virtually all cell colonies were disintegrating by day 10 of culture.

### *T-Helper and T-suppressor activity of colony cells*

Evaluation of T-helper and T-suppressor functions of pooled T-colony cells was kindly carried out by Dr. F. and Ms. M. Siegal at the Sloan-Kettering Institute, New York. T-Colony cells were harvested from 40 to 50 plates, and all of the cells removed with cold saline were used. No effort to select individual colonies was made. Each study was carried out only once. The addition of T-colony cells ($\cong 10^6$ cells) from subject 1 (Table I) to control mononuclear cells caused an 86% suppression of plasma cell differentiation (from 59 plasmacytoid cells/1,000 F/H cells to 8 plasmacytoid cells/1,000 F/H cells). Prior irradiation to 1,000 rads of the colony cells from subject 1 abrogated the suppression from 86 to 17%. When pooled T-colony cells from subject 7 were tested on control mononuclear cells a 371% increase in plasma cell number was found. Irradiation of the pooled cells before incubation caused a 665% increase in plasma cell number compared with control cells alone. These data suggest that pooled T-colony cells could be shown to have suppressor or helper activity when their ability to regulate plasma cell production was studied (21).

*Synthetic prostaglandins.* The addition of synthetic $PGE_2$ to the cell cultures caused a dose-dependent inhibition of T-colony formation, with a 50% point of inhibition ($I_{50}$) = 0.178 $\mu$M. $PGE_1$ was equivalent to $PGE_2$ in inhibiting colony formation and so the $PGE_1$ and $PGE_2$ data were pooled (Fig. 3). The inhibition caused by PGE could not be removed by washing the cells with fresh media before agar plating. In contrast, $PGD_2$ had no effect on colony formation. $PGF_{2\alpha}$ augmented colony formation from 1 nM to 0.1 $\mu$M in dose-dependent fashion; no further increase was noted at 1 $\mu$M and it appeared to inhibit at 10 $\mu$M (Fig. 4).

*Monocyte modulation of colony formation.* The addition of adherent monocytes to the culture dishes before the placement of the agar overlayers resulted in a progressive reduction of colony formation in those plates (Table II). This inhibition was presumably effected through the release of a diffusable inhibitor because cell-cell contact did not occur. The addition of cell-free media conditioned by $10^6$ monocytes cultured for 25 h caused inhibition of colony formation in F/H preparations when tested from 5 to 15% final (vol/vol) concentrations; augmentation of colony formation was not found (Table II). When increasing numbers of autologous monocytes were added to the F/H mononuclear cells at the initiation or after 24 h of liquid culture, i.e., immediately before agar plating, progressive inhibition of T-colony formation was found. This latter inhibition caused by augmenting endogenous monocyte numbers could be abrogated in most cases if IM (1 $\mu$M) was present in the cultures containing $<10^5$ monocytes; these differences were significant (Table III). There appeared to be some increase of colony formation at the lowest number of added monocytes when they were added at the inception of culture; this difference was not significant.

*PGE levels in lymphocyte culture media.* Endogenous PGE levels were measured by radioimmunoassay in cell-free aliquots taken from the 24-h liquid culture medium before agar plating. The measured PGE levels were compared with the T-colony counts

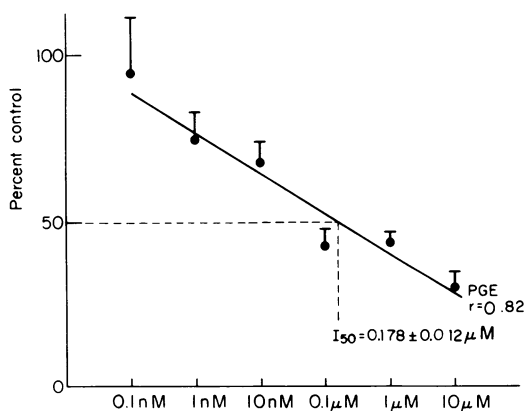


FIGURE 3 Dose-dependency curve for the combined data of $PGE_1$ and $PGE_2$ is shown. The ability of synthetic PGE to inhibit colony formation when added to F/H cell culture is expressed as percentage of control cultures which contained no inhibitor. The $I_{50}$ for PGE is shown. The inverse correlation is highly significant, $P < 0.001$.

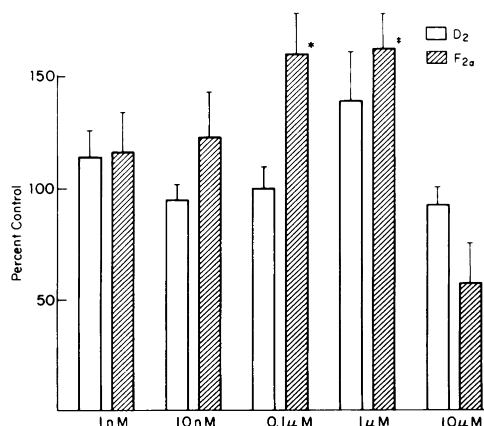


FIGURE 4 Colony counts expressed as percentage±SEM of control colony number formed by $10^6$ F/H cells plotted as a function of increasing $PGD_2$ and $PGF_{2\alpha}$ concentration. $PGF_{2\alpha}$ at 0.1 and 1 $\mu$M were significantly different compared with control. * $P < 0.05$; ‡ $P < 0.01$.

TABLE II
*Effect of Diffusable Factors from Monocyte on T-Colony Formation*

| Number F/H cells plated* | Colony formation percentage of control | Percentage of monocyte conditioned medium | Colony formation percentage of control |
|---|---|---|---|
| | % | % | % |
| 0 | 100 (728±146)‡ | 0 | 100 (2127±130)‡ |
| 2.4 × 10⁶ | 94 | 5 | 19 |
| 4.7 × 10⁶ | 35 | 10 | 7 |
| 9.4 × 10⁶ | 14 | 15 | 3 |

* F/H cells plated into 35-mm dishes. After 1.5 h the plates were washed vigorously to remove nonadherent cells, then adherent monocytes were overlayed with agar, as described in Methods.
‡ Mean±SEM colony numbers of control.

TABLE III
*Effect of Added Monocytes on Colony Formation by F/H Cells**

| Number of added monocytes | Day 1‡ | +IM§ | Day 2‡ | +IM§ |
|---|---|---|---|---|
| Control | 100±3 | 115±20‖ | 100±10 | ND |
| 10,000 | 128±15¶ | 98±10‖ | 80±6** | 119±6‡‡ |
| 25,000 | 74±8§§ | 112±17‖‖ | 68±15§§ | 115±9¶¶ |
| 50,000 | 57±12** | 132±29‖‖ | 29±9*** | 78±13‖‖ |
| 100,000 | 43±7*** | 25±4§§ | 18±2*** | 23±13*** |

* Mean as percentage of control±SEM. Each value represents the mean of six experimental plates, containing F/H cells with their usual concentration of endogenous monocytes (≅14%) to which additional adherent cells have been added.
‡ Monocytes added at inception of liquid culture (day 1) or immediately before plating (day 2).
§ IM at 1 μM in liquid F/H and monocyte cultures.
‖ Difference±IM not significant.
¶ Difference with control not significant.
** Difference with control significant, $P < 0.01$.
‡‡ Difference±IM significant, $P < 0.01$.
§§ Difference with control significant, $P < 0.02$.
‖‖ Difference±IM significant, $P < 0.05$.
¶¶ Difference±IM significant, $P < 0.025$.
*** Difference with control significant, $P < 0.001$.

which were determined in those cultures. A significant correlation ($r = 0.71$, $P < 0.001$) was found when the nontreated data were analyzed by linear regression analysis. In Fig. 5 the data are plotted as the log of colony counts vs. prostaglandin concentration. The higher levels of PGE were consistently found in those cultures to which additional autologous monocytes had been added.

## DISCUSSION

It would appear from the data presented that colonies of mature, functional T lymphocytes which grow in soft agar can arise from peripheral blood mononuclear cells, including E-rosetted subfractions, and from nontumor-involved lymph nodes. The number of colonies which were obtained from blood agreed with previously published studies with similar methods (26–28). Greater numbers of colonies arose from nontumor-involved human lymph nodes than had previously been reported for the mouse (28), or for the human spleen (23). These colonies are presumed to develop as a result of clonal expansion of a small population of precursor T cells which appear to be pluripotent with regard to producing various functional T lymphocytes, though only B-helper/suppressor function and T-killer activities have been tested to date. We hope to report more fully on these latter functional activities where data from individual clones can be presented.

The addition of IM to the F/H cells during the liquid culture phase resulted in an average increase in colony counts of 23%. This finding of augmented proliferation was similar to the mean increase of 32% in $^3$H-tdr uptake measured in six of the normal subjects, but is lower than the mean increase of 58% reported by Goodwin et al. (7) for 15 normal subjects. Whereas it is probable that $^3$H-tdr uptake and colony formation report similar components of mitogen-induced T-lymphocyte proliferation, they may also reflect different aspects. Many more cells incorporate $^3$H-tdr and undergo blast transformation compared with the number of cells which

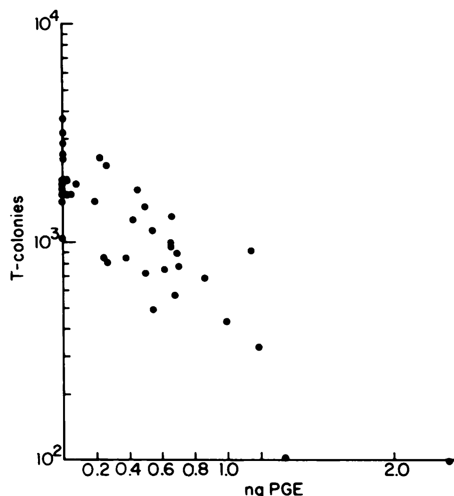


FIGURE 5 PGE levels measured by radioimmunoassay in the cell-free supernates from the 24-h cultures of F/H (human mononuclear) cells just before agar plating. These are compared with the T-colony counts which were subsequently scored for those cultures. A highly significant correlation ($P < 0.001$) was obtained for the 40 cultures which were so measured with the correlation coefficient $r = 0.71$.

can undergo clonal proliferation to produce colonies in soft agar. It is conceivable that the clonal proliferation assay is measuring selected subpopulations of lymphoid cells. On the other hand, it may just reflect the more stringent conditions imposed by growth on soft agar. It is clear however, that the colony assay measures true cell proliferation and offers the possibility of isolating and studying the clones of responding cells. Furthermore, the colony assay can avoid the artifactual interpretation of decreased proliferation as a consequence of the release of nonspecific agents (e.g., of cold thymidine by stimulated macrophages).

The addition of synthetic $PGE_2$ caused a dose-dependent inhibition of colony formation; this inhibition was not removed by subsequently lowering $PGE_2$ level by cell washing. Furthermore, the addition of $PGE_2$ 24 h after PHA initiation of T-lymphocyte proliferation also caused dose-dependent inhibition of T-colony formation, which would suggest that precursor cell activation and proliferation as well as clonal cell division could be inhibited by $PGE_2$. The dose-response curve of $PGE_2$-mediated inhibition extended over several logs with a 50% inhibition at 0.178 $\mu$M. The inhibition curve and $I_{50}$ are quite similar to the results of $PGE_2$ inhibition of $^3$H-tdr uptake previously reported (7). Testing other prostaglandins in the colony assay revealed additional complexities. $PGE_1$ was found to be equivalent in its inhibitory activity as $PGE_2$. This differs from the CFU-c system where $PGE_1$ was reported to be more potent than $PGE_2$ (1). $PGD_2$ was found to have no effect on T-colony formation, which demonstrates the sensitivity of the precursor T cells to the stereospecificity of the constituents about the pentane ring portion of the prostaglandin molecule. As further evidence of the complexity of prostaglandin effect, the addition of $PGF_{2\alpha}$ to the mononuclear cell cultures gave a different response. Whereas low concentrations of $PGF_{2\alpha}$ showed dose-dependent stimulation of colony formation, at the higher concentrations tested $PGF_{2\alpha}$ (10 $\mu$M) was inhibitory. This inhibition may have been a result of $PGE_2$ contamination or a $PGE_2$-like effect seen at high doses of $PGF_{2\alpha}$.

Inhibition of T-colony proliferation consistently occurred when autologous monocytes were added to the F/H cultures. This inhibition seemed likely to be mediated in part through monocyte elaboration of $PGE_2$. Several earlier reports have identified the monocyte/macrophage as the prime immune-cell producer of $PGE_2$ (4–7) in guinea pig, mouse, and human; and $PGI_2$(5) in the mouse. When taken together, our data showed (*a*) that cyclooxygenase inhibitors such as IM enhanced colony formation and abrogated monocyte-mediated inhibition of colony formation at low monocyte numbers, and (*b*) that synthetic PGE mimicked the effect of monocytes. These findings would appear to implicate monocyte prostaglandin production as one of the physiologically relevant regulatory mechanisms of T-lymphocyte proliferation. Such a conclusion would be consistent with previously published evidence that monocyte-derived PGE inhibits CFU-c and B-lymphocyte clonal expansion (2, 3, 9). It should be noted that the dose-response curve for synthetic PGE extended over several log orders (Fig. 3) whereas much greater inhibition of colony formation was found at considerably lower measured PGE levels in the cultures to which monocytes had been added (Fig. 5). These experiments are not truely comparable because, as shown in Fig. 3, only synthetic PGE was added, whereas when monocytes were added other, more potent, cyclooxygenase products (e.g., $PGI_2$) may have been coincidentally released. In this latter case, there would still be a correlation of colony inhibition with measured PGE levels and IM would abrogate the inhibition. It is also possible that PGE was metabolized to PGA, PGB, or $PGF_{2\alpha}$ (29) during the incubation period resulting in lower measured levels of PGE. Finally, it would appear that other nonprostaglandin inhibitors of T-lymphocyte proliferation are produced by monocytes. When $>0.5 \times 10^6$ monocytes were added to the cultures, IM at a dose 100 times that necessary to inhibit cyclooxygenase could not reverse the inhibition.

The clonal expansion of the circulating precursor T lymphocytes gives rise to several types of effector T cells. With an established system that measured B-lymphocyte maturation by their ability to synthesize immunoglobulins (21) it could be shown that pooled T-colony cells expressed both B-helper and suppressor activity. Moreover, in a system in which killer T cells were generated it can be demonstrated that macrophage inhibition could be mimicked by PGE and abrogated by IM (30). Other functional subsets of T lymphocytes are being studied to ascertain whether monocyte/macrophage synthesis of prostaglandins modulates immunoresponsiveness by selectively inhibiting the clonal expansion of functional T-lymphocyte subpopulations.

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